A RE-EXAMINATION OF THE ALKALOIDS OF *LUPINUS COSENTINII*

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ABSTRACT.—A re-examination has been made of the leaf and seed alkaloids of *Lupinus cosentinii* Guss. (incorrectly designated *L. pilosus*, *L. varius* or *L. digitatus* in earlier literature). The previous findings of epilupinine (**1**), epilupinine *N*-oxide (**4**) and multiflorine (**2**) in both leaf and seed have been confirmed but, in contrast to the earlier reports, it has been shown that the leaf alkaloid consists largely of epilupinine esters. The esterifying acids are acetic acid, unidentified acids related to truxinic or truxillic acid, ferulic acid and p-coumaric acid and the rhamnosides of ferulic acid and p-coumaric acid. The acetate ester of epilupinine *N*-oxide and mixed esters of 13-hydroxymultiflorine are also present in the leaf.

Both leaf and seed contain a new tricyclic alkaloid $C_{15}H_{22}N_2O$ which is shown to have structure **19** based on the rhombifoline skeleton.

Lupinus cosentinii Guss. (the Western Australian "Sandplain Lupin", erroneously referred to as *L. pilosus*, *L. digitatus* or *L. varius* in earlier literature (1)) is of Mediterranean origin. It has become naturalized on the coastal plains of south western Australia where it has been widely cultivated to improve soil fertility (2). The leaves are too bitter to be palatable to stock, but the seeds are relatively low in toxic alkaloids and provide valuable summer forage for sheep; the seeds, however, are too toxic to be used commercially in pig or poultry feeds.

Because the species is ecologically well adapted to the coastal soils of south western Australia, Gladstones and Francis (3) have begun to develop chemically-induced mutants of low alkaloid content for possible use as human and stock feed. Health authorities now demand detailed information concerning the alkaloids in lupin products for such use, but little is known of the alkaloids of this species. Earlier investigators (4, 5) showed that epilupinine, epilupinine *N*-oxide and multiflorine were the main alkaloids, but other unidentified alkaloids were also present; of these, three were isolated and coded as LV2, LV3 and LV4[2]. The present study was undertaken to provide a basis for current plant-breeding programs and for future toxicological studies. It is also hoped that the data will throw light on the biosynthesis and metabolism of bicyclic lupin alkaloids.

RESULTS

SEPARATION OF ALKALOIDS.—Figure 1 is a diagramatic representation of the tlc pattern observed for alkaloidal extracts of *L. cosentinii* leaves. We have adopted a coding system similar to that used by the earlier workers. The prefix LC indicates *L. cosentinii*, and the previously undescribed alkaloids are numbered in order of decreasing R_f; eLi and Mf are the known constituents epilupinine (**1**) and multiflorine (**2**).

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[2]LV indicates *L. varius*, the name then used for the species.

Extensive chromatographic fractionation has given LC2, LC4, LC5, LC6, LC7, LC8, epilupinine and multiflorine as pure crystalline solids and LC1 as a pure colorless oil. LC3 and LC9, although giving single spots on tlc[3], have been shown to be mixtures of closely related substances. Because of the complexity of the mixture of alkaloids having R_f values similar to epilupinine, not all were isolated, but only a relatively small amount of the total alkaloids remains unaccounted.

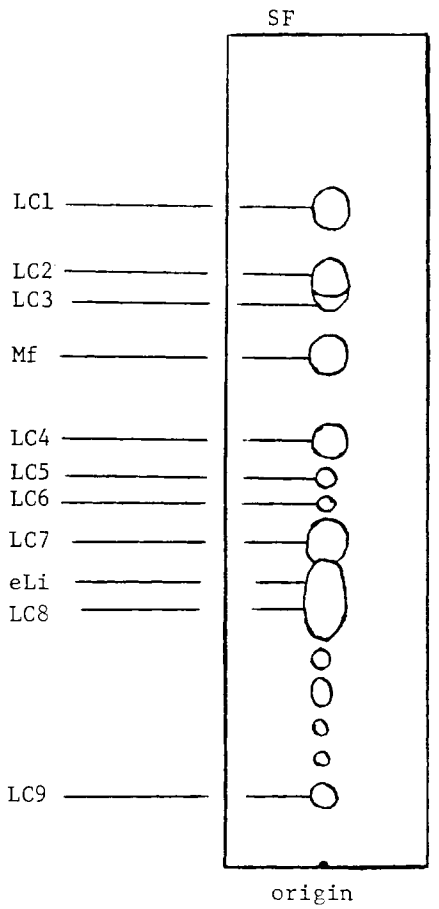


FIG. 1. Tlc of *L. cosentinii* leaf alkaloids. (System 1a, see Experimental)

The seed alkaloids showed a more simple pattern and contained predominantly multiflorine with small amounts of LC2 and epilupinine and a trace of material which appears to be 13-hydroxymultiflorine.

We have confirmed the earlier findings (4) that both leaf and seed contain water-soluble epilupinine *N*-oxide **4** (0.25 and 0.9% on dry matter respectively).

Structural studies have established LC1, LC4–6, LC7, LC8 and LC9 as derivatives of epilupinine, all of which are reported here for the first time from natural

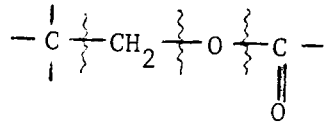
[3]Multiple development tlc gives an indication of more than one spot for LC9.

sources. LC2 and LC3 are new alkaloids related to multiflorine. Evidence for the structures of the new alkaloids is presented below.

EPILUPINYL ESTERS.—The simple epilupinyl esters LC1, LC4, LC5 and LC6 were readily recognized to be respectively the acetate **3**, the *trans*-ferulate **5** and the *trans*- and *cis*-*p*-coumarate derivatives **6** and **7** from a consideration of the spectroscopic and combustion analysis data. Chemical confirmation of the identification was not attempted with LC5 and LC6 which were available in only very small quantities but support for the assignment of the LC1 and LC4 structures was obtained by alkaline hydrolysis which readily afforded epilupinine as the sole base. Ferulic acid was also obtained in the acid fraction from LC4.

