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ALKALOIDS OF LEUCONOTIS GRIFFITHII AND L. EUGENIFOLIA (APOCYNACEAE)

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ABSTRACT

Leuconotis griffithii and **L. eugenifolia** provided a variety of Strychnos and Aspidosperma-Hunteria alkaloids as well as novel ring-opened indole alkaloids including leuconolam (**6**), rhazinilam (**8**) and 5,21-dihydrorhazinilam (**9**). Facile acid and base reactions of leuconolam lead to ring reclosure products. Physical and spectral data of norfluorocurarine (**1a**), norfluorocurarine-N_b-oxide (**1b**), eburnamine (**2**), kopsinine (**5**) and epileuconolam (**7**) as well as some derivatives of leuconolam are also provided.

INTRODUCTION

Leuconotis, Jack is a small genus of climbing shrubs of the family **Apocynaceae**. The genus comprises 10 species altogether[1] but in Peninsula Malaysia, it is represented by three species only. They are L. eugenifolia, D.C., L. griffithii, Hook and L. maingayi, Dyer. All are woody climbers. L. eugenifolia is also found in Sumatra and Borneo and in Peninsular Malaysia, it is restricted to the north-west region. Medicinally, its latex was once used for the treatment of yaws by applying it on the infected skin. It was also used for treating worm infection[2].

L. griffithii is a climber confined to Peninsular Malaysia and so is L. maingayi. The latter species however could not be distinguished from L. griffithii[3], despite comparing leaf specimens of the two species available at the herbarium of the Forest Research Institute of Malaysia.

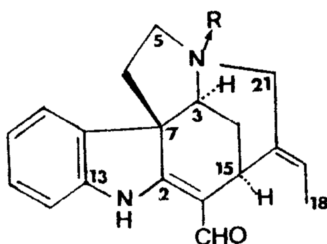
The presence of alkaloids in L. eugenifolia has been reported in Burkill[2] and later confirmed by Douglas and Kiang[4]. The presence of alkaloids in L. griffithii was reported by Kiang et. al[5]. However, no chemical work on the alkaloids has been carried out on any of the Leuconotis species, the only study reported was on L. eugenifolia[6,7] from which a few triterpenes, including a new triterpenoid compound called "leuconol", were isolated[6]. Further investigation[8] however, showed "leuconol" to be a mixture of bauerenol, α-amyrin

and β-amyrin. Other compounds isolated were n-nonacosane, n-hentriacontane, lupenyl acetate and two new triterpenoid esters, viz β-amrenyl behenate and β-amrenyl eicosonoate.

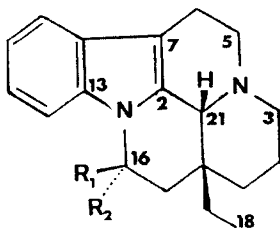
Herein we report a detailed investigation of the alkaloidal extracts of *L. eugenifolia* and *L. griffithii* (Apocynaceae). These species furnish several indole alkaloids including a number of ring-opened indole alkaloids; their structures and ring reclosure reactions are examined.

RESULTS AND DISCUSSION

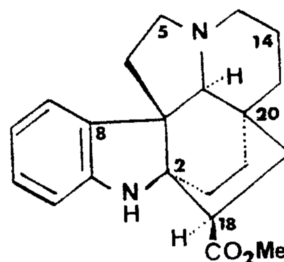
Initial studies of the alkaloid content of *L. griffithii* show one new as well as five known alkaloids. The known alkaloids are norfluorocurarine (**1a**), eburnamine (**2**), O-methyleburnamine (**3**), O-methylisoeburnamine (**4**) and kopsinine (**5**), whereas norfluorocurarine-N_b-oxide (**1b**) is new[9,10]. Further investigation on *L. griffithii* and *L. eugenifolia* resulted in the isolation of four ring-opened indoles :- leuconolam (**6**), epileuconolam (**7**), rhazinilam (**8**) and 5,21-dihydrorhazinilam (**9**) as reported in our preliminary results[9-11]. From both these plants another alkaloid presently identified as Alkaloid 376 (**11**) has its structure partially elucidated.



(1) (a) R = :
(b) R = O



(2) R_1 = OH; R_2 = H
(3) R_1 = OMe; R_2 = H
(4) R_1 = H; R_2 = OMe



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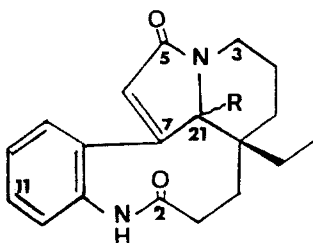
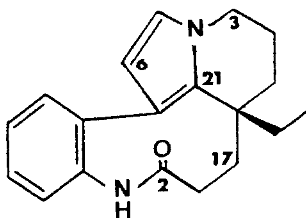
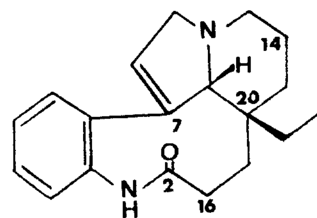






(6) R = β-OH (Leuconolam)
(7) R = α-OH (Epileuconolam)

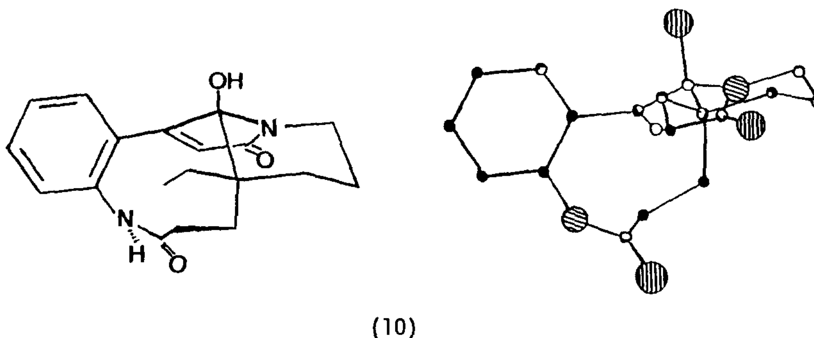
(8) (Rhazinilam)

(9) 5,21-Dihydrorhazinilam

The structure of leuconolam (**6**), a dilactam alkaloid containing an unusual nine-membered ring, was initially identified spectroscopically[9] and eventually confirmed unambiguously by X-ray diffraction[12]. The structure of leuconolam given with the perspective drawing shown as (**10**), can now be rationalised and a detailed interpretation made to its H-NMR and ^{13}CMR spectra. Similarly, we detail here spectroscopic assignments for related structures epileuconolam (**7**), rhazinilam (**8**) and 5,21-dihydrorhazinilam (**9**) also isolated from Leuconotis species.

Structure of leuconolam (6)

The hydroxydilactam compound (-)-leuconolam (**6**), isolated from L. griffithii and L. eugenifolia, forms monoclinic crystals from aqueous alcohol with m.p. 263 - 264° C and $[\alpha]_D$ = - 28.3^o ($CDCl_3$). Crystals of leuconolam (**6**) (from ethanol) possess the space group $P2_12_12_1$ with a = 8.07, b = 11.38 and c = 20.64 Å[12]. As shown below, leuconolam (**10**) has the unusual structural feature in which the two N-C=O planes are out-of-plane with the benzene ring, therefore minimizing conjugation. The hydroxy group is shown to be in the β-position.



(10)

The 90 MHz proton NMR spectrum in $CDCl_3$ shows the presence of a sharp one-proton singlet at 5.8 ppm, corresponding to the olefinic H-6 signal. The OH and NH groups are shown by broad one-proton singlets at 5.13 and 7.95 ppm respectively and were exchangeable with D_2O. The triplet methyl centred at 0.55 ppm is indicative of an ethyl group under the influence of benzene anisotropic effect.

The high resolution 400 MHz HNMR spectrum (Figure 1) in $CDCl_3$ reveals the structure of a pure compound existing in only one preferential conformation. Assignment of the resolved lower-field signals to methylene hydrogens was based on their splitting patterns as well as by spin decoupling experiments. Two hydrogens are at 3.98 ppm (dd, J = 4, 12 Hz), assigned to H-3_α, and 2.96 ppm (dt, J = 4, 12 Hz), assigned to H-3_β. The dissymmetric shape and splitting pattern of the signals at 2.14 (dd, J = 6, 12.5 Hz) and 2.00 ppm (approx. t, J = 12.5 Hz) belongs to the two geminal protons H-16. The aromatic protons were assigned based on those previously made for oxindole alkaloids[13]: 7.20 (dd, J=2, 6 Hz, H-9), 7.33 (m, H-10 & H-11) and 7.92 (dd, J = 2, 6 Hz, H-12).

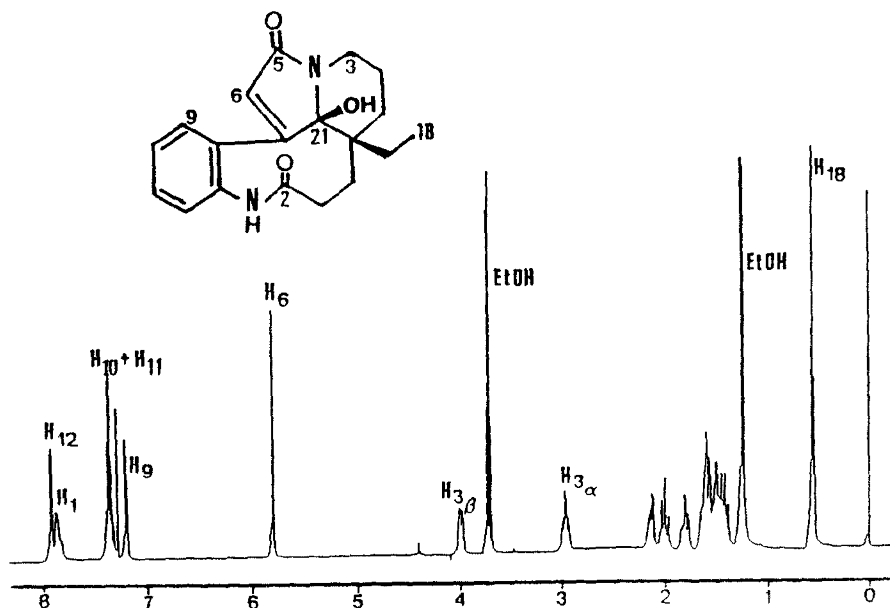


Figure 1 : 400 MHz HNMR Spectrum of Leuconolam in $CDCl_3$

The completely H-decoupled ^{13}C spectrum confirms the presence of 19 carbons in the molecule, 11 carbons being in the low field and 8 in the high field regions. The assignments are shown in Table 1.

