*Phytochemistry*, Vol. 25, No. 5, pp. 1143–1150, 1986.
Printed in Great Britain.

0031-9422/86 $3.00 + 0.00


# GC/MS EXAMINATION OF FOUR *LYCOPODIUM* SPECIES FOR ALKALOID CONTENT

ROBERT V. GERARD and DAVID B. MACLEAN*

Department of Chemistry, McMaster University, Hamilton, Ontario, Canada, L8S 4M1

(*Received* 4 *September* 1985)

**Key Word Index**—*Lycopodium australianum*; *L. clavatum*; *L. deuterodensum*; *L. fastigiatum*; Lycopodiaceae; alkaloids; GC/MS; chemotaxonomy.

**Abstract**—A GC/MS examination of extracts of *Lycopodium clavatum* var. *borbonicum* and *L. deuterodensum* revealed alkaloids which had not been previously observed in these species. New alkaloids have been found in *L. australianum* and in *L. fastigiatum*, two species which had not been investigated before.

## INTRODUCTION

There are about 100 alkaloids of established structure derived from some 20 different ring systems in *Lycopodium* [1–3]. Braekman *et al.* [4, 5] have discussed the possibility of using the various ring systems as markers for the classification of *Lycopodium* species since their taxonomy has been a matter of controversy [6 and references therein]. It has been noted by Braekman *et al.* [4, 5] that only about 10% of the extant *Lycopodium* species have been examined for alkaloid content and, therefore, much more work is required to test the reliability of alkaloid content for purposes of chemotaxonomy. It was with this consideration, among others, in mind that we were led to apply a GC/MS method for the rapid screening of *Lycopodium* extracts for their alkaloids. The method is able to recognize new alkaloids as well as those of established structure and is applicable to small amounts of plant material. Here we report its application to the investigation of two previously examined species. *L. clavatum* var. *borbonicum* [7, 8] and *L. deuterodensum* Herter [9] ( = *L. densum* Labill. [6]) and to two species, *L. australianum* (Herter) Allan and *L. fastigiatum* R. Br., which are examined here for the first time.

## RESULTS AND DISCUSSION

The results of the examination of the various extracts for their alkaloid content are recorded in Tables 1–4. Retention indices were determined on 33 authentic samples of alkaloids available to us and these were used to help identification. The values for the authentic samples (ARI) and the values found on examination of the extracts (RI) by GC are both recorded in the Tables. A reference library of mass spectra was prepared using 39 authentic samples and this was supplemented by entering into the data base spectra of 36 other alkaloids obtained from the literature. In an actual run on an extract a library search program (VG data system 2000) was used to search the data base to find the best match to a sample spectrum. Three different measures of fit were used in the search program, a purity fit, a mixture fit and a reverse fit (*vide infra*), and each measure of fit is recorded in the Tables. The closer the values are to 1000 the better the unknown matches the library spectrum. In all cases the line spectra were retrieved from the data system and compared visually with those of authentic samples or with literature spectra. This proved to be a more convincing measure of identity than the numbers derived from the library search. In the case of chromatographic peaks containing more than a single component, spectra were taken from the leading and trailing edges of the peak and also from the central portion. In most cases clean spectra were obtained for each component in this way. In a few cases where mixtures were recognized to contain known alkaloids, resort was taken to subtracting the spectrum of the known alkaloid from that of the mixture to provide a more representative spectrum of the second component. An estimate of the relative percent of each alkaloid in the mixture is also recorded in the Tables; where two or more alkaloids coeluted a combined value is given. The alkaloids are listed in the Tables in order of their elution from the column.