The signals in the nmr spectra due to the vinyl protons of the substituted cinnamate groupings in LC4, LC5 and LC6 show that the compounds are each single geometric isomers. LC4 and LC5 have signals near δ6.2 and 7.6 with a mutual coupling (J 16Hz) which is indicative of a *trans* stereochemistry, whereas LC6 has signals at δ5.71 and 6.78 with a coupling (J 12Hz) attributable to the *cis* stereochemistry.

The ms of LC4, LC5 and LC6 each show ions at *m/e* 138, 152 and 168 corresponding to the alkaloidal fragment from the cleavages



in the region of the ester functionality and are characteristic for epilupinine derivatives (6). For the acetate ester, LC1, the ions are at *m/e* 138, 152 and 169. The complementary ions *m/e* 207, 193 and 177 are prominent in the spectrum of the ferulate ester, LC4.

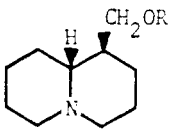
The most polar of the alkaloid fractions, LC9, showed ir absorption (1715 cm^{-1}) and ms ions typical of epilupinine esters. Mild acid treatment afforded a base fraction shown by tlc to be a mixture of LC4, LC5 and LC6. This provides an explanation for the presence in the nmr spectrum of LC9 of signals for both *trans*- and *cis*- substituted cinnamate groupings. Only one of the vinyl proton signals of each grouping was observable (δ6.28, J 15Hz; δ5.82, J 12Hz) the other being obscured by the aromatic proton signals. In addition, rhamnose was obtained from the acid hydrolysis and identified by tlc comparison. Nmr signals at δ1.27 (methyl), 5.55 (anomeric proton, $w_{1/2}$ 5Hz) and 3.4–4.3 in the spectrum of LC9 are attributed to the sugar moiety. It thus follows that the LC9 fraction is a mixture of rhamnosides of epilupinyl *trans*-ferulate **8**, epilupinyl *trans*-*p*-coumarate **9** and epilupinyl *cis*-*p*-coumarate **10**. Judging from the yields of LC4, LC5, and LC6 from acid hydrolysis, these are present in the approximate proportion of 1:3:2.

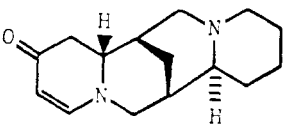
The weak ions at *m/e* 461 and 491 in the ms are attributable to the molecular ions of the coumarate and ferulate esters respectively. The uv spectrum has long wavelength absorption at a similar position to those for LC4, LC5 and LC6, but this is unaffected by alkali as is to be expected for phenolic glycosides.

Upon alkaline hydrolysis, the LC9 fraction afforded epilupinine as the sole base together with an acid fraction which was methylated with diazomethane. The mixed methyl esters could be separated into two fractions which have properties consistent with their formulation as methyl rhamnosyl coumarate (*cis* and *trans*) **11** and methyl rhamnosyl *trans*-ferulate **12**. Upon separate mild acid hy-

drolysis, these gave *cis*- plus *trans*-methyl *p*-coumarate **13** and methyl *trans*-ferulate **14**, respectively, together with rhamnose in each case.

LC7 is probably identical with the substance LV3 isolated by Crow and Michael (4) as shown by the similarity of melting point, uv spectra and analytical data. The analytical data was previously taken as evidence for the molecular formula

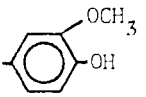


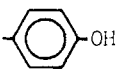


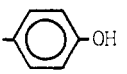
2

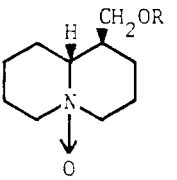
1 R = H

3 R = -$COCH_3$

5 R = -COCH$\overset{t}{=}$CH- 

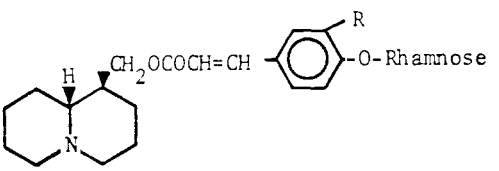
6 R = -COCH$\overset{t}{=}$CH- 

7 R = -COCH$\overset{c}{=}$CH- 



4 R = H

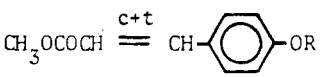
16 R = -$COCH_3$

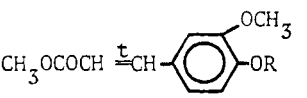


8 R = OCH_3, trans d.b.

9 R = H, trans d.b.

10 R = H, cis d.b.





11 R = Rhamnose

12 R = Rhamnose

13 R = H

14 R = H

$C_{20}H_{27}NO_4$ with one methoxyl group. This now needs to be modified to $C_{40}H_{54}N_2O_8$ with two methoxyl groups because it has been found that the ms shows M^+ at *m/e* 690. The methoxyl groups are shown by a sharp singlet at δ3.89 in the nmr spectrum.

LC7 has weak uv absorption maxima at 234 and 283 nm; the addition of alkali causes a broadening and diminution of the absorbance at 283 nm and the appearance of a new absorption shoulder near 250–260 nm. This is similar to the spectral properties of eugenol and is suggestive of a 1-alkyl-4-hydroxy-3-methoxybenzene. Consistent with this formulation is a broad singlet at δ6.89 in the nmr spectrum due to six aromatic hydrogens and absorption in the ir spectrum due to hydroxyl groups. Ir absorption at 1720 cm^{-1} suggests an ester grouping. This is confirmed by alkaline hydrolysis, which gives epilupinine and an unidentified acid fraction showing two spots on tlc.

A feature of the ms is the dominant abundance of ions at *m/e* 345 and below with a striking resemblance of this region to the total spectrum of epilupinyl ferulate. The molecular formula of LC7 is that of a dimer of LC4, and it is postulated that the compound has a cyclobutane group corresponding to a (2+2) addition between the olefinic bonds of 2 molecules of LC4. In the mass spectrometer, bond fission in the reverse manner would then lead to the observed similarities of ms. Similarly, on gc the same retention times observed for LC4 and LC7 probably indicate a splitting of the dimeric form. This analysis would suggest that LC7 is the diester **15** between a substituted truxillic acid or truxinic acid and epilupinine. The most favored of these is an α-truxillic acid derivative because plant sources have previously yielded santiaguine, a diamide of α-truxillic acid (7) and thesine (8) and hoveine (9) which are diesters of 4,4'-dihydroxy-α-truxillic acid.