The assignments were also simplified by the clear differentiation of methyl, methylene, methine and quaternary carbons using partially and off-resonance decoupled spectra of leuconolam **(6)**. The quartet at 6.9 ppm clearly shows it to be the terminal C-18 methyl group. The singlet at 44.9 ppm is assigned to C-20 as it is the only quaternary high field carbon present in the molecule. The individual assignments of the aromatic carbons were based on previous assignments on Aspidosperma alkaloids[13]. The lowest field quaternary carbon at 155.6 ppm is assigned to C-7, whilst those at 135.0 ppm and 133.1 ppm are assigned to C-13 and C-8 respectively. The singlet at 93.6 ppm is indicative of a carbon bonded to both an oxygen and a nitrogen and therefore assigned to C-21. The signals at 177.8 and 166.5 ppm are assigned to the two amidocarbonyls, C-2 and C-5 respectively. The assignment of the higher field signal of 166.5 ppm to C-5 is due to the effect of conjugation on the carbonyl and is in agreement with assignments made on model compounds[14]. The remaining methylene carbons are distinguished from the other carbons since they appear as triplets. The lowest field signal at 35.3 ppm is assigned to the amino-methylene C-3 carbon while the rest are assigned as in Table 1. Other spectroscopic information for leuconolam **(6)** (IR, UV and e.i-mass spectra[9]) are consistent with the structure as shown.

TABLE 1: Chemical Shifts of Leuconolam (**6**), Epileuconolam (**7**), Rhazinilam (**8**) and 5,21-Dihydrorhazinilam (**9**)[a]

Carbon	(**6**)	(**7**)	(**8**)	(**9**)
C-2	177.8 (s)	175.8 (s)	177.2 (s)	179.1 (s)
C-3	35.3 (t)	37.0 (t)	46.0 (t)	50.3 (t)
C-5	166.5 (s)	173.2 (s)	118.9 (d)	58.3 (t)
C-6	128.1 (d)	118.1 (d)	109.5 (d)	130.2 (d)
C-7	155.6 (s)	164.1 (s)	117.2 (s)	141.0 (s)
C-8	133.1 (s)	123.4 (s)	130.5 (s)	136.2 (s)
C-9	129.3[b] (d)	124.2 (d)	127.8 (d)	127.9[d] (d)
C-10	126.6[c] (d)	121.4 (d)	127.0 (d)	127.7[d] (d)
C-11	129.4[b] (d)	131.4 (d)	131.3 (d)	129.2 (d)
C-12	126.3[c] (d)	115.8 (d)	126.7 (d)	126.6 (d)
C-13	135.0 (s)	148.6 (s)	140.2 (s)	138.7 (s)
C-14	19.7 (t)	16.8 (t)	19.4 (t)	21.1 (t)
C-15	24.5 (t)	26.1 (t)	28.1 (t)	26.3[e] (t)
C-16	32.1 (t)	34.1 (t)	36.5 (t)	37.2 (t)
C-17	25.4 (t)	30.4 (t)	30.1 (t)	27.4[e] (t)
C-18	6.9 (q)	8.2 (q)	8.1 (q)	6.8 (q)
C-19	27.3 (t)	33.0 (t)	33.1 (t)	29.2[e] (t)
C-20	44.9 (s)	44.5 (s)	38.8 (s)	41.2 (s)
C-21	93.6 (s)	93.6 (s)	138.0 (s)	75.9 (d)

[a] - In $CDCl_3$ solutions (ppm downfield from TMS)
[b,c] - May be interchanged
[d,e] - May be interchanged

Structure of Epileuconolam (7)

Extractions of L. griffithii and L. eugenifolia with methanol in the presence of dilute sulphuric acid gave rise to another new compound, $[\alpha]_D = +30^o$, which is assigned as the α-hydroxy-isomer of leuconolam (**6**). This isomer, named epileuconolam (**7**), was obtained together with leuconolam (**6**) from the acidic extract. However, epileuconolam was not isolated from neutral or basic extracts of the plant[11] and is therefore not a natural product. The structure was determined mainly by comparison of its spectroscopic data with those of leuconolam (**6**). IR, UV and e.i.-mass spectra are consistent with the assigned structure[11].

The 90 MHz and 270 MHz proton spectra of epileuconolam (**7**) show some features which in many ways display the compound's isomeric relationship with leuconolam (**6**). A sharp singlet at 6.20 ppm integrating for one proton shows the presence of an isolated olefinic

proton corresponding to H-6 while the triplet centred at 0.73 ppm indicates the presence of an ethyl group in the molecule. All other assignments were made from analysis of the 270 MHz spectrum as given in the Experimental Section.

The completely decoupled ^{13}C spectrum of epileuconolam (**7**) accounts for all the 19 carbons with 11 carbons in the low field and 8 carbons in the high field regions. The quaternary carbons C-2, C-7, C-8, C-13, C-20 and C-21 are differentiated from the other carbons by being singlets in the partially-decoupled spectrum . The assignments of the carbonyl carbons were made by comparison with the ^{13}C assignments of leuconolam (**6**). The C-2 carbonyl remains quite unchanged at 175.8 ppm but the C-5 amide carbonyl is shifted downfield to 173.2 ppm, from 166.5 ppm in leuconolam (**6**). The aromatic quaternary carbons, C-8 and C-13, are at 123.4 and 148.6 ppm respectively whereas the olefinic quaternary C-7 is at 164.1 ppm. The C-20 signal is at 44.5 ppm since it is the only quaternary high field carbon present in the molecule. The remaining singlet at 93.6 ppm is indicative of the C-21 quaternary type carbon. The doublet at 118.1 ppm is assigned to C-6 as it clearly differs from the broader aromatic CH resonances. The quartet at 8.2 ppm is assigned to the terminal methyl group while the assignments of the rest of the methylene carbons are given in Table 1.

Structure of Rhazinilam (8)

A compound with m.p. 204-206° C, $[\alpha]_D = -146.6°$ ($CHCl_3$), molecular formula $C_{19}H_{22}N_2O$ and identified as rhazinilam (**8**) was isolated from both L. griffithii and L. eugenifolia[11]. Identification of **8** was by spectroscopic comparison to leuconolam (**6**), epileuconolam (**7**)[11] and previously reported information[15,16]. The ^{13}C spectral assignments are given in Table 1.

Structure of 5,21-Dihydrorhazinilam (9)

A new compound identified as 5,21-dihydrorhazinilam (**9**), was obtained together with rhazinilam (**8**)[11]. On prolonged exposure to air **9** gave rhazinilam (**8**); in fact all chromatographic fractions of **9** were observed to contain traces of **8** even after careful TLC separations[11]. The e.i.-mass spectrum of **9** gave a molecular weight of 296 with a molecular formula of $C_{19}H_{24}N_2O$. Analysis of the spectral data led to the conclusion that the compound was the 5,21-dihydro-derivative of rhazinilam (**8**). The structure was determined mainly by correlation of the spectroscopic data with those of leuconolam (**6**) and rhazinilam (**8**).

The 90 MHz proton NMR spectrum shows a triplet at 0.58 ppm corresponding to an ethyl group and an exchangeable proton at 8.1 ppm due to an amide. The multiplet at 5.6 ppm is assigned to the olefinic proton H-6. The doublet at 3.73 ppm, integrating for one proton, is assigned to $H\text{-}5_\alpha$ while the non-symmetrical triplet at 3.33 ppm is assigned to $H\text{-}3_\beta$. The four aromatic protons are grouped together from 7.20 to 7.28 ppm. The other CH_2 protons fall in the range from 1.25 to 3.0 ppm. The ^{13}C spectrum assignments are shown in Table 1.

The ultraviolet spectrum shows some similarities with that of rhazinilam (**8**) with absorptions at 213 (18,210), 227 (4,500) and 261 nm (2,670). The infrared spectrum shows an absorption at 1640 cm^{-1} due to the amide carbonyl group. The e.i.-mass spectrum shows

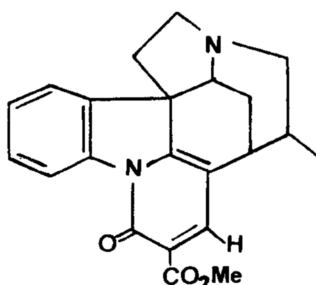
similarities with that of rhazinilam (**8**). In fact, there was an initial loss of two protons to give rhazinilam (m/z 294). The base peak at m/z 265 represents the loss of an ethyl group from rhazinilam (**8**) followed by a further loss of a carbonyl group (m/z 237), confirming the structure of the dihydro-compound to be **9**.

Alkaloid 376 (11)

Alkaloid 376 (**11**) was obtained from both L. griffithii and L. eugenifolia. The alkaloid has an R_f value of 0.40 with silica gel in 10%MeOH/$CHCl_3$ (v/v) and gave a very intense green coloration under long-wave (366 nm) UV light. The e.i.-mass spectrum gives a molecular ion at 376.1787 from which the molecular formula $C_{23}H_{24}N_2O_3$ is obtained. The presence of 23 carbons is confirmed by the completely decoupled carbon spectrum. The partially decoupled spectrum shows the low field carbons to be made up of 6 quaternary, 4 aromatic and 1 olefinic carbons. The singlets at 165.7 and 161.9 ppm suggest the presence of two carbonyl carbons. The presence of carbonyl in the molecule is also shown in the infrared spectrum from the absorption at 1660 cm^{-1}. The 90 MHz proton spectrum showed a strongly deshielded olefin proton as a sharp singlet at 7.9 ppm; a methoxy group (3.9 ppm) and an ethyl group (unsymmetrical triplet at 1.1 ppm) were also present.

The mass fragments at m/z 130, 143 and 144, derived from the indole system, suggest aromatic carbons 9 to 12 are unsubstituted. The presence of ions m/z 121 and 122 is characteristic of the lower-mass range fragments of akuammicine-type alkaloids[17]. The methylene indole system is confirmed by the presence of doublets and singlets at 128.1, 126.6, 119.9 and 117.6 ppm while the high-field region of the carbon spectrum provides support for the akuammicine-type structure with the presence of one quaternary carbon (55.6 ppm), two amino-methylenes (51.3 and 54.2 ppm) and three methines (61.9, 38.6 and 36.1 ppm). The ultraviolet spectrum shows absorptions at 205 (21,530), 220 (15,600), 278 (2,300) and 372 nm (6,094), which are quite characteristic for methylene-indole type of chromophore. The carbon singlet at 158.8 ppm fitted well for C-2 carbon signal in such systems as well as for those found earlier in norfluorocurarine (**1a**) and norfluorocurarine-N_b-oxide (**1b**)[9].

From the above spectroscopic information, structure for Alkaloid 376 is assigned below as **11**.



(11)

Another non-indolic alkaloid designated Alkaloid 308 was isolated and determined to be a dilactam quinolizidine alkaloid but the structure was not determined because of insufficient information.

Ring-Opened Indole Alkaloids of Leuconotis

The major alkaloid of L. griffithii, leuconolam (**6**), provides the first instance where a ring-opened indole alkaloid has been found in moderate quantities. Although it was assumed to be an artefact, extraction of the fresh bark under neutral and alkaline conditions also provided the alkaloid showing that it is indeed a natural product. Unsuccessful attempts were made to find possible precursors of **6** by extracting the fresh plant materials with methanol and chloroform and then analysing the extract by TLC for the presence of other major components present in the fresh extract and not present in the dried extract.