In a previous examination of the alkaloids of *L. clavatum* var. *borbonicum* [7] by more conventional methods the following alkaloids were reported; anhydrolycodoline (**1**), lycopodine (**2**), dihydrolycopodine (**3**), acetyldihydrolycopodine (**4**), lycodoline (**5**), lycoflexine (**6**), borbonicine (**7**), lycodiflexine (**8**) and $N_a$-acetyl-$N_b$-methylphlegmarine (**9**). The structures of borbonicine and lycodiflexine have not been clarified but their masses are known [7]. In this study (Table 1) we have detected all but lycodiflexine and in addition we have found lycodine (**10**), flabelliformine (**11**), alkaloid L20 (**12**) and an alkaloid of *m/z* 279 whose identity has not been firmly established. Lycodiflexine may not have been detected because of its involatility; its molecular weight of 562 is well above that of other components in the extract and a different set of operating conditions might be required for its elution. This re-investigation of *L. clavatum* var. *barbonicum* reveals very clearly that the method employed is suitable for the examination of *Lycopodium* extracts, particularly for those alkaloids with

*Author to whom correspondence may be addressed.

Table 1. Alkaloids of *L. clavatum* var. *borbonicum*

| Component | Alkaloid | RI | ARI | Measure of fit: pure | mixture | reverse | % |
|---|---|---|---|---|---|---|---|
| A | lycodine (**10**) | 1935 | 1930 | 913 | 913 | 993 | 3.5 |
| B | anhydrolycodiline (**1**) | 1935 | — | 536 | 536 | 905 | |
| C | lycopodine (**2**) | 2000 | 2030 | 846 | 866 | 923 | 80.8 |
| D | dihydrolycopodine (**3**) | 2000 | 2000 | 810 | 927 | 951 | |
| E | flabelliformine (**11**) | 2073 | 2070 | 688 | 737 | 743 | 0.4 |
| F | acetyldihydrolycopodine (**4**) | 2086 | 2085 | 885 | 885 | 995 | 10.0 |
| G | lycodoline (**5**) | 2120 | 2133 | 568 | 568 | 937 | 1.6 |
| H | L20 (**12**) | 2149 | 2154 | 767 | 857 | 880 | 0.7 |
| I | unknown 279 | 2182 | — | — | – | — | 0.9 |
| J | lycoflexine (**6**) | 2263 | — | 622 | 849 | 725 | 0.2 |
| K | borbonicine | 2278 | – | – | — | — | 0.5 |
| L | $N_a$-acetyl-$N_\beta$-methyl phlegmarine | 2380 | – | 603 | 628 | 951 | 1.4 |

Table 2. Alkaloids of *L. deuterodensum*

| Component | Alkaloid | RI | ARI | Measure of fit: pure | mixture | reverse | % |
|---|---|---|---|---|---|---|---|
| A | lycodine (**10**) | 1936 | 1930 | 754 | 808 | 933 | 1.1 |
| B | anhydrolococoline (**1**) | 1954 | — | 324 | 443 | 724 | |
| C | lycopodine (**2**) | 2018 | 2030 | 711 | 829 | 927 | 93.6 |
| D | flabelliformine (**11**) | 2091 | 2070 | 615 | 615 | 994 | 0.1 |
| E | lycodoline (**5**) | 2145 | 2133 | 524 | 524 | 989 | 3.4 |
| F | unknown 271 | 2145 | — | — | — | — | |
| G | lycoflexine | 2286 | – | 459 | 509 | 857 | 0.7 |
| H | clavolonine | 2308 | 2300 | 767 | 767 | 980 | |
| I | flabelline | 2384 | 2422 | 591 | 865 | 665 | 0.2 |
| J | unknown 279 | 2416 | — | — | – | — | 0.4 |
| K | unknown 304 | 2416 | – | — | — | – | |
| L | unknown 272 | 2526 | — | — | — | – | 0.5 |

Table 3. Alkaloids of *L. australianum*

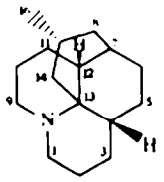
| Component | Alkaloid | RI | ARI | Measure of fit: pure | mixture | reverse | % |
|---|---|---|---|---|---|---|---|
| A | lycodine (**10**) | 1921 | 1930 | 811 | 841 | 915 | 3 |
| B | cernuine (**15**) | 2268 | — | 678 | 716 | 932 | 82 |
| C | unknown | 2422 | — | – | — | - | 15 |

a basic skeleton of 16 carbon atoms and 1 or 2 nitrogen atoms.

