STUDIES ON DIBENZ[*d,f*]AZECINE AND OTHER PHENETHYLISOQUINOLINE-DERIVED ALKALOIDS

by

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CONTENTS

ABSTRACT

STUDIES ON DIBENZ[*d,f*]AZECINE AND OTHER PHENETHYLISOQUINOLINE-DERIVED ALKALOIDS

The initial goal of this study was to develop a targetted and systematic search method for new examples of the known groups of phenethylisoquinoline-derived alkaloids with special emphasis on the least represented group, the dibenz[*d,f*]azecines. A considerable part of the work was devoted to the successful development of a search procedure using the combined gas chromatography-mass spectrometry (GC-MS) technique as the main tool. This included establishing working parameters for the GC-MS screening of this family of alkaloids; synthesizing some dibenz[*d,f*] azecines from possible new sources; and re-screening some known sources, with special attention being given to the minor components.

Re-screening of *Dysoxylum lenticellare*, the only reported source of a naturally occurring dibenz[*d,f*]azecine alkaloid, along with six other species of *Dysoxylum* showed the absence of dibenz[*d,f*]azecines and other phenethylisoquinoline-derived bases. Doubts have now been raised as to the actual identity of the plant. *Athrotaxis selaginoides*, a homoerythrina-containing plant species, was shown to contain an extremely minor component of a dibenz[*d,f*]azecine base. The very low concentration did not allow its isolation.

Attention was shifted to the Family Liliaceae, from which the majority of the reported phenethylisoquinoline-derived alkaloids have

been isolated. Known phenethylisoquinoline-containing plants, and more than thirty other liliaceous species, were screened for dibenz[*d,f*]azecines. Their presence was detected only in species of *Wurmbea* and *Baeometra*.

At least eight new dibenz[*d,f*]azecine bases were detected and one named wurmbazecine, isolated pure from *Wurmbea dioica* ssp. *dioica*. The amount of this isolate limited the n.m.r. experiments that could be done on it leaving some ambiguity in its substitution pattern. Probable structures were therefore synthesized to confirm its structure, using the eliminative ring destruction approach from tetracyclic amino ketone precursors. A biogenesis of wurmbazecine is also proposed.

The conversion of the known homoerythrina alkaloid homoerythratine has led to the formation of some A-ring reduced dibenz[*d,f*]azecine derivatives whose mass spectral fragmentation patterns are distinct from those where the A-ring is oxidized.

As a result of the alkaloid screening work, over twelve new, as well as known, wurmbaeiodeae alkaloids such as the 1-phenethylisoquinoline, homomorphines, homoaporphines, homoproaporphines, and colchicine-types from *Wurmbea dioica* ssp. *dioica* were isolated and their structures determined. Known alkaloids were also isolated from *Gloriosa plantii*, *Wurmbea pygmaea*, *Baeometra uniflora* and *Colchicum autumnale*. Other phenethylisoquinoline-type alkaloids were detected, but not isolated, from other plant species.

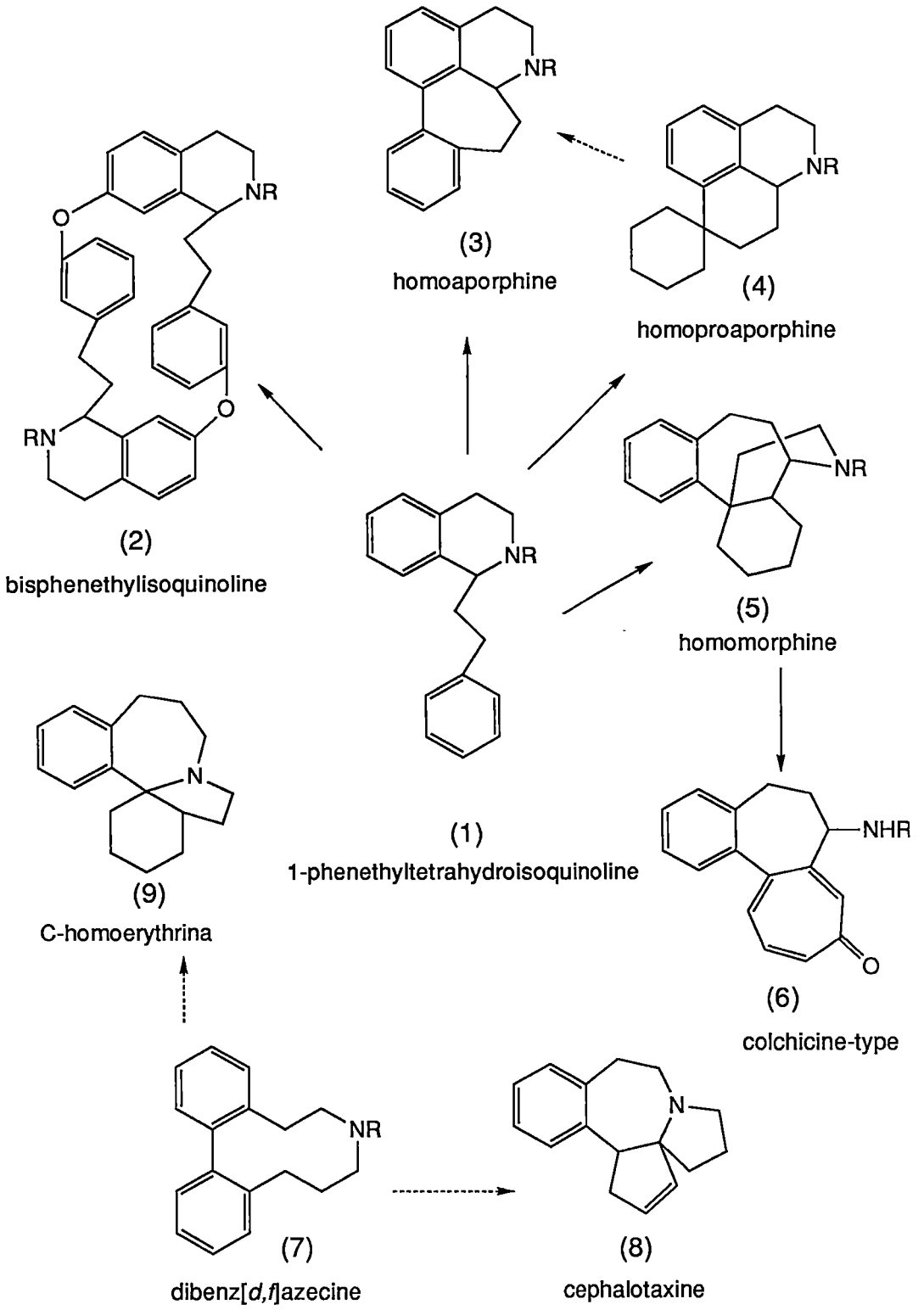
Twelve homoerythrina-type alkaloids were also isolated and characterized from the liliaceous plants *Burchardia umbellata* and *Burchardia multiflora* as well as from *Athrotaxis selaginoides* (Taxodiaceae). The ring D modification of the two new bases isolated from *Burchardia umbellata* is the first of its kind; their structures were established by spectroscopic methods.

The search for the dibenz[*d,f*]azecine alkaloids from the family Liliaceae has allowed an in-depth study of the family's alkaloid profile. A summary of this is briefly discussed in the last discussion Chapter of the thesis, together with some possible chemotaxonomic implications.

Chapter 1
THE PHENETHYLISOQUINOLINE - DERIVED ALKALOIDS

1.1 General Introduction

The phenethylisoquinoline-derived alkaloids represent a group of alkaloids of widely diverse structures and functionality based on the simple 1-phenethyltetrahydroisoquinoline system. Although not recognized as such until the early 1970's[1], colchicine was the first representative of the group, being isolated in the 1920's. In the mean time, during the early 1960's and onwards, other members of this group have been found in nature and characterized. Nine subgroups have been identified from terrestrial plant sources (Scheme 1.1): the simple 1-phenethyltetrahydroisoquinoline (1), bisphenethylisoquinoline (2), homoaporphine (3), homoproaporphine (4), homomorphine (5), colchicine (6), dibenz[*d,f*]azecine (7), cephalotaxine (8), and C-homoerythrina (9) types. Of the nine groups known, the colchicine, cephalotaxine, and the homoerythrina groups are so far well represented. The homoproaporphine group has about twenty two representatives, while the homoaporphines and the homomorphinanes have sixteen each. To date, there is only one example of the dibenz[*d,f*]azecine, two of the bisphenethylisoquinoline, and six for the 1-phenethylisoquinoline bases (Table 1.1). The recently reported 1-phenethylisoquinoline bases from *Colchicum szovitsii*[2] were the first trioxygenated alkaloids of this type.



Scheme 1.1 Skeletal Interconversions of the Phenethylisoquinoline-Derived Alkaloids (·····► proposed interconversion but not fully backed up by tracer studies).

Table 1.1 Distribution of the Phenethylisoquinoline-Derived Alkaloids

Family	Genus	HE	DB	PE	BP	HA	HP	HM	CO	CE	REFERENCES
Liliaceae	*Baeometra*								+		3
	Camptorrhiza								+		4
	Iphigenia					+	+	+	+		4, 5, 6
	Ornithoglossum						+	+	+		5
	Neodregea								?		7, 8
	Dipidax								+		3, 5
	Wurmbea								+		3
	Kreysigia					+	+	+	+		6, 9-13
	Androcymbium				+			+	+		3, 14-17
	Bulbocodium						+		+		4, 18, 19
	Colchicum			+		+	+	+	+		2, 5, 9, 18, 20-48
	Merendera				+	+	+	+	+		3, 5, 9, 18, 49-59
	Gloriosa					+	+		+		18, 20, 60, 61
	Littonia								+		20, 62
	Sandersonia								+		62
	Schelhammera	+									63-66
Meliaceae	*Dysoxylum*	+	+	+							67-70
Taxodiaceae	*Athrotaxis*	+									71-72
Phellinaceae	*Phelline*	+									73-81
Cephalotaxaceae	*Cephalotaxus*	+								+	83

HE homoerythrina; **DB** dibenz[*d,f*]azecine; **PE** 1-phenethylisoquinoline; **BP** bisphenethylisoquinoline;**HA** homoaporphine; **HP** homoproaporphine; **HM** homomorphine; **CO** colchicine and derivatives; **CE** cephalotaxus alkaloids

Except for the homoerythrina, cephalotaxine, 1-phenethylisoquinoline, and the dibenz[*d,f*]azecine bases, the rest of the known phenethylisoquinoline-derived alkaloids are restricted to the family Liliaceae, specifically in the subfamily Wurmbaeoideae[84]. The phenethylisoquinoline-derived wurmbaeoideae alkaloids were classified by Santavy[85] into two main subgroups: the tropolonic alkaloids consisting of the colchicine alkaloids and their derivatives, and the non-tropolonic group, which included the simple 1-phenethylisoquinolines, homomorphines, homoproaporphines, and homoaporphines. Two genera of the subfamily Wurmbaeoideae were also reported to contain aporphine-type alkaloids[3,4],but their relationship with the other phenethylisoquinoline-derived alkaloids has not been established.

There have been several reviews of these alkaloids. One, published by Kametani and Koizumi[86] in 1973, covered most of the known non-tropolonic phenethylisoquinoline skeletal type, except the dibenz[*d,f*]azecine type; a follow up to this review was published in 1989[87]. Separate reviews by Santavy[85], covering the tropolonic and the non-tropolonic alkaloids of the subfamily Wurmbaeoideae, and by Tojo[88], on the homoaporphine alkaloids were also published. A review on the erythrina and the homoerythrina alkaloids was published by Dyke and Quessy in 1981[89], and followed up by Chawla and Jackson[90a,90b] who briefly covered some newer homoerythrina alkaloids, including the only naturally-occurring dibenz[*d,f*]azecine alkaloid, dysazecine. A current review of the homoerythrina alkaloids has been completed by Bick and Panichanun[91].

1.2 Distribution

The phenethylisoquinoline-derived alkaloids have so far been isolated from very diverse plant sources covering five unrelated plant families (Table 1.1) namely: the Liliaceae (Monocotyledon), Cephalotaxaceae (Gymnosperm), Taxodiaceae (Gymnosperm), Meliaceae (Dicotyledon), and Phellinaceae (Dicotyledon). The homoerythrina-type alkaloids are the only ones that have so far been found in all the five families. The Tasmanian endemic *Athrotaxis* spp. (Taxodiaceae), some species of *Cephalotaxus* (Cephalotaxaceae), *Phelline* (Phellinaceae), *Schelhammera* (Liliaceae) and *Dysoxylum* (Meliaceae) have all yielded some homoerythrina alkaloids. However, a current re-investigation of the species *Dysoxylum lenticellare* (Chapter 2), which was reported to contain the first naturally-occurring dibenz[*d,f*]azecine[68], casts doubts on the actual identity of this plant.

The simple 1-phenethyltetrahydroisoquinolines have been isolated from the families Liliaceae (subfamily Wurmbaeoideae) and Meliaceae only, although they could also be present in the other plant families containing phenethylisoquinoline-derived alkaloids but in trace amounts. The tropolonic and the non-tropolonic wurmbaeoideae bases have been isolated or detected in the genera *Gloriosa, Littonia, Sandersonia, Ornithoglossum, Camptorrhiza, Iphigenia, Colchicum, Androcymbium, Bulbocodium, Merendera, Baeometra, Dipidax,* and *Wurmbea*, all of the subfamily Wurmbaeoideae (Liliaceae). The isolation of some homomorphine, homoproaporphine, and homoaporphine alkaloids from the genus *Kreysigia* is chemotaxonomically significant in that Santavy[7,8] has now included this genus in the subfamily Wurmbaeoideae.

Of interest is the fact that although the alkaloids of the homoerythrina-type are found in the family Liliaceae, they have not been found to co-occur with the wurmbaeoideae alkaloids, thus, they are treated as a separate group. To date, among the homoerythrina-containing species, only the reported *Dysoxylum lenticellare*, whose identification is now in question, was found to contain other phenethylisoquinoline-derived alkaloids such as the simple 1-phenethylisoquinoline and the dibenz[*d,f*]azecine types[68].

1.3 Pharmacology

Particular interest is focussed on the phenethylisoquinoline derivatives, as they are homologous to the important class of alkaloids, the benzylisoquinolines, which are known for their very wide range of pharmacological activities[92,93] as in the important applications of the morphine alkaloids. There are already reports on halogenated simple 1-phenethyltetrahydroisoquinoline derivatives as analgesics and antitussives[94]. Depending on the mode of dosage, methopoline, a chlorinated 1-phenethylisoquinoline can be as strong as codeine as an analgesic, and it is non-addictive[95]. In both reports the enantiomer with the R-configuration was found to be more active. Some homoproaporphine alkaloids have been reported as useful drugs acting on the central nervous system[96], and as drugs possessing analgesic and sedative properties[97]. On the other hand, the total base of *Tripladenia cunninghamii* (*Kreysigia multiflora*), composed of colchicine, homoaporphine and homomorphine alkaloids was also found to have

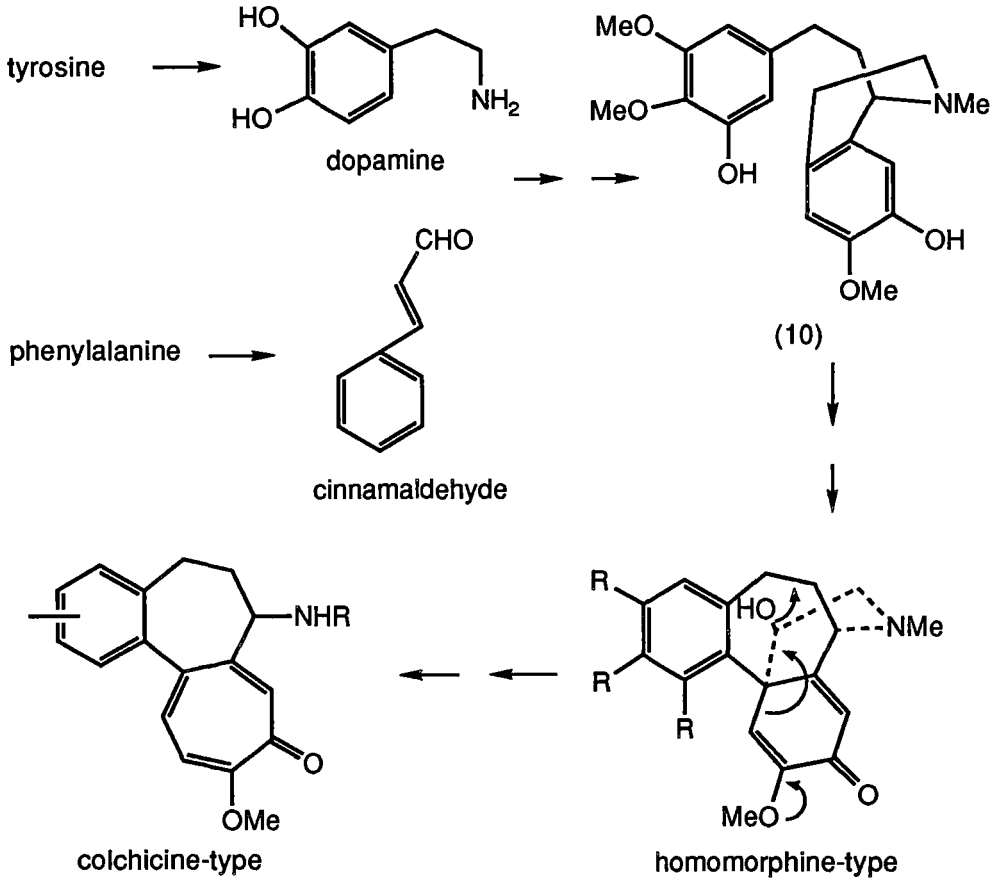
some weak central nervous system activity (CNS) as well as analgesic and antipyretic properties[98].

Some homoerythrina alkaloids from *Dysoxylum lenticellare* were found to possess a moderate molluscicidal activity[67]. The total base from *Kuntheria pedunculata* (*Schelhammera pedunculata*) and the alkaloids schelhammeridine and schelhammerine were found to act as antipyretics and as weak analgesics; they also possess some weak CNS activity[98]. Colchicine on the other hand has long been used, and continues to be used, to treat gout[99,100]. It also exhibits very potent antimitotic activity and thus, has been considered as a potential anti-cancer agent. The colchicine-derived compounds have attracted a lot of research studies to explore potency, tubulin binding ability, and toxicity with a view to developing more effective and more selective anti-gout and antitumour agents. Likewise, the cephalotaxine alkaloids and their derivatives have also been widely studied. Clinical trials are on-going to maximize their application as antitumour agents[83].

1.4 Biosynthesis

As the name implies, the common structural feature of the phenethyltetrahydroisoquinoline alkaloids is the tetrahydroisoquinoline moiety. In some groups, as in the homoaporphine alkaloids, this structural unit is easily discernible; in others, as in colchicine and derivatives, it is obscured in the complexity of the structure. Biosynthetically, this tetrahydroisoquinoline moiety is derived from the common precursor of this class of alkaloids, a 1-phenethylisoquinoline-type alkaloid such as autumnaline (10) (Scheme 1.2). Initial

experiments on the biosynthesis of colchicine, conducted by Leete[101,102] and Battersby[103] and co-workers with *Colchicum autmnale* and *Colchicum byzantinum* supported the theory that autumnaline (10) is biosynthesized from phenylalanine and tyrosine by way of cinnamic acid and dopamine, respectively. It was later established by Herbert and Knagg[104], in a feeding experiment carried out with corms of *Colchicum byzantinum,* that it is cinnamaldehyde from cinnamic acid which condenses with dopamine to form autumnaline or its derivatives.



Scheme 1.2 Biogenetic pathway of colchicine-type alkaloids

The different groups of the phenethylisoquinoline series of alkaloids are in turn derived from the 1-phenethylisoquinoline precursor by phenolic oxidative coupling involving the formation of C-C or C-O

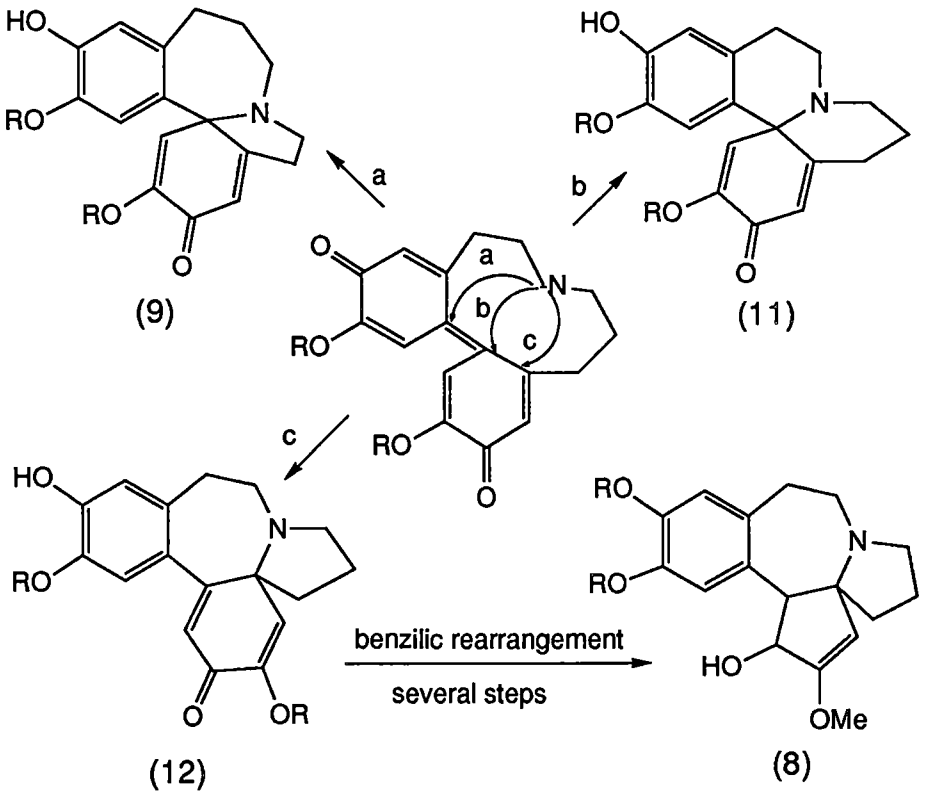
bonds. Thus bisphenethylisoquinolines may be biosynthesized by phenolic oxidative coupling involving one of the phenolic oxygen atoms[105].

By analogy with the biosynthesis of the aporphines, there are two most probable biosynthetic pathways for the homoaporphines. The phenolic oxidative coupling of autumnaline (10) or its derivatives may form homoaporphine-type alkaloids directly. Alternatively, a homoproaporphine-type product may be formed, which may then rearrange to a homoaporphine skeleton. Tracer studies with *Kreysigia multiflora*[106, 107] showed that homoaporphines in this plant are derived directly from autumnaline by ortho-para coupling.

The biosynthesis of the homomorphines was indirectly verified[1,103, 108.109,110] as a result of the study of the biosynthesis of colchicine. Various tracer experiments conducted by Battersby and co-workers[1] confirmed the proposed biogenetic route from autumnaline to colchicine with a homomorphinandienone as a key intermediate (Scheme 1.2). This was supported further by the isolation of a homomorphine-type alkaloid, O-methylandrocymbine from *Androcymbium melanthioides*[111]. This homomorphinandienone intermediate was proposed to undergo ring expansion to form the tropolone ring of the colchicine skeleton[112].

The isolation of the base schelhammeridine[63], from *Schelhammera pedunculata* provided a clue to the biogenetic origins of the C-homoerythrina alkaloids. The incorporation experiments by Battersby and co-workers[113] showed that its immediate precursor was a 1-phenethylisoquinoline-type as phenylalanine was incorporated in the

A-ring of schelhammeridine, while tyrosine was incorporated in the B-ring. By comparison with the erythrina group of the benzylisoquinoline series of alkaloids, a dibenz[*d,f*]azecine-type base was proposed as a biogenetic intermediate to the C-homoerythrina alkaloids[63,68,73]. In support of this proposal, a dibenz[*d,f*]azecine named dysazecine was isolated from *Dysoxylum lenticellare* along with some simple 1-phenethylisoquinoline and some C-homoerythrina alkaloids[68]. No further biosynthetic studies have been performed to verify this proposal.



Scheme 1.3. Proposed biogenetic interconversions to the Homoerythrina-type and the Cephalotaxus Alkaloids

Powell[114] speculated on common biogenetic origins of the homoerythrina and the cephalotaxine alkaloids based on the co-existence of these two types of alkaloids in the genus *Cephalotaxus*. He proposed that both alkaloid types are divergent products of a

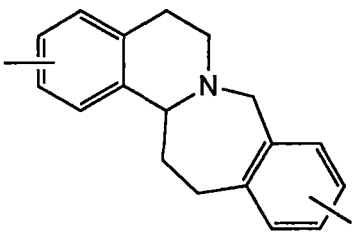
common dibenz[*d,f*]azecine-type precursor (Scheme 1.3). The ring closure of a dibenz[*d,f*]azecine intermediate via *mode a* gives rise to a C-homoerythrina skeleton (9), or via *mode b* to form a B-homoerythrina-type product (11). An alternative route as in *mode c* could lead to the formation of an intermediate (12) which undergoes benzilic rearrangement with subsequent ring contraction due to loss of a carbonyl group to form the cephalotaxine skeleton. This speculation was confirmed by a precursor incorporation experiment conducted by Parry and co-workers[115,116].

1.5 Hypothetical Phenethylisoquinoline Alkaloids

By analogy with the very diverse benzylisoquinoline group of alkaloids, more skeletal types might be expected to be derived from the 1-phenethylisoquinoline-type precursors. Their oxidation and condensation derivatives could be modified further to more complex skeletal systems as in the case of colchicine and cephalotaxine. This is more so in the case of systems like homoprotopine and homoprotoberberine, whereas in contrast to their protopine and protoberberine analogues, an additional carbon in the aliphatic bridge may induce more pronounced deformations, hence an increase in transannular or other steric interactions.

In the past, some phenethylisoquinoline-type alkaloids were predicted to exist in nature. Some of these alkaloids were later isolated and characterized. The existence of the dibenz[*d,f*]azecine alkaloids was first predicted by Fitzgerald and co-workers[63] in their report on the isolation of some homoerythrina alkaloids. This alkaloid type has now

been isolated from *Dysoxylum lenticellare* along with some homoerythrina-type alkaloids. Despite the isolation of the C-homoerythrina bases from various plant sources, no B-homoerythrina-type alkaloid (11) has ever been isolated. The homoprotoberberine skeleton (13), which may be derived biosynthetically from a 1-phenethylisoquinoline precursor via a Mannich-type condensation, was anticipated by Battersby[117] and was synthesized[118-121] following various routes. Although a homoprotoberberine (14) was isolated[210] from *Berberis acticantha* it cannot possibly be derived from a Mannich-type condensation of a 1-phenethylisoquinoline-type precursor as the berberine bridge of the proposed structure has two carbon atoms instead of one , while the bridge opposite to it has only one . This base could be a benzylisoquinoline-derived alkaloid like most of the alkaloids of *Berberis* species.



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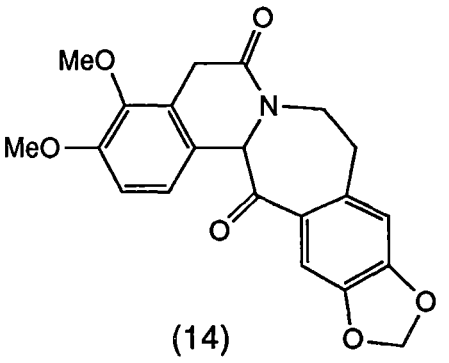
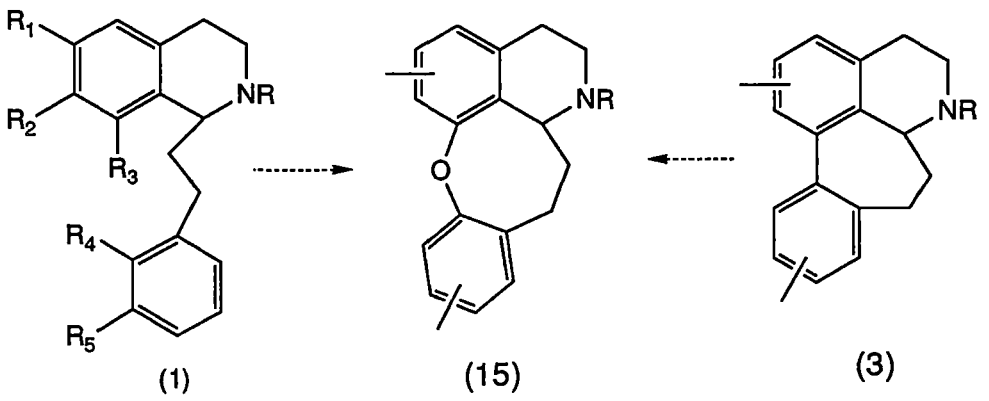


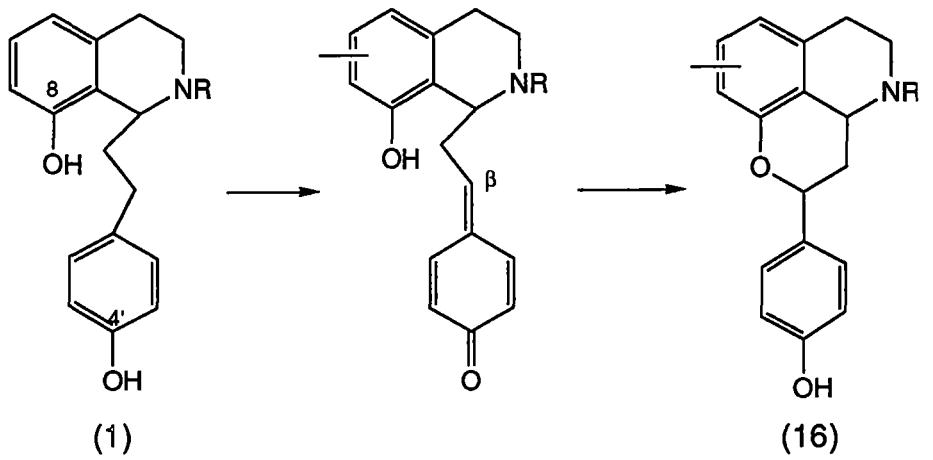
(14)

More oxidation or condensation products might be expected from the 1-phenethylisoquinoline derivatives as in cases analogous to the benzylisoquinoline class of alkaloids. The additional one carbon in the 1-alkyl substituent of the isoquinoline could hinder possible ring closures due to ring size effects, however, plants are generally able to overcome this by enzymatic catalysis. A homocularine-type base (15) might therefore be biosynthesized from a phenolic oxidation of a hydroxy

derivative (1; R_4 = OH) of a 1-phenethylisoquinoline precursor (Scheme 1.4). Alternatively, it may be derived from the oxidation of a homoaporphine skeleton (3). Although no alkaloid of this skeleton has been isolated, it has however been synthesized via an intramolecular Ullmann reaction of a brominated phenethylisoquinoline and predicted by Kametani and Terui[87] as possibly naturally-occurring. If the phenethylisoquinoline-type precursor (1) has 8- and 4'- hydroxy groups, and if $C_{2'}$ or $C_{1'}$ is oxidized, a homologue of the quettamine[122] skeleton (16) may also be considered possible (Scheme 1.5).



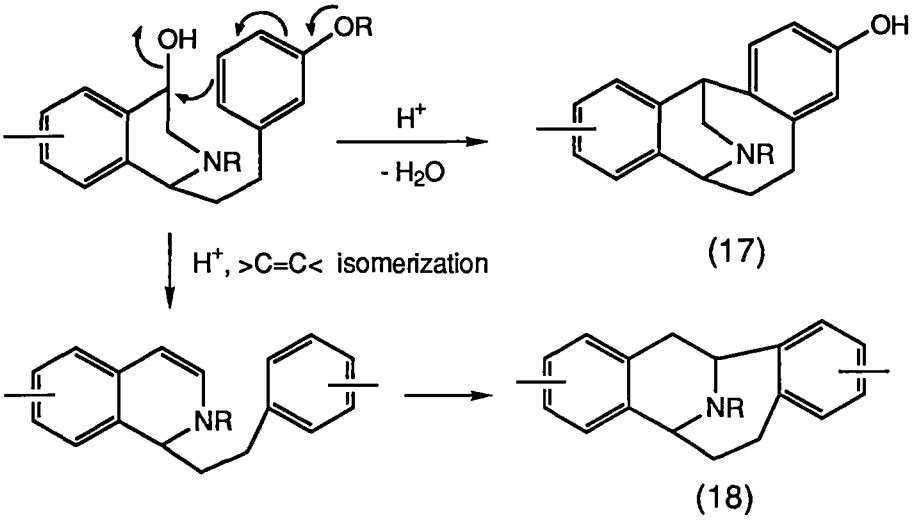
Scheme 1.4



Scheme 1.5

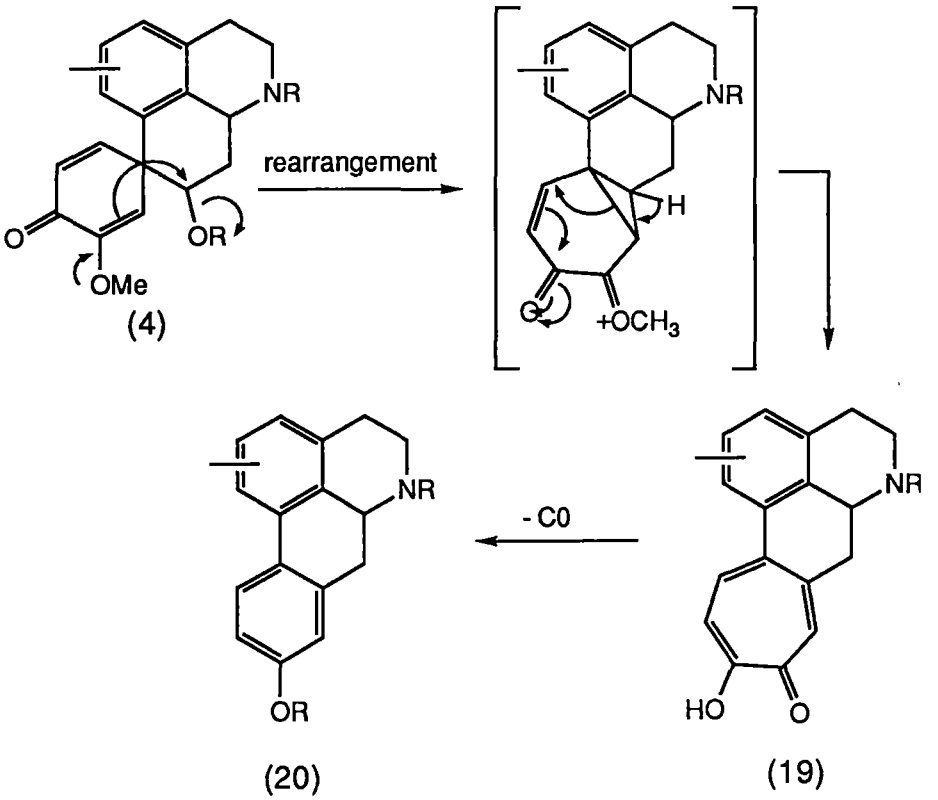
As in 1-benzylisoquinolines, 1-phenethylisoquinoline derivatives where C-4 is oxygenated may cyclize directly to form a homoisopavine

skeleton (17) or undergo dehydration at C-4 and subsequent double bond isomerization to cyclize into a homopavine skeleton (18) instead (Scheme 1.6). Several examples of unnatural homopavine and homoisopavine alkaloids have been synthesized and published[124,125,126,127].



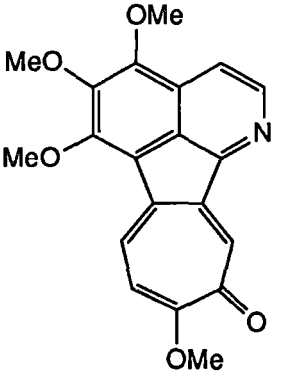
Scheme 1.6

The cyclization of a 1-phenethylisoquinoline derivative into a homoproaporphine skeleton by phenolic oxidative coupling may also yield intermediates not only for the possible formation of homoaporphine-type bases but could possibly give rise to other rearrangement products (Scheme 1.7). The migration of one of the carbons at the spiro center of the homoproaporphine skeleton which may be induced by a nitrogen atom in ring B or by oxygenated substituents at rings C and D, brings about the expansion of ring D thereby forming a homoproazafluoranthene-type product (19). This skeleton (19) may undergo the loss of a carbonyl group to form a homoazafluoranthene whose skeleton is identical with that of an aporphine base (20). Although there has been no precedence of the



Scheme 1.7

carbonyl group being lost from a tropolone ring, the isolation of imerubine (21)[128] and its co-occurrence with azafluoranthenes in *Abuta* spp., supported Silverton and co-workers' proposed[128] biogenetic route in which the tropolone ring of the imerubine-type skeleton loses a carbonyl group to form the six-membered D-ring of the azafluoranthene (22). This is very significant biosynthetically as the aporphine bases, corydine and isocorydine have been isolated from *Baeometra collumellaris* Salisb.(Syn. *Baeometra Uniflora* (Jacq.) Lewis)[3] and *Camptorrhiza strumosa*[4] which are known to biosynthesize phenethylisoquinoline-derived alkaloids.



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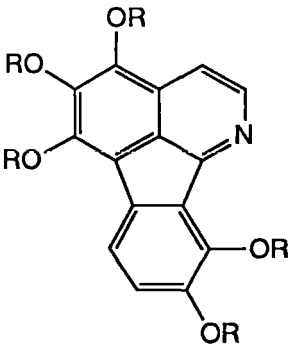
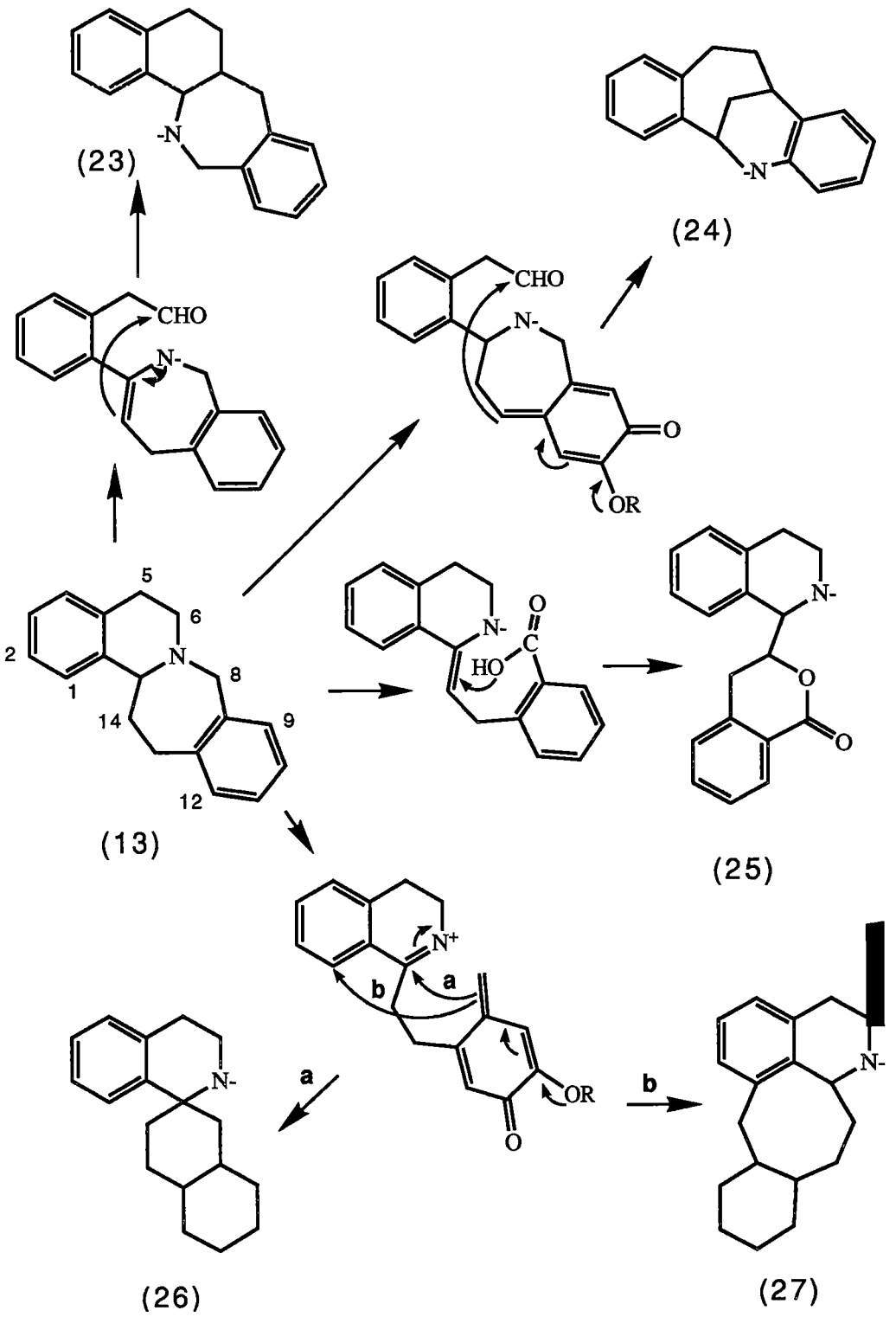


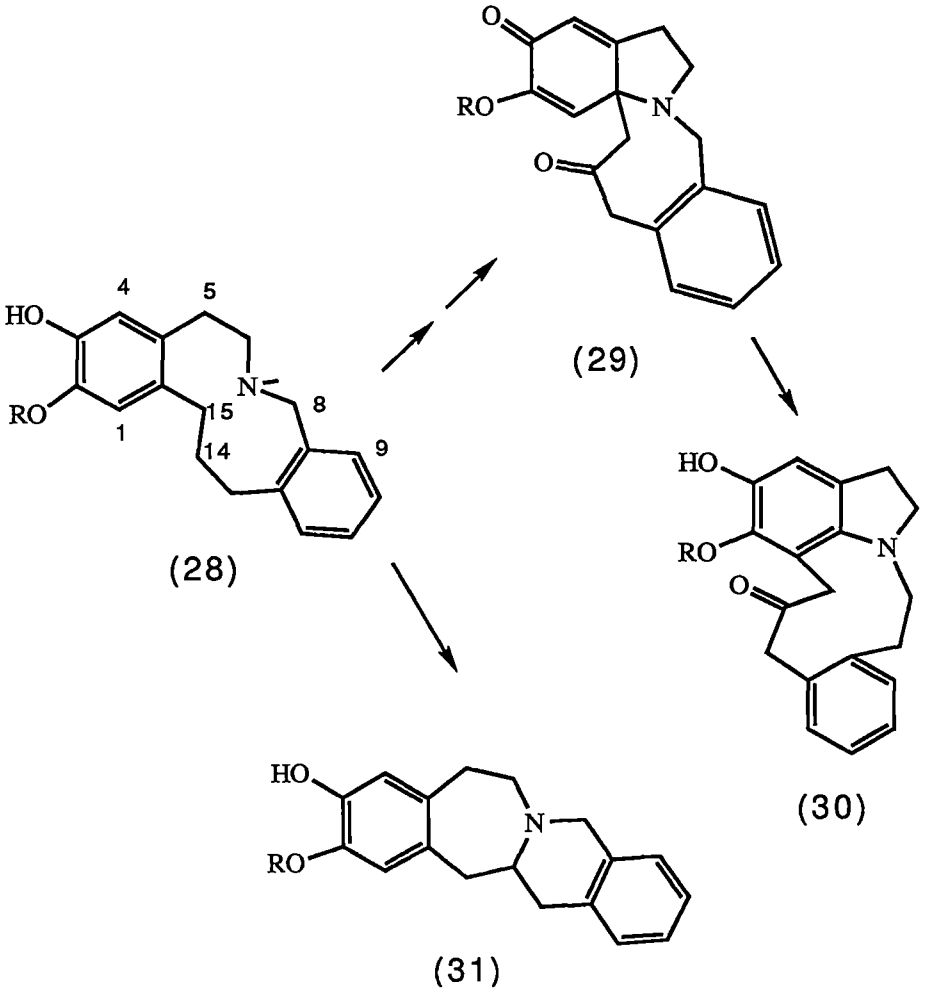
(22)

Protoberberine and the protopine analogues are important intermediates in the biosynthesis of a wide variety of benzylisoquinolines[129]. Consequently, the C-homoprotoberberines (13) and their ring-opened derivatives, the homoprotopines (27), may also give rise to a whole new range of phenethylisoquinoline-derived bases. As in benzophenanthridines, a homoprotoberberine skeleton may undergo an oxidative cleavage at the C_6-N bond and subsequent condensation of C_6 and C_{14} giving rise to a homobenzophenanthridine (23) (Scheme 1.8). Alternatively, oxidation of ring D after C_6-N cleavage may lead to C_6-C_{13} ring closure by condensation to form a homoisobenzophenanthridine (24). The berberine bridge of the homoprotoberberine skeleton may also be oxidized at C_8 which could then condense with C_{14} to form a homophthalideisoquinoline base (25). Alternatively, C_8 may also condense with C_{14a} where the ring B is oxidized, to give rise to a spirophenethylisoquinoline-type skeleton (26) or instead, with C_1, by methoxy or hydroxy-assisted nucleophilic addition to form the new skeleton (27) which may also be naturally-occurring.



Scheme 1.8

The homoprotopine (28) base may behave in a similar manner to the dibenz[*d,f*]azecines where nitrogen could be involved in an intramolecular Michael-type addition after ring oxidation to form a new spiro skeleton (29) (Scheme 1.9). This spiro skeleton may rearrange to form a 9-membered heterocyclic ring (30). Also an unsaturated center at C_{14}-C_{15}, possibly formed from the dehydration of C_{15}-hydroxylated homoprotopine, may allow addition of the nitrogen to C_{14} giving rise to a B-homoprotoberberine (31).



Scheme 1.9

1.6 Purpose of the Study

While there is a sustained interest in the newly emerging group of phenethylisoquinoline-derived alkaloids in general, this study has focussed primarily on the dibenz[*d,f*]azecine systems. The significant application of some medium-sized heterocycles such as 1,4-benzodiazepine[130a] and 2,5-benzoxazocine[130b] directed considerable attention to the fused medium-sized and related heterocycles. Along this line, the isolation of new medium-sized heterocycles is seen as an anchor to look at the chemistry and activity of medium sized heterocycles in general and to provide some basis for designing new pharmacologically active compounds. Biosynthetically, the dibenz[*d,f*]azecine system has already demonstrated its fascinating chemistry in the formation of the C-homoerythrina and cephalotaxine alkaloids. Moreover, it is of interest to look at the pharmacological activity of the dibenz[*d,f*]azecines, as some of its nine-membered ring analogues, the dibenz[*d,f*]azonines have been reported to have a wide range of activities including central nervous system antiarrhythmic, local anaesthetic and hypotensive activities[131]. Thus there is a significant possibility for this group of compounds to be biologically active as well.

There is only one naturally occurring dibenz[*d,f*]azecine known so far. The main objective of this study was to identify plant sources of these alkaloids, and to isolate and characterize further examples of the known as well as new groups of phenethylisoquinoline-derived bases. It was also planned to study their spectroscopic and pharmacological properties where possible. The isolation of dibenz[*d,f*]azecines from other sources would also provide the possibility of further insights into the biosynthesis of this group of alkaloids. Furthermore, the search for

possible sources of this alkaloid group could provide a vehicle for the development of targetted search methods for minor new alkaloids and hence, for minor new metabolites in general.

In Chapter 2, the alkaloid search methodology is discussed. The latter part of the chapter describes the results of the GC-MS screening for the phenethylisoquinoline-derived alkaloids in general and the dibenz[*d,f*]azecines in particular. The isolation and characterization of the dibenz[*d,f*]azecines detected in *Wurmbea* species is given in Chapter 3. The chapter also includes the synthesis of some dibenz[*d,f*]azecine derivatives to confirm the structure of an isolated dibenz[*d,f*]azecine alkaloid named wurmbazecine as well as to establish their fragmentation patterns. Based on the isolated dibenz[*d,f*]azecine, a biogenetic route to these types of bases is proposed. The chemical transformations of some known alkaloids to dibenz[*d,f*]azecine derivatives are also included in this chapter.

The structural elucidation of the other wurmbaeoideae alkaloids isolated from *Wurmbea* species is described in Chapter 4. Results of the re-investigation of the alkaloids of *Baeometra collumellaris* (syn. *Baeometra uniflora*), *Colchicum autumnale*, *Gloriosa plantii*, *Littonia modesta*, and *Sandersonia aurantica* are briefly included in this chapter. Chapter 5 discusses the isolation and structural elucidation of the homoerythrina-type alkaloids isolated from *Athrotaxis selaginoides*, *Burchardia umbellata*, and *Burchardia multiflora.* Finally, Chapter 6 gives an overview of the alkaloid distribution in the family Liliaceae. Experimental procedures are described in Chapter 7.

Chapter 2

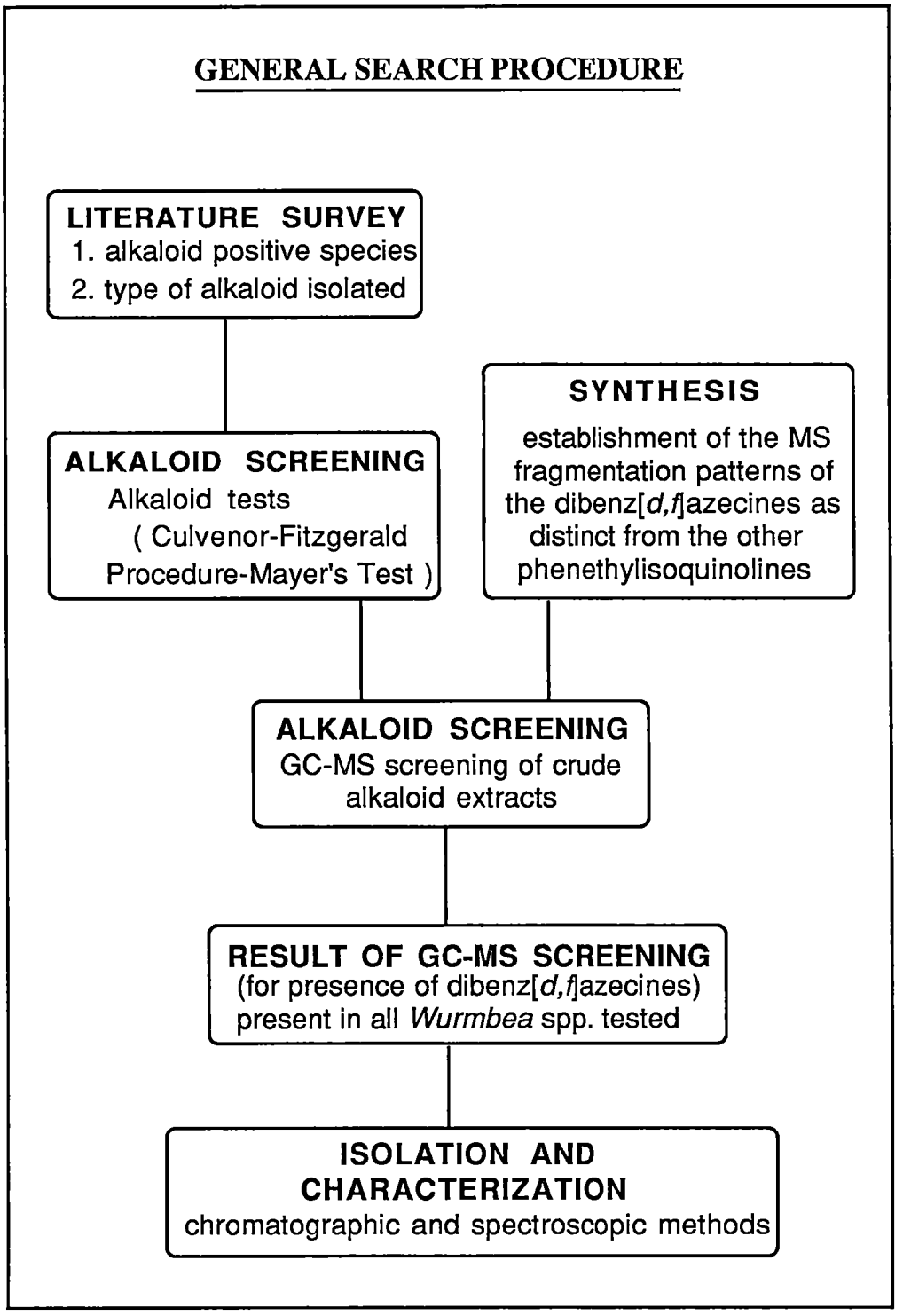
THE SEARCH FOR THE DIBENZ[*d,f*]AZECINE SYSTEMS

2.1. General Search Procedure

In the search for the dibenz[*d,f*]azecine alkaloids possible sources of these bases must be identified and a systematic search method (Scheme 2.1) followed. Plant families previously known to elaborate phenethylisoquinoline-type alkaloids and their related families need to be identified and related genera and species looked at. This is to establish the pattern of phenethylisoquinoline-derived alkaloid occurrences in terrestrial plants, in order to direct the search for the dibenz[*d,f*]azecines. A literature search and a study of the relationships in the plants of interest was therefore required.

Plants suspected to contain these bases were tested for alkaloids. Screening for the alkaloids using the gas chromatography-mass spectrometry (GC-MS) technique was seen as the main preliminary tool to study the components of the new alkaloid positive species, once an alkaloid extract was made available. This tool was also used to re-investigate some plants previously known to elaborate phenethylisoquinoline-type bases, with particular attention given to the minor alkaloidal components. This called for a knowledge of the mass spectral fragmentation patterns of the phenethylisoquinoline alkaloids in general, the dibenz[*d,f*]azecines in particular, and of some related benzylisoquinoline alkaloids. While the MS fragmentation patterns of most of the phenethylisoquinoline groups could be established from their reported data, those of the dibenz[*d,f*]azecine-type could not be fully established from the limited amount of data available at the time this

work commenced. Consequently, some dibenz[*d,f*]azecines were synthesized. Once a dibenz[*d,f*]azecine source was found, alkaloid isolation and characterization then followed.



Scheme 2.1

2.2. Literature Study and Alkaloid Screening

From among the five phenethylisoquinoline-containing plant families identified, only three families were covered in this study as the families Cephalotaxaceae and Phellinaceae were not readily available. However, in the process of screening for alkaloids, species from other families were also randomly looked at. The initial work was directed towards the known and available C-homoerythrina-containing plants in the families Meliaceae and Taxodiaceae. This was based on the proposal that the alkaloid of interest, the dibenz[*d,f*]azecine, is an intermediate in the biosynthesis of the C-homoerythrina group of alkaloids[63,113].

Two known homoerythrina-containing genera were obtained for re-investigation of their alkaloids. The genus *Athrotaxis* (Taxodiaceae) is endemic to Tasmania whereas the rest of the members of the family Taxodiaceae are confined to Eastern Asia and North America. Aside from the homoerythrina alkaloids isolated from the *Athrotaxis* species, no other alkaloid types were isolated from these species nor from the other genera of this family[132,133,134,135]. On the other hand, the genus *Dysoxylum*, the species *lenticellare* in particular, is the only member of the family Meliaceae where homoerythrina alkaloids were found and where the only naturally occurring dibenz[*d,f*]azecine was isolated[68]. This genus is composed of about sixty species distributed from the tropical Asian regions, to Australia, New Guinea, Polynesia, and New Zealand. Some thirty species have been tested for alkaloids[136-142] of which, only very few, (mainly the ones tested in Australia[141,142]) showed weakly positive results. A chromone-type alkaloid has however been

isolated from *Dysoxylum binectariferum*[143]. Some related genera of *Dysoxylum* such as *Didymocheton* and *Epicharis* [144] could not be screened as they were not available. The same was true with the close relatives of the Tasmanian endemic *Athrotaxis* species. The fact that more phenethylisoquinoline-type alkaloids have been isolated previously from a number of liliaceaous plants, that there are more of them available in Australia, and that there was a reasonable success in the initial screening of this plant family, prompted a more concentrated study of this group of plants.

A study of the relationships in the plants belonging to the family Liliaceae was complicated by the diversity in the classifications of this family ranging from the more classical ones, as in the classification by Hutchinson[145], and Cronquist's, as used in the 'Flora of Australia'[146], to the most recent taxonomy by Dahlgren and co-workers[147] and by Conran[148] (Table 2.1). In Dahlgren's classification most of the traditionally classified liliaceous plants were classified under a superorder Liliiflorae, which included the families Orchidaceae and Iridaceae. In this classification, the family Liliaceae was narrowed down to 13 genera of 385 species. The known phenethylisoquinoline-containing plants were excluded from the family Liliaceae and were classified into two separate families, the families Colchicaceae and Uvulariaceae. Members of these two families are mainly found in the African and the European continents, in the Mediterranean regions, and extending to Australia with a few genera such as *Iphigenia* and *Gloriosa* scatterred in Eastern and Western Asia, the Philippines, and New Zealand[7,8].

In Hutchinson's classification (Scheme 2.2), the genera in Dahlgren's families Uvulariaceae and Colchicaceae, and in Buxbaum's

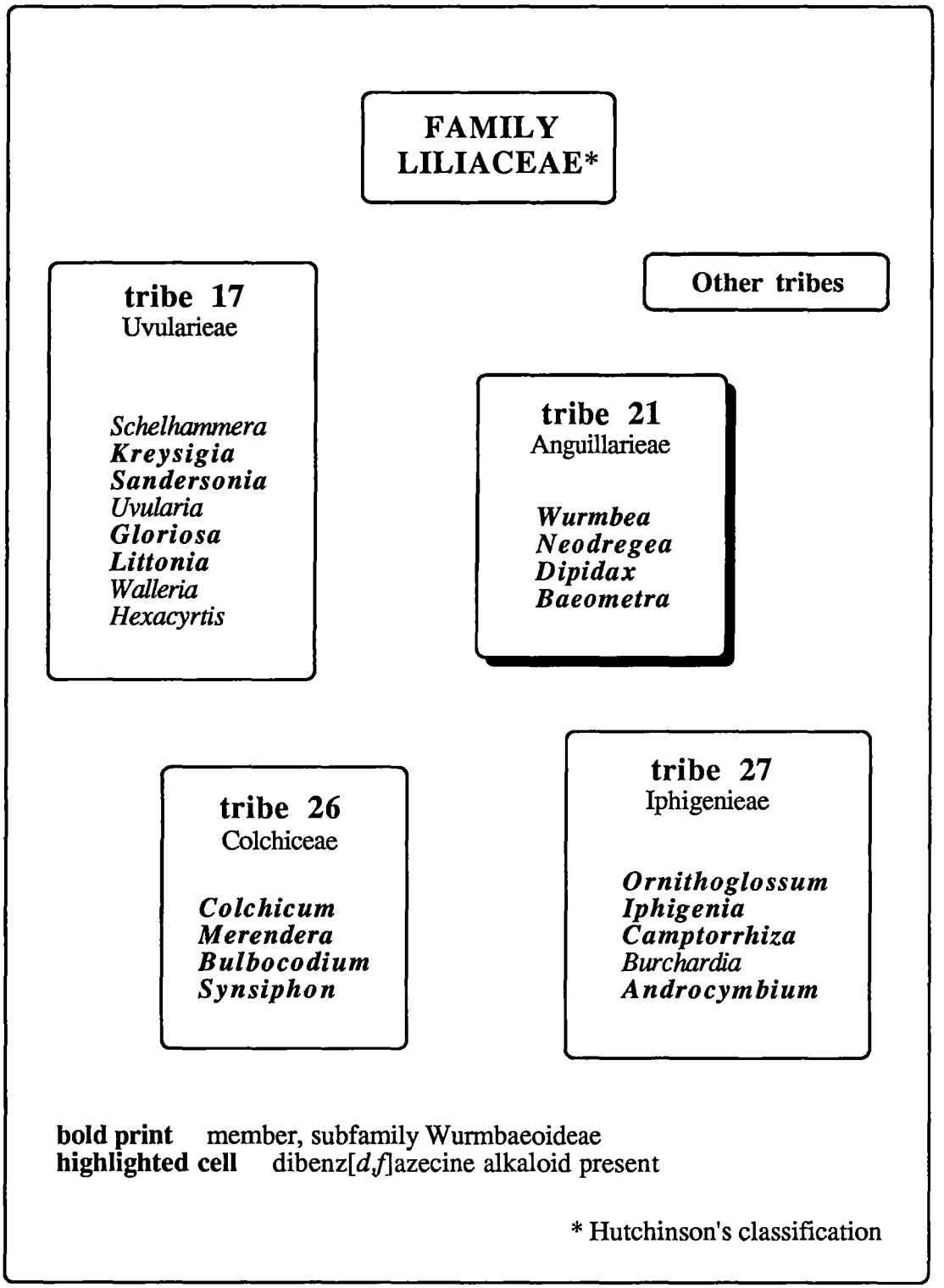
Table 2.1 Comparison of DifferentTaxononmic Classification of some Liliaceous Plants

Genus	**Hutchinson** Tribe	**Dahlgren** Family	**Dahlgren** Tribe	**Conran** Family	**Buxbaum**[b] Tribe	**Buxbaum**[b] Subfamily
Tricyrtis	Tricyrtideae	Uvulariaceae	Tricytidaceae	Uvulariaceae		
Disporum	Polygonatae	Uvulariaceae	Uvularieae	Uvulariaceae		
Clitonia	Polygonatae	Uvulariaceae	Uvularieae	Uvulariaceae		
Prosartes		Uvulariaceae	Uvularieae	Uvulariaceae		
Streptopus	Polygonatae	Uvulariaceae	Uvularieae	Uvulariaceae		
Scoliopus	Trilliaceae[a]	Uvulariaceae	Uvularieae	Uvulariaceae		
Medeola	Trilliaceae[a]	Uvulariaceae	Uvularieae	Uvulariaceae		
Walleria	Uvularieae	Tecophillaeaceae				
Uvularia	Uvularieae	Uvulariaceae	Uvularieae	Uvulariaceae		
Schelhammera	Uvularieae	Uvulariaceae	Uvularieae	Uvulariaceae		
Kreysigia	Uvularieae	Uvulariaceae	Uvularieae	Uvulariaceae	Kreysigieae	Wurmbaeoideae
Gloriosa	Uvularieae	Colchicaceae	Iphigenieae	Uvulariaceae	Glorioseae	Wurmbaeoideae
Littonia	Uvularieae	Colchicaceae	Iphigenieae	Uvulariaceae	Glorioseae	Wurmbaeoideae
Sandersonia	Uvularieae	Cochicaceae	Iphigenieae	Uvulariaceae	Glorioseae	Wurmbaeoideae
Hexacyrtis	Uvularieae	Colchicaceae	Iphigenieae			

Table 2.1 Continued

Genus	**Hutchinson** Tribe	**Dahlgren** Family	**Dahlgren** Tribe	**Conran** Family	**Buxbaum**[b] Tribe	**Buxbaum**[b] Subfamily
Ornithoglossum	Iphigenieae	Colchicaceae	Iphigenieae		Iphigenieae	Wurmbaeoideae
Iphigenia	Iphigenieae	Colchicaceae	Iphigenieae		Iphigenieae	Wurmbaeoideae
Camptorrhiza					Iphigenieae	Wurmbaeoideae
Burchardia	Iphigenieae					
Baeometra	Anguillarieae	Colchicaceae			Baeometreae	Wurmbaeoideae
Wurmbea	Anguillarieae	Colchicaceae	Anguillarieae		Wurmbaeae	Wurmbaeoideae
Dipidax	Anguillarieae	Colchicaceae	Anguillarieae		Neodregeae	Wurmbaeoideae
Neodregeae	Anguillarieae	Colchicaceae	Anguillarieae		Neodregeae	Wurmbaeoideae
Colchicum	Colchiceae	Colchicaceae	Colchiceae		Colchiceae	Wurmbaeodeae
Merendera	Colchiceae	Colchicaceae	Colchiceae		Colchiceae	Wurmbaeoideae
Bulbocodium	Colchiceae	Colchicaceae	Colchiceae		Colchiceae	Wurmbaeoideae
Synsiphon	Colchiceae	Colchicaceae	Colchiceae			

[a] Family; [b] as complemented by Santavy



Scheme 2.2

subfamily Wurmbeaoideae, were spread out to four tribes namely Uvularieae, Anguillarieae, Colchiceae, and Iphigenieae. The search in the present study was therefore directed to members of these four tribes

and their relatives, whose alkaloid profile had not been reported previously. This included species of *Uvularia*, *Hexacyrtis, Burchardia, Walleria,* and *Synsiphon* which were classified by Hutchinson within the four tribes. Some related genera like *Tricyrtis, Disporum, Clintonia, Prosartes, Streptopus, Scolius,* and *Medeola* also warranted investigation as they were included by Dahlgren and co-workers in the family Uvulariaceae (Table 2.1). Buxbaum, according to Vikova and co-workers[9], had also mentioned a possible relationship between *Tricyrtis stolonifera* and *Romulea rosea* with the subfamily Wurmbaeoideae. However, in a study by Vikova's group, no phenethylisoquinoline-derived alkaloids were detected from these two species. Clifford and Conran[146] also identified the genera *Tripladenia and Kuntheria* together with *Schelhammera* as members of the family Uvulariaceae. *Tripladenia cunninghamii* (*Kreysigia multiflora* Reichb.), *Kuntheria pedunculata* and two *Schelhammera* species have already been found to contain phenethylisoquinoline-derived alkaloids. Related to these three genera was the genus *Drymophila*. This genus, together with the genus *Campynema*, which has been ambiguously classified in many different families including the family Colchicaceae[146], were considered as prospective sources of the phenethylisoquinoline-derived alkaloids.