Attempts to prepare a (2+2) dimer of ferulic acid by irradiation brought about only *trans* to *cis* isomerization of the olefinic bond.

OTHER ALKALOIDS.—LC8 forms colorless highly hygroscopic crystals. Combustion analysis corresponded to $C_{12}H_{21}NO_8 \cdot \frac{1}{2}H_2O$, and the indicated molecular formula was confirmed by high resolution mass measurements of the molecular ion. Ir absorption at 1735 cm^{-1} and signals at δ2.04 and 4.07 in the nmr suggested a primary acetate group. The δ4.07 signal was enhanced, possibly by a contribution from water in the sample. In comparison with the epilupinyl acetate spectrum, there is a downfield spread of nmr signals for the ring protons to nearly δ3.5 similar to the situation for epilupinine *N*-oxide. The possibility, thus indicated, that the new alkaloid is epilupinyl acetate *N*-oxide **16**, is supported by the ms which shows a close relationship to that of epilupinine *N*-oxide. The interpretation of the spectrum was aided by high resolution measurements. The ion most significant in the characterization of the *N*-oxide functionality of LC8 has the composition $C_5H_{10}NO$ (*m/e* 100.076). This is considered to represent the atoms of the unsubstituted ring in structure **16** arising from fission of a C–C and a C–N bond together with H transfer to the ring fragment. Other ions at *m/e* 168 and 154 arise from simple fission of the ring to side chain bond and of the adjacent C–O bond, whereas ions at *m/e* 152, 150 and 137 result from accompanying loss of *N*-oxide oxygen.

Chemical evidence for the proposed structure was obtained by reduction of the alkaloid with Zn–H_2SO_4 at 0°; this afforded epilupinyl acetate identical with the substance LC1 already isolated from the plant.

LC2 is a stable colorless crystalline solid with the molecular formula $C_{15}H_{22}N_2O$.

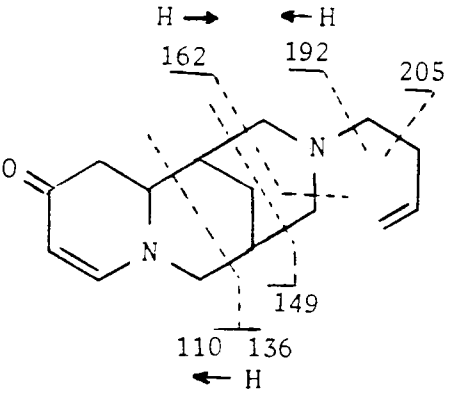
Absorption in the uv spectrum at 335 nm and ir spectrum at 1580 and 1625 cm^{-1} together with one proton doublets at δ4.92 and 6.86 in the nmr spectrum are comparable to the situation with multiflorine and indicate the $-\overset{|}{N}-CH=CH-C=O$ grouping. A $-CH=CH_2$ grouping is indicated by ir absorption at 920 and 990 cm^{-1} and by a complex three proton multiplet in the vinyl proton region of the nmr spectrum.

These data have led to the consideration of four possibilities for the structure of LC2. The possibilities are that the alkaloid has a skeleton based on the rhombifoline (10), the tinctorine (11) or the "dehydroalbine" (12) structures or, finally, that it may be identical with *N*-methylalbine, an alkaloid previously isolated from *L. albus* (13, 14).

The reported properties of *N*-methylalbine (mp 67.5°, [α]D −559°) are similar to those of LC2 (mp 60°, [α]D −520°). Furthermore, extracts of *L. albus* show a spot which is indistinguishable from LC2 in all tlc systems tested. We have been unable to obtain a sample for comparison, but the identification was not considered likely because we were unable to assign convincingly an *N*-methyl signal in the nmr spectrum of LC2. The most closely related substance available to us, *N*-methyl angustifoline, shows a *N*-methyl signal at δ2.21, while in tinctorine the *N*-methyl signal is reported at δ2.16 (11). The reported clean hydrogenation of *N*-methyl albine (13) is in marked contrast to the complex mixture obtained with LC2 and gives a further indication that the two compounds are not identical.

The second and third possible structures are also considered unlikely as these, too, would require an *N*-methyl group.

The ms of LC2 provided some support for the first alternative. The interpre-

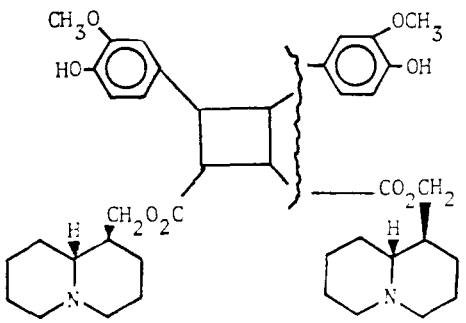


tation is presented in the following structure and gains support from the fact that tetrahydrorhombifoline has analogous fragment ions in the ms. It is noteworthy that the loss of 41. a.m.u. is characteristic of the monosubstituted vinyl group in this class of substance.

This was confirmed by a direct comparison of the previously reported completely reduced desoxy derivatives of *N*-methyl angustifoline (13) and tetrahydrorhombifoline (10) with the corresponding derivative from LC2. Vigorous catalytic hydrogenation of LC2 afforded a mixture of products from which a completely reduced desoxy derivative **17** was obtained after careful chromatographic separation. This product was identical with the reduced product **17** from tetrahydrorhombifoline but was different from the product **18** derived from *N*-

methyl angustifoline. Furthermore the *N*-methyl signal was clearly defined (δ2.25) for the product from *N*-methyl angustifoline but was absent for the product from LC2. LC2 thus has the structure **19**.

LC3 was obtained as a pale brown gum. Nmr and ms studies indicated it to be a mixture, although only one spot was observed on tlc. The uv spectrum (λmax 230 and 328nm) and nmr spectrum (AB pattern at δ4.81 and 6.73) indicated the chromophore $-\overset{|}{N}-CH=CH-\overset{|}{C}=O$ as in LC2 and multiflorine. Alkaline hydrolysis afforded a base which, on catalytic hydrogenation, gave the same compound (13-hydroxysparteine **21**) as that similarly produced (16) from 13-hydroxylupanine **22**. The evidence thus indicates that the base is 13-hydroxymultiflorine **20** reported previously from *L. albus* as an incompletely-characterized gum (16).