*Lycopodium deuterodensum* has been examined previously [9] but only three alkaloids were reported, namely lycopodine (**2**), L34 (clavolonine, **13**) [10] and an alkaloid assigned the formula $C_{14}H_{21}NO_2$, and designated L35. In our examination we have found lycopodine, which accounts for *ca* 94% of the alkaloids detected, and clavolonine but none of L35. We have, however, detected a host of alkaloids of established structure present in minor amount which have not previously been reported. They are, in order of elution, lycodine (**10**), anhydrolycodoline (**1**), flabelliformine (**11**), lycodoline (**5**), lycoflexine (**6**), flabelline (**14**) and three minor components that have not been characterized showing molecular ions at *m/z* 271, 279 and 272. Component K would appear to be a mixture of several compounds of which that of highest mass has a molecular weight of 304. Component E has been identified as lycodoline but this assignment must be considered tentative because the isomeric alkaloid L23 (epimeric with **5** at C-12) has the same peaks in its mass spectrum and the two spectra differ only in intensity. However, the spectrum of component E more closely resembles the

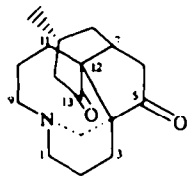
Table 4. Alkaloids of *L. fastigiatum*

| Component | Alkaloid | RI | ARI | Measure of fit: pure | mixture | reverse | % |
|---|---|---|---|---|---|---|---|
| A | lycodine (**10**) | 1948 | 1930 | 525 | 819 | 575 | 0.9 |
| B | anhydrolycodoline (**1**) | 1956 | - | 388 | 530 | 726 | |
| C | lycopodine (**2**) | 2010 | 2030 | 897 | 964 | 926 | 72.0 |
| D | dihydrolycopodine (**3**) | 2010 | 2000 | 440 | 702 | 605 | |
| E | flabelliformine (**11**) | 2059 | 2070 | 504 | 750 | 618 | |
| F | acetyldihydrolycopodine (**4**) | 2095 | 2085 | 806 | 806 | 950 | 9.5 |
| G | lycodoline (**5**) | 2171 | 2133 | 620 | 620 | 976 | 0.8 |
| H | lycoflexine (**6**) | 2349 | --- | 618 | 724 | 836 | 6.0 |
| I | unknown 273 | 2349 | - | | - | — | |
| J | clavolonine (**13**) | 2349 | 2300 | 524 | 648 | 581 | |
| K | des-*N*-methylfastigiatine | 2514 | — | — | — | - | |
| L | fastigiatine | 2514 | — | - | — | — | 10.8 |
| M | α-obscurine | 2514 | 2422 | 825 | 825 | 998 | |

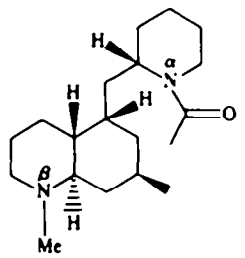


Lycopodane skeleton

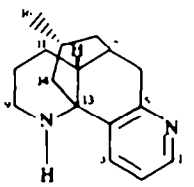
| | Functionality |
|---|---|
| Anhydrolycodoline (**1**, $C_{16}H_{23}NO$) | 5,=O; $\Delta^{11,12}$ |
| Lycopodine (**2**, $C_{16}H_{25}NO$) | 5,=O |
| Dihydrolycopodine (**3**, $C_{16}H_{27}NO$) | 5,OH (*R*) |
| Acetyldihydrolycopodine (**4**, $C_{18}H_{29}NO_2$) | 5,OAc (*R*) |
| Lycodoline (**5**, $C_{16}H_{25}NO_2$) | 5,=O, 12,OH (*S*) |
| Flabelliformine (**11**, $C_{16}H_{25}NO_2$) | 5,=O; 4,OH (*R*) |
| L 20 (**12**, $C_{16}H_{25}NO_2$) | 5,=O; 6,OH (*S*) |
| Clavolonine (**13**, $C_{16}H_{25}NO_2$) | 5,=O; 8,OH (*R*) |
| Flabelline (**14**, $C_{16}H_{28}N_2O$) | $\Delta^{4,5}$; 5, NHAc |