2.3. Testing for the Presence of Alkaloids

The alkaloid testing was done with a slight modification of Culvenor and Fitzgerald's Mayer's test procedure for alkaloid field testing. In some cases where this method showed negative results, and where there was enough plant material available, the plant samples were extracted exhaustively with methanol. Any alkaloid present was extracted from the concentrated methanol extract following the usual acid-base extraction

procedure (Chapter 7, Section 7.5.2). The presence of the alkaloids was confirmed, where possible, by analytical thin layer chromatography (TLC) of the crude alkaloid extract and by spraying the plates with Schlittler's (potassium iodoplatinate) or Dragendorff's reagent. The crude alkaloid extracts and their fractions were then screened for the dibenz[*d,f*]azecine-type and related alkaloids by the GC-MS technique.

Alongside the initial collection and alkaloid screening of *Dysoxylum,* and *Athrotaxis* species, members of Hutchinson's tribes Uvularieae, Anguillarieae, Colchiceae, Iphigenieae, and their related genera were also screened. The screening was extended to families other than the five families already identified and to any plant traditionally classified under the family Liliaceae. Most plants were tested for alkaloids in the field, or otherwise immediately after the samples reached the laboratory. The plant samples were collected from Tasmania, Western Australia, Victoria, Queensland and the Philippines. Three batches of crude methanol extracts of *Dysoxylum lenticellare* were obtained from Fiji, where the reported *Dysoxylum lenticellare* was sampled[68]. The third batch was collected from the same area (near the Boy Scout camp, Coli-Suva, Fiji) where the reported *Dysoxylum* species was collected. The results of the alkaloid testing covering the families Meliaceae, Taxodiaceae, and Liliaceae are discussed in Section 2.6 and summarized in Tables 2.2 and 2.3.

2.4. Mass Spectral (MS) Fragmentation Patterns

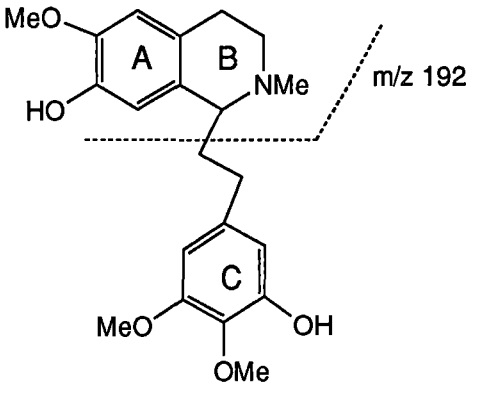
To establish the unique features of the mass spectral fragmentation pattern of the dibenz[*d,f*]azecine systems, the mass spectra of the other

phenethylisoquinoline-derived alkaloids and some of their analogues were surveyed. The MS fragmentation pattern of the homoerythrina alkaloids is well established[91]. The $\Delta^{1(6)}$ series of the homoerythrina alkaloids could be very easily recognized from their mass spectra. The intense peaks at m/z 178 or increments of it (according to the oxygenation pattern at C_2), including the weak peaks at m/z 135, 146, 165, due to the cleavage of the C-ring, and the fragments M^+-58 and M^+-59 due to a retro Diels-Alder type of cleavage of ring A, followed by loss of protons, are all diagnostic of this alkaloid type. On the other hand, the 1,6-diene, $\Delta^{2(1)}$ alkene including the oxo and the epoxy members of these series and those with a modified D-ring, do not necessarily have a distinct mass spectrum. High mass fragments resulting from the loss of methyl, methoxy, formaldehyde or hydroxyl groups are usually observed as intense peaks, if not base peaks. In the case of the $\Delta^{2(1)}$ alkene series, a moderate to strong peak at M^+-45 is diagnostic. In contrast, the cephalotaxine alkaloids undergo more complex fragmentations, depending on the position of the double bond in ring C. Fission or recyclization of ring B and C or other alternative pathways may result in a number of high mass fragments[149].

Of the MS fragmentation patterns of the other phenethylisoquinoline alkaloids, those of the colchicine-type have long been established[99]. A survey of the mass spectral properties of the homoaporphines, homoproaporphines, and homomorphines showed that alkaloids of these types, with an N-methyl group, generally undergo a retro Diels-Alder fission of the B-ring losing a methyleneimine fragment (m/z 43). The peak corresponding to this is of moderate to weak intensity. A range of high mass fragments corresponding to the loss of various small groups such as methyl or methoxy groups or a hydrogen atom can also be

observed. The homomorphines can be distinguished from the other groups in that, the parent ion is generally the base peak while the high mass fragment peaks are usually of weak to moderate intensity.

A distinct feature of many homoproaporphine alkaloids is that they give a weak to strong M^+-1 peak which can sometimes be the base peak[150,151]. The nature of the spirane ring such as its degree of saturation, the type of substituents and their stereochemistry bring about a large variation in the primary as well as in secondary fragmentations of this group of alkaloids.



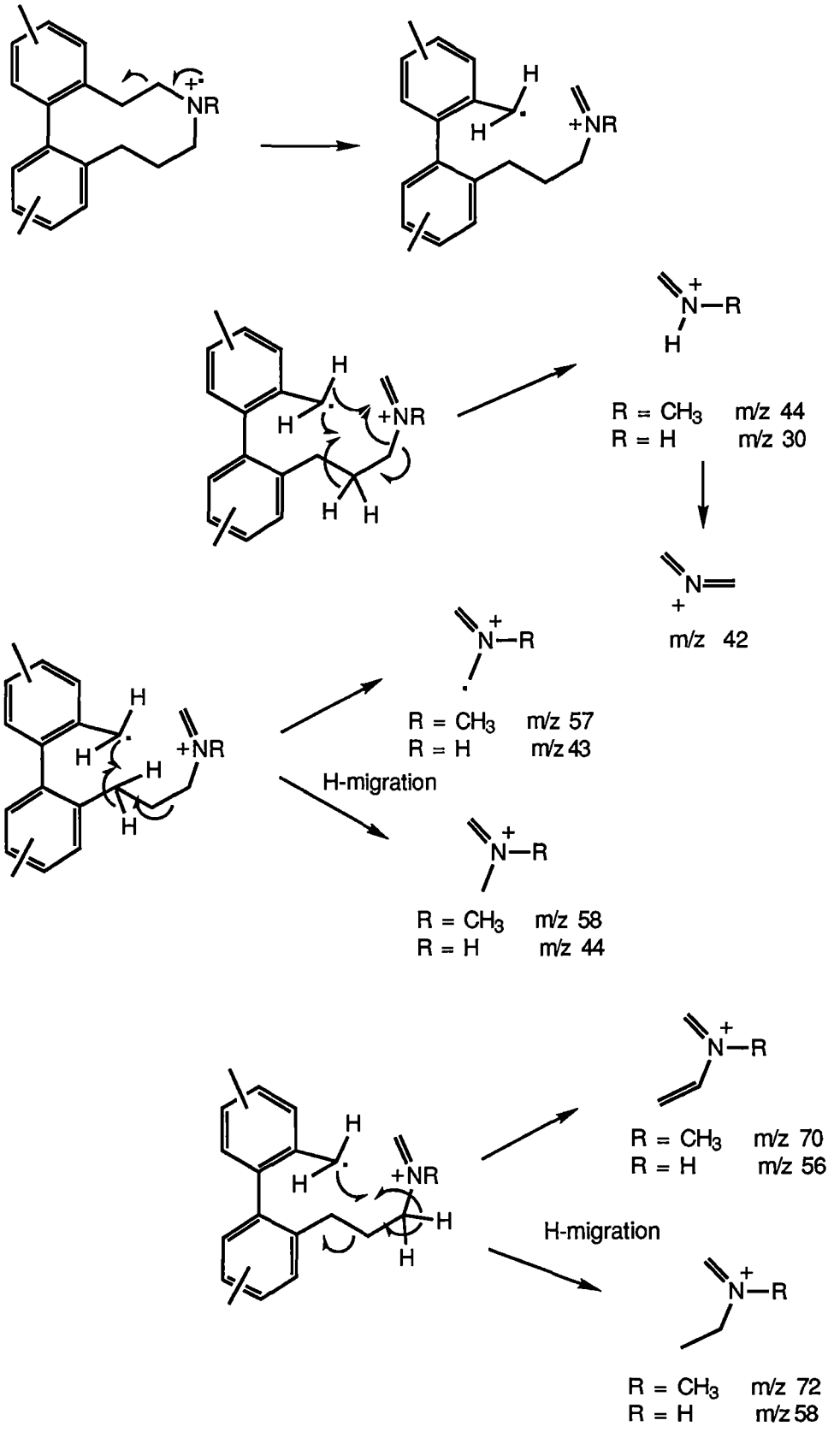
(10)

The simple 1-phenethylisoquinoline alkaloids have unique mass spectra in that most of the peaks including those of the parent ion are very weak as contrasted to the very intense base peak. This base peak corresponded to the fragment resulting from the cleavage of the isoquinoline moiety (ring A and B) giving rise to a stable isoquinolium ion. In autumnaline (10) the base peak corresponds to m/z 192, the aromatic ring A being substituted with a methoxy and a hydroxy group. On the other hand, the homoaporphines have moderately strong to weak parent ions. The base peak is generally due to the loss of the substituent at C-1 which is normally a methoxy or a hydroxy group[46,151]. This is

generally accompanied by weak or moderately intense peaks due to the fragments M^+-19 (2H, OH), M^+-33 (H_2O, CH_3), and M^+-43 (CH_2NCH_3).

The MS fragmentation pattern of the dibenz[*d,f*]azecines was established by comparing the fragmentation patterns of some analogous systems such as the dibenz[*d,f*]azonine alkaloids[152,153,154], related benzylisoquinoline bases, and of other phenethylisoquinoline derivatives, with some dibenz[*d,f*]azecine systems. The dibenz[*d,f*]azecine data available were those of dysazecine, two dibenz[*d,f*]azecine systems synthesized by Tsuda and co-workers[155,156] and a number of dibenz[*d,f*]azecine derivatives synthesized in this work (Chapter 3, Section 3.3.1). Where both the A- and the C-rings are aromatic, two sets of fragmentation patterns distinct from the other phenethylisoquinolines and the dibenz[*d,f*]azonines were recognized for the dibenz[*d,f*]azecines. That is, the mass spectrum of the dibenz[*d,f*]azecine systems with the N-methyl group could be easily distinguished from those with an -NH group in the heterocyclic ring.

The primary mode of cleavage in the dibenz[*d,f*]azecine systems was thought to proceed via a radical initiated alpha cleavage characteristic of the amines (Scheme 2.3). Secondary fragmentation could include further radical-induced cleavages possibly accompanied by hydrogen shifts in some cases, which could then lead to fragmentations involving the loss of nitrogenous moieties. This proposal was backed up by high resolution mass spectrometric results of the characteristic fragments. It is presumed that both the N-methylated and the N-demethylated dibenz[*d,f*]azecine derivatives undergo similar



Scheme 2.3 Proposed Mode of Fragmentation of Reduced Dibenz[*d,f*]azecine Derivatives

modes of cleavage; the variation in the relative peak intensities of the fragment ions of the N-methylated and the N-demethylated systems cannot be fully accounted for, although the stabilizing effect of the methyl group in the N-methylated systems could be partly responsible for this. For instance the mass fragment at m/z 70 in the N-methylated systems was always an intense peak while the corresponding fragment of the N-demethylated system, m/z 56, was extremely weak. The high resolution mass spectrum of one characteristic peak of the N-demethylated system (M^+-32) revealed the loss of a nitrogenous fragment rather than loss of methanol. This was thought to be due to the loss of 30 mass units followed by loss of two protons (Scheme 2.3). The corresponding fragment of the N-methylated derivative at m/z 44 was of moderate intensity. An equally moderate peak at m/z 42 could be due to loss of two protons from the fragment at m/z 44.

These fragmentations therefore allowed the recognition of the two systems as distinct from each other due to their consistent relative peak intensities. In the high mass range, the system with an N-methyl group in the heterocyclic ring is recognized by a weak peak at M^+-58, and a weak to moderately intense peak at M^+-72 (Figure 2.1). With the N-demethylated system (Figure 2.2), the peak due to M^+-32 is diagnostic in the high mass range. The peaks at M^+-58 and M^+-44 equivalent to those of the N-methylated systems (M^+-72, and M^+-58 respectively) were also observed (Scheme 2.3). The lower mass range of the N-demethylated derivative has very weak peaks except for the characteristic intense peak due to m/z 43 which is generally the base peak. In the N-methylated system, the lower mass range is a diagnostic region of the spectrum, in which moderate to strong peaks due to m/z 70, 58, 57, 44 and 42 were

always observed. The base peak could be any of the peaks at m/z 57, 58, 70 or the parent ion.

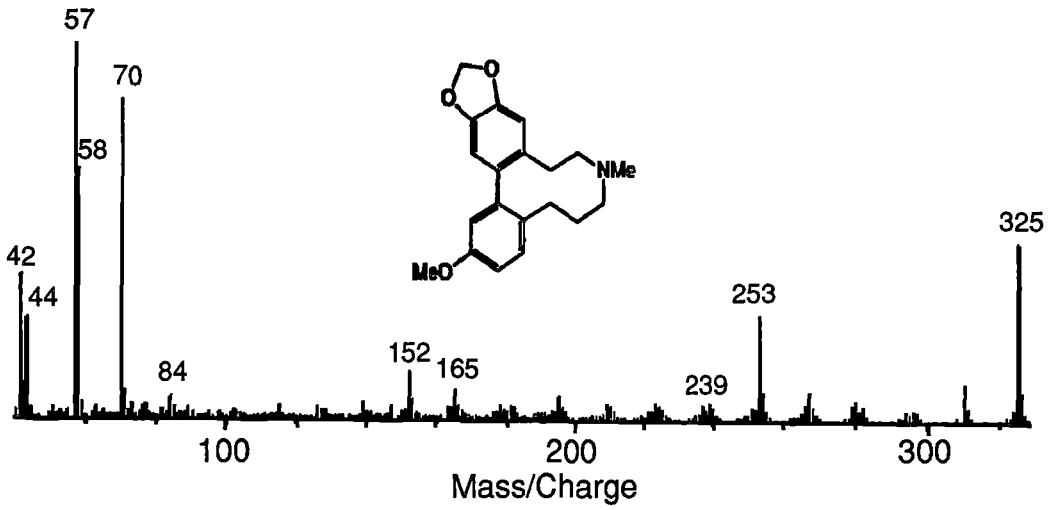


Figure 2.1 Mass Spectrum of Dibenz[*d,f*]azecine Derivative (32e)

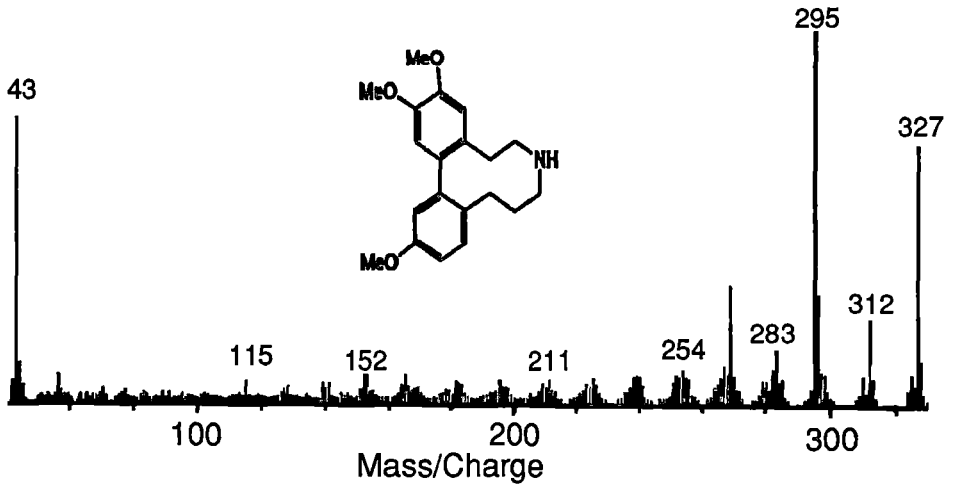


Figure 2.2 Mass Spectrum of Dibenz[*d,f*]azecine Derivative (42a)

The mass spectra of the dibenz[*d,f*]azecine derivatives are also distinct from their dibenz[*d,f*]azonine analogues. The dibenz[*d,f*]azonine with an N-methyl group in the heterocyclic ring (Figure 2.3) does not have intense fragments in the lower mass range. The intense peaks are instead observed in the high mass range. A cluster of strong to

moderately intense peaks at M^+-56, M^+-57, and M^+-58 along with some less intense peaks due to M^+-74, M^+-87 M^+-43, and M^+-15 are generally observed. The base peak of the N-demethylated dibenz[*d,f*]azonine analogue has the parent ion for a base peak (Figure 2.4). All other peaks are of very weak intensity except for two peaks in the high mass range, M^+-42 and M^+-73. This difference in the mode of fragmentation in the nine and ten-membered ring systems can be explained, at least in part, by the presence of the extra methylene group in the latter, resulting in other fragmentation possiblities (Scheme 2.3).

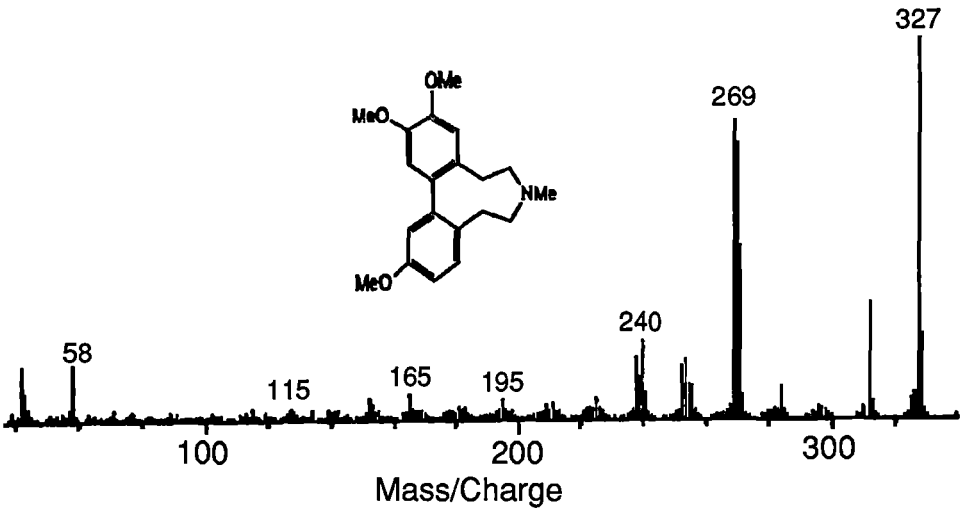


Figure 2.3 Mass Spectrum of Laurifonine

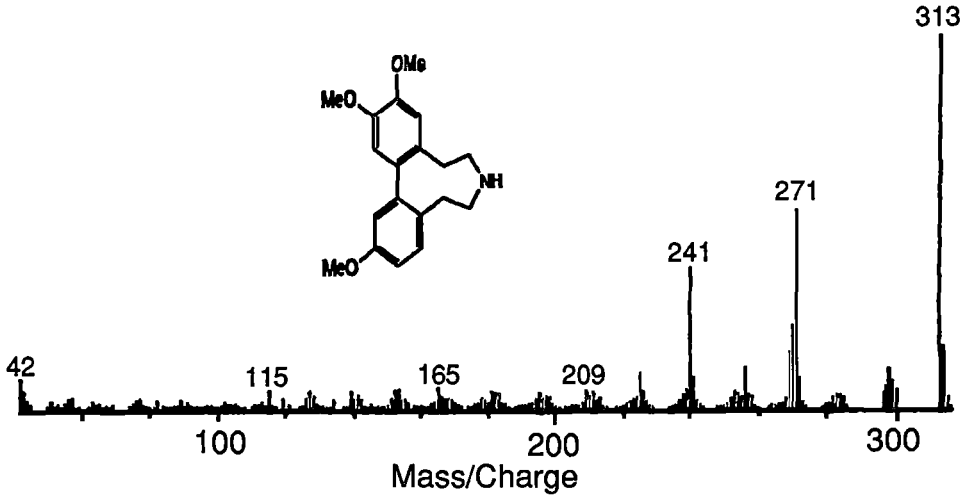


Figure 2.4 Mass Spectrum of Laurifine

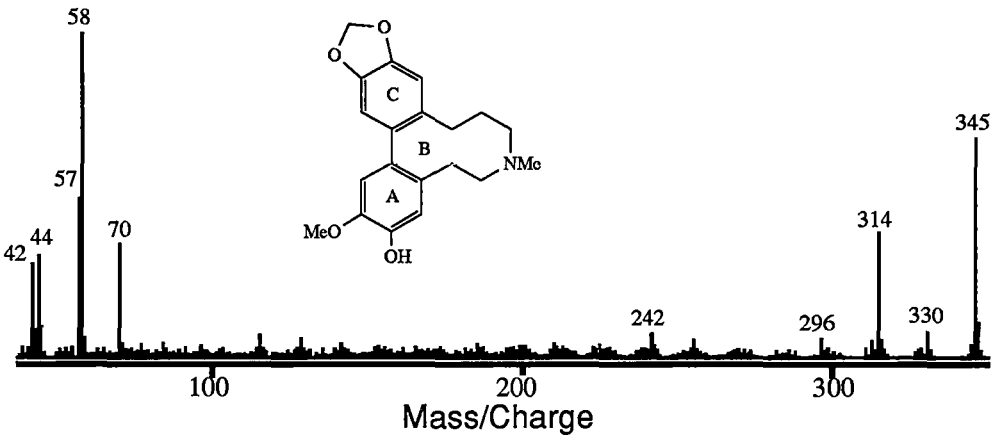


Figure 2.5 Mass Spectrum of the Dibenz[d,f]azecine Derivative (64)

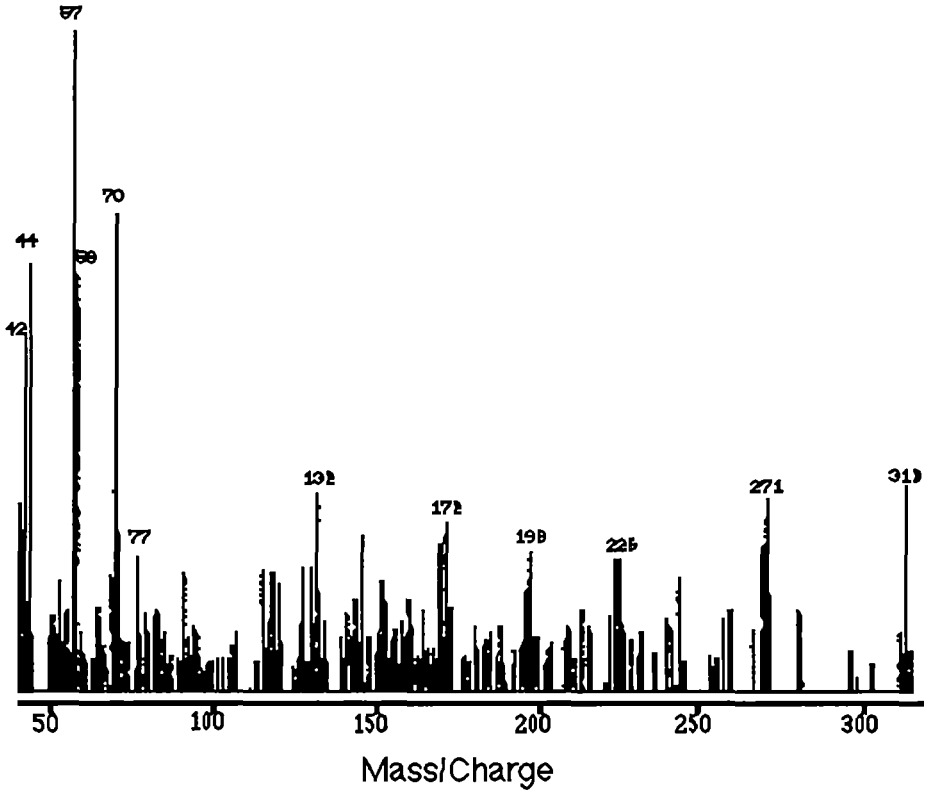


Figure 2.6 Mass Spectrum of the Dibenz[*d,f*]azecine Component of *Athrotaxis selaginoides*

In two cases, where the C-ring in the dibenz[d,f]azecine derivatives was not aromatized, the high mass fragmentation pattern established in the aromatized system, that is, the peaks due to M^+-58 (weak) and M^+-72 (moderate), was not observed (Figure 2.5, Chapter 3

Section 3.4.1). However, the lower mass fragments remained the same. A similar pattern was observed in one of the trace alkaloid components of *Athrotaxis selaginoides* (Figure 2.6).

2.5. GC-MS Screening for Alkaloids

The combined analytical methods of gas chromatography and mass spectrometry (GC-MS) have proved to be a very powerful tool for screening purposes. Recent advances in instrumentation have brought about the development of a reliable and easy-to-operate instrument which requires minimal sample preparation. It has also allowed the use of a capillary column which greatly improves the separation capability of the gas chromatograph and minimized column bleeding. The present ability of this technology to scan trace quantities of sample to a very low detection limit over an expanded boiling point range has widely increased its application in the area of natural products chemistry.

However, the few limitations of this instrument which are mainly centred on the gas chromatograph, present some difficulty in screening for the very minor as well as polar materials. Although in the initial work with the homoerythrina-type alkaloids the boiling range of the compounds of interest was found to be within the detectable range, they required acquisition temperatures very close to the limits of the instrument. There is also a chance that some trace components, although not very polar, will be degraded in the column. This problem is even more pronounced at higher temperatures where detection becomes more difficult due to an increase in noise level. Some of these problems may be partially overcome by derivatization of the samples.

However, if the GC-MS technique was to be used as a routine tool, it was deemed necessary that sample preparation time be kept to a minimum, thus doing away with derivatization of samples as much as possible. There was therefore a need to develop a method which would optimize the separation capability of the gas chromatograph over short run times with respect to the compounds to be screened.

The GC-MS acquisition parameters for the purposes of screening phenethylisoquinoline-type alkaloids were established initially with the homoerythrina alkaloids from *Athrotaxis selaginoides*. The major alkaloids of this plant have been previously identified [71] and their mass spectral fragmentation patterns characterized. Most of the alkaloids under study were found to elute from the column at a temperature above 250°. Two method files were established initially, set to scan the mass range from 40 to 500 atomic mass units. The first file was an intermediate oven temperature program from 50° to 290°. The program had two levels to minimize run time. The first level was 50° to 250° at a rate of 40° per minute since the alkaloids were eluted above 250°, and the second level was from 250° to 290° at 10° per minute. This method file was then applied in the split or splitless mode to screen both the crude alkaloid extracts and the partially purified fractions. The total ion chromatogram (TIC) using the intermediate temperature program from 50° to 250° displayed most of the lower boiling, and generally non-alkaloidal components of the extract thus enabling one to estimate the purity of the sample. Analyses using this method file also gave an idea of the retention times of the alkaloids under study so that for the relatively pure fractions, the succeeding analyses could then be done at a higher temperature with consequent faster run times.

In the second method file, the oven temperature was programmed from 250° to 290°. This provided a very quick run time ranging from 8 to 15 minutes. It was found ideal for the routine analysis of relatively pure fractions where trace components were of lesser concern, enabling the problems of detectability due to molecular degradation and increased noise level at higher temperatures to be disregarded. However, this was not applicable to very low concentrations of alkaloids from very limited amounts of plant samples since splitless injection could not be employed due to the high initial temperature conditions. The intermediate temperature splitless injections were therefore preferred for routine screening of crude alkaloid extracts of relatively low concentrations.

A comparison of the GC-MS analyses of the crude alkaloid extract from batch 10 of *Wurmbea dioica* ssp. *dioica* (Figure 2.7) shows the relative detection ability using different modes of injection (for the components detected beyond a retention time of fifteen minutes for split and splitless injection, and twenty two minutes for cold on-column injection). The selective loss of peaks in the splitless mode could be rationalized as due to the longer time the sample spent in the injector before being flushed into the column, and its subsequent slow release in the column. The chromatographic resolution in the split mode could therefore be expected to be more efficient based on the above arguments. Improvement of the resolution by cold on-column injection accounted for the negative effect of high temperature on the chromatography of different substances. Consequently, for extracts or fractions where the components had not been ascertained properly, screening was best done using more than one mode of injection. This

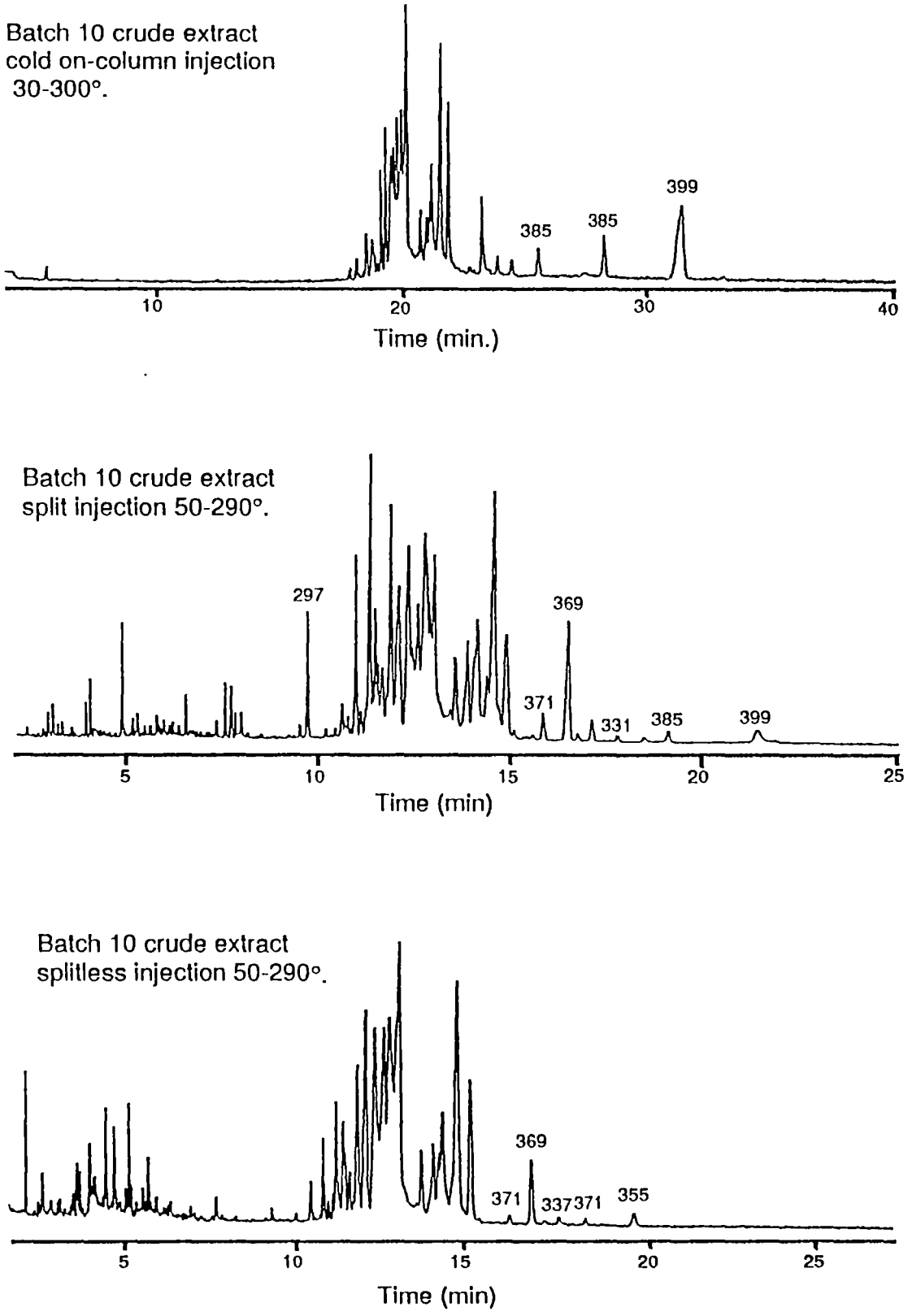


Figure 2.7 TIC of the Crude Alkaloid Extract of *Wurmbea dioica* ssp. *dioica* (Batch 10; Different Modes of Injection)

meant a much longer total GC-MS screening time in these cases. Where the results from different injections remained ambiguous, a direct probe MS was best employed. In cases like this, poor resolution of certain components could not simply be due to factors like oven temperature and mode of injection, but there could be a basic chromatographic problem inherent to the substance in question.

Scan 450 of Data: 10A10 (from batch 10 *Wurmbea dioica* ssp.*dioica)*

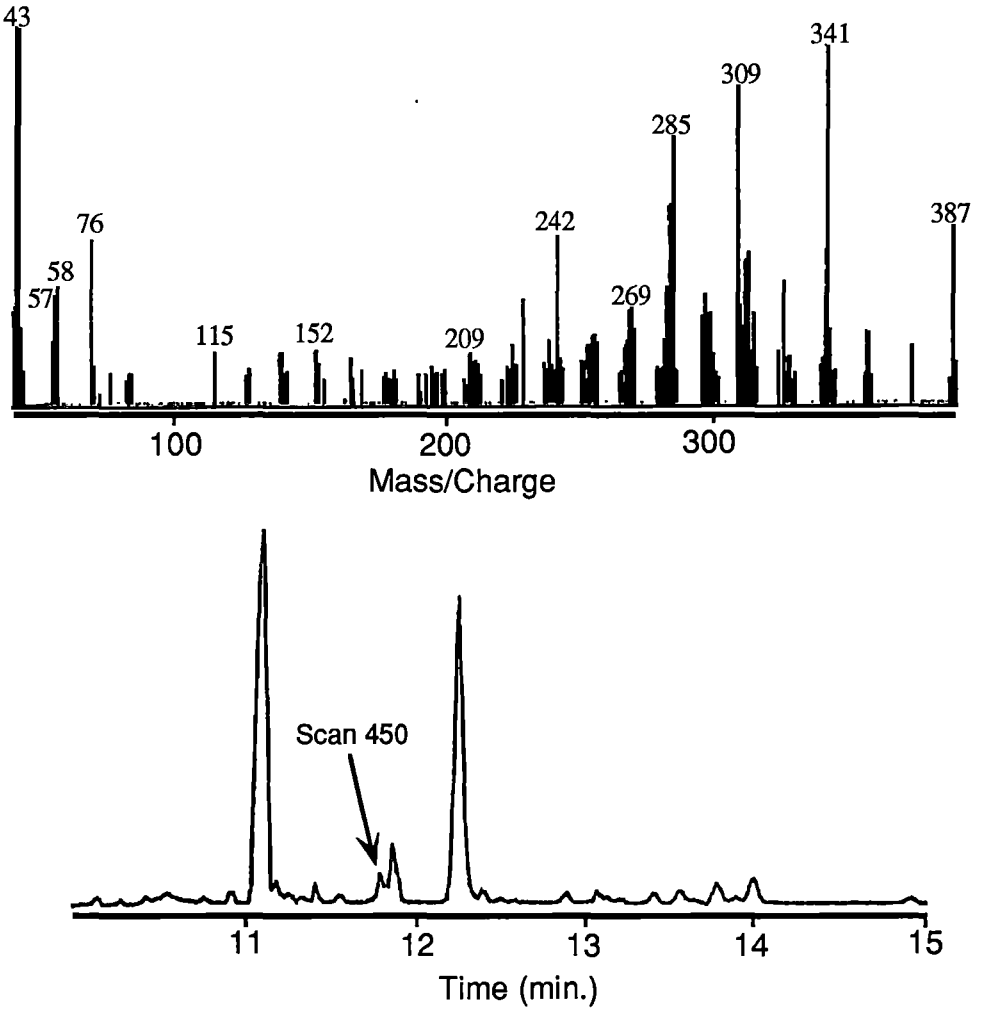


Figure 2.8.1 TIC of Fraction 10 (Batch 10) of *Wurmbea dioica* ssp. *dioica* and the Mass Spectrum Corresponding to Scan 450

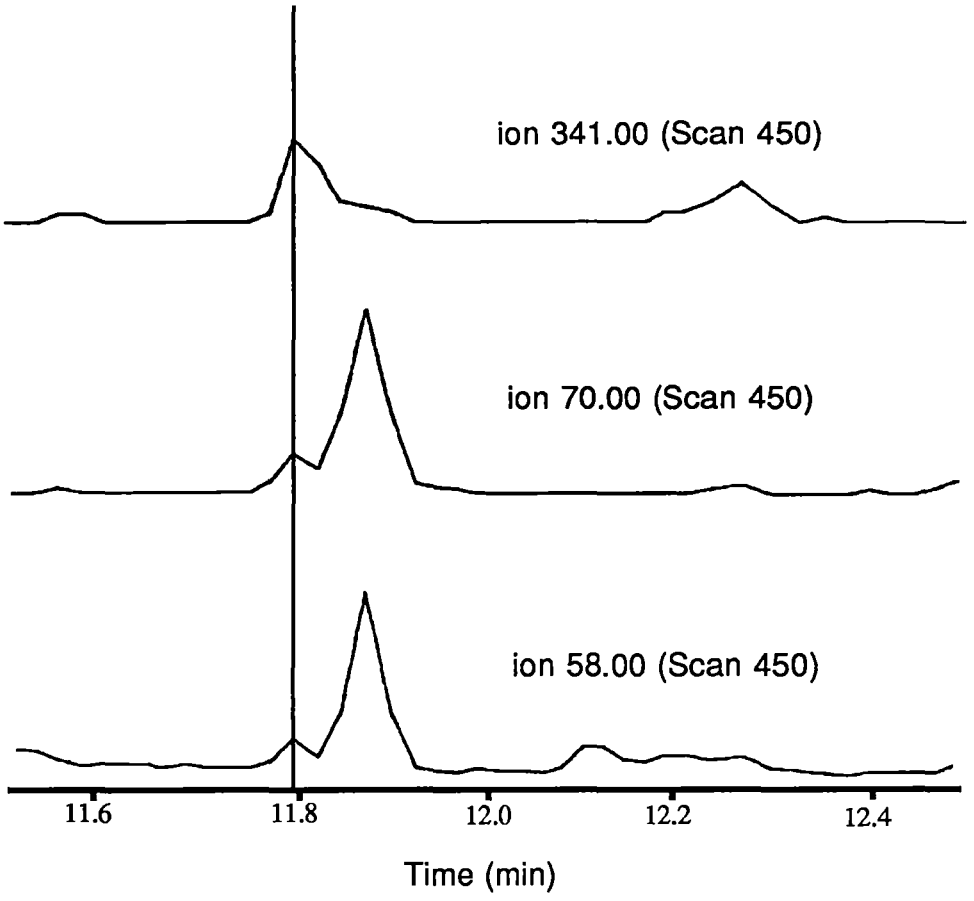


Figure 2.8.2

In the search for some minor to trace components such as the dibenz[*d,f*]azecine systems, repeated fractionation of the sample was generally done and each fraction monitored by GC-MS analysis. Where GC-MS peaks overlapped, distinguishing mass spectral fragment ions were drawn out of the total ion chromatogram and compared against one another. In the case of the dibenz[*d,f*]azecine alkaloids, any of the intense peaks such as those at m/z 57, 58, 70, and the suspected molecular weight, were generally used for comparison. Figure 2.8.1 shows the mass spectrum corresponding to a GC-MS ion trace (scan 450) which appeared to be an overlap of two components of molecular masses 387 and 341. Figure 2.8.2 shows the drawn chromatograms of a suspected dibenz[*d,f*]azecine derivative of molecular weight 341. The coincidence of the parent ion (341) with the characteristic

dibenz[*d,f*]azecine peaks drawn (m/z 58 and 70) showed that these peaks (m/z 58 and 70) were derived from the parent ion 341, which was therefore assigned as a dibenz[*d,f*]azecine derivative.

2.6. Results: Alkaloid Testing and GC-MS Screening

A summary of the results of the alkaloid testing and the GC-MS screening for possible dibenz[*d,f*]azecine derivatives from the families Meliaceae, Taxodiaceae and Liliaceae is given in Tables 2.2 and 2.3. Over thirty genera of fifty one species were tested, out of which nineteen species were found to be alkaloid positive. These were then screened for dibenz[*d,f*]azecines and the other alkaloid types by the GC-MS technique. Out of the nineteen species, fifteen were found to contain phenethylisoquinoline-derived bases.

From among the seven species of *Dysoxylum* tested, *Dysoxylum decandrum* was the only species found to contain alkaloids. A GC-MS scan of the crude alkaloid extract of *D. decandrum* collected from Queensland indicated the absence of phenethylisoquinoline-derived alkaloids even after several fractionations. Its alkaloidal components were very polar and difficult to separate and were not investigated further. The three batches of *Dysoxylum lenticellare* samples obtained from Fiji, where the reported dibenz[d,f]azecine containing species *Dysoxylum lenticellare* was sampled, proved to be devoid of alkaloids even after acid-base re-extraction and analysis by GC-MS and direct probe MS. This raises some doubts as to the actual identity of the plant material reported to contain the phenethylisoquinoline-type alkaloids[68].

Table 2.2 Results of Alkaloid Testing and GC-MS Screening
(Re-investigation of known Generic Sources of Phenethylisoquinoline-Derived Alkaloids)

Family (Subfamily) Tribe Species	A	B	C	D	E
Liliaceae (Wurmbaeoideae) Baeometreae *Baeometra Uniflora*	-	+	+	+	+
Wurmbaeae *Wurmbea dioica ssp. dioica*	-	+	+	+	
Wurmbea dioica ssp. *alba*	-	+	+	+	
Wurmbea pygmaea	-	+	+	+	
Wurmbea uniflora	-	+	+	+	
Kreysigieae *Kreysigia multiflora*	-	+	+	-	-
Colchiceae *Colchicum autumnale*	-	+	+	-	-
Colchicum byzantinum	-	+	+	-	-
Bulbocodium foliosus	-	+	+	-	-
Glorioseae *Gloriosa superba*	-	+	+	-	-
Gloriosa plantii	-	+	+	-	-
Littonia modesta	-	+	+	-	-
Sandersonia aurantica	-	+	+	-	-
Taxodiaceae *Athrotaxis selaginoides*	+	-	-	+	-
Athrotaxis cupressoides	+	-	-	-	-
Athrotaxis laxifolia	+	-	-	-	-
Meliaceae *Dysoxylum decandrum*	-	-	-	-	+
Dysoxylum altissimum	-	-	-	-	-
Dysoxylum arborescens	-	-	-	-	-
Dysoxylum floribundum	-	-	-	-	-
Dysoxylum fraseranum	-	-	-	-	-
Dysoxylum richii	-	-	-	-	-
Dysoxylum lenticellare	-	-	-	-	-

A Homoerythrina; **B** Tropolonic Wurmbaeoideae Alkaloid; **C** Non-tropolonic Wurmbeaodeae alkaloids; **D** Dibenz[d,f]azecine Alkaloids; **E** Others; [a] -ve results for all A, B, C, D and E implies -ve alkaloid test result; [b] species not studied previously

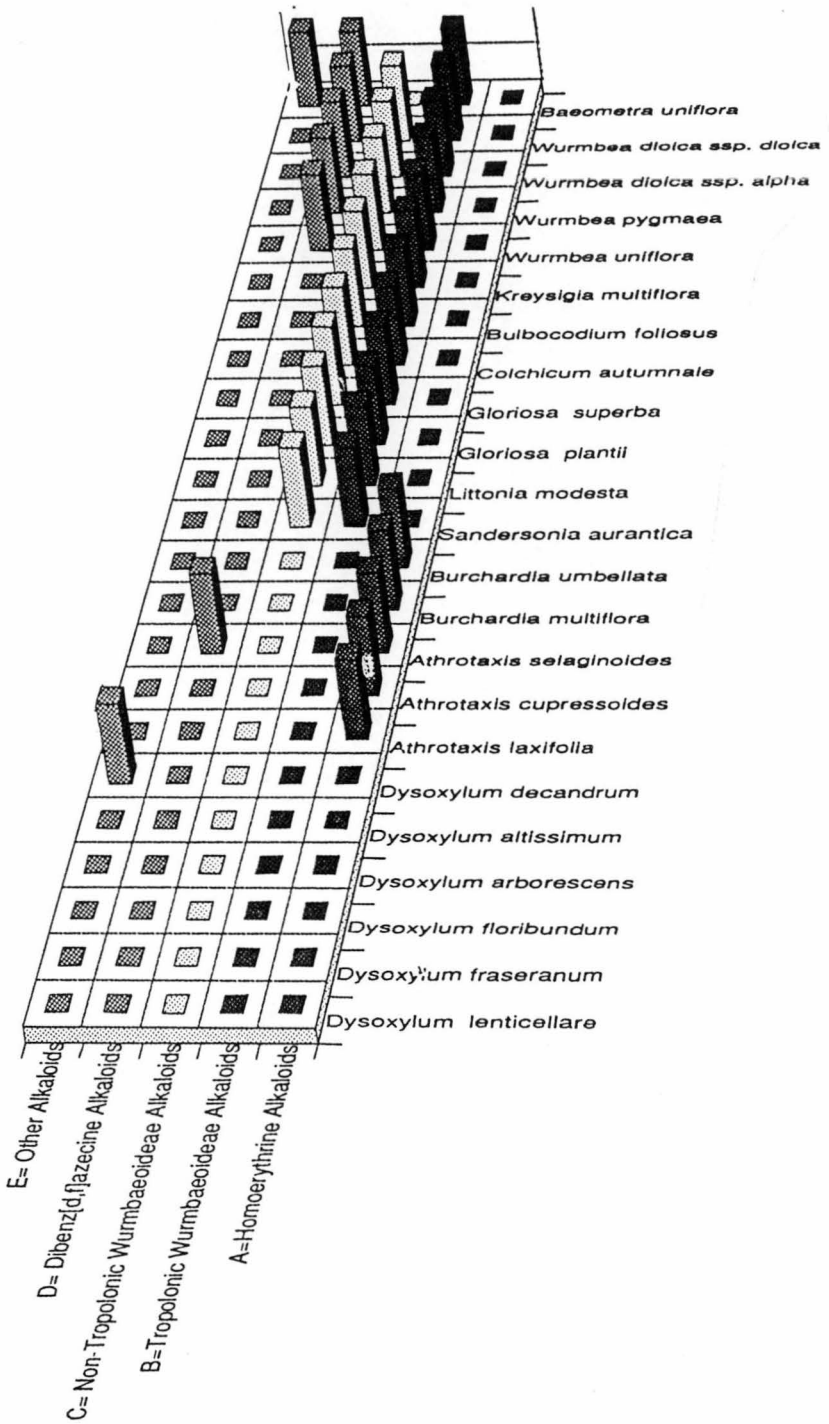


Table 2.3 Alternative Presentation of Alkaloid Testing and GC-MS Results

TABLE 2.4 Other Plants Screened for Phenethylisoquinoline-Derived Alkaloids

Species (Liliaceae)	A	B	C	D	E	Date	Place of Collection
Arthropodium milleflorum	-	-	-	-	+	8/1/88	Orford, Tas.
Asphodelus sp.	-	-	-	-	-[a]	5/10/87	Richmond, Tas.
Astelia alpina	-	-	-	-	-	29/10.87	South of Doughboy's Hill, Tas.
Bessera elegans	-	-	-	-	-	5/11/89	Kalorama, Victoria
Blandfordia punicea	-	-	-	-	-	30/11/86	Blue Lagoon, Southport, Tas.
Bulbine bulbosa	-	-	-	-	-	6/10/87	Rosny Hills (near Hobart), Tas.
Burchardia umbellata	+	-	-	-	-	17/7/87	South of Epping Forest, Tas.
Burchardia multiflora	+	-	-	-	-	12/9/88	Perth, Western Australia
Caesia caliantha	-	-	-	-	-	12/12/87	South of Epping Forest, Tas.
Caesia parviflora	-	-	-	-	-	12/12/87	4 km from Conara Junction, Tas.
Campynema lineare	-	-	-	-	-	17/1/90	Mt. King William I, Tas.
Chorophytum variegiatum	-	-	-	-	-	2/9/88	Private Garden, Sandy Bay, Tas.
Convallaria majalis	-	-	-	-	-	3/10/87	Private Garden, Taroona, Tas.
Corynotheca micrantha	-	-	-	-	+	14/6/88	Perth,Western Australia
Dianella revoluta	-	-	-	-	-	3/10/86	Upper Domain, Hobart, Tas.
Dianella tasmanica	-	-	-	-	-	5/10/86	Capricorn One Farm, Huonville, Tas.
Dichopogon strictus	-	-	-	-	+	5/11/89	South of Epping Forest, Tas.

Table 2.4 continued							
Drymophila cyanocarpa	-	-	-	-	-	27/8/88	near Solomon's Cave, Tas.
Hemerocallis sp.	-	-	-	-	+		Salamance Market (P.S.)[b], Tas.
Hosta sp.	-	-	-	-	-	10/2/88	Hobart Botanical Garden, Tas.
Johnsonia lupulina	-	-	-	-	-	14/9/88	Perth, Western Australia
Lilium tigrenum	-	-	-	-	+	4/2/88	Salamanca Market (P.S.), Tas.
Lomandra longifolia	-	-	-	-	-	15/11/87	New Norfolk, Tas.
Milligania densiflora	-	-	-	-	-	2/1/88	Lake Will, Northwest Coast, Tas.
Milligania longifolia	-	-	-	-	-		Northwest Coast, Tas.
Ophiopogon japonicum	-	-	-	-	-	10/2/88	Hobart Botanical Garden, Tas.
Ornithogalum umbellatum	-	-	-	-	-	9/4/90	New Town Station Nursery, Tas.
Protasparagus oficinalis	-	-	-	-	-	5/12/88	Private Garden, Hobart, Tas.
Protasparagus plumosus	-	-	-	-	-	13/9/88	Dimbulah, Qld.
Sansevieria tritasciata	-	-	-	-	-	23/8/88	University of Tasmania
Scilla peruviana	-	-	-	-	-		Salamanca Market (P.S.), Tas.
Stypandra caespitosa	-	-	-	-	-	21/1/87	Near Binalong Bay, Tas.
Stypanda glauca	-	-	-	-	-	29/10/87	Gippsland, Victoria
Stypandra umbellata	-	-	-	-	-	2/11/86	Tasman Peninsula, Tas.
Tricoryne elatior	-	-	-	-	+	14/9/88	Perth, Western Australia
Sowerbeae juncea	-	-	-	-	-	14/9/88	Perth, Western Australia

A Homoerythrina **B** Tropolonic Wurmbaeoideae Alkaloids **C** Non-tropolonic Wurmbaeoideae Alkaloids **D** Dibenz[*d,f*]azecines **E** Others; [a] negative results for all of **A,B,C,D** and **E** implies -ve alkaloid test result [b] Private Supplier

The alkaloids of *Hemerocallis* sp. and *Lilium tigrenum* were simply identified as different from the phenethylisoquinoline-derived bases by GC-MS and were not characterized further (Table 2.4). On the other hand the components of *Arthropodium milleflorum* were identified as of the solanidine-type steroidal alkaloids. There has been no previous report of any steroidal alkaloid in the genus *Arthropodium*. Some plant samples of *Narcissus* sp., *Valotra speciosa, Muscari* sp., *Hemerocallis* sp., and *Dichopogon strictus* were found to be alkaloid positive, but GC-MS screening showed that their alkaloid contents were other than the known phenethylisoquinoline alkaloids. Despite a previous report[135] that a *Hemerocallis* sp. and a *Tulipa* sp. contained colchicine alkaloids, no alkaloids of this type were detected in these plants.

The crude alkaloid extract of *Athrotaxis selaginoides* was obtained from a fresh sample of this plant and analyzed by the GC-MS technique. Most of the observed fragmentation patterns of the different alkaloidal components of the extract showed the diagnostic fragments of the $\Delta^{1(6)}$-homoerythrina-type alkaloids. Some alkaloids not reported previously from this plant, of molecular weights 303, 313, 315, 345, 361, 373, were also observed in the total ion chromatogram (TIC) of the crude alkaloid extract. There was no indication of any dibenz[*d,f*]azecine-type alkaloids in the crude extract. To further check this, the GC-MS injections were purposely overloaded in the hope of increasing the concentrations of the trace components; although in the process the activity of the column was sacrificed. Alternatively, the crude extract was fractionated by preparative TLC several times to enhance the detectability of the minor components. Still, in both cases, none of the sought for dibenz[*d,f*]azecines, where both rings A and B are aromatic, were observed. However, one of the fractions showed a very minor peak in the TIC which exhibited

the characteristic dibenz[*d,f*]azecine lower mass fragments of m/z 57, 58, 70 (Figure 2.6). As described earlier, this pattern suggested a possible dibenz[*d,f*]azecine derivative in which either ring A or C was non-aromatic. The possibility of it being an artifact after repeated chromatography on a silica support was ruled out as its MS fragmentation pattern indicated an N-methylated dibenz[d,f]azecine derivative. This component was not characterized further as it was present as a trace component of a 13 mg fraction of a 1.49 kg crude alkaloid extract obtained from 61 kg of fresh plant material.

As in the *Athrotaxis* spp., results of the GC-MS screening of the crude alkaloid extracts of *Burchardia umbellata* and *Burchardia multiflora* indicated the presence of homoerythrina alkaloids. This is chemotaxonomically significant suggesting a re-classification of the genus with other homoerythrina-containing liliaceous species (Chapter 6). The two *Burchardia* species were consequently investigated for the possible presence of the dibenz[*d,f*]azecines. However, no alkaloids of this type were detected. In the process of fractionating the crude alkaloid extract in the search for dibenz[*d,f*]azecine alkaloids, some new homoerythrina bases were isolated and their structures elucidated (Chapter 5).

The result of the GC-MS study of two batches of *Wurmbea pygmaea* could be of biosynthetic significance. The GC-MS study of a crude alkaloid extract from the first batch of a very mature plant sample showed the presence of a dibenz[*d,f*]azecine of molecular weight 387 as the major alkaloidal component, with traces of a few other alkaloids including one with a molecular weight of 371. A second batch of samples, collected one month earlier than the first collection but a year

later, was obtained to isolate the major component (M^+ 387) detected in the first batch of materials. The GC-MS trace of its crude alkaloid extract did not indicate the presence of the component of molecular weight 387, instead, the alkaloid of molecular weight 371 was detected as the major component. As observed with other *Wurmbea* species (Chapter 4, Section 4.2), there was an apparent variation in the alkaloid content and composition of the plant according to age, and locality.

2.7. Conclusions

The GC-MS technique employing a split or splitless injection on either a high or low temperature program, was found to be an excellent and effective tool to routinely detect most phenethylisoquinoline alkaloids in crude, partially purified, and pure samples with only a few exceptions. For some components, the mode of injection (split or splitless; high or low oven temperature program) was a major consideration. A cold on-column injection greatly improved the detection sensitivity. In a few cases, the GC-MS technique needed to be supplemented by direct probe mass spectrometric analysis.

Three new generic sources of the dibenz[*d,f*]azecine-derived alkaloids were found in this work: the genera *Wurmbea* (Liliaceae), *Baeometra* (Liliaceae), and *Athrotaxis* (Taxodiaceae). Based on the GC-MS screening results of some other members of the subfamily Wurmbaeoideae, it appeared that in the family Liliaceae, the dibenz[*d,f*]azecine-type alkaloids could be confined to Hutchinson's tribe Anguillarieae (tribe 21). Other members of this tribe such as the genera *Neodregea* and *Dipidax* (*Onixotis*), which occur in South Africa, remain to be studied. The dibenz[*d,f*]azecine component of *Athrotaxis*

selaginoides appeared different from those found in *Wurmbea* and *Baeometra* spp. in that, either ring A or C of the dibenz[*d,f*]azecine skeleton could be non-aromatic.

Although the co-occurrence of the homoerythrina and the dibenz[*d,f*]azecine alkaloids was demonstrated by their isolation from *Dysoxylum lenticellare*, none of the *Dysoxylum* species screened in this work have so far been found to contain any of the phenethylisoquinoline-derived alkaloids. More importantly, a re-investigation of three batches of samples of *D. lenticellare* obtained from Fiji, including a collection obtained from the same site where the reported *Dysoxylum lenticellare* was collected, did not give a positive alkaloid test. There is a need to verify the identity of the *Dysoxylum lenticellare* reported as a source of 1-phenethylisoquinolines, homoerythrina, and dibenz[*d,f*]azecine alkaloids. Efforts directed towards this verification are now being undertaken by others[156] as a result of this work.

In the process of screening members of the family Liliaceae for dibenz[*d,f*]azecines, the genus *Burchardia* was found as a new generic source of the homoerythrina alkaloids. Alkaloids other than the phenethylisoquinoline-type were also detected. Steroidal alkaloids have been detected and were later isolated and characterized from *Arthropodium milleflorum*.

Further screening of the liliaceous plants and their relatives could provide useful information for the taxonomy of these plants.

Chapter 3

THE DIBENZ*[d,f]*AZECINE ALKALOIDS

3.1 Introduction

The isolation of homoerythrina alkaloids from *Schelhammera* species has led to the proposal that dibenz[*d,f*]azecine-type systems may serve as immediate precursors to the homoerythrina alkaloids[63,113], analogous to the relationship between the dibenz[*d,f*]azonine systems and the erythrina alkaloids[131]. The reported[68] co-occurrence of a dibenz[*d,f*]azecine with homoerythrina alkaloids, as well as with simple 1-phenethylisoquinoline alkaloids, in *Dysoxylum lenticellare* also strongly supported this proposal. Yet to date, no further new dibenz[*d,f*]azecine-type alkaloids have been reported. However, there have been reports[155,156-160] on the syntheses of systems of this type. Most of these reports gave ^{1}H n.m.r. data, but very few gave details on mass spectral (MS) fragmentations. Overall, only two compounds with an NH group[155,160], and another two with an NMe[68,155] group in the heterocyclic ring were described.

This chapter discusses the results of the search for further dibenz[*d,f*]azecine alkaloids, including their detection and isolation from various plant sources, plus spectroscopic characterization and biogenetic proposals. Syntheses of various dibenz[*d,f*]azecine bases, mainly to establish the characteristic MS fragmentation patterns and to confirm the structure of wurmbazecine, are also described. A discussion of the chemical transformation of some known alkaloids to dibenz[d,f]azecine derivatives is also included.

3.2 Detection, Isolation and Characterization

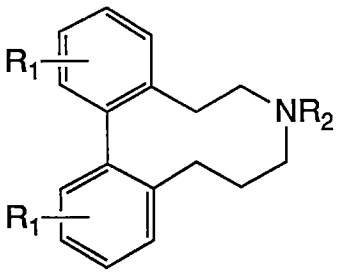
The GC-MS screening for dibenz*[d,f]* azecine alkaloids from various plant sources (Chapter 2, Section 2.6) indicated at least three genera containing this alkaloid type: the genera *Wurmbea* and *Baeometra* in the family Liliaceae, and *Athrotaxis* in the family Taxodiaceae. The dibenz[*d,f*]azecine component of the *Athrotaxis* sp. indicated a ring A or ring C-reduced dibenz[*d,f*]azecine derivative, whose isolation was not pursued further as its concentration was extremely low. Likewise, no further separation and isolation work on the dibenz*[d,f]* azecine from *Baeometra uniflora* (Jacq.) G. Lewis was conducted due to the unavailability of this plant. The study was therefore concentrated on the *Wurmbea* species.

Table 3.1 Plant Sources of Dibenz[*d,f*]azecine Alkaloids as Detected by the GC-MS Technique.

Plant Species	Dibenz[*d,f*]azecine Alkaloids Detected (Molecular Weights)
Baeometra uniflora	387[a]
Wurmbea uniflora	373[a], 387[a]
Wurmbea pygmaea	373[a], 387[a]
Wurmbea dioica ssp. *alba*	357[a], 373[a], 387[a]
Wurmbea dioica ssp. *dioica*	341[a], 341[b], 355[a], 357[a], 371[a], 373[a], 401[b]
Athrotaxis selaginoides	313[a]

[a] NMe in the heterocyclic ring; [b] NH in the heterocyclic ring

Table 3.2 Proposed Structures of Dibenz[*d,f*]azecine Alkaloids Detected from *Wurmbea* and *Baeometra* spp.



MW'S	Substituents
341	R_1 OCH_2O, 2(OMe) R_2 H
355	R_1 2(OCH_2O), 1(OH) or 1(OCH_2O), 2(OMe) R_2 Me
357	R_1 1(OCH_2O), 1(OMe), 2(OH) or 1(OH), 3(OMe) R_2 Me
371	R_1 1(OCH_2O), 2(OMe), 1(OH) R_2 Me
373	R_1 3(OMe), 2(OH) R_2 Me
387	R_1 4(OMe), 1(OH) R_2 Me
401	R_1 4(OMe), OCH_2O R_2 H

There are some forty species of *Wurmbea* known, nineteen of which are native in Australia[146]. From previous studies[3], *Wurmbea capensis* Thunb., *W. purpurea* Banks, *W. spicata* (Burm) Dur et Schinz,

and *W. dioica* F. Muell. were found to contain tropolonic as well as non-tropolonic bases. Some of the tropolonic bases, such as colchicine, were positively identified, whereas the non-tropolonics were only detected as being present by TLC analysis. In this work, *Wurmbea dioica* (R.Br.) F.Muell. ssp. *dioica*, *W. dioica* ssp. *alba*, T. Macfarlane, *W. pygmaea* (Endl.) Benth., and *W. uniflora* (R.Br.) T. Macfarlane were screened by GC-MS and found to contain dibenz*[d,f]*azecine-type alkaloids (Table 3.1), together with other alkaloid types. Of the four species studied, only *Wurmbea dioica* ssp. *dioica* could be obtained in sufficient quantities to allow more detailed separation and isolation of the alkaloid components, especially the dibenz*[d,f]*azecines. Bases of this type were generally found as minor components of the crude alkaloid extract.

3.2.1 Dibenz*[d,f]*azecine Alkaloids from *Wurmbea dioica* ssp. *dioica*

Figure 3.1

Wurmbea dioica ssp. *dioica*, commonly known as Early Nancy, is a small bulbous plant which grows in pastures, grassland and light forest[161] (Figure 3.1) in all states of Australia except the Northern Territory. It flowers from early to late spring, and stands 10-20 cm in height (Figure 3.2) in clusters or scattered populations of dioecious, subdioecious, or hermaphrodite plants[162](Figure 3.4). The tepals are ordinarily white with purple nectaries although some pink and green ones also occur (Figure.3.3). The flowers of the plant give off a strong cloying scent.

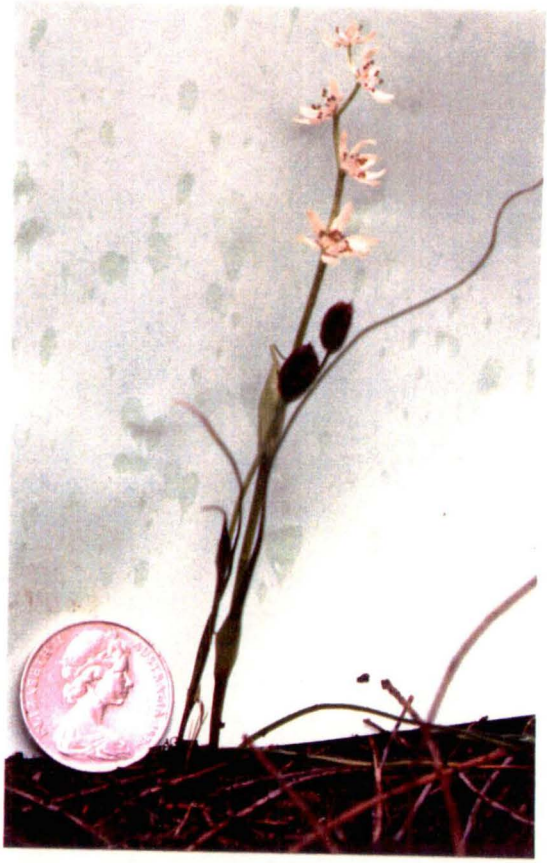


Figure 3.2

Figure 3.3

Figure 3.4

Samples of this plant were collected from various places in Tasmania as well as Victoria[163] from mid-September to late October. The plants in the northern part of Tasmania, as at Epping Forest, mature earlier than those in the southern part, as at Rosny Point near Hobart. In most cases, due to its small size and patchy distribution, the quantity of the plant sample collected was not sufficient for further separation but enough for GC-MS screening. The crude alkaloid extract of this plant was estimated to contain about thirty to fifty alkaloidal components based on its GC-MS ion trace. Consequently, a lot of overlapping of the ion peaks was observed. The extent of overlapping was also dependent on the activity of the column being used. Figure 3.5 illustrates the overlapping of peaks, as clearly seen in the area between 20.75 to 21 minutes, by recalling from the total ion chromatogram ion traces of the different fragments indicated in the mass spectrum and overlapping them.

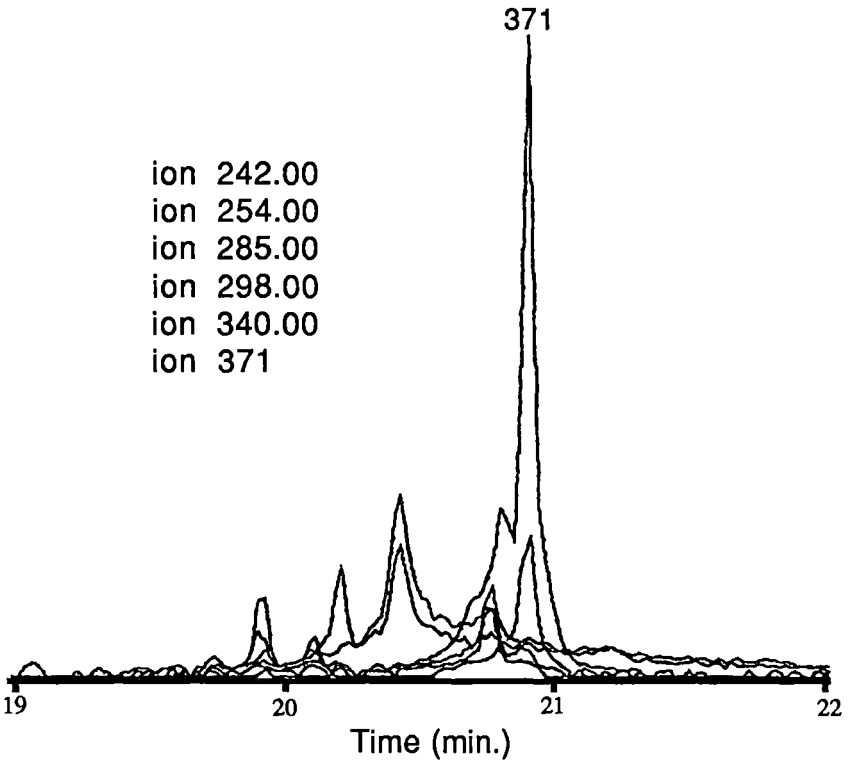


Figure 3.5 Overlap of GC-MS Ion Chromatograms

Table 3.3 Dibenz[*d,f*]azecine Alkaloid Profile of *W. dioica* ssp. *dioica*

Sample Number[a]	Dibenz[*d,f*]azecines detected (MW'S)								Remarks[b]
	341	341	355	357	371	373	387	401	
1[c]									Y
2			+ve	+ve	+ve	+ve	+ve		VY
3			+ve			+ve	+ve		M
4			+ve		+ve	+ve	+ve		Y
5			+ve			+ve	+ve		M
6	+ve		+ve	+ve	+ve	+ve	+ve		M
7			+ve		+ve		+ve		?[d]
8							+ve		?
9	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	M
10			+ve			+ve	+ve		VM

[a] Place of collection: 1. Hobart Domain; 2. Tunbridge; 3. Hobart Domain; 4. Epping Forest; 5. Rosny Hill; 6. Rosny Point; 7. Sandon, Victoria; 8. 3 km E of the Victoria-South Australia border 9. Epping Forest; 10. Gordon's Hill; [b] **VY** very young; **Y** young; **M** mature; **VM** very mature; [c] crude alkaloid extract not screened; [d] received as dried plant sample.