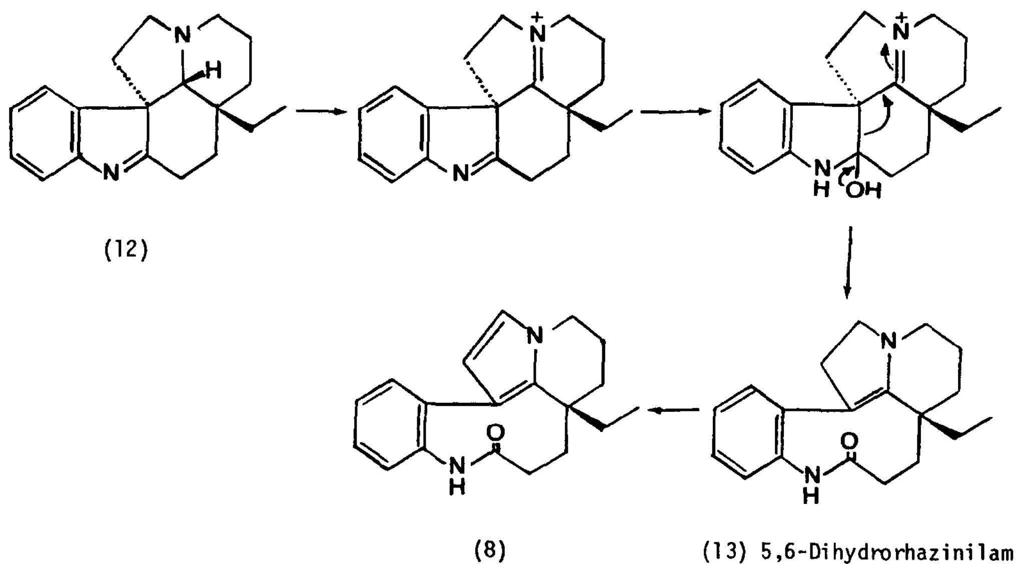
The isomeric compound, epileuconolam (**7**), can be obtained in significant amounts from the extraction of the fresh bark of L. griffithii with methanol under acidic conditions. Epileuconolam (**7**) in this instance is considered an artefact since it was not isolated from basic or neutral extracts of L. griffithii.

Basic extraction of fresh bark of L. griffithii with methanol, on the other hand, provided two other ring-opened indole alkaloids, rhazinilam (**8**) and 5,21-dihydrorhazinilam (**9**). These compounds were not obtained from the dried bark extracts of L. griffithii indicating that they are less stable. Rhazinilam (**8**) was considered an artefact previously obtained from the basic fractions of Rhazya stricta[15] whereas 5,21-dihydrorhazinilam (**9**) is new and a likely precursor to **8**. Indeed 5,21-Dihydrorhazinilam (**9**) on prolonged exposure to air gave **8**.

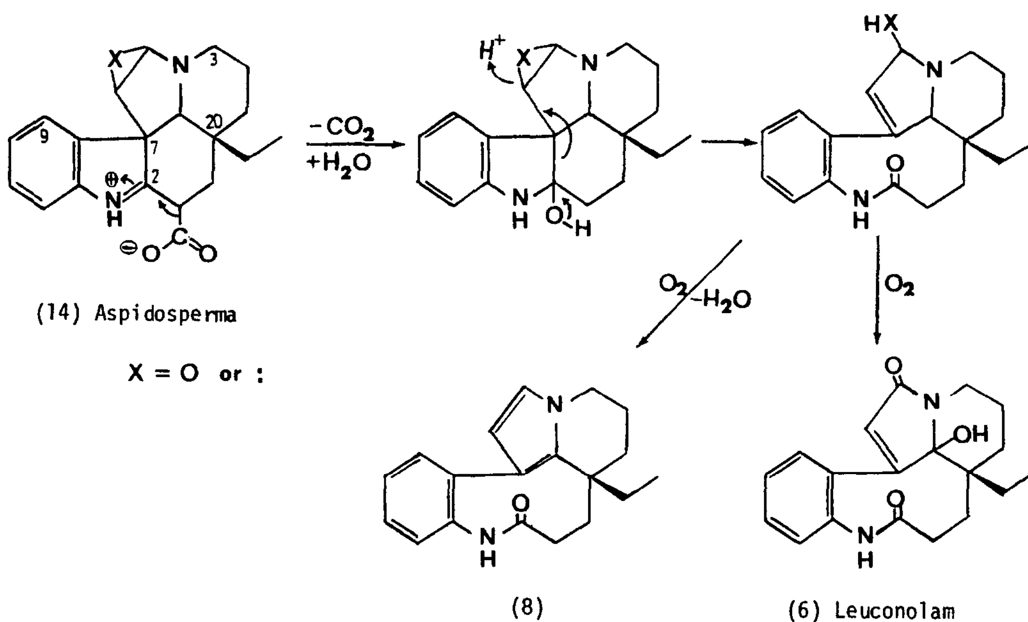
Basic extraction of the fresh bark of L. eugenifolia with methanol also gave ring-opened indole alkaloids leuconolam (**6**), rhazinilam (**8**) and 5,21-dihydrorhazinilam (**9**) and Alkaloid 376 (**11**), as well as epileuconolam (**7**), previously only obtained from the acidic extraction of the fresh barks of L. griffithii.

Rhazinilam (**8**) was first isolated from Melodinus australis[18] and later from R. stricta[16] and Aspidosperma quenracho-blanco[19,20]. Work by two independent groups gave the structure of rhazinilam as **8**[15,16]. The latter group[16] obtained the structure from spectral and chemical considerations while the former group[15] obtained an x-ray structure of rhazinilam.

The formation of ring-opened indole alkaloids such as leuconolam (**6**), epileuconolam (**7**), rhazinilam (**8**) and 5,21-dihydrorhazinilam (**9**) was postulated earlier as arising from the rearrangement of Aspidosperma alkaloids[11]. This proposal considers the oxidation of an Aspidosperma alkaloid **12** as the precursor and hydroxylation of C-2 position is followed by the breaking of the C-2 to C-7 bond to give 5,6-dihydrorhazinilam as an intermediate (Scheme 1). Our present results however suggested that the intermediate could well be 5,21-dihydrorhazinilam (**9**), instead of 5,6-dihydrorhazinilam (**13**). An alternative scheme to the formation of **9** and leuconolam (**6**) from an Aspidosperma alkaloid **14** is given (Scheme 2).



SCHEME 1 : Formation of Rhazinilam[13] (8)

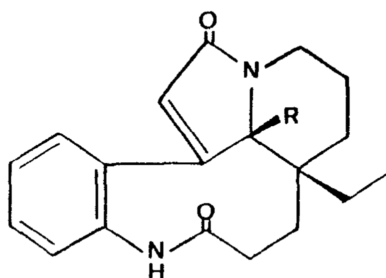


SCHEME 2 : Formation of Open-Ring Indole Alkaloids[11]

Reactions of leuconolam (6)

Under normal acetylation condition, acetic anhydride in pyridine, leuconolam (**6**) produced O-acetylleuconolam (**15a**). The proton and ^{13}C spectra confirmed the presence of acetate group and the rest of the spectra remains very much the same as the precursor.

The reaction of leuconolam (**6**) with dilute hydrochloric acid in methanol at room temperature produced O-methylleuconolam (**15b**). However, under more vigorous reaction conditions, e.g. with concentrated hydrochloric acid in methanol, two transannular cyclisation products resulted, viz 6α-chlorodiazaspiroleuconolam (**16**) and 6β-chlorodiazaspiroleuconolam (**17**) in 35% and 45% respectively. Their structures are described below. The ring closure reaction occurs via a transannular attack of nitrogen upon the

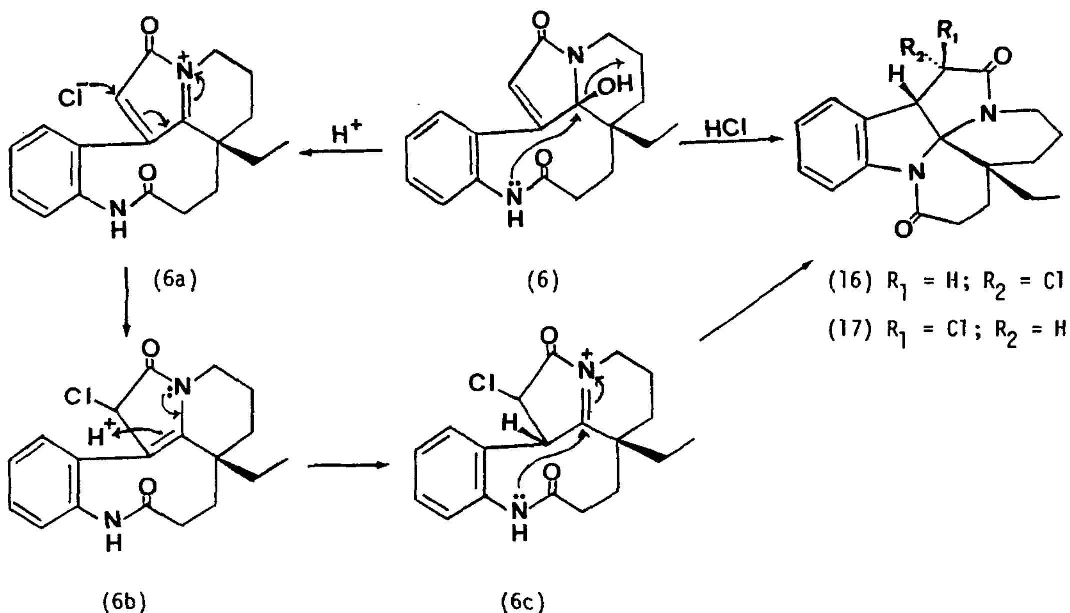


(15) (a) R = OCOMe
(b) R = OMe

incipient carbocationic centre followed by a non-stereospecific anti-Markovnikoff addition of HCl yielding 6α and 6β-chlorodiazaspiroleuconolam (Scheme 3). Alternatively, intermediates **6a** – **6c** may be involved in the reaction mechanism. Bromine in CCl_4 causes a similar ring closure with subsequent addition of Br_2 to produce 6β, 7β-dibromodiazaspiroleuconolam (**18**). Under basic conditions, e.g. KOH in methanol, leuconolam (**6**) undergoes an internal Michael addition to yield (80%) a pentacyclic (Melodinus-type) alkaloid, 5-oxo-14, 15, 18, 19-tetrahydro-21-hydroxymeloscine (**19**). This facile reaction provides an alternate pathway for the biogenesis of Melodinus alkaloids[21] found in this subfamily.