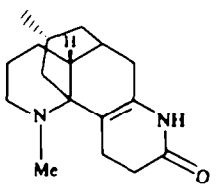
Lycoflexine (**6**)
$C_{16}H_{25}NO_2$



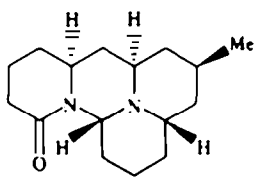
$N_\alpha$-Acetyl-$N_\beta$-methylphlegmarine (**9**)
$C_{19}H_{34}N_2O$



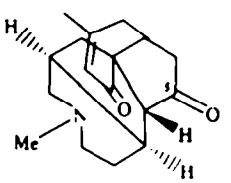
Lycodine (**10**)
$C_{16}H_{22}N_2$



α-Obscurine (**16**)
$C_{15}H_{24}N_2O$



Cernuine (**15**)
$C_{16}H_{26}N_2O$



Magellanimone (**17**)
$C_{15}H_{23}NO_2$

spectrum of authentic lycodoline than that of authentic L23. The unknown with a molecular ion at *m/z* 279 has a spectrum that is very similar to that of alopecuridine and similar to that observed for component I of *L. clavatum* (*vide supra*). The spectra of the other unknowns in *L. deuterodensum* do not resemble the spectra of any of the

known alkaloids. The alkaloids of unknown structure represent about 4% of the total alkaloids based upon the GC examination and we did not attempt to isolate them.

The results of the examination of the extract of *L. australianum*, a species not examined before, are recorded in Table 3. Cernuine (**15**) is the major alkaloid of the plant but it also elaborates lycodine and a new alkaloid, component C, which comprises about 15% of the total alkaloids eluted in the GC examination. The retention index and mass spectrum of 'C' do not correspond to any of the known *Lycopodium* alkaloids. The EI mass spectrum has a single intense ion at *m/z* 166, but is otherwise uniformative. The composition of the ion at *m/z* 166 as measured by high resolution mass spectrometry is $C_{11}H_{20}N$ (found 166.164; calc. 166.160). A probe spectrum run on a sample of C isolated by liquid chromatography showed an ion of low intensity at *m/z* 344 ( < 1% of *m/z* 166) and a CI spectrum of component C using methane as reagent gas showed an $[M+H]^+$ ion at *m/z* 345 which substantiates the EI data with respect to the molecular ion. The fragment ion of *m/z* 166 was still however the most intense ion in the CI mass spectrum. An ion of *m/z* 166 is a feature of the mass spectra of phlegmarane alkaloids [7] that have a methyl group at $N_\beta$, such as **9** shown in Scheme 1. It is possible therefore that component C may contain a part structure similar to that giving rise to ion *m/z* 166 in the phlegmarane alkaloid shown in Scheme 1. However, with the data available and the small sample at hand it was not possible to define the structure of component C and further examination must await the collection and extraction of fresh plant material.