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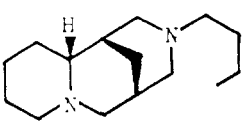

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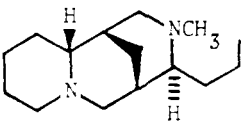

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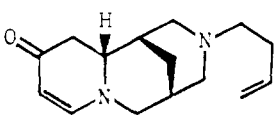
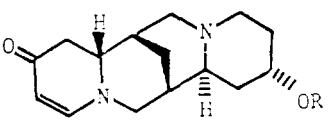
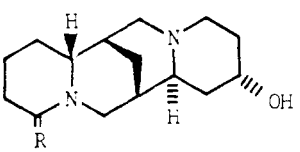


19



20 R = H

23 R = COC_6H_5



21 R = H_2

22 R = O

The acid fraction recovered from hydrolysis of LC3 was almost pure benzoic acid, but examination of the nmr spectrum of LC3 indicated that only approximately one-third of this alkaloid fraction is the benzoate ester. The nmr spectrum of LC3 also showed a broad absorption in the region δ5.0–5.4, apparently due to overlapping –C(H)–O– signals. The molecular ion of the benzoate ester has *m/e* 366 in the ms, whereas an ion at *m/e* 344 is attributed to the unidentified component and indicates the composition $C_5H_8O_2$ for an esterifying acid. However, this could not be reconciled with other spectral data. LC3 therefore contains 13-benzoyloxy-multiflorine **23**, but the nature of the remaining, apparently volatile, esterifying acid remains to be established.

DISCUSSION

Our findings for leaf alkaloids show considerable differences from those of Crow and his colleagues (4). Much of the difference is related to the fact that these workers did not realize that the greater part of the epilupinine was present in ester form. Crow (pers. comm.) has pointed out that their counter-current distribution (ether–2% NaOH) occupied several days; this would have allowed for considerable hydrolysis of esters and would account for the high proportion of epilupinine found by this method. The presence of multiflorine, epilupinine *N*-oxide and free epilupinine has been confirmed, but we have also established the presence of two new alkaloids in addition to the epilupinine esters and the related *N*-oxide **16**. These are the benzoate ester **23** and the ring-cleaved alkaloid **19** which may both derive biosynthetically from multiflorine through hydroxylation followed either by esterification or ring fragmentation and reduction. The alkaloid previously designated LV3 appears to be the same as our LC7 but we were unable to find any alkaloid corresponding to the previously reported LV4, which from combustion analyses, could have been an epilupinine ester of a trihydroxy benzoic acid. In addition we were not able positively to demonstrate the presence of sparteine; if actually present in our extracts, it was in exceedingly small amounts. Both of these two alkaloids were found by Crow and Michael (4) only after their counter-current separation, and they may be artifacts.

The amount of free leaf alkaloid found in the early studies (4) was very much lower than in the current investigation (0.8%, as against 3.5%). Our observations show that ester material could have been lost during the extraction of lipids with chloroform in acid solution, but this would not fully account for the difference.

Although our findings for the seed alkaloids largely confirm the earlier studies (4), we found very much less free epilupinine (7% of crude free alkaloids as against 30%). While we cannot positively identify the previously reported alkaloid LV2, we are of the opinion that it is 13-hydroxymultiflorine. The published combustion analysis of LV2 approximates that of 13-hydroxymultiflorine, and the reported chromatographic behaviour is consistent with this assignment.

Tlc on free alkaloids of leaf indicate that, apart from raised levels of LC4 in very young plants, there is no very marked change of alkaloids during growth up to seed maturity. In the absence of detailed studies, it would seem that multiflorine and LC2 are transported to the seed with little change. By contrast, all the epilupinine esters and most of the free epilupinine of the leaf are metabolized to epilupinine *N*-oxide with only a small amount of residual free epilupinine.

Esters of 13-hydroxymultiflorine are also absent from the seed and appear to have been converted, at least in part, to the free base.

Published data on North American lupins (17) and our own unpublished data on other Mediterranean lupins suggest that alkaloid patterns may vary considerably within an apparently homogeneous species. By contrast *L. cosentinii* seems to be a species with a stable alkaloid pattern. The tlc pattern for free leaf alkaloids from three ecotypes from Morocco, three from Tunisia, and one from Spain was indistinguishable from that of the local strain, which has been naturalized in Western Australia for 100 years.

Reports of C_{10} esters of ferulic (18), coumaric (19), and rhamnosylcoumaric acids (20) among the *L. luteus* alkaloids parallel our observations with *L. cosentinii*, although the alkaloid esters derive from lupinine in the former plant and epilupinine in the latter.

EXPERIMENTAL[4]

PLANT MATERIAL.—Leaves were collected at the early flowering stage from wild plants growing at South Perth in August 1974, 1976, and 1977. Seeds were collected from the same area in the same years. A voucher specimen has been deposited at the Western Australian Herbarium (Perth).

Tlc observations were also made on leaf extracts from plants of the same species grown from seeds of ecotypes collected by Dr. J. S. Gladstones from Mediterranean localities and from leaf samples of the local wild strain collected through the growing period. The tlc patterns are described in the Discussion section.

THIN LAYER CHROMATOGRAPHY.—For silica gel G plates, the solvent system chloroform-methanol saturated with ammonia gas (ca7N)-methanol was used routinely. A ratio of 93:5:2 was used for general purposes (system 1a), 88:5:7 for LC7–LC8 alkaloids (system 1b) and 70:15:15 for stable polar or highly basic alkaloids (system 1c). For cellulose MN300 plates, *n*-butanol: acetic acid-water (80:3:17) was used (system 2); this system gave R_f values higher than those obtained by paper chromatography in the early studies, (4) but the relative R_f values are comparable. For the less stable, fully hydrogenated desoxy C_{15} alkaloids derived from LC2, tetrahydrorhombifoline and *N*-methyl angustifoline, we have used System 2 or air-dried, neutral aluminium oxide (Woelm) plates with benzene-methanol (4:1) (system 3); the acetates from Sephadex LH20 separations may be used on these two systems without prior reversion to free bases. Plates were routinely developed to 12.5 or 15 cm and viewed under uv light immediately and after drying. Potassium iodoplatinate was used to visualize alkaloids, and silica gel plates were subsequently exposed to iodine vapor to visualize alkaloids which gave little or no color with the iodoplatinate on this adsorbent (LC1, LC8 and eLi).