Structures of Chlorodiazaspiroleuconolam Isomers

Two isomeric derivatives, 6α-Chlorodiazaspiroleuconolam (**16**) and 6β-chlorodiazaspiroleuconolam (**17**), were obtained from the reaction of leuconolam (**6**) with concentrated hydrochloric acid in 35% and 45% yield respectively (Scheme 3). Both isomers have the molecular weight of 344 and formula $C_{19}H_{21}N_2O_2Cl$. The presence of one chlorine is demonstrated by the presence and intensity of the M+2 peak in the mass spectrum, with both isomers exhibiting identical fragmentation pattern.



SCHEME 3 : Formation of Chlorodiazaspiroleuconolam[11]

The spectral data, IR, UV and ^{13}CMR (Table 2) for both isomers are also almost identical, making it extremely difficult to distinguish between them unambiguously. In the 90 MHz HNMR, however, isomers (**16**) and (**17**) exhibit very distinguishable 6-H to 7-H coupling constants of <7 and 0.2 Hz respectively, differences being attributable to differences in dihedral angles as expected from the structures shown.

The structure of the 6α-chloro isomer (**16**) was confirmed by X-ray crystallography[22] and found to belong to the space group $\underline{P2_1}$, with $\underline{a}$ = 9.903, $\underline{b}$ = 7.513 and $\underline{c}$ = 11.399 Å. The perspective drawing of **16** shown as **20**, depicts both the H-6 and H-7 protons in the β-positions.

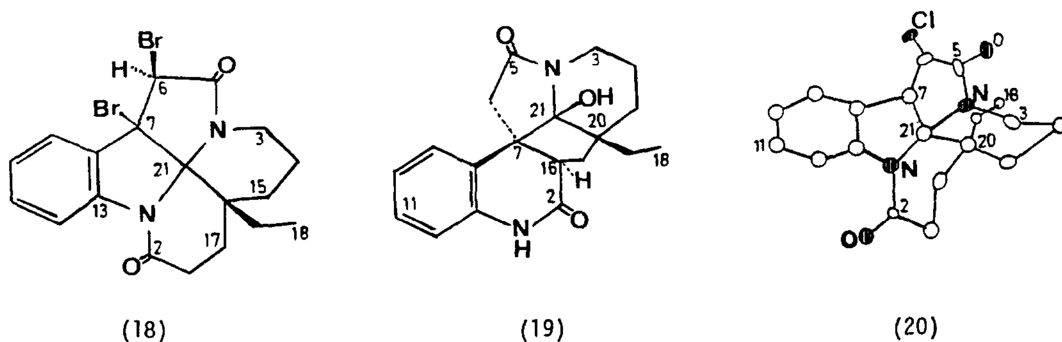


TABLE 2: ^{13}C-Spectral Data of Diazaspiroleuconolam Derivatives **16, 17** and **18**

Carbon	**16**	**17**	**18**
C-2	172.7 (s)	172.6 (s)	172.1 (s)
C-3	37.5 (t)	36.6 (t)	38.5 (t)
C-5	166.5 (s)	166.8 (s)	164.3 (s)
C-6	57.3 (d)	57.6 (d)	50.5 (d)
C-7	47.5 (d)	51.1 (d)	63.7 (s)
C-8	128.7 (s)	131.5 (s)	136.8 (s)
C-9	128.1[a] (d)	125.6 (d)	126.3 (d)
C-10	124.5 (d)	124.1 (d)	123.6 (d)
C-11	128.3[a] (d)	129.0 (d)	130.1 (d)
C-12	119.4 (d)	119.6 (d)	120.7 (d)
C-13	142.5 (s)	141.4 (s)	139.0 (s)
C-14	19.9 (t)	19.2 (t)	19.5 (t)
C-15	26.7 (t)	27.8 (t)	24.4[c] (t)
C-16	29.3 (t)	30.6[b] (t)	29.2[d] (t)
C-17	26.7 (t)	27.8 (t)	25.4[c] (t)
C-18	7.2 (q)	7.7 (q)	6.8 (q)
C-19	27.1 (t)	30.4[b] (t)	28.0[d] (t)
C-20	38.2 (s)	37.1 (s)	39.1 (s)
C-21	89.9 (s)	92.9 (s)	93.2 (s)

[a,b] - May be interchanged
[c,d] - May be interchanged

Structure of 6β, 7β-Dibromodiazaspiroleuconolam (18)

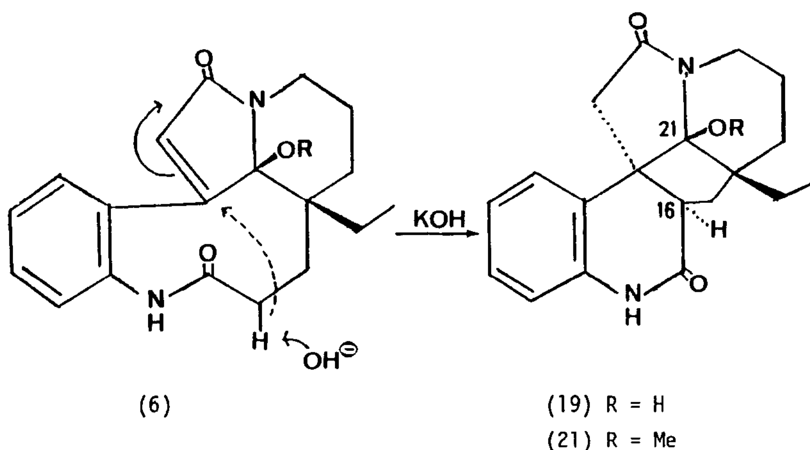
Reaction of leuconolam (**6**) with bromine in CCl_4 produced a compound of molecular weight 466 in 95% yield. The intensities of M, M+2 and M+4 peaks in the ratio of 1:2:1 suggest the presence of two bromine atoms in the molecule. Comparison of the spectral (HNMR, CMR (Table 2), UV, IR and Mass) data with those of compounds **13** and **14** showed it to be 6β, 7β-dibromodiazaspiroleuconolam (**18**), with the bromine atoms attached to C-6 and C-7 positions.

The 90 MHz proton spectrum shows the presence of a singlet at 5.17 ppm corresponding to the lone H-6 proton. The triplet at 0.93 ppm is assigned to the methyl group while the doublet of doublet at 4.06 ppm to $H\text{-}3_\beta$. The aromatic protons are found at 7.5 ppm (H-12) and ca. 7.2 ppm (H-9, 10 and 11) while the methylenes are distributed from 2.6-2.9 ppm (3H) and 1.6-2.4 ppm (8H). Homonuclear spin decoupling experiments indicate that $H\text{-}3_\alpha$ and $H\text{-}3_\beta$ are at 2.72 and 4.06 ppm respectively. Irradiation at 2.72 ppm simplified the signal at 4.06 ppm to an approximate triplet and the signal at 1.70 ppm a singlet. At the same time, the H-6 singlet at 5.17 ppm shows a 30% increase in intensity. NOE effects on this signal is also observed on irradiation at around 7.2 ppm.

It can therefore be concluded that bromine atoms are in the β-position, and that a cis-addition of bromine to dehydroxyleuconolam intermediate as in the HCl reaction (Scheme 3) has occurred on the more exposed β-face to yield **18.**

Meloscine Derivative 19

Leuconolam (**6**) undergoes a surprising facile reaction with potassium hydroxide to yield (-)-5-oxo-14,15,18,19-tetrahydro-21-hydroxymeloscine (**19**), the optical antipode of 21-hydroxy derivative of (+)-meloscine. The structure was assigned based on the internal Michael addition of the anion from leuconolam as shown in Scheme 4. The mechanism of formation requires a pseudorotation of N(1)-C(2)=O bond, anion formation and an internal Michael addition on the α-side leading to 16-H_α stereochemistry in **19.** The hydroxy function can be converted to the methoxy derivative **21** by HCl in methanol.



SCHEME 4: Formation of 5-oxo-14,15,18,19-tetrahydro-21-hydroxymeloscine (**19**)

Alkaloid Diversity in Leuconotis

We have from our present investigation of the alkaloid extracts of the two *Leuconotis* species shown the presence of a diversity of indole alkaloids such as of *Strychnos*, *Aspidosperma-Hunteria*, and novel ring-opened indole alkaloids as well as a non-indolic (probably quinolizidine) type alkaloid. Such diversity in alkaloidal content is found in only a few species such as *R. stricta*[15,16], *R. orientalis*, *M. australis*[18] and *Kopsia singaporensis*[23]. It is noteworthy that this is the first instance whereby a variety of ring-opened alkaloids have been isolated in significant quantities.

EXPERIMENTAL

Materials

The stems of L. griffithii were collected from Bukit Nanas Forest Reserve, Kuala Lumpur. Leaf specimens were kept at the herbarium of the Forest Research Institute of Malaysia (FRIM) as sample FRI 25859. The stems of L. eugenifolia were collected from Field 36 of the Forest Research Institute of Malaysia. Leaf samples were kept at FRIM as herbarium sample FRI 25855.

General

Various grades of silica gel used for column (G 60, 230 - 400 mesh ASTM), analytical and preparative thin layer chromatography (GF_{254} for TLC) were purchased from Merck (Darmstadt, Germany). TLC plates (thickness 0.5 mm) were prepared using a mixture of SiO_2 (GF_{254}) and water in the ratio of 1:2 (w/v). All chemicals were of analytical or reagent grades and were used without further purification. All solvents were redistilled before use. Dragendorff spray reagent was prepared according to the method described by Munier[24].

Proton (90 MHz) and carbon-13 (22.5 MHz) nuclear magnetic resonance (NMR) spectra were recorded on a Jeol JNM-FX90Q Fourier Transform NMR spectrometer in deuterochloroform solutions (unless otherwise stated) with tetramethylsilane (TMS) as internal standard. Chemical shifts are reported as δ ppm downfield from TMS. Ultraviolet spectra and absorbances were obtained from a Perkin-Elmer Model 554 UV-VIS spectrometer with a 10 mm cell, infrared spectra were recorded in chloroform solutions using a Perkin-Elmer IR Model 399B spectrometer while mass spectra were determined by a Kratos MS 3074 Mass Spectrometer with a DS 55 Data System. Melting points were determined on a Gallenkamp M.P. apparatus and are uncorrected. High field 200 MHz, 300 MHz and 400 MHz proton NMR and high resolution mass spectra were obtained by courtesy of various sources.

Extraction of L. griffithii

The powdered bark (5 kg) was soaked in methanol (2 L/kg) over 3 days. The methanol extract was drained after one day and fresh methanol added. The process was repeated until the methanol extract gave a negative alkaloid test with Meyer's reagent. The methanol extracts were combined and then evaporated under reduced pressure, furnishing about 500 gm of crude extract. The crude extract was then mixed with water (500 ml) and allowed to homogenise by rotating in the flask of the rotary evaporator. The solution was then filtered and the filtrate, with pH adjusted to 7.5-8, was extracted with chloroform until it gave a negative alkaloid test. The combined chloroform extracts were evaporated to a smaller volume (about 500 ml) and then extracted with 10% sulfuric acid (500 ml) three times. The acid solution was then basified with concentrated ammonia solution until pH 7.5-8 and the free bases then reextracted out with chloroform.