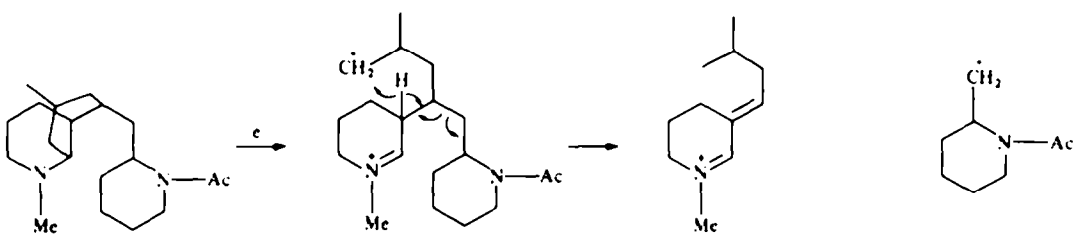
The extract of *L. fastigiatum*, also examined for the first time, gave the results shown in Table 4. A total of 13 alkaloids were recognized of which 10 are known compounds. The molecular ion of component I was examined by HRMS (found: 273.172; calc. for $C_{17}H_{23}NO_2$, 273.173). The composition corresponds to that of magellaninone (**17**) [11] but unfortunately we were unable to obtain an authentic sample for comparison. Components K and L, which have been given the trivial names, des-*N*-methylfastigiatine and fastigiatine, respectively, and which coelute with α-obscurine, are new alkaloids which have been separated by liquid chromatography. Their compositions were established by HRMS: des-*N*-methylfastigiatine, $C_{18}H_{26}N_2O$, and fastigiatine, $C_{19}H_{28}N_2O$. Components K and L showed $[M+1]^+$ ions at *m/z* 287 and 301, respectively, in their CI mass spectra run with methane or ammonia as reagent gas. Des-*N*-methylfastigiatine was converted to fastigiatine by *N*-methylation with formaldehyde and sodium borohydride thereby establishing the structural relationship between the two alkaloids. The low resolution spectrum of fastigiatine was characterized by ions at *m/z* 176 and 124 which together accounted for the mass of the molecule. In des-*N*-methylfastigiatine the ion of *m/z* 176 was still present but the ion present at *m/z* 124 in fastigiatine had shifted to *m/z* 110 in the spectrum of des-*N*-methylfastigiatine. The spectra were unlike the spectra of any other *Lycopodium* alkaloids containing two nitrogen atoms and yielded little structural information.

The $^1$H NMR spectrum of fastigiatine indicated the presence of an NMe group (δ2.32, *s*, 3H), an NAc group (δ2.15, *s*, 3H), a CHMe group (δ0.92, *d*, 3H, $J$ = 6.4 Hz) and a single vinylic proton in a trisubstituted double bond (δ5.20, *dd*, 1H, $J$ = 1.1 and 5.5 Hz). There were also signals corresponding in area to three protons which were distinctly separated from the vinyl proton and the bulk of the aliphatic protons. These signals were attributed to protons situated on carbon atoms geminal to nitrogen. However, the rest of the complex spectrum did not lend itself to simple analysis. The IR spectrum of fastigiatine showed an amide absorption at 1620 cm$^{-1}$ and a UV absorption, $\lambda_{max}^{MeOH}$ 224 nm, log ε 3.78, which suggested that the vinyl group and the acetamide group were present as an enamide. With the composition and the number and nature of the functional groups established it was apparent that the alkaloids were pentacyclic.

The methyl ester of ferulic acid was detected in the alkaloid extract of *L. fastigiatum* by GC/MS. Ferulic acid itself has been previously found in *L. clavatum*, *L. selago* and *L. annotinum* [12]. We have also detected methyl ferulate in extracts of *L. lucidulum* prepared and examined by the procedures reported in this investigation [unpublished results].

In the Wilce classification [6] of the Lycopodiaceae, *L. deuterodensum* and *L. fastigiatum* are both placed in the subgenus *Lycopodium* of the genus *Lycopodium*. Braekman *et al.* [4 5] have noted that members of this subgenus are rich in alkaloids of the lycopodane ring system and this is evident with the species examined here. In both cases alkaloids of other ring systems are present only in minor amount. *Lycopodium australianium* on the other hand has been classified in the *Urostachys* subgenus, *Selago* section, by Wilce [6]. This is the first reported isolation of a cernuane alkaloid from this subgenus. Previously cernuane alkaloids have been found only in the subgenus *Lepidotis* [4, 5].