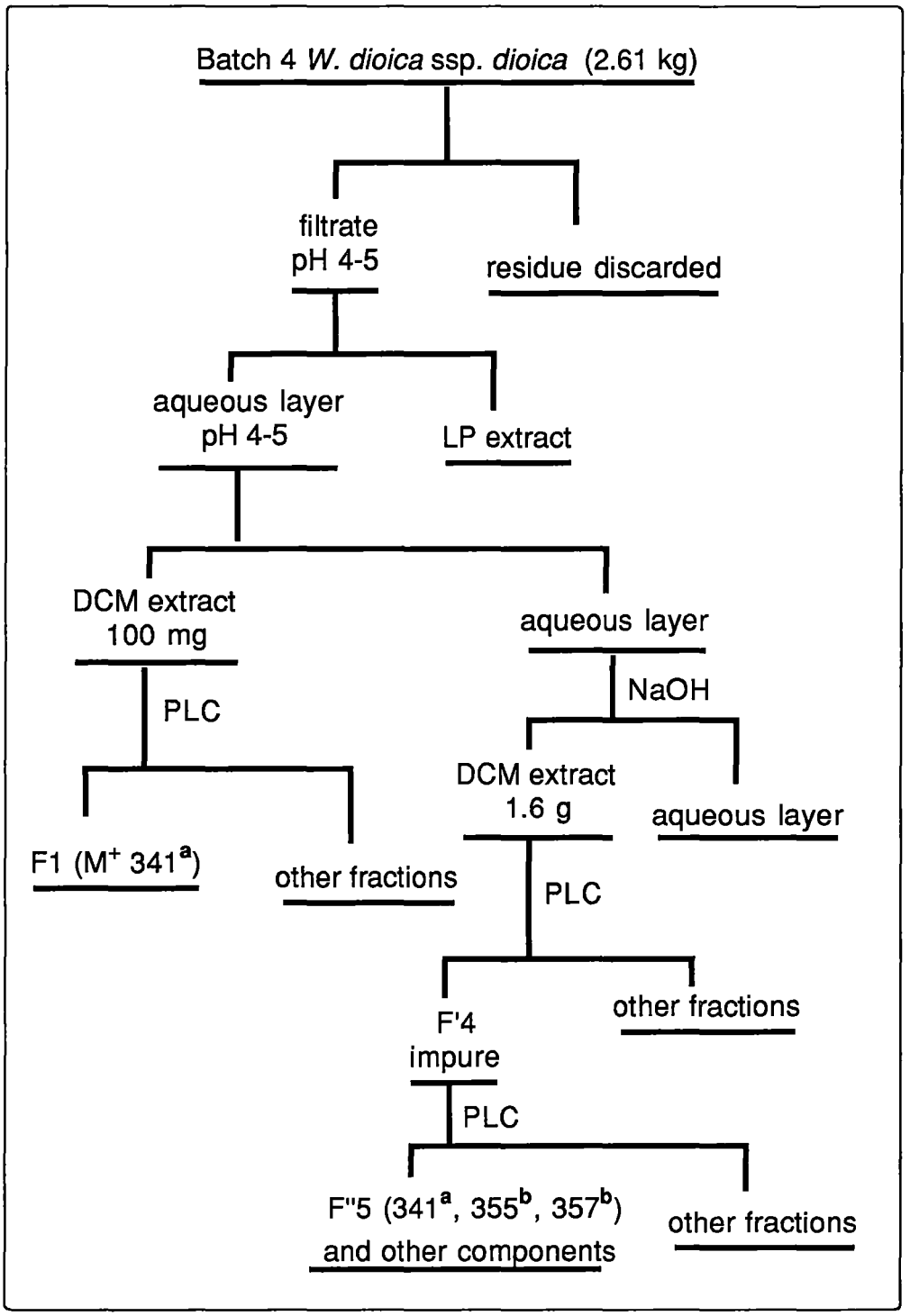
A summary of the dibenz*[d,f]* azecines detected from different batches of samples of *Wurmbea dioica* ssp. *dioica* by the GC-MS technique is given in Table 3.3. Overall, eight dibenz[*d,f*]azecine-type alkaloids were detected, and Table 3.2 shows their possible substitution patterns. The nature of the R_2 substituent, however, was assigned with certainty based on their MS fragmentation patterns. Six of these dibenz[*d,f*]azecine bases were found to have an N-Methyl group in the heterocyclic ring while two, of molecular weights 341 and 401, had an -NH group. The dibenz[*d,f*]azecine alkaloids of molecular weights 355,

371, 373, and 387 were generally detected as minor, yet distinct, components in the crude alkaloid extract. Those of molecular weights 341(2), 357, and 401 were detected only after chromatographic fractionation except in a few cases, as for the dibenz[*d,f*]azecine component of molecular weight 357. Concentrations of the dibenz[*d,f*]azecine derivatives in the crude alkaloid extract, as well the overall alkaloid profiles, varied from one sample to another.

3.2.2. Isolation and Characterization

Scheme 3.1 outlines the extraction and isolation of alkaloids from *Wurmbea dioica* ssp. *dioica* collected from Epping Forest (Batch 4) from which the the dibenz[*d,f*]azecine-type alkaloid wurmbazecine was first detected and isolated. The total ion chromatogram (TIC) of the dichloromethane extract (100 mg) at pH 4-5 showed the presence of the dibenz[*d,f*]azecines of MW 355, 371, and 387. In the preparative layer chromatography (PLC) of this extract, all of the bands were collected and analyzed by GC-MS technique. All the fractions showed a number of components as indicated by their TIC except for the first fraction. The main component of this fraction (1.5 mg) had a molecular weight of 341 whose fragmentation pattern was indicative of a dibenz[*d,f*]azecine-type system, with a methyl group on nitrogen, in the heterocyclic ring; that is, the mass spectrum of this isolate gave peaks at M^+-58, M^+-72, m/z 70, 58, 57, 44, and 42. Peak broadening made the ^1H n.m.r spectrum of this compound difficult to interpret, so the fraction was basified with ammonia, re-extracted with dichloromethane and passed through an alumina plug. A cleaner sample later named wurmbazecine was obtained (< 1 mg). The presence of three aromatic methoxy groups, an

N-methyl group, and five aromatic protons was deduced from its ^1H n.m.r. spectrum. Some impurity peaks obscured



a wurmbazecine, b other dibenz[d,f]azecine alkaloids detected

Scheme 3.1 Isolation and Detection of Wurmbazecine

part of the aliphatic region of the spectrum. However, the ^{1}H n.m.r. and the mass spectra suggested a dibenz[*d,f*]azecine skeleton in which rings A and C are aromatic (Figure 3.6). Coupling of the aromatic protons at δ 7.19 (d; J 8.5) and δ 6.89 (dd; J 8.5, 2.7) indicated two aromatic protons adjacent to each other . A third aromatic proton at δ 6.6 (d; J 2.7) must be in a meta position relative to the proton at δ 6.89. From this information, four possible structures of this isolate could be deduced (Figure 3.7).

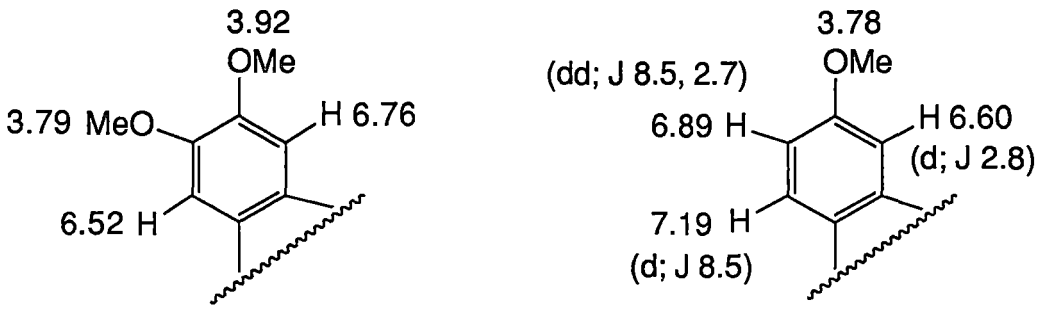


Figure 3.6 Substitution Pattern of Wurmbazecine

The ^{1}H n.m.r. and the ^{13}C n.m.r. data could not be used confidently to distinguish the structure of wurmbazecine from any of its isomers. In theory, the nuclear Overhauser (nOe) difference spectroscopy or long range proton-proton correlation experiments (COSYLR) could be used for this purpose by establishing proton disposition or connectivities. Difficulties were experienced in obtaining good n.m.r. experimental results on the isolate due to its extremely low concentration. A ^{13}C n.m.r. spectrum was obtained using an insert tube, which allowed observation of most of the methyl, methylene and methine carbon atoms but not the quaternary carbon atoms, after three days of spectral accumulation. The resolution of the ^{1}H n.m.r. spectrum of the sample in the insert tube was too poor to allow good n.O.e. or 2-D experiments. It was therefore decided to synthesize these possible systems for comparison.

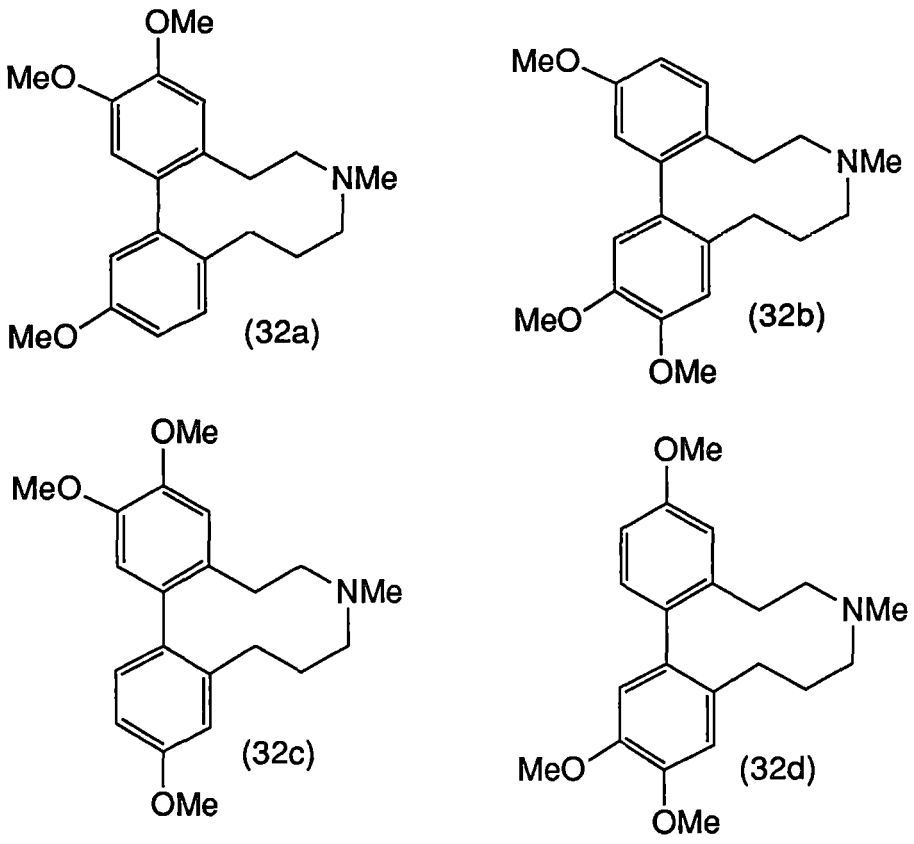
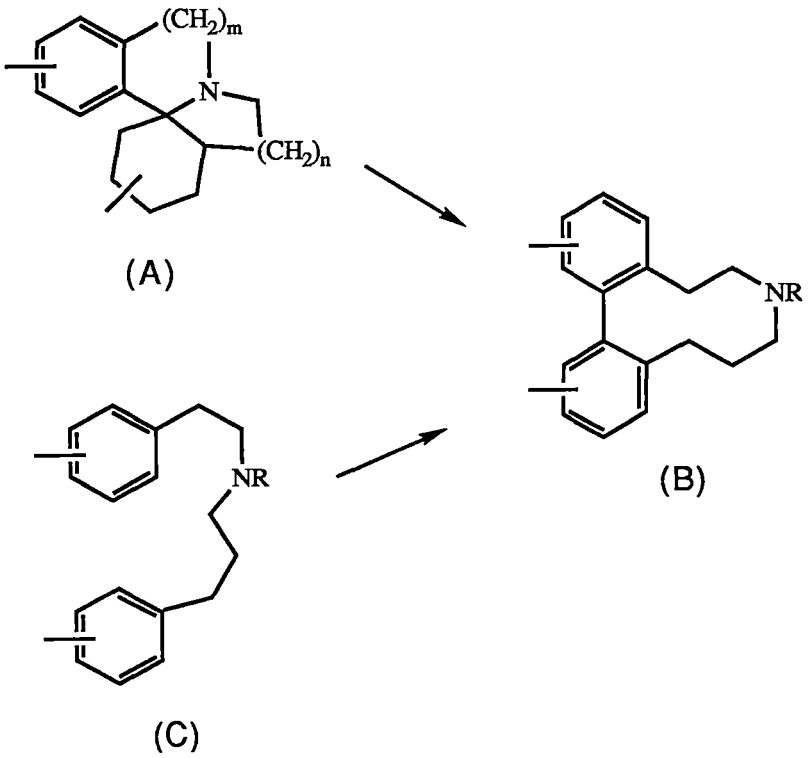


Figure 3.7 Possible Structures of Wurmbazecine

3.3 Synthesis

The reported syntheses of the dibenz[*d,f*]azecine systems have basically involved two approaches (Scheme 3.2). The first approach, was via a homoerythrina-type intermediate as in the work of McDonald and Suksamrarn[157], and by Tsuda, Murata, and Kiuchi[156], where ring destruction of a benzylic C-N bond of a homoerythrina-type skeleton (A) gave rise to a dibenz*[d,f]*azecine skeleton (B). Alternatively, McDonald and Wylie[158], and Tanaka and co-workers[159,160], synthesized these systems by direct oxidative coupling of acyclic phenethyl-3-phenylpropylamine-type derivatives (C).

The synthesis of dibenz[*d,f*]azecine systems was called for to fully establish the mass spectral fragmentation patterns of this group of alkaloids. Most of the synthesized systems have aromatic A- and C-rings as in the case of the naturally-occurring dibenz[*d,f*]azonine derivatives. Their synthesis was also made to confirm the structure of wurmbazecine, the dibenz*[d,f]*azecine alkaloid isolated from *Wurmbea dioica* ssp. *dioica*. Attempts were also made to obtain these systems from some known alkaloids such as the homoerythrina and the homomorphine alkaloids.



Scheme 3.2 Synthetic Routes to Dibenz[*d,f*]azecine Derivatives

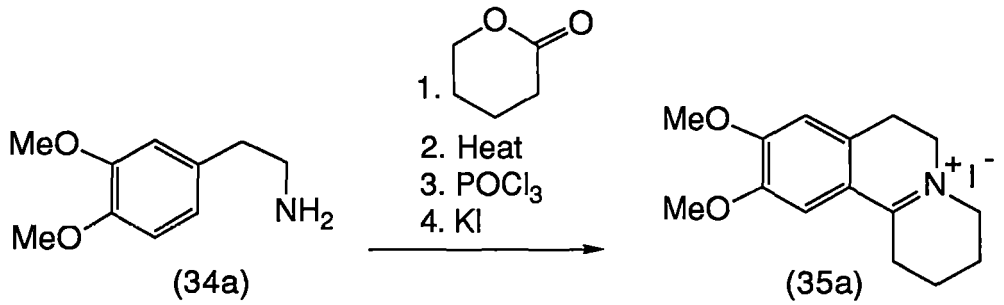
The initial approach to the synthesis of dibenz[*d,f*]azecines was via a homoerythrina-type intermediate. The route followed was parallel to the previous synthesis of some dibenz[*d,f*]azonine systems by Bremner and Dragar[164]. A similar approach involving direct annulation of an endocyclic enamine precursor to further elaborate the ring system, followed by ring expansion by cleavage of a benzylic C-N bond, was

recently reported [165]. Cleavage of the benzylic C-N bond of B-homoerythrina or C-homoerythrina-type precursors could lead to the dibenz[d,f]azecine derivatives (32a) or (32b), respectively. However, this route does not provide an easy approach to variations in the oxygenation pattern of the A- or C-ring. An alternative approach to the synthesis of these bases via oxidative coupling of some phenethyl-3-phenylpropylamine derivatives was also attempted as in the synthesis by McDonald and Wylie [158].

3.3.1. Synthesis of Wurmbazecine and Related Systems

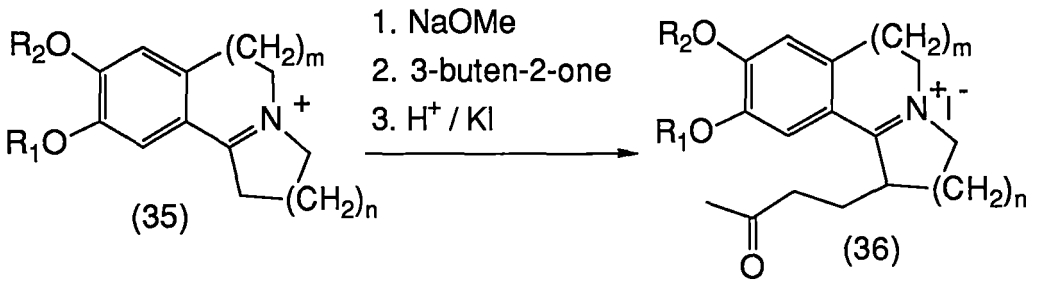
Alkylation of the iminium salts (35a) and (35b)

The iminium salt (35a), which has been synthesized[166] via the Bischler-Napieralski reaction of the amide derived from the corresponding amine (34a) and 5-pentanolide, was used as a starting material in this study (Scheme 3.3). Treatment of the iminium iodide salt (35a) with sodium methoxide base formed the enamine which was then reacted with methyl vinyl ketone allowing a Michael-type addition of the enamine to this ketone (Scheme 3.4). Initially, the yield of (36a) obtained was very poor. Some black to reddish tarry materials immediately formed upon addition of the methyl vinyl ketone indicating the rapid formation of various side products. The presumed formation of by-products was effectively decreased by decreasing the concentration of the enamine through the use of methanol instead of acetonitrile as solvent and working at a lower temperature (*ca.* -10°) using an ice-salt mixture. The iodide salt of this alkylated system (36a) could then be prepared in yields up to 85%.



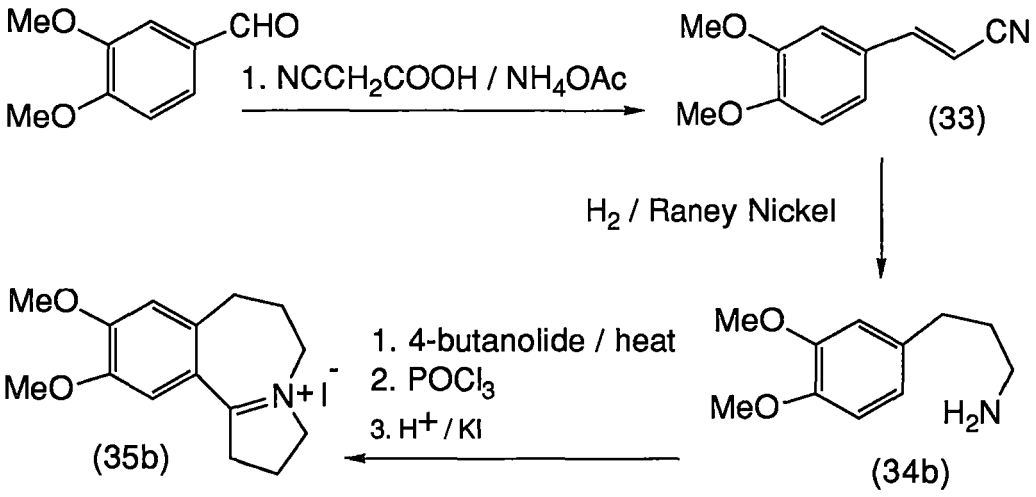
Scheme 3.3

Elemental analysis of (36a) was consistent with the empirical formula $C_{19}H_{26}NO_3I$. The ^1H n.m.r. peaks at δ 6.81 (H_{11}), 7.81 (H_8), 4.00 (OCH_3), 4.04 (OCH_3), and 2.19 ($COCH_3$) were consistent with the structure (36a) and compared very closely with the non-alkylated precursor (35a). The ^{13}C n.m.r. spectrum showed the presence of two additional methylene carbons and the carbonyl carbon (δ 207.50) of the alkyl side chain. The presence of the side chain was further supported by the presence of a resonance signal at δ 18.1, assigned to the C-methyl group.



	R_1	R_2	m	n
a	Me	Me	1	2
b	Me	Me	2	1
c	$-CH_2-$		1	2

Scheme 3.4



Scheme 3.5

The iminium salt (35b) was synthesized following the same methodology as for the iminium salt (35a). The amine (34b) was prepared from the condensation of 3,4-dimethoxybenzaldehyde with cyanoacetic acid to form the carbonitrile intermediate (24), which was then reduced to the desired amine (34b) by hydrogenation over Raney nickel (Scheme 3.5). The desired iminium salt (35b) was then obtained from the Bischler-Napieralski reaction on the amide from the amine (34b) and 4-butanolide. The yield of benzazepinium salt (35b) was very low even after optimization of the reaction conditions. This low yield could be attributed to the relative difficulty in cyclizing a seven-membered ring. The empirical formula $C_{15}H_{20}NO_2I$ for this compound was in accord with the measured molecular mass. The absorption band at 1622 cm^{-1} in the IR spectrum, and the ^{13}C n.m.r. signal at d 184.51 corresponded to the $C=N^+$ bond of the salt. The rest of the ^1H n.m.r. and ^{13}C n.m.r. data were consistent with the given structure of (35b).

Alkylation of the iminium salt (35b) was done in the same way as for (35a) except for the use of acetonitrile instead of methanol as solvent. The resulting salt (36b) was obtained in good yield. The ^1H n.m.r. and

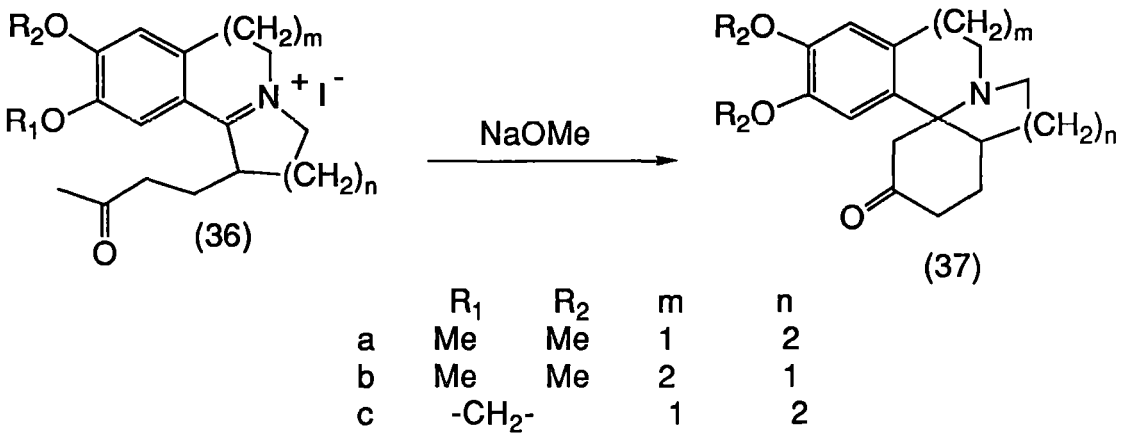
the ^{13}C n.m.r. spectrum compared very closely with that of the iminium salt (36a).

Likewise, an iminium salt (36c) was prepared to serve as a precursor to other dibenz[*d,f*]azecine derivatives with a different oxygenation pattern. The ^{1}H n.m.r. and ^{13}C n.m.r. of the alkylated iminium salt (36c) obtained in yields of up to 97%, from (35c) , were also consistent with the given structure and compared very closely with (36a).

Cyclization of the iminium salts (36a) and (36b)

The alkylated iminium iodide salt (36a) was converted efficiently to an oxo-homoerythrina derivative (37a) by portionwise addition of sodium methoxide to methanolic solution of the iodide salt at 0° (Scheme 3.6). TLC and GC-MS analysis of the crude product after partial purification through an alumina quick column, indicated that the complete conversion of (36a) to (37a) had been achieved. The yield of 95% was an improvement to the previous unreported yield[167] of 50%. The spectroscopic properties of (37a) confirmed the cyclization of the alkyl side chain as desired. Its ^{1}H n.m.r. spectrum indicated the absence of the $COCH_3$ group. Effective cyclization at the benzylic carbon was also indicated by a quaternary carbon resonance at δ 63.32 for the spirocarbon. Furthermore, eight methylene carbons were identified in the ^{13}C DEPT n.m.r., the methyl carbon in (36a) being converted to a methylene carbon. The mass spectrum, showed an intense peak due to a loss of 57 mass units, supported a characteristic homoerythrina N-induced α cleavage resulting in the fragmentation of the A-ring[168]. A complete assignment of the ^{1}H and ^{13}C n.m.r. chemical shifts on the basis of the homonuclear shift correlated 2-D n.m.r (COSY) and

heteronuclear shift correlated 2-D (XHCORR) experiments was also made.



	R_1	R_2	m	n
a	Me	Me	1	2
b	Me	Me	2	1
c	$-CH_2-$		1	2

Scheme 3.6

Cyclization of (36b) and (36c) also took place with relative ease. The mass spectra of the cyclized products (37b) and (37c) were very similar to (36a), where the main fragment was m/z 57, due to the N-induced cleavage of the A-ring. The assignments of their ^{1}H and ^{13}C n.m.r. chemical shifts were made by comparison with (37a).

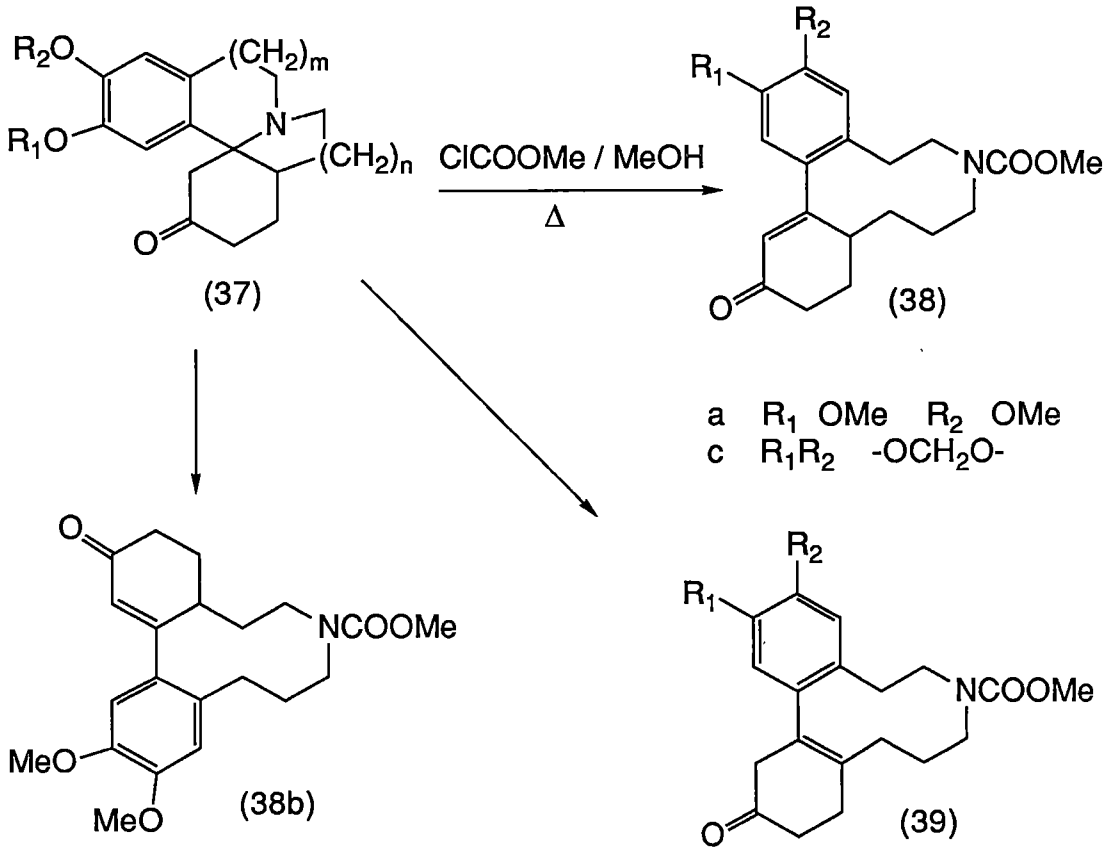
Formation of the Medium Ring Systems (38a) and (38b)

Von Braun cleavage of the benzylic C-N bond of a homoerythrina alkaloid was reported previously by Langlois, Das and Potier[77] Their approach was applied in this work to the homoerythrina alkaloid taxodine (55) (Section 3.4) whose reaction with cyanogen bromide took place very slowly as monitored by GC-MS analysis. This was consistent with previous observations[167] and was not pursued further. However, when instead of cyanogen bromide excess methyl chloroformate was used, the benzylic C-N bond cleavage occurred efficiently giving rise to

the cyclohexenone-annulated 3-benzazecine (38a) (Scheme 3.7); this reaction is believed to occur via the N-methoxycarbonylammonium salt intermediate followed by the abstraction of a proton alpha to the keto group. The apparent difference in the rates of reaction of cyanogen bromide and methyl chloroformate could be due to the relative ease of addition of the nitrogen atom to the unsaturated carbon center in methyl chloroformate as opposed to cyanogen bromide. The GC-MS analysis of the crude product showed the benzazecine (38a) (M^+373) as the major product. A minor amount of a component of the same molecular weight (M^+ 373) was also present. This was separated by PLC to give (38a) in 84% yield. The minor component (M^+ 373), which was not characterized further, may be the isomer (39) of (38a), analogous to that obtained in the synthesis of laurifonine[167].

The high resolution mass spectrum of (38a) confirmed the expected molecular mass (373.1893) and the corresponding elemental composition ($C_{21}H_{27}NO_5$). The mass spectrum also indicated the loss of a fragment containing the carbamate fragment m/z 102 [$CH_2N(CH_2)COOMe$]. The ^1H n.m.r. and the ^{13}C n.m.r. spectra of (38a) showed the broadening and doubling up of peaks which was expected of carbamates due to hindered rotation about the amide bond. However, the ^1H n.m.r. chemical shifts for the two aromatic protons (δ 6.81 and δ 6.50) the olefinic proton (δ 5.92), the two methoxy groups (δ 3.92 and δ 3.86), and the carbamate methoxy group (broad, δ 3.53) could be clearly distinguished. The ^{13}C n.m.r. spectrum also indicated the presence of a cyclohexenone ring, the chemical shift of the carbonyl carbon being 199.48 ppm[192] as contrasted to the ^{13}C n.m.r. chemical shift of the carbamate carbonyl carbon at 157.49 ppm. This was supported by an IR

absorption band at 1672 cm^{-1} for the cyclohexenone carbonyl carbon and 1699 cm^{-1} for the carbamate[169].

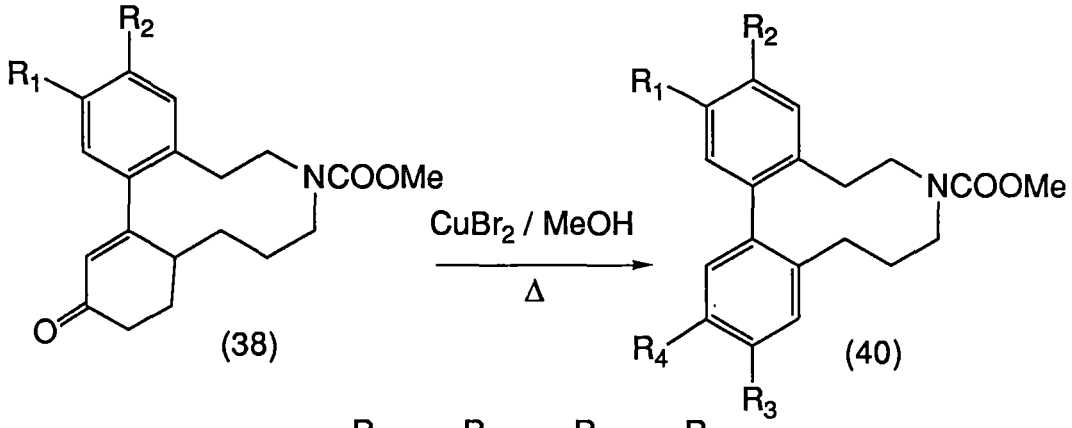


Scheme 3.7

The same methyl chloroformate procedure was applied to the cleavage of the benzylic C-N bond of the homoerythrina bases (37b) and (37c). GC-MS analysis of the crude product from (37b) indicated the presence of the expected medium ring system (38b) along with some minor components (M^+ 373 and M^+ 389). This crude product was used for the aromatization reaction without purification. On the other hand, ring destruction of (37c) using methyl chloroformate afforded only one product (38c) along with some unreacted precursor (37c). This was also used for the next reaction without purification.

Aromatization of (38a) (38b), and (38c)

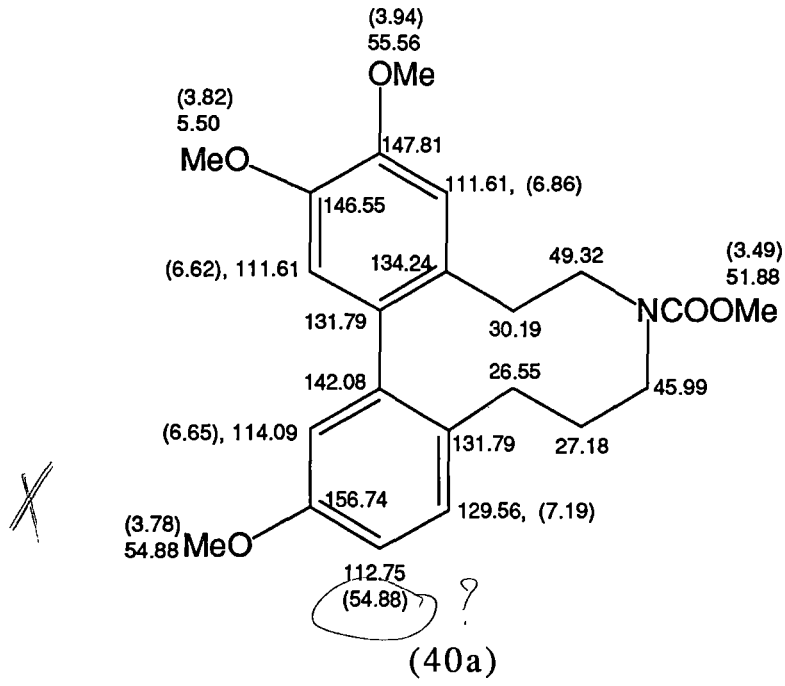
The use of copper (II) bromide in methanol or water-methanol solvent systems was applied by Fort[170], Bondon, Pietrasanta, and Pucci[171], and by Bremner and Dragar[164] in the aromatization of some cyclohexenone systems. This led to the formation of phenols or their methyl ethers depending on the solvent used. When applied to the cyclohexenone (38a), using dry methanol as solvent, the aromatized methyl ether derivative (40a) was obtained in very high yield (Scheme 3.8). Its mass spectrum showed peaks corresponding to the loss of masses m/z 102 and 116 [$CH_2CH_2N(CH_2)COOMe$] which are characteristic carbamate-containing fragments from cleavage of the medium ring.



	R_1	R_2	R_3	R_4
a	OMe	OMe	H	OMe
b	OMe	H	OMe	OMe
c	-OCH_2O-		H	OMe
d	OMe	OMe	H	OH

Scheme 3.8

Although doubling up and broadening of the ^{1}H and ^{13}C n.m.r. spectra were once again observed, it was still possible to get a full interpretation of the spectra of the carbamate derivative (40a). The aromatization of the cyclohexenone ring was indicated by the coupling patterns of the new aromatic protons at δ 7.19 (broad s, H_{11}), 6.86 (broad dd; J 8.39, 2.72; H_{12} overlapped with H_4), and 6.65 (broad s; H_{14}). A full assignment of the ^{1}H and ^{13}C n.m.r. was made possible by comparison with (32a) and (32d) where more n.m.r. data was obtained from COSY 2-D, XHCORR 2-D (C-H correlation), and XHCORR (with long range enhancement effects) experiments.

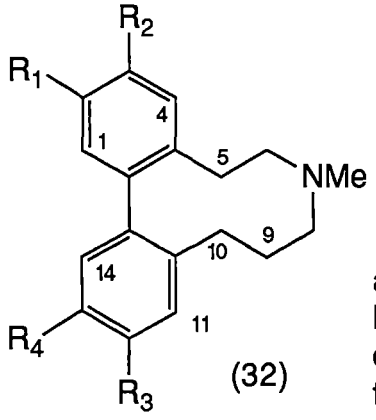


(40a)

The crude product containing (40b) was obtained in the same way as (40a). Complete aromatization leading to the formation of (40b) was presumed to have taken place as indicated by its GC-MS ion trace. However, other ions were observed due to impurities which were initially present with the homoerythrina base (37b). The crude product was used for the next reaction without purification.

Aromatization of (38c) led to two products: the expected product (M^+ 369) as the major component with a minor one of molecular weight 399. The major product was separated by PLC and was fully characterized by comparison with (38a). A separate sample of (38a) was also reacted with copper (II) bromide using water instead of methanol as solvent. Analysis by GC-MS showed some unreacted (38a) in minor amounts and the expected hydroxylated derivative (40d) as the sole product. Its ^1H and ^{13}C n.m.r. data compared closely with those of (40a) and (40c).

Reduction of (40a), (40b), (40c), and (40d)



(32)

	R_1	R_2	R_3	R_4
a	OMe	OMe	H	OMe
b	OMe	H	OMe	OMe
c	-OCH_2O-		H	OMe
f	OMe	OMe	H	OH

Figure 3.8

The carbamate derivative (40a) was reduced using lithium aluminum hydride to afford a basic product. GC-MS analysis of the crude product indicated complete reduction, with the dibenz[*d,f*]azecine (32a) (M^+ 341) as the sole product (Figure 3.8). Its mass spectrum gave the characteristic dibenz[*d,f*]azecine fragmentation pattern showing peaks due to loss of 15, 58, and 72 mass units, and the more intense

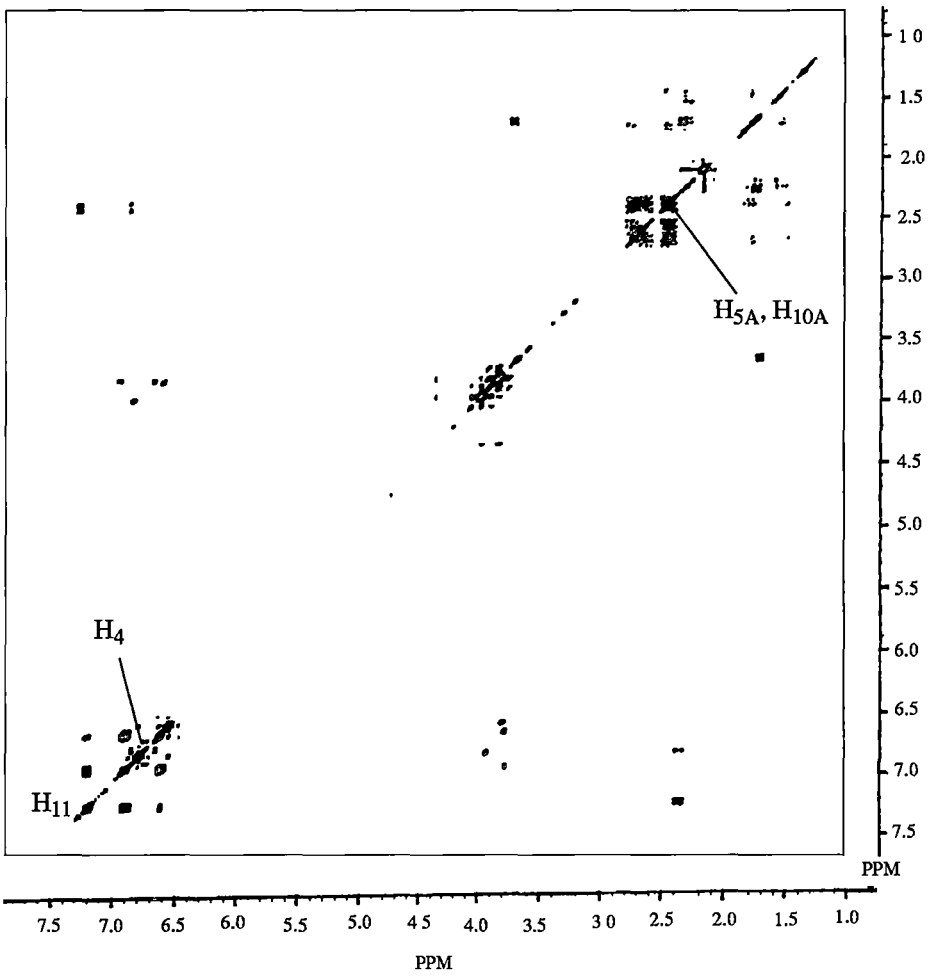


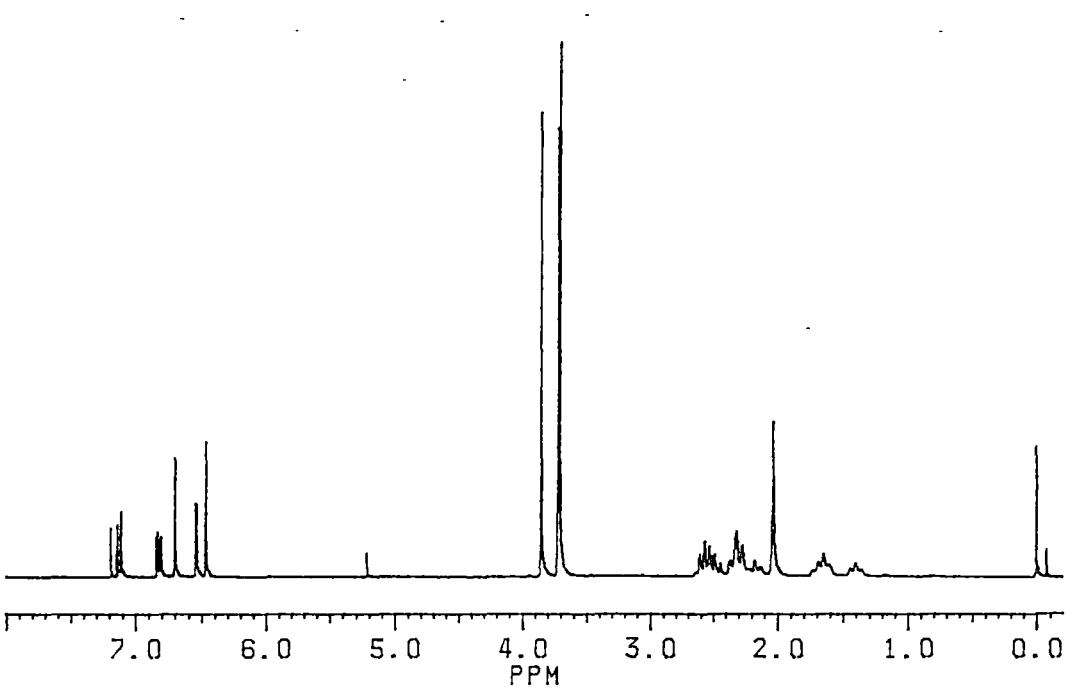
Figure 3.9 COSYLR Spectrum of Dibenz[*d,f*]azecine (32a)

peaks due to fragments at m/z 70, 58 57, 42 and 44. The possiblity of the C-ring methoxy group being attached to C_{12} instead of C_{13} was ruled out based on the COSYLR 2-D (COSY with enhancement of long range effects) experiments (Figure 3.9). The second coherence period (D2) was set for 0.08 milliseconds in which normal COSY cross peaks as well as long range effects were observed. The aromatic proton at C_{11} (δ 7.19) showed cross peaks with an aliphatic proton (δ 2.38) presumably attached to C_{10}. A cross peak was also observed between the aromatic proton at C_4 (δ 6.77) and an aliphatic proton (δ 2.38).

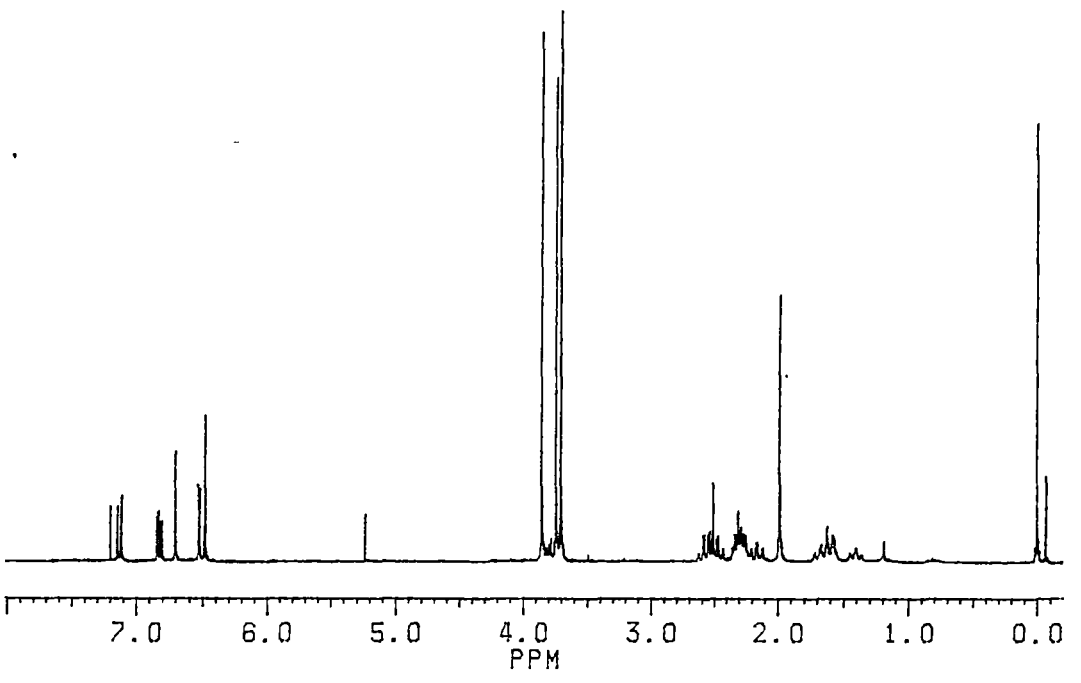
The position of the methoxy group at C_{13} was also well supported by the coupling patterns of the aromatic protons. A 2-D correlation of carbon and hydrogen (XHCORR) experiment supplemented by a COSY 2-D experiment made possible the assignment of most of the protonated carbon atoms and the corresponding protons attached to them. Their complete assignment including quaternary carbon atoms was made by comparison with (32f) whose XHCORR (with long range effects) data were also obtained. The ^{13}C n.m.r. chemical shifts of the methine carbons of the biaryl system differed significantly from those noted by Pande and Bhakuni[154] and by Bremner and Dragar[167] in the analogous dibenz[*d,f*]azonine derivatives.

The carbamate (40b) was reduced with lithium aluminum hydride without further purification. This gave the desired product (32b) as a major component which was separated by PLC. Its mass spectrum exhibited the characteristic peaks of the dibenz[*d,f*]azecine systems with an N-methyl group in the heterocyclic ring. The ^{1}H n.m.r. spectrum of this compound was almost indistinguishable from (32a), but slight differences were noted in their ^{13}C n.m.r. spectra (Figure 3.10 and 3.11, Table 3.3).

Similarly, the carbamates (40c) and (40d) were also reduced, affording products (32e) and (32f), respectively. Most peaks in their ^{1}H and ^{13}C n.m.r spectra were assigned by comparison with their carbamate precursors (40c) and (40d), and with the reduced systems (32a) and (32b). However, the chemical shifts of the quaternary carbons were only fully established from the C-H correlation (with long range effects) data.

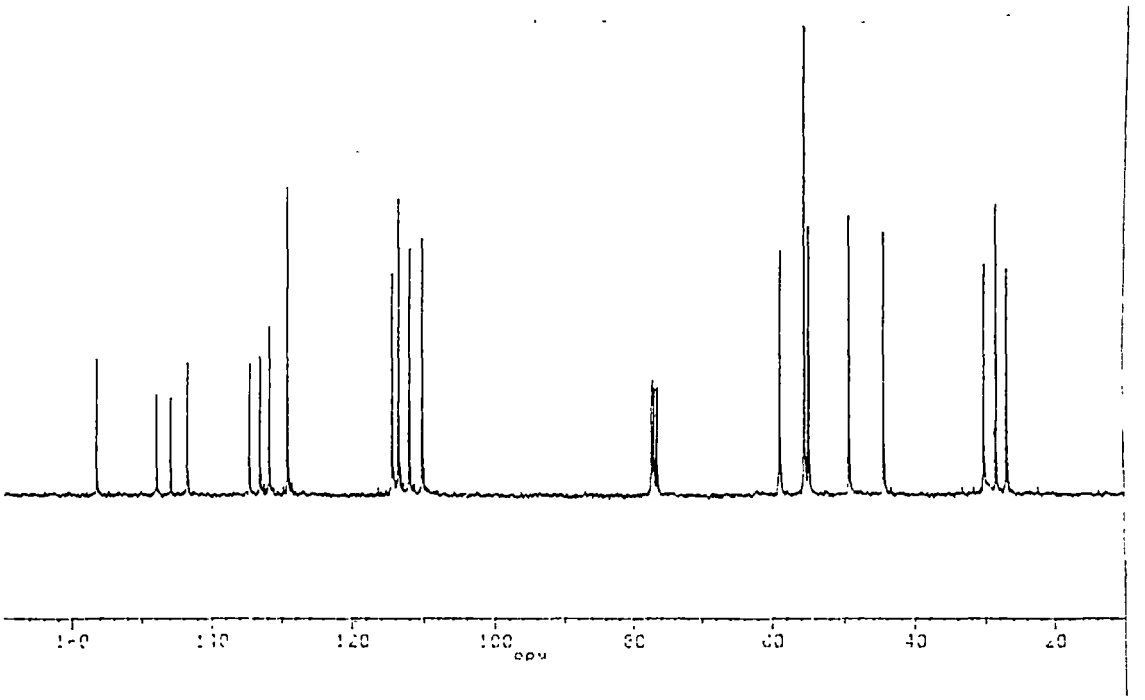


(32a)

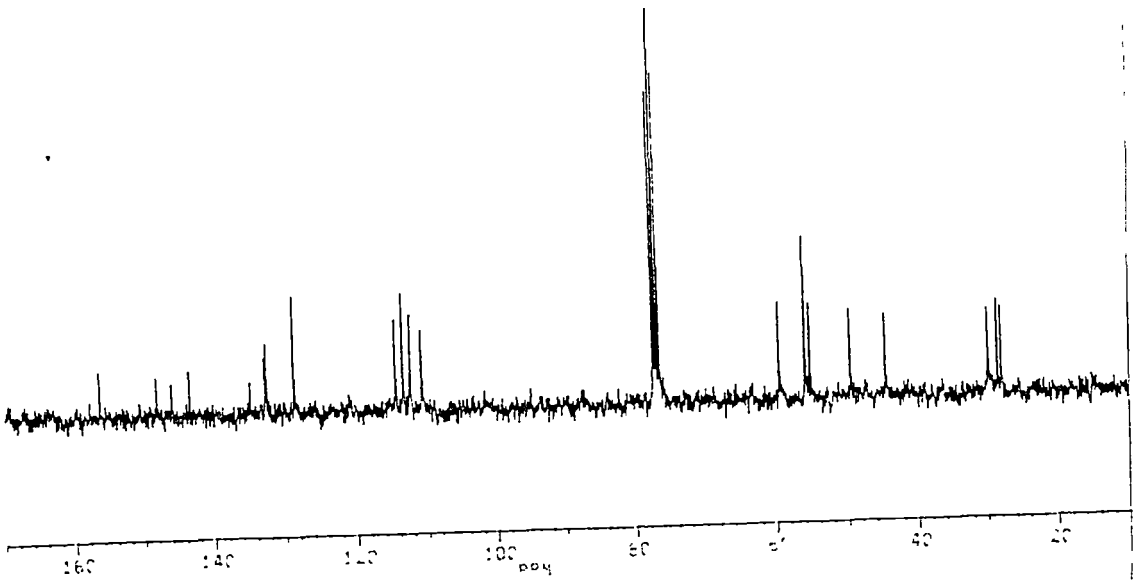


(32b)

Figure 3.10 ^{1}H n.m.r. of (32a) and (32b)

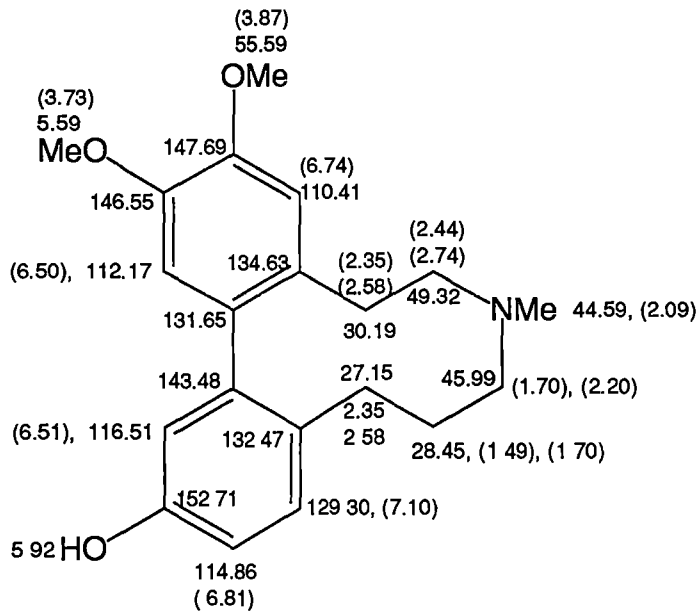


(32a)



(32b)

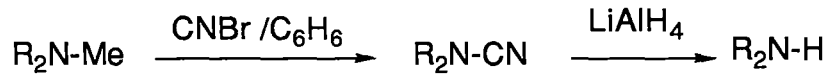
Figure 3.11 ^{13}C n.m.r. of (32a) and (32b)



(32f)

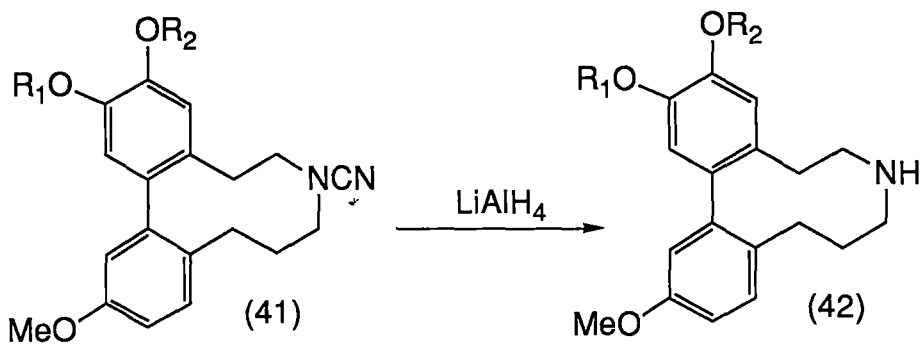
N-Demethylation of the Dibenz[*d,f*]azecine Derivatives (32a) and (32e).

Von Braun's demethylation procedure (Scheme 3.9) was applied previously in the synthesis of laurifine from laurifonine[164] and has been successfully applied to the dibenz[*d,f*]azecine derivatives (32a) and (32e) in this study. Reaction of cyanogen bromide with the dibenz[*d,f*]azecine (32a), which was monitored by GC-MS, afforded the product (41a) in very high yield. The high resolution mass spectrum of this product was in accord with the composition ($C_{21}H_{24}N_2O_3$) and the molecular weight (352) of the given structure (41a). Its ^1H n.m.r. spectrum confirmed the removal of the N-methyl group, while the presence of the nitrile group was indicated by the ^{13}C n.m.r. resonance at δ 117.57 and an IR absorption band at 2204 cm^{-1}.



Scheme 3.9

The carbonitrile derivative (41a) was then reduced to the dibenz[*d,f*]azecine (42a) using lithium aluminum hydride (Scheme 3.10). Its molecular weight and elemental composition were consistent with that of the desired product (42a), while the IR spectrum showed the appearance of an absorption band at 3394 cm^{-1} corresponding to the NH group. The dibenz[*d,f*]azecine (42b) was also synthesized from the corresponding dibenz[*d,f*]azecine (32e) following the same procedure and was fully characterized. GC-MS analyses of these demethylated products indicated complete reduction; the moderate yields obtained indicated that some of the products were lost during work up. These two demethylated compounds (42a) and (42b) were then used as model compounds in the study of the MS fragmentation patterns of the N-demethylated dibenz[*d,f*]azecine derivatives (Chapter 2, Section 2.4).



	R_1	R_2
a	Me	Me
b	$-CH_2-$	

Scheme 3.10

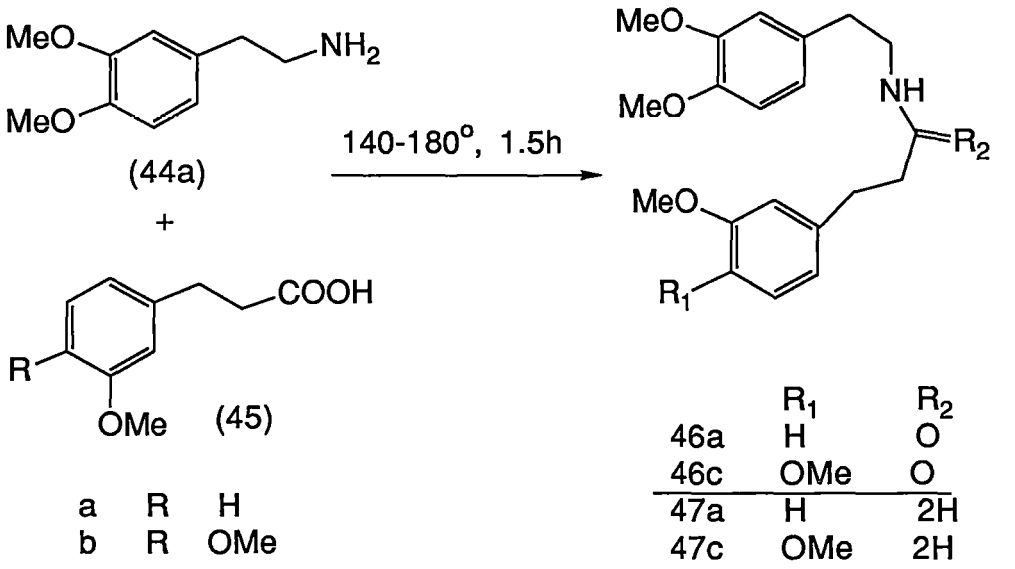
3.3.2. Synthesis of Wurmbazecine and/or Isomers Via Oxidative Cyclization with Thallium (III) Trifluoroacetate

The reported[158] high yield of a dibenz[*d,f*]azecine derivative via a direct oxidative coupling of an acyclic phenethyl-3-phenylpropylamine derivative seemed to be a practical approach to the synthesis of the alternative structures (32c) and (32d) of wurmbazecine (Section 3.2.2, Figure 3.7). The initial synthesis of (32c) from the amide (48a) via oxidation with thallium (III) trifluoroacetate in trifluoracetic acid was unsuccessful. Synthesis of (32g), the system synthesized by McDonald and Wylie[158], was then undertaken to check their procedure. The results of this work are described below.

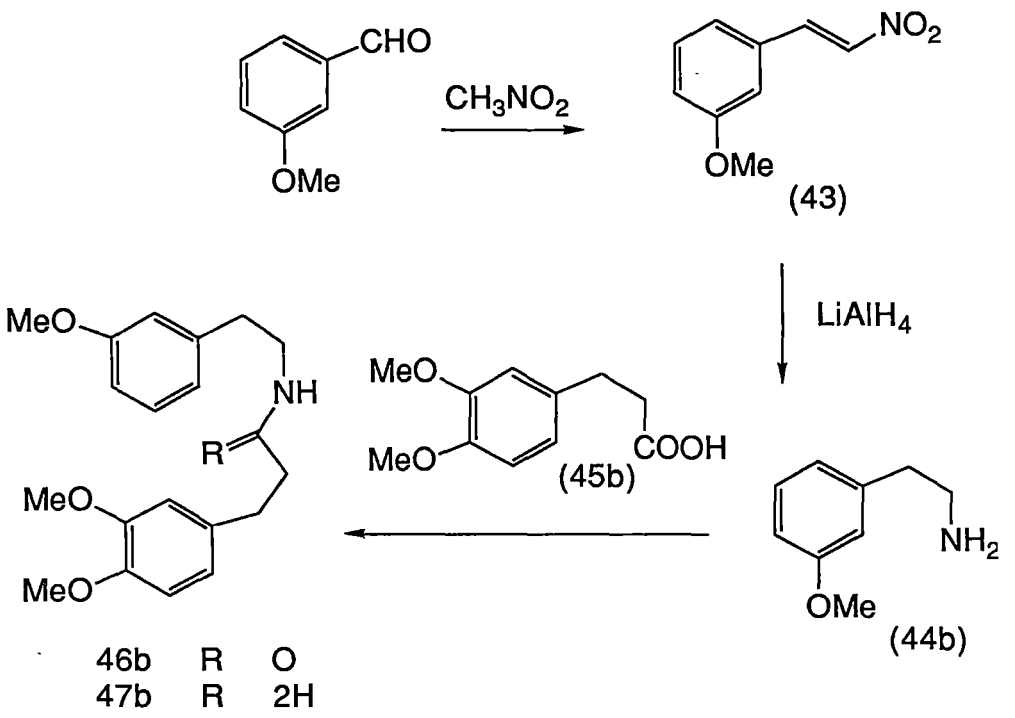
Synthesis of the amides (46a), (46b) and (46c).

To synthesize the amide (46a), 3-methoxycinnamic acid was catalytically hydrogenated over platinum oxide affording 3-(3-methoxyphenyl)propanoic acid (45a). This acid (45a) and homoveratryl-amine were melted together at 140-180° to form the amide (46a) (Scheme 3.11). The molecular weight and the elemental composition of this product were in accord with the the expected product. In the ^{1}H n.m.r. spectrum of (46a), signals indicating seven aromatic protons at δ 7.17 (1H) and δ 6.77-6.63 (6H), one -NH at δ 5.78 (1H), three methoxy groups at δ 3.83, 3.82, and 3.75, and eight aliphatic protons at δ 3.44 (2H), 2.91 (2H), 2.68 (2H), and 2.42 (2H) were identified. The ^{13}C n.m.r. resonance at δ 171.96 was indicative of an amide carbonyl carbon. In combination with ^{13}C DEPT experiments, the expected signals for the methyl,

methylene, methine and quaternary carbon atoms were accounted for and distinguished.



Scheme 3.11



Scheme 3.12

In the same manner, amide (46b) was synthesized from its corresponding acid (45b) and amine (44b) in very high yield (Scheme 3.12). The amine (44b) was synthesized from 3-methoxybenzaldehyde and nitromethane and the resulting product, the 3-methoxy-β-nitrostyrene (43), reduced with lithium aluminum hydride. Amide (46c) was also synthesized in the same manner as for (46a), from homoveratrylamine and 3,4-dimethoxyphenylpropanoic acid (Scheme 3.11). These amides were characterized by their MS, ^{1}H n.m.r. and ^{13}C n.m.r. spectra which compared very closely with the spectra from (46a). Each of the mass spectra of the three amides (46a), (46b), and (46c), gave weak to moderately intense parent ions, and peaks at m/z 209, 164, and 151, the intensities of which varied presumably according to the oxygenation pattern on the aromatic ring systems. The peaks at m/z 164 and 151 could arise from the fragmentation of benzylic or phenethyl moieties of the amides (Figure 3.12). The presence of a methoxy substituent in a <u>para</u> position relative to the aliphatic bridge seemed to enhance these fragmentations.