The residue from the above filtration was treated with glacial acetic acid (200 ml) and allowed to rotate in a 1 L flask for 1 hour. The filtered acid solution was then diluted with water (500 ml), basified with concentrated ammonia solution to pH 7.5-8 and then extracted three times with chloroform. The chloroform solutions were then treated with 10% sulphuric acid as above and the acid phase after neutralisation reextracted with chloroform. The chloroform extracts were then combined and evaporated under reduced pressure to give 10 gm (0.2%) of crude alkaloids.

Replacement of the chloroform in the crude extract with methanol and concentrating produced a precipitate on cooling (0° C) over 2 weeks. The precipitate, after filtration and washing with methanol, provided 200 mg of a white powder. This product, identified as leuconolam (**6**), was recrystallised from aqueous ethanol, giving colourless plates, m.p 263-

264°C. The rest of the crude alkaloids not precipitated out were first separated using column chromatography with Silica Gel 60 (230 - 400 mesh ASTM). Typically, 0.5 g of crude alkaloids were separated on a 25 X 100 cm column with 60 gm SiO_2. Initially, the solvent used was hexane, then hexane with increasing amounts of chloroform, then chloroform and finally with increasing amounts of methanol in chloroform. Fractions (50 ml) were collected and each fraction was checked with TLC (silica gel 60 GF_{254}) using the following solvent systems: chloroform : methanol (19 : 1, 10 : 1 and 5 : 1, v/v) and hexane : chloroform : methanol (3 : 16 : 1); plate sizes: 5 X 20 cm and 20 x 20 cm; thickness: 0.5 mm for analytical TLC and 1 mm for preparative TLC. The alkaloid spots were first detected by ultraviolet light (254 and 366 nm) then confirmed by spraying with Dragendorff Reagent. Fractions having spots with the same R_f values and stains were combined and treated as a group. The alkaloids were further separated by preparative thin layer chromatography using silica gel 60 GF_{254} material and solvent systems as given above. The alkaloid bands were located under UV light (254, 366 nm) and scraped off the plate. The silica gel containing the purified alkaloids were eluted in methanol/chloroform solution (1:1, v/v), filtered off and the filtrate evaporated to dryness. The alkaloids isolated are summarised in Table 3 below.

TABLE 3: The Alkaloids of L. griffithii

Alkaloid	R_f	Yield (%)	$[\alpha]_D$
Norfluorocurarine	0.25	55 mg (0.0011)	
Norfluorocurarine-N_b-oxide	0.10	35 mg (0.0007)	
Leuconolam	0.30	400 mg (0.008)	-28.3°
Eburnamine	0.35	25 mg (0.0005)	-90°
O-Methyleburnamine	0.70	20 mg (0.004)	-67.3°
O-Methylisoeburnamine	0.75	18 mg (0.00036)	+72.7°
Alkaloid 376	0.40	40 mg (0.0008)	
Kopsinine	0.45	50 mg (0.001)	
Alkaloid 308	0.50	60 mg (0.0012)	

Physical and spectral characteristics of the alkaloids isolated are as follows:

Leuconolam (6).-- M.p. 263-264°C; $[\alpha]_D$ = -28.3° (c = 0.7, $CHCl_3$); PMR (90 MHz, $CDCl_3$): δ 7.95 (1H, s, exchangeable with D_2O, NH), 7.8-7.9 (1H, m, ArH), 7.2-7.3 (3H, m, ArH), 5.8 (1H, s, = CH, H-6), 5.13 (1H, br. s, exchangeable with D_2O, OH), 3.96 (1H, br. d, CHN), 1.1-2.3 (11H, m, CH_2), 0.55 triplet (3H, t, Me); (400 MHz $CDCl_3$) : δ 7.92 (1H, dd, J = 2, 6 Hz, H-12), 7.89 (1H, br. s, NH), 7.33 (2H, dt, J = 2, 6 Hz, H-10 & H-11), 7.20 (1H, dd, J = 2, 6 Hz, H-9), 5.79 (1H, s, H-6), 3.98 (1H, dd, J = 4, 12 Hz, H-3_α), 2.96 (1H, dt, J = 4, 12 Hz, H-3_β), 2.14 (1H, dd, J = 6, 12.5 Hz, H-16_α), 2.00 (1H, unsym. t, J = 12.5 Hz, H-16_β), 1.79 (1H, dt, J = 5, 12.5 Hz, H-15_α), 1.65 to 1.37 (7H, m, H-14, H-15_α, H-17 and H-19) and 0.55 (3H, t, J = 8 Hz, Me); CMR is given in Table 1; IR ν_{max} (KBr): 3460 (m, OH), 3190 (m, NH), 2950 (s), 1690 (s, C=ON), 1650 (s, C=ON), 1600 (m, C=C), 1420 (s) and 1300 cm^{-1} (m); UV λ_{max} (EtOH): 205 (8,960), 218 (9,060) and ca 292 nm (sh, 1,390); mass spectrum EI-MS: m/z 326.1629 (100%, M^+, calcd. for $C_{19}H_{22}N_2O_3$, 326.1630), 308 (6, M-H_2O), 297 (7, M-C_2H_5), 279 (4, M-H_2O-C_2H_5), 186 (26), 172 (16), 145 (34), 144 (24), 143 (5), 130 (6), 118 (11), 110 (4) and 91 (12).

Norfluorocurarine (1a).-- PMR (90 MHz, $CDCl_3$): δ 10.25 (1H, br. s, exchangeable with D_2O, NH), 9.27 (1H, s, CHO), 6.91-7.30 (4H, m, ArH), 5.40 (1H, q, J = 6.8 Hz, H-19), 4.08 (1H, s, NCH), 3.73 (1H, d, J = 4.1 Hz, H-21_α), 1.7 - 3.9 (8H, m, CH_2) and 1.60 (3H, d, J = 5.1 Hz,

Me); CMR (22.5 MHz, $CDCl_3$) : δ 188.0 (C=O, d), 169.0 (C-2, s), 142.9 (C-20, s), 139.6 (C-13, s), 130.0 (C-8, s), 127.9 (C-11, d), 122.1 (C-9, d), 120.7 (C-19, d), 121.0 (C-10, d), 114.3 (C-16, s), 110.5 (C-12, d), 61.9 (C-3, d), 58.4 (C-7, s), 56.9 (C-5, t), 56.7 (C-21, d), 46.6 (C-6, t), 31.4 (C-15, d), 31.0 (C-14, t) and 13.0 (C-18, q); IR ν_{max} (KBr, $CDCl_3$): 3680 (weak, NH), 3620 (medium), 3400 (m, broad), 3020 (s), 2980 (s) 1640 (m, amide in chloroform), 1610 (m), 1540 (s), 1475 (s), 1465 (s), 1420 (w), 1380 (w), 1210 (s), 1110 (m), 1010 (s) and 930 cm^{-1} (m, sh); UV λ_{max} (EtOH): 205 (17,050), 228 (11,390), 284 (2,980) and 361 nm (9,110); mass spectrum EI-MS : m/z 292.1566, (calcd. for $C_{19}H_{20}N_2O$, 292.1576), 249 (16), 247 (10), 208 (14), 194 (21), 180 (11), 170 (12), 169 (10), 168 (15), 167 (34), 156 (10). 154 (12), 121 (97) and 106 (33).

Norfluorocurarine-N_b-oxide (1b).-- PMR (90 MHz, $CDCl_3$): δ 10.2 (1H, broad s, exchangeable with D_2O, NH), 9.2, (1H, s, CHO), 7.5 - 7.6 (1H, d, J = 6.9 Hz, H-12), 6.91 - 7.28 (3H, m, ArH), 5.67 (1H, d, J = 6.8 Hz, H-19), 4.47 (1H, m, H-21_α), 4.36 (1H, s, H-3), 2.5-3.8 (8H, m, CH_2), 1.65 (3H, d, J = 5.7 Hz, Me); CMR (22.5 MHz, $CDCl_3$): δ 188.4 (C=O, d), 164.6 (C-2, s), 142.3 (C-20, s), 134.5 (C-13, s), 133.9 (C-8, s), 129.3 (C-11, d), 126.0 (C-19, d), 121.9 (C-9, d), 121.1 (C-10, d), 111.3 (C-16, s), 111.0 (C-12, d), 78.9 (C-3, d), 74.5 (C-5, t), 69.6 (C-21, t), 54.6 (C-7, s), 41.0 (C-6, t), 29.8 (C-15, d), 28.8 (C-14, t) 14.3 (C-18, q); UV λ_{max} (EtOH); 202 (14,630), 228 (9,390), 288 (3,700) and 360 nm (14,300); IR ν_{max} (KBr): 3340 (br, m), 3010 (s), 1660 (s), 1615 (m), 1550 (s), 1470 (s), 1380 (m) and 1220 cm^{-1} (s); mass spectrum EI-MS: m/z 308.1531 (4%, M^+, calcd. for $C_{19}H_{20}N_2O_2$, 308.1524), 292 (16), 290 (11), 263 (4), 249 (7), 220 (4), 208 (7), 180 (18), 168 (39), 156 (19), 122 (29), 121 (100) and 107 (26).

Eburnamine (2). -- $[\alpha]_D$ = - 90° (c = 0.15, $CHCl_3$); PMR (90 MHz, $CDCl_3$): δ 7.6 (1H, m, H-12), 7.4 (1H, m, H-9), 7.10 - 7.28 (2H, H-10 & H-11), 5.54 (1H, dd, J = 5.2, 9.5 Hz, H-16), 3.76 (1H, broad, s, H-21), 2.35 (1H, m, H-17_β), 2.18 (1H, m, H-17_α), 1.25-3.4 (11H, m, CH_2), 1.14-1.6 (2H, m, H-19 & H-19'), 0.87 (3H, t, J = 7.6 Hz, CH_2CH_3); CMR (22.5 MHz, $CDCl_3$): δ 136.7 (C-2, s), 132.8 (C-13, s), 128.7 (C-8, s), 121.3 (C-11, d), 120.1 (C-9, d), 118.0 (C-10, d), 112.0 (C-12, d), 105.8 (C-7, s), 76.7 (C-16, d), 58.8 (C-21, d), 50.9 (C-5, t), 44.4 (C-17, t), 43.7 (C-3, t), 36.9 (C-20, s), 28.7 (C-19, t), 25.4 (C-15, t), 20.6 (C-14, t), 16.9 (C-6, t) and 7.6 (C-18, q); UV λ_{max} (EtOH): 231 (16,340), 280 (6,390) and 287 nm (5,150); mass spectrum EI-MS : m/z 296.182 (2%, M^+, calcd for $C_{19}H_{24}N_2O$, 296.188), 279 (8), 278 (41), 250 (16), 249 (90), 248 (10), 220 (5), 218 (4), 208 (100), 206 (15) and 193 (16).