COLUMN CHROMATOGRAPHY.—Preliminary fractionation of crude alkaloids was made by gradient elution on acid-washed Davison silica gel 923 (100–200 mesh) using toluene with increasing amounts of methanol and finally with pure methanol. The eluents were made alkaline with 1% methanol saturated with NH_3.

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[4]Melting points are uncorrected. Uv spectra were determined with a Varian-Techtron 623 spectrophotometer. Ir spectra were determined on a Perkin Elmer 823 spectrophotometer using Nujol or CS_2 solutions. Nmr spectra were determined at 60 MHz with a Varian A-60-A or with a Hitachi Perkin Elmer R-24B spectrophotometer using $CDCl_3$ solutions. A Bruker HX-90 instrument was used for determinations at 90 MHz. Mass spectra were determined with Varian MAT CH7 and MAT 311 instruments. Optical rotations were determined with a Perkin Elmer 141 polarimeter using methanol solutions (c, 1.0) except for LC7 for which a methanol-chloroform (1:1) solution was used.

Gas chromatography was carried out on a Packard 7400 instrument with f.i.d. and N_2 as a carrier gas. Columns were packed with Chromosorb W. HP containing 3% OV 101. Injection temperature 260°. Initial temperature 120°, 6° per min to 280°.

Microanalyses were performed by the Australian Microanalytical Service, CSIRO, Melbourne.

Samples of multiflorine, epilupinine and epilupinine *N*-oxide were prepared by the procedures described under the Extraction and Fractionation headings. The physical constants agreed closely with those found in the earlier investigations (4, 25). 13-Hydroxylupanine was obtained from *L. angustifolius* (21). Tetrahydrorhombifoline and *N*-methyl angustifoline were prepared by published methods (15).

Base hydrolyses were carried out by refluxing with 5% methanolic KOH for 75 min under N_2.

Successful separation of most individual alkaloids was achieved by the short column chromatography (scc) procedure of Hunt and Rigby (22). Acid-washed silica gel H (Merck) was re-activated at 105° for several hours. Solvent systems were chloroform-methanolic ammonia mixtures similar to those used for silica gel tlc but of much lower polarity. In contrast to results reported with scc on silica gel HF_{254} (23), we did not obtain satisfactory separations unless sufficient ammonia was present to neutralize all acidic sites on the prepared gel H. Optimum separations were obtained with isocratic elution. Chloroform preserved with 1% methanol gave elution mixtures which were too polar for the satisfactory separations of LC1–3. In these cases the methanol was removed by distillation immediately before use and 0.4–0.8% methanolic ammonia added to the resulting chloroform for elution of the alkaloids.

For highly basic or highly polar alkaloids, satisfactory separations were not always obtained by scc on silica gel with chloroform-methanol-ammonia elution, and there was some indication of decomposition of the fully-hydrogenated desoxy C_{15} alkaloids derived from LC2 tetrahydrorhombifoline and *N*-methylangustifoline. With such alkaloids, we obtained useful separations on Sephadex LH20 with methanol-water-acetic acid-ethyl acetate (100:20:1:379 for LC8 and 120:20:1:259 for 13-hydroxymultiflorine, 13-hydroxysparteine and the fully hydrogenated C_{15} alkaloids); the hydrogenation products of LC2 required a second separation with a solvent ratio of 70:20:1:309. The alkaloids were applied as acetates equivalent up to 6 mg free base per g LH20. With a 25 mm diameter column, a flow rate of 1 ml per min was used.

Gas chromatography has been of use in confirming the purity of the separated simpler alkaloids. With some of the more complex alkaloids (LC7, LC8, LC9 and epilupinine *N*-oxide) there are obvious indications of breakdown under the high temperatures used on the column. However, in some of these cases the fingerprint chromatogram has provided a useful indication of the nature of the parent compound.

The chromatographic data routinely used to identify individual alkaloids are summarized in table 1.

TABLE 1. Chromatographic data on *L. cosentinii* alkaloids.

Alkaloid	GC Retention time (min)			TLC data Relative R_f values[b]	
				System 1a	System 2
LC1	5.3			0.75	0.62
LC2	16.3			0.71	0.45
LC3	25.6	26.7	31.0	0.70	0.69
Mf	18.3			0.65	0.35
LC4	26.6			0.51	0.84
LC5	25.4			0.45	0.85
LC6	25.4			0.42	0.86
LC7	26.6[a]			0.40	0.65
eLi	3.8			0.36	0.47
LC8	5.3[a]	6.1[a]	16.7[a]	0.30	0.73
13-Hydroxymultiflorine	22.1			0.28	0.24
Epilupinine *N*-oxide	3.8[a]			0.12	0.62
LC9	3.8[a]	25.4[a]	26.6[a]	0.10	0.81

Fluorescence, 350nm (system 1a); LC3, Mf, 13-hydroxymultiflorine dark; LC2 transient yellow; LC4 bright blue; LC5, LC6, LC9 purple; LC7 bluish.

Colors with iodoplatinate (system 1a): LC3–9, purplish brown; LC2, blue; Mf, purplish blue; eLi, pale blue; 13-hydroxymultiflorine, epilupinine *N*-oxide greenish blue; LC1 and LC8 barely visible.

[a]These peaks are probably breakdown products.

[b]R_f measured from center of spot.

EXTRACTION AND FRACTIONATION OF LEAF ALKALOIDS.—500 g of green leaves without petioles (*ca* 70 g dried weight) were placed in 2.5 liters boiling 85% (v/v) ethanol and kept boiling for 10 minutes. After cooling, the leaves were macerated and re-extracted with boiling 70% ethanol. The combined extracts were evaporated under vacuum to remove ethanol, and the solid residue was discarded. The pH was brought to 2, and the lipids were removed with ethyl ether. (If chloroform is used to extract lipids, LC3–6 are removed almost quantitatively while LC1, LC7 and LC9 are partially extracted). Alkaloids were extracted sequentially with chloroform at pH 9, 10.5 and 12 and the extracts combined to give crude free alkaloids (*ca* 3.5% of leaf, dried weight). The aqueous phase was examined for *N*-oxides by reduction as

described for seeds (see below). The reduction products were identified as epilupinine (1974 collection) and 9:1 epilupinine, epilupinyl acetate (1976 collection). The presence of epilupinyl acetate is attributed to the incomplete extraction of the *N*-oxide (LC8) by chloroform.