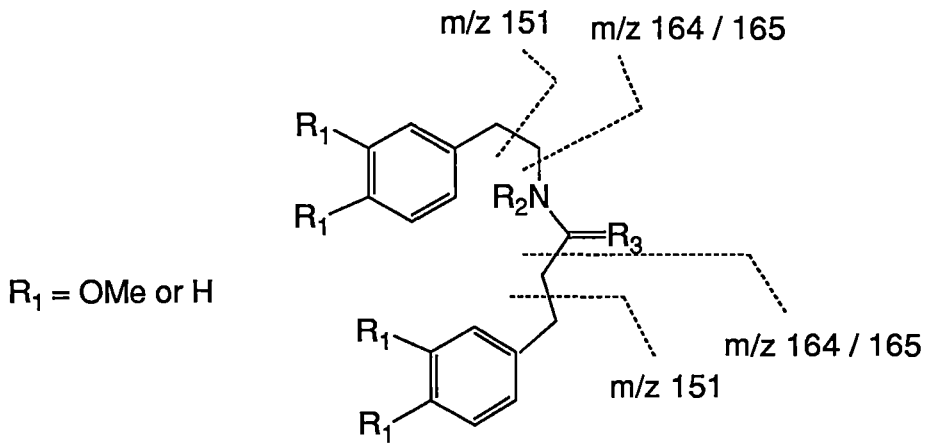


Figure 3.12

Reduction of Amides (46a), (46b), and (46c).

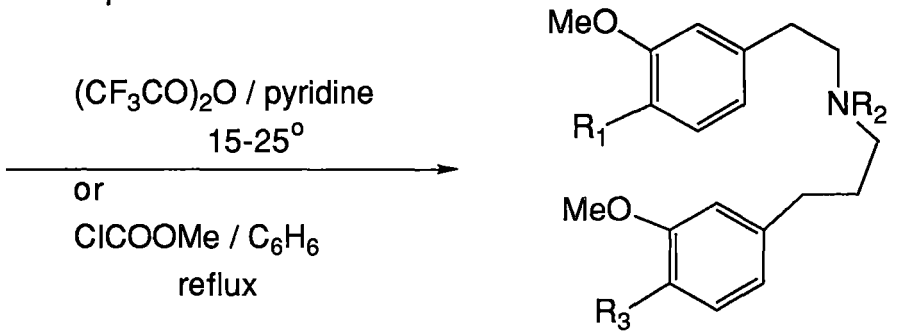
The reduction of the amide (46a) with lithium aluminum hydride was straightforward and gave high a yield of the amine (47a). The molecular composition $C_{20}H_{27}NO_3$ of (47a) was established by high resolution mass spectroscopy. Its ^1H n.m.r. spectrum showed two additional aliphatic protons due to the reduction of the carbonyl carbon, while the chemical shift due to the carbonyl carbon was absent in the ^{13}C n.m.r. spectrum of (47a).

The amines (47b) and (47c) were obtained from the amides (46b) and (46c) respectively, following the same procedure as for (47a). Their MS data were in accord with the given structures (47b) and (47c), while the ^1H and ^{13}C n.m.r. chemical shifts were assigned in comparison with those for (47a). The MS fragmentation patterns of the three amines (47a), (47b), and (47c) were very similar and followed the patterns for the amide precursors except for an increase in the peak intensities at M+-151, and at m/z 165 (Figure 3.12). The parent ions of these amine derivatives could only be detected by positive chemical ionization mass spectroscopy.

N-Trifluoroacetyl and Methyl N-Carboxylate Derivatives of Amines (47a), (47b), and (47c).

The need to derivatize the amines prior to oxidation was mainly to avoid their oxidative cyclization to the homoerythrina-type skeleton and other by-products which could arise from oxidation at nitrogen[158]. Trifluoroacetyl derivatives were made based on the work of McDonald

and Wylie[158]. The difficulty and uncertainty encountered in the cyclization of the trifluoroacetyl derivatives (48a) and (48c) prompted the use of alternative derivatives such as the methyl carbamate derivatives. This would also allow the formation of systems whose spectroscopic properties, especially the MS fragmentions, had been established previously (Section 3.3.1). Moreover, reduction of the methyl carbamate derivatives with lithium aluminum hydride would provide a one step and high yielding route to the N-methyl derivatives in contrast to the two steps which would be required in the case of the trifluoroacetamide derivatives.

(47) 

	R_1	R_2	R_3
48a	OMe	$COCF_3$	H
48b	H	$COCF_3$	OMe
48c	OMe	$COCF_3$	OMe
49a	OMe	COCMe	H
49b	H	COCMe	OMe
49c	OMe	COCMe	OMe

Scheme 3.13

The amine (47a) was trifluoroacetylated by the addition of trifluoroacetic anhydride to a solution of the amine in pyridine (Scheme 3.13). The product (48a) was obtained in high yield. Its mass spectrum gave peaks of very weak intensities including the parent ion, except for the intense peaks at m/z 151 and m/z 164. The ^1H n.m.r. spectrum showed seven aromatic protons, one triplet (δ 7.18) which integrated for one proton, and the other overlapping signals at δ 6.79-6.67 integrated

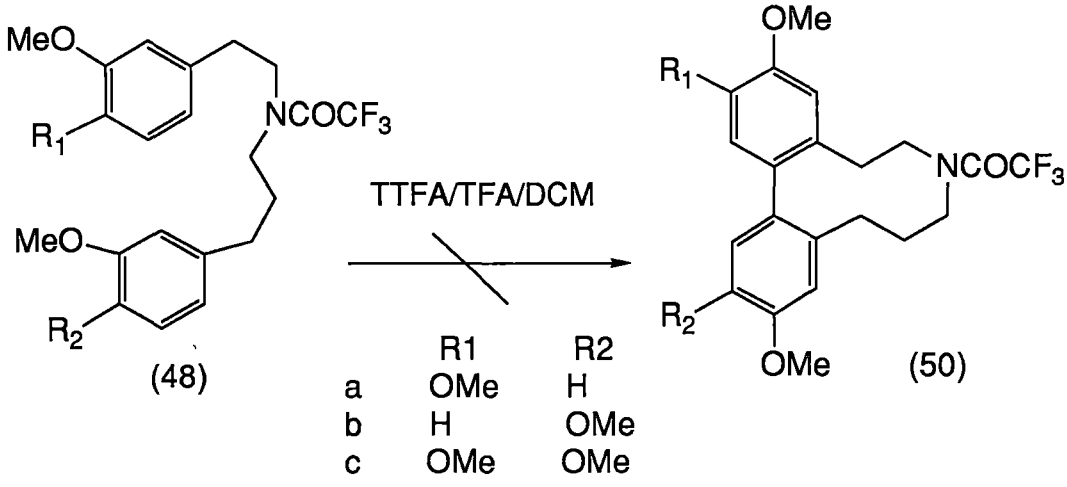
for six protons. Three methoxy groups (δ 3.80 X 2, 3.74), and ten aliphatic protons at δ 3.52 (2H), 3.32 (1H), 3.20 (1H), 2.72 (2H), 2.55 (2H), 1.90 (2H) were also present in the ^{1}H n.m.r. spectrum. Although some doubling up of peaks was observed in the ^{13}C-n.m.r. spectrum due to hindered rotation in the amide bond, all the signals corresponded well with the expected structure. A distinct quartet at δ 156.47 due to the trifluoroacetyl carbon was also observed.

Trifluoroacetylation of amines (47b) and (47c) was done in a similar manner as for the trifluoroacetamide (48a). The resulting trifluoroacetyl derivatives (48b) and (48c) were characterized by their spectroscopic data and by comparing them with the trifluoroacetamide (48a). The mass spectra of the trifluoroacetamide derivatives showed mainly the intense peaks at m/z 151 and m/z 164 and some peaks of moderate intensity at m/z 165, M^{+}-164, and M^{+}-151.

The reaction of the amine (47a) with methyl chloroformate gave a high yield of the carbamate derivative (49a) (Scheme 3.13). GC-MS analysis of the derivative indicated a relatively clean product; thus, this was used for the next reaction without further purification. The characteristic mass fragment at m/z 102, due to an N-methylcarboxylate-containing fragment was observed as the base peak in the mass spectrum of (49a), while the peak at m/z 165 was weak. A peak of moderate intensity was also observed at m/z 236 (M^{+}-151). The methyl N-carboxylate derivatives of (47b) and (47c) were prepared using the same procedure as for (49a) affording the derivatives (49b) and (49c), respectively, in high yields.

Cyclization of the Trifluoroacetyl and the Methyl N-Carboxylate Derivatives.

McDonald's[158] cyclization procedure via direct oxidative coupling of the trifluoroacetamide derivative (48c, M^+ 455) using thallium (III) trifluoroacetate (TTFA) was followed as described in their report (Scheme 3.14). The oxidation of the trifluoroacetamide derivative (48c) with thallium (III) trifluoroacetate afforded a tarry product. GC-MS analysis of the crude product indicated the presence of two components of molecular weights 453 and 471. The fact that the bulk of the product were components of very high molecular weight was confirmed by direct probe mass spectrometry. Although the molecular weight 453 indicated an oxidized product (455 to 453), the intense mass fragments of m/z 151 and 164 of the oxidized component suggested an uncyclized product.

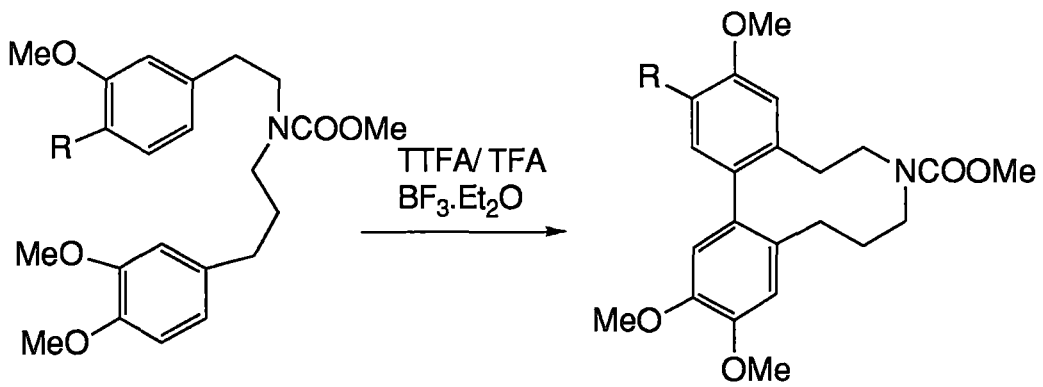


Scheme 3.14

The oxidation of the trifluoroacetamide derivatives (48a) and (48b) was also unsuccessful. The bulk of the crude product obtained after the work up from the oxidation of (48a) was very polar as observed by TLC.

The less polar components were separated by an alumina quick column. Mass spectrometric analysis of each of the four fractions collected indicated the presence of polymeric materials. No cyclized product was detected in any of the fractions.

A similar result was observed in the oxidation of the trifluoroacetamide (48b). However, the first fraction indicated the presence of a minor amount of an oxidized product (M^+ 423) mixed with the unreacted amide (48b, M^+ 425). The oxidized product was hardly detectable in the GC-MS ion trace; however, its presence was confirmed by direct probe mass spectrometry. The fragmentation pattern of the oxidized product could not be distinguished from the unoxidized component.



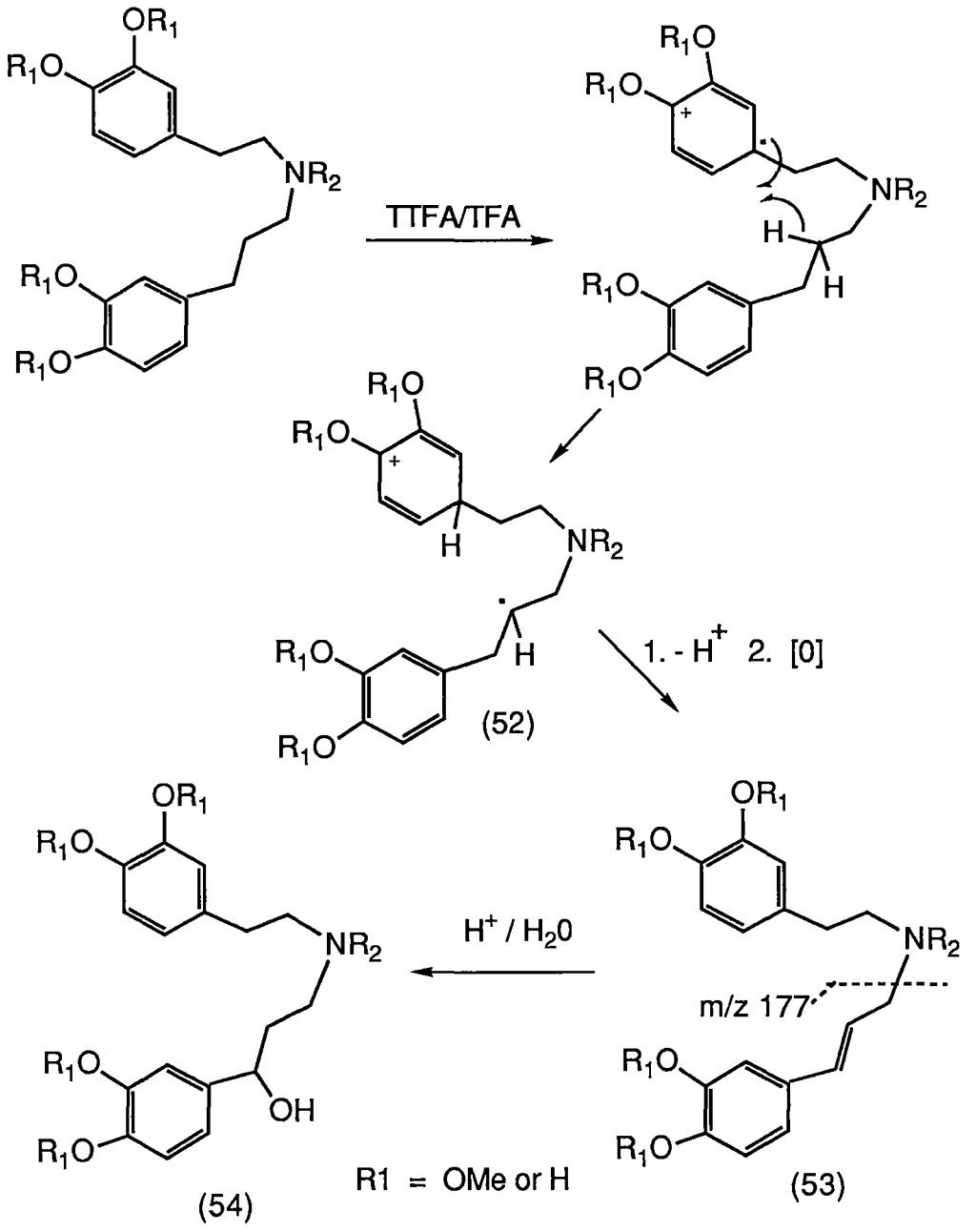
(49) b R = H (51)
c R = OMe

Scheme 3.15

The oxidation procedure described above was repeated on the carbamate derivative (49c) varying the reaction time, reaction temperature, solvent system and concentration of TTFA (Scheme 3.15). The variation of reaction conditions (Chapter 7, Section 7.3) was based on previous reports on the oxidation of some biaryl systems[172-175]. The

results of the present work were consistent with some of the points raised by McKillop, Turrel, Young and Taylor[172]. Firstly, the use of excess TTFA could further oxidize the initial oxidation product in competition with the unoxidized substrate. This was indicated by the presence of tarry materials which varied in nature and in quantity from one experiment to another. Secondly, it was observed that the yield of the less polar product was increased when the reaction time was reduced. Quenching the reaction with water before it went to completion minimized further oxidation of the inital oxidation products. In addition, the presence of the Lewis acid boron trifluoride etherate appeared to have increased the yield of the desired product, although not very significantly. Increasing the solvent volume decreased the formation of the tarry products while the use of acetonitrile in place of trifluoroacetic acid resulted in more tarry materials being formed.

Overall, in all the oxidation experiments with (49c) at different reaction conditions (Chapter 7, Section 7.3), the more favoured oxidation product after the polymeric materials was an uncyclized product which was not fully characterized, but was thought to have been oxidized in the aliphatic chain based on the mass spectroscopic data. This was fractionated by PLC affording two fractions. The first fraction was composed mainly of the uncyclized oxidation product tentatively assigned as (53c) (M^+ 415) with a minor unidentified component of molecular weight 433. This minor component was the major component of the second fraction. A minor amount of the uncyclized oxidation product was also present in the second fraction. GC-MS monitoring showed that the component of molecular weight 433 was initially absent



Scheme 3.16

in the crude product. It is therefore proposed that the cyclization of systems (48) and (49) is generally not favoured due to inherent entropy effects against ten-membered ring formation, so that the initial oxidation of the aromatic ring system could instead lead to the oxidation of the aliphatic carbon atom via abstraction of an aliphatic hydrogen by the phenyl radical (Scheme 3.16). An olefinic derivative (53) could then be formed from the radical intermediate (52) by loss of a proton and further

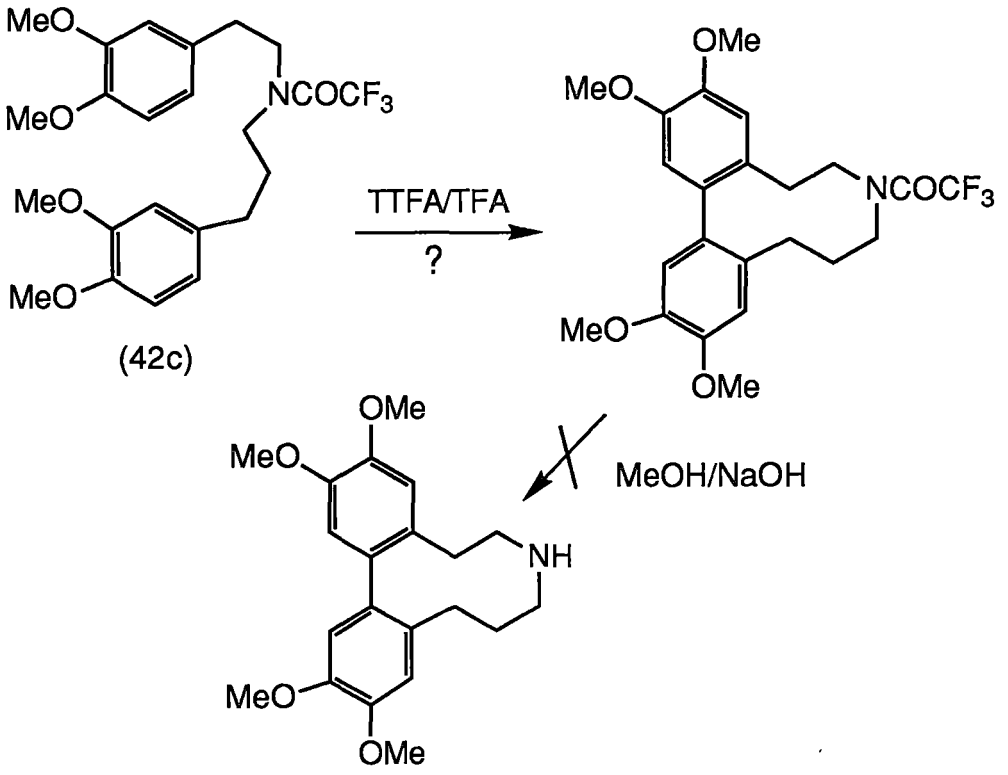
oxidation. The component of the oxidation product detectable only after PLC separation (M^{+}+ 18) was then presumed to have arisen by acid-catalyzed addition of water to the double bond, in the presence of silica. Structure (54) was therefore proposed for this presumed hydration product. Although this has not been fully substantiated by spectroscopic data due to time and purity constraints, the presence of an intense peak at m/z 177 in the uncylized oxidation product (53) is consistent with a substituted styrene system.

The yield of the desired cyclized product (51c) together with the uncyclized system (53c) remained relatively low although increased dilution significantly increased the yield. The reaction could possibly be shifted to favour the formation of (51c) by further modification of the reaction conditions. This could not be pursued further due to time constraints. Although the targetted cyclized product (51c) was not separated pure, its cyclization was substantiated by its mass spectrum showing the characteristic fragments M^{+}-102, and M^{+}-116, and m/z 102. The mass spectrometric properties of the N-carboxylate derivatives of dibenz[*d,f*]azecines were established previously for this type of compound (Section 3.3.1).

Hydrolysis of the Trifluoroacetamide derivative (50c).

The crude product (50c) from the thallium trifluoroacetate oxidation of the trifluoroacetamide derivative (48c) was hydrolyzed and the resulting product analyzed by GC-MS (Scheme 3.17). The expected molecular ion (M^{+} 357) was not detected, nor were any of the characteristic mass spectral fragments of a dibenz[*d,f*]azecine derivative with an NH group in the heterocyclic ring observed. Direct probe MS

indicated the presence of ions in the high mass range indicative of polymeric materials. Acid-base re-extraction of the crude product, and GC-MS analysis of the dichloromethane extract at pH 8, gave the same result.



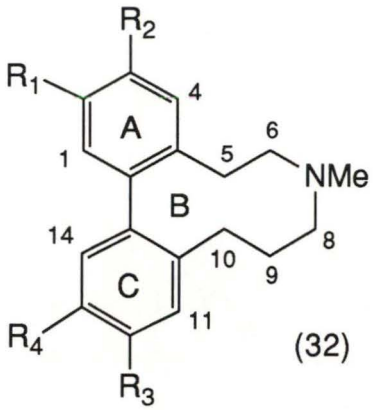
Scheme 3.17

The successful cyclization of the carbamate derivative (49c) was indicated by the mass spectral fragments of the resulting oxidation product (51c). In the absence of other spectroscopic data, lithium aluminum hydride reduction of the crude product containing the cyclized carbamate derivative (51c), provided further support to the assumption that the desired cyclization had taken place. The GC-MS ion trace of the crude product from the reduction reaction showed a minor component of the expected molecular weight of 371. Its mass spectrum gave the fragments M^+-58, M^+-72, m/z 70, 58, 57, 44, and 42 which are characteristic fragments of a dibenz[*d,f*]azecine derivative with an N-

methyl group in the heterocyclic ring. This supported the expected structure (32g). The isolation of this product was not possible due to the limited sample size in which it was a minor component.

3.3.3 The Structure of Wurmbazecine

Although the syntheses of the alternative wurmbazecine structures (32c) and (32d) were not successful, the spectroscopic properties of wurmbazecine agreed quite closely with (32a). Both (32a) and wurmbazecine have methoxy signals at δ 3.92, 3.79 and 3.78 as opposed to that of (32b) at δ 3.92, 3.81, and 3.78. While the ^{1}H n.m.r. spectra of (32a) and (32b) were almost indistinguishable (Figure 3.10), their ^{13}C n.m.r. spectra gave some significant variations (Figure 3.11). These variations were consistent in the different ^{13}C n.m.r. spectra obtained for each of (32a) and (32b), in an ordinary 5 mm diameter n.m.r. tube and in an insert tube. An obvious difference was noted in the chemical shift of the aliphatic carbon atoms C_9 and C_{10} of both (32a) and (32b). In (32a), the difference between the chemical shift of these two carbon atoms is 1.33 ppm, which is about the same for (32e) and (32f), while in (32b), the difference was only 0.55 ppm. The significance of the variation of the chemical shift of each carbon was determined by comparing the pattern of chemical shifts of wurmbazecine not only with (32a) and (32b) but also with (32e) and (32f) (Table 3.4). It must be noted that the oxygenation pattern of rings A and C of (32b) is the exact opposite to those of (32a), (32e), and (32f). The chemical shifts of $C_{11(4)}$, $C_{12(3)}$, $C_{4(11)}$, and C_{10} in wurmbazecine, (32a), (32e), and (32f) were consistently and significantly different from those of (32b). For all the other carbon atoms, there was no apparent uniformity in the



(32)

	R_1	R_2	R_3	R_4
a	OMe	OMe	H	OMe
b	OMe	H	OMe	OMe
e	-OCH_2O-		H	OMe
f	OMe	OMe	H	OMe

Table 3.4 ^{13}C N.m.r. Resonances of Wurmbazecine and Related Systems (ppm)

Carbon	Isolate	32a	32b	32e	32f
11(4)	129.38	129.37	**128.76**	129.37	129.30
14(1)	114.66	114.65	114.53	114.60	116.51
12(3)	113.76	113.75	**113.47**	113.78	114.86
1(14)	112.26	112.22	112.36	109.37	112.17
4(11)	110.38	110.43	**110.80**	107.52	110.41
6	59.10	58.86	59.42	59.12	58.70
OCH_3	55.81	55.77	55.85		55.59
OCH_3	55.25	55.20	55.16	55.22	
8	49.63	49.56	49.56	49.69	49.38
NCH_3	44.66	44.59	44.56	44.68	44.59
5	30.48	30.30	29.77	30.64	30.01
9		28.56	28.47	28.68	28.45
10	27.21	27.23	**27.92**	27.12	27.15

chemical shifts. Despite the inability to ascertain the C_9 chemical shift of wurmbazecine due to some impurity peaks, the chemical shifts of this carbon in all the four derivatives were not significantly different. The

difference in the chemical shift of C_9 and C_{10} is dependent on the chemical shift of C_{10}. In this case the C_{10} signal of (32b) is distinctly more upfield as compared with the other derivatives including wurmbazecine. Wurmbazecine is therefore tentatively assigned the structure (32a) based on the above arguments.

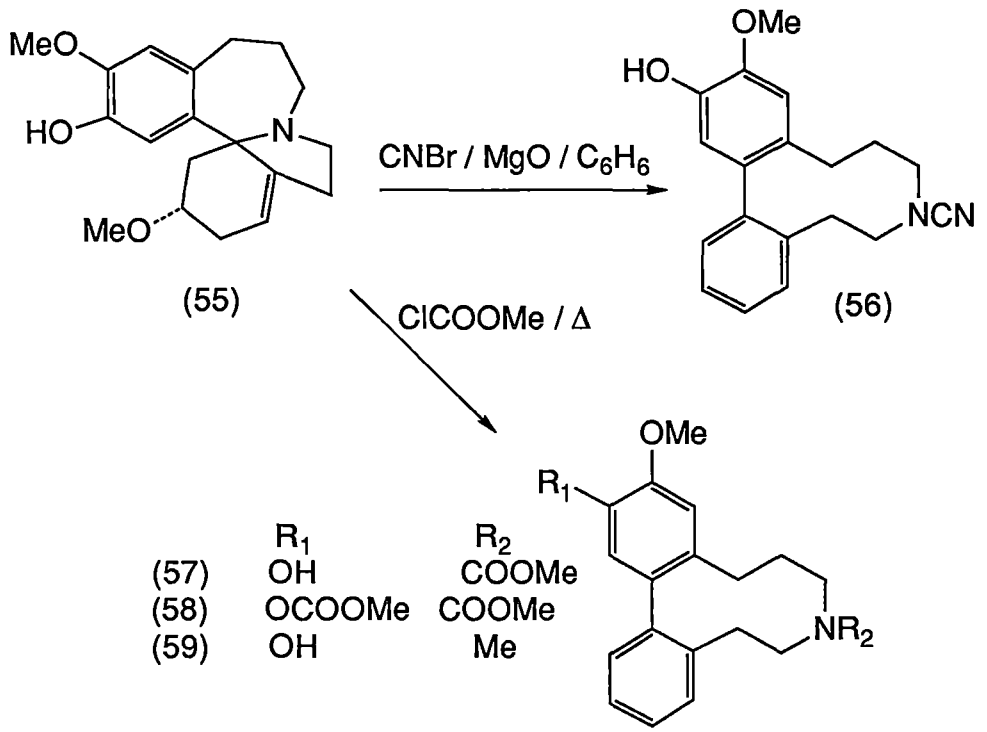
3.4 Dibenz[*d,f*]azecine Derivatives from Known Alkaloids

Some of the isolated phenethylisoquinoline-derived alkaloids were envisaged as practical sources of dibenz[*d,f*]azecine systems. In a few steps, and depending on their substitution patterns, new dibenz[*d,f*]azecine bases could be obtained from them. The availability of the dibenz[*d,f*]azecines of different saturation and substitution patterns could then be used to study the variation of the MS fragmentation patterns of the dibenz[*d,f*]azecines. The possibility of obtaining dibenz[*d,f*]azecine derivatives from homoerythrina and homomorphine alkaloids was therefore investigated.

3.4.1. Dibenz[*d,f*]azecine Derivatives from Homoerythrina Alkaloids

The search for some dibenz[*d,f*]azecines from *Athrotaxis selaginoides* allowed the re-isolation of some of their known components including taxodine (55) and homoerythratine (60). Attempts were then made to convert these alkaloids to dibenz[*d,f*]azecine derivatives. Von Braun cleavage[170] of taxodine (55) took place very slowly but gave a single product as seen in the GC-MS trace (Scheme 3.18); the structure (56) was assigned to this product on the basis of the mass spectrum.

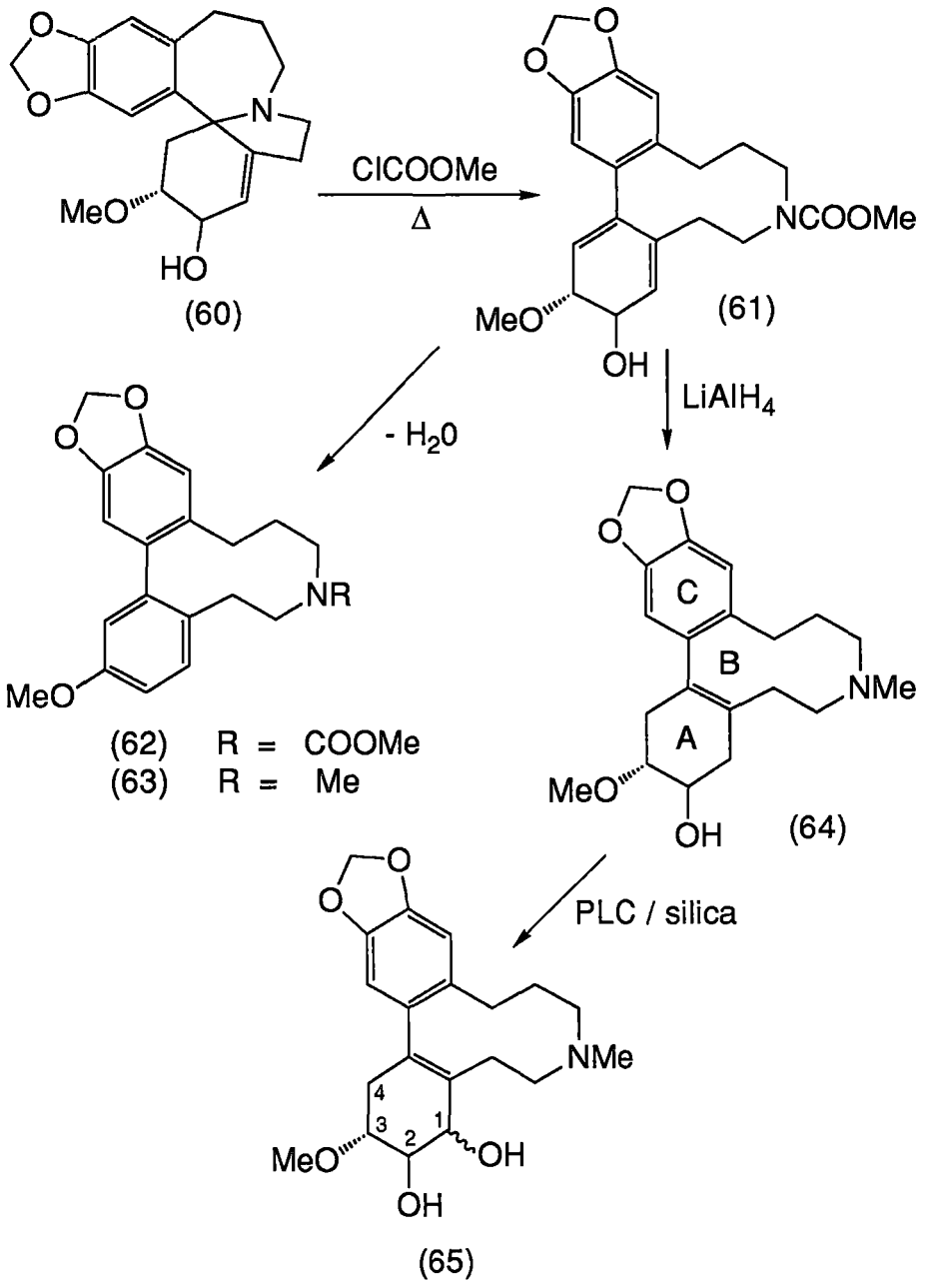
Further characterization of the product or investigation of the reaction was not undertaken in view of the very slow rate of formation and the very small amount of the precursor (55) available.



Scheme 3.18

Alternatively, following the same procedure as in cleavage of the homoerythrina system (37), taxodine (55) was refluxed with methyl chloroformate (Scheme 3.18). The presence of two cleavage products along with some unreacted taxodine was indicated in the TIC of the crude product. Structures (57) (M^+ 341) and (58) (M^+ 399) were proposed for these two products. Without further purification, the crude product was reduced with lithium aluminum hydride. The GC-MS trace of the reaction product mixture showed the major component with the expected molecular weight of 297; the unreduced carbamate (57) and taxodine(55) were also present. The reduced product had the characteristic fragmentation pattern of a dibenz[*d,f*]azecine with an N-

methyl group in the heterocyclic ring. Although this was not isolated pure due to sample size limitations, it is proposed tentatively to have the structure (59) based on its mass spectral data.



Scheme 3.19

In a similar manner, homoerythratine (60) was reacted with methyl chloroformate (Scheme 3.19). After work up, the sample was allowed to stand for a few days before it was analyzed by GC-MS. Homoerythratine

(60) was completely converted to two products (M^+387 and M^+369) for which structures (61) and (62) respectively were proposed. An attempt to separate the two components by PLC was unsuccessful, possibly due to reaction on the plate. Further separation could not be carried out due to sample size limitations.

A second batch of homerythratine (60) was reacted with methyl chloroformate and the product reduced with lithium aluminum hydride immediately following work up. The GC-MS trace of the product showed one major component of molecular weight 345 with two minor components (M^+ 295 and M^+ 297), and a trace amount of a component of molecular weight 325 (63). The structure of (63) was proposed based on the chemical transformations it underwent and, more importantly, on its mass spectral fragmentation pattern.

The presence of (63) in trace amount indirectly implied a low concentration of the proposed carbamate precursor (62); it is therefore presumed that upon standing, (61) was slowly dehydrated to (62) since the original product of the initial ring cleavage of (60) showed a relatively higher concentration of (62). PLC separation of the crude product afforded nine fractions, with fractions 3 (M^+ 345) and 4 (M^+ 361) being relatively pure. Their dibenz[*d,f*]azecine skeletons were derived from their mass spectrum where the intense peaks at m/z 70, 58, and 57 characteristic of dibenz[*d,f*]azecine derivatives, were observed. However, the other characteristic peaks in the high mass range (M^+-58, and M^+-72) of the dibenz[*d,f*]azecine derivatives with two aromatic rings were not observed.

The molecular weight of fraction 3 (M^+ 345) suggested that the substituents of the original homoerythrina alkaloid had remained intact. Its ^1H n.m.r. spectrum confirmed the presence of N-methyl (δ 2.23), methylenedioxy, (δ 5.92), and methoxy (δ 3.39) groups. The ^{13}C n.m.r. spectrum complemented by ^{13}C DEPT revealed six quaternary carbon atoms, four of which were assigned to the substituted carbon atoms of the aromatic ring. The remaining two quaternary carbon atoms implied the presence of a double bond which could only be the bond adjoining rings A and B. This was supported by the presence of 7 methylene carbon atoms and the absence of an olefinic proton. Structure (64) was tentatively proposed, which presumably was derived from the homoerythratine ring cleavage product (61). The formation of the dibenz[*d,f*]azecine derivative (64) indirectly confirmed the proposed structure of (61) since allylic alcohols are known[176,177] to be reduced by lithium aluminum hydride.

The component of fraction 4 with M^+ 361 was not present initially in the crude reduction product of (61) as monitored by GC-MS. This could therefore be a transformation product from (64) when the crude product was separated by PLC. The ^1H n.m.r. spectrum showed three oxygenated methine groups corresponding to the chemical shifts at δ 4.45 (d, *J* 3.90, H-1), 3.84 (br, H-2), and 3.68 (m, H-3). A local spin network of a homonuclear shift-correlated 2-D n.m.r. (COSY) experiment showed the connectivity of the protons at δ 4.45 and at δ 3.84; the latter in turn showed a cross peak with the proton at δ 3.68 (Figure 3.13). A cross peak was also observed between δ 3.68 and the methylene protons at δ 2.62 and δ 2.08. This connectivity series supported the assignment of the hydroxy group at C-1 and not at C-4, ; the assignment of the methoxy substituent at C_3 and the other hydroxy group at C_2 was derived from the

substitution pattern of the precursor alkaloid (60). Consequently, structure (65) is proposed for fraction 4. The mechanism of the hydroxylation of (64) to (65) is not fully understood although an allylic free radical oxidation and subsequent peroxide cleavage and hydrogen abstraction is considered a possibility.

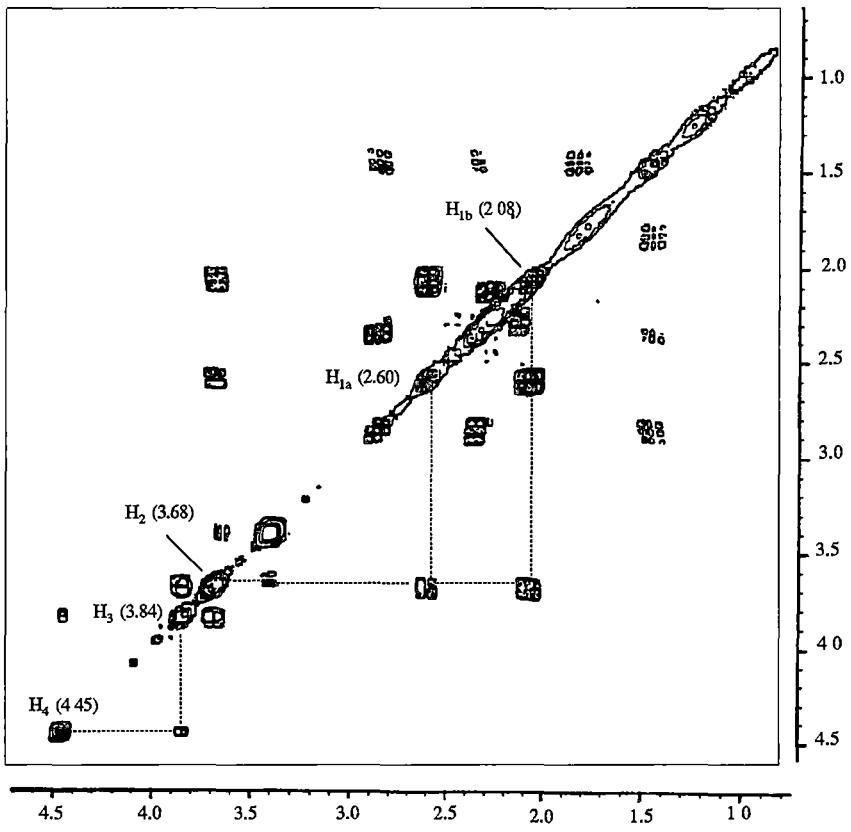
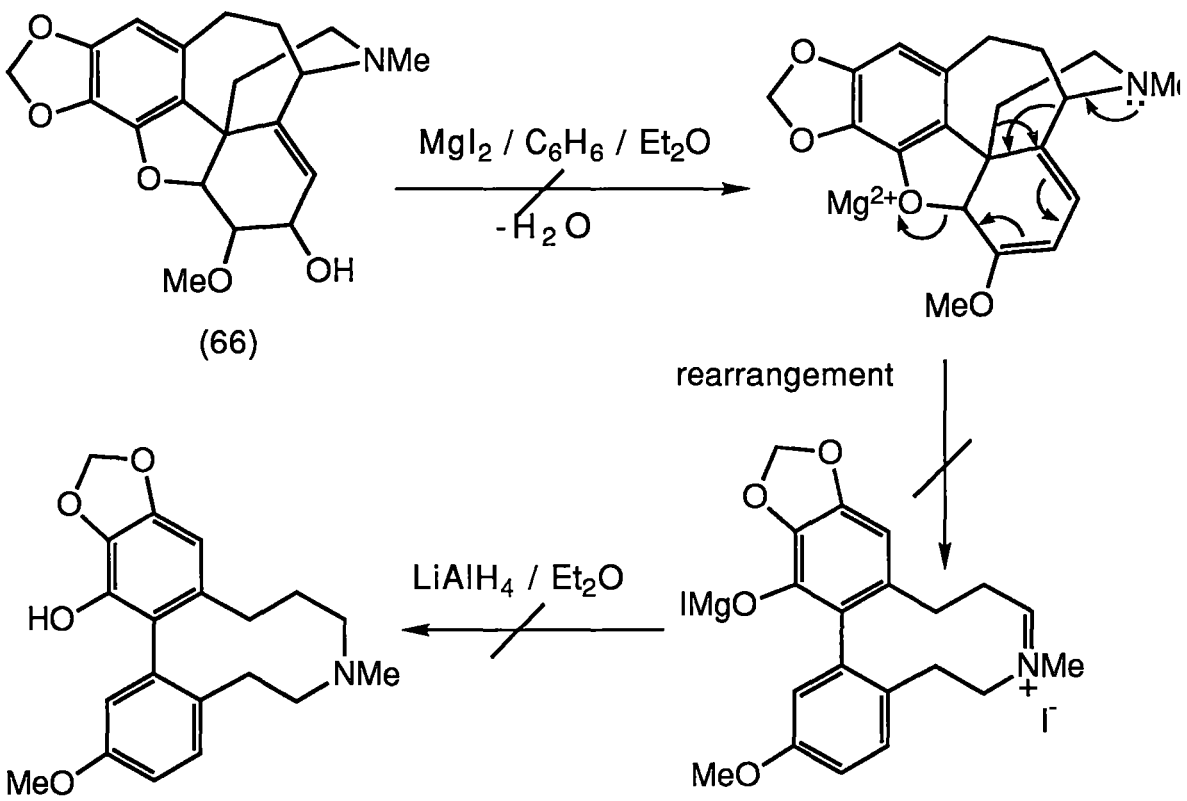


Figure 3.13 COSY 2-D Spectrum of Dibenz[*d,f*]azecine derivative (65)

3.4.2. Attempted Conversion of a Homomorphine-Type Alkaloid to a Dibenz[*d,f*]azecine Derivative.

Some dibenz[*d,f*]azonine derivatives have been obtained by Grignard reagent[178-182] and Lewis acid-promoted rearrangement[153,183] of thebaine and its derivatives. In trial experiments, the latter reaction was repeated on thebaine using magnesium bromide and magnesium iodide in separate reactions. In both cases, the products were

immediately reduced with lithium aluminum hydride. GC-MS monitoring of the products showed the presence of a number of by-products when magnesium bromide was used. A relatively clean product was obtained with magnesium iodide where the only trace impurity was the unreacted thebaine. It was therefore decided that magnesium iodide be used on the homomorphine alkaloid (66) in an attempt to promote after dehydration, an analogous rearrangement which could lead to a dibenz[*d,f*]azecine-type derivative. The resulting salt could then be reduced with lithium aluminum hydride (Scheme 3.20).



Scheme 3.20

The homomorphine alkaloid (66) was thus refluxed with freshly prepared magnesium iodide in dry benzene. A small sample of the reaction mixture was taken after three hours and analyzed by GC-MS; no reaction was shown to have taken place. The reaction was repeated using a large excess of magnesium iodide and reduced with lithium

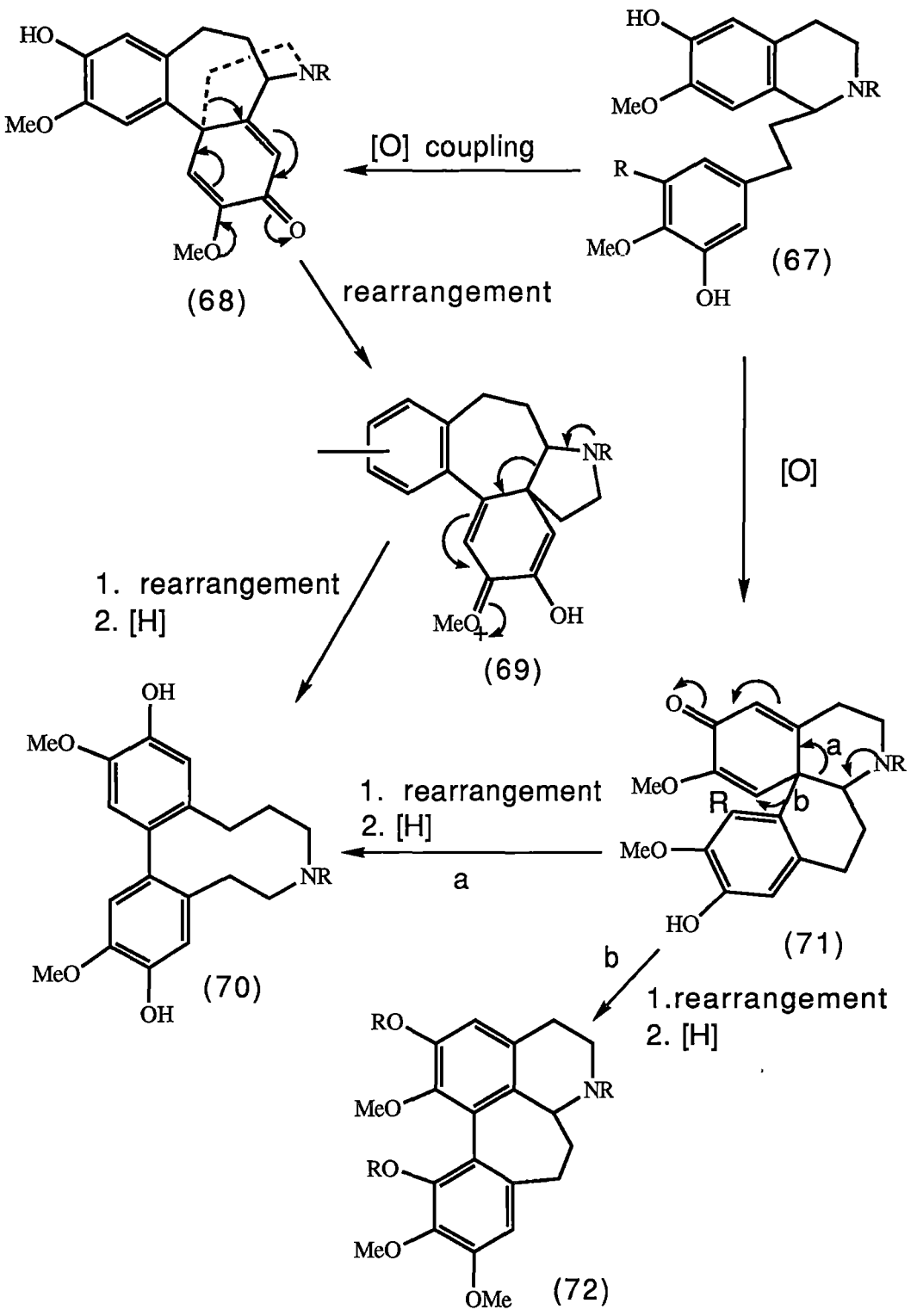
aluminum hydride. GC-MS analysis showed only one peak corresponding to the unreacted homomorphine (66). It is possible that conditions for the initial dehydration were not strong enough and, hence, the required rearrangement could not take place. Further investigation was not undertaken.

3.5 Biosynthesis of Wurmbazecine

[Note: In these thesis the terms biosynthesis and biogenesis are used synonymously, as indicated for example in "The Vocabulary of Organic Chemistry" by Orchin, M., Kaplin, F., Macomber, R.S., Marshall Wilson, R., and Zimmer, H., John Wiley and Sons, New York, 1980, p.456.]

To date no further proposals have been put forward regarding the biogenetic significance of the dibenz[*d,f*]azecines other than their being precursors of the homoerythrina alkaloids[63, 113] and the cephalotaxus alkaloids[83]. This was the basis for the original search for the dibenz[*d,f*]azecines from the homoerythrina-containing terrestial plant species. Their detection and isolation from the subfamily Wurmbaeoideae, where no homoerythrina-type alkaloid was previously detected (Table 1.1 and Table 2.2), is considered significant. This raises the question as to the biogenetic role of the dibenz[*d,f*]azecines in relation to other wurmbaeoideae alkaloids. It would be interesting to establish the identity of their immediate precursors and to ascertain the possibility of their being metabolized further to some known or yet unknown skeletal systems.

The dibenz[*d,f*]azecine alkaloids might follow a biogenetic route analogous to the dibenz[*d,f*]azonines[131] (Scheme 3.21). They could be derived via biotransformations which might initially involve oxidative coupling of a 1-phenethylisoquinoline-type precursor such as (67) to homomorphine (68) or homoproerythrina-type (71) bases. A dienone-



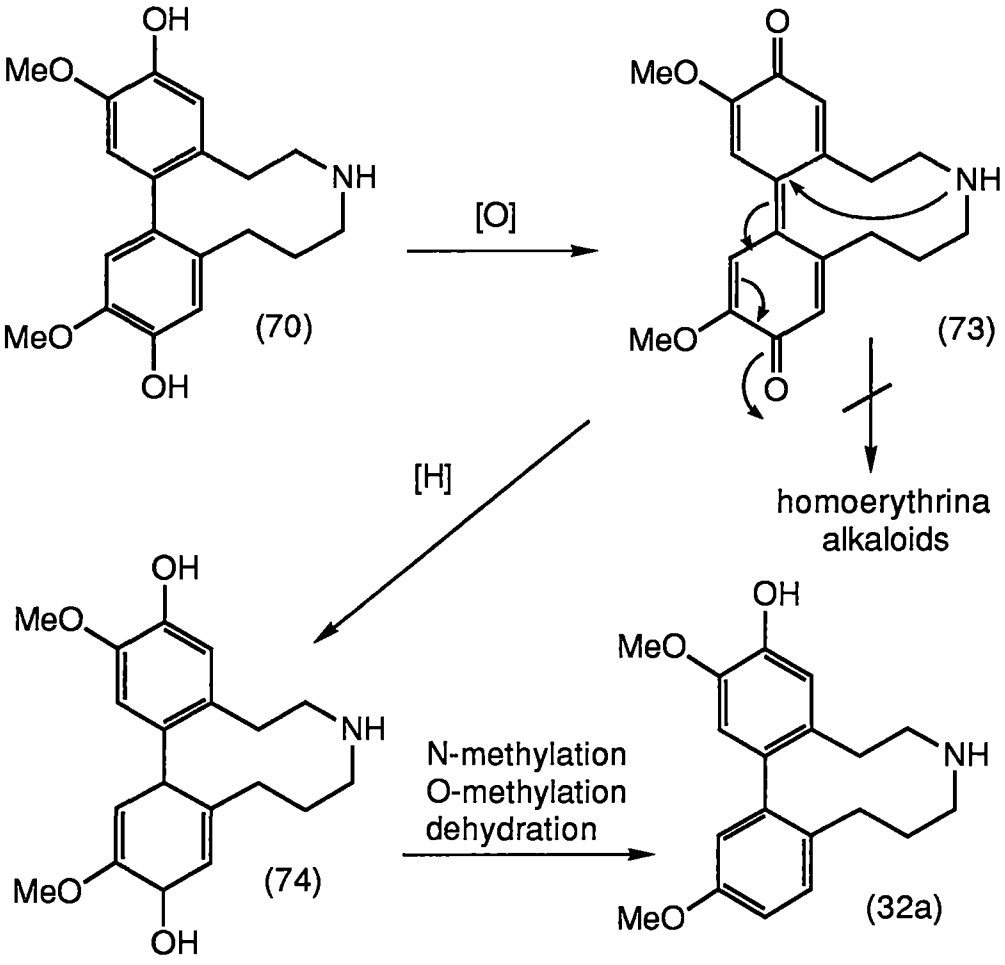
Scheme 3.21

phenol rearrangement of the intermediate (68) could give rise to a homoneospirene-type skeleton (69); this could further rearrange to a tetraoxygenated dibenz[*d,f*]azecine base (70). The same intermediate

(70) could also be biosynthesized through the rearrangement of a homoproerythrina-type intermediate (71). Alternatively, the intermediate (71) could rearrange to a homoaporphine skeleton such as (72).

Wurmbazecine could be derived via the dibenz[*d,f*]azecine (70) as illustrated in Scheme 3.22. Further oxidation of (70) could then give rise to a diphenoquinone-type intermediate (73), a transformation analogous to the biogenesis of erythrina alkaloids in which the proposed[184,185] diphenoquinone intermediate was supported by ^{14}C-labelling experiments[186]. Although homoerythrina-type alkaloids could be obtained by cyclization of (73) via an internal Michael-type addition as in the biosynthesis of the erythrina alkaloids, no alkaloid of this type was detected to co-occur with wurmbazecine nor with any other wurmbaeoideae alkaloids. This could possibly be due to the absence of the necessary enzymes to catalyze this transformation. Alternatively, the reduction of (73) to a dienol (74) could in turn lead to wurmbazecine (32a) by dehydration, O-methylation, and N-methylation.

The isolation of O-methylandrocymbine from *Wurmbea dioica* ssp. *dioica*, (Chapter 4, Section 4.2.3) and therefore its co-occurrence with the dibenz[*d,f*]azecine wurmbazecine, supported the proposed biosynthetic pathway. The enzyme composition of the plant may preferrably transform a diphenoquinone intermediate (73) to a dibenz[*d,f*]azecine (74). In *Wurmbea pygmaea*, it was observed that the dibenz[*d,f*]azecine profile varied with age. The enzymatic control involved in the alternative biosynthesis of a homoaporphine and dibenz[*d,f*]azecine-type alkaloid was typified in the alkaloid profile of this plant. While the more mature plant sample contained a dibenz[*d,f*]azecine alkaloid(M^+ 387) as a major



Scheme 3.22

component, and the homoaporphine (M^+ 371) as a trace component, the less mature plant sample had the homoaporphine (M+371) as the major component . No dibenz[*d,f*]azecine derivative was detected in the latter sample (Chapter 4, Section 4.3). It is possible that in the later stage of plant development the enzyme profile changes such that a homoproerythrina intermediate (70) (Scheme 3.20) could rearrange to a dibenz[*d,f*]azecine instead of a homoaporphine skeleton. This would be analogous to the biogenesis of *Dicentra* alkaloids in which the the aporphine alkaloids were presumed to be formed via proerythrinadi-enone-type intermediates, based on the results of incorporation experiments[187].

3.6. CONCLUSIONS

Dibenz[*d,f*]azecine alkaloids have been successfully obtained via three approaches: by isolation from a plant source, by synthesis, and through chemical transformations of some known alkaloids. The number of dibenz[*d,f*]azecine alkaloids synthesized was instrumental in establishing the mass spectral fragmentation pattern of this group of compounds. The second approach to the synthesis of the skeleton via the oxidative coupling of the corresponding acyclic amine derivatives, provided more information on the chemistry of thallium (III) as an oxidizing agent.

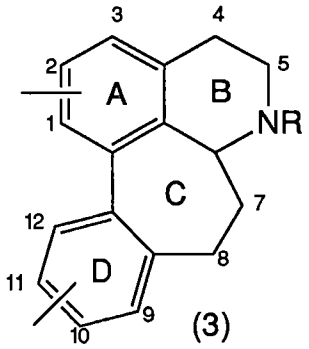
The dibenz[*d,f*]azecine alkaloid named wurmbazecine, was isolated from *Wurmbea dioica* ssp. *dioica*. Its structure was assigned from its spectroscopic data, in comparison with other dibenz[*d,f*]azecine derivatives synthesized in this work. This is only the second naturally-occurring dibenz[*d,f*]azecine alkaloid known, and the first of this type detected and isolated in the subfamily Wurmbaeoideae, and in particular, in the genus *Wurmbea*. Its co-occurrence with the phenethylisoquinoline-derived alkaloids other than the homoerythrina alkaloids has not been reported previously and is of biosynthetic significance. The biosynthetic precursors of wurmbazecine, and possibly the identification of further products elaborated from it could be a subject of further investigation. A proposed biosynthetic pathway for wurmbazecine has been put forward.

The transformation of the homoerythrina alkaloids to dibenz[*d,f*]azecine derivatives provided model compounds for the study of the mass spectrometric properties.

Chapter 4
THE WURMBAEOIDEAE ALKALOIDS

4.1. Introduction

The phenethylisoquinoline-derived alkaloids in the family Liliaceae can be divided into two classes according to their sources. The first class is that of the wurmbaeoideae alkaloids, composed of the colchicine alkaloids and their derivatives, the homoaporphines, homoproaporphines, and homomorphines. These alkaloids were restricted to Buxbaum's subfamily Wurmbaeoideae. The homoerythrina alkaloids, which comprise the second class, had been isolated from some liliaceous genera whose taxonomic interrelationships had not been fully established. This class of alkaloids has also been isolated from other families such as the Cephalotaxaceae, Meliaceae, Taxodiaceae, and Phellinaceae, and is therefore classified separately from the wurmbaeiodeae alkaloids of the family Liliaceae. The wurmbaeoideae alkaloids were in turn sub-classified by Santavy[8,85] into the tropolonic (colchicine and their derivatives) and the non-tropolonic alkaloids [homoaporphines (3), homoproaporphines (4), and homomorphines (5)]. The other phenethylisoquinoline-derived bases such as the simple 1-phenethylisoquinoline, and dibenz*[d,f]*azecines are not confined to these two classifications, nor to the family Liliaceae, and will be discussed wherever they are encountered. The bisphenethyl-isoquinoline-type, which so far has only two naturally-occurring examples[55,117], could fall into the same category as the simple 1-phenethylisoquinoline and the dibenz[*d,f*]azecine-type alkaloids. Since a lot of work has been done on the tropolonic alkaloids[99,100,188] not much attention was given to this alkaloid group in this study.



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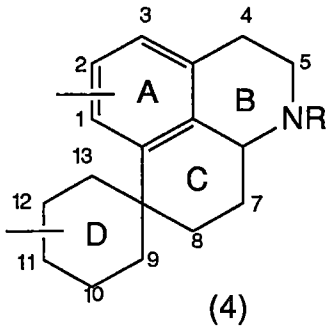

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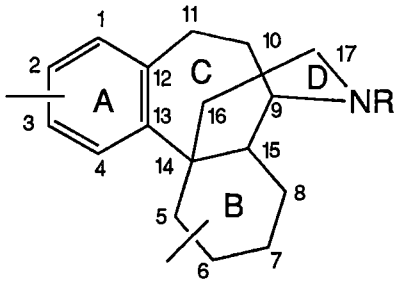
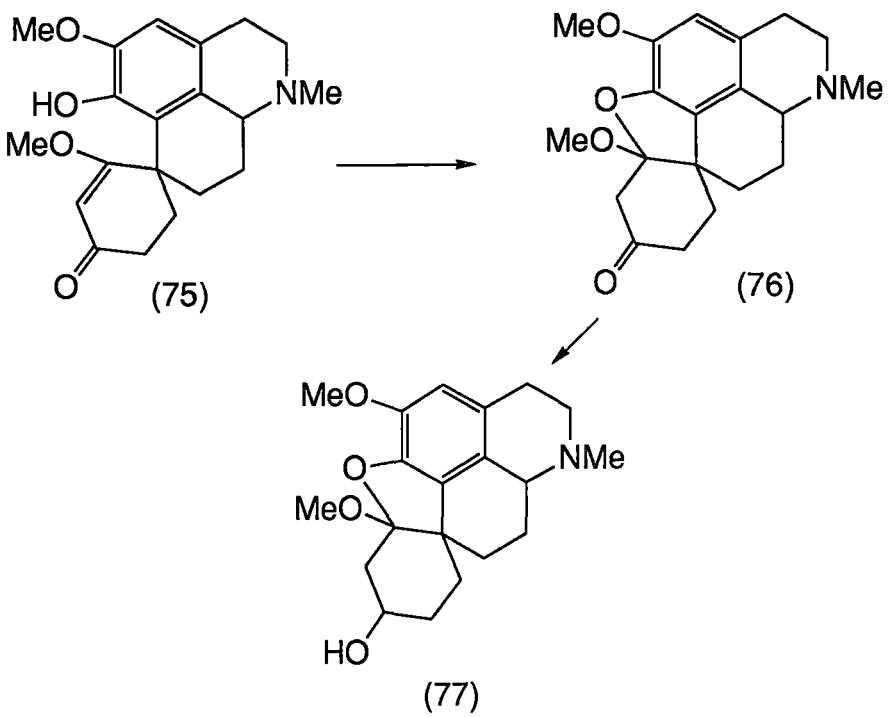


(5)

The phenethylisoquinoline-derived alkaloids are defined by a C-17 tetracyclic carbon skeleton based on the simple 1-phenethylisoquinoline structure. In the case of homoaporphines(3), rings A and D are always aromatic and are in a twisted biphenyl configuration. They are usually pentaoxygenated at carbons 1, 2, 10, 11, and 12. A general trend in which the chemical shift of the C_3 aromatic proton is always shifted downfield more than the C_9 equivalent has recently been established by n.m.r. experiments on a related system[17]. The various homoaporphine alkaloids isolated vary mainly in their oxygenated substituents.

Different types of homoproaporphines(4) arise from the variation in the substitution pattern as well as the oxidation level of ring D. Carbon 11 is generally alcoholic or ketonic. A few homoproaporphine alkaloids have been isolated with cyclic hemiacetals linking C_1 and C_{12}. One exception to these cyclic hemiacetal systems is luteicine (77) in which

the acetal bridge was shown to be between C_1 and C_{13}. The actual structure of this alkaloid is, however, in doubt. The reported partial synthesis of this alkaloid from the cyclization of luteidine (75) to (76) and its subsequent reduction to (77) (Scheme 4.1) was used to support the proposed structure, specifically the hemiacetal bridge[43]. The reported result indicated an identical ^1H nmr spectrum for (77) and luteicine. The correction of the structure of luteidine[39,41] in which the position of the methoxy group was moved from C_{13} to C_{12} implied the formation of the acetal bridge at C_{12} and not at C_{13}.



Scheme 4.1

The homoproaporphine alkaloids can be recognized by their mass spectra. The facile loss of a proton and the relative stability of the resulting (M^+-1) ion is generally observed. High mass fragments due to the loss of methoxy (M^+- 31) or hydroxy groups (M^+-17) are also indicated in the mass spectrum. In addition, some intermediate mass

fragments are usually observed as a result of the facile fragmentation of the C- and D- rings.

All morphine-like homologues, regardless of the presence or absence of an ether bridge and of the saturation of the B-ring are referred to as homomorphine-type alkaloids in this work. Homomorphine alkaloids are ordinarily tetracyclic, or pentacyclic in the presence of an ether ring. Ring B is generally oxidized with an alcoholic or ketonic functionality at C-11. Like some tropolonic alkaloids, their molecular ion is always the base peak. They can be easily distinguished from the tropolonic alkaloids by their ^{1}H n.m.r. spectra.

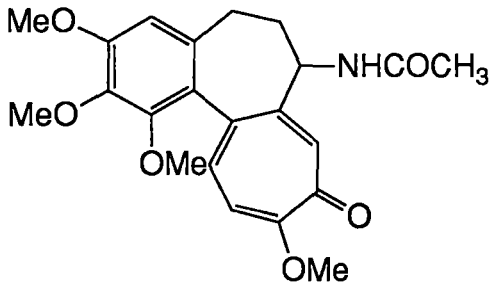
4.2. Alkaloids of *Wurmbea dioica* ssp. *dioica*

This plant has become the focus of interest in this study having been found to elaborate not only the sought-for dibenz[*d,f*]azecine alkaloids but also a wide range of skeletal systems. In comparison with the other members of the subfamily Wurmbaeoideae investigated in this study, this plant has by far the highest number of alkaloidal components as described in Chapter 2. As a result, the isolation of pure components has in most cases required repeated fractionation due to the overlapping of bands. Fractions containing tropolonic alkaloids were ordinarily set aside except in a few cases in which they were contained in fractions with other alkaloids of interest. While nine different batches of this plant were collected from various locations, the sample size limited a more extensive separation, isolation and characterization to only three batches of samples. Most of the other batches were separated at least once, by chromatographic procedures to monitor the alkaloid profile with special

attention given to the minor components. In the PLC separation, distinct bands as detected by UV light absorption and by alkaloid spray reagents were collected in one case, but in all the other succeeding separations, all bands were collected to avoid losing the minor components. Although the main aim in the study of this plant was to isolate the dibenz*[d,f]*azecine-type components, other phenethylisoquinoline-derived alkaloids present were also isolated in the process.

4.2.1. Alkaloids of Batch 1 of *Wurmbea dioica* ssp. *dioica*

This work was performed prior to the establishment of the mass spectral fragmentation patterns for the dibenz[*d,f*]azecine derivatives. As a consequence, the initial isolation was mainly aimed at any alkaloidal components of this plant. Since this sample size was small, the isolation was focussed mainly on the relatively major components.



(78)

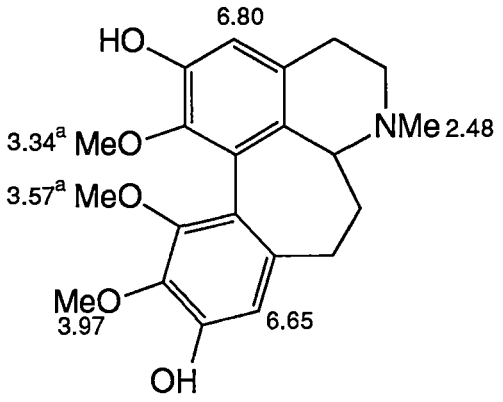
Separation of the crude extract by PLC afforded thirteen fractions as detected by UV absorption as well as alkaloid positive reactions by spraying with Schlittler's reagent or Dragendorf's reagent. Each fraction was then analyzed by GC-MS. The first three fractions did not contain any alkaloids. The twelfth fraction (fraction 1L) contained mainly

colchicine (78), its identity being confirmed by comparison with an authentic sample.