O-Methyleburnamine (3).-- $[\alpha]_D$ = -67.3° (c = 0.26, $CHCl_3$); PMR (90 MHz, $CDCl_3$): δ 7.43-7.62 (2H, m, H-9 & H-12), 7.10-7.24 (2H, m, H-10 & H-11), 5.52 (1H, dd, J = 9.2, 5.5 Hz, H-16), 3.90 (1H, broad s, H-21), 3.33 (3H, s, OMe), 1.25-3.24 (14H, m, CH_2), 0.92 (3H, t, J = 7.5, H-18); CMR (22.5 MHz, $CDCl_3$) : δ 136.7 (C-2, s), 133.2 (C-13, s), 129.0 (C-8, s), 121.4 (C-11, d) 120.1 (C-9, d), 118.1 (C-10, d), 112.0 (C-12, d), 105.9 (C-7, s), 82.4 (C-16, d), 58.8 (C-21, d), 50.9 (C-5, t), 50.6 (OMe, q), 44.3 (C-3, t), 36.3 (C-17, t), 36.3 (C-20, s), 28.9 (C-19, t), 25.3 (C-15, t), 20.5 (C-14, t), 16.9 (C-6, t) and 7.6 (C-18, q); UV λ_{max} (MeOH): 229 (23,200), 280 (6,130) and 290 (sh) nm (5,060); mass spectrum EI-MS : m/z 310.2038 (17%, M^+, calcd. for $C_{20}H_{26}N_2O$, 310.2045), 311 (6), 278 (33), 277 (4), 250 (23), 249 (84), 248 (12), 247 (6), 220 (11), 209 (17), 208 (100), 207 (6), 206 (23), 194 (6), 193 (23) and 191 (9).

O-Methylisoeburnamine (4).-- $[\alpha]_D$ = + 72.7° (c = 0.22, $CHCl_3$); PMR (90MHz, $CDCl_3$): δ 7.40 - 7.45 (1H, m, H-12), 7.07-7.25 (3H, m, H-9, H-10 & H-11), 5.45 (1H, dd, J = 4.1, 1.1 Hz, H-16), 3.83 (1H, broad s, H-21), 3.50 (3H, s, OMe), 1.22 - 3.33 (14H, m, CH_2), 0.92 (3H, t, J = 7.5 Hz, H-18); CMR (22.5 MHz, $CDCl_3$): δ 135.6 (C-2, s), 131.3 (C-13, s), 128.9 (C-8, s), 121.0 (C-11, d), 119.9 (C-9, d), 118.2 (C-10, d), 110.5 (C-12, d), 105.5 (C-7, s), 83.1 (C-16, d), 59.2 (C-21, d), 55.7 (OMe, q), 51.3 (C-5, t), 44.8 (C-3, t), 35.2 (C-17, t), 34.5 (C-20, s), 29.1 (C-19, t), 25.8 (C-15, t), 21.1 (C-14, t), 16.9 (C-6, t) and 7.6 (C-18, q); UV λ_{max} (MeOH): 226 (21,750), 275 (5,760) and 289 (sh) nm (4,740); mass spectrum EI-MS m/z: 310.2022 (10%, M^+, calcd. for $C_{20}H_{26}N_2O$, 310.2045), 309 (34), 281 (12), 279 (14), 278

(22), 250 (20), 249 (100), 220 (3), 209 (6), 208 (97), 206 (31), 193 (26) and 180 (10).

Kopsinine (5).-- PMR (90 MHz, $CDCl_3$): δ 6.60-7.29 (4H, m, ArH), 3.75 (3H, s, OMe), 3.32 (1H, m, NCH), 3.00 (1H, m, NCH), 2.56-3.41 (7H, m, CH_2) and 1.07-1.97 (10H, m, CH_2); CMR (22.5 MHz, $CDCl_3$) : 174.6 (C=O, s), 149.0 (C-13, s), 140.6 (C-8, s), 126.5 (C-11, d), 121.5 (C-9, d), 119.6 (C-10, d), 110.8 (C-12, d), 68.3 (C-21, d), 66.5 (C-2, s), 57.9 (C-7, s), 51.8 (OMe, q), 50.6 (C-6, t). 47.5 (C-3, t), 43.8 (C-18, d), 36.4 (C-6, t), 35.4 (C-19, t), 34.8 (C-17, t), 32.0 (C-16, t), 31.8 (C-20, s) and 17.2 (C-14, t); IR ν_{max} (KBr): 2930 (s), 1720 (s, C=O), 1610 (m), 1450 (m) and 1350 cm^{-1} (m); mass spectrum EI-MS : m/z 338.1975 (11%, M^+, calcd. for $C_{21}H_{26}N_2O_2$, 338.1994), 308 (35), 280 (12), 279 (43), 252 (14), 251 (36), 237 (12), 224 (15), 223 (33), 214 (15), 209 (13), 195 (10), 184 (12), 171 (13), 156 (24), 124 (100) and 109 (54).

Alkaloid 376 (11).-- PMR (90 MHz, $CDCl_3$): δ 8.52 - 8.61 (1H, m, ArH, H-12), 7.89 (1H, s, =CH), 7.25-7.45 (3H, m, ArH), 4.07 (1H, m, NCH), 3.94 (3H, s, OMe), 2.83 - 3.05 (3H, m, CH_2), 1.75 - 2.35 (4H, m, CH_2), 1.2-1.5 (5H, m, CH_2) and 1.1 (3H, t, J = 6 Hz, CH_3); CMR δ (22.5 MHz, $CDCl_3$): 165.7 (C=O, s), 161.9 (C-17, s), 158.8 (C-2, s), 145.6 (C-23, d), 140.2 (C-13, s), 139.4 (C-8, s), 128.1 (C-11, d), 126.6 (C-9, d), 120.1 (C-22, s), 119.9 (C-10, d), 117.6 (C-12, d), 113.8 (C-16, s), 61.9 (C-3, d), 55.6 (C-7, s), 54.2 (C-5, t), 52.2 (OMe, q), 51.3 (C-21, t), 44.7 (C-6, t), 38.6 (C-20, d), 36.1 (C-15, d), 31.2 (C-19, t), 26.3 (C-14, t) and 11.4 (C-18, q); IR ν_{max} (KBr): 2950 (s), 1745 (s), 1700 (s), 1650 (s), 1610 (m), 1540 (s), 1455 (m) and 1270 cm^{-1} (m); UV λ_{max} (EtOH) : 205 (21,530), 220 (15,600), 278 (2,300), 372 nm (6,090); mass spectrum EI-MS : m/z 376.1787 (19%, M^+, calcd. for $C_{23}H_{24}N_2O_3$ 376.1787), 375 (73), 346 (5), 291 (16), 259 (15), 245 (21), 203 (4), 123 (9), 122 (100), 121 (4) and 109 (20).

Alkaloid 308.-- PMR (90 MHz, $CDCl_3$) : δ 4.14 - 4.33 (m, CH_2), 2.46 - 2.76 (m, CH_2), 1.46 - 2.28 (m, CH_2) 1.19 (d, J = 7 Hz, Me) and 1.11 (d, J = 7 Hz, Me); CMR (22.5 MHz, $CDCl_3$) : 174.9 (s), 173.6 (s), 75.2 (d), 74.8 (d), 68.6 (t), 66.8 (t), 47.1 (q), 43.9 (q), 41.6 (t), 41.5 (t), 41.4 (t), 41.0 (t), 34.4 (t), 33.7 (d), 32.5 (d), 29.3 (t), 13.5 (q) and 12.5 (q); UV λ_{max}, (MeOH) : 205 (11,550), 225 (sh, 6,600) and 270 nm (920); mass spectrum EI-MS : m/z 308.1381 (6%, M^+), 279 (4), 251 (4), 222 (13), 210 (5), 171 (12), 170 (13), 152 (19), 141 (30), 139 (45), 124 (49), 123 (12), 111 (48), 109 (24) and 99 (60).

Other Extraction Methods

Freshly ground bark (1 kg) of *L. griffithii* was placed in a soxhlet and then extracted with methanol (2 L) for 8 hours. The methanol extract was then evaporated to dryness. Examination by thin layer chromatography showed the presence of the alkaloidal compounds described above as well as two others, rhazinilam (**8**) and 5,21-dihydrorhazinilam (**9**).

The ground fresh bark (1 kg) was also soxhlet extracted with acidic methanol (100 ml 10% aqueous sulphuric acid added to the bark) for 8 hours. The acidic extract afforded similar alkaloidal components as above as well as another new compound, epileuconolam (**7**). Extractions under neutral or basic conditions did not produce epileuconolam (**7**). The three new compounds isolated are described below:-

Epileuconolam (**7**), 60 mg (0.006%), R_f 0.45, $[\alpha]_D$ = +30.0°; Rhazinilam (**8**), 65 mg (0.0065%), R_f 0.50, $[\alpha]_D$ = -146.6°; 5,21-Dihydrorhazinilam (**9**), 25 mg (0.0025%), R_f 0.40.

Epileuconolam (7).-- $[\alpha]_D$ = +30° (c = 0.65, $CHCl_3$); PMR (90 MHz, $CDCl_3$) : δ 8.1-8.2 (1H, m, ArH), 7.0-7.5 (3H, m, ArH), 6.20 (1H, s, = CH), 4,4 (1H, m, NCH), 1.25-3.79 (11H, m, CH_2)

and 0.73 (3H, t, CH_3); (270 MHz, $CDCl_3$): δ 8.16 (1H, br. d, J = 8.5 Hz, H-12), 7.45 (1H, br. d, J = 8.5 Hz, H-9), 7.33 (1H, approx. t, J = 8.5 Hz, H-10), 7.12 (1H, approx. t, J = 8.5 Hz, H-11), 6.20 (1H, s, =CH, H-6), 4.44 (1H, dt, J = 10, 4.4 Hz, H-3_β), 3.16 (1H, dd, J = 5.5, 2.5 Hz, H-16_α), 3.07 (1H, dt, J = 10, 4.4 Hz, H-3_α), 2.66 (1H, dd, J = 5.5, 2.5 Hz, H-16_β), 2.03 - 2.13 (2H, dd, J = 5.5, 2.5 Hz, H-17), 1.3 - 1.79 (6H, m, CH_2) and 0.73 (3H, t, J = 7 Hz, CH_3); CMR is given in Table 1; IR ν_{max}(KBr): 3400 (br, s, OH & NH), 1680 (s, CO), 1600 (m, CO), 1500 (m) and 1370 cm^{-1} (m); UV λ_{max} (EtOH): 203 (12,980), 251 (16,700), 342 nm (3,540); mass spectrum EI-MS : m/z 326.158 (100%, M^+, calcd. for $C_{19}H_{22}N_2O_3$, 326.1630) 308 (68, M-H_2O), 297 (6, M-Et), 279 (6, M-H_2O-Et), 261 (12), 251 (18), 186 (43), 159 (50) and 145 (56).