A methanol solution of the crude alkaloids (10 g per 100 ml) was kept for 1 week at 4° to give an insoluble fraction (3.5% of crude alkaloids) which after recrystallization (methanol-chloroform) gave a mixture of LC7 and closely related alkaloids.

The soluble fraction was partially separated on Davison 923 silica gel to give four mixed fractions: (a) LC2 and LC3 (ca 5%), (b) LC1 with LC4–6 (15–20%), (c) Mf and LC9 (20–25%), and (d) a complex mixture of alkaloids (ca 50%) with R_f 0.12-0.40 on system 1 (see fig. 1). Fractions a-c were separated into individual alkaloids by scc. Fraction (d) was converted to the mixed acetates and separated on Sephadex LH20 to give LC8 (subsequently purified by scc), a mixed ester fraction and epilupinine with a trace of epilupinine *N*-oxide. The mixed ester fraction was reverted to free bases and treated with methanol to give further insoluble LC7 alkaloids together with a mixture of unidentified soluble epilupinine esters which showed no evidence of *N*-oxide or glycosidic functionality.

Pure LC7 was obtained from the insoluble alkaloids by scc. Later fractions from the column (ca 50%) formed a highly insoluble mixture which could not be separated further. Multiple development tlc showed some residual LC7 and two other components. On gc two peaks were obtained corresponding to those of LC4 and LC5 (or LC6). The ms showed peaks *m/e* 690, 660, 497, 345, 315, 152. The mixture may thus contain an isomer of LC7 and a crossed dimer of LC4 and LC5 (or LC6).

The approximate overall recovery of individual alkaloids from the original crude alkaloid extract was LC1 5%, LC2 3%, LC3 1%, LC4 11%, LC5 and LC6 traces, insoluble LC7 type alkaloids 10%, LC8 5%, LC9 11%, multiflorine 2%, epilupinine 20% and unidentified methanol-soluble epilupinine esters 6%.

EXTRACTION AND FRACTIONATION OF SEED ALKALOIDS.—Finely crushed seeds (−1 mm) were allowed to stand with 5 lots of 70% (v/v) ethanol at 4° over 5 days with intermittent shaking. The combined extracts were processed as for the leaves. After removal of the free alkaloids (0.4%), the aqueous phase was acidified to 1N with sulphuric acid, and the *N*-oxide was reduced (Zn dust, 4°, 4–6 hr) to give free epilupinine (0.8%, tlc). In a separate experiment the aqueous phase was extracted with *n*-butanol (4) to give a crude *N*-oxide extract which, after purification by chromatography on Davison 923 silica gel followed by scc on silica gel H, afforded pure epilupinine *N*-oxide. The free alkaloids were separated by scc to give, in order of elution, LC2 (ca 2%), multiflorine (ca 60%), epilupinine (ca 7%) and a trace of an alkaloid showing the same R_f, fluorescence and iodoplatinate reaction as 13-hydroxymultiflorine. The epilupinine fraction contained small amounts of an unidentified alkaloid staining brown with iodoplatinate.

The following are the detailed properties of the individual alkaloids.

Epilupinyl acetate (LC1, **3**) [Found: C, 68.09; H, 9.92; N, 6.89. $C_{12}N_{21}NO_2$ requires: C 68.21; H, 10.02, N, 6.63%]; $[\alpha]^{25}D+33°$; ir, ν max (film or CCl_4) 1750cm^{-1}; nmr δ (CCl_4), 1.97 (3H, s, CH_3CO), 3.95 (2H, br s, $-CH_2O-$); ms, *m/e* 211(M^+), 169, 152, 138, 136, 119, 117, 110, 98, 97, 96, 95. Base hydrolysis gave epilupinine (co-tlc).

Epilupinyl *trans*-ferulate (LC4, **5**) mp 119° (pale yellow crystals from acetone-cyclohexane) [Found: C, 69.38; H, 7.88; N, 4.34. $C_{20}H_{27}NO_4$ requires: C 69.54; H, 7.88; N, 4.05%]; $[\alpha]^{25}D+35°$, ir, ν max (Nujol) 3500, 1700, 1630, 1595cm^{-1}; uv, λ max (MeOH) 327 (ϵ=20300), Sh. 295, 235 (ϵ=11760); (MeOH+KOH) 378 (ϵ=29700), Sh. 308, 250 nm (ϵ=8240); nmr, δ 6.28 and 7.61 (2H, AB pattern, J=16Hz, *trans* CH=CH), 6.78–7.14 (3H, m, ArH), 4.17 (2H, br s, $-CH_2O-$), 3.92 (3H, s, OCH_3); ms, *m/e* 345 (M^-), 207, 193, 177, 168, 152, 138, 136, 122, 117, 110, 98, 96, 89, 84, 83, 56, 55, 41. Hydrolysis with KOH gave epilupinine (mp, mmp, ir, co-tlc) and ferulic acid (mp, mmp, co-tlc).

Epilupinyl *trans-p*-coumarate (LC5, **6**) mp 165° (off-white crystals from acetone-cyclohexane) [Found: C, 72.38; H, 8.17; N, 4.05. $C_{19}H_{25}O_3N$ requires: C 72.35; H, 7.99; N, 4.44%]; ir, ν max (Nujol) 1700, 1640, 1610, 1590cm^{-1}; uv, λ max (MeOH) 313 (ϵ=26500), 225 (sh), 208 (ϵ=14200); λ max (MeOH-KOH) 360 (ϵ=32800), 239 (sh), 205 nm (ϵ=21500); nmr, δ 4.11 (2H, br s, $-CH_2O-$), 6.19 and 7.57 (2H, AB pattern, J=16Hz, *trans* CH=CH–), 6.6–6.9 and 7.2–7.5 (4H, A_2B_2 pattern, ArH); ms, *m/e* 315 (M^+), 168, 152, 147, 138, 136, 124, 123, 122, 111, 110, 109, 98, 97, 96, 91, 84, 83, 70, 69, 68, 67, 65, 55, 41.