Fraction 1D, which contained traces of impurities, showed a molecular ion of 371 for the major component; high resolution mass spectrometry confirmed a molecular formula of $C_{21}H_{25}NO_5$ for this component. The mass spectrum of this major component showed only high mass fragments with the peak due to the loss of a methoxy fragment (M^+- 31) as the base peak. This was indicative of a homoaporphine skeleton with a methoxy group at C_1. Although the ^1H n.m.r. spectrum was not very clean, integration suggested the presence of three methoxy groups at δ 3.97, 3.57, and 3.34; an N-methyl group at δ 2.48; and two singlet aromatic protons at δ 6.80 and δ 6.65 for the major component. Since the oxygenation patterns of all the homoaporphines reported to date have been confined to C_1, C_2, C_{10}, C_{11}, and C_{12}, the aromatic protons at δ 6.80 and δ 6.65 were tentatively assigned to H_3 and H_9 respectively. This was later confirmed based on the chemical shift trend recently established by Tojo and co-workers[17]. The mass spectrum of a trimethylsilyl derivative of this isolate gave a molecular mass of 515 confirming the presence of two hydroxy substituents. This was supported by a sharp GC-IR absorption band at 3564 cm^{-1}.

A COSYLR 2-D experiment for which a delay of 0.5 sec. was established to pick up long range coupling between a vicinal proton and a methoxy group in an aromatic system, did not show any cross peaks between any of the methoxy groups and the two aromatic protons at δ 6.80 and δ 6.65. This implied the absence of methoxy substituents at C_2 and at C_{10}. Each of carbon atoms 1,11, and 12 should therefore be substituted with methoxy groups. The upfield positions of two methoxy

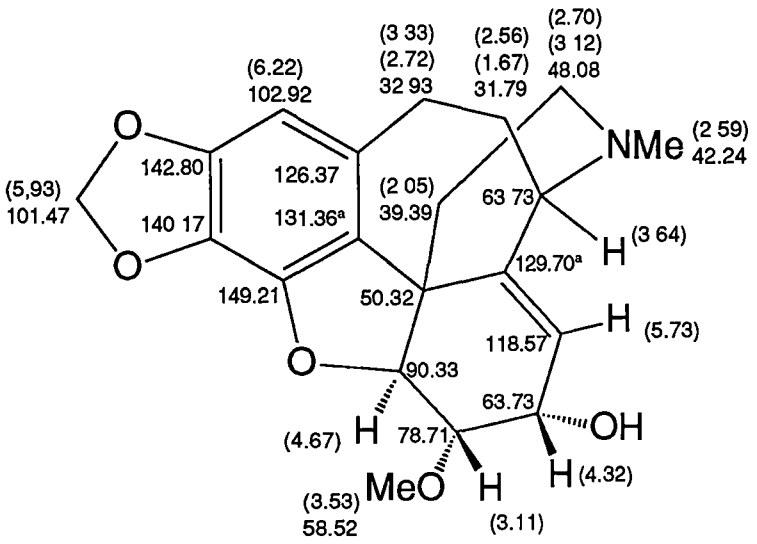
groups (δ 3.57 and δ 3.34) supported their positions as being at C_1 and C_{12}, respectively or *vice versa* . Based on the above arguments, structure (79) was assigned for this isolate. The chemical shifts of the rest of the methylene protons could not be fully assigned due to the presence of impurities. However, a COSY 2-D experiment established the connectivity of the H_{6a} proton to the aliphatic protons at δ 1.95 and δ 1.50. Recently, androbine, whose oxygenation pattern is the same as isolate (79), was reported as being isolated from *Androcymbium palaestinum*.[17]. The chemical shifts of the aromatic, methoxy and N-methyl protons of the two isolates were in close agreement. Those of H_{6a} and H_7 were, however, significantly different. This difference could be due to a difference in the configuration at C_{6a} and the chirality of the biphenyl system



(79)

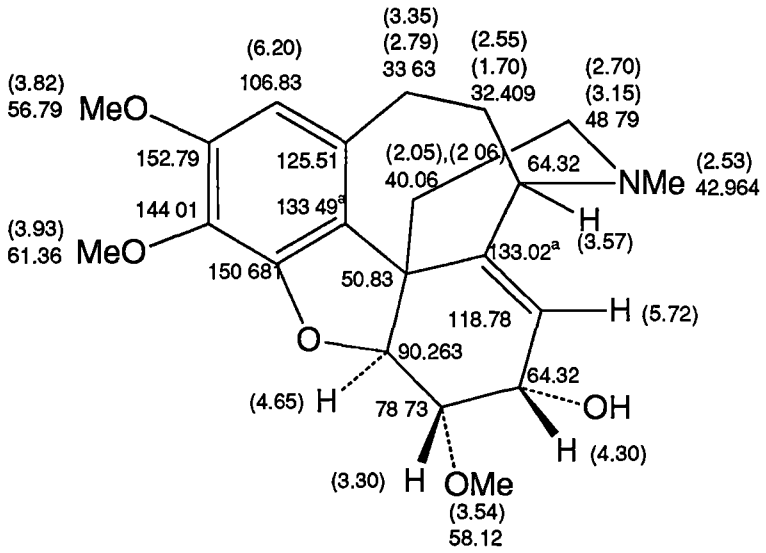
Fraction 1E was a mixture of (79) and the component of fraction 1F, and was not separated further. Yellow needle-like crystals were obtained from the recrystallization of fraction 1F from dichloromethane-ether. The high resolution mass spectrum indicated a molecular formula of $C_{20}H_{23}NO_5$. The presence of methylenedioxy (δ 5.93, ABq), methoxy (δ 3.33, s) and N-methyl (δ 2.59, s) groups in the ^{1}H-n.m.r. spectrum supported a C-17 skeleton characteristic of the phenethylisoquinoline-

derived bases. This skeletal structure was predicted to be of the homomorphine type based on its mass spectrum where the parent ion was the base peak, its ^1H n.m.r. spectrum being different from the tropolonic alkaloids. The presence of a hydroxy substituent was indicated by a loss of 17 mass units in the mass spectrum; this was confirmed by the mass spectrum of its trimethylsilyl (TMS) derivative (M^+ 429), and by the IR absorption band at 3600-3100 cm^{-1}. Ring D of this molecule was elucidated from the proton coupling data of an isolated spin system as indicated in the 1-D ^1H n.m.r. and COSY spectra. The index of hydrogen deficiency (10), complemented by the n.m.r. data, indicated the presence of six rings. Four of these rings would be due to the basic homomorphine skeleton, and the fifth due to a methylenedioxy ring system. The ^1H n.m.r. chemical shift of H_5 (δ 4.68) and the corresponding ^{13}C n.m.r. signal at δ 90.33 indicated the presence of an ether linkage[169] between rings A and D, a system analogous to the morphine alkaloids. This was further indicated by the IR absorption band at 1068 cm^{-1}, which is a characteristic symmetric stretching vibration for unsaturated ethers. This accounted for the sixth ring system.



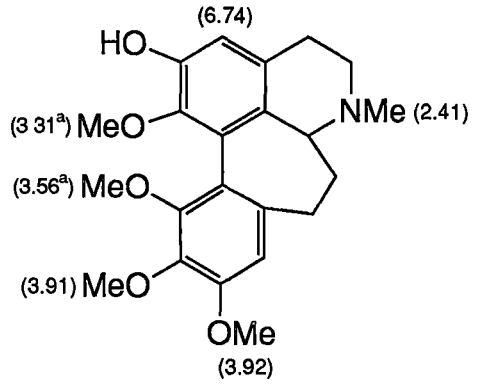
(66)

Based on the n.m.r. data and biogenetic arguments, structure (66) was proposed for this isolate, named wurmbeanine, which proved to be very similar to the reported homomorphine alkaloid kreysiginine (80) isolated from *Kreysigia multiflora*[189]. A comparison of their n.m.r. data showed that this isolate is identical with kreysiginine[190] including the configuration at C_5, C_6, and C_7 except for their A-ring substitution. The magnitude of the coupling (J 9.5) between the C_5 proton (δ 4.67) and the C_6 proton (δ 3.11) is consistent with a *trans* diaxial configuration for these two protons. A *cis* axial-equatorial configuration between the protons at C6 and C7 was also derived from a coupling constant of 3.7. This is similar to the established configuration in the B ring of kreysiginine. A combination of COSY and XHCORR experiments was used to assign the chemical shifts of the proton and the carbon atoms. The quaternary carbon assignments were made by comparing its chemical shifts with other isoquinoline systems such as thebaine[191]. In comparison with this alkaloid, a complete ^1H n.m.r. and ^{13}C n.m.r. chemical shift assignment for kreysiginine (80) was also made for the first time.

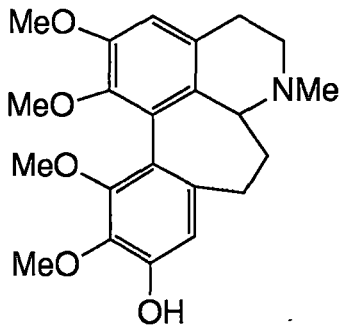


(80)

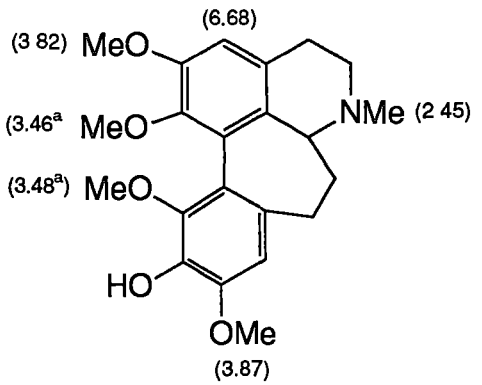
Fraction 1G was not obtained pure, but spectroscopic data was obtained on the major component. Its molecular weight was measured by high resolution MS as 385.1892 which was calculated as $C_{22}H_{27}NO_5$ (385.1888). The mass spectrum showed the loss from the molecular ion of 15 and 31 mass units, the latter being the base peak. From this MS data, a homoaporphine skeleton was derived. In the ^1H n.m.r. spectrum, the two singlets at δ 6.70 and δ 6.53 were assigned to the aromatic protons H_3 and H_9, respectively. An N-methyl (δ 2.37) and four methoxy groups (δ 3.92, 3.91, 3.56, and 3.31) were also observed from the ^1H n.m.r. spectrum. The mass spectrum of the trimethylsilyl derivative of this sample (M^+ 457) confirmed the presence of a hydroxy group. This was also supported by an IR absorption band at 3312 cm^{-1}.



(81)



(82)



(83)

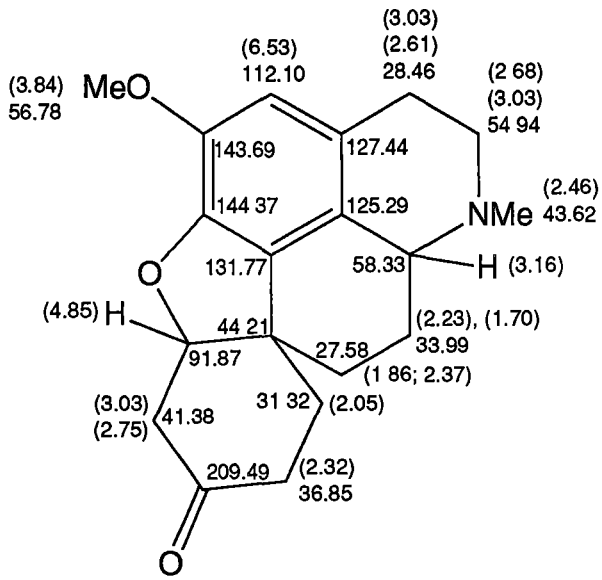
The upfield shift in the signal of two of the methoxy groups (δ 3.31 and δ 3.56) indicated the presence of methoxy groups at C_1 and C_{12}. The methoxy group at C_1 was also indicated by the mass spectrum base peak at (M^+-31). This left C_2, C_{10}, and C_{11} as possible positions for the hydroxy group. The small sample size (5mg) did not allow a good COSYLR experiment to verify the immediate neighbors of the two aromatic protons. Comparison of the the ^1H n.m.r. signals with (+)-androcine (81)[17], (+)-androcimine (82), and (+)-11-hydroxy-1,2,10,12-tetramethoxyhomoaporphine (83)[88], allowed the tentative assignment of the fraction 1G substituents as in androcine (83).

4.2.2 Alkaloids of Batch 4 of *Wurmbea dioica* ssp. *dioica*.

This sample was collected from Epping Forest, Tasmania, in two lots and the crude alkaloid extract was obtained from the combined freeze-dried material as in batch 1. The separation of the dichloromethane extract at pH 2-4 was described in Chapter 3, Section 3.2.2. The initial separation of the crude alkaloid extract obtained at pH 8-9 afforded nine fractions (Fraction 4A to 4I) on PLC, all of which contained a number of alkaloids as indicated by the GC-MS ion trace. Each of these fractions was further fractionated, except for fraction 9 which contained mostly polar materials This was done in an effort to isolate the dibenz[*d,f*]azecine-type alkaloids. In the subsequent separation of these fractions by PLC on silica, all of the silica was removed to ensure that even trace amounts of components were isolated. These trace components may not be easily detected as a distinct band even when sprayed with Schlitler's reagent. Each fractionation was monitored by GC-MS.

Separation of fraction 4A

Thirteen fractions were collected from the PLC separation of fraction 4A. No alkaloidal components were detected in the first and the last fractions. All the other fractions still contained a number of alkaloids except for fractions 4A4 and 4A5. Both contained an alkaloid of molecular weight 313.1685, corresponding to $C_{19}H_{23}NO_3$. The mass spectrum gave a moderately intense M^+-1 peak which was comparable



(84)

with the parent ion in intensity. This is a characteristic fragment of the homoproaporphine alkaloids. An aromatic proton (δ 6.53), a methoxy group (δ 3.84), and an N-methyl group (δ 2.46) were identified from the ^1H n.m.r. spectrum. A combination of ^{13}C n.m.r. and ^{13}C DEPT n.m.r. experiments identified seven methylene, two methine, and seven quaternary carbon atoms including a carbonyl carbon (δ 209.49), and a saturated quaternary carbon (δ 44.21).

The number of olefinic carbon atoms suggested a five-ring system to satisfy the index of hydrogen deficiency of 9. The oxygen atom unaccounted for was presumed to be part of a fifth ring system, the basic homoproaporphine skeleton being tetracyclic, and the absence of a hydroxy substituent being indicated in the IR spectrum. The chemical shift of a methine carbon (δ 91.87) with a corresponding proton at δ 4.85 was indicative of an ether bridge as in kreysiginine (80) and in wurmbeanine (66). Structure (84) was thus assigned for this isolate and was fully backed up by its spectroscopic data. The position of the carbonyl carbon was initially based on biogenetic arguments.

Although most of the reported homoproaporphine alkaloids of this type had the acetal bridge at C_1 and C_{12}, the bridge of this isolate is proposed to be at C_{13} instead of C_{12}. The coupling pattern of the methine proton of the ether bridge with the vicinal methylene carbon could not be used to distinguish a bridge at C_{12} or C_{13}. A carbon-proton shift correlated 2-D n.m.r. (XHCORR) with long range effects experiment on this isolate was done to confirm the proposed structure (Figure 4.1). Optimum polarization of the C-H delay periods in the pulse sequence was set for 50 milliseconds, while the delay for the anti-phase C-nucleus multiplet was 33 milliseconds. This allowed a long range coupling of a carbon atom two to three bonds away from a proton ($^{2,3}J$ ^{1}H-^{13}C). The generally greater magnitude of the ^{3}J over ^{2}J and ^{4}J allowed an assignment of the chemical shifts of the quaternary carbon atoms. The coupling of the aliphatic carbon atom where the ether bridge was attached (91.87 ppm) to the protons of C_9 (δ 2.05), but not with the C_{10} protons (δ 2.32), supported an ether bridge being at C_{13} and not at C_{12}. In addition, the quaternary carbon, C_{8a}, was coupled to the methylene protons adjacent to the methine proton of the ether bridge; this coupling

would not have been observed if the methylene protons were at C_{13}. Also, the rest of the cross peaks observed from the C-H correlation experiment supported unambigously the assignment of the chemical shifts of the carbon atom. This conclusively supported the proposed structure (84) for this isolate named dioicinone; this represents a new structural type within the homoproaporphine group of alkaloids.

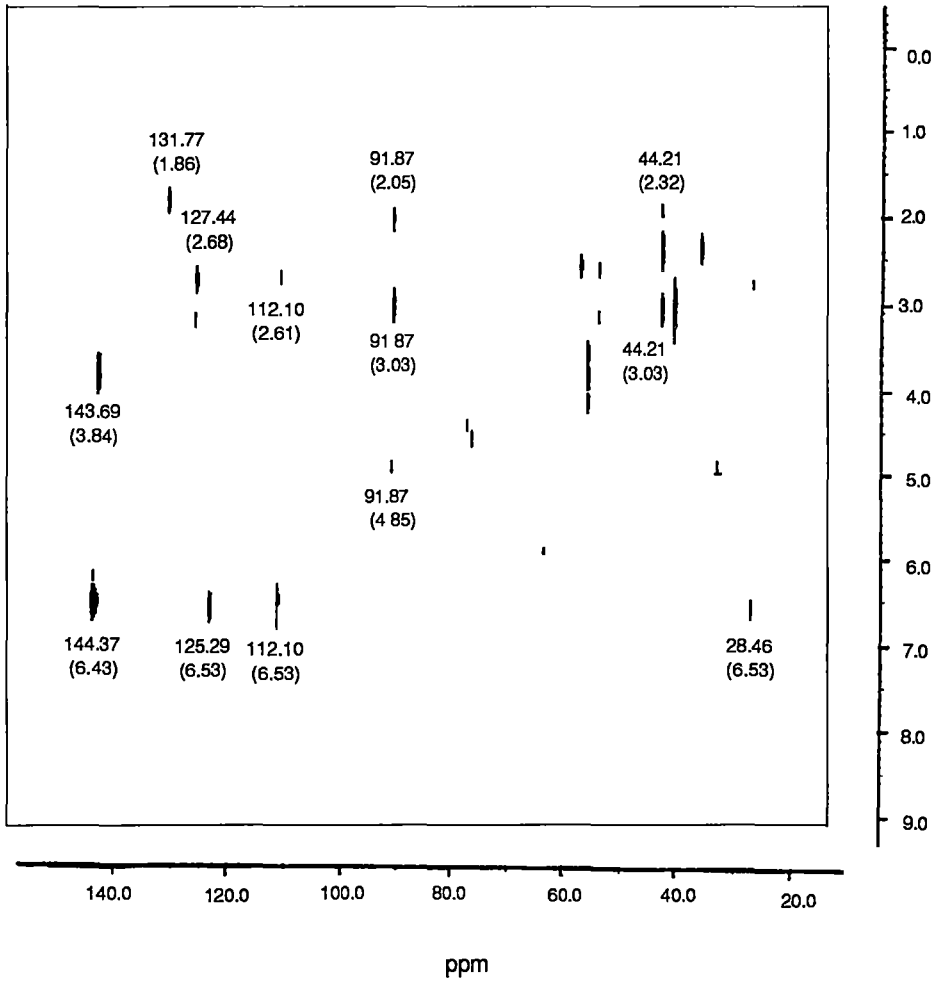
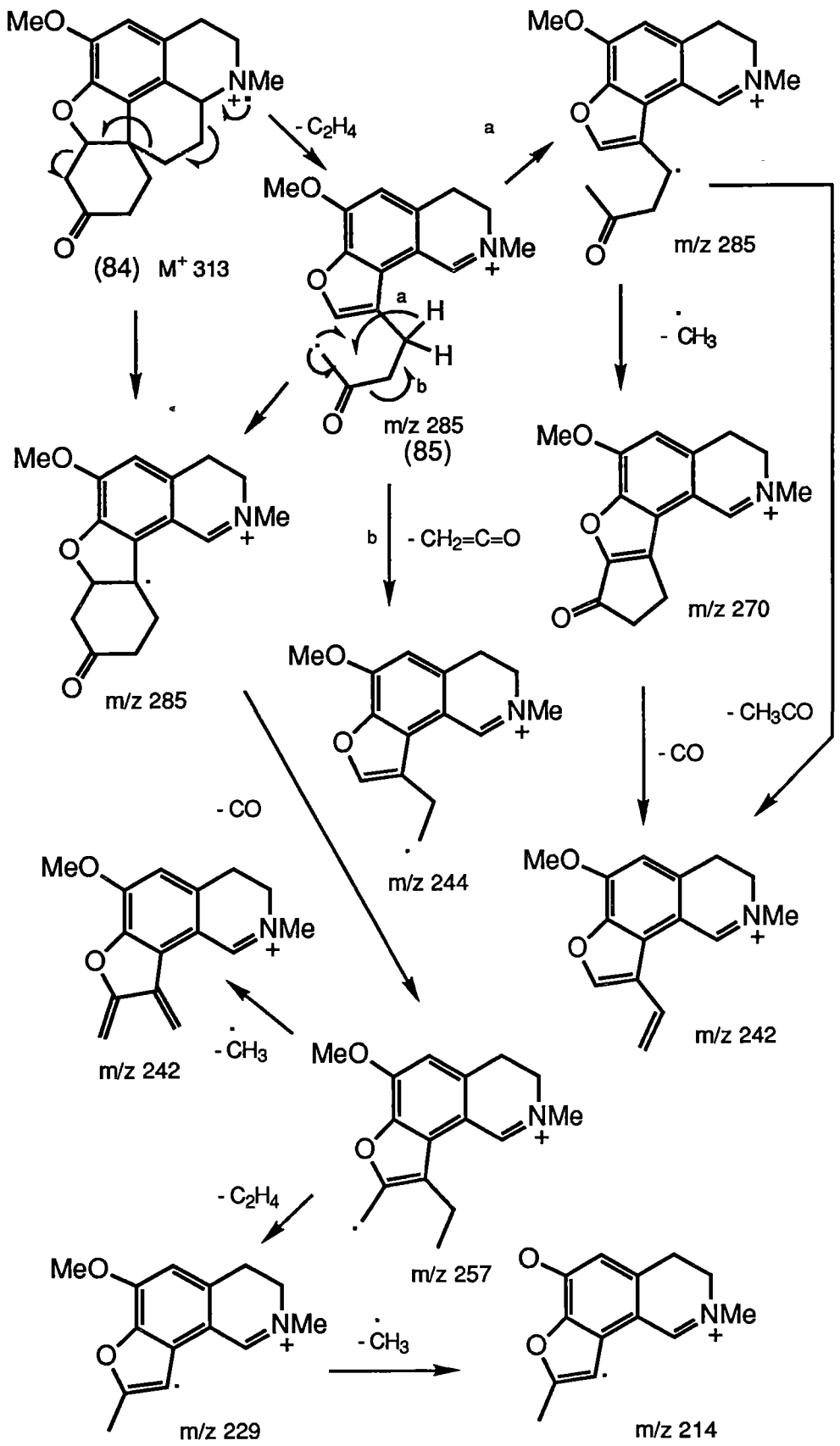


Figure 4.1. XHCORRLR of dioicinone (84)



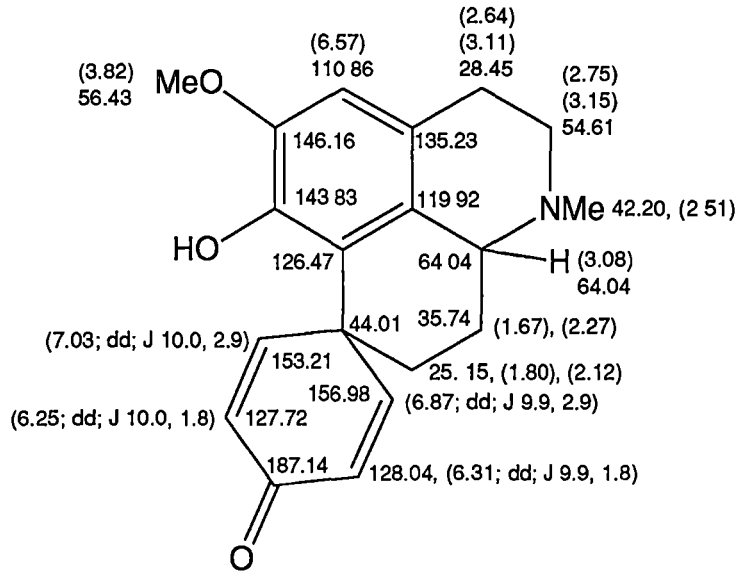
Scheme 4.2 Proposed Mass Spectral Fragmentation Pattern of (84)

This structural assignment for dioicinone (84) was supported further by the mass spectrum, where a facile loss of a C_2H_4 fragment giving rise to a base peak of M^+-28 ($C_{17}H_{19}NO_3$) was observed. This intense peak could be due to the formation of a specially stable benzofuran-type fragment (85) (Scheme 4.2). The proposed fragmentation pattern was derived by direct analysis of parent ion (m^*) determination (Barber-Elliot method). Biosynthetically, it is conceivable that an ether bridge joining the aromatic ring and ring D could be formed via a Michael-type addition of a hydroxy oxygen at C_1 to an enone system in ring D. The co-occurrence of dioicinone (84) with a homoproaporphine alkaloid (86) containing a cyclohexadienone ring system, and a hydroxy substituent at C_1 (86), lent support to this proposed mode of ether bridge formation.

Separation of fractions 4B and 4C

Further separation of fraction 4B by PLC allowed the re-isolation of dioicinone (84). All the other fractions still contained a number of alkaloidal components. No dibenz[*d,f*]azecine-type alkaloid was detected in these fractions, hence, no further separation was done on them. Fraction 4C afforded one relatively pure fraction on separation by PLC. Since the other fractions did not contain any dibenz[*d,f*]azecine-type alkaloids, no further separation was undertaken on the rest of the fractions. In the mass spectrum of the pure fraction 4C5, a moderately intense M^+-1 peak which was more intense than the parent ion, suggested a homoproaporphine skeleton assignment. The M^+-1 fragment was calculated as $C_{19}H_{20}NO_3$ (310.1441). A chemical ionization mass spectrum indicated an MH^+ ion at 312. The base peak in the mass spectrum was due to a loss of 28 mass units (m/z 283). An

N-methyl (δ 2.51) and a methoxy group (δ 2.82) were identified in the upfield region of the ^{1}H n.m.r. spectrum, while a total of five olefinic and the aromatic region protons were present. One was a singlet (δ 6.57), and each of the other four was all coupled as doublets of doublets. Their coupling constants supported the proposed cyclohexadienone system for ring D, consistent with a ketonic carbon signal at 187.14 ppm of the ^{13}C n.m.r. spectrum.



(86)

The ^{13}C n.m.r. coupled with ^{13}C DEPT experiments confirmed the presence of an aromatic methine carbon atom along with five others, one of which is in the aliphatic region of the spectrum. Four methylene, one methoxy, one N-methyl and six quaternary carbon atoms were also identified in the ^{13}C n.m.r spectrum further supporting the proposed structure (86). The complete assignment of the n.m.r. chemical shift was made by comparison with an aporphinoid alkaloid glaziovine[191a] and with a cyclohexenone system[192]. The methoxy position was confirmed by COSYLR experiments. This alkaloid has not been isolated previously from natural sources, but had been synthesized by an oxidative

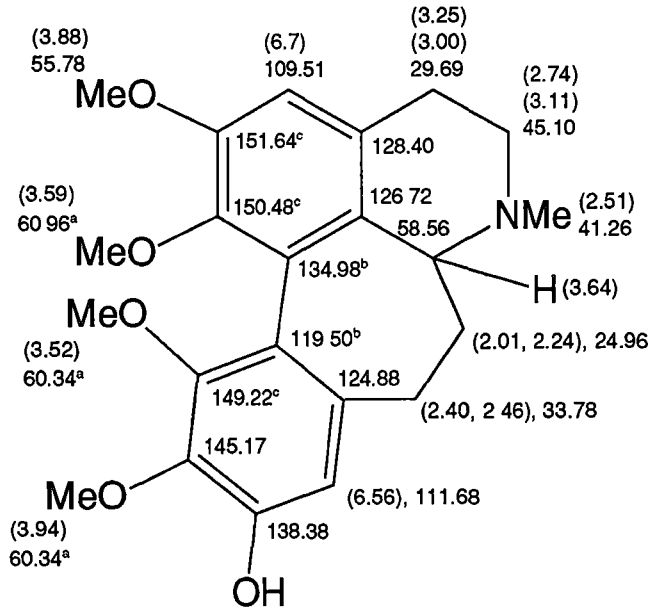
coupling reaction by Kametani and co-workers[193]. Its possible conversion to dioicinone (84) on PLC was ruled out as the latter had been detected as a major non-tropolonic alkaloidal component of the crude extract; the former was present in trace amount.

Separation of fraction 4D

Twenty five further fractions, weighing from 1 to 16 milligrams each except for one (50 mg), were collected from fraction 4D by PLC. Fractions 4D2 and 4D3 did not contain any alkaloid. The GC-MS ion trace of fraction 4D1 gave one peak of molecular weight 355. However, the ^{1}H n.m.r. spectrum of this isolate indicated the presence of significant amounts of impurities. These may be polar impurities not detected in the GC-MS scan. The amount of this fraction was too small to allow further fractionation. Fractions 4D9 and 4D10 contained the same alkaloid of molecular weight 385 and were combined together. Fraction 4D18 as well as fractions 4D20 and 4D21 were also isolated pure. Fractions 4D20 and 4D21 both contained dioicinone (84). All the other fractions still contained a number of alkaloidal components. Fractions 4D4 and 4D5 contained the dibenz[*d,f*]azecine, wurmbazecine (32a), in minor amounts. Dibenz[*d,f*]azecine alkaloids of molecular weights 355, 357, and 373 were also present as minor components of fractions 4D5 and 4D16.

The mass spectrum of the main component of the combined fractions 4D9 and 4D10 was characteristic of the homoaporphine alkaloid type. The base peak (M^+-31; m/z 354) was indicative of a methoxy substituent at C_1. This was supported by the ^{1}H n.m.r. signals

of two upfield methoxy groups at δ 3.52 and δ 3.59. This implied that both C_1 and C_{12} were substituted with methoxy groups. Two other methoxy groups were identified in the ^1H n.m.r. spectrum. Nuclear Overhauser (nOe) difference spectroscopy and COSYLR spectral data showed an interaction of the aromatic proton at δ 6.70, with a methoxy substituent at δ 3.88. The assignment of the two aromatic protons at δ 6.70 and δ 6.59 as H_3 and H_9 respectively is in agreement with the established trend observed in homoaporphines[17]. The fourth methoxy group was assigned at C_{11} since the aromatic proton at δ 6.59 showed no interaction with a methoxy group in the nOe nor in COSYLR experiment. This implied the presence of a hydroxy substituent adjacent

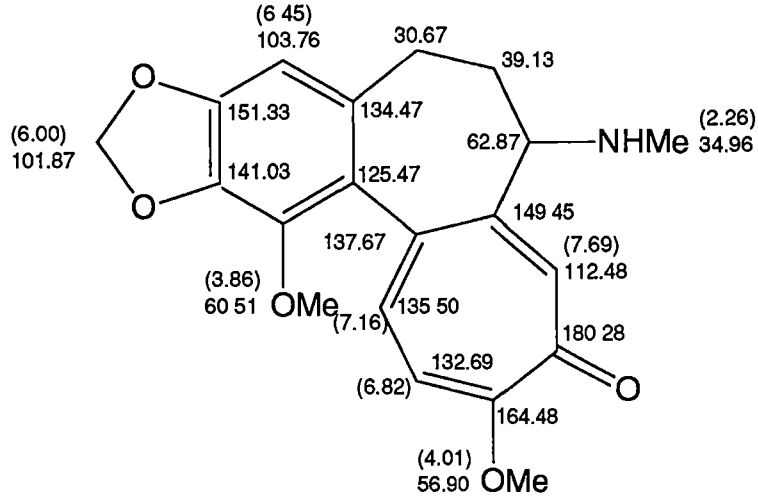


(82)

to the aromatic proton. A broad absorption band at 3234 cm^{-1} of the IR spectrum confirmed the presence of a hydroxy group. Structure (82) was therefore proposed for this alkaloid. Recently, an alkaloid of the same structure, from *Androcymbium palestinum*, was reported by Tojo and co-workers[17]. The ^1H n.m.r. assignments derived from the COSY 2-D spectrum compared very closely with those for androcimine although

some slight differences in the chemical shifts of the aliphatic protons were noted; a difference in the configuration at C_{6a} is possible, but this was not investigated further.

The relatively polar isolate from fraction 4D18 was found to have a molecular weight of 355.1421 which was calculated for $C_{20}H_{21}NO_5$. High mass fragments due to the loss of masses 29 and 59, and a base peak at m/z 191, did not resemble any of the non-tropolonic alkaloid structural types. The ^1H n.m.r spectrum, especially the olefinic and the aromatic region of the spectrum, indicated a colchicine-type skeleton for this isolate. A methyl group at δ 2.25 was indicative of an N-methyl group rather than an acetyl methyl of the colchicine group of alkaloids. The reported[99] base peak of demecolcine at m/z 207 was 16 units higher than the base peak of this isolate. The presence of a methylenedioxy substituent (δ 6.00; 101.87 ppm) and an N-Methyl substituent (δ 2.26) instead of an acetyl group (δ 1.9), accounted for the



(87)

base peak at m/z 191, a fragment composed of rings A and B[99]. Structure (87) is thus proposed for this isolate. The position of the

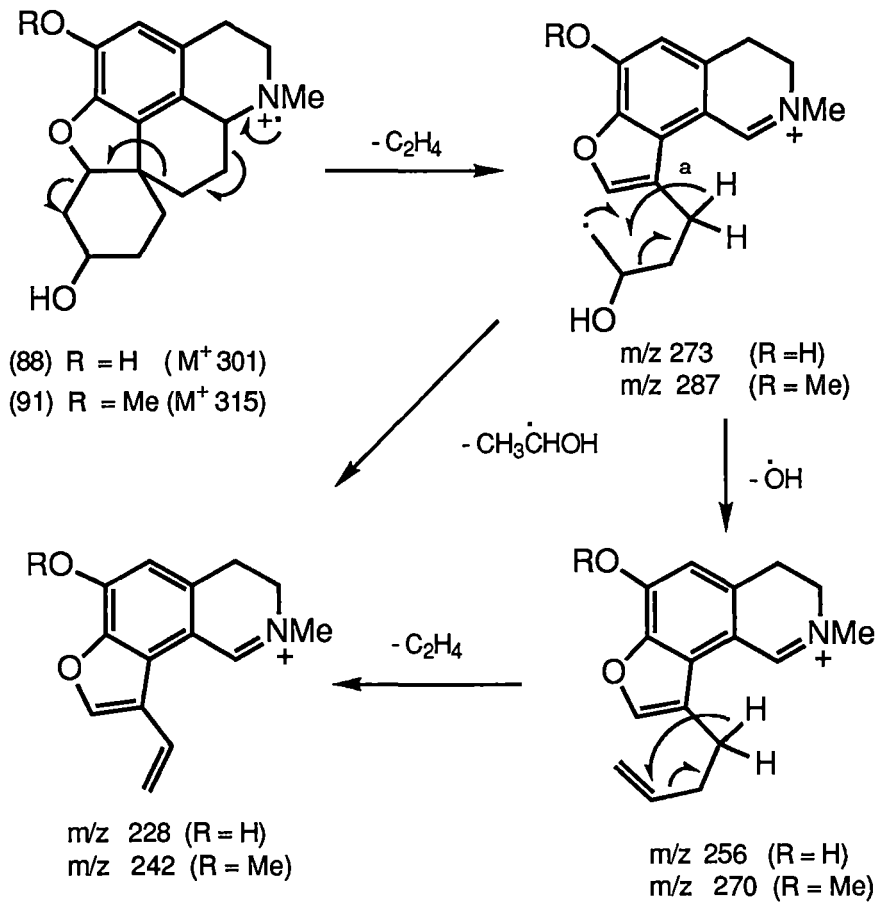
methoxy substituent at C_1 was confirmed by the through-space interaction of the methoxy group with an olefinic proton H_{11} (δ 6.82) by an nOe difference experiment; the rest of the olefinic protons were assigned on the basis of their coupling patterns. The complete assignment of the ^{13}C n.m.r. chemical shifts was made in comparison with isodemecolcine[99] and other alkaloids[191b].

Separation of fraction 4E, 4F, and 4G.

Attempted further separation of fractions 4E and 4F did not give any pure compounds.

Out of the thirteen fractions of 4G, fractions 4G1 to 4G5 and 4G11 to 4G13 did not contain any alkaloids. The sixth fraction (4G6) contained about five alkaloidal components. Recrystallization of this fraction from dichloromethane-ether allowed the isolation of white needle-like crystals. The molecular mass of this isolate was found to be 301.1652, which corresponded to the molecular formula, $C_{18}H_{23}NO_3$ (301.1676). Although the molecular ion was very weak (7%), the peak due to the M+-1 fragment was comparable with it. A homoproaporphine skeleton was tentatively assigned to this isolate based on the mass spectrum. The lone carbon-containing substituent, an N-methyl group identified in the ^1H n.m.r. spectrum, was in accord with a C_{17} basic skeleton of a homoproaporphine. The molecular ion (m/z 303) of a deuterated sample of this isolate indicated the presence of two hydroxy substituents leaving one oxygen atom unaccounted for. The IR spectrum showed a strong absorption band at 3453 cm^{-1}. Since there was only one aromatic proton in the ^1H n.m.r. spectrum, two of the oxygen atoms had to be in the aromatic ring. The downfield shift of a methine carbon (δ 93.25) was

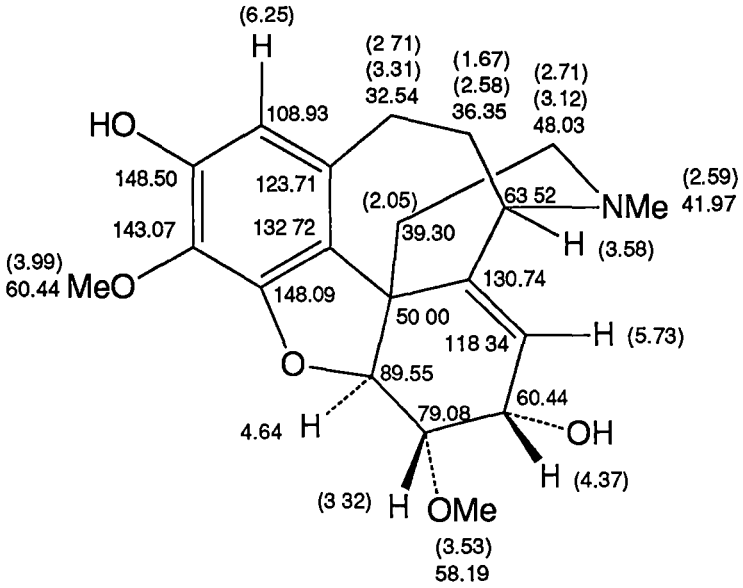
indicative of an ether bridge. The ^{1}H n.m.r, as well as the ^{13}C n.m.r., complemented by ^{13}C DEPT editing, accounted for all the protons and the carbon atoms of the proposed structure (88) for this isolate (dioicinine). The proposed mass spectral fragmentation pattern, showing the loss of an ethylene fragment (M^{+}-28) was supported by high resolution mass spectrometry and the parent ion (m*, B/E method) and daughter ion (m*, d.a.d.i.) analysis of the key mass fragments of this isolate (Scheme 32).



Scheme 4.3

The possibility of the ether bridge being at C_{12} instead of C_{13} was ruled out, based on the ^{1}H n.m.r data and the MS fragmentation pattern. Both the methine protons at C_{11} (δ 4.28) and C_{13} (δ 4.46) were coupled to the same methylene protons (δ 2.13 and δ 2.54) as illustrated in its

COSY 2.D n.m.r. spectrum. This position of the ether bridge also supported the facile loss of a C_2H_4 fragment in the mass spectrum, as in (84). The above data fully established the proposed gross structure (88).



(89)

The mass spectrum of fraction 4G7 indicated a homomorphine-type component, the parent ion being the base peak, and the ^1H n.m.r. spectrum being different from those of the colchicine derivatives. High resolution mass spectroscopy was in accord with the empirical formula $C_{20}H_{25}NO_5$ (359.1731). The proton n.m.r. spectrum was very similar to that of wurmbeanine (66). The main difference was the absence of methylenedioxy protons in this isolate. In its place was observed a methoxy group (δ 3.99). Its IR spectrum showed an absorption band at 3406 cm^{-1} indicating the presence of a hydroxy group. Nuclear Overhauser effect (nOe) difference spectroscopy revealed a through space interaction of the aromatic proton at δ 6.25 with an aliphatic proton at δ 2.71. No interaction between this aromatic proton and the methoxy

group was observed. The olefinic proton at δ 5.73 showed an interaction with an aliphatic proton at δ 3.58. Since both the ^{1}H n.m.r. and the ^{13}C n.m.r. spectra were similar in most respects to (66), except for the aromatic ring substitution, structure (89) was proposed for this isolate. The aliphatic proton assignment was derived from the COSY spectrum of this molecule; the rest of the n.m.r. chemical shift assignments were made in comparison with wurmbeanine (66).

4.2.3. Alkaloids of Batch 6 of *Wurmbea dioica* ssp. *dioica*.

This collection of *Wurmbea dioica* ssp. *dioica* was obtained from Rosny Point near Hobart, Tasmania over a period of six days in September, 1988. These plant samples were taller (ca. 6-7cm) than the ones collected from the south of Epping Forest (ca. 3-4cm). Three main extracts were obtained from the methanol extract of this plant sample. Extract 6B was the light petroleum solubles at pH 2-4. Extraction with dichloromethane at the same pH 2-4 gave extract 6C, and the dichloromethane solubles at pH 8-10 was named extract 6D. Extract 6C was not separated further as its GC-MS ion trace showed mostly non-alkaloidal peaks. In the succeeding separations, the main targets were to isolate the dibenz[*d,f*]azecine-type alkaloids and to re-isolate wurmbazecine (32a). Only the fractions obtained in reasonable amounts were subjected to further separation. While other fractions did not contain this alkaloid group, they were separated when other unidentified phenethylisoquinoline-derived alkaloids looked reasonably separable.

Alkaloids of Extract 6B

This extract was separated by multiple development radial layer chromatography (RLC) using solvents of an increasing polarity. Nineteen fractions were collected, of which only fractions 6B13 and 6B14 were pure. Fraction 14 contained dioicinone (84). Fraction 6B17 contained three dibenz[*d,f*] azecine-type alkaloids. The quantity of this fraction did not allow further separations, however. The GC-MS ion trace (different modes of injection) showed a dibenz[*d,f*]azecine (M^+ 387) as the major component (Figure 4.2). A second component (M^+385) appeared in trace amounts. The ^1H n.m.r spectrum did not give signals corresponding to the possible structures of a dibenz[*d,f*]azecine alkaloid of molecular weight 387. A GC-MS by on column injection of this fraction showed an increased peak area but the dibenz[*d,f*]azecine base remained the major component.

A direct probe MS analysis of this fraction (6B13) showed mainly the component of molecular weight 385. The dibenz[*d,f*]azecine alkaloid was hardly detected in this spectrum. The calculated molecular mass was consistent with the empirical formula $C_{22}H_{27}NO_4$. The parent ion of this molecule was also the base peak; a homomorphine skeleton was predicted based on the mass spectrum. The ^1H and the ^{13}C n.m.r. spectra revealed an O-methylandrocymbine structure (90). The ^1H n.m.r chemical shifts compared well with the assignment of Freyer and co-workers[44]. The carbon chemical shift was assigned from the ^{13}C n.m.r complemented by ^{13}C DEPT experimental data and by comparison with wurmbeanine (66) and (89).

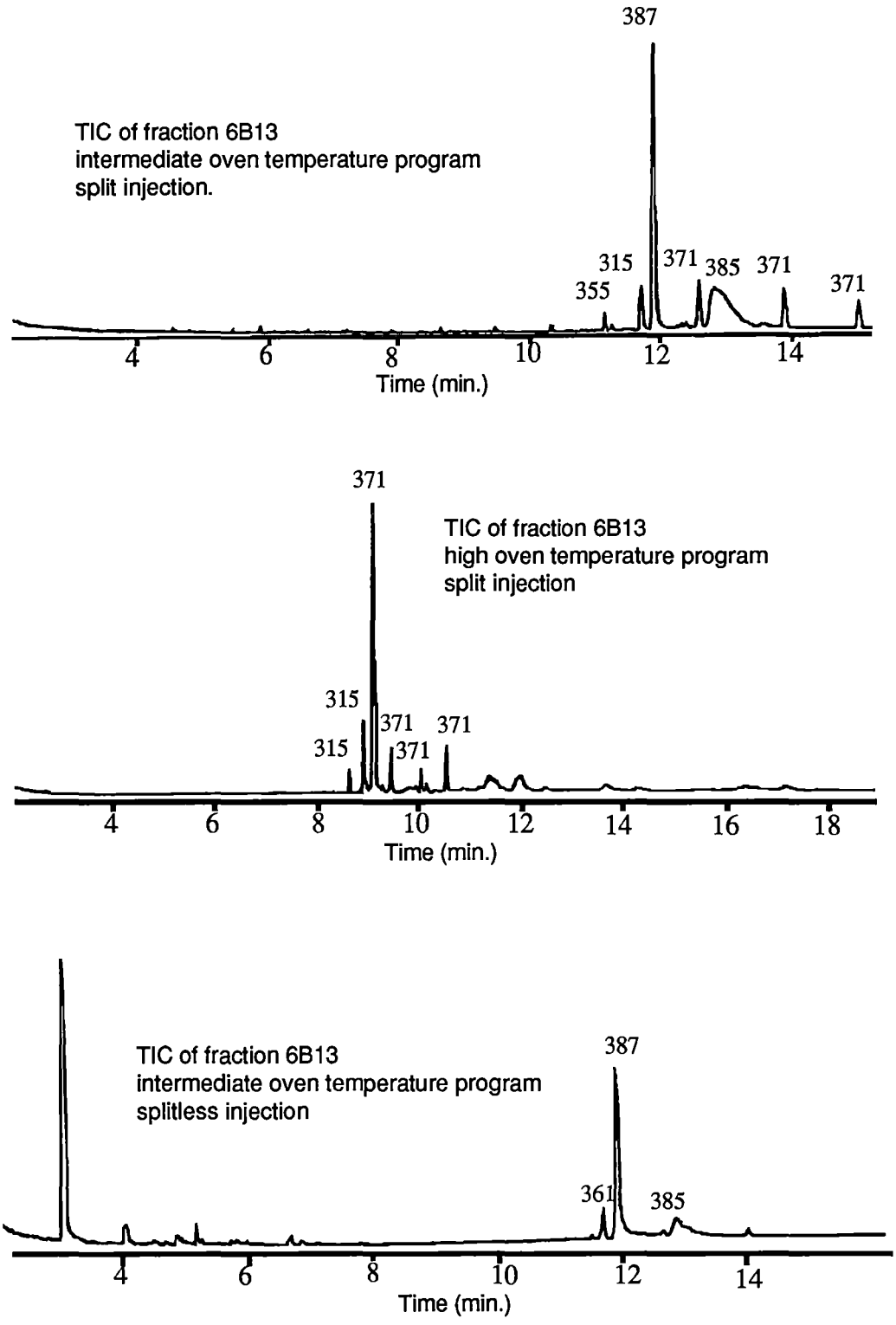
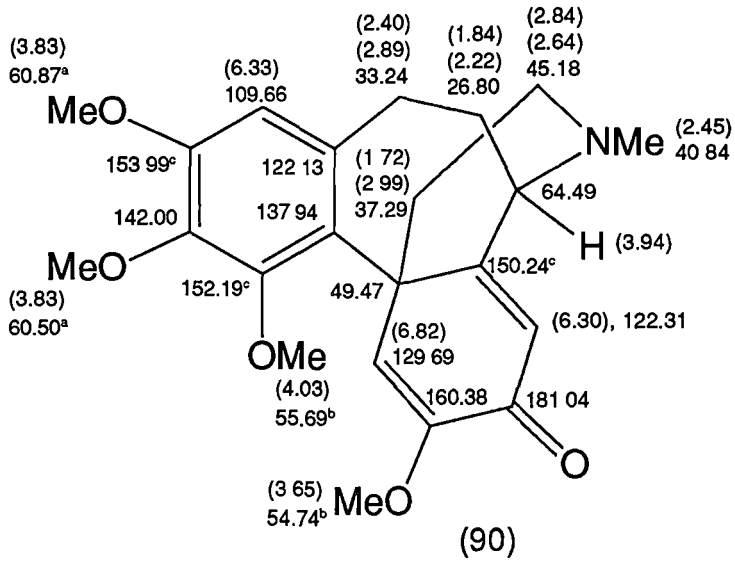


Figure 4.2 Total Ion Chromatograms of Fraction 6B13



(90)

The poor chromatographic behavior of O-methylandrocymbine (90) is not understood. However, it does provide some insight into the problems with the isolation of the dibenz[*d,f*]azecine-type alkaloids. While a number of fractions obtained separately from different *Wurmbea* species were shown to be relatively pure as indicated by their GC-MS ion trace, their ^{1}H n.m.r. and ^{13}C n.m.r. spectral data were always complex and inconsistent with the expected structure as in the case of fraction 6B13. Repeated attempts to isolate the dibenz[*d,f*]azecine alkaloids of molecular weights 355, 373 and 387, including further fractionation of combined fractions from which each of these alkaloids was detected, were unsuccessful. It seemed that the dibenz[d,f]azecine alkaloids were actually present in trace amounts, and that they were always isolated with some other major components with very poor chromatographic properties. A bigger sample size and an alternative separation method need to be employed if these alkaloids are to be looked at further. High pressure liquid chromatography could prove beneficial in this area; this technique was investigated in a preliminary fashion but good separations were difficult to achieve.

Alkaloids of Extract 6D

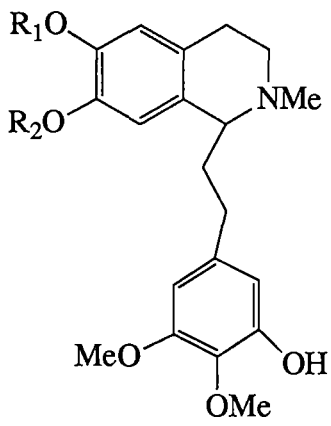
Extract 6D was separated further on the basis of different solvent solubilities giving rise to extracts 6D1, 6D2, 6D3, and 6D4. Fraction 6D4 contained mainly the new alkaloid wurmbeanine (66), with various colchicine-derived alkaloids, as well as a few other non-tropolonic alkaloids. This mixture was not separated further; fractions 6D3 and 6D2 were combined together based on their alkaloid profile.

Quick column separation of fraction 6D1 afforded nine impure fractions. The first fraction 6D1A was separated by multiple development PLC. The homoproaporphine dioicinone and the homomorphine wurmbeanine (66) were re-isolated in the process. Another interesting fraction, 6D1D1A1, of molecular weight 385 was isolated relatively pure but in very small quantity (<1 mg.). A loss of a $C_5H_6O_3$ fragment (M^+ 271) comprised the base peak. This base peak, along with the high mass fragments due to the loss of masses 28 and 59, is quite unique. None of the other known phenethylisoquinoline-derived alkaloids have this fragmentation pattern. Two dimensional n.m.r. experiments were needed to further elucidate the structure; but the sample size, however, did not permit these. The structure of this alkaloid remains to be determined.

Another fraction 6D1D1I4 proved to be a new homoproaporphine alkaloid. Its molecular weight was measured as 315.178 in accord with the empirical formula $C_{19}H_{25}NO_3$. Like dioicinone (84) and dioicinine (88), its base peak was due to the loss of a C_2H_4 fragment. The ^1H n.m.r. was very similar to that for dioicinine (88) except for the presence of a methoxy group and some differences in the chemical shifts of the

aliphatic protons. Structure (91, O-methyldioicinine) (Scheme 4.3) was proposed for this alkaloid. This structure assignment, and its close similarity with the homoproaporphine alkaloid dioicinine (88), was further supported by its MS fragments (Scheme 4.3).

The combined fractions of 6D1F and 6D1E were subjected to RLC separation with multiple development. A number of fractions were collected and combined together into a total of five fractions. Fractions 6D1F2 and 6D1F3 were separated one more time by PLC. The homomorphine wurmbeanine (66) was re-isolated; fraction 6D1F2 also contained traces of wurmbazecine.



	R_1	R_2
(10)	Me	H
(92)	H	Me

Radial layer chromatographic separation of fraction 6D2 afforded seventeen fractions. One of the fractions, 6D2J, was separated by PLC. The alkaloids wurmbeanine (66) and dioicinone (84) were re-isolated, together with a 1-phenethylisoquinoline-type alkaloid. The skeletal system of the 1-phenethylisoquinoline alkaloid was recognized from its mass spectrum, which showed a base peak at m/z 192; most of the other peaks were of very weak intensities. The molecular mass was consistent with the molecular formula $C_{21}H_{27}NO_5$ (373.183) . Its ^1H n.m.r spectrum was almost identical to the reported chemical shifts of

autumnaline(10)[44]. Since the differences in the chemical shifts compared with isoautumnaline (92)[44] were very slight, the relative positions of the methoxy groups were identified by COSY 2-D experiments with long range enhancement effects. The result conclusively identified this isolate as the known alkaloid autumnaline (10).

4.3. Alkaloids of *Wurmbea pygmaea*

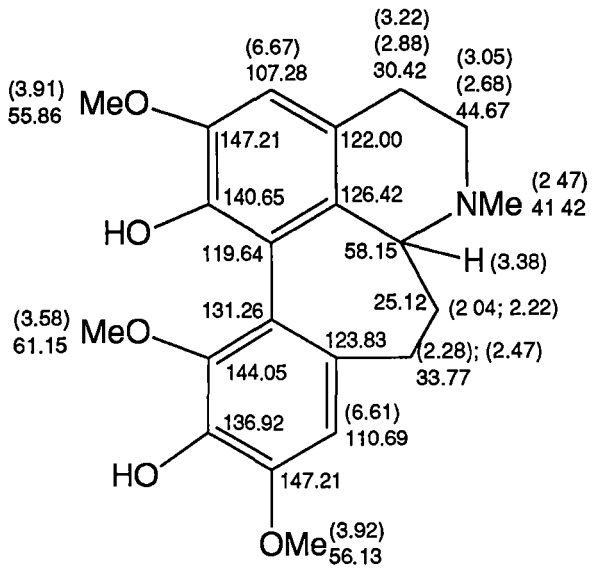
Eight stalks of very mature *Wurmbea pygmaea* were collected from an area south of Perth, Western Australia in mid-September. The alkaloids were extracted immediately after collection following the usual procedure. A GC-MS scan of the crude extract was made the next day at the University of Western Australia. The total ion chromatograph showed at least three alkaloidal components. The major component was a dibenz[*d,f*]azecine of molecular weight 387. The other two minor components were not identified due to their low concentrations. A repeat GC-MS scan of this crude extract was later done in the University of Tasmania to identify the other two components. One of the minor components was a dibenz[*d,f*]azecine of molecular weight 373. The other one (M^+ 371) appeared to be a homoaporphine with a hydroxy substituent at C_1, as the base peak was due to a loss of 17 mass units. The ^1H n.m.r. spectrum of this isolate, however, indicated the presence of a tropolonic alkaloid in major quantity. The GC-MS run program did not pick up the colchicine-type components, presumably due to volatility differences. This situation was also observed in many of the other fractions from *Wurmbea dioica* ssp. *dioica.*

In an attempt to isolate the dibenz[*d,f*]azecine (M^+ 387), a second batch of sample was collected in Western Australia the next year but a month earlier (first week of August). This was younger than the previous collection although it was already fruiting. The fruit was treated separately from the leaves. The alkaloid profile of this sample was distinctly different from the first batch. In both the fruit and the leaf extracts, no dibenz[*d,f*]azecine alkaloids were detected. The major component of the leaf extract at basic pH was an alkaloid of molecular weight 371. This was a minor component of the first batch of samples, which was thought to be a homoaporphine.

Three components of the alkaloid extract from the fruits of molecular weight 341, 313, and 369, had M^+-1 for their base peaks and could therefore be presumed to be of the homoproaporphine type. Another component of molecular weight 385 could be a colchicine derivative. A comparison of the results of the GC-MS analysis indicated a higher concentration of these alkaloids in the fruits than in the leaves and stems. The leaves and stems were however observed to contain mainly a homoaporphine-type alkaloid of molecular weight 371.

The dichloromethane extract of the basified extract from the leaves and stems was passed through an alumina quick column from which 22mg of the main component (M^+371) were collected and characterized spectroscopically; the high resolution mass spectrum indicated a molecular formula of $C_{21}H_{25}NO_5$. The three methoxy groups as well as the N-methyl group observed in the ^1H n.m.r. spectrum supported a C_{17} basic skeleton for a homoaporphine. This indirectly suggested that the other two oxygen atoms could be hydroxy groups. The molecular mass measurement (M^+ 373) of a deuterated sample confirmed the presence

of two hydroxy groups. One of the hydroxy groups was assigned at C_1 as a result of the facile loss of 17 mass units in the mass spectrum. A through space interaction of the two methoxy groups (δ 3.92 and δ 3.91) with the aromatic protons at δ 6.61, 6.67 respectively, was observed in the nOe spectrum of this sample. Knowing the assignment of the two aromatic protons based[17] on their chemical shifts, the methoxy substituents were assigned to C_2 and C_{10}. The upfield-shifted signal of the third methoxy group (δ 3.58) allowed its assignment to C_{12}. This left C_{11} as the only possible position of the second hydroxy group. The derived structure (93) proved to be the known homoaporphine, multifloramine[6,12]. However, this is the first assignment of the ^1H and ^{13}C chemical shifts for this alkaloid. This assignment was derived from the proton-proton coupling established by COSY as well as COSYLR experiments, by carbon-proton coupling (XHCORR), and by comparison with baytopine[57,194]. and with domesticine[191].



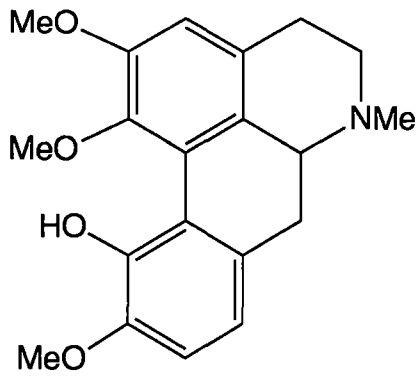
(93)

4.4. Alkaloids of *Baeometra uniflora* (Jacq.) G.Lewis.

A few stalks, including bulbs, of this plant were collected in Western Australia and the alkaloids of the bulbs and the stalks were extracted separately. In both the leaf and the bulb extracts, a dibenz[*d,f*]azecine of molecular weight 387 was detected as a very minor component. Both extracts also contained a major component of molecular mass 341. Other components of the bulb extract were two alkaloids whose base peaks were at m/z 192, and whose other peaks were of very weak intensity; this is characteristic of a simple 1-phenethyl-isoquinoline-type alkaloid. One of these did not show any molecular ion peak while the other had a molecular mass of 385. It was not possible to separate these alkaloids due to sample size limitations. A 1-phenethyl-isoquinoline-type alkaloid with three methoxy and one methylenedioxy substituents is proposed for the component of molecular mass 385.

The other components of the dichloromethane extract from the leaves included alkaloids with molecular masses of 385, 355, 383, and 397. The first component (M^+ 385) appeared to be N-methyldemecolcine based on its mass fragmentation pattern[99]. The other two (M^+ 355 and M^+ 383) showed comparable if not more intense M^+-1 peaks than the molecular ion. These could be homoproaporphine-type alkaloids. Collumellarine (M^+ 355; $C_{21}H_{25}NO_4$), along with isocorydine, have been reported[3] previously from this plant, although the latter was not characterized. Although the isomer corydine was also reported to be present in this plant, the GC-MS result did not indicate its presence unless it had the same retention time as isocorydine and was present in trace amount, as the ^1H n.m.r. result indicated signals only due to isocorydine. The fourth component had very weak peaks in the mass

spectrum except for a very intense peak at m/z 58; no alkaloid skeleton could be deduced for this component.



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The structure of isocorydine (94), the main component obtained from the leaves and stems of the extract at pH 8-10 was completely established by comparison of its ^1H n.m.r. and mass spectroscopic data with an authentic sample. Its re-isolation has brought to attention its co-existence with other alkaloids of 1-phenethylisoquinoline origin and its biosynthetic significance. In the subfamily Wurmbaeoideae, this aporphine-type alkaloid had only been reported from this plant and from a close relative, *Camptorrhiza strumosa*[4]. The only other alkaloid of a non-phenethylisoquinoline origin, in the subfamily Wurmbaeoideae, was a carboline alkaloid, isopylorine, isolated from *Gloriosa superba*[60].

4.5. Alkaloids of *Colchicum autumnale* and *Colchicum byzantinum*

GC-MS analysis of the crude alkaloid extract of the bulbs of *Colchicum autumnale* and leaves of *Colchicum byzantinum* indicated the presence mainly of colchicine and its derivatives (Table 4.1). Demecolcine (M^+ 371) and colchicine (M^+ 399) were isolated and were

Table 4.1 Alkaloids of *Colchicum* species

SOURCE-pH	M^+ [base peak (bp); name if any]
Colchicum autumnale	
roots-acid	399 [bp 312; colchicine]
roots-basic	colchicine, 371 [bp 207; demecolcine
bulb-acid	colchicine, demecolcine
bulb-basic	demecolcine
leaves-acid	colchicine (major), [317 bp 316], demecolcine, 383 [bp296], 385 [bp 312], 369 [bp 296]
leaves-basic	317 [bp 316; major], colchicine, demecolcine, 357 [bp 193], 385 [bp 312], 369 [bp 296]
Colchicum byzantinum	
leaves-basic	317 [bp 316], demecolcine, colchicine, 383 [bp 296], 369 [bp 296]

identified from their n.m.r. and mass spectral data. Overall, the alkaloid profiles of both plant samples were consistent with the observation that colchicine-type alkaloids (tropolonic alkaloids) are concentrated in the bulb, whereas more of the non-tropolonic wurmbaeoideae alkaloids could be found in the leaves and stems. Thus, the alkaloid extract from the bulbs of *Colchicum autumnale* contained mainly the tropolonic alkaloids. The leaves of *Colchicum byzantinum,* however,contained some non-tropolonic alkaloids which could be of the homoproaporphine-type as the mass spectrum showed an M^+-1 fragment as the base peak. The leaves of *Colchicum autumnale* were just developing from the bulb

and could possibly be used to explain the presence of more tropolonic alkaloids in its crude alkaloid extract.

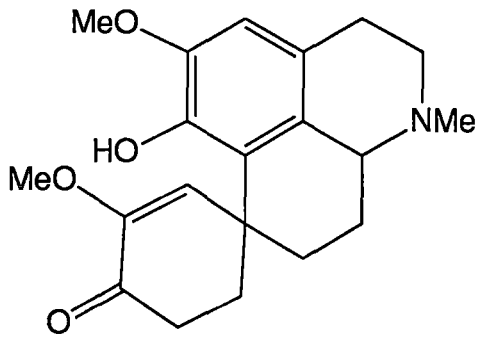
The assignment of the structure of colchicine and demecolcine was based on their mass spectrum, and the ^1H n.m.r. data obtained from the root extract at pH 2-4, and from the bulb extract at pH 8-10, respectively. In the basic leaf extract, the major non-tropolonic component of molecular mass 317 could not be further purified. Its MS fragmentation pattern indicated a homoproaporphine skeleton. No homoproaporphine of this molecular mass has been reported from this species. Regelamine (M^+ 317) has however been reported[28] from *Colchicum kesselringii*. The component of molecular mass 357 (bp 193) should be a tropolonic alkaloid. A base peak at m/z 193 was observed in 2- and 3-demethyl-N-methyldesacetylcolchiceine[99].

4.6. Alkaloids of *Gloriosa plantii, Sandersonia aurantica* and *Littonia modesta*

The genera *Gloriosa*, *Sandersonia*, and *Littonia* have consistently been classified in one tribe (see Chapter 6). Aside from the interest in finding dibenz[d,f]azecine-type alkaloids, interest was also focussed on the overall alkaloid profile of these three genera. The plant samples were collected at the same time and from the same source, and were all bearing fruits. However, the fruits of *Sandersonia* were most mature and of those of *Gloriosa* were very young.

GC-MS analysis of the crude alkaloid extract showed that the major components of the extracts from these three plants were mainly tropolonic. The organic extract at pH 2-4 of *Littonia modesta* and

Sandersonia aurantica contained a lot of material which chromatographed poorly in GC. In contrast, *Gloriosa plantii* contained not only the tropolonic alkaloid types in the pH 2-4 extract but also alkaloids of the non-tropolonic type as recognized from their mass spectra. The most mature plant, *Littonia modesta,* did not have any alkaloids while *Sandersonia aurantica* had more of the tropolonic alkaloids. The youngest sample, *Gloriosa plantii,* contained not only tropolonic alkaloids, but also non-tropolonic alkaloids. This possible pattern of variation in the alkaloid profile according to age needs to be fully established as it might prove useful in the search for specific alkaloid types such as the dibenz[d,f]azecine alkaloids. The alkaloid profile of two batches of *Wurmbea pygmaea* at two different levels of maturity had also demonstrated significant variation in alkaloid profile which involved the dibenz[d,f]azecine-type alkaloids.



(95)

The nontropolonic alkaloids of *Gloriosa plantii* of molecular masses 343, 345, and 347 gave intense peaks corresponding to M^+-1 indicative of homoproaporphine type alkaloids. The major component of the basic extract was isolated pure by alumina quick column separation. Its mass spectrum showed a weak parent ion (M^+ 343) with an M^+-1 peak of equally weak intensity; the base peak was at m/z 244. ^1H n.m.r.

and ^{13}C n.m.r. data indicated a structure (95) for this isolate which compared closely to that reported data for luteidine[39,41].