Rhazinilam (8).-- M.p. 204 - 206° C; $[\alpha]_D$ = - 146.6° (c = 0.15, $CHCl_3$); PMR (90 MHz, $CDCl_3$): 7.20 - 7.46 (4H, m, ArH), 6.62 (1H, broad singlet, exchangeable with D_2O, NH), 6.51 (1H, d, J = 2.6 Hz, H-5), 5.76 (1H, d, J = 2.6 Hz, H-6), 3.92 (1H, t, J = 5.4 Hz, H-3_β), 3.78 (1H, d, J = 5.4, H-3α), 2.35 - 2.49 (2H, m, H-16), 1.79 (1H, m, H-14_α) 1.10 - 1.83 (8H, m, CH_2), and 0.71 (3H, t, J = 7.3 Hz, H-18); CMR is given in Table 1, IR ν_{max} (KBr): 3200 (m, NH), 2950 (s), 1655 (s, CO), 1600 (m), 1495 (m), 1450 (m) and 1340 cm^{-1} (m); UV λ_{max} (EtOH): 205 (29,660), 222 (sh) (16,190), and 270 nm (sh) (3,150); mass spectrum EI-MS : m/z 294.1739 (17%, M^+, calcd. for $C_{19}H_{22}N_2O$, 294.1732), 293 (3), 266 (18), 265 (100), 238 (5), 237 (29), 209 (17), 197 (3), 168(3), 149 (12), 123 (2) and 118 (4).

5,21-Dihydrorhazinilam (9).-- PMR (90 MHz, $CDCl_3$): δ 8.1 (1H, s, exchangeable with D_2O, NH), 7.20-7.28 (4H, m, ArH), 5.62 (1H, m, = CH, H-6), 3.73 (1H, m, H-5_α), 3.33 (1H,. s, H-3_β), 1.25 - 3.10 (13H, m, CH_2) and 0.58 (3H, t, Me); CMR is given in Table 1; UV λ_{max} (EtOH): 213 (18,210), 227 (4,500) and 261 nm (2,670); mass spectrum EI-MS : m/z 296.1823 (60%, M^+; calcd. for $C_{19}H_{24}N_2O$, 296.1888), 294 (16), 279 (6), 266 (10), 265 (100), 253 (6), 238 (32), 237 (62), 209 (52), 197 (3), 171 (2), 149 (5), 123 (11) and 118 (15).

Extraction of L. eugenifolia

The ground bark (1 kg) was first soxhlet extracted with hexane (2 L) for 8 hours and then extracted with methanol (2 L) for another 8 hours (0.5 kg bark/L methanol). The hexane extract (10 gm) did not contain any alkaloid and therefore was not examined further. The methanol extract (10 gm) was evaporated to dryness, redissolved in chloroform and then separated by column and preparative thin layer chromatography as above.

The methanol extract gave leuconolam (**6**), epileuconolam (**9**), rhazinilam (**10**), 5,21-dihydrorhazinilam (**11**) and Alkaloid 376. The compounds were identified based on their R_f values under several TLC solvent systems and compared to those previously isolated from L. griffithii.

Methylation of Leuconolam (6)

Leuconolam (40 mg) was dissolved in 50 ml of dry methanol. Concentrated hydrochloric acid was added (3 drops). The O-methylleuconolam (**15b**) formed (35 mg, 87.5%) was separated by preparative TLC (SiO_2, 10% MeOH/$CHCl_3$, v/v) and recrystallised from methanol as colourless crystals, m.p. 155 - 156°C.

O-Methylleuconolam (15b).-- The following are the spectral characteristics : PMR (90 MHz, $CDCl_3$) : δ 8.59 (1H, s, exchangeable with D_2O, NH), 7.19-7.52 (4H, m, ArH), 6.35 (1H, s, 6-H), 4.18 (1H, m, H-3), 3.14 (3H, s, OMe), 2.40 - 3.03 (2H, m, CH_2), 2.02 - 2.12 (2H, m, CH_2), 1.03 - 1.85 (7H, m, CH_2) and 0.56 (3H, t, Me, H-18); CMR (22.5 MHz, $CDCl_3$): δ 178.4 (C-2, s), 166.8 (C-5, s), 150.9 (C-7, s), 135.8 (C-13, s), 133.1 (C-8, s), 132.9 (C-11, d), 131.9 (C-6, d), 129.9 (C-9, d), 128.6 (C-10, d), 128.0 (C-12, d), 97.4 (C-21, s), 49.9

(OMe, q), 45.5 (C-20, s), 35.9 (C-3, t), 32.6 (C-16, t), 28.0 (C-19, t), 26.2 (C-17, t), 24.1 (C-15, t), 19.6 (C-14, t) and 7.3 (C-18, q); UV λ_{max} (EtOH): 208 (8,500), 218 (9,230) and 290 nm (1,250); IR ν_{max} (KBr): 3620 (m), 3440 (m), 2690 (s), 1685 (s), 1640 (s), 1610 (m) cm^{-1}; mass spectrum EI-MS : m/z 340.1785 (71%, M^+, calcd. for $C_{20}H_{24}N_2O_3$, 340.1787), 326 (20), 325 (100), 309 (29), 308 (13), 280 (5), 297 (8), 279 (6), 251 (6), 201 (9), 186 (7), 172 (19), 154 (15), 144 (13) and 130 (11).

Acetylation of Leuconolam.

Pyridine (4 drops) was added to 40 mg of leuconolam placed in a 50 ml round bottomed flask and 2 ml of acetic anhydride were then added dropwise. The solution was stirred for 4 days at room temperature, after which ice (2 mg) was added, followed by concentrated ammonia solution to pH 8 and the mixture extracted with chloroform. Removal of the solvent and purification by preparative TLC (SiO_2, 10% MeOH/$CHCl_3$, v/v) gave O-acetylleuoconolam (**15a**) (25 mg, 62.5 %).

O-Acetylleuconolam (15a).-- The compound recrystallised from methanol as colourless crystals m.p. 235 - 236°C. PMR (90 MHz, $CDCl_3$): δ 7.9 (1H, m, ArH, H-12), 7.4 (1H, m, ArH, H-9), 7.2 (2H, m, ArH, H-10, H-11), 5.66 (1H, s, H-6), 4.30 (1H, m, H-3) 2.55 (3H, s, COMe), 1.1 - 2.2 (12H, CH_2) and 0.53 (3H, t, J = 7 Hz, Me); CMR (22.5 MHz, $CDCl_3$): δ 177.5 (C-2, s), 174.8 (C=0, s), 166.5 (C-5, s), 154.9 (C-7, s), 136.4 (C-13, s) 132.8 (C-8, s), 130.0 (C-11, d), 129.8 (C-9, d), 128.9 (C-6, d), 127.9 (C-10, d), 127.6 (C-12, d), 93.9 (C-21, s), 45.0 (C-20, s), 35.8 (C-3, t), 32.3 (C-16, t), 31.4 (C-19, t), 27.2 (C-17, t), 27.1 (Me, q), 24.3 (C-15, t), 19.9 (C-14, t) and 7.3 (C-18, q); mass spectrum EI-MS : m/z 368.1730 (19%, M^+ calcd. for $C_{21}H_{24}N_2O_4$, 368.1736), 352 (6), 340 (3), 326 (33), 308 (19), 297 (11), 279 (21), 251 (30), 186 (24), 172 (41), 145 (94), 144 (86), 143 (46), 136 (63), 130 (49), 123 (100) and 121 (57).

Reaction of Leuconolam with Hydrochloric Acid

Leuconolam (100 mg) was first dissolved in a minimum amount (2 ml) of dry methanol. Concentrated hydrochloric acid was then added dropwise (4 drops). The solution was stirred continuously and left to react overnight. After neutralisation with concentrated ammonia solution to pH 7.5 - 8 the products were extracted by chloroform and separation by preparative TLC (SiO_2, 10% MeOH/$CHCl_3$, v/v) afforded two products, 6α-chlorodiazaspiroleuconolam (**16**) (R_f = 0.75, 35 mg, 35%) and 6β-chlorodiazaspiroleuconolam (**17**) (R_f=0.8, 45 mg, 45%).

6α-Chlorodiazaspiroleuconolam (16).-- The α-chloro has m.p. 248-249°C; $[\alpha]_D$ = + 46.9° (c = 0.48, $CHCl_3$); PMR (90 MHz, $CDCl_3$): δ 7.7-7.8 (2H, m, H-9, H-12), 7.1-7.3 (2H, m, H-10, H-11), 5.07 (1H, d, J = 7 Hz, H-6), 4.20 (1H, d, J = 7 Hz, H-7), 4.08 (1H, m, H-3), 1.0-1.7 & 2.5-2.7 (11H, m, CH_2) and 0.94 (3H, t, J = 7Hz, Me); CMR is given in Table 2; IR ν_{max} (KBr, $CHCl_3$): 3020 (s), 2980 (s), 1710 (s, CO), 1680 (s, CO), 1590 (m), 1480 (m), 1450 (m), 1400 (m) and 1220 cm^{-1} (s); UV λ_{max} (EtOH): 205 (27,600), 238 (s) (8,310) and 278 (s) nm (2,470); mass spectrum EI-MS : m/z 344.1300 (100%, M^+, calcd. for $C_{19}H_{21}N_2O_2Cl$, 344.1286), 346 (35, M+2), 316 (17), 309 (65), 301 (10), 281 (9), 280 (9), 265 (5), 253 (5), 207 (11), 171 (17), 157 (12), 144 (6) and 128 (13).

6β-Chlorodiazaspiroleuconolam (17).-- The β-chloro isomer has m.p. 210-211°C; $[\alpha]_D$ = -31.4° (c = 0.16, $CHCl_3$); PMR (90 MHz, $CDCl_3$): δ 7.89-7.98 (1H, m, ArH, H-12), 7.15-7.29 (3H, m, ArH), 4.63 (1H, s, H-6), 4.18 (1H, s, H-7), 4.02 (1H, m, H-3_β), 1.0 - 1.9 & 2.59-2.85 (11H, m, CH_2) and 0.94 (3H, t, Me); (300 MHz, $CDCl_3$): δ 7.94 (1H, br. d, J = 8 Hz, H-12), 7.27 (2H, m, H-10, H-11), 7.18 (1H, br. d, J = 8 Hz, H-9), 4.65 (1H, s, H-6), 4.18 (1H, s, H-7), 4.02 (1H, dd, J = 4, 10 Hz, H-3_β), 2.82 (1H, dd, J = 4, 10Hz, H-3_α), 2.7 (1H, dd, J = 2, 6 Hz, H-16_β), 2.5 (1H, dd, J = 2, 6 Hz, H-16_α), 1.9 - 2.05 (2H, m, J = 7, 2 Hz, H-17), 1.5 -

1.8 (6H, m, CH_2) and 0.95 (3H, t, J = 7.5 Hz, Me); CMR is given in Table 2; IR ν_{max} (KBr): 3050 (s), 2960 (s), 1670 (br. s), 1600 (m), 1470 (m), 1450 (m), 1400 (m), 1350 (m) and 1210 cm^{-1} (s); UV λ_{max} (EtOH): 205 (26,100), 244 (sh) (6,630), 280 nm (sh) (1,460); mass spectrum EI-Ms : m/z 344.1293 (100%, M^+, calcd. for $C_{19}H_{21}N_2O_2Cl$, 344.1286), 346 (35, M+2), 316 (16), 309 (85), 301 (22), 281 (10), 280 (10), 265 (7), 253 (5), 207 (6), 171 (14), 157 (8), 144 (4) and 128 (13).