Epilupinyl *cis-p*-coumarate (LC6, **7**) mp 144° (off-white crystals from acetone-cyclohexane) [Found: C, 72.59; H, 8.10; N, 4.08. $C_{19}H_{25}O_3N$ requires: C, 72.35; H, 7.99; N, 4.44%]; ir, ν max (Nujol) 1710, 1620, 1595cm^{-1}; uv, λ max (MeOH) 308 (ϵ=15200); 207 (ϵ=15200), λ max (MeOH-KOH) 359 (ϵ=21200), 225 (sh), 204nm (ϵ=12700); nmr, δ 4.03 (2H, br s, $-CH_2O-$), 5.71 and 6.78 (2H, AB pattern J=12Hz, *cis* –CH=CH–), 6.55–6.80 and 7.37–7.62 (4H, A_2B_2 pattern, ArH); ms, *m/e* 315 [M^-], 168, 152, 147, 138, 136, 124, 123, 122, 111, 110, 109, 98, 96, 91, 84, 83, 70, 69, 68, 67, 65, 55, 41.

LC7 (**15**) mp 228° (methanol-chloroform) [Found: C, 69.74; H, 7.72; N, 4.36. $C_{40}H_{54}N_2O_8$ requires: C 69.54; H, 7.88; N, 4.05%]; $(\alpha)^{25}D+23°$; ir, ν max (Nujol), 3470, 1720, 1606, 1600cm^{-1}; uv, λ max (MeOH) 283 (ϵ=4830), 234 (ϵ=12350) nm; nmr, δ ($CDCl_3$) 3.89 (6H, s, 2 x OCH_3), 6.89 (6H, br s, ArH); δ (C_5D_5N), 3.78 (H, s, 2 x OCH_3); ms, *m/e* 690 [M^-], 672, 497, 395, 345,

209, 194, 177, 168, 152, 138, 136, 122, 110, 98, 96, 83, 82, 55, 41. High resolution ms, *m/e* 345.1980 ($C_{20}H_{27}NO_4$ requires: 345.2021), 194.0590 ($C_{10}H_{10}O_4$ requires: 194.0601), 177.0573 ($C_{10}H_9O_3$ requires: 177.0594), 168.1394 ($C_{10}H_{18}NO$ requires: 168.1400), 152.1438 ($C_{10}H_{18}N$ requires: 152.1437). Base hydrolysis gave epilupinine (ir, co-tlc) and an acid fraction which showed two spots on tlc (silica gel, toluene: acetic acid, 4:1).

LC9 was a mixture (**8**, **9**, **10**) which could not be separated by scc or by Sephadex LH20. Ir, ν max (Nujol) 1604, 1635, 1715cm^{-1}; uv, λ max 295, 230nm unchanged by alkali; nmr, δ 1.27, 3.4, 4.7 and 5.55 (-CH_2O- and OH and sugar protons), 3.42 (OCH_3), 5.85 (J=12Hz, *cis* -CH=CH-C(=O)-), 6.25 (J=15Hz, *trans* -CH=CH-C(=O)-), 6.7-7.1 and 7.2-7.7 (ArH and *cis* and *trans* -CH=CH-C(=O)-); ms, *m/e* 491, 461, 345, 315, 208, 168, 166, 152, 138, 136, 124, 122, 110, 98, 97, 96, 85, 83. Mild acid hydrolysis (3% HCl, 18 hr, 40-50°) gave a mixture which contained LC4, LC5, LC6, some free epilupinine, some unchanged LC9 and rhamnose. Estimation of the amounts of the individual alkaloids from scc fraction weights and by visual assessment of tlc spots indicated LC9 to contain LC4, LC5 and LC6 rhamnosides in approximate ratios of 1:3:2 respectively. Rhamnose was identified by co-tlc with multiple development. (Solvent system modified from ref. 24).

After base hydrolysis of LC9 in methanol a small amount of water was added and the methanol removed under vacuum. The basic product was extracted with chloroform and identified as epilupinine (ir and co-tlc). The aqueous solution was diluted with an excess of methanol and the KOH removed on a macroreticular resin (A15-H$^-$, Rohm and Haas). The eluate was evaporated under vacuum and methylated with diazomethane. The resulting methyl esters were separated by scc on silica gel H, eluting with cyclohexane-ethylacetate-methanol (12:12:1.1-1.4). The first fractions contained free methyl esters presumably formed by hydrolysis on the A15 column; this was followed by a mixed fraction of *cis*- plus *trans*-isomeric methyl rhamnosyl *p*-coumarates (ca 50%) and finally by a small amount of crude methyl rhamnosyl *trans*-ferulate (ca 15%). The rhamnosides were purified by re-chromatography.

Methyl rhamnosyl *p*-coumarate (**11**), thus obtained had ir, ν max (Nujol) 3500, 1700, 1605, 1600cm^{-1}; uv, λ max (MeOH) 295, 219 (sh); λ max (MeOH+KOH) 295, 215nm; nmr, δ 1.25, 3.4-4.8 and 5.42 ($W_{1/2}$ 5Hz, OH and sugar protons), 3.60 (OCH_3), 5.75 (J=12Hz cis -CH=CH-CO), 6.16 (J=15Hz trans -CH=CH-CO), 6.6-7.7 (ArH and *cis* and *trans* -CH-CH-CO); ms, *m/e* 324 (M$^+$), 275, 220, 191, 189, 178, 159, 156, 147, 137, 133, 129, 119, 111, 101, 91, 85, 71, 65, 60, 42, 39; high resolution ms, *m/e* 324.1212 ($C_{16}H_{20}O_7$ requires: 324.1208), 178.0625 ($C_{10}H_{10}O_3$ requires: 178.0630). Acid hydrolysis gave rhamnose (tlc) and mixed *cis*-+*trans*- isomeric methyl *p*-coumarates (tlc). uv, λ max (MeOH) 310, λ max (MeOH-KOH) 350nm.