4. 7. Other Sources of Wurmbaeiodeae Alkaloids

The crude alkaloid extracts obtained from the following plants included in this section were screened for the presence of dibenz[*d,f*]azecine alkaloids and the wurmbaeoideae alkaloids in general. All the plant species included here are members of Buxbaum's subfamily Wurmbaeoideae. The two species of *Wurmbea* have not been studied previously. The rest of the species were screened in search for the dibenz[*d,f*]azecine- type alkaloids.

Two subspecies of*Wurmbea dioica* are recognized by Macfarlane[162]. Some differences between the sub-species include the colour of the nectaries which in the case of the former, are generally white instead of the dominant purple nectaries in the subspecies *dioica*. Also subspecies *alba* flowers later than the subspecies *dioica*. A clear cut comparison of the alkaloid profile of the two subspecies was not possible owing to the variation in age and locality of the samples collected. The crude alkaloid extract obtained contained a great deal of polar material that interferred in the isolation of pure components of the crude extract; the number of alkaloids detected was not as many as in the subspecies *dioica*. The majority of these alkaloidal components showed M^+-1 peak of intensity comparable to the parent ion and were therefore presumed to be of the homoproaporphine type alkaloids. There were also a number of homoaporphine alkaloids as indicated by

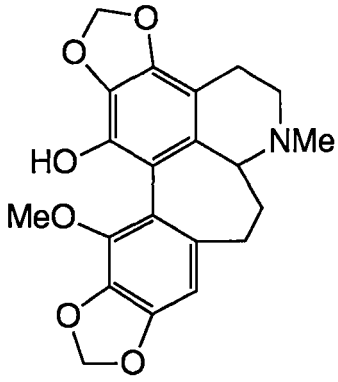
their mass spectra. The dibenz[*d,f*]azecine alkaloids detected are given in Table 3.1 (Chapter 3, Section 3.2).

Other alkaloids detected by GC-MS in this plant sample belonged to the homomorphine-type. The mass spectral fragmentation of one of the alkaloids, of molecular weight 373, was the same as from kreysiginine (M^+ 373) including the intensities, and was therefore assumed to be kreysiginine. Another component, M^+ 343, was assumed to be the homoproaporphine luteidine from its mass spectrum. A 1-phenethylisoquinoline alkaloid, whose molecular mass could not be easily determined, was also detected. A more interesting group of components of molecular masses 431, 373, and 359 had novel mass fragmentation patterns. All three components showed peaks due to the loss of 15 and 31 mass units. The base peak (M^+-45) of each of these three alkaloids had not been observed in other non-tropolonic phenethylisoquinoline-derived bases. However, these alkaloids were not isolated pure enough to allow full characterization.

A crude alkaloid extract obtained from five tiny stalks of *Wurmbea uniflora* obtained from Lake Leake road in Tasmania allowed the study of its alkaloid profile by the GC-MS technique. Aside from the two dibenz[*d,f*]azecine alkaloids, M^+ 373 and M^+ 387, five others of molecular masses 399, 385 (bp 314), 315 (bp 272), 385, and 371, were also detected. However, their skeletal make-up could not be ascertained from their mass spectrum.

The phenethylisoquinoline alkaloids kreysiginine (M^+ 373), floramultine (M^+ 371), kreysigine (M^+ 385), multifloramine (M^+ 371), and kreysiginone (M^+ 341) have been isolated previously from *Kreysigia*

multiflora[6,9-13]. In the present study, the crude alkaloid extract from this plant was re-evaluated in a search for the dibenz[*d,f*]azecine alkaloids. The plant sample consisted of a cut stem collected from New South Wales through the auspices of the Society for Growing Native Plants in Tasmania. A significant variation in the alkaloid profile was observed. Of the reported alkaloids isolated from this plant, only kreysiginine and kreysigine were present in this extract; however, two unreported alkaloids were detected. One, which was a major component, had a molecular mass of 401 with a base peak at m/z 366 (M^+-35). All the other peaks other than M^+, M^+-17, and the base peak, were of extremely low intensity, The second constituent, (M^+ 383) had a base peak due to a loss of 17 mass units (M^+-17) and the rest of the ms peaks were of extremely low intensities. These mass fragmentation patterns were indicative of homoaporphine-type alkaloids.



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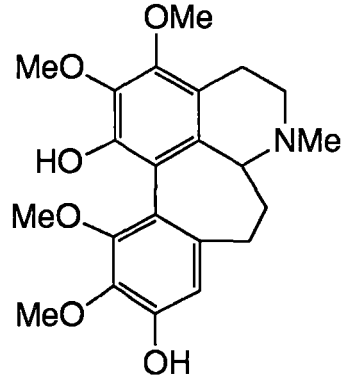


(97)

Although the component of molecular weight 401 did not give a base peak due to a loss of a hydroxy or methoxy substituent at C1, the peak due to the loss of a hydroxy group was relatively intense. This could also be a homoaporphine alkaloid whose fragmentation could have been influenced by its substitution pattern. The proposed structures (96) and (97) for the first and second components respectively, would be

the first homoaporphine alkaloids with C_3 oxygenation. The position of the substituents are all tentative, however, except for the hydroxy group at C_1. The alkaloid profile of this plant seemed to vary very significantly, not only in the concentrations but also in its actual composition. An unreported extraction conducted by a CSIRO group in Victoria[13] identified floramultine as the major component of their extract followed by kreysigine and kreysiginine. In this study, the major components were kreysiginine and the unknown alkaloid of molecular mass 401. The alkaloid of molecular weight 383 was a minor component while kreysigine was present in trace amount. All these results however, indicated that the main alkaloid components of *Kreysigia multiflora* are of the homoaporphine type; there was no indication of any of the dibenz[*d,f*]azecine-type alkaloids.

4.8 Conclusions

Fourteen alkaloids from *Wurmbea dioica* ssp. *dioica* have been isolated and characterized. These alkaloids represented six different skeletal systems namely: the dibenz[*d,f*]azecine, homoaporphine, homoproaporphine, homomorphine, 1-phenethylisoquinoline, and the colchicine types. Initially, eleven of these alkaloids were characterized for the first time; however, very recently three of the homoaporphine alkaloids were reported from *Androcymbium palaestinum*[17]. The three homoproaporphine alkaloids dioicinone(84), dioicinine (88) and O-methyldioicinine (91) are the first three examples of the homoproaporphine alkaloid type with an ether bridge at C_{13}. This structural assignment was unambiguously identified from their spectroscopic data. A probable precursor to this new homoproaporphine

group, the homoproaporphine (86), was also isolated and the structure elucidated by spectroscopic means.

Other known alkaloids mostly of phenethylisoquinoline origin, such as multifloramine, isocorydine, luteidine, and some colchicine derivatives, were re-isolated from different plant sources, in the search for the dibenz[*d,f*]azecine-type alkaloids.

The variation of the alkaloid profile according to age was re-affirmed by analysis of the alkaloids of *Wurmbea pygmaea*, *Gloriosa plantii, Sandersonia aurantica*, and *Littonia modesta*.

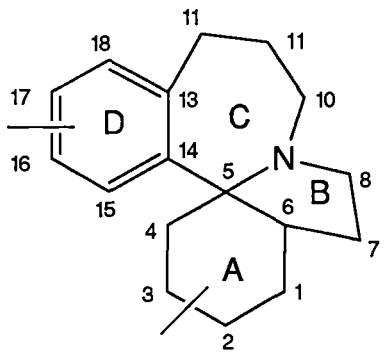
The inability to isolate further examples of the dibenz[*d,f*]azecine alkaloids was partially identified as due to the presence of components with similar Rf values as the dibenz[*d,f*]azecines, but with poor chromatographic behaviour. This was exemplified by the failure to detect O-methylandrocymbine in the GC-MS so that the minor component, the dibenz[d,f]azecine of molecular weight 387, appeared to be the major component. There remains a need to identify new separation procedures to isolate the dibenz[d,f]azecine alkaloids in *Wurmbea dioica* ssp. *dioica*.

Chapter 5

THE HOMOERYTHRINA ALKALOIDS

5.1. Introduction

Among the different classes of phenethylisoquinoline-derived alkaloids, the homoerythrina group has been the most represented. To date, there are approximately forty nine alkaloids of this type that have been reported[89-91]. Like the other phenethylisoquinoline-derived alkaloids, the homoerythrina alkaloids are basically composed of a C-17 tetracyclic skeleton. Carbon atoms 2, 3, 16, and 17 are generally oxygenated, and in a few cases, C_{18} may also be oxygenated. Epoxidation and carbonyl group formation has also been observed in the A-, B- and C-rings. In contrast with the erythrina alkaloids, the C_3 substituents in the homoerythrina alkaloids are normally epimeric. Although both the C-homoerythrina (1) and B-homerythrina-type (2) bases could be expected from the Michael-type



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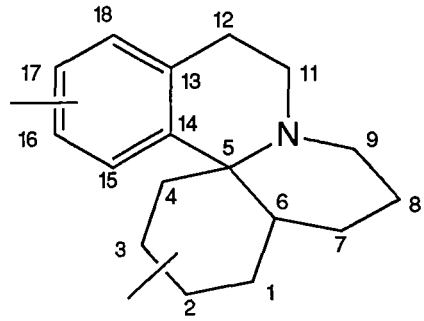

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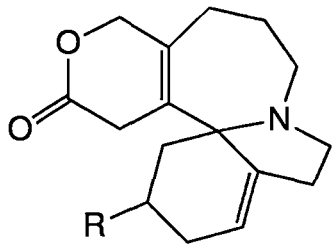
addition of a nitrogen to the unsaturated center of the biaryl system of a dibenz[*d,f*]azecine intermediate, only the C-homoerythrina-type have

been isolated from nature. The name homoerythrina will be used in the following discussion to refer to the C-homoerythrina alkaloids unless otherwise specified. The Chemical Abstracts numbering system is used in this discussion.



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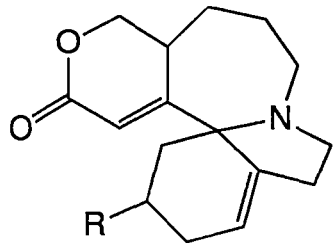

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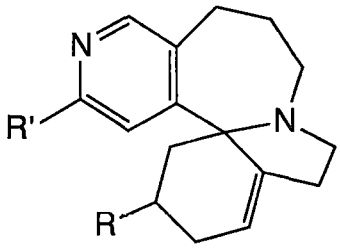

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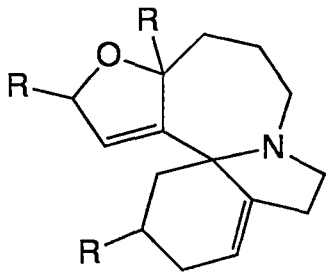

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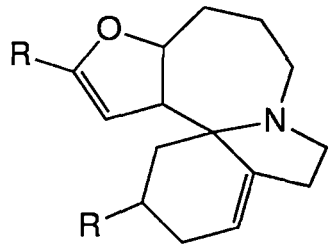
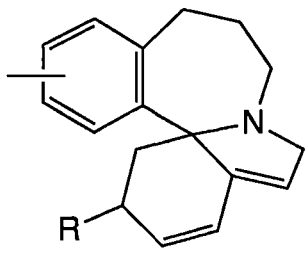


(102)

The homoerythrina alkaloids can be classified into two groups. The main group is composed of the C-homoerythrina systems in which ring D is aromatic (9). The less represented group includes those systems in which the D-ring has been modified. Three types of modifications have so far been identified in the reported alkaloids namely: the homoerythroidine types (98) and (99), the homoazaerythrina type (100), and the homoisococculolidines (101) and (102). Both groups of alkaloids are also classified according to their unsaturation or their oxygenation pattern. So far, 1,6-diene series (103), $\Delta^{1(6)}$ alkene series (104), and $\Delta^{2(1)}$ alkene series (105) can also be classified separately according to the unsaturation pattern of the A- and B- rings. The epoxy

and the oxo derivatives of these series can also be treated as separate types. A few representatives of the 6,7-epoxy $\Delta^{2(1)}$ series and three representatives of the 1,6-epoxy alkane series have been reported[73,79,195-6]. Two oxo derivatives, where the carbonyl group is located at C_{12} in one and at C_8 in the other, have also been identified.



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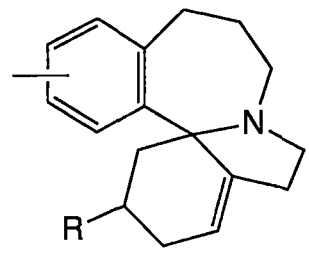

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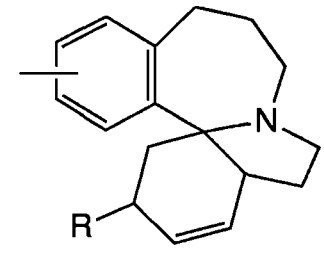


(105)

This chapter discusses the homoerythrina alkaloids isolated and characterized as a result of the search for the dibenz[*d,f*]azecine bases. Although GC-MS screening was done on the crude alkaloid extract of the three species of *Athrotaxis* (Taxodiaceae) only those from *Athrotaxis selaginoides* were fractionated more extensively. New sources of homoerythrina alkaloids were found in the liliaceous plants *Burchardia umbellata* and *Burchardia multiflora*. These are the first known alkaloids from this genus.

5.2. Alkaloids of *Athrotaxis selaginoides* D. Don

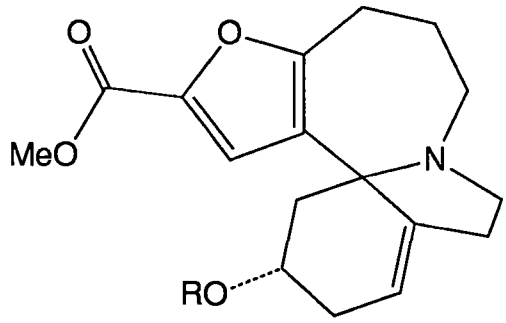
A phytochemical study of the Tasmanian endemic tree, *Athrotaxis selaginoides,* was previously undertaken by Panichanun and Bick. Eight homoerythrina alkaloids, all of the Δ1(6) alkene-type, were characterized and reported[39]. The present GC-MS screening of the crude alkaloid extract of this plant indicated the presence of a number of other

unidentified alkaloids. Over ten other homoerythrina akaloids of the 1,6-diene and $\Delta^{2(1)}$-alkene series were detected mass spectrometrically. Some of these alkaloids were present in reasonable amounts but were not purified further as the main interest was the dibenz[*d,f*]azecine-type alkaloids. However, in the process of repeated fractionation to detect minor components, some of the alkaloids such as taxodine and homoerythratine were re-isolated. In addition, two more unreported $\Delta^{1(6)}$-type homoerythrina alkaloids were isolated pure and their structures elucidated.

The crude alkaloid extract was subjected to preliminary fractionation by PLC on a silica support. Nine impure fractions (fractions A1 to A9) were collected. Each of these fractions was fractionated further by PLC to allow detection of the minor components. A trace amount of a dibenz[*d,f*]azecine-type alkaloid was detected in the twelfth fraction of A5. No further attempts were made to separate this component due to the minute quantities involved.

The ninth fraction of A5 was an alkaloid of molecular weight 303.1461 by high resolution mass spectroscopy; this indicated a molecular formula of $C_{17}H_{21}NO_4$. This alkaloid was detected as a minor component in the original crude extract. The mass spectrum of this fraction (A5I) was distinctly different from the 1,6 diene-, $\Delta^{2(1)}$ alkene-, and $\Delta^{1(6)}$ alkene-series of homoerythrina alkaloids. It was, however, very similar to that of selaginoidine (106) which had been isolated from this plant previously. A comparison of the ^1H n.m.r. spectra of this sample and that of an authentic sample of selaginoidine revealed one main difference: the absence of the C_3-methoxy group in the spectrum of A5I. The molecular weight difference of these two compounds,

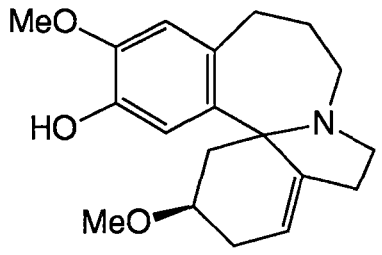
supported by the n.m.r. data, as well as by its IR spectrum, pointed to a hydroxy group at C_3. The structure of this new alkaloid is thus proposed as 3-demethylselaginoidine (107).



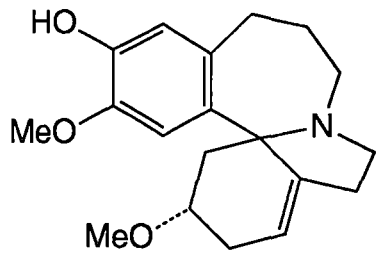
(106) R Me
(107) R H

One of the fractions of A7 which was present in reasonable quantity contained two $\Delta^{1(6)}$ homoerythrina-type alkaloids both of molecular weight 315. One of these was assigned as the known alkaloid taxodine (55) based on its mass spectrum. The close similarity of the mass spectra of the two components implied that the second component could be isomeric with taxodine. The two components were separated by double development PLC on silica and their n.m.r spectra obtained. The ^1H n.m.r spectrum of the second component ruled out its being a C_3-epimer of taxodine by comparison with the spectrum of 3-epitaxodine[197] (108; alkaloid II). A triplet at δ 1.57 for an axial C_4 proton and the chemical shift of the C_3-methoxy group (δ 3.21) supported a 3R-methoxy stereochemistry at C_3. While the chemical shifts of the two aromatic protons of taxodine (55) were reasonably separated, they overlapped in the isomeric component in deuterochloroform. However, two distinct peaks were observed in deuterated pyridine (300 MHz), and in a 500 MHz ^1H n.m.r. spectrum in deuterochloroform. A nOe experiment (500 MHz) confirmed an isotaxodine structure (109) for this alkaloid. The aromatic proton at δ 6.720 (500 MHz) showed an nOe effect with an

aliphatic proton at δ 2.77 but not with the methoxy group. An nOe effect was also observed between the aromatic proton δ 6.704 (500 MHz) and the methoxy group (δ 3.84). This isolate is therefore a new alkaloid isotaxodine (109).



(108)



(109)

Fraction 12 had a major component of molecular weight 331 and a number of minor components. One of the very minor components (M^+ 313) had the characteristic fragmentation pattern of an N-methylated dibenz[*d,f*]azecine alkaloid. The presence of moderate to intense peaks at m/z 70, 58, 57, 44, and 42, and the absence of the peaks due to M^+-58 and M^+-72, are indicative of a dibenz[*d,f*]azecine derivative in which either ring A or C are not aromatic. The possibility that this component might be an artifact was ruled out due to the fact that the system is N-methylated, as indicated by its mass spectrum.

5.3. Alkaloids of the Genus *Burchardia*

Although the genus *Burchardia* (Liliaceae) was not included in Buxbaum's subfamily Wurmbaeoideae, it was suspected to contain phenethylisoquinoline-derived bases as Hutchinson had classified it with other genera (tribe 27), all known to contain wurmbaeoideae alkaloids. There has been no report on isolated alkaloids from this genus, except for a report on the absence of the tropolonic alkaloids such as

colchicine[162]. This genus is composed of five herbaceous species, all of which are endemic in Australia[162]. Most of these species are restricted to Western Australia except for *B. umbellata* which is widely distributed throughout Australia including Tasmania. They are generally recognized by their white, attractive, honey scented flowers.

5.3.1. Alkaloids of *Burchardia umbellata* R. Br.

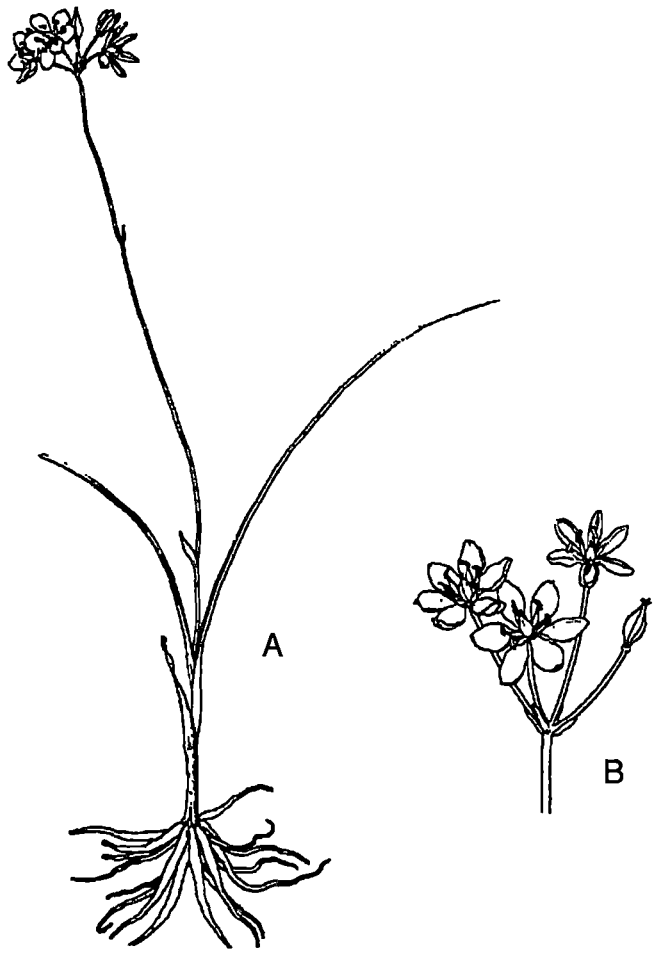


Figure 5.1 Sketch *B. umbellata* (**A** habit, **B** umbel)

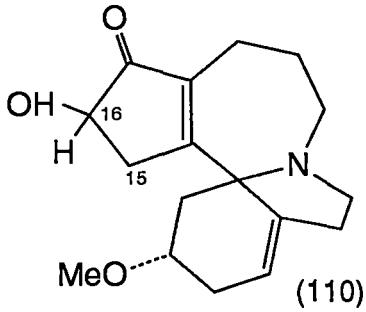
Alkaloids were extracted from each of the four batches, and the crude alkaloid extracts were screened by the GC-MS technique. An

apparent difference was observed in the alkaloid profile of the sample collected from Western Australia and those collected from Tasmania. Most of the alkaloidal components of the Western Australian sample were also detected in the Tasmanian samples. In addition, the Tasmanian samples contained at least six other alkaloidal components. All the four sample batches were fractionated in search of the dibenz[*d,f*]azecine alkaloids. However, only the fractionation which led to the isolation of pure samples will be described in this section.

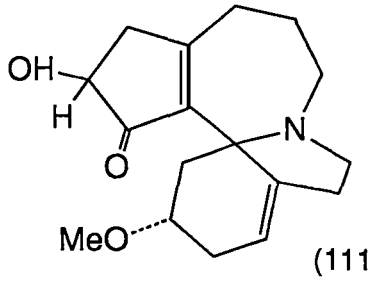
The crude alkaloid extract obtained, at basic pH, from the sample collected from Lake Leake road in Eastern Coast of Tasmania, was fractionated by PLC on silica giving rise to nine fractions (fractions B1 to B9). The third (B3), fourth (B4), and fifth (B5) fractions were obtained pure. Fraction 6 contained an alkaloid of molecular weight 347, which looked reasonably pure as indicated by its GC-MS trace. Its skeleton appeared to be unique from the mass spectrum. Doubling up of ^{1}H n.m.r and ^{13}C n.m.r peaks made interpretation of the structure difficult. More samples of this isolate are needed for further investigation of the structure. On the other hand, fraction B3 proved to be the known alkaloid taxodine (55) by comparison of its MS, ^{1}H n.m.r. and ^{13}C n.m.r. spectra with those of an authentic sample.

Fractions B4 and B5 contained the same alkaloid and were combined together. The molecular formula of this alkaloid was $C_{17}H_{23}NO_3$, on the basis of the high resolution mass spectrum. The mass spectrum showed weak peaks corresponding to m/z 178, m/z 165 and m/z 146, which are diagnostic of a $\Delta^{1(6)}$ homoerythrina type in which a methoxy group at C_3 is the only oxygenated carbon in both the A- and B-rings. A C-16 skeletal system was deduced from the ^{1}H n.m.r.

spectrum which indicated the presence of the methoxy substituent at C_3 (δ 3.34). Since the mass spectrum indicated the usual skeletal system for the A-, B- and C-rings of a $\Delta^{1(6)}$-type of homoerythrina alkaloid, a modified D-ring was considered. This possibility was supported by the absence of an aromatic ring system in both the ^1H n.m.r and ^{13}C n.m.r. spectra of this isolate. A COSY 2-D experiment on this sample revealed a cross peak between the methine proton at δ 4.21, with two protons at δ 2.73 and δ 2.32 of an isolated methylene group. An IR absorption peak at 1701 cm^{-1} and a ^{13}C n.m.r. chemical shift at 208.88 ppm were indicative of an α,β-unsaturated five-membered ring ketone[192]. The carbonyl carbon could either be at C_{15} or C_{17} considering the coupling pattern of the methine and the methylene protons. Four possible structures (110), (111), (112), and (113) were deemed possible.



(110)



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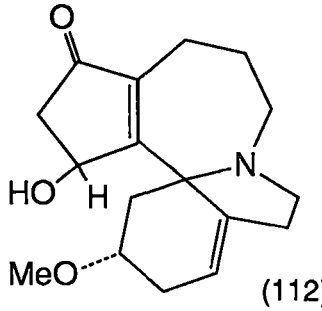

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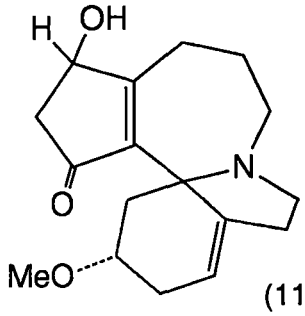
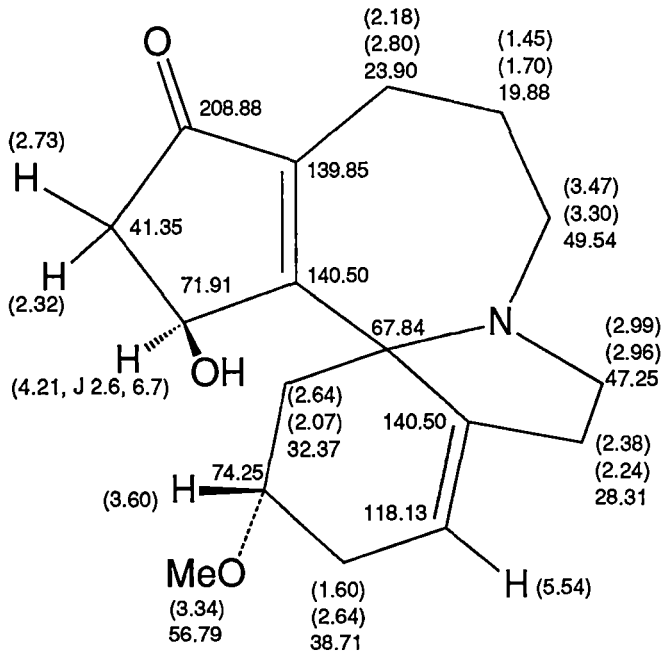


(113)

A proton-carbon correlation experiment (with long range enhancement effects) did not give satisfactory results. However, an nOe experiment, irradiating the methine proton (H_{15}; δ 4.12) showed a

relatively strong nOe effect with the A-ring methine proton (δ 3.60). This narrowed down the possible structures to (110) or (112). A computer generated model (PC model[198]) of each of the possible positions of the D-ring methine proton was obtained, from which approximate proton distances could be calculated. This modelling indicated (110) and (112) as the most probable structures consistent with the nOe result (Figure 5.2). However the preferred structure is (112) whereby the two methine protons (δ 4.12 and δ 3.60) are most proximate as illustrated in the model. The calculated coupling constant (^3J) between H_{15} and H_{16} for (110) is 9 Hz whereas in (112), ^3J is 7. The latter (112), is in close agreement with the ^1H n.m.r. data obtained for these protons (J 6.7 Hz), thus this isolate, named burchardine, was tentatively assigned the structure (112). The model of (112) also supported the 3R-methoxy stereochemistry at C_3. The rest of the proton and carbon chemical shift assignments were derived from the COSY and XHCORR experiments.



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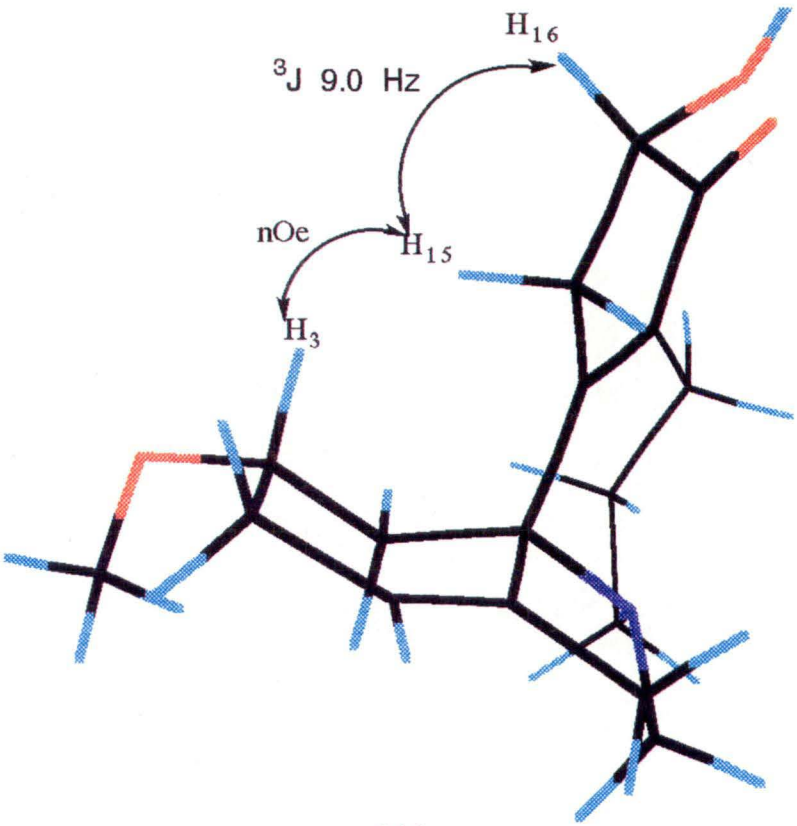

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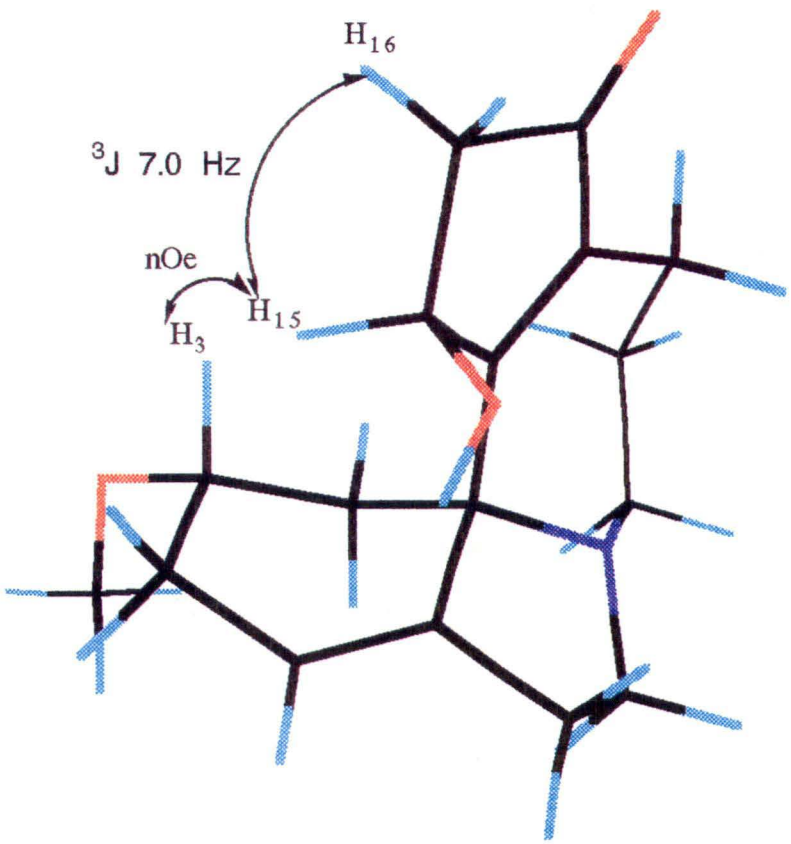
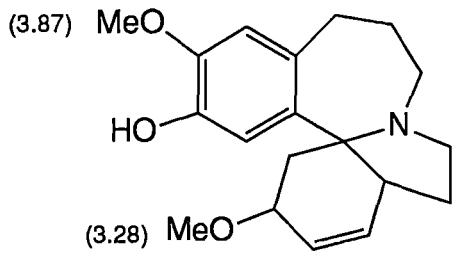


112

Figure 5.2 Computer Generated Models of Burchadine (112) and Isomer (110).(Blue = Hydrogen, Purple = Nitrogen and Red = Oxygen)

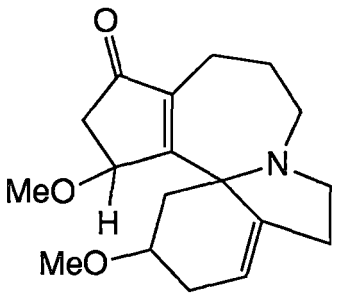
Further fractionation of B7 afforded one clean fraction (B7G). This proved to be the reported homoerythrina alkaloid lucidinine[81] by comparison of the mass and ^1H n.m.r. spectra. The previously unspecified position[81] of the hydroxy and the methoxy substituents of the D-ring have now been assigned as in (114). The assignment was made by comparison with the chemical shifts of taxodine (55) and the new alkaloid isotaxodine (109).



(114)

The dichloromethane extract of the acidic aqueous solution from *Burchardia umbellata* was subjected to preliminary separation by quick column chromatography on silica. All of the five fractions collected were impure. The second fraction A2 was separated further by PLC affording six fractions. The first fraction A2A contained one of the major fractions of the crude extract. This was relatively pure as assessed by GC-MS analysis and by its ^1H n.m.r. spectrum. The molecular mass was determined to be 303.181 corresponding to the formula $C_{18}H_{25}NO_3$. Its mass spectrum was very similar to that of (112) indicating a $\Delta^{1(6)}$ homoerythrina-type component. There were some slight differences between the ^1H n.m.r. and ^{13}C n.m.r.spectra of (112) and this isolate. A second methoxy group (δ 3.46) was identified, which was assigned to C_{15} in comparison with (112) (H_{15}, δ 4.21) and based on the chemical shift of the methine proton (H_{15}, δ 3.78) of this isolate . Structure (115) is therefore proposed for this new alkaloid.

PLC of the second fraction A2B afforded a clean fraction of the main component of molecular weight 313 ($C_{19}H_{23}NO_3$). This was identified as the known alkaloid schelhammericine[64] (116) from its spectroscopic data.



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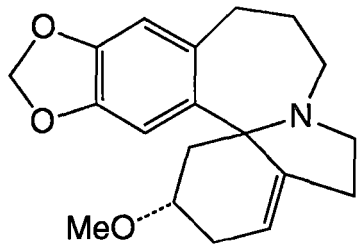


(116)

5.3.2. Alkaloids of *Burchardia multiflora* Lindley.

The Western Australian endemic plant, *Burchardia multiflora* Lindley, resembles *B. umbellata* very closely in terms of habitat and appearance (Figure 5.3). In the actual collection site, it was found growing a few metres away from *B. umbellata*. This plant is, however, generally shorter but with bigger stems than *B. umbellata*. It also has more pinkish white tepals with a deep pink mid-stripe; alkaloids were extracted from one portion in the University of Western Australia, while a second portion was air-dried and processed in Hobart (University of Tasmania). GC-MS screening showed no distinct differences in the alkaloid profiles of the two batches of extracts, nor from the acidic or basic solutions. The GC-MS ion trace showed at least seven alkaloidal components. Preliminary separations were done on all extracts, and, as in other plant extracts, most of the fractions were monitored by GC-MS to

identify the alkaloid profile and to search for the presence of dibenz[*d,f*]azecine alkaloids. This section describes the isolation and structure elucidation of pure isolates only.

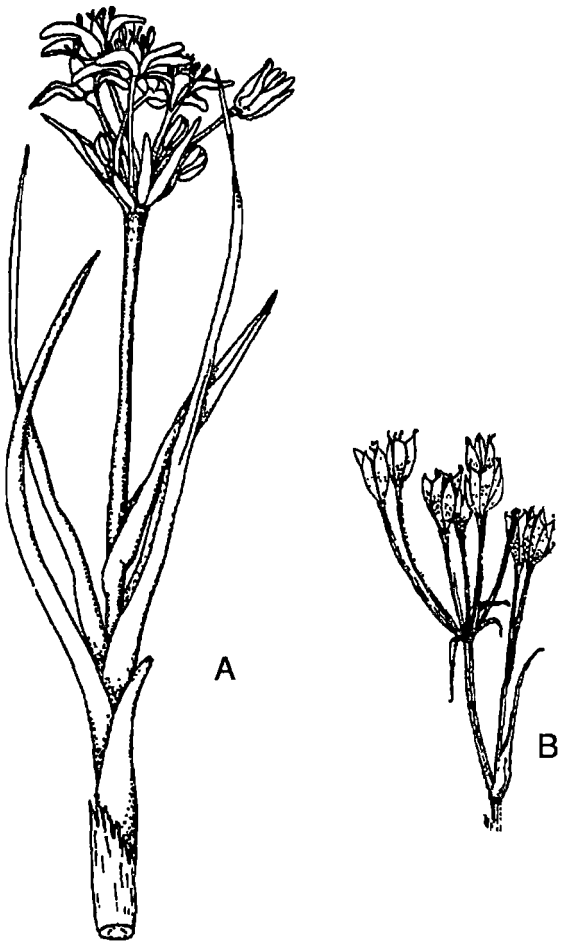


Figure 5.3 Sketch *B. multiflora* (A flowering shoot, B fruiting umbile)

A portion of the dichloromethane extract from a basic aqueous solution was separated by quick column chromatography. The third fraction (1C) contained the major component of the crude extract (M^+ 329.164; $C_{19}H_{23}NO_4$) with smaller amounts of the other components. Recrystallization from dichloromethane-ether afforded pale yellow needle-like crystals of the major component. The mass spectrum did not

show the usual pattern for any of the 1,6-diene-, $\Delta^{1(6)}$-, or $\Delta^{2(1)}$-series of homoerythrina alkaloids. Two aromatic protons (δ 6.61 and δ 6.60; singlets), two methylenedioxy protons (δ 5.92, dd), a methoxy group (δ 3.35), along with the aliphatic protons were indicated in the ^{1}H n.m.r. spectrum. The ^{13}C n.m.r. suggested the absence of unsaturated centers other than those associated with the aromatic ring. A sixth ring was thus indicated to satisfy the index of hydrogen deficiency. An epoxy ring was proposed to account for the other oxygen atom, and the presence of three downfield methine groups (δ 3.34/76.24, 3.45/54.2, 3.59/55.60) in the ^{1}H n.m.r. and ^{13}C n.m.r. spectra of this compound. These chemical shifts were consistent with epoxy methine groups.

A COSY 2-D spectrum complemented by a nOe difference experiment indicated an isolated coupling pattern of four methine protons including a methylene proton. This isolate named florine, was assigned a 1,2-epoxyhomoerythrina structure (117) based on spectroscopic data, and supported by biogenetic arguments. The rest of the homoerythrina skeleton was confirmed from MS data and the proton coupling patterns. The characterisitic axial proton at δ 1.52 (t) and the chemical shift of the methoxy group (δ 3.34) were indicative of a 3R-methoxy group at C_3, and defined an equatorial (δ 2.23, J 12.2, H_4) axial (δ 3.18, J 11.0, H_3) configuration for protons at C_4 and C_3 (Figure 5.4). The coupling constant of 4.0 between H_3 and H_2 (δ 3.45) is consistent with an pseudo axial-equatorial configuration between these protons. A *cis* equatorial-equatorial configuration was also deduced for H_1 and H_2 (J 9.8), and a *cis* equatorial-axial for H_2 and H_6. A complete assignment of the n.m.r chemical shifts was made by obtaining a proton-carbon correlation spectrum (XHCORR), to support the proton connectivities established from the COSY spectrum. The stereochemistry

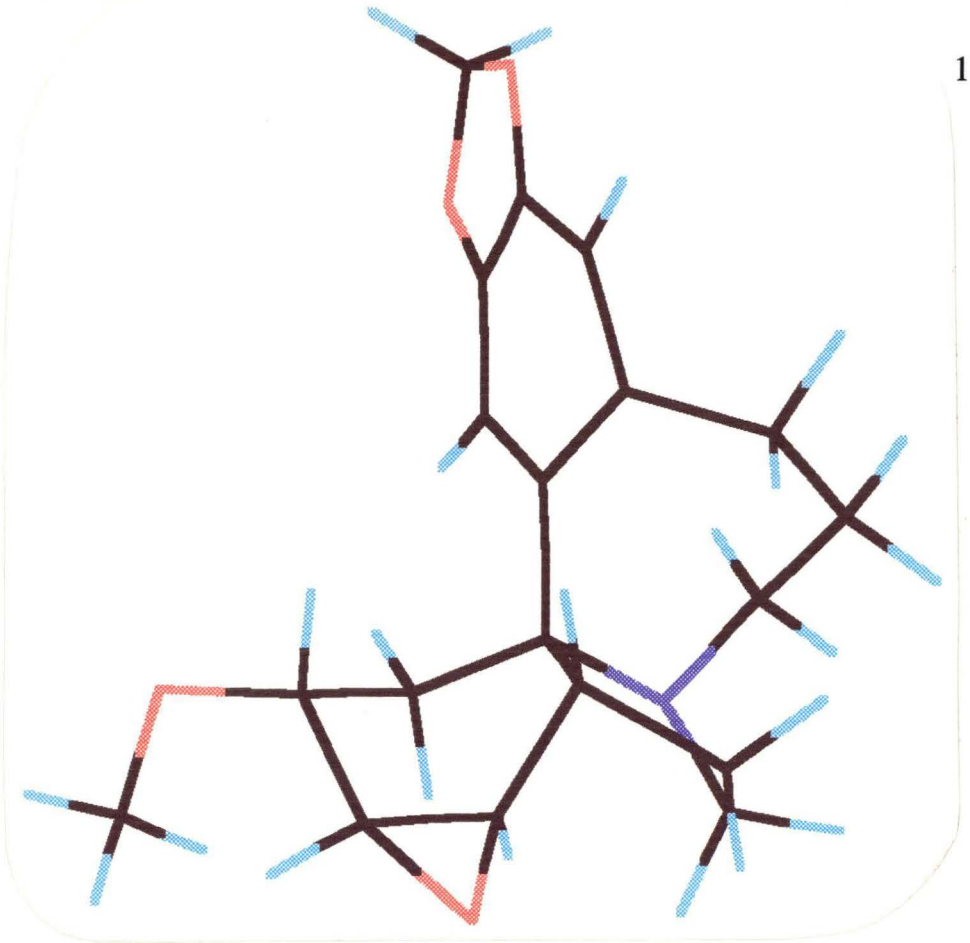
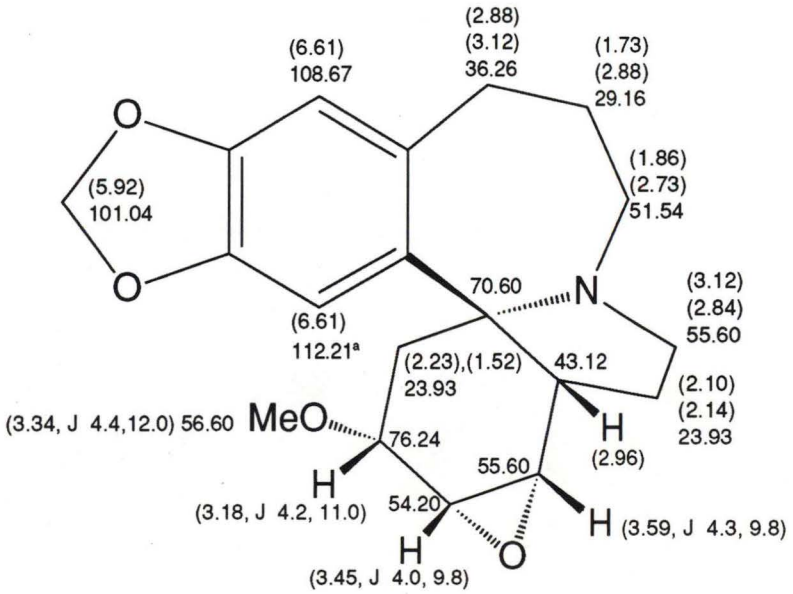


Figure 5.4 Computer generated model of florine (117)

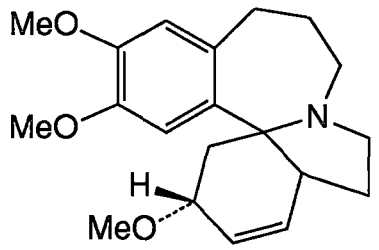


(117)

at C_5 was presumed as such (5S) by comparison with other homoerythrina alkaloids[151] having negative and low amplitude Cotton

effects at 262 and 293 nm. A strong positive Cotton effect observed at 205 nm for this alkaloid, was also comparable to homoerythrina alkaloids with a 3R-hydroxy group[199].

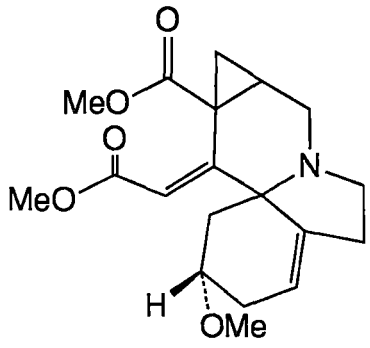
A further batch of the crude alkaloid extract was separated by double development PLC on silica. Eight bands were collected, three of which were relatively pure. One of these pure fractions (fraction 2B) was the known alkaloid schelhammericine (116)[64], while the second one, fraction 2C, was the new 1,2-epoxyhomoerythrina alkaloid (117). The seventh fraction (fraction 2G) also proved to be a known alkaloid comosidine[80] (118, M+ 329), on the basis of mass and ^{1}H n.m.r spectral comparison.



(118)

The major component of the last fraction (fraction 2I) was an alkaloid of molecular weight 347, whose GC-MS retention time was equivalent to the other major component of the crude extract. Extra interest was focussed on this component due to its unique MS fragmentation pattern. Instead of separating fraction 2I further, a new batch of crude extract was separated by PLC, not only to isolate the major component of molecular mass 347, but also to survey any possible minor, but more polar, components of the crude extract. Five fractions were collected from this work up. The first two fractions contained the alkaloid of interest (M+ 347) along with a previously undetected alkaloid

(M+ 363). The second fraction was re-separated by PLC on silica to afford a relatively clean isolate (fraction 2"B2) of this isolate. The molecular ion was found to have a mass of 347.172, corresponding to the formula $C_{19}H_{25}NO_5$. As in the homoerythrina alkaloid (112), the ^1H n.m.r. and the ^{13}C n.m.r. spectra indicated the absence of an aromatic ring system. A study of its proton-proton coupling pattern revealed the skeleton of the newly reported homoerythrina alkaloid lenticellarine[67,70]. Comparison of other spectroscopic properties such as MS, ^{13}C n.m.r. and ^1H n.m.r data, proved this isolate to be lenticellarine (119).



(119)

5.4. Conclusions:

The search for the dibenz[*d,f*]azecine-type alkaloid from *Athrotaxis selaginoides* has allowed the isolation of two new homoerythrina alkaloids of the $\Delta^{1(6)}$-type, and the re-isolation of the known alkaloids taxodine and homoerythratine. These two known compounds were then transformed to the dibenz[d,f]azecine derivatives (Chapter 3, Section 3.4.1).

Known homoerythrina alkaloid such as taxodine, lucidinine, and schelhammericine were re-isolated from *Burchardia umbellata* in minor

amounts. The substitution pattern of the D-ring of lucidinine was assigned, for the first time, in comparison with the ^1H n.m.r. chemical shifts of taxodine and the new alkaloid, isotaxodine. Two new homoerythrina alkaloids, burchardine and O-methylburchardine were also isolated from *B. umbellata*, and characterized unambiguously. These alkaloids were found to have a new ring D modification. The cyclopentenone ring (ring D) could arise from the oxidation of the aromatic ring between C16 and C_{17} and subsequent condensation of C_{18} with C_{16}, followed by elimination and reduction reactions on C_{16}.

A new homoerythrina alkaloid, the first example of a 1,2-epoxy-type homoerythrina alkaloid, was also isolated from *Burchardia multiflora*, and was fully characterized. This was presumed to arise from the oxidation of a $\Delta^{2(1)}$-type homoerythrina precursor. Three more known alkaloids were also isolated from *Burchardia multiflora,* namely, schelhammericine, comosidine, and lenticellarine.

Chapter 6

ALKALOID PROFILE OF THE FAMILY LILIACEAE

6.1. Introduction

Chemical investigation of the secondary metabolites in plants allows the structural determination of their components and the isolation of compounds of potential pharmacological interest. Used carefully, such investigations can also provide new insights into taxonomic classification at different levels, providing clues to the taxonomic and evolutionary relationships between closely related plants. Metabolites such as the flavonoids, chelidonic acids, iridoids, steroidal sapogenins, alkaloids and a few other compounds, have been used for this purpose[201]. An accurate definition of plant taxa can in turn allow a more systematic search for specific metabolites. However, in the family Liliaceae, the use of established taxa for this purpose could not be fully utilized due to a prevalent movement of groups within the system.

The widely diverse approaches to the classification of the family Liliaceae exemplified the need for a more accurate delimitation and description of plant taxa, and reflected the inadequacy of the taxonomic markers available to plant taxonomists. For instance, the use of leaf flavonoids as taxonomic markers did not justify most of Dahlgren's new families[200]. In contrast, a phenetic and cladistic study of *Drymophila* by Conran[148] did not lend support to Dahlgren's classification. In some cases, the use of alkaloids as taxonomic markers has offered some convincing proof to delimit plant groups. The alkaloid profiles of the families Liliaceae and Amaryllidaceae for instance, have allowed a more

definitive separation of these two families[201]. Buxbaum's delimitation of some members of the family Liliaceae into the subfamily Wurmbaeoideae has now been strongly supported by their alkaloid profile, the wurmbaeoideae alkaloids being restricted to this subfamily. So far, only the Australian endemic genus *Kreysigia* has been added to the original taxon proposed by Buxbaum.

In the succeeding discussion, unless otherwise specified, the more classical classification by Hutchinson[145] will be used as a point of reference. Of the twenty eight tribes in this classification, fifteen were found to be alkaloid positive. Although the alkaloidal components of most of the liliaceous plants reported have not been isolated and characterized, two major alkaloid groups were identified in the family Liliaceae: the phenethylisoquinoline-derived alkaloids and the steroidal alkaloids.

6.2. The Phenethylisoquinoline-Derived Alkaloids

The presence of phenethylisoquinoline-derived alkaloids such as neutral tropolonic alkaloids, basic tropolonic alkaloids and non-tropolonic alkaloids, have allowed Santavy[8] to correct and complement Buxbaum's developmental scheme for the different tribes in the subfamily Wurmbaeoideae (Table 6.1). Within the tribe Colchiceae, the subgenera *Eucolchicum, Archicolchicum,* and *Merendera* were also recognized.

In Hutchinson's classifcation, the phenethylisoquinoline alkaloids were restricted to tribes Uvularieae (Tribe 17), Anguillarieae (Tribe 21), Colchiceae (Tribe 26), and Iphigenieae (Tribe 27) (Scheme 10). Most of

the members of the tribe Uvularieae contained phenethylisoquinoline-derived alkaloids except for *Uvularia* and *Walleria*. The genus *Schelhammera* contained only the homoerythrina-type alkaloids in contrast to the genera *Kreysigia, Littonia*, and *Sandersonia* which contained the wurmbaeoideae alkaloids.

Table 6.1 Tribes of the Subfamily Wurmbaeoideae

TRIBE	GENERA/SUBGENERA
Glorioseae	*Gloriosa, Littonia, Sandersonia*
Iphigenieae	*Iphigenia, Camptorrhiza, Ornithoglossum*
Baeometreae	*Baeometra*
Colchiceae	*Colchicum*, *Androcymbium* [*Eucolchicum* (Ia), *Archcolchicum* (IIa), *Merendera* (IIIa)]
Kreysigieae	*Kreysigia*
Neodregeae	*Dipidax, Neodregea*
Wurmbaeae	*Wurmbea*

All the genera (*Baeometra, Anguillaria, Neodregea, Dipidax,* and *Wurmbea*) classified under the tribe Anguillarieae contained the characteristic wurmbaeoideae alkaloids. The genera *Colchicum, Merendera,* and *Bulbocodium* of tribe Colchiceae also contained the wurmbaeoideae alkaloids except for the genus *Synsiphon*. Although the genera *Iphigenia, Ornithoglossum, Camptorrhiza, Burchardia,* and *Androcymbium* of tribe Iphigenieae have been found to contain phenethylisoquinoline alkaloids, those of *Burchardia* are distinct, being of the homoerythrina type, and suggesting that *Burchardia* should be re-classified.

It would be of interest to re-investigate the genera *Iphigenia* and *Ornithoglossum*, both of tribe Iphigenieae, for aporphine-type alkaloids, as one member of this tribe, the genus *Camptorrhiza* has been reported

to contain this alkaloid type. The only other source of this alkaloid type in the subfamily Wurmbaeoideae is *Baeometra* (Tribe Baeometreae). The alkaloid profile of these two tribes may be utilized to establish their taxonomic relationship. The co-occurrence of the aporphine-type alkaloids with the phenethylisoquinoline-derived alkaloids could be biosynthetically significant. In addition, attention has been focussed on the genus *Baeometra,* not only because of its aporphinoid alkaloids, but also due to the presence of the dibenz[*d,f*]azecine alkaloids as detected in this study.

The detection and/or isolation of the dibenz[*d,f*]azecine-derived alkaloids from *Wurmbea* and *Baeometra* species, has supported Hutchinson's classification of these two genera under one tribe. Dahlgren also expressed a close relationship between the tribes of *Baeometra* and of *Anguillaria* (*Wurmbea*). Further investigation of the alkaloid profile of the other members of tribe Anguillarieae, the genera *Dipidax* and *Neodregea*, should be of interest. If such close a relationship exists, it is possible that these two genera might contain the dibenz[*d,f*]azecine alkaloids.

Little is known about the liliaceous plant taxa that may contain the homoerythrina-type alkaloids. The taxonomic relationship of the two homoerythrina alkaloids-containing genera, *Schelhammera* and *Burchardia,* is yet to be established. The use of different markers seemed to include *Burchardia* and *Schelhammera* in different families[145,147,148]. Table 6.2 shows some alternative groupings of these two genera.

Table 6.2

Conran (IC[a])	(A)	*Uvularia,* ***Burchardia,*** ***Schelhammera,*** *Kreysigia*
	(B)	*Gloriosa, Sandersonia, Littonia*
Conran (GAC[b])	(A)	*Kreysigia, Uvularia,* ***Burchardia***
	(B)	***Schelhammera,*** *Tricyrtis*
	(C)	*Gloriosa, Sandersonia, Littonia*
Schulze		***Schelhammera,*** *Drymophila* (Family Polygonatae)
Hutchinson	(A)	***Schelhammera,*** *Uvularia, Kreysigia, Littonia, Sandersonia*
	(B)	*Ornithoglossum, Iphigenia, Camptorrhiza,* ***Burchardia,*** *Androcymbium*
Dahlgren		*Clitonia, Disporum, Medeola, Kreysigia, Tricyrtis, Uvularia,* ***Schelhammera,*** Prosartes, etc.

Although the homoerythrina-type alkaloids can not be used confidently as a taxonomic marker, as their reported occurrence is non-predictive, their presence in *Schelhammera* and *Burchardia* suggest a taxon distinct from the other wurmbaeoideae alkaloid-containing plants as they are biosynthetically divergent groups. They should not be classified with *Kreysigia, Ornithoglossum, Camptorrhiza, Androcymbium, Littonia, Sandersonia,* and *Gloriosa* which are known to contain the wurmbaeoideae-type alkaloids. Conran's grouping of *Littonia,*

Sandersonia, and *Gloriosa* is, however, consistent with Buxbaum's tribal classification. In most reports, the genera *Uvularia* and *Tricyrtis* were consistently negative for alkaloid content. This data could be used as a basis to classify *Uvularia* and *Tricyrtis* as distinct from *Schelhammera* and *Burchardia*.

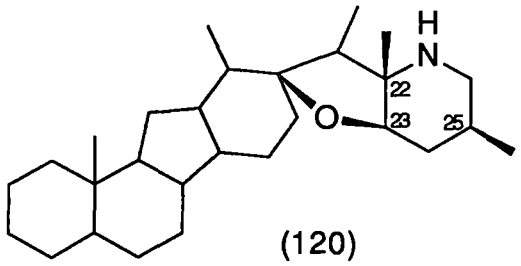
Cronquist's opinion is that families of the order Liliales (with a few exceptions) are derived from the family Liliaceae. This, together with the fact that the homoerythrina alkaloids are present in all the known phenethylisoquinoline-containing families offers the possibility of the homoerythrina alkaloid being found in other Liliaceous genera as well as in new genera from other families. It would be worthwhile to investigate the other members of the family Uvulariaceae (Dahlgren) and the families Tecophilaeaceae and Trillaciae for homoerythrina-type alkaloids. The family Tecophilaeaceae has been identified as a distinct link between the Liliaceae and Iridaceae. Investigation of the alkaloid profile of the family Iridaceae might also be significant. The family Trilliaceae includes such genera as *Scoliopus* and *Medeola* which are included by Dahlgren in the family Uvulariaceae.

6.3. The Steroidal Alkaloids

A separate group of toxic steroidal alkaloids whose biogenesis is unrelated to the phenethylisoquinoline-derived alkaloids has been reported from the liliaceous plants. So far, the reported steroid-containing species included plants from the families Solanaceae, Buxaceae, Apocynaceae and Liliaceae[132,133,202]. Modified steroidal alkaloids have also been isolated from salamanders and from

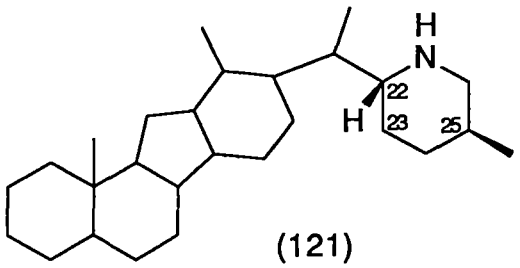
frogs[132,133,202]. In the family Liliaceae, the steroidal alkaloids were restricted to two tribes. This included species of *Schoenocaulon, Amaianthium, Zigadenus,* and *Veratrum* of tribe Veratreae, and *Frittilaria,* and *Lilium* of tribe Tulipeae. The genera *Rhinopetalum, Petilium,* and *Korolkowia* have also been reported to contain this alkaloid type, but these were not included in Hutchinson's classification; *Index Kewensis*[203] has however referred to these genera as synonymous with *Frittilaria*. In the process of screening the liliaceous plants for the phenethylisoquinoline-type alkaloids in this work, a new source of steroidal alkaloid was identified. *Arthropodium milleflorum* was found to contain a solanidine-type steroidal alkaloid. This is the first known steroidal alkaloid from the genus *Arthropodium* and from the tribe Asphodeleae.

The reported steroid alkaloids of plant origin have contained the basic steroid skeletal system from which Pelletier[204] identified two general types. The first type included pregnane-derived steroid alkaloids (x) in which C_3 and/or C_{20} were substituted with an amino group. Steroid alkaloids isolated from the family Apocynaceae are mainly of this type. The other type is composed of steroid alkaloids derived from 20-piperidylpregnanes such as the alkaloids of the families Solanaceae, Buxaceae, and Liliaceae. Tomko and Voticky[205] subclassifed the latter group as follows: the jervanine group (120), veratramine group (121) cervanine group (122), solanidanine group (123), the 5a-cholestane derivatives such as veralkamine (124), and derivatives of rearranged tomatidine, such as veramine (125).



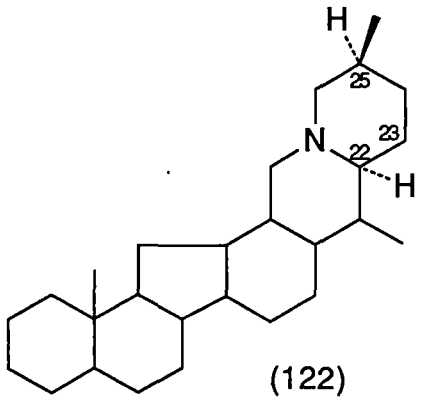
(120)

(22S, 23R, 25S)-5α-Jervanine



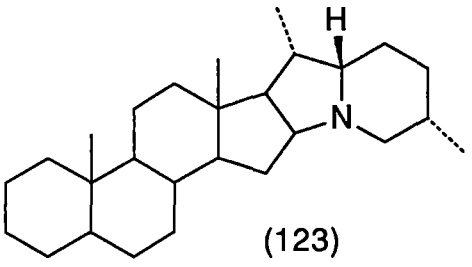
(121)

(22R, 25S)-5α-Veratramine



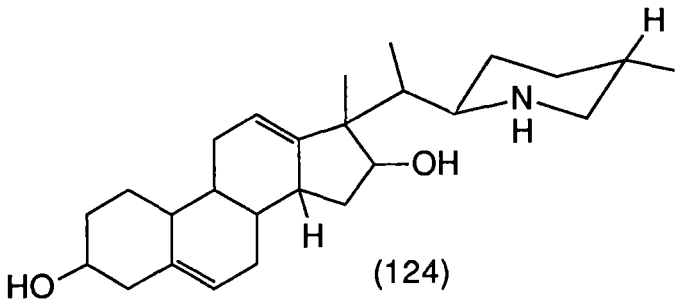
(122)

(22S, 25S)-5α-Cevanine



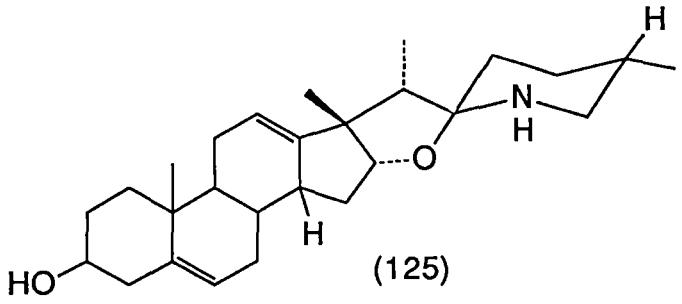
(123)

(22S, 25S)-5α-Solanidanine



(124)

Veralkalmine



(125)

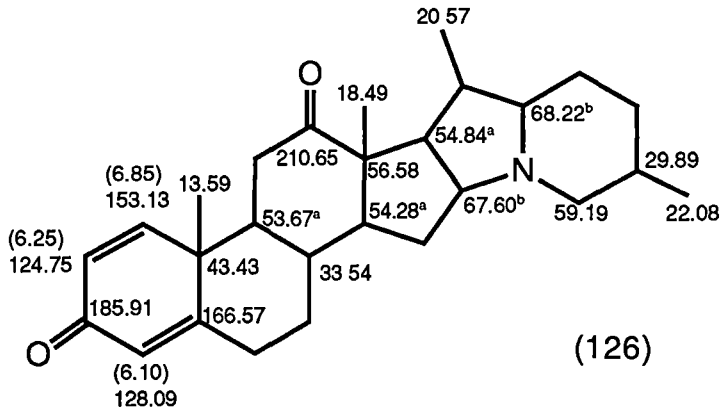
Veramine

6.4 Steroidal Alkaloids of *Arthropodium milleflorum*.

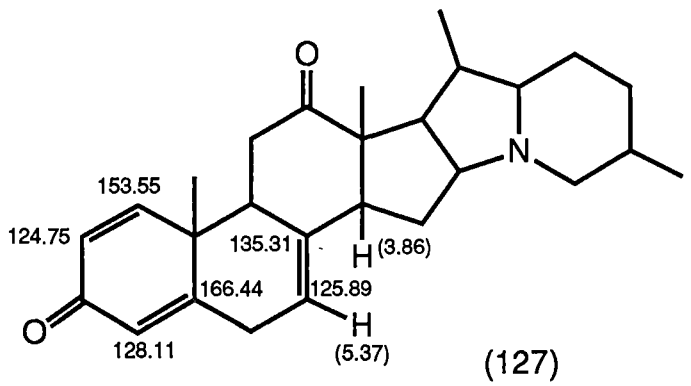
The presence of alkaloids in *Arthropodium milleflorum* was identified as a result of the general screening for the dibenz[d,f]azecine alkaloids from liliaceous plants. The alkaloids were extracted, and separated initially by a quick column, then by PLC; two relatively clean fractions were obtained. The first fraction (A3D1) had a molecular weight consistent with the molecular formula $C_{27}H_{37}NO_2$ from high resolution mass spectrometry. The absence of high mass fragments and a moderate to strong M^+-1 peak suggested a steroidal skeleton for this isolate. A solanidine-type skeleton was deduced from the presence of an intense peak at m/z 150 (base peak), and a weak peak at m/z 204[202a]. The coupling pattern of the olefinic protons indicated a cyclohexadienone ring system. This was supported by the IR absorption bands at 1663 cm^{-1} characterisitic of an α,β–α',β'-unsaturated ketone[206], and by the ^{13}C n.m.r signal at δ 185.91[192]. A second carbonyl group in a saturated six-membered ring system was deduced on the basis of an IR absorption band at 1711 cm^{-1}, and a ^{13}C n.m.r. resonance at 210.65 ppm. This alkaloid was therefore tentatively assigned the structure (126) as indicated by its spectroscopic data; some ^{13}C n.m.r. and ^1H n.m.r. assignments are indicated on the structure. The position of the carbonyl group was based on the position of oxidation in rubijervine[202b] but was not verified further. The alternative position of the second double bond in the B-ring can not be ruled out.

The spectroscopic properties of the second fraction (A3D2) were very similar to (126) except for the presence of a third double bond whose position was tentatively assigned as in (127). The possibility of

the double bond being in the F-ring was ruled out based on the mass spectroscopic data; the base peak at m/z 150 was indicative of saturated rings E and F. Time constraints precluded further work on these alkaloids, which both appear to be new.