Reaction of Leuconolam with Bromine

To a solution of leuconolam (20 mg) in chloroform, 5 drops 20% solution of bromine in CCl_4 were added. The solution was left to react at room temperature overnight. Preparative TLC (SiO_2, 10% MeOH/$CHCl_3$, v/v) of the solution gave 6β, 7β-dibromo-diazaspiroleuconolam (**15**) (R_f = 0.85, 18 mg, 95%).

6β,7β-Dibromodiazaspiroleuconolam (18).-- The dibromo-derivative had the following characteristics: m.p. 109 - 110° C; $[\alpha]_D$ - 32° (c = 0.5, $CHCl_3$); PMR (90 MHz, $CDCl_3$): δ 7.32 - 7.84 (1H, m, ArH, H-12), 7.12 - 7.44 (3H, m, ArH), 5.17 (1H, s, H-6), 4.06 (1H, m, H-3_β), 2.72 (1H, m, H-3_α), 1.70 (1H, m, H-14_α), 1.07 - 2.06 (2H, m, H-19 & H-19'), 1.4 - 2.2 (7H, m, CH_2) and 0.93 (3H, t, J = 7 Hz, Me); CMR is given in Table 2; UVλ_{max} (MeOH): 212 (13,920), 222 (sh) (12,850) and 285 (1,590); mass spectrum EI-MS: m/z 465.9870 (7%, M^+, calcd. for $C_{19}H_{20}N_2Br_2$, 465.9892), 470 (6, M+4), 468 (13, M+2), 389 (99), 387 (100), 308 (88), 307 (59), 279 (62), 251 (35), 237 (17), 223 (12), 209 (5), 196 (6), 171 (16) and 156 (17).

Reaction of Leuconolam with Potassium Hydroxide

Leuconolam (50 mg) was dissolved in methanolic ethanol (50 ml) and 2 pellets (0.5 mg) of potassium hydroxide were added to the solution which was stirred continuously for 6 hours. The solution was neutralised by aqueous HCl and the product extracted with $CHCl_3$. Purification by preparative TLC (SiO_2, 10% MeOH/$CHCl_3$, v/v) and crystallisation from methanol gave the product as colourless plates (40 mg, 80%) and identified as (-)-5-oxo-14,15,18,19-tetrahydro-21-hydroxymeloscine (**19**).

5-oxo-14,15,18,19-Tetrahydro-21-hydroxymeloscine (19).-- The compound has the following physical and spectral characteristics: m.p. 175 - 177°; $[\alpha]_D$ = - 14.3° (c= 0.35, $CHCl_3$); PMR (90 MHz, $CDCl_3$): δ 9.16 (1H, br. s, exchangeable with D_2O, NH), 7.36-7.45 (1H, m, ArH, H-12), 7.09 - 7.27 (2H, m, ArH), 6.67 - 7.02 (1H, m, ArH), 4.04 - 4.18 (1H, m, NCH), 1.68 (1H, s, exchangeable with D_2O, OH), 1.6-2.95 (12H, m, CH_2) and 0.72 (3H, t, J = 7 Hz, CH_3); (300 MHz, $CDCl_3$) : δ 8.86 (1H, br. s, NH), 7.40 (1H, dd, J = 1.5, 8 Hz, H-12), 7.25 (1H, dt, J = 1.5, 8 Hz, H-11), 7.11 (1H, dt, J = 8, 1.5 Hz, H-10), 6.81 (1H, dd, J = 8, 1.5 Hz, H-9), 4.13 (1H, dt, J = 1.5, 14 Hz, H-3_α), 3.05 (1H, dd, J = 1.5, 18 Hz, H-6_β), 2.95 (1H, m, J = 1.5, 14 Hz, H-3_β), 2.89 (1H, dd, J = 10, 2.5 Hz, H-16), 2.69 (1H, d, J = 18 Hz, H-6_β), 2.32 (1H, dd, J = 14, 2.5 Hz, H-17_β), 0.9-2.2 (6H, m, CH_2) and 0.75 (3H, t, J = 7.3 Hz, Me); CMR (22.5 MHz, $CDCl_3$): δ 171.1 (C-2, s), 170.9 (C-5, s), 135.9 (C-13, s), 129.1 (C-11, d), 128.7 (C-9, d), 123.6 (C-10, d), 121.9 (C-8, d), 116.1 (C-12, d), 100.8 (C-21, s), 51.6 (C-3, t), 50.6 (C-7, s), 49.7 (C-16, d), 46.6 (C-20, s), 37.1 (C-6, t), 32.4 (C-17, t), 27.8 (C-19, t), 26.2 (C-15, t), 19.5 (C-14, t) and 7.3 (C-18, q); IR ν_{max} (KBr, $CDCl_3$): 3480 (m), 3365 (m), 1700 (br. s), 1590 (m), 1410 (m) and 1380 cm^{-1} (m); UVλ_{max} (EtOH): 212 (30,000), 251 (9,720), 258 (8,420), 284 (2,620) and 293 nm (2,180); mass spectrum EI-MS : m/z 326.1667 (45%, M^+, calcd. for $C_{19}H_{22}N_2O_3$, 326.1630), 309 (8), 297 (4), 255 (7), 242 (7), 228 (13), 186 (100), 167 (30), 160 (11) and 159 (79).

5-Oxo-14,15,18,19-Tetrahydro-21-methoxymeloscine (21)

Reaction of 5-oxo-14,15,18,19-tetrahydro-21-hydroxymeloscine (25 mg) in 1 ml dry methanol with concentrated hydrochloric acid (2 drops) at room temperature for 4 hours, neutralisation with NH_3 solution (pH 7.5 - 8) and purification by preparative TLC (SiO_2, 10% MeOH/$CDCl_3$) afforded a non-crystalline compound (10 mg), identified as 5-oxo-14,15,18,19-tetrahydro-21-methoxymeloscine (**21**); PMR (90 MHz, $CDCl_3$): δ 8.71 (1H, br, s, exchangeable with D_2O, NH), 7.44-7.52 (1H, m, ArH), 7.07-7.31 (2H, m, ArH), 6.74-6.84 (1H, m, ArH), 4.15 - 4.27 (1H, d, J = 11.3, NCH), 3.42 (3H, s, OMe), 1.2 - 2.89 (12H, m, CH_2) and 0.67 (3H, t, J = 7.3 Hz, Me).

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REFERENCES

1. Whitmore, T.C. "The Tree Flora of Malaya", Vol. 2, Longman, London, 1972.

2. Burkill, I.H. "A Dictionary of the Economic Products of the Malay Peninsula", Government of the Straits Settlement, London, 1935.

3. Ridley, H.N. "The Flora of the Malay Peninsula", Vol 2, L. Reeve & Co., London, 1932.

4. Douglas, B.; Kiang, A.K. Malayan Pharm. J. 1957, Vol VI, 138.

5. Kiang, A.K.; Douglas, B.; Morsingh, F. J. of Pharm. Pharmacog. 1961, 98-104.

6. Chatterjee, A.; Das, B.; Roy, S.K. J. Ind. Chem. Soc. 1959, 36, 92-4.

7. Das, B.; Mukherjee, R. J. Sci. Ind. Res. 1962, 21B, 506.

8. Sim, K.Y.; Mukherjee, R.; Toubiana M.J.; Das, B. Phytochemistry. 1971, 10 (11), 2803-6.

9. Goh, S.H.; Chen Wei; Abdul Razak Mohd Ali. Tetrahedron Letters. 1984, 25, 3483-3484.

10. Goh, S.H.; Abdul Razak Mohd Ali. "Proceedings of the 5th Asian Symposium on Medicinal Plants and Spices", Seoul 1984.

11. Goh, S.H.; Abdul Razak Mohd Ali. Tetrahedron Letters. 1986, 27, 2501-2504.

12. Chen Wei; Abdul Razak Mohd Ali; Goh, S.H.; Sinn, E.; Butcher, R.J. Acta Cryst. 1986, C42, 349-351.

13. Phillipson, J.D.; Rungsiyakul D.; Shellard, E.J. Phytochemistry. 1973, 12, 2043-2048.

14. Wenkert, E.; Cochran, D.W.; Hagaman, E.W.; Schell, F.M.; Neuss, N.; Katner, A.S.; Potier, P.; Kan, C.; Plat, M.; Koch, M.; Mehri, H.; Poisson, J.; Kunesch N.; Rolland, Y. J. Am. Chem. Soc. 1973, 95 (15), 4990-4995.

15. De Silva, K.T.; Ratcliffe, A.H.; Smith, G.F.; Smith, G.N. Tetrahedron Letters. 1972, 913 -916.

16. Banerji, A.; Majumder, P.L.; Chatterjee, A. Phytochemistry. 1970, 9, 1491.

17. Budzikiewicz, H.; Djerassi, C.; Williams, D.H. "Structural Elucidation of Natural Products by Mass Spectroscopy", Volume 1: Alkaloids, Holden-Day Inc., San Francisco, 1964.

18. Linde, H.H.A. Helv. Chim. Acta. 1965, 48, 1822-1842.

19. Abraham, D.J.; Rosenstein, R.D.; Lyon R.L.; Fong, H.H.S. Tetrahedron Letters. 1972, 909-912.

20. Lyon, R.L.; Fong, H.H.S.; Farnsworth N.R.; Svoboda, G.H. J. Pharm Sci. 1973, 62, 218.

21. Gordell, G.A. In "The Alkaloids" (Manske, R.H.F.; Rodrigo R. Eds.), Vol XVII, 287-288, Academic Press, 1979.

22. Chen Wei; A. Razak Mohd Ali; Goh, S.H.; Mak, T.C.W. Acta Cryst. 1986, C42, 1554.

23. Thoison, O.; Guenard, D.; Sevenet, T.; Kan-Fan, C.; Quirion, J.C.; Husson, H.P.; Deverre, J.R.; Chan, K.C.; Potier, P. C.R. Acad. Sc. Paris. 1987, 34(II), 157.

24. Munier, R. Bull. Soc. Chim. Biol. 1953, 35, 1225.