Methyl rhamnosyl *trans*-ferulate (**12**) was obtained from the hydrolysis in impure form in small amount only. Ir, ν max (Nujol) 3500, 1700, 1605, 1600cm^{-1}; uv, λ max (MeOH) 316, 291 and 230 (sh) unaltered by KOH; nmr, δ 1.27, 3.3-4.9 and 5.51 ($W_{1/2}$ 5Hz, -OH and sugar protons), 3.33 and 3.83 (2X OCH_3), 6.30 and 7.59 (J=16Hz, *trans* -CH=CH), 6.8-7.1 (ArH); ms, *m/e* 208, 177, 167, 147, 129, 120, 118, 116, 105, 100, 87, 85, 74, 71, 60, 58, 52, 41, 38; high resolution ms, 208.0719 ($C_{11}H_{12}O_4$ requires 208.0735). Acid hydrolysis gave methyl ferulate, rhamnose, and an unidentified compound which showed an R_f greater than that of rhamnose and stained blue with 1:3 dihydroxynaphthalene.

Epilupinyl acetate *N*-oxide (LC8, **16**) mp 192° (acetone-cyclohexane) [Found: C, 61.36; H, 9.50; N, 5.93. $C_{12}H_{21}NO_3 \cdot \frac{1}{2}H_2O$ requires C, 60.99; H, 9.39; N, 5.93%]; $[\alpha]^{25}D$+14°; ir, ν max (film) 1735cm^{-1}; nmr δ 2.04 (3H, s, CH_3-CO-), 4.07 (4H, d, -CH_2O-); ms, *m/e* 227 (M$^+$), 211, 210, 209, 168, 154, 152, 150, 137, 122, 112, 100, 96, 67, 55, 43, 41; high resolution ms, *m/e* 227.1516 ($C_{12}H_{21}NO_3$ requires 227.1511), 168.1389 ($C_{10}H_{18}NO$ requires 168.1390), 154.1225 ($C_9H_{16}NO$ requires 154.1218), 152.1443 ($C_{10}H_{18}N$ requires 152.1447), 150.1284 ($C_{10}H_{16}N$ requires 150.1285), 137.1185 ($C_9H_{15}N$ requires 137.1166), 100.0759 ($C_5H_{10}NO$ requires 100.0756). Reduction with Zn-1N H_2SO_4, 0°, 4 hrs gave LC1 (co-tlc, gc).

LC2 (**19**) mp 60° (cyclohexane) [Found: C, 73.24; H, 9.26; N, 11.67. $C_{15}H_{22}N_2O$ requires: C, 73.13; H, 9.00; N, 11.37%]; $[\alpha]^{25}D$-520°; ir, ν max (Nujol) 1580, 1625cm^{-1}; uv, λ max (MeOH) 335nm (ϵ=14100) not altered by NaOH; nmr, δ 6.86 and 4.92 (2H, 2 x d, J=7Hz, -CH=CH-), 4.77-5.18 (2H) and 5.53-6.00 (1H); ms, *m/e* 246 (M$^+$), 205, 192, 162, 149, 136, 134, 122, 110, 108, 94, 82, 80, 67, 58, 42, 41.

Catalytic hydrogenation overnight in 2N HCl with *ca* 1 mg platinum oxide catalyst per mg alkaloid afforded a mixture of four products from which the fully hydrogenated desoxy derivative (**17**, *ca* 20%) was isolated after chromatography on Sephadex LH20 (see general experimental section). Similar catalytic hydrogenation of tetrahydrorhombifoline gave a mixture of two products from which the fully reduced desoxy derivative (**17**, *ca* 25%) was similarly recovered. The products from the two reductions were identical (ir, ms, tlc, R_f= 0.30 with system 3, bright pink color with iodoplatinate).

N-Methylangustifoline was similarly converted to the fully hydrogenated desoxy derivative (**18**) in significantly higher yield (>60%), nmr δ 2.25 (N-Me). This differed from the above product **17** in the ir and ms. Tlc (system 3) gave a spot of higher R_f (0.42) which stained mauve with iodoplatinate.

The fully hydrogenated desoxy derivatives are rapidly oxidized in air but are relatively stable in acetic acid medium.

LC3 was obtained as a mixture showing one spot on tlc; ir, ν max (CCl_4) 1605, 1660, 1720cm^{-1}; uv, λ max (MeOH) 328, 230 not altered by NaOH; nmr, δ 1.1–3.5 (ring protons), 4.81 and 6.73 (J=8Hz, –CH=CH–), 5.0–5.4 (mixed >C*H*O–), 7.3–7.6 and 7.9–8.1 (ArH), and sharp peaks at 0.93, 1.01, 1.11, 1.18; ms, *m/e* 366, 344, 254, 245, 243, 234, 232, 217, 216, 215, 203, 201, 188, 175, 174, 164, 162, 149, 147, 134, 132, 122, 120, 111, 110, 96, 95, 82, 67, 55.

Base hydrolysis gave an alkaloid fraction which was a highly hygroscopic gum showing one spot on tlc. The properties of this alkaloid correspond to those previously reported for 13-hydroxymultiflorine (16). $[\alpha]^{25}D-335°$; uv, λ max (MeOH) 326nm (ϵ=14100) not altered by alkali[5]; nmr, δ 1.1–3.2 (ring protons), 4.11 ($W_{\frac{1}{2}}$ 7Hz, >C*H*O–), 4.86 and 6.73 (J=7Hz, –CH=CH–); ms, *m/e* 262 (M^+), 244, 164, 163, 150, 134, 126, 122, 119, 117, 111, 110, 100, 96, 94, 86, 84, 83, 82, 67, 55, 41. Catalytic hydrogenation (Pt catalyst in 2NHCl) gave a base (mp 153–154°) identical with 13-hydroxysparteine formed by the hydrogenation of 13-hydroxylupanine (ir, mp, mmp).

Crystallization of the acid fraction from the hydrolysis gave benzoic acid (mp, mmp and nmr). Additional nmr signals at δ 1.0–1.9 and 3.3 in the crude acid fraction had a total integration one quarter of that for the aromatic proton region.

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[5]Multiflorine shows λ max (MeOH) 326nm (ϵ 15800), λ max (cyclohexane 302nm (ϵ 13400). λ max values near the latter value have been reported (4, 16) without specifying solvent. Comin and Deulofeu (25) give λ max (EtOH) 326nm.

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