(126)



(127)

Chapter 7

EXPERIMENTAL

7.1.General Notes

Microanalyses of samples were performed either by the Canadian Microanalytical Service Ltd., Vancouver, Canada or by using the EA1108-Elemental Analyzer (Carlo-Erba Instruments).

Melting points were determined on a Yanagimoto Seisakusho Micro-Melting Point apparatus and were uncorrected.

The infrared (IR) spectra were recorded on a Digilab FTS-20E Fourier Transform spectrometer (FT) and refer to thin films of liquid unless otherwise specified. GC-IR was carried out on a Hewlett Packard 5880A series gas chromatograph coupled to the DIGILAB FTS-20E Fourier Transform spectrometer. Absorption bands were described as strong (s), medium (m), weak (w) and broad (br).

Direct probe mass spectrometry was carried out on a Vacuum Micromass 7070F spectrometer using the direct insertion technique with a source temperature of 200^{o} and electron beam energy of 70eV.

All Gas Chromatography-Mass Spectrometric analyses (GC-MS) were performed on HP 5790 Mass Selective Detector coupled to a Hewlett Packard 5890 gas chromatograph fitted with a direct capillary interface. An HP-5 (crosslinked 5% Ph Me Silicone; 25 m X 0.31mm i.d.) capillary column was used in most analysis and an HP-1 (polymethylsiloxane; 25 cm) capillary column in a few cases. Helium was used as the carrier gas at 2ml/min flow rate. Peak intensities given in parentheses are expressed as a percentage (5) of the base peak (100%). In figures 2.1 to 2.6 and 2.8.1, the vertical axes represent this abundance.

Cotton effects were measured on a Jobin Yvon Dichograph 3. Proton nuclear magnetic resonance (^{1}H n.m.r.) spectra were measured at 300 MHz on a Bruker AM-300 and, for a few samples, at 500 MHz on a Bruker AM-500. Chemical shifts were measured in parts per million (ppm) relative to the internal standard tetrametylsilane (TMS) and are given in delta values (δ); coupling constansts (J) are in Hertz (Hz); and the resonances are described as singlet (s), doublet (d), quartet (q), multiplet (m), doublet of doublet (dd), and broad (br).

Carbon nuclear magnetic resonance (^{13}C n.m.r.) spectra were recorded at 75.5 MHz on a Bruker AM-300. Chemical shifts (δ) were measured in ppm relative to the internal standard tetramethylsilane. The DEPT.AUR microprogram was used to determine the degree of protonation of the observed carbon resonances of the ^{13}C n.m.r. spectra.

For both the ^{1}H n.m.r. and ^{13}C n.m.r. data, chemical shift assignments with superscripted letters indicate interchangeable assignments within each group defined by these letters. All two-dimensional (2D) n.m.r. experiments were performed using standard Bruker microprograms unless otherwise specified.

The nuclear Overhauser experiments were carried out at 300 MHz and/or at 500 MHz using the Bruker microprogram NOEDIFF.AUR and NOEMULT.AUR with multiple irradiation for multiplets. A preacquisition delay (D4) *ca* 0.1 sec was inserted in the NOEMULT.AUR program.

Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60 GF_{254} precoated glass or aluminum based plates of 0.25 mm thickness. A Camag DSF-5 silica gel was used for preparative layer chromatography (PLC) and quick column column chromatography. Aluminum oxide G (Type E) was used for other quick column chromatography and for cleaning up crude alkaloid extracts. Quick

column chromatography refers to a medium pressure technique, applying pressure by suction on a dry packed column.

Radial layer chromatography (RLC) was carried out on a chromatotron model 7924T using Kieselgel 60PF_{254} with $CaSO_4$.1/2 H_2O (Merck Art 7749) under a nitrogen atmosphere.

The reagents and solvents used were purified and dried by standard techniques[207]. Technical grade solvents used for plant extractions were normally re-distilled. Dichloromethane, chloroform, and ether extracts were generally dried over anhydrous sodium sulfate, and solvents were evaporated under reduced pressure on a rotary evaporator. Light petroleum used refers to the hydrocarbon fraction of boiling point range 40-60°. All solvents used in preparative thin layer chromatography (PLC) are expressed in percentage by volume (%v/v); methanol-dichloromethane solvent systems used for PLC development were always ammoniacal unless otherwise specified.

Ultrasonication, used to assist in the acid-base extraction of the alkaloids and in extracting the alkaloids from silica, was carried out on a Lucas Dawe Ultrasonics Sonicleaner model B-1200 E4 (HF output power 30w, working frequency 47 kHz) ; Branson Cleaning Equipment Company, Shelton, CT, U.S.A. For bulk extraction, a KLN ultrasonicator (output power 300w, working frequency 50 kHz) was used.

Fresh plant samples were blended in a vitamizer or crushed in a compost shredder. Other plant samples were dried on an Ly-5-FM Bredda Scientific freeze drier and powdered using an Allen West electric grinder.

7.2. Synthesis I (Chapter 3, Section 3.3.1)

3,4-Dimethoxycinnamonitrile (33)

3,4-Dimethoxybenzaldehyde (83g, 0.50 mol) in toluene-pyridine (150 ml, 2:1) was treated with cyanoacetic acid (0.55 mol) and ammonium acetate (1.5 g, 0.02 mol), and refluxed with separation of water for two days. The solvent was evaporated *in vacuo* and the residue dissolved in dichloromethane. It was then washed successively with hydrochloric acid (50 ml, 5%w/v) then with water (2x50 ml). The crude product was obtained after passing the solution through a column of silica gel eluting with dichloromethane. The desired product was recrystallized from chloroform-light petroleum as yellow-greenish crystals (80.0g, 0.42 mol, 56%, m.p. 88-89^{o}).

3-(3,4-Dimethoxyphenyl)-1-propanamine (34b)

To the crude acrylonitrile derivative (33) (27 g, 0.14 mol) in dry methanol (200 ml), and saturated with dry ammonia at 0^{o}, was added activated Raney nickel (ca. 25g). The mixture was hydrogenated at 60 p.s.i. until hydrogenation was complete, then filtered and the filtrate evaporated *in vacuo*. The desired amine (34b) was collected by vacuum distillation as a colorless oil (b.p. 122-126^{o}, 0.5 mm Hg).

1,2,3,5,6,7-Hexahydro-9,10-dimethoxypyrrolo [2,1-a] [2] benzazepinium iodide (35b)

3-(3,4-Dimethoxyphenyl)-1-propanamine (34b) (8.65g, 44.0 mmol) was treated with 4-butanolide (5.20 g., 0.06 mol) and stirred at 140^{o} under nitrogen for 3h. Excess lactone and water were removed by drying *in vacuo* at *ca* 90^{o}. The amide residue was taken up in a water-

ether mixture (75 ml, 2:1) and added dropwise to a refluxing solution of phosphoryl chloride (12 ml, 0.48 mol) in dry butanenitrile (50 ml), under nitrogen. The resulting mixture was stirred for another 3h after the addition was complete. After the solvent was evaporated *in vacuo* , the residue was taken up in a water-acetone mixture (150 ml, 2:1). The solution was stirred at room temperature for another 1h to insure complete hydrolysis.The aqueous mixture was washed with diethylether (50 ml) and basified to pH 7 with concentrated ammonia solution. The mixture was allowed to stand for 30 min, and basified further with sodium hydroxide (40% w/v) to pH 10. The resulting solution was partitioned with dichloromethane. The residue from the organic layer was taken up with methanol and acidified with concentrated hydrochloric acid to pH 4. Water (60 ml), then potassium iodide solution (50 ml, saturated solution) were added. The mixture was extracted with dichloromethane and the desired product, (35b), recrystallized from ethanol (1.8 g, 4.9 mmol, 8%; m.p. 203-205°)[167].

Anal. Calcd. for $C_{15}H_{20}NO_2I$: C, 48.27; H, 5.40; N, 3.75. Found: C, 47.97; H, 5.39; N, 3.70; **IR** : 1622 cm^{-1} ($C=N^+$).

1,2,3,4,6,7-Hexahydro-9,10-dimethoxy-1-(3-oxobutyl)-benzo[a]quinolizinium iodide (36a)

To a stirred suspension of the iminium salt (35a) (1.33 g, 3.56 mmol) in dry methanol (20 ml), kept at 0°, was added a methanolic sodium methoxide solution (3.0 ml, 2.5M, 7.5 mmol) and the mixture stirred until all of the salt was dissolved. To the resulting solution was added 3-buten-2-one (1.0 ml, 0.012 mol) under nitrogen. Stirring was continued on for 2h. Upon completion of the reaction, the mixture was allowed to cool to 0° and concentrated hydrochloric acid (2 ml.) was

added. The residue obtained after evaporation of solvent was re-dissolved in water (5 ml). A saturated solution of potassium iodide (10 ml) was added to the solution and then extracted with dichloromethane to give a crude alkylated iminium iodide salt (36a) (1.361 g, 3.07 mmol, 86%, m.p. 210-211°).

Anal. Calcd. for $C_{19}H_{26}NO_3I$: C, 51.48; H, 5.91; N, 3.16. Found: C, 51.70; H, 6.13; N, 3.30.

^{1}H n.m.r. δ ($CDCl_3$): 7.81 (s; H_{11}); 6.81 (s; H8); 4.52 (m; H_1); 4.08 (s; C-O$\underline{C}H_3$); 4.00 (s; C-O$\underline{C}H_3$).

^{13}C n.m.r. δ ($CDCl_3$): 207.5 (C_3); 177.67 (C_{11b}); 156.5 (C_9); 149.4 (C_{10}); 133.6 (C_{7a}); 118.7 (C_{11a}); 112.4 (C_8); 111.3 (C_{11}); 57.5 (C-O$\underline{C}H_3$); 57.3 (C-O$\underline{C}H_3$); 56.6 (C_4); 53.6 (C_6); 39.6 (C_2); 34.5 (C_1); 31.0 ($C_{4'}$); 28.1 (C_1); 26.6 (C_7); 21.3 (C_2); 18.1 (C_3).

IR (KBr disk): 1710 cm^{-1} (s, >C=O); 1638 cm^{-1} (m, $C=N^+$).

<u>1,2,3,5,6,7-Hexahydro-9,10-dimethoxy-1-(3-oxobutyl)-pyrrolo[2,1-a][2] benzazepinium iodide (36b)</u> (Prepared by Dr. C. Dragar)

A suspension of the iminium salt (35b) (0.746g, 2 mmol) in dry acetonitrile (10 ml) was treated as in the preparation of (36a). The crude product was recrystallized from ethanol-ethyl acetate-ether to give (36b) as aggregates of yellow prisms (757 mg, 1.71 mmol, 85%, m.p. 172-173°).

Anal. calcd. for $C_{19}H_{26}NO_3I1/2H_2O$: C, 50.45; H, 6.02; N, 3.10. Found: C, 50.70; H, 5.83; N, 3.22.

^{1}H n.m.r. δ ($CDCl_3$): 7.46 (s, H_{11}); 6.9 (s; H_8); 4.02 (C-O$\underline{C}H_3$); 4.01 (C-O$\underline{C}H_3$), 2.18 (s; CO$\underline{C}H_3$);

^{13}C n.m.r. δ ($CDCl_3$): 207.45 ($C_{3'}$); 187.87 ($\underline{C}$=N^+); 154.27 (C_9); 148.08 (C_{10}); 137.82 (C_{7a}); 117.92, (C_{11a}); 113.71 (C_8); 113.11 (C_{11}); 61.47 (C_3); 51.00 (C_5); 48.69 (C_1); 39.95 ($C_{2'}$); 33.90 (C_7); 30.58 (C_6); 30.12 ($CO\underline{C}H_3$); 26.59 ($C_{1'}$); 25.19 (C_2).

IR: (Nujol, Beckmann): 1705 cm^{-1} (>C=O), 1625 cm^{-1} (C=N^+).

1,2,3,4,6,7-Hexahydro-9,10-methylenedioxy-1-(3-oxobutyl)-benzo[a]quinolizinium iodide (36c)

This compound was prepared in a like manner to (36a) from the corresponding iminium salt (35c) (1.94 g, 5.43 mmol). The alkylated salt was obtained as yellow powder in 97% yield (m.p. 186-187°).

Anal. Calcd. for $C_{18}H_{22}NO_3I$: C, 50.60; H, 5.19; N, 3.28. Found: C, 50.79; H, 5.47; N, 3.42

^{1}H n.m.r. δ ($CDCl_3$): 6.77 (s, H_{11}); 6.68 (s, H_8); 6.02 (dd, J 3.74, 1.23, OCH_2O); 2.21 (s, $H_{4'}$);

15,16-Dimethoxy-B-homoerythrinan-3-one (37a)

To a stirred suspension of the keto iminium salt (36a) (1.1g, 2.5 mmol) in dry methanol (35 ml), cooled to 0°, was added methanolic sodium methoxide (6.0 ml, 0.015 mol). This was kept stirred until all of the yellow salt was completely dissolved. Glacial acetic acid (0.35 ml, 0.006 mol) was added to the mixture and the solvent evaporated *in vacuo* at room temperature. The residue was re-dissolved in a dichloromethane-ether mixture (1:1) and filtered through basic alumina. The solution was evaporated *in vacuo* to give the crude product (37a), which was recrystallized from ether (745 mg, 2.37 mmol, 95%, m.p. 136-137°).

MS: m/z 315 (M^+, 33, accurate mass 315.1839, $C_{19}H_{25}NO_3$ requires 315.1833); 314 (31); 272 (20); 259 (25); 258 (100); 245 (36); 244 (39).

^{1}H n.m.r. δ ($CDCl_3$): 6.80 (s; C_{18}); 6.58 (s; H_{15}); 3.89 (s; C_{17}-OC$\underline{H}_3$); 3.86 (s; C_{16}-OC$\underline{H}_3$); 3.65 (m; H_{11a}); 3.0 (m; H_{12a}); 2.81 (m; H_{4a}, H_{11b}); 2.78 (m; H_6); 2.72 (m; H_{1a}); 2.62 (m; H_{9a}, H_{9b}); 2.47 (m; H_{12b}); 2.45 (m; H_{2a}, H_{2b}); 2.35 (m; H_{4b}); 1.91 (m; H_{1b}, H_{8a}); 1.76 (m; H_{7a}); 1.49 (broad d; J 12.35; H_{7b}); 1.28 (m; H_{8b}).

^{13}C n.m.r. δ ($CDCl_3$): 209.87 (C_3); 147.33 (C_{16}, C_{17}); 130.77 (C_{14}); 125.79 (C_{13}); 112.14 (C_{18}); 107.58 (C_{15}); 63.32 (C_5); 56.05 (C_{17}-O$\underline{C}H_3$); 55.56 (C_{16}-O$\underline{C}H_3$); 54.23 (C_4); 48.35 (C_9); 43.85 (C_{11}); 40.44 (C_2); 36.02 (C_6); 27.09 (C_1); 24.80 (C_7); 21.27 (C_{12}); 20.11 (C_8).

<u>15,16-Dimethoxy-C-homoerythrinan-3-one</u> (37b) (Prepared by Dr. C. Dragar).

The title compound (37b) was prepared in a similar manner to (37a), as a colorless gum (150 mg, 0.48 mmol, 48%).

MS: m/z 315 ($M^{+\cdot}$, 10, accurate mass 315.1843, $C_{19}H_{25}NO_3$ requires 315.1833); 313 (100); 270 (22); 258 (33); 256 (100); 225 (91); 210(20).

IR (Nujol): 1705 cm^{-1} (>C=O);

^{1}H n.m.r. δ ($CDCl_3$): 6.77 (S, H_{18}); 6.59 (S, H_{15}); 3.84 (S, C-OC$\underline{H}_3$).

^{13}C n.m.r. δ ($CDCl_3$): 211.87 (C_3); 146.67 (C_{15}); 146.42 (C_{16}); 137.83 (C_{12}); 133.12 (C_{13}); 114.50 (C_{17}); 111.57 (C_{14}); 71.60 (C_5); 56.10 (C-O$\underline{C}H_3$); 55.67 (C-O$\underline{C}H_3$); 49.87 (C_4); 48.48 (C_8); 46.28 (C_{10}); 44.22 (C_6); 37.20 (C_2); 34.36 (C_1); 27.95 (C_7, C_{11a}); 25.57 (C_{11}).

15,16-Methylenedioxy-B-homoerythrinan-3-one (37c)

This was synthesized in the same manner as for the synthesis of (37a). Cyclization of the alkylated salt (36c) (1.00 g, 2.34 mmol) afforded the title compound (37c) as a gum (0.859 g, 2.01 mmol) in 86% yield.

MS: m/z 299 (M^+, 29, accurate mass 299.1526, $C_{18}H_{21}NO_3$ requires 299.1520); 242 (100); 229 (21); 228 (34).

^{1}H n.m.r. δ (CDCl3): 6.81 (s, H_{18}); 6.54 (s, H_{15}); 5.90 (d,J 4.7, OCH_2O).

^{13}C n.m.r. δ (CDCl3): 208.65(C_3); 146.15 (C_{17}); 145.90 (C_{16}); 131.95 (C_{14}); 126.70 (C_{13}); 109.21 (C_{18}); 104.38 (C_{15}); 100. 53 ($O\underline{C}H_2O$); 63.53 (C_5); 54.21 (C_4); 48.37 (C_9); 43.72 (C_{11}); 40.42 (C_2); 36.08 (C_6); 27.05 (C_1); 24.71 (C_7); 21.95 (C_{12}); 20.19 (C_8).

Methyl-5,6,8,9,10,10a,11,12-Octahydro-2,3-Dimethoxy-13-oxo-dibenz[*d,f*]azecine-7(13H)-carboxylate (38a)

To a solution of the amino ketone (37a) (586 mg, 1.86 mmol) in benzene (20 ml) was added anhydrous potassium carbonate (3.56 g, 26.0 mmol) and excess methyl chloroformate (2.5 ml, 32.3 mmol). The mixture was refluxed with stirring, under nitrogen, for 12h and kept stirred for another 10h. After evaporation of the organic solvent *in vacuo,* the residue was dissolved in water (20 ml) and the resulting solution was partitioned with dichloromethane. Removal of the organic solvent *in vacuo* gave the desired product (38a) as a gum (0.740 g, 1,98 mmol, 84%).

MS: m/z 373 (M^+, 100, accurate mass 373.1893, $C_{21}H_{27}NO_5$ requires 373.1887); 358 (4); 342 (6); 302 (10); 257 (38); 228 (16); 115 (9); 102 (24); 59 (26).

^{1}H n.m.r. δ ($CDCl_3$): 6.81 (s, H_4); 6.50 (s, H_1); 5.93 (br, H_{14}); 3.93 (s, C_3-OC$\underline{H}_3$); 3.86 (s, C_2-OC$\underline{H}_3$); 3.53 (br, COOC$\underline{H}_3$).

^{13}C n.m.r. δ ($CDCl_3$): 199.48 (C_2); 157.49 ($\underline{C}$OOCH_3); 148.37 (C_3); 147.21 (C_2); 133.49 (C_{4a}); 130.28 (C_{14}); 128.37 (C_{1a}[a]); 128.04 (C_{14a}); 112.96 (C_1); 109.43 (C_4); 55.8 (C_2-O$\underline{C}H_3$, C_3-O$\underline{C}H_3$); 52.45 (COO$\underline{C}H_3$); 49.47 ($\underline{C}H_2$); 48.18 (CH_2); 39.24 (N$\underline{C}H_3$); 32.46 ($\underline{C}H_2$); 31.82 ($\underline{C}H_2$); 24.78 ($\underline{C}H_2$ X 2).

Methyl 5,8,9,10-tetrahydro-2,3,13-trimethoxydibenz[*d,f*]azecine -7(6H)-carboxylate (40a)

A solution of the cyclohexenone derivative (38a) (280 mg, 0.75 mmol) and copper (II) bromide (0.9 g, 4.0 mmol) in methanol (20 ml) was refluxed for 1.5h. The volume of the resulting solution was then reduced to approximately 1 ml by evaporation of the solvent *in vacuo* at room temperature. To the concentrated solution was added a saturated solution of ammonium chloride (15 ml), and the resulting mixture partitioned with dichloromethane. The organic layer was dried with anhydrous sodium sulfate and the solvent was evaporated *in vacuo* to give (40a) as a white solid (282 mg, 0.73 mmol, 98%, m.p. 109-111°).

MS: m/z 385 (M^+ 100, accurate mass 385.1897, $C_{22}H_{27}NO_5$ requires 385.1889); 283 (50); 269 (72); 102 (10); 88 (8); 43 (19); 42 (20).

^{1}H n.m.r. δ, ($CDCl_3$): 7.19 (br, s, H_{11}); 6.86 (s, H_4); 6.86 (dd, J 8.4, 2.7, H_{12}); 6.65 (br, s, H_{14}); 6.62 (s, H_1); 3.95 (s, C_3-OC$\underline{H}_3$); 3.82 (s, C_2-OC$\underline{H}_3$); 3.78 (s, C_{13}-OC$\underline{H}_3$); 3.49 (br, s, COOC$\underline{H}_3$).

^{13}C n.m.r. δ ($CDCl_3$): see (40a)

Methyl 5,8,9,10-tetrahydro-2,3,-methylenedioxy-13-methoxydibenz[*d,f*]azecine -7(6H)-carboxylate (40c)

An excess of methyl chloroformate (2.5 ml, 32.3 mmol) and the amino ketone (37c) (0.86 g, 2.88 mmol) was refluxed in dry toluene (60 ml) for 14h, and then kept stirred for another 10h at room temperature. The resulting methyl N-carboxylate derivative (38c) obtained after the work up was dried *in vacuo* and immediately reacted with copper (II) bromide (3.3 g, 14.7 mmol) in methanol (60 ml) without purification. This afforded the carbamate derivative (40c) as the major product, along with a number of other products, as indicated by GC-MS. This was separated by PLC using methanol-dichloromethane (5%) for development. The title compound (40c) was obtained as a gum (0.56 g, 1.52 mmol, 53%).

MS: m/z 369 (M^+, 100, accurate mass 369.1595, $C_{21}H_{23}NO_5$ requires 369.1574); 267 (72); 253 (72); 152 (10); 102 (9); 88 (9).

^{1}H n.m.r. δ ($CDCl_3$): 7.17 (br, d, J 7.4; H_{11}); 6.84 (br, dd, J 8.4, 2.7; H_{12}, H_4); 6.60 (d, J 2.6; H_{14}); 6.57 (br, s, H_1); 5.95 (dd, J 3.3, 1.00; OCH_2O); 3.76 (s, C_{13}-OCH_3); 3.50 (br, s,$COOCH_3$).

^{13}C n.m.r. δ ($CDCl_3$): 156.99 (C_{13}, COOMe); 146.88 (C_3); 145.43 (C_2); 142.19 (C_{14a}); 135.18 (C_{4a}); 131.86 (C_{10a}[a]); 131.09 (C_{1a}[a]); 129.81 (C_{11}); 114.33 (C_{14}); 112.92 (C_{12}); 108. 59 (C_1, C_4); 100.74 (OCH_2O); 55.04 (C_{13}-OCH_3); 51.82 ($COOCH_3$); 49.65 (C_6);46.34 (C_8); 30.68 (C_5); 28.03 (C_9); 26.72 (C_{10})

Methyl-8,9,10-trihydro-2,3-dimethoxy-13-hydroxy-5H-dibenz[*d,f*]azecine-7(6H)-carboxylate (40d).

The carbamate derivative (38a) (210 mg, 0.56 mmol) was reacted with copper (II) bromide (0.7 g, 3.12 mmol) using water (20 ml) instead of

methanol. The crude product showed the aromatized product (40d) with some unreacted (38a) as indicated in its GC-MS ion trace. Double development PLC (methanol-dichloromethane, 4%) of the crude product afforded the desired product (40d) as a white solid (182 mg, 0.49 mmol, 88%, m.p. 206-208°).

MS: m/z 371 (M^+, accurate mass 371.1748, $C_{21}H_{25}NO_5$ requires 371.1731); 269 (44); 255 (93); 102 (7).

^{1}H n.m.r. δ ($CDCl_3$-CD_3OD): 7.09 (br, H_{11}); 6.91 (s, H_4); 6.77 (br, dd, J 9.03, 2.68; H_{12}); 6.63 (s, H_1); 6.54 (br, H_{14}); 3.94 (s, C_3-OC$\underline{H}_3$); 3.82 (s, C_2-OC$\underline{H}_3$); 3.43 (br, COOMe).

^{13}C n.m.r. δ ($CDCl_3$-CD_3OD): 157.16 (C$\underline{O}$OMe); 153.88 (C_{13}); 147.70 (C_3); 146.55 (C_2); 141.95 (C_{14a}); 134.49 (C_{4a}); 130.55 (C_{10a}); 129.88 (C_{11}[a]); 129.42 (C_{1a}[a]); 115.87 (C_{14}); 114.14 (C_{12}); 111.41 (C_1, C_4); 55.58 (C_2-O$\underline{C}H_3$,C_3-OMe); 52.01 (COO$\underline{C}H_3$); 50.29 (C_6); 46.16 (C_8); 30.30 (C_5); 28.47 (C_9); 26.57 (C_{10}).

5,6,7,8,9,10-hexahydro-2,3,13-trimethoxy-7-methyldibenz[*d,f*]azecine (32a)

To a solution of the carboxylate derivative (40a) (282 mg, 0.73 mmol) in dry tetrahydrofuran (20 ml) was added lithium aluminum hydride (500 mg, 13.3 mmol), and the mixture refluxed for 1.5h. The resulting mixture was cooled in ice after which, sodium hydroxide (1.5 ml, 10% w/v) was added dropwise with vigorous stirring until effervescence was minimal. A few drops of water were added, and the resulting mixture was acidified with dilute hydrochloric acid, then filtered. The filtrate was washed with light petroleum, basified with sodium hydroxide (2N), and extracted with dichloromethane. Evaporation of the organic solvent *in*

vacuo gave the desired product (32a) as a pale yellow crystals (245 mg, 0.72 mmol, 98%, m.p. 110-112°).

MS: m/z 341 (M^+, 40, accurate mass 341.1982,.$C_{21}H_{27}NO_3$ requires 341.1990); 326 (17.5); 283 (8); 269 (24); 70 (84); 58 (90); 57 (100); 44 (33); 42 (24).

^{1}H n.m.r. δ ($CDCl_3$): 7.19 (d, J 8.53, H_{11}); 6.89 (dd, J 8.51, 2.78, H_{12}); 6.77 (s, H_4); 6.61 (d, J 2.78, H_{14}); 6.54 (s, H_1); 3.92 (s, C_3-OC$\underline{H}_3$); 3.79 (s, C_2-OC$\underline{H}_3$); 3.78 (s, C_{13}-OC$\underline{H}_3$); 2.11 (s, NC$\underline{H}_3$).

^{13}C n.m.r. δ ($CDCl_3$): 156.30 (C_{13}); 147.69 (C_3); 145.68 (C_2); 143.28 (C_{14a}); 134.43 (C_{4a}); 132.92 (C_{10a}); 131.52 (C_{1a}); 128.97 (C_{11}); 114.23 (C_{14}); 113.32 (C_{12}); 111.83 (C_1); 110.04 (C_4); 55.35 (C_2-O$\underline{C}H_3$, C_3-O$\underline{C}H_3$); 54.76 (C_{13}-O$\underline{C}H_3$); 49.14 (N$\underline{C}H_3$); 58.70 (C_6); 44.26 (C_8); 30.07 (C_5); 28.36 (C_9); 26.80 (C_{10}).

5,6,7,8,9,10-hexahydro-2,12,13-trimethoxy-7-methyldibenz[*d,f*] azecine (32b)

The title compound was obtained from the amino ketone (37b) (45 mg, 0.143 mmol) following the same procedure as in the synthesis of (32a), without isolating the intermediates. The crude product (33 mg) was fractionated by PLC (methanol-dichloromethane, 5%) and the desired product (32b) was isolated as a brown gum (20 mg, 0.059 mmol, 40%).

MS: m/z 341 (M^+, 100, accurate mass 341.2010, $C_{21}H_{27}NO_3$ requires 341.1989); 326 (25); 283 (15); 269 (55); 70 (45); 58 (45); 57 (55).

^{1}H n.m.r. δ ($CDCl_3$): 7.19 (d, J 8.4, H_4); 6.89 (dd, J 8.5, 2.78, H_3); 6.76 (s, H_{11}); 6.59 (d, J 2.8, H_1); 6.54 (s, H_{14}); 3.92; (s, C_{12}-OC$\underline{H}_3$); 3.81 (s, C_{13}-OC$\underline{H}_3$); 3.78 (s, C_2-OC$\underline{H}_3$); 2.06 (s, NC$\underline{H}_3$).

^{13}C n.m.r. δ ($CDCl_3$): 156.50 (C_2); 148.17 (C_{12}); 146.08 (C_{13}); 143.48 (C_{1a}); 134.87 (C_{11a}); 132.68 (C_{4a}); 132.52 (C_{14a}); 128.68 (C_4);

114.45 (C_1); 113.4 (C_3); 112.30 (C_{14}); 110.73 (C_{11}); 59.38 (C_6); 49.49 (C_8); 29.73 (C_5); 28.42 (C_9); 27.84 (C_{10}).

5,6,7,8,9,10-hexahydro-2,3,methylenedioxy-13-methoxy-7-methyldibenz[*d,f*]azecine (32e)

The carbamate (40c) (310 mg; 0.84 mmol) was reduced with lithium aluminum hydride in similar manner as for (40a) affording the title compound (32e) as pale yellow crystals, in high yield (266 mg; 0.78 mmol, 93%, 124-126°).

MS: m/z 325 (M^+, 66, accurate mass 325.1672, $C_{20}H_{23}NO_3$ requires 325.1676); 310 (16); 267 (9); 253 (43); 165 (12); 152 (21); 70 (78); 58 (66); 57 (100).

^{1}H n.m.r. δ ($CDCl_3$): 7.17 (d, J 8.5, H_{11}); 6.87 (dd, J 8.5, 2.8, H_{12}); 6.74 (s, H_4); 6.57 (d, J 2.7, H_{14}); 6.50 (s, H_1); 5.95 (s, $OC\underline{H}_2O$); 3.77 (s, C_{13}-$OC\underline{H}_3$); 2.08 (s, $NC\underline{H}_3$).

^{13}C n.m.r. δ ($CDCl_3$): 156.75 (C_{13}); 146.86 (C_3); 144.77 (C_2); 143.63 (C_{14a}); 135.60 (C_{4a}); 133.32 (C_{10a}); 129.37 (C_{1a}, C_{11}); 114.60 (C_{14}); 113.78 (C_{12}); 109.27 (C_1); 107.52 (C_4); 59.12 (C_6); 55.22 (C_{13}-$O\underline{C}H_3$); 49.69 (C_8); 44.68 ($N\underline{C}H_3$) 30.64 (C_5); 28.68 (C_9); 27.12 (C_{10}).

5,6,7,8,9,10-hexahydro-13-hydroxy-2,3-dimethoxy-dibenz[*d,f*]azecine (32f).

Reduction of carbamate derivative (40d) (182 mg., 0.49 mmol) with lithium aluminum hydride (230 mg., 7.4 mmol) under similar conditions as for the preparation of (32a) afforded the title compound (32f) as a gum (138 mg, 0.42 mmol, 86%).

MS: m/z 327 (M^+, 49, accurate mass 327.1842, $C_{20}H_{25}NO_3$ requires 327.1831); 312 (20); 255 (40, accurate mass 255.100, $C_{16}H_{15}NO_3$ requires 255.1020); 165 (10); 152 (10); 84 (14); 71 (17); 70 (88, accurate

mass 70.067, C_4H_8N requires 70.0656); 58 (75, accurate mass 58.066, C_3H_8N requires 58.0656); 57 (100, accurate mass 57.058, C_3H_7N requires 57.0578).

^{1}H n.m.r. and **^{13}C n.m.r.** δ ($CDCl_3/CD_3OD$): see (32f)

5,8,9,10-tetrahydro-1,2,13-trimethoxydibenz[*d,f*]azecine-7(6H)-carbonitrile (41a).

To the dibenz[*d,f*]azecine (32a) (112 mg., 0.328 mmol) dissolved in anhydrous chloroform (3.5 ml) was added cyanogen bromide (98 mg, 0.925 mmol). The resulting mixture was stirred at room temperature for 24h after which the solvent was evaporated *in vacuo* . Passage of the crude product through an alumina plug eluting with dichloromethane and subsequent solvent evaporation afforded the desired product (41a) as a waxy solid (109 mg, 0.31 mmol, 95%).

MS: m/z 352 (M^+, 100, accurate mass 352.1464, $C_{21}H_{24}N_2O_3$ requires 352.1458).

^{1}H n.m.r. δ ($CDCl_3$): 7.21 (d, J 8.53, H_{11}); 6.92 (dd, J 8.58, 2.45, H_{12}); 6.79 (s, H_4); 6.61 (d, J 2.61, H_{14}); 6.58 (s, H_1); 3.93 (s, C_3-O$\underline{C}H_3$); 3.82 (s, C_2-O$\underline{C}H_3$); 3.79 (s, C_{13}-O$\underline{C}H_3$).

^{13}C n.m.r. δ ($CDCl_3$): 157.06 (C_{13}); 148.61 (C_3); 147.07 (C_2); 142.88 (C_{14a}); 134.66 (C_{4a}); 130.95 (C_{10a}); 129.28 (C_{11}); 127.83 (C_{1a}); 117.56 ($\underline{C}N$); 114. 71 (C_{14}); 114.05 (C_{12}); 112.34 (C_2); 110.66 (C_4); 55.92 (C_3-O$\underline{C}H_3$); 55.77 (C_2-O$\underline{C}H_3$); 55.17 (C_{13}-O$\underline{C}H_3$) 53.58 (C_6); 44.01 (C_8); 29.19 (C_{11}); 27.50 (C_9); 26.07 (C_{10}).

FT-IR: 2208 cm^{-1} (s, -CN).

5,8,9,10-tetrahydro-13-methoxy-2,3-methylenedioxydibenz[*d,f*]-azecine-7(6H)-carbonitrile (41b).

The title compound (41b) was prepared from the dibenz[*d,f*]azecine (32e) (100 mg, 0.31 mmol) and cyanogen bromide (70 mg, 7.09 mmol) in dry chloroform (10 ml). The crude product was passed through an alumina plug eluting with dichloromethane, which upon evaporation of solvent gave (41b) as white crystals (92 mg, 0.27 mmol, 87%, m.p. 139-140°).

MS: m/z 336 (M^+, 100, accurate mass 336.1461, $C_{20}H_{20}N_2O_3$ requires 336.1473); 254 (16); 253 (28); 152 (14).

^{1}H n.m.r. δ ($CDCl_3$): 7.17 (d, J 8.5; H_{11}); 6.89 (dd, J 8.5, 2.6, H_{12}) 6.75 (s, H_4); 6.57 (d, J 2.6; H_{14}); 6.53 (s, H_1); 5.98 (br, s; $OC\underline{H}_2O$); 3.77 (s, C_{13}-$OC\underline{H}_3$);

^{13}C n.m.r. δ ($CDCl_3$): 157.03 (C_{13}); 147.26 (C_3); 145.74 (C_2); 142.74 (C_{14a}); 135.47 (C_{4a}); 130.74 (C_{10a}); 129.20 (C_{1a}^a); 129.09 (C_{11}^a); 118.00 ($\underline{C}N$); 114.04 (C_{14}^b); 114.00 (C_{12}^b); 109.39 (C_1); 107.70 (C_4); 100.94 ($O\underline{C}H_2O$); 55.06 (C_{13}-$O\underline{C}H_3$); 53.55 (C_6); 43.89 (C_8); 29.25 (C_5); 27.32 (C_9); 25.84 (C_{10}).

5,6,7,8,9,10-Hexahydro-2,3,13-trimethoxy-dibenz[*d,f*]azecine (42a).

To a solution of the carbonitrile (41a) (120 mg, 0.34 mmol) in dry tetrahydrofuran (6 ml) was added lithium aluminum hydride (150 mg, 4.8 mmol) and the mixture refluxed for 2h. After the usual work-up, as for (32a), the mixture was filtered and passed through an alumina plug eluting with dichloromethane. Evaporation of the solvent gave (42a) as an oil which formed into a gum on standing (80 mg, 0.25 mmol, 74%).

MS: m/z 327 (M^+, 70, accurate mass 327.1831, $C_{20}H_{25}NO_3$ requires 327.1832), 312 (23); 295 (100, accurate mass 295.131, $C_{19}H_{19}NO_3$ requires 295.1333); 296 (30); 283 (15); 269 (31); 43 (100).

^{1}H n.m.r. δ ($CDCl_3$): 7.19 (d, J 8.5, H_{11}); 6.91 (dd, J 8.5, 2.8, H_{12}); 6.76 (s, H_4); 6.60 (d, J 2.9, H_{14}); 6.57 (s, H_1); 3.92 (s, C_3-OC$\underline{H}_3$); 3.82 (s, C_2-OC$\underline{H}_3$); 3.78 (s, C_{13}-OC$\underline{H}_3$).

^{13}C n.m.r. δ ($CDCl_3$): 156.88 (C_{13}); 148.46 (C_3); 146.62 (C_2); 143.04 (C_{14a}); 135.28 (C_{4a}); 131.50 (C_{1a}); 129.64 (C_{11}); 129.31 (C_{10a}); 114.76 (C_{14}); 113.92 (C_{12}); 112.35 (C_1); 111.01 (C_4); 55.84 (C_2-O$\underline{C}H_3$); 55.78 (C_3-O$\underline{C}H_3$); 55.15 (C_{13}-O$\underline{C}H_3$) 48.51 (C_6); 41.43 (C_8); 29.47 (C_5, C_9); 26.58 (C_{10}).

5,6,7,8,9,10-Hexahydro-13-methoxy-2,3-methylenedioxy-dibenz[*d,f*]azecine (42b).

Reduction of (41b) (155 mg, 0.46 mmol) with lithium aluminum hydride in tetrahydrofuran as for (42a), gave the title compound (42b) (86 mg, 0.28 mmol, 61%) as a gum.

MS: m/z 311 (M^+, 32, accurate mass 311.1519, $C_{19}H_{21}NO_3$ requires 311.1519); 296 (15); 279 (42); 267 (8); 253 (13); 165 (8); 152 (13); 56 (6); 44 (15); 43 (100).

^{1}H n.m.r. δ ($CDCl_3$): 7.15 (d, J 8.5, H_{11}); 6.87 (dd, J 8.5, 2.7; H_{12}); 6.73 (s, H_4); 6.56 (d, J 2.7, H_{14}); 6.53 (s, H_1); 5.94 (br, s, OC$\underline{H}_2$O); 3.75 (C_{13}-OC$\underline{H}_3$).

^{13}C n.m.r. δ ($CDCl_3$): 156.86 (C_{13}); 147.16 (C_3); 145.29 (C_2); 142.96 (C_{14a}); 136.12 (C_{4a}); 131.42 (C_{10a}, C_{1a}); 129.53 (C_{11}); 114.70 (C_{14}); 113.82 (C_{12}); 109.36 (C_1); 108.19 (C_4); 100.81 (O$\underline{C}H_2$O); 55.01 (C_{13}-O$\underline{C}H_3$); 48.58 (C_6); 41.45 (C_8); 29.53 (C_5, C_9); 23.86 (C_{10}).

7.3. Synthesis II: Wurmbazecine and/or Isomers by Thallium (III) Trifluoroacetate Oxidation (Chapter 3, Section 3.3.2)

3-methoxy-β-nitrostyrene (43)

A mixture of 3-methoxybenzaldehyde (13.6 g, 0.01 mol), nitromethane (12.2 g, 0.20 mol), ammonium acetate (10 g, 0,13 mol), and glacial acetic acid (100 ml) was refluxed for 1h. The resulting solution was then allowed to cool and was partitioned between water and dichloromethane (100 ml, 1:1). The aqueous layer was extracted exhaustively with dichloromethane and the combined organic layer was neutralized with a saturated solution of sodium bicarbonate until the effervescence ceased. The mixture was washed with water, and dried over anhydrous sodium sulfate. After the solvent was evaporated, the resulting brown residue was re-crystallized from methanol, affording light green flakes of the desired product (43) (12.46 g, 70 mmol, 70%, m.p. 87-88°).

3-methoxyphenyl-1-propanoic acid (45a)

3-methoxycinnamic acid (4.5 g, 0.025 mol) in dry methanol (25 ml) was hydrogenated under pressure (60 p.s.i.), over platinum oxide (0.025 mg) until the hydrogen uptake ceased. The resulting mixture was filtered and the filtrate evaporated *in vacuo*. Re-crystallization of the residue from diethyl ether gave (45a) as white needles (4.13 g, 0.023 mol, 91%). **MS**: m/z 180 (M^+, 97, accurate mass 180.077, $C_{10}H_{12}O_3$ requires 180.0785); 137 (100); 121 (76).

3-methoxyphenyl-1-ethanamine (44b)

To a solution of lithium aluminum hydride (0.5 g, 0.013 mol) in tetrahydrofuran (15 ml), was added dropwise the solution of nitrostyrene (43) (1.8 g, 0.01 mol) in dry tetrahydrofuran (20 ml) and the mixture refluxed for 1.5h. The amine ((44b) was obtained as a yellow oil (1.45 g, 9.6 mmol, 93%) after the usual work up.

MS: m/z 151 (M^+, 5, accurate mass 151.101, $C_9H_{13}NO$ requires 151.0996); 122 (22); 30 (100).

Synthesis of the amides (46a), (46b) and (46c)

The amide derivative (46a) was prepared by heating together homoveratrylamine (1.5 g, 8.3 mmol) and 3-(3-methoxyphenyl)-1-propanoic acid (45a) (1.4 g, 7.8 mmol) in toluene (20 ml) at 140°-180°. After 2h the remaining solvent was evaporated *in vacuo* and the residue recrystallized from toluene to give (46a) as pale yellow crystals (2.02 g, 6 mmol, 77%, m.p. 65-66°)

MS: m/z 343 (M^+, 4, accurate mass 343.180, $C_{20}H_{25}NO_4$ requires 343.1781); 164 (100, accurate mass 164.083, $C_{10}H_{12}O_2$ requires 164.0837); 151 (30, accurate mass 151.076, $C_9H_{11}O_2$ requires 151.0758).

^{1}H n.m.r. δ ($CDCl_3$): 7.17 (t, J 6.2, 1 ArH); 6.77-6.63 (m, 6H, ArH); 5.78 (s, br, NH); 3.83 (s, 3H, OCH_3); 3.82 (s, 3H, OCH_3); 3.75 (s, 3H, OCH_3); 3.44 (q, J 6.4, 2H); 2.91 (t, J 7.7, 2H); 2.68 (t, J 7.0, 2H); 2.42 (t, J 7.7, 2H).

^{13}C n.m.r. δ ($CDCl_3$): 171.96 (>C=O); 159.45 (=C<); 148.70 (=C<); 147.33 (=C<); 142.26 (=C<); 131.14 (=C<); 129.26 (=CH); 120.40 (=CH X 2), 113.78 (=CH); 111.51 (=CH); 111.27 (=CH); 110.99 (=CH); 55.55 (OCH_3 X 2); 54.85 (OCH_3); 40.53 (CH_2); 38.06 (CH_2); 34.98 (CH_2); 31.52 (CH_2).

The amide (46b) was prepared in a similar manner as (46a), from an unpurified sample of the amine (44b) (1.4 g, 9.27 mmol) with 3-(3,4-dimethoxyphenyl)-propanoic acid (2.05 g, 9 mmol). The resulting mixture showed five spots on TLC (methanol-dichloromethane, 5%) and was passed through a quick silica column eluting with methanol-dichloromethane (3%). The fraction containing the amide was recrystallized from toluene to give (46b) as yellow needle-like crystals (1.2 g, 4 mmol, 39%, m.p. 66-67^{o}).

MS: m/z 343 (M^+, 39, accurate mass, 343.179, $C_{20}H_{25}NO_4$ requires 343.1781); 209 (63), 164 (22), 151 (100).

^{1}H n.m.r. δ ($CDCl_3$): 7.15 (t, J 4.0), 1H Ar<u>H</u>); 6.75-6.66 (m, 6H, Ar<u>H</u>); 6.14 (s, br, N<u>H</u>); 3.79 (s, 3H, OC<u>H</u>$_3$); 3.79 (s, 3H, OC<u>H</u>$_3$); 3.72 (s, 3H, OC<u>H</u>$_3$); 3.45 (br, 2H, C<u>H</u>$_2$); 2.87 (t, J 7.6, 2H, C<u>H</u>$_2$); 2.71 (t, J 7.1, 2H, C<u>H</u>$_2$); 2.41 (t, J 7.6, 2H, C<u>H</u>$_2$).

^{13}C n.m.r. δ ($CDCl_3$): 171.91 (>C=O); 159.33 (>C=); 148.41 (=C<); 146.96 (=C<); 140.11 (=C<); 133.19 (=C<); 129.14 (=<u>C</u>H); 120.43 (=<u>C</u>H); 119.75 (=<u>C</u>H); 113.82 (=<u>C</u>H); 111.26 (=<u>C</u>H); 111.12 (=<u>C</u>H); 110.89 (=<u>C</u>H); 55.41 (O<u>C</u>H$_3$); 55.32 (O<u>C</u>H$_3$); 54.65 (O<u>C</u>H$_3$); 40.17 (<u>C</u>H$_2$); 38.14 (<u>C</u>H$_2$); 35.28 (<u>C</u>H$_2$); 30.94 (<u>C</u>H$_2$).

Condensation of homoveratrylamine (1.8 g, 0.01 mol) with 3-(3,4-dimethoxyphenyl)-propanoic acid (2.10 g, 0.01 mol) following the same procedure as for (46a) afforded a brown gum. Re-crystallization from dichloromethane/diethyl ether gave (46c) as pale yellow crystals (3.46 g, 93%, m.p. 95.5-96.5^{o}C).

MS: m/z 373 (M^+, 6, accurate mass 373.188, $C_{21}H_{27}NO_5$ requires 373.1887); 209 (19); 164 (100); 151 (69).

^{1}H n.m.r. δ ($CDCl_3$): 6.60-6.78 (m, 6H, Ar<u>H</u>), 5.75 (s, br, N<u>H</u>); 3.83 (s, 12H, 3 X OC<u>H</u>$_3$); 3.45 (q, 2H, C<u>H</u>$_2$); 2.89 (t, J 7.6, 2H, C<u>H</u>$_2$); 2.70 (t, J 7.0, 2H, C<u>H</u>$_2$); 2.42 (t, J 7.2, 2H, C<u>H</u>$_2$).

^{13}C n.m.r. δ ($CDCl_3$): 171.94 (>C=O); 148.70 (=C<); 148.59 (=C<); 147.36 (=C<); 145.15 (=C<); 133.26 (=C<); 131.09 (=C<); 120.35 (=<u>C</u>H); 119.91 (=<u>C</u>H); 111.54 (=<u>C</u>H); 111.40 (=<u>C</u>H<); 111.01 (=<u>C</u>H X 2); 55.59 (O<u>C</u>H$_3$ X 4); 40.46 (<u>C</u>H$_2$); 38.46 (<u>C</u>H$_2$); 34.98 (<u>C</u>H$_2$); 31.09 (<u>C</u>H$_2$).

<u>Reduction of the Amides (46a), (46b), and (46c)</u>

To a stirred solution of the amide (46a) (1.8 g, 5.24 mmol) in dry tetrahydrofuran (30 ml) was added lithium aluminum hydride (500 mg, 13.17 mmol) and the mixture refluxed for 1.5h. After the usual work up, the amine (47a) was obtained as a yellow oil (1.5 g, 4.6 mmol, 92%).

MS: m/z 329 (M^+, 4, 329.198, $C_{20}H_{27}NO_3$ requires 329.1989); 165 (91); 164 (100); 151 (72). **CIMS**: m/z 330 (MH^+).

^{1}H n.m.r. δ ($CDCl_3$): 7.13 (t, J 7.8, 1H Ar<u>H</u>), 6.76-6.67 (m, 6 Ar<u>H</u>); 3.80 (s, 3H, OC<u>H</u>$_3$), 3.77 (s, 3H, OC<u>H</u>$_3$); 3.70 (s, 3H, OC<u>H</u>$_3$); 3.04 (s, br, 1H, N<u>H</u>), 2.83-2.72 (m, 3H, C<u>H</u>$_2$); 2.62-2.53 (m, 3H, C<u>H</u>$_2$); 1.80-1.65 (m, 2H, C<u>H</u>$_2$).

^{13}C n.m.r. δ ($CDCl_3$): 158.85 (=C<), 148.13 (=C<); 146.67 (=C<); 142.75 (=C<); 131.63 (=C<); 128.51 (=<u>C</u>H); 119.94 (=<u>C</u>H); 119.82 (=<u>C</u>H); 113.32 (=<u>C</u>H); 111.21 (=<u>C</u>H); 110.57 (=<u>C</u>H); 110.24 (=<u>C</u>H); 54.97 (O<u>C</u>H$_3$ X 2); 54.15 (O<u>C</u>H$_3$); 50.32 (<u>C</u>H$_2$); 48.33 (<u>C</u>H$_2$); 34.86 (<u>C</u>H$_2$); 32.81 (<u>C</u>H$_2$); 30.46 (<u>C</u>H$_2$).

The amine (47b) (546 mg, 1.66 mmol) was obtained as a yellow oil from the reduction of the amide (46b) (0.865 g, 2.52 mmol) with lithium aluminum hydride in the same manner as for (47a).

MS: m/z 329 (M^+, 3, accurate mass 329.200, $C_{20}H_{27}NO_3$ requires 329.1989); 165 (19); 151 (72). **CIMS**: m/z 330 (MH^+).

^{1}H n.m.r. δ ($CDCl_3$): 7.20 (m, 1H, ArH); 6.81-6.66 (m, 6H, ArH); 3.85 (s, 3H, OCH_3); 3.84 (s, 3H, OCH_3); 3.78 (s, 3H, OCH_3); 2.89-2.83 (m, 4H, CH_2); 2.67-2.54 (m, 4H, CH_2); 1.78 (m, 2H, CH_2).

^{13}C n.m.r. δ ($CDCl_3$): 159.61 (=C<); 148.66 (=C<); 147.00 (=C<); 141.54 (=C<); 134.56 (=C<); 129.35 (=CH); 120.98 (=CH); 119.99 (=CH); 114.37 (=CH); 111.50 (=CH); 111.32 (=CH); 111.05 (=CH); 55.79 (OCH_3); 55.69 (OCH_3); 55.03 (OCH_3); 50.69 (CH_2); 49.17 (CH_2); 36.25 (CH_2); 33.09 (CH_2); 31.68 (CH_2).

Reduction of the amide (46c) (1.17 g, 3.12 mmol) with lithium aluminum hydride as for (47a) afforded the amine derivative (47c) as a yellow oil (0.98 g, 2.73 mmol, 88%).

MS: m/z 359 (M^+, 2, accurate mass 359.209, $C_{21}H_{29}NO_4$ requires 359.2095); 222 (75), 208 (38); 165 (15); 164 (10); 151 (57); **CIMS**: m/z 360 (MH^+).

^{1}H n.m.r. δ ($CDCl_3$): 6.81-6.66 (m, 6H, ArH); 3.84 (12H, OCH_3 X 4); 2.89-2.54 (m, 8H, CH_2); 1.80 (m, 2H, CH_2).

^{13}C n.m.r. δ 148.62 (=C<); 148.53 (=C<); 147.15 (=C<); 146,85 (=C<); 134.42 (=C<); 132.36 (=C<); 120.31 (=CH); 119.87 (=CH); 111.68 (=CH); 111.38 (=CH); 111.01 (=CH X 2); 55.61 (OCH_3 X 3); 51.03 (CH_2); 49.06 (CH_2); 35.67 (CH_2); 32.96 (CH_2); 31.59 (CH_2).

Trifluoroacetylation Reactions

To a solution of the amine (47a) (1.3 g, 3.95 mmol) in dry pyridine (40 ml) was added trifluoroacetic anhydride (1.51 g, 7.2 mmol) and the mixture stirred at 15-20°, under nitrogen. After 3h, the solvent was evaporated *in vacuo* and the residue was partitioned between

dichloromethane and dilute sulfuric acid (2.5%). The organic layer was washed with water (2X50 ml) and dried over anhydrous sodium sulfate. Evaporation of the solvent *in vacuo* afforded a brown oil, which was passed through an alumina quick column using dichloromethane as eluant. The desired product (48a) was obtained as a pale brown oil (1.59 g, 3.74 mmol, 95%) and was used for the next reaction without further purification.

MS: m/z 425 (M^+, 7, accurate mass 425.180, $C_{22}H_{26}F_3NO_4$ requires 425.1811); 164 (100); 151 (78).

^{1}H n.m.r. δ ($CDCl_3$): 7.18 (t, J 7.8, 1H, ArH); 6.77-6.67 (m, 6H, ArH); 3.80 (s, 6H, OCH_3 X 2); 3.74 (s, 3H, OCH_3); 3.52 (t, br, 2H, CH_2); 3.32 (t, br, 1H, CH_2); 3.20 (t, br, 1H, CH_2); 2.79 (m, 2H, CH_2); 2.55 (m, 2H, CH_2); 1.90 (m, 2H, CH_2).

^{13}C n.m.r. δ ($CDCl_3$): 159.58 (=C<), 156.47 (q, C=O); 148.60 (=C<); 147.57 (=C<); 142.27 (=C<); 130.45 (=C<); 129.36 (=CH); 120.46 (=CH X 2); 114.41 (CF_3); 113.90 (=CH); 111.77 (=CH); 111.66 (=CH); 111.27 (=CH); 55.61 (OCH_3 X 2); 54.88 (OCH_3); 49.13 (CH_2); 46.83 (CH_2); 32.41 (CH_2); 29.75 (CH_2); 27.90 (CH_2).

FT-IR: 1678 cm^{-1} (s, C=O.)

In a similar fashion to the above, the trifluoroacetamide derivative (48b) was obtained from the trifluoroacetylation of amine (47b) (1.35 g, 4.10 mmol) with excess trifluoroacetic anhydride (2.25 g, 10.68 mmol). A brown oil (1.44 g, 3.39 mmol, 83% yield) was obtained after passing the crude product through an alumina plug.

MS: m/z 425 (M^+, 42, accurate mass 425.182, $C_{22}H_{26}F_3NO_4$ requires 425.1811); 304 (78); 291 (30); 177 (24); 164 (82); 151 (100).

^{1}H n.m.r. δ ($CDCl_3$): 7.17 (m, 1H, ArH); 6.79-6.69 (m, 6H, ArH); 3.81 (s, br, 6H,)CH_3 X 2); 3.72 (s, 3H, OCH_3); 3.52 (m, 2H, CH_2); 3.40 (m, 1H,

$C\underline{H}_2$); 3.20 (m, 1H, $C\underline{H}_2$); 2.79 (m, 2H, $C\underline{H}_2$); 2.51 (m, 2H, $C\underline{H}_2$); 1.86 (m, 2H, $C\underline{H}_2$).

^{13}C n.m.r. δ ($CDCl_3$): 159.34 (=C<); 156.25 (q, C=O); 148.37 (=C<); 146.85 (=C<); 139.11 (=C<); 132.92 (=C<); 129.25 (=C<); 120.37 (=$\underline{C}$H); 119.59 (=$\underline{C}$H); 117.99 ($\underline{C}F_3$); 114.09 (=$\underline{C}$H); 111.47 (=$\underline{C}$H); 110.97 (=$\underline{C}$H); 110.85 (=$\underline{C}$H X 2); 55.10 ($O\underline{C}H_3$ X 2); 54.33 ($O\underline{C}H_3$); 48.71 ($\underline{C}H_2$); 47.18 ($\underline{C}H_2$); 34.87 ($\underline{C}H_2$); 31.82 ($\underline{C}H_2$); 29.54 ($\underline{C}H_2$).

FT-IR: 1689 cm^{-1} (s, C=O).

The trifluoroacetamide derivative (48c) was also obtained from the corresponding amine (47c) (648 mg, 1.8 mmol), by reaction with trifluoroacetic anhydride (379 mg, 1.8 mmol). The product (48c) was obtained as yellow needle-like crystals (478.2 mg, 1.05 mmol, 58%, m.p. 67-68°) after PLC separation (methanol-dichloromethane, 4%).

MS: m/z 455 (M^+, 26, accurate mass 455.189, $C_{23}H_{29}F_3NO_5$ requires 455.1917); 440 (4); 304 (19); 291 (39); 177 (10); 165 (58); 164 (99); 151 (100).

^{1}H n.m.r. δ ($CDCl_3$): 6.82-6.63 (m, 6H, ArH); 3.861 (s, 6H, $OC\underline{H}_3$ X 2); 3. 858 (s, 6H, $OC\underline{H}_3$ X 2); 3.54 (t, br, 2H, $C\underline{H}_2$); 3.43 (t, br, 1H, $C\underline{H}_2$); 3.20 (t, br, 1H, $C\underline{H}_2$); 2.81 (m, 2H, $C\underline{H}_2$); 2.60 (m, 2H, $C\underline{H}_2$); 1.91 (m, 2H, $C\underline{H}_2$).

^{13}C n.m.r. δ ($CDCl_3$): 156.46 (q, C=O); 148.93 (=C<); 147.84 (=C<); 147.57 (=C<); 147.30 (=C<); 133.20 (=C<); 130.45 (=C<); 120.43 (=$\underline{C}$H); 119.87 (=$\underline{C}$H); 114.39 ($\underline{C}F_3$); 111.74 (=$\underline{C}$H); 111.59 (=$\underline{C}$H); 111.32 (=$\underline{C}$H); 111.14 (=$\underline{C}$H); 55.65 ($O\underline{C}H_3$ X 4); 49.14 ($\underline{C}H_2$); 47.65 ($\underline{C}H_2$); 32.38 ($\underline{C}H_2$); 29.97 ($\underline{C}H_2$); 28.14 ($\underline{C}H_2$).

Carbamate derivatives of amines (47a), (47b), and (47c).

Methyl chloroformate (0.5 ml, 6.5 mmol) was added to a mixture of the amine (47a) (389 mg, 1.18 mmol), dry toluene (20 ml), and finely ground anhydrous potassium carbonate (1.8 g, 13.1 mmol) and the mixture refluxed. After 3h, the mixture was allowed to cool, filtered and the filtrate evaporated to dryness *in vacuo*. The desired product (49a) (432.4 mg, 1.12 mmol, 95%) was obtained as a pale yellow oil which turned into a waxy solid upon standing.

MS: m/z 387 (M^+, 63, accurate mass 387.202, $C_{22}H_{29}NO_5$ requires 387.2044); 236 (45); 102 (100).

^{1}H n.m.r. δ ($CDCl_3$): 7.16 (t, br, 1H, ArH); 6.77-6.72 (m, 6H, ArH); 3.82 (s, 3H, $OC\underline{H}_3$); 3.79 (s, 3H, $OC\underline{H}_3$); 3.73 (s, 3H, $OC\underline{H}_3$); 3.41 (s, br, 2H, $H_{1'}$); 3.21 (s, br, 2H, H_1); 2.75 (s, br, 2H, $H_{2'}$); 2.54 (s, br, 2H, H_3); 1.82 (s, br, 2H, H_2).

^{13}C n.m.r. δ ($CDCl_3$): 159.05[a](=C<); 156.06 (C=O); 148.26 (=C<); 146.92 (=C<); 142.56 (=C<); 131.01 (=C<); 128.68 (=$\underline{C}$H); 120.10 (=$\underline{C}$H); 119.70 (=$\underline{C}$H); 113.39 (=$\underline{C}$H); 110.70 (=$\underline{C}$H); 110.49 (=$\underline{C}$H); 55.13 ($O\underline{C}H_3$ X 2); 54.32 ($O\underline{C}H_3$); 51.73 ($O\underline{C}H_3$); 48.70 ($C_{1'}$); 46.68 (C_1); 33.81 ($C_{2'}$); 32.53 (C_3); 29.16 (C_2).

FT-IR: 1701 cm^{-1} (s, >C=O).

Following the above procedure, carbamate derivative (49b) (1.146 g, 2.96 mmol, 95%) was obtained from its amine derivative (47b) (1.026 g, 3.12 mmol) as a waxy solid which was used for the next reaction without further purification.

MS: m/z 387 (M^+, 33, accurate mass 387.206, $C_{22}H_{29}NO_5$ requires 387.2044); 266 (44); 165 (37); 151 (23); 102 (100).

^{1}H n.m.r. δ ($CDCl_3$): 7.20 (t, J 7.9, 1H, ArH); 6.60-6.72 (m, 6H, ArH); 3.86 (s, 3H, OCH_3); 3.85 (s, 3H, OCH_3); 3.78 (s, 3H, OCH_3); 3.69 (s, 3H, $COOCH_3$); 3.42 (s, br, 2H, $H_{2'}$); 3.22 (s, br, 2H, H_1); 2.79 (s, br, 2H, $H_{2'}$); 2.53 (s, br, 2H, H_3); 1.62 (s, br, 2H, H_2).

^{13}C n.m.r. δ ($CDCl_3$); 159.51 (=C<); 156.59 (C=O); 148.62 (=C<); 147.01 =C<); 140.49 (=C<); 134.07 (=C<); 129.29 (=CH); 120.96 (=CH); 119.85 (=CH); 114.35 (=CH); 111.40 (=CH X 2); 111.03 (=CH), 55.72 (OCH_3 X 2); 54.98 (OCH_3); 52.30 ($COOCH_3$); 49.05 ($C_{2'}$); 47.19 (C_1); 34.78 ($C_{2'}$); 32.45 (C_3); 29.93 (C_2).

FT-IR: 1701 cm^{-1} (s, >C=O).

A reaction of the amine derivative (47c) (340 mg, 0.95 mmol) with methyl chloroformate (0.5 ml, 6.5 mmol) as for (41a) , afforded the methyl N-carboxylate derivative (49c) (367.2 mg, 0.88 mmol, 93%) as a yellow gum.

MS: m/z 417 (M^+, 47, accurate mass 417.213, $C_{22}H_{29}NO_5$ requires 417.2148); 266 (38); 191 (7); 165 (23); 151 (15); 102 (100).

^{1}H n.m.r. δ ($CDCl_3$): 7.17 (m, 1H, ArH); 6.80-6.71 (m, 5H, ArH); 3.86 (s, 6H, OCH_3 X 2); 3.84 (s, 6H, OCH_3 X 2); 3.69 (s, br, 3H, $COOCH_3$); 3.43 (s, br, 2H, $H_{1'}$); 3.21 (s, br, 2H, H_1); 2.75 (s, br, 2H, $H_{2'}$); 2.52 (s, br, 2H, H_3); 1.82 (s, br, 2H, H_2).

^{13}C n.m.r. δ ($CDCl_3$): 156.55 (C=O); 148.64 (=C<); 147.31 (=C<); 146.97 (=C<); 133.95 (=C<); 131.41 (=CH); 128.80 (=C<); 127.99 (=C<); 120.48 (=CH); 111.78 (=CH); 111.37 (=CH); 111.02 (=CH X 2); 55.64 (OCH_3 X 4); 52.23 ($COOCH_3$); 49.16 ($C_{1'}$); 47.14 (C_1); 34.23 ($C_{2'}$); 32.39 (C_3); 30.05 (C_2).

FT-IR: 1701 cm^{-1} (s, >C=O).

Oxidative Cyclization of the Trifluoroacetamide Derivatives (48a), (48b), and (48c).

To a cooled solution (ice-salt mixture, -18°) of the thallium (III) trifluoroacetate (460 mg, 0.86 mmol) in dry trifluoroacetic acid (9.8 ml), under nitrogen, was added a pre-cooled solution (ice-salt, -18°) of the trifluoroacetamide derivative (48c) (326 mg, 0.72 mmol) in dry triflouroacetic acid (7 ml) and dry dichloromethane (3.1 ml) and the mixture stirred for 30 min at -18°. The resulting mixture was poured into water (25 ml) and extracted with dichloromethane (3x40 ml), and the combined organic extracts washed with brine solution (2x40 ml) and dried over anhydrous sodium sulfate. Evaporation of solvent *in vacuo* and passing the crude product through an alumina plug eluting with dichloromethane, afforded a tarry brown gum (165 mg). Two major components, of molecular masses 453 and 471 were detected by GC-MS analysis. This crude product was used for the next reaction without purification.

MS: m/z 453 (M^+, 16); 302 (M^+-151, 13); 289 (M^+-154, 19); 177 (26), 164 (100); 151 (75).

MS: m/z 471 (M^+, 15); 320 (M^+-151, 17); 164 (100); 151 (81).

To a solution of thallium (III) trifluoroacetate in trilfluoroacetic acid (10 ml) was added the trifluoroacetamide derivative (48a, M^+ 425) (300 mg) in trifluoroacetic acid (5 ml) and dry dichloromethane (2.2 ml) in the same manner as for (48c). After the usual work up, a brown gum (200 mg) was obtained and analyzed by GC-MS and by direct probe MS. The result of the analysis indicated mostly polymeric products with traces of less polar product of molecular weights 425; no oxidized product was detected from the crude product. The crude product was fractionated on

an alumina quick column affording four fractions. All the fractions contained components of molecular weight of 425.

The trifluoracetamide (48b) (425 mg, 1.05 mmol) in dry trifluoroacetic acid (10 ml) and dry dichloromethane (4.4 ml) was oxidized with thallium (III) trifluoroacetate (250 mg) in dry trifluoroacetic acid (7 ml) as above, which after the usual work afforded a brown powder as product (165 mg). GC-MS analysis of the crude product showed only very weak ion peaks in the expected retention time range (4-10 min, 250-290°); direct probe mass spectrometry indicated high molecular weight products as the major component of the extract. It also contained minor amount of the unreacted trifluoroacatide derivative (48b). The crude product (300 mg) was passed through an alumina quick column eluting with dichloromethane, then methanol-dichloromethane (3%). The first fraction (18 mg) contained two less polar components, the unreacted trifluoroacetamide derivative (M^+ 425) and a second component of molecular weight 423. These two components could not be separated further.

Oxidative Cyclization of the Methyl N-Carboxylate Derivatives (49b) and (49c)

A degassed solution of the carbamate derivative (49c) (194 mg, 0.47 mmol) in dry trifluoroacetic acid (20 ml), cooled at -22°C in dry ice, was added in one lot to a degassed, stirred, and pre-cooled solution (-22°C) of thallium (III) trifluoroacetate (300 mg, 0.56 mmol) in dry trifluoroacetic acid (40 ml). Boron trifluoride etherate (2 ml) was added immediately to the mixture. After 15 min, the resulting mixture was poured into water (10 ml) and partitioned with dichloromethane (2x25

ml). The combined organic layer was washed with water (2x50 ml), then brine solution (50 ml) and dried over anhydrous sodium sulfate. The crude product was obtained as a gum after evaporation of the solvent *in vacuo*. GC-MS analysis of the crude product indicated the presence of the cyclized product along with the starting material, the oxidized but uncyclized product (M^+ 415, bp 177°), a component of molecular mass 357 (major component) and a few other components. The crude product was fractionated by PLC (methanol-dichloromethane, 5%) affording four fractions.

The first fraction (12 mg) was a mixture of an oxidized product (M^+ 415) along with the unreacted carbamate precursor (M^+ 417)
GC-MS: m/z 415 (M^+, 58); 177 (100); 164 (28); 151 (28).

The second fraction (28 mg) was also not pure but contained a major component of molecular weight 357. The desired product (51c) (10 mg) was detected as a major component of the third fraction, along with some very minor components; the ^1H n.m.r. data could not be fully interpreted due to broadening of peaks.
GC-MS: m/z 415 (M^+ 100); 313 (M^+-102, 36); 299 (M^+-116, 16); 102 (54).

Oxidative cyclization of the carbamate derivative (49b) (203 mg, 0.52 mmol) with thallium (III) trifluoroacetate (191 mg, 0.36 mmol) as above, afforded a crude product which by GC-MS analysis indicated the presence of the desired product as a minor component. The crude product was passed through an alumina quick column eluting with dichloromethane, affording four fractions. Fraction 1 contained the unreacted carbamate precursor (M^+ 387) while each of the other three

fractions was a mixture of the unreacted carbamate and the desired component of molecular weight 385. This could not be isolated pure, and was used directly for the next reaction.

GC-MS: m/z 385 (M^+, 37); 283 (M^+-102, 15); 269 (M^+-116, 15); 102 (100).

Several other oxidation experiments were done under different conditions and are summarized briefly in Table 7.1.

Hydrolysis of Trifluoroacetamide Derivative Cyclization Product.

A solution of the crude product suspected to contain (50c) (162 mg) in methanol (15 ml) and dilute sodium hydroxide (3N, 15 ml) was stirred at room temperature under nitrogen for 1.5h. The resulting mixture was poured to a brine solution (25 ml) and extracted with dichloromethane (2x40 ml). The combined organic extracts were washed with water twice, then with brine solution. Evaporation of the solvent *in vacuo* afforded a brown-black tarry product, which by analytical tlc showed mostly polar products. Direct probe MS analysis did not show a distinct parent ion, but gave peaks beyond 800 mass units. The crude product was re-extracted with acid, (2.5 % H_2SO_4), washed with light petroleum at that pH, then re-basified with sodium hydroxide (2N). The dichloromethane extract of the basified solution was dried over anhydrous sodium sulfate and the solvent evaporated *in vacuo,* The resulting brown gum (45.5 mg) was analyzed by direct probe MS. The mass spectrum was the same as before.

Table 7.1- Oxidative Cyclization Reaction Conditions and Results

[Substrate]	Solvent	Conditions	[TTFA]/[Solvent]	Results (MW's of prod.)/Remarks
[**1**]. (48c) (0.72 mmol)	7 ml TFA 2.2 ml DCM	ice-salt -18°C 9.8 ml TFA	1.05 mmol 0 ml TFA	major non-tarry products: 453 bp 164 and 371 bp 164°C
[**2**]. (49c) (0.77 mmol)	7.6 ml TFA 3.3 ml DCM	ice-salt -18°C 3.5 hrs.	1.1 mmol 11 ml TFA	415 bp 177°C (major); unreacted material; 415 (desired product), others.
[**3**]. (49c) (0.72 mmol)	7.5 ml TFA 3.0 ml DCM	ice-salt -18°C 5.5 hrs.	0.28 mmol 10 ml TFA	no reaction
[**4**]. (49c) (0.55 mmol)	5 ml TFA 2.2 ml DCM 2.0 ml $BF_3.Et_2O$	ice-salt -18°C 30 min.	0.75 mmol 7 ml TFA	373(2), 371 415 (desired product) with 417; 415 bp 177
[**5**]. (49c) (0.61 mmol)	5 ml TFA 2.5 ml DCM 3 ml $BF_3.Et_2O$	ice-salt 18°C -22°C 1 hr	(0.83 mmol) 7.8 ml TFA	mostly tarry products; direct probe MS: polymeric products
[**6**]. (6c) (0.54 mmol)	5 ml TFA 2.2 ml DCM 2 ml $BF_3.Et_20$	ice-salt 18°C 15 min.	(0.75 mmol) 7 ml TFA	less tarry products than [**5**]

Table 7.1 continued.

[substrate]	solvent	rxn conditions	[TTFA]/[solvent]	Results (MW's of prod.)/Remarks
[**7**]. (49c) 0.53 mmol	50 ml TFA 2.2 ml DCM	ice-salt -18°C degassed 30 min.	(0.75 mmol) 10 ml TFA 10 ml DCM	1/2 portion (with $BF_3.Et_2O$): 415 bp 177°C with trace of desired product etc. 1/2 portion (no $BF_3.Et_2O$) no desired prod
[**8**]. (49c) (0.47 mmol)	5 ml CH_3CN 3 ml $BF_3.Et_2O$	dry ice-CCl_4 -22°C degassed	0.53 mmol 10 ml CH_3CN	1/2 portion (20 min.) - tarry prods. 1/2 portion (40 min) - tarry prods
[**9**]. (6c) (0.54 mmol)	30 ml TFA	degassed 20 min.	0.79 mmol 20 ml TFA 2 ml $BF_3.Et_2O$	1/2 portion (room temp.) ; 1/2 portion (dry ice -22°C) - similar results: 415 (desired prod;) in trace amounts; minimum. polar material
[**10**]. (49c) (0.47 mmol)	20 ml TFA	degassed dry ice -22°C 15 min.	0.56 mmol 40 ml TFA 2 ml $BF_3.Et_2O$	357 bp 206°C, 415 bp 177°C, 415 (desired product); best result.
[**11**]. (16b) (0.52 mmol)	20 ml TFA	degassed; dry-ice -22°C 15 min.	0.36 mmol 40 ml TFA 6 ml $BF_3.Et_2O$	387 (major; unreacted material); 385 (desired product; minor component); others.

Reduction of the Carbamate Derivative (51c)

A solution of the crude product containing carbamate (51c) (200 mg) in tetrahydrofuran (10 ml) was added to a stirred solution of lithium aluminum hydride (150 mg) in tetrahydrofuran (15 ml) and refluxed for 1.5h. A gum was obtained after the usual work up and passed through a quick column on a silica support. GC-MS analysis indicated the presence of the desired dibenz[*d,f*]azecine derivative (32g) as a minor component of the the third fraction, along with other components. The sample size did not allow its isolation. The bulk of the product was composed of very polar materials.

GC-MS: m/z 371 (M^+, 100); 299 (M^+-72, 65); 70 (34), 58 (36); 57 (36).

7.4. Dibenz[*d,f*]azecine Derivatives from Known Alkaloids.

Reaction with Cyanogen Bromide

To a stirred mixture of the homoerythrina alkaloid taxodine (55) (10 mg, 0.03 mmol), dry methanol-dry chloroform (1:1, 0.7 ml) and magnesium oxide (100g, 0.5 mol) was added cyanogen bromide (16 mg, 0.15 mmol) and kept stirred at room temperature. At the end of 12h, then 36h, samples were drawn from the reaction mixture and analyzed by TLC and GC-MS. The homoerythrina alkaloid remained unchanged as analyzed by GC-MS.

Cyanogen bromide (52 mg, 0.49 mmol) was added to a separate mixture of the alkaloid taxodine (55) (15 mg, 0.05 mmol) in dry benzene (0.5 ml) and stirred at room temperature. A reaction mixture was drawn after 2 days, 57h, then 112h, to monitor the reaction. Approximately one half of the reactant was converted to the desired product (56) at the end

of 112h as indicated in the total ion chromatogram of the crude product. This crude product was not isolated pure.

MS: m/z 308 (M^+, 100), 226 (11), 211 (10).

Reaction with methyl chloroformate

A mixture of taxodine (55) (15 mg, 0.05 mmol), potassium carbonate (6.7 mg), methyl chloroformate (0.2 ml) and dry benzene (1.5 ml), under nitrogen, was refluxed with stirring for 8h and stirred for another 12h at room temperature. The solvent was evaporated *in vacuo* and the crude product was passed through an alumina plug eluting with dichloromethane. After evaporation of the solvent *in vacuo*, the residue obtained as a gum (12.5 mg) was analyzed by GC-MS. Approximately 75 percent of the taxodine alkaloid was converted to two products of molecular weights 341 (57) and 399 (58) (2:1 respectively).

MS: m/z 341 (M^+, 100), 239 (16), 225 (30), 165 (14), 102 (21).

MS: m/z 399 (M^+,100), 283 (6), 222 (11), 165 (13), 102 (32).

Homoerythratine (60) (18 mg, 0.05 mmol) was reacted with methyl chloroformate (0.3 ml) in the same manner as for taxodine (55). A brown gum (18 mg) was obtained after the usual work up. GC-MS analysis of the crude product indicated the presence of homoerythratine in trace amounts, and two products of molecular weights 369 (62) and 387 (61) in approximately 2 to 1 ratio.

MS: m/z 387 (M^+, 100), 355 (68), 253 (47), 239 (96), 102 (82).

MS: m/z 369 (M^+, 100), 267 (94), 253 (74), 102 (12).

Separation of the crude product by PLC on an alumina support, developed with dichloromethane, afforded eight fractions. The first fraction was a mixture of product of mass 369 and 387, while the second

fraction was composed mainly of a product of mass 387. The rest of the fractions contained compounds which were not detected in the original crude product. The ^{1}H n.m.r. and ^{13}C n.m.r data of the second fraction (M^+ 387) were too complex to allow interpretation.

A second batch of homoerythratine (20 mg, 0.06 mmol) was refluxed with methyl chloroformate (0.5 ml) as above. Work-up of the mixture afforded a yellow gum (19 mg). This was immediately used for the next reaction without purification.

Reduction of (61) and (62) with Lithium Aluminum Hydride

To a solution of the crude product of the first batch of homoerythratine containing the carbamate (62) was added lithium aluminum hydride (*ca* 10 mg) and the resulting mixture refluxed for 1.5h. After the usual work up the crude product obtained (7.5 mg) was analyzed by GC-MS. Three major components of molecular weights 297, 315 and 341, and a minor component of molecular weight 399 were identified from the GC trace; the components of masses 315, 341 and 399 were the same components from the crude reactant; 297 was the expected reduction product (63).

MS: m/z 297 (M^+, 74), 282 (21), 254 (16), 239 (11), 225 (29), 165 (21), 70 (46), 58 (45), 57 (100).

The crude product obtained from the reaction of methyl chloroformate and the second batch of homoerythratine was reduced with lithium aluminum hydride in the same manner as above. The crude product obtained (14 mg) was found to contain three components of molecular weights 295, 297, and 345 (64, major component).

MS: 297 (M^+, 59), 296 (100), 70 (13), 58 (88), 57 (20), 44 (18), 42 (25).

MS: 295 (M^+, 72), 223 (22), 165 (31), 70 (51), 58 (50), 57 (100), 44(27), 42 (40).

MS: m/z 345 (M^+, 70), 314 (38), 70 (35), 58 (100), 57 (50), 44 (35), 42 (33).

Double development PLC separation of the crude product (methanol-dichloromethane, 4%) afforded eight fractions which by GC-MS analysis was found to contain a number of components not initially present in the crude product. Two fractions (fractions 3 and 4) were isolated pure. Fraction 3 was obtained as a pale yellow gum (2.5 mg) after evaporation of the solvent *in vacuo*.

MS: m/z 345 (M^+, 70, accurate mass 345.192, $C_{20}H_{27}NO_4$ requires 345.1938) see Figure 2.5, Chapter 2.

^{1}H n.m.r. δ (CDCl3): 6.67 (s, 1H, $H_{10}{}^a$), 6.44 (s, 1H, $H_{13}{}^a$), 5.92 (dd, J 0.45, 3.7, OCH_2O), 3.94 (m, 1H, H_3); 3.39 (s, OCH_3); 2.23 (s, br, 3H NCH_3).

^{13}C n.m.r. δ (CDCl3): 147.30 (=C<); 146 (=C<); 136.62 (+C<); 130.96 (=C<); 130.56 (=C<); 127.29 (=C<); 108.88 (=CH X 2); 101.43 (OCH_2O); 81.93 (-OCH, C_3); 71.04 (-OCH, C_2); 57.22 (OCH_3); 54.63 (CH_2); 50.40 (CH_2); 45.44 (NCH_3); 37.89 (CH_2); 34.35 (CH_2); 31.58 (CH_2); 29.00 (CH_2); 27.20 (CH_2).

FT-IR: 3406 cm^{-1} (br, OH^-).

Evaporation of the organic solvent from fraction 4 afforded a yellow gum (3 mg). GC-MS analysis of this isolate indicated a molecular weight of 361 which was not detected in the crude extract.

MS: m/z 361 (M^+, 78, accurate mass 361.188, $C_{20}H_{27}NO_5$ requires 361.1887); 330 (19); 312 (31); 70 (40); 58 (100); 57 (59); 44 (40); 42 (33).

^{1}H n.m.r. δ ($CDCl_3$): 6.67 (s, 1H, $H_{10}{}^a$); 6.52 (s, 1H, $H_{13}{}^a$); 5.92 (dd, 0.5, 3.7, 2H, OCH_2O); 4.45 (d, J 3.9, 1H, H_4); 3.84 (s, br, 1H, H_3); 3.68 (m, 1H, H_2); 3.40 (s, 3H, C_2-$OC\underline{H}_3$); 2.26 (s, br, 3H, $NC\underline{H}_3$).

^{13}C n.m.r. δ (CDCl3): 147.55 (=C<); 146.53 (=C<); 136.05 (=C< X 2); 133.22 (=C<); 132.97 (=C<); 108.70 (=CH, $C_{13}{}^a$), 108.61 (=CH, $C_{10}{}^a$); 101.52 (OCH_2O); 76.45 (-$\underline{C}_4H$); 73.65 (-$\underline{C}_3H$); 66.81 (-$\underline{C}_2H$); 57.43 (C_2-OCH_3); 54.56 ($\underline{C}H_2$); 50.55 ($\underline{C}H_2$); 38.42 ($\underline{C}H_2$); 28.78 ($\underline{C}H_2$); 28.24 ($\underline{C}H_2$); 27.91 ($\underline{C}H_2$).

FT-IR: 3204 cm^{-1} (br, s, OH^-).

Reaction of the Homomorphine (68) with Magnesium Iodide.

A mixture of freshly prepared magnesium iodide (39.2 mg), homomorphine (66) (50 mg) and dry benzene (3 ml) was refluxed together under nitrogen for 3h and stirred at room temprature for another three hours. The solvent was evaporated *in vacuo* and dry ether (5 ml) was added to the residue; lithium aluminum hydride was then added and the mixture refluxed for 1.5h. After the usual work up, a yellow brown powder was obtained (40 mg). The crude product contained only the unreacted homomorphine as indicated in its GC-MS data.

This reaction was repeated on the recovered homomorphine (40 mg) increasing the concentration of magnesium iodide five times. Work up after reduction with lithium aluminum hydride afforded a pale yellow oil which solidified on standing. GC-MS analysis indicated the presence of the unreacted homomorphine (66) only.

7.5. Extraction, Isolation and Characterization of Alkaloids

7.5.1 Alkaloid Testing: Mayer's Test

This was performed following the procedure of Culvenor and Fitzgerald[208] with some slight modifications. Approximately five grams of ground fresh plant material was extracted with ammoniacal dichloromethane. The solvent was very carefully drawn off with a Pasteur pipette and, where necessary, filtered through a cotton plug, directly into a 10 ml test tube. The filtered dichloromethane extract was partitioned with 4 to 5 drops of sulfuric acid (2M), then 3-4 drops of the aqueous layer was drawn off and added with a drop or two of Mayer's reagent . The amount of alkaloid was assessed as +1 for faint turbidity to +4 for heavy turbidity.

In cases where Mayer's test showed negative results, the ground fresh material was also extracted exhaustively with cold methanol and the concentrated extract was taken up with minimal amounts of dichloromethane, then filtered. The dichloromethane extract was concentrated, then partitioned with a few drops of dilute sulfuric acid (2.5%) and tested with Mayer's reagent. Where more plant sample was available, the alkaloid was separated from the crude methanol extract by acid base extraction. It was subjected to analytical tlc and sprayed with Schlittler's or Dragendorff's reagent to confirm the presence of alkaloids.

No separate tests were made for the water-soluble alkaloids, the very weak bases, and the amide-type alkaloids.

7.5.2. General Procedure for Alkaloid Extraction.

The blended fresh plant or powdered freeze-dried plant materials were extracted exhaustively with cold methanol. The methanol extract was then concentrated *in vacuo*, an equal volume of 2.5% sulfuric acid-methanol mixture (1:1), added and the mixture filtered. The acidic filtrate was defatted with light petroleum (b.p. 40-60^{o}). The pH of the aqueous layer was then adjusted to pH 2-4, and/or pH 8-10 using sodium hydroxide (2M), and partitioned with dichloromethane in either or both pH ranges. In the case of bulk extractions, the aqueous layer was partitioned with dichloromethane by ultrasonication to minimize frothing. In other cases where heavy precipitation took place upon basification of the aqueous layer, the organic layer was separated by centrifugation. All extracts, including that of the petroleum ether from defatting, were tested for the presence of alkaloids by spraying analytical TLC plates with Schlittler's or Dragendoff's reagents.

In some cases, the crude alkaloid extracts were cleaned by re-dissolving them in dichloromethane and partitioning with sulfuric acid (2.5%). The alkaloidal component was then re-extracted from either or both pH ranges (pH 2-4 or pH 8-10). All the crude alkaloid extracts were monitored for alkaloidal components by the GC-MS technique, and by spraying analytical TLC plates with alkaloid spray reagents such as Schlittler's or Dragendorff's reagent. Results of the alkaloid testing are given in Tables 2.2, 2.3, and 2.4 (Chapter 2).

Herbarium specimens for all plants worked on have been lodged with the Tasmanian Herbarium, Sandy Bay Campus, University of Tasmania.

7.5.3 General GC-MS Screening Procedures

Most of the crude alkaloid extracts used for GC-MS analysis were initially separated from the polar components by passing through an alumina plug. The nature of the solvent system for elution varied according to the nature of the alkaloidal components and their impurities. In most cases, solvents such as dichloromethane or chloroform were used for initial elution followed by a slightly more polar solvent system such as 1 to 3% methanol in dichloromethane or in chloroform, depending on their chromatographic behaviour on silica. Where a possible loss of the more polar alkaloids on the alumina support was suspected, the alkaloid profile of the crude extract was monitored by direct probe mass spectrometry. The suitability of the resulting fractions for GC-MS analysis was assessed from their thin layer chromatogram.

The method files used in the GC-MS analysis of the crude alkaloid extracts and the resulting fractions are given in Table 7.2. In all these files, the injection port was set at 260^{o}C and the transfer line at 290^{o}C. Method files 1 and 2 were applied in either split or splitless mode and were used for general screening. Method file 1 was most applicable to crude alkaloid extract analysis, while method file 2 was employed in the analysis of most fractions from the crude alkaloid extract, and to monitor products from synthesis. The cold on-column injection (method file 3) was used whenever components were suspected to be temperature sensitive. Where the the detection problem was chromatographic, instead of being temperature sensitive, a direct MS probe was used for analysis.

Table 7.2 GC-MS Method File Parameters

Method File	Initial Temp. ^{o}C	Initial Time (min)	Rate ^{o}C/min.	Final Temp. ^{o}C	Final Time (min)
1	50	1.00	40.00 10.00	250 290	0 10.00
2	250	0	10	290	
3	30	0	30 10	150 300	0 5.00

Chromatographic sensitivity of the capillary column was regularly monitored using a standard activity mix. Whenever necessary, the column was regenerated by very slowly flushing it with N, O-*bis*(trimethylsilyl) trifluoroacetamide (BSTFA) to silylate the column.

7.5.4. Alkaloids of *Wurmbea dioica* ssp. *dioica* (Chapter 3, Section 3.2 and Chapter 4, Section 4.2)

Several sample batches of this plant were collected from ten different places in Australia. Most collections from the same source in the same collection season were treated as one batch (Table 3.3, Chapter 3). The amounts of each collection determined the extent of the separation of the crude extract. However, alkaloids were extracted from each of the ten batches of samples, separated by chromatographic procedures, and all of the resulting fractions analyzed by GC-MS in the search for the dibenz[*d,f*]azecine-type alkaloids.

7.5.4.1 Alkaloids of Batch 1 of *W. dioica* ssp. *dioica*

This first batch of sample (453 g, wet weight) was collected on 3 October 1986 in Upper Domain, Hobart. The fresh plant sample was blended and soaked in ammoniacal dichloromethane overnight, then four more times. The marc, as indicated by the Mayer's test, remained alkaloid positive. It was then extracted exhaustively with a dichloromethane-methanol mixture (1:1). The alkaloidal components of the combined crude extract were extracted following the usual acid-base extraction procedures. Based on the polarity of the major component, as indicated by the analytical thin layer chromatogram, the crude alkaloid extract (39 mg) was separated by PLC on silica using 10% methanol in dichloromethane to develop the plate. Thirteen bands, (1A to 1M) as detected by the UV light and/or as indicated alkaloid positive by spraying with Schlittler's reagent, were collected.

The first three fractions did not contain any alkaloids. Fraction 1D (7 mg) contained an alkaloid of molecular weight 371 (79) with trace amounts of other components.

MS: m/z 371 (M^+, 22, accurate mass 371.1741, $C_{21}H_{25}NO_5$ requires 371.1731); 356 (14); 340 (100). **MS** TMS derivative: m/z 515 (M^+).

^{1}H n.m.r. δ ($CDCl_3$): see (79)

GC-IR: 3564 cm^{-1}, (s, OH^-).

Fraction 1E was not obtained pure. Fraction 1F was re-crystallized from dichloromethane-diethyether as straw yellow needle-like crystals (66) (10 mg, m.p. 202-203^o-dec).

MS: m/z 357 (M^+, 100, accurate mass 357.1552, $C_{20}H_{23}NO_5$ requires 357.1574); 342 (9); 340 (14); 326 (28); 282 (18), 256 (10), 181 (18). **MS** TMS derivative: m/z 429 (M^+).

^{1}H n.m.r. and **^{13}C n.m.r.** δ ($CDCl_3$): see (66).

IR : 3600-3100 cm^-, (br, OH^-).

Fraction 1G contained an alkaloid of molecular weight 385, together with some very minor alkaloidal components, and was obtained as a yellow gum after evaporation of the solvent *in vacuo* (83) (5 mg).

MS: m/z 385 (M^+, 18, accurate mass 385.1892, $C_{22}H_{27}NO_5$ requires 385.1888); 370 (13); 354 (100). **MS** TMS derivative: 457 (M^+).

^{1}H n.m.r. δ ($CDCl_3$): see (83)

FT-IR : 3312 cm^{-1}, (OH^-)

The rest of the fractions were impure except for fraction 12, which contained colchicine (78), the major component of the crude alkaloid extract. The identity of this isolate was confirmed by comparison of its spectroscopic properties with an authentic sample of colchicine. A dibenz[*d,f*]azecine-type alkaloid (M^+ 373), was detected as a minor component of fraction 11.

MS: m/z 373 (M^+, 100); 358 (40); 242 (43); 315 (19); 301 (37); 269 (33); 70 (69); 58 (61); 57 (63).

7.5.4.2 Alkaloids of Batch 4 of *W. dioica* ssp. *dioica*.

This plant sample (2.61 kg, wet weight) was collected in two lots (4/10/87; 10/10/87) from the southernmost part of Epping Forest, by the side of Midland Highway. The bulbs (90 g) were treated separately from the stems, flowers and leaves. The alkaloids were extracted by the usual acid-base alkaloid extraction procedure, at both pH 4 and 10. GC-MS

analysis of the acidic extract indicated the main component to be colchicine (78), with trace amounts of two components whose MS, fragments were characteristic of the dibenz[*d,f*]azecine-type alkaloids.

MS: m/z 355 (M^+, 100), 340 (9); 283 (19), 70 (90); 58 (63); 57 (88).

MS: 387 (M^+, 78); 372 (13); 356 (18); 315 (20); 70 (100); 58 (67); 57 (63).

At least 25 major and minor alkaloidal components were identified in the GC-MS analysis of the crude alkaloid extract at basic pH (48 mg). Four components showed the characteristic mass spectrum of the dibenz[*d,f*]azecine systems, of molecular weights 355, 373, 387, and 357 the first three having been identified above.

MS: m/z 357 (M^+, 100); 342 (36); 285 (30); 70 (76); 58 (55); 57 (70).

The alkaloids of the freeze-dried sample of leaves, stems and flowers (2.5 kg) were extracted with dichloromethane in the usual manner affording an alkaloid extract at pH 4 (100 mg) and at pH 8-10 (1.7 g). These alkaloid extracts were not cleaned-up by the usual acid-base re-extraction procedures.

Alkaloid Extract at pH 2-4

PLC separation of the extract at acidic pH, which was overdeveloped with methanol in dichloromethane (7%), afforded nine fractions which by GC-MS analysis were shown to be impure, except for the first fraction. The first fraction (1.5 mg) was found to contain a major component whose MS fragmentation suggested a dibenz[*d,f*]azecine-type skeleton with just a trace of other components. However, its ^1H n.m.r. spectrum showed very broad signals. This isolate was then cleaned up by basifying with ammonia, and re-extracting with

dichloromethane. The organic extract was dried over anhydrous sodium sulfate, and passed through an alumina plug eluting with dichloromethane, giving rise to a pale yellow gum (32a) (< 1mg).

MS: m/z 341 (M^+, 50, accurate mass 341.1983, $C_{21}H_{27}NO_3$ requires 341.1989); 326 (12), 283 (8); 269 (36); 70 (70); 58 (70); 57 (100).

^{1}H n.m.r. δ (CDCl3): 7.19 (d, J 8.5, 1H, H_{11}); 6.89 (dd, J 8.5, 2.7, 1H, H_{12}); 6.76 (s, 1H, H_4); 6.60 (d, J 2.8, 1H, H_{14}); 6.52 (s, 1H, H_1); 3.92 (s, 3H, C_3-OC$\underline{H}_3$); 3.79 (s, 3H, C_2-OC$\underline{H}_3$); 3.78 (s, 3H, C_{13}-OC$\underline{H}_3$); 2.59 (m, 3H, C$\underline{H}_2$); 2.36 (m, 3H, C$\underline{H}_2$); 2.20 (m, 1H, C$\underline{H}_2$); 2.08 (s, 3H, NC$\underline{H}_3$); 1.77 -1.45 (m, 2H, C$\underline{H}_2$).

^{13}C n.m.r. δ (CDCl3): see Table 3.4 (Chapter 2).

Alkaloid extract (pH 2-4) of batch 4 stems, leaves and flowers

GC-MS analysis of the basic alkaloid extract from stems, leaves and flowers of batch 4 of *W. dioica* ssp. *dioica* indicated the presence of the dibenz[*d,f*]azecines-type systems described earlier including one of molecular mass 371.

MS: m/z 371 (M^+, 96); 356 (20); 340 (22); 313 (18); 299 (45); 70 (100); 58 (72); 57 (75).

Separation of the basic extract (1.6 g) by PLC developed with 6% methanol in dichloromethane, in five batches gave rise to a number of fractions. GC-MS analysis of each of the fractions allowed the combination of these fractions to a total of nine fractions (fractions 4A to 4I). Each of these fractions was fractionated further by PLC in search for wurmbazecine (M^+ 341), and other dibenz[*d,f*]azecine-type alkaloids.

Alkaloids of Fraction 4A and 4C

Fractionation of the first fraction 4A (254 mg) afforded pure isolates in fractions 4A4 and 4A5 only, which were combined together. Its structure was elucidated as (84) from the spectroscopic data. This new alkaloid was named dioicinone (84).

MS: m/z 313 (M^+, 40, accurate mass 313.1685, $C_{19}H_{23}NO_3$ requires 313.1676); 312 (30); 286 (19); 285 (100, accurate mass 285.136, $C_{17}H_{19}NO_3$ requires 285.136); 284 (33); 270 (17, accurate mass 257.138, $C_{16}H_{19}NO_2$ requires 257.1414); 256 (17); 244 (17); 243 (16); 242 (64, accurate mass 242.116, $C_{15}H_{16}NO_2$ requires 242.1180); 229 (34, accurate mass 229.110, $C_{14}H_{15}NO_2$ requires 229.1101).

^{1}H n.m.r., and **^{13}C n.m.r.** δ ($CDCl_3$) see (84)

FT-IR: 1722 cm^{-1}, (s, >C=0).

Fraction 4C (156 mg) was fractionated by PLC, double developed with methanol-dichloromethane (4%) allowing the isolation of a new alkaloid (86) in fraction 4C5 (yellow gum, 12 mg).

MS: m/z 311 (M^+, 22); 310 (M^+-H, 37, accurate mass 310.14410, $C_{19}H_{20}NO_3$ requires 310.1441); 284 (18); 283 (100); 268 (14); 244 (7); 240 (9). **CIMS** 312 (M^++ H, 100).

^{1}H n.m.r. and **^{13}C n.m.r.** δ ($CDCl_3$) see (86)

FT-IR: 1656 cm^{-1} (s, >C=O).

Separation of Fraction 4D

Fraction 4D (181 mg) was separated by PLC with triple development using methanol-dichloromethane system (3.5%), from which twenty five fractions were isolated and analyzed by GC-MS technique. The first fraction (9 mg) appeared to be relatively pure with a

major component of molecular weight 355, and whose mass spectrum was characteristic of a dibenz[*d.f*]azecine-type system. However, the ^{1}H n.m.r. spectrum indicated an impure sample; this was not purified further. All the other fractions were also impure except for fractions 4D9, 4D10, 4D18, 4D20. The last two fractions contained the new homoproaporphine alkaloid dioicinone (84) isolated earlier in this work. Fractions 4D9 and 4D10 were combined together and characterized spectroscopically as the homoaporphine alkaloid (82).

MS: m/z 385 (M^{+}, 16, accurate mass 385.1871, $C_{22}H_{27}NO_5$ requires 385.1888); 370 (19); 356 (21); 354 (100).

^{1}H n.m.r. and **^{13}C n.m.r.** see (82)

FT-IR: 3234 cm^{-1} (br, OH^{-}).

Fraction 4D18 was obtained as a pale yellow gum (16 mg), and was characterized as (87) from its spectroscopic properties.

MS: m/z 355 (M^{+}, 43, accurate mass 355.1421, $C_{20}H_{21}NO_5$ requires 355.1418); 326 (20); 312 (12); 298 (15); 296 (27); 192 (14); 191 (100); 83 (14).

^{1}H n.m.r. and **^{13}C n.m.r.** see (87).

FT-IR; 1632 cm^{-1}, (s, >C=O).

Separation of Fraction 4G

Further fractionation of fraction 4G (160 mg) afforded thirteen fractions; only fractions 4G6 to 4G9 contained alkaloids. Fractions 4G6 and 4G7 had a major component of molecular weight 359 although fraction 4G6 contained more impurities. Re-crystallization of 4G6 (48 mg) over dichloromethane-ether afforded white needle-like crystals (4G6C) (12 mg, m.p. 197-198-dec).

MS: m/z 301 (M^+, 7, accurate mass 301.1652, $C_{18}H_{23}NO_3$ requires 301.1676); 300 (6); 274 (17); 273 (100); 272 (27); 256 (22); 228 (22).
MS: 4G6C + D_2O: m/z 303 (9), 302 (7), 275 (100).
^{1}H n.m.r. and **^{13}C n.m.r.** δ ($CD_3OD/CDCl_3$): see (88)
FT-IR: 3453 cm^{-1} (br, s, OH^-).

Fraction 4G7 obtained as yellow gum (24 mg), was characterized spectroscopically as (89).
MS: m/z 359 (M^+, 100, accurate mass 359.1742, $C_{20}H_{25}NO_5$ requires 359.1731); 344 (16); 342 (11); 328 (25); 284 (25); 181 (15); 115 (8).
^{1}H n.m.r. and **^{13}C n.m.r.** δ ($CDCl_3$): see (89)
FTIR: 3650-3300 cm^{-1} (br, OH^-); 1057 cm^{-1} (s, =C-O-C).

7.5.4.3. Alkaloids of Batch 6 of W. dioica ssp. dioica.

More samples of this plant were collected to re-isolate wurmbazecine (32a) and the other dibenz[*d,f*]azecine-type alkaloids detected previously. A total of 5.69 kg of mature plants were collected over a period of six days (21, 23, 25, and 26 September 1988), from Rosny Point in Hobart, Tasmania. The freeze dried plant sample was extracted exhaustively with methanol and the alkaloids extracted in the usual manner. Three main extracts were obtained: the petroleum ether extract at acid pH (6B; 850 mg), the dichloromethane extract at acid pH (6C; 1.10 g), and the dichloromethane extract at basic pH (6D; 7.51 g). Analytical TLC of these extracts indicated the presence of very polar components. Extract 6C was found to contain mostly non-alkaloids as shown by its thin layer chromatogram and the GC ion trace.

Extract 6B (360 mg) was separated by multiple development radial layer chromatography eluting with an increasing solvent polarity

(methanol in dichloromethane, 2 to 50%). Fraction collection was monitored by analytical TLC, combining fractions of similar Rf values. A total of nineteen fractions were collected all of which were found to contain a number of alkaloidal components. Alkaloid components of molecular weights 387, 373, and 355, whose MS spectra were characteristic of dibenz[*d,f*]azecine-type systems, were detected in fractions 6B13 to 6B18. Fraction 6B14 contained the homopropaporphine alkaloid (10) with trace amounts of the dibenz[*d,f*]azecine alkaloid (MW 387).

Initial GC-MS analysis of fraction 6B13 (yellow gum, 35 mg) showed a dibenz[*d,f*][azecine-type alkaloid, of molecular weight 387 as the major component. Direct probe mass spectroscopy, however, showed a molecular mass of 385. This was identified as the known compound O-methylandrocymbine (90) from its spectroscopic data.

MS: m/z 385 (M^+, 100, accurate mass 385.1879, $C_{22}H_{27}NO_4$ requires 385.1888); 354 (52); 327 (30); 284 (28).

^{1}H n.m.r. and **^{13}C n.m.r.** δ ($CDCl_3$): see (90)

Alkaloids of extract 6D

Extract 6D (7.51 g) was re-dissolved in dichloromethane and light petroleum slowly added causing precipitation. The precipitate (precipitate 1) was separated from the supernatant liquid by filtration, and the supernatant liquid added with more light petroleum ether. Filtration gave rise to a light yellow solution which was concentrated *in vacuo* (Extract 6D1; 3.83 g), and a reddish black dichloromethane soluble precipitate (Extract 6D2; 1.40 g). Dissolution of precipitate 1 with minimal amounts of dichloromethane and cooling the resulting mixture in the freezer for six hours afforded extract 6D3 (1.65 g) from the concentrated

supernatant liquid, and 6D4 (620 mg) from the resulting dichloromethane insoluble precipitate. Extracts 6D2 and 6D3 were combined together based on their alkaloid profile. Further attempts to separate the dibenz[*d,f*]azecine-type alkaloids, particularly those of molecular weights 355, 387, and 373, and those containing the pure isolates are described below. No details are given for the other impure fractions.

Fractionation of extract 6D1 by quick column chromatography eluting initially with dichloromethane-petroleum ether (1:1), then increasing the solvent polarity up to 20% methanol in dichloromethane. Fractions 6D1D to 6D1G contained dibenz[*d,f*]azecine-type alkaloids. The combined fractions of 6D1D and 6D1E were separated further by PLC using methanol-dichloromethane (5%) for development. PLC separation of the first fraction 6D1D1 (251 mg) afforded six fractions, which were subjected to further fractionation. The resulting fraction 6D1D1A afforded seven fractions on PLC separation using methanol/dichloromethane (6%) for development. Most fractions were not pure, however, the first fraction 6D1D1A1 (yellow gum, 1.5 mg) showed only traces of impurities, was passed in an alumina plug and analyzed spectroscopically. Sample size limitation did not allow characterization of this isolate.

MS: m/z 385 (M^+, 65, accurate mass 385.1485, $C_{21}H_{23}NO_6$ requires 385.1525); 357 (14); 326 (21); 271 (100, accurate mass 271.118, $C_{16}H_{17}NO_3$ requires 271.1206); 270 (70); 258 (48); 254 (38).

^{1}H n.m.r. δ ($CDCl_3$): 6.29 (s, 1H); 5.99 (s, 1H); 5.92 (dd, J 1.5, 10.6, 2H, $OC\underline{H}_2O$); 3.87 (s, 3H, $OC\underline{H}_3$); 3.60 (s, 3H, $OC\underline{H}_3$), 2.17 (s, 3H, $NC\underline{H}_3$).

Double development PLC separation of 6D1D1G using methanol-dichloromethane (5%) for development afforded eight fractions. The first

fraction, 6D1D1G1 (7 mg) contained the targetted dibenz[*d,f*]azecine alkaloid (M^+ 355) as a minor component, but could not be separated further. Fraction 6D1D1H afforded nine fractions after PLC separation using methanol-dichloromethane (8%) for development. Only fractions 8 (fraction 6D1D1H8) (15 mg) and 9 (fraction 6D1D1H9) (26 mg) contained the desired dibenz[*d,f*]azecine alkaloid (M^+ 387), as minor component. Further attempts to isolate this from the major component (dioicinone) were unsuccessful.

Fraction 6D1D1I was separated by PLC, developing the plates four times with 5% methanol in dichloromethane, affording eight fractions. The last fraction (6D1D1I8, 25 mg) contained the targetted dibenz[*d,f*]azecine alkaloid (M^+ 373) which appeared to be the major component in the GC trace. The ^1H n.m.r. spectrum of this isolate was very complex indicating an impure fraction. Attempts to further purify this fraction, along with other fractions containing this alkaloid of interest were also unsuccessful.

One pure fraction 6D1D1I4 (9 mg) was however obtained pure and characterized as a new homoaporphine O-methyldioicinine (91).

MS: m/z 315 (M^+, 10, accurate mass 315.178, $C_{19}H_{25}NO_3$ requires 315.1834); 287 (100); 286 (40); 270 (20); 242 (28).

^1H n.m.r. δ ($CDCl_3$): 6.49 (s, 1H, H_3); 4.37 (m, 1H, H_{12}); 4.12 (m, 1H, H_{11}); 3.86 (s, 3H, C_2-OC$\underline{H}_3$); 3.15 (s, br, 1H, H_{6a}); 3.07 (m, 2H, H_{4a}, H_{13a}); 3.04 (m, 1H, H_{5a}); 2.72 (m, 2H, H_{13b}; H_{5b}); 2.49 s, 3H, NC$\underline{H}_3$); 2.35 (m, 1H, C$\underline{H}_2$); 1.96 - 1.44 (m, 8H, C$\underline{H}_2$).

^{13}C n.m.r. δ ($CDCl_3$): 144.46 (=C<, C_1); 143.78 (=C<, C_2); 136.24 (=C<, C_{1a}); 126.99 (=C<, C_{3b}); 124.80 (=C<, C_{3a}); 111.06 (=$\underline{C}$H, C_3); 93.38 (O$\underline{C}$H, C_{13}); 65.68 (O$\underline{C}$H, C_{11}); 59.78 ($\underline{C}$H, C_{6a}); 56.88 (O$\underline{C}H_3$);

54.83 ($\underline{C}H_2$, C_5); 43.98 ($N\underline{C}H_3$); 40.82 (C_{8a}); 32.60 ($\underline{C}H_2$); 29.30 ($\underline{C}H_2$ x 2); 28.86 ($\underline{C}H_2$); 28.41 ($\underline{C}H_2$ x 2).

Fractions 6D1F2 and 6D1F3 from the radial layer chromatography with multiple development, of the combined fractions 6D1F and 6D1G were separated by PLC developed four times with methanol-dichloromethane (4%). The resulting fractions 6D1F2C (5 mg) and 6D1F2G (16 mg) contained a number of alkaloidal components including trace amounts of the desired dibenz[*d,f*]azecine-type alkaloids wurmbazecine (M^+ 341) and the other dibenz[*d,f*]azecine alkaloid of molecular weight 357, respectively. It was not possible to isolate these dibenz[*d,f*]azecine alkaloids pure.

Alkaloids of Extract 6D2

Double development PLC separation of fraction 6D2J (methanol-dichloromethane, 7%), obtained from the radial layer chromatography of extract 6D2, resulted in the re-isolation of dioicinone (84), wurmbeanine (66), and the isolation for the first time from this source of the known alkaloid autumnaline (10).

7.5.4.4. Alkaloids of Batch 9 of *W. dioica* ssp. *dioica*.

This sample batch of mature *Wurmbea dioica* ssp. *dioica* (1.10 kg) was collected from Epping Forest on 1 October, 1989. Acid base extraction of the concentrated methanol extract afforded a dichloromethane alkaloid extract at basic pH (1.9 g). This extract was passed through a quick column giving rise to four combined fractions 9A to 9D. Each of these fractions was fractionated further to screen for wurmbazecine (32a) and other dibenz[*d,f*]azecine-type alkaloids.

Fraction 9B (700 mg) was separated by PLC with triple development using 4 % methanol in dichloromethane. All the fractions collected contained a number of components. Fraction 9B3 contained a dibenz[*d,f*]azecine alkaloid (M^+ 355) in trace amounts, while fraction 9B4 and 9B5 contained a dibenz[*d,f*]azecine alkaloid (M^+ 401) that has not been detected in any of the previous *W. dioica* ssp. *dioica* batches. Its proposed structure is given in Table 3.2.

MS: m/z 401 (M^+, 100, 386 (38); 370 (53); 329 (58); 298 (35); 70 (69); 58 (49); 57 (47).

The dibenz[*d,f*]azecine alkaloid of molecular weight 371 was detected in fractions 9B8 and 9B9, while that of molecular weight 387 was detected in fractions 9B9 and 9B10. Fractions 9B11 to 9B14 contained the dibenz[*d,f*]azecine alkaloid of molecular weight 355. Fraction 9B14 also contained another dibenz[*d,f*]azecine alkaloid of molecular weight 341, which was detected for the first time and its proposed structure is given in Table 3.2.

MS: m/z 341 (M^+, 83); 326 (28); 309 (72); 297 (22); 283 (28); 43 (100).

7.5.5. Alkaloids of *Wurmbea pygmaea*.

Initial sample of *W. pygmaea* consisted of five stalks of mature plant material collected from South of Perth on 12 September 1988. The fresh plant sample was blended and extracted with methanol in the University of Western Australia (UWA) and the alkaloids were extracted following the usual procedure. The crude alkaloid extract (WP1) (4.5 mg) was analyzed by GC-MS both in UWA and in the University of Tasmania. The presence of three alkaloidal components was indicated by GC-MS

analysis. The major one had a molecular weight of 387 and two minor ones had molecular weights of 373 and 371.

MS: m/z 387 (M^+, 26); 372 (10); 356 (10); 315 (9); 284 (9); 70 (100); 58 (55); 57 (53).

MS: m/z 373 (M^+, 57); 358 (14); 342 (15); 301 (13); 269 (14); 70 (100); 58 (76); 57 (70).

MS: m/z 371 (M^+, 49); 354 (100); 340 (13); 338 (15).

The second batch of *Wurmbea. pygmaea* (85 g) was a combined collection from Welshpool Road, 500 meters west of the intersection of Crystal Brook Road, and from a place 500 meters east of Mills road, all in Western Australia. The material was collected on 5 August, 1989 and received in Hobart on 7 August, 1989. It was immediately extracted with methanol upon receipt. The fruits (30.25 g) were treated separately from the leaves and stems. The alkaloid extract of the basic solution of the fruits (WP2A; 8 mg) contained four alkaloidal components:

MS: m/z 385 (M^+, 38); 368 (100); 310 (20); 383 (30); 192 (77).

MS: m/z 371 (M^+, 40); 354 (100); 342 (20); 338 (22).

MS: m/z 341 (M^+, 78); 340 (100); 326 (12); 324 (20).

MS: m/z 313 (M^+, 50); 312 (100); 298 (80).

The sample size of WP2A did not allow further fractionation. The alkaloid extract of the acidic solution (pH 4) (WP2B, 5 mg) contained only colchicine and another alkaloid of molecular weight 369 which was not investigated further.

MS: m/z 369 (M^+, 40); 368 (100); 352 (18).

The alkaloid extract of the leaves and stems (54.7 g) at acidic pH (WP3B, 6 mg) had the same components as extract WP2B. The basic

pH alkaloid extract (WP3A, 40 mg) contained the same alkaloid identified in extract WP2A (M^+ 341) as a major component. A second component of molecular weight 385 was present in a trace amount. This extract was passed through an alumina quick column affording a relatively clean fraction containing the major component as a yellow-green gum (22 mg). **MS**: 341 (M^+, 43, accurate mass 371.174, $C_{21}H_{25}NO_5$ requires 371.1731). This proved to be the known alkaloid multifloramine. A complete nmr chemical shift assignment is given in structure (93).

7.5.6. Alkaloids of *Baeometra uniflora*

This plant sample (320 g, wet weight) was collected from the South of Perth, Western Australia on 16 September, 1988, when the flowers were just starting to bloom. The sample was extracted on the same day it was collected following the usual acid-base extraction procedure for alkaloids, treating the bulbs separately from the leaves and stems.

The alkaloid profiles of the leaves and stems extracts at both pH 2-4 and pH 8-10 were very similar and were combined together (53 mg). The major component of this extract had a molecular mass of 341. Other components, present in very minor concentrations, included the dibenz[*d,f*]azecine-type alkaloid of molecular weight 387 as in *W. dioica* ssp. *dioica*, and other alkaloids, whose mass fragmentation patterns could not be fully interpreted due to poor chromatography. The isolation of the major component was carried out by PLC developed with methanol in dichloromethane (5%). Fraction 1 contained the major component as a pale green gum which was not re-crystallyzed (40 mg). Its mass spectrum and the proton and carbon n.m.r. spectra established

this alkaloid to be the known aporphine alkaloid, isocorydine (94). Its identity was confirmed by comparison with an authentic sample of isocorydine.

MS: m/z 341 (M^+, 25, accurate mass 341.161, $C_{20}H_{23}NO_4$ requires 341.1625).

The dichloromethane extract at pH 2-4 of the bulb did not chromatograph well on GC-MS analysis. The major component, isocorydine, and a minor component of molecular weight 387 with a characteristic mass fragments of a dibenz[d,f]azecine type alkaloid, were, however, identified. GC-MS analysis of the pH 8-10 alkaloid extract showed that it contained mainly the alkaloid isocorydine (94) with trace amounts of other components. Two of the trace components showed extremely weak ms peaks, except for the base peak at m/z 192. One had a molecular weight of 385 while the parent ion of the other could not be identified.

7.5.7. *Colchicum autumnale* and *Colchicum byzantinum*

The sample of *C. autumnale* was obtained from a private supplier in Salamanca market on 19 May, 1990. Alkaloid extracts at both acidic and basic pH of the roots, bulbs, and the very young leaves were obtained following the usual alkaloid extraction procedure. Crude alkaloid extract was also obtained from a few leaves of *C. byzantinum*, collected from Hobart Botanical garden on 18 September, 1990. Results of the GC-MS analysis of each of these extracts is given in Table 4.1 (Chapter 4). The major non-tropolonic alkaloid component was found in the leaves only.

MS: m/z 317 (M^+, 39); 316 (100); 288 (20); 274 (40).

7.5.8. *Gloriosa plantii, Sandersonia aurantica, and Littonia modesta.*

Freshly cut fruit-bearing stems of *Gloriosa plantii* (121.8 g) *Sandersonia aurantica* (90.8 g fruits; 117.3 g leaves and stems) and *Littonia modesta* (71.0 g leaves and stems; 61.0 g fruits) grown in a nursery in Kalorama, Victoria, Australia, were delivered to Hobart airport on the same day they were collected (27 April, 1990)[209]. The material was extracted with methanol immediately upon receipt and the alkaloid extracts analyzed by TLC and GC-MS. Table 7.3 summarizes the results of the GC-MS screening of these extracts.

Table 7.3

Gloriosa plantii	
leaves, stems (pH 4)	colchicine, Major, 385 (bp 312), 345 (bp 344), 357 (bp 207), 343 (bp 244), 315 (bp 314)
leaves stems (pH 8)	343 (bp 244, major), 345 (bp 344), 343 (bp 342), 347 (bp 346)
S. aurantica	
leaves, stems (pH 4)	no alkaloid
leaves, stems (pH 8)	demecolcine (major), colchicine, 357 (bp 193), trace of non-tropolonic alkaloid
fruits (pH 4)	colchicne (major), 385 (bp 207), demecolcine
fruits (pH 8)	demecolcine (major), colchicine,
Littonia modesta	
leaves, stems (pH4)	mostly polar materials
leaves, stems (pH 8)	303, 331 (bp 330), polar materials
fruits (pH 4)	colchicine (major), 385 (bp 312, major), 385 (bp 298)
fruits (pH 8)	same as pH 4, plus 373 (bp 373), 371 (bp 354)

The major component of the basic extract from the leaves of *G. plantii* was isolated by quick column on an alumina support eluting with methanol in dichloromethane (3%). This was obtained as a yellow green gum (28 mg)

MS: m/z 343 (M^+, 18); 328 (45); 300 (32); 244 (100).

7.5.9. Other Sources of Wurmbaeoideae Alkaloids

Very mature plant samples of *Wurmbea dioica* ssp. *alba* (1.77 kg) were collected in Medina, Western Australia on 18 September, 1988, freeze dried, and the powdered sample extracted exhaustively with methanol. Both the dichloromethane extracts at pH 4 and 8 behaved very poorly in the gas chromatograph. The combined extract was separated by radial layer chromatography with multiple development eluting with dichloromethane initially, then increasing the solvent concentration stepwise with methanol up to a concentration of 25 % methanol in dichloromethane. Most of the fractions obtained still chromatographed very poorly in GC. At least 15 to 20 different alkaloids were identified in the GC ion trace. A number of fractions whose GC ion trace looked reasonably clean all proved to be very impure as indicated by their ^1H n.m.r. specta. The dibenz[*d,f*]azecine alkaloids of molecular weight 357, 373 and 387 were, however, detected in a number of fractions. Several further attempts to isolate the dibenz[*d,f*]azecine alkaloid (M^+373) from the combined fractions containing this alkaloid were unsuccessful. No further studies were performed on this alkaloid extract.

Eight tiny stalks of flowering *Wurmbea uniflora* were collected from Lake Leake Road on 19 November, 1988, and the alkaloids extracted

using the usual acid-base extraction procedure, affording enough alkaloid extract for GC-MS analysis. The main alkaloidal component, as detected by the GC-MS technique were the two dibenz[*d,f*]azecine-type alkaloids (M^+ 373 and M^+ 387) formerly detected in the other *Wurmbea* species, and an alkaloid of molecular weight 315.

MS: 315 (M^+, 18); 300 (42); 287 (32); 272 (100).

A stalk of *Kreysigia multiflora* collected from New South Wales was obtained from New South Wales under the auspices of the Tasmanian Society for Growing Native Plants, on 5 November, 1989. As in *W. uniflora* enough alkaloid extract was obtained for GC-MS analysis. Kreysiginine was present as a major component while kreysigine appeared as a very minor component. The other two alkaloidal components were unreported.

MS: m/z 383 (M^+, 49); 366, M^+-17, 100), 340 (19).

MS: m/z 401 (M^+, 19); 384 (78); 366 (100).

7.5.10. Alkaloids of *Athrotaxis selaginoides*. (Chapter 2, Section 2.6, and Chapter 5, Section 5.2).

A total of sixty six kilograms of this plant sample was collected from New Town Dam Site near Henty River in Tasmania on 25 May 1987. The fresh plant sample was brought back in the laboratory right after its collection, shredded, and covered with methanol to quench the suspected enzymatic degradation of the alkaloidal components. The alkaloids were extracted at basic pH from the crude methanol extract by the usual acid-base extraction procedures (Section II). Acid-base re-extraction of the crude alkaloid extract afforded 1.55 grams of clean alkaloid extract. Preliminary separation was done by quick column

(Fractions A1 to A9). Each fraction was analyzed by analytical thin layer chromatography including spraying the TLC plates with alkaloid spray reagents, and by GC-MS technique. None of this fractions were obtained pure.

Separation of fraction A5

PLC on silica support separation of fraction 5 (198 mg) using benzene-ethylacetate-diethylamine (7:2:1) solvent system for development afforded fifteen fractions. Fractions 1 to 5, 14 and 16 were non-alkaloidal. All the other fractions contained more than one alkaloidal component except fraction 9 which was obtained as a gum (15 mg).

MS: m/z 303 (M^+, 4, accurate mass 303.1461, $C_{17}H_{21}NO_4$ requires 303.1468); 259 (100), 258 (82).

^{1}H n.m.r. δ ($CDCl_3$): 6.78 (s, 1H, H_{15}); 5.46 (s, br, 1H, H_1); 3.83 (s, 3H, OCH_3).

IR: 3439 cm^{-1} (br, OH^-); 1712 cm^{-1} (s, >C=O).

Fraction 12 (13 mg) contained a major component of molecular mass 331 along with a number of very minor components. One of these minor components (M^+ 313) has the characteristic low mass fragments of a dibenz[*d,f*]azecine-type base.

MS: m/z 313 (M^+, 29); 271 (28); 70 (70); 58 (61); 57 (100).

Separation of fraction A7

From fraction A7 (291 mg), ten fractions were obtained by double development PLC on silica using methanol in dichloromethane (4%) for development. GC-MS analysis revealed one non-alkaloidal fraction

(fraction 1), and two pure fractions (fractions 8 and 9). No dibenz[*d,f*]azecine-type alkaloid was detected from any of these fractions. Fraction A78 was obtained as a creamy needle-like crystals (99 mg, m.p. 154-155^{o}).

MS: m/z 315 (M^{+}, 23, accurate mass 315.1840, $C_{19}H_{25}NO_3$ requires, 315.1834); 284 (41); 257 (95); 256 (59); 242 (34); 178 (100); 165 (35); 146 (42).

^{1}H n.m.r. δ ($CDCl_3$): 6.70 (6.7197 in 500 MHz; s, 1H, H_{18}); 6.70 (6.7036 in 500 MHz; s, 1H, H_{15}); 5.54 (s, br, 1H, H_1); 3.79 (s, 1H, C_{16}-OCH_3); 3.30 (m, 1H, H_3); 3.22 (s, 3H, C_3-OCH_3); 2.77 (m. 2H, CH_2); 1.58 (t, J 11.5, 1H, H_4 axial).

^{13}C n.m.r. δ ($CDCl_3$): 143.72 (=C<); 143.59 (=C<); 142.71 (=C<); 134.86 (=C<); 132.54 (=C<); 117.96 (=CH); 116.05 (=CH); 114.47 (=CH); 74.24 (-OCH); 68.67 (-C-); 56.01 (OCH_3); 55.65 (OCH_3); 49.83 (CH_2); 46.21 (CH_2); 38.64 (CH_2); 36.64 (CH_2); 31.95 (CH_2); 27.50 (CH_2); 23.05 (CH_2).

Fraction A79 was found identical with the known alkaloid taxodine (55).(109 mg, m.p. 152-153^{o}).

MS: m/.z M^{+} 315 accurate mass 315.1842, Calculated for $C_{19}H_{25}NO_3$, 315.1834).

7.5.11. Alkaloids of *Burchardia umbellata*

The alkaloids of *Burchardia umbellata* (800 g, 12 September 1988) from South of Perth, Western Australia was extracted at basic pH only. The GC-MS analysis of the crude extract (319 mg) indicated the presence of six alkaloidal components of molecular weights 303(3), 317, 313 and 315. Another sample of this plant (660 g) was collected from Lake Leake Road on 19 November 1988. The crude alkaloid extract (pH

9) obtained (287 mg) was analyzed by GC-MS, and indicated the presence of ten different alkaloids (MW 303, 289, 317, 313, 315(2), 347, 301, and 331(2). This crude extract was subjected to PLC, developed with 7% methanol in dichloromethane, affording nine fractions (fractions B1 to B9) which were screened by GC-MS technique. The first two fractions were impure, while the third one (B3) contained the major alkaloidal component of the crude extract (63 g) which was identified as the known alkaloid taxodine (55).

MS: m/z 315 (M^+, accurate mass 315.180, $C_{19}H_{25}NO_3$ requires 315.1834).

Fractions B4 and B5 were relatively pure and were combined together then re-crystallized over diethylether-dichloromethane, as creamy needle-like crystals (22mg, m.p. 169-172^o). This was identified as the new homoerythrina alkaloid burchardine (112) from its spectroscopic data.

MS: m/z 289 (M^+, 72, accurate mass 289.167, $C_{17}H_{23}NO_3$ requires 289.1676); 258 (100); 231 (47); 230 (58); 178 (14); 146 (16).

^{1}H n.m.r. and **^{13}C n.m.r.** δ ($CDCl_3$): see (112)

FT-IR: 3151 cm^{-1}, (br, OH^-), 1701 cm^{-1}, (s, >C=O).

Fraction B6, obtained as a yellow gum, (4 mg, M^+ 347) was also relatively pure based on its GC ion trace; its ^{1}H n.m.r. spectrum was very complex. Its structure remained to be elucidated. Fraction B7 whose major constitution was the same as fraction 6 was fractionated further, from which one clean fraction (pinkish gum, 2 mg) was obtained. this was characterized as the known alkaloid lucidinine (114).

MS: m/z 315 (M^+, 43, accurate mass 315.1840, $C_{19}H_{25}NO_3$ requires 315.1834); 300 (100); 284 (89); 270 (94).

The dichloromethane extract at pH 2-4 (123 mg) was fractionated by quick column chromatography affording five impure fractions (fractions A1 to A5). Fraction A2 was separated by PLC, double developed with 4% methanol in dichloromethane, giving rise to six fractions. The first fraction (A2A) was obtained as a white gum (18 mg). Its structure was elucidated as O-methylburchardine (115) from its spectroscopic data.

MS: m/z 303 (M^+, 27, accurate mass 303.1811, $C_{18}H_{25}NO_3$ requires 303.1804); 288 (6); 273 (100); 272 (84); 245 (28); 244 (64); 178 (40); 146 (65).

^{1}H n.m.r. δ ($CDCl_3$): 5.45 (s, br, 1H, H_1); 3.78 (dd, J 2.3, 6.4, 1H, H_{15}); 3.46 (s, 3H, C_{15}-OCH_3); 3.39 (m, 1H, H_3); 2.91-2.65 (m, 4H, CH_2); 2.61-2.48 (m, 5H, CH_2); 2.28-1.86 (m, 4H, CH_2); 1.61-1.40 (m, 3H, CH_2).

^{13}C n.m.r. δ ($CDCl_3$): 175.82 (C=O); 140.53 (=C<); 139.56 (=C<); 116.94 (CH); 78.91 (CH); 73.66 (CH); 66.74 (-C-); 58.07 (CH_3); 56.11 (CH_3); 48.79 (CH_2); 46.49 (CH_2); 38.98 (CH_2); 38.19 (CH_2); 31.83 (CH_2); 27.73 (CH_2); 22.86 (CH_2); 19.17 (CH_2).

Further fractionation of fraction 2 by double development PLC using 5% methanol for development resulted in the reisolation of the known alkaloid schelhammericine (116).

MS: m/z 313 (M^+, accurate mass 313.167, $C_{19}H_{23}NO_3$ requires 313.1676);

7.5.12. Alkaloids of *Burchardia multiflora*

The fresh plant sample of *Burchardia multiflora* collected from the South of Perth Western Australia on 12 September 1988, was blended and extracted exhaustively with methanol. The alkaloids extracted from

the concentrated methanol extract was analyzed by GC-MS technique which showed that the alkaloid content of the dichloromethane extract at pH 4 and pH 9 were very similar that they were treated as one. A portion of the alkaloid extract (500 mg) was separated by an alumina quick column, eluting with a series of solvents (light petroleum, dichloromethane, to 10% methanol in dichloromethane). The third fraction (1C) was re-crystallyzed over dichloromethane-ether affording pale yellow needle-like crystals (156 mg, m.p. 135-137^{o}). The structure (117) was assigned to this isolate on the basis of its spectroscopic data.

MS: m/z 329 (M^{+}, 3, accurate mass 329.164, $C_{19}H_{23}NO_4$ requires 329.1625); 298 (41); 270 (100); 228 (21).

^{1}H n.m.r. and **^{13}C n.m.r.** δ ($CDCl_3$) see (117)

[a]$_D$ (3.22 mg/10 ml, EtOH): +205^{o}, -262^{o}, -293^{o}.

A separate batch of the crude alkaloid extract (300 mg) was fractionated by double development PLC (methanol in dichloromethane, 5%) affording nine fractions(fractions 2A to 2I). GC-MS analysis of each fractions indicated three relatively pure fractions . Fraction 2B (15 mg) proved to be the known alkaloid schelhammericine (116).

MS; m/z 313 (M^{+} 313, 20, accurate mass 313.1670, $C_{19}H_{23}NO_3$ requires 313.1676).

The third fraction (fraction 2C; 18 mg) was a re-isolation of the new 2,3-epoxyhomoerythrina alkaloid florine (117). The major component of the seventh fraction which was obtained as a brown gum (fraction 2G; 2.4 mg) was also a known alkaloid comosidine (118).

MS: m/z 329 (M^{+} , 44, accurate mass 329.162, $C_{19}H_{23}NO_3$ requires 329.1625)

The GC ion trace of the ninth fraction (fraction 2I) showed the presence of an alkaloid of molecular weight 347 whose mass fragmentation pattern was unique as compared to the other homoerythrina alkaloids known. A separate portion of the crude alkaloid extract (300 mg) was fractionated by PLC (methanol in dichoromethane, 8%) affording five fractions. GC-MS analysis indicated the presence of the alkaloid of molecular weight 347 in fractions 3A and 3B along with other alkaloidal components. The combined fraction 3A and 3B (65 mg) was fractionated by double development PLC (methanol in dichloromethane, 4%). The desired component was detected in the second fraction (fraction 3A2) which after evaporation of the solvent *in vacuo*, was obtained as a yellow gum (20 mg). Spectroscopic characterization of this alkaloid proved this alkaloid to be lenticellarine (119).

MS: m/z 347 (M^+, 48, accurate mass 347.172, $C_{19}H_{25}NO_5$ requires 347.1730).

7.5.13 Alkaloids of *Arthropodium milleflorum*

A collection of *Arthropodium milleflorum* from Margate (10/12/88; 490 g), Taroona (27 and 29/12/88; 1.35kg), Orford (30/12/88; 335g), and Mt. Nelson (6 and 7/1/89; 3.9kg) was freeze-dried and the alkaloids extracted at pH 8-9 following the usual alkaloid extraction procedure. The crude alkaloid extract (A3) was separated on a silica quick column affording six combined fractions (Fractions A3A-A3F) which were all impure. Fraction A3D (170 mg) was separated by PLC [2 x methanol-dichloromethane (5%); once, methanol dichloromethane (7%)] from which the first (A3D1) and the second (A3D2) were relatively pure.

Fraction A3D1 was obtained as white needle-like crystals (20 mg, m.p. 239-242°).

MS: m/z (407, 15); 406 (24, accurate mass 406.275, $C_{27}H_{36}NO_2$ requires 406.2743); 204 (8); 151 (20); 150 (100)

^{1}H n.m.r. δ ($CDCl_3$): 6.85 (d, J 10.15, 1H); 6.25 (dd, J 10.12, 1.82, 1H), 6.10 (s,br, 1H), 1.33 (s, 3H, $C\underline{H}_3$) 1.17 (s, 3H, $C\underline{H}_3$), 1.11 (d, J 6.67, 3H CH_3), 1.00 (d, J 6.65, 3H, CH_3).

^{13}C n.m.r. δ ($CDCl_3$): see (126)

FT-IR: 1711 cm^{-1} (s, >C=O), 1663 cm^{-1} (s, >C=C-$\underline{C=O}$)

Fraction A3D2 was also obtained as needle-like crystals (32 mg, m.p. 248-250°).

MS: m/z 405 (M^+, 40, accurate mass 405.265, $C_{27}H_{35}NO_2$ requires 405.2666); 404 (28); 390 (63); 150 (100).

^{1}H n.m.r. δ ($CDCl_3$): 6.86 (d, J 10.14, 1H), 6.25 (dd, J 10.17, 1.86, 1H), 6.11 (t, J 1.3, 1H), 5.37 (br, 1H), 3.86 (br, 1H), 1.32 (s, 3H, CH_3), 1.18 (s, 3H, CH_3), 1.01, (d, J 5.47, CH_3).

^{13}C n.m.r. δ (CDCl3): 210 (>C=O), 185.82 (>C=O), 166.444 (=C<); 153.55 (=C<), 135.31 (=C<), 128.11 (=CH), 125.90 (=CH), 124.76 (=CH), 61.55 (-CH), 61.13 (CH_2), 55.36 (-C-), 53.99 (-CH), 53.27 (-CH), 50.36 (-CH), 43.32 (-C-), 39.67 (CH_2), 38.42 (CH_2), 34.74 (-CH), 32.43 (CH_2), 31.92 (CH_2), 30.50 (-CH), 29.07 (CH_2), 20.78 (CH_3), 18.83 (CH_3), 18.45 (CH_3), 14.70 (CH_3).

FT-IR: 1707 cm^{-1} (s, >C=O), 1663 cm^{-1} (s, >C=C-$\underline{C=O}$).

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