The GC/MS method described here has proved to be useful for screening extracts from *Lycopodium* species. By using the library of reference spectra it is possible to recognize and identify those components of the extract




*m/z* 166

Scheme 1.

whose spectra are recorded in the library and by exclusion compounds which may be new. The method also lends itself to quantitation so that an estimate of the amount of plant material required for isolation and characterization of new alkaloids can be readily calculated.

## EXPERIMENTAL

*Materials and methods.* The three *Lycopodium* species collected in New Zealand were obtained through the Department of Scientific and Industrial Research, Botany Division, Christchurch, New Zealand. *Lycopodium australianum* (Herter) Allan (75 g) was collected at Travers Range, Nelson and Malte Brun Range at Mt. Cook, South Island, New Zealand. *Lycopodium deuterodensum* Herter (2259 g) was collected at Huia, Auckland, North Island, New Zealand. *Lycopodium fastigiatum* R. Br. (2077 g) was collected at Mt. Robert, Nelson Lakes National Park, South Island, New Zealand. *L. clavatum* var. *borbonicum* (5100 g) was obtained through Dr. J. C. Braekman; the plant material was collected in Zaire, Africa, by Dr. L. Nyembo.

The source of 39 alkaloids used in compiling the library of spectra is indicated in Table 5. The alkaloids are listed in order of molecular weight. The source of the alkaloids is given along with literature refs to previous mass spectral examination. When a source reference is absent literature data were entered in the library. The $^1$H NMR spectra were run on a Bruker WM250 FT spectrometer or on a Bruker WP80 spectrometer. The IR spectrum was run in $CHCl_3$ soln and the UV spectrum in MeOH soln. Neutral alumina (activity I) and silica gel (Kieselgel 60, 230–400 mesh) were used for CC and alumina plates (1.5 mm) for preparative layer chromatography.

*Extraction of plant material.* Dried plant material was extracted with MeOH in a Soxhlet apparatus for 48 hr. The MeOH extract was then filtered and taken to dryness in $N_2$. The residue was heated on a steam bath with 5% HCl and left to stand for up to 24 hr then filtered over Celite. The Celite was washed with 5% HCl until the filtrate was negative to Dragendorff's reagent. The filtrate was then basified with conc. $NH_3$(aq) and extracted with $CHCl_3$. Removal of the $CHCl_3$ gave a crude alkaloid extract which was filtered through a pad of alumina (activity I) using an EtOAc MeOH (9:1) mobile phase. Removal of the solvent under red. pres. yielded an alkaloid extract, which was dissolved in MeOH (1 mg/ml) for GC/MS analysis.

*Gas chromatography.* One $\mu$l each of authentic samples of the *Lycopodium* alkaloids (1 mg/ml) were coinjected with the even numbered *n*-alkanes $C_{16}$–$C_{36}$ (1 mg/ml in hexane) to determine their RI. These experiments were repeated in triplicate using on-column injection onto a 30 m × 0.32 mm i.d. wide bore fused silica capillary column coated with the methylsilicon phase (0.1 $\mu$) DB-1 (J&W). Conditions: temp. prog. 50–300°, 10°/min; carrier gas: He, 1.0 bar. A gas chromatograph with a home built on-column injector was used for retention index determination.

All extracts were examined on a gas chromatograph equipped with a dual detector system, FID/NPD. The column eluent was split into two streams, and the output from each detector was recorded on the same chart recorder. The conditions used were the same as above, with the exception of the use of a commercial on-column injector.

*Mass spectrometry.* The MS of authentic samples were recorded using a Vacuum Generator (VG) Micromass 7070F mass spectrometer. An electron energy of 70 eV was used with the source heated to 200° and an accelerating voltage of 4 kV. The mass range, 40–500, was scanned at 2 sec per decade. Samples were introduced via a sample probe.

The MS of the samples, along with literature MS of the alkaloids were entered into a library data base for computer identification of GC/MS data. The soft-ware used was VG data system 2000 running on a Digital PDP 8 computer. Data were stored on Digital RLO2 disk packs. The software uses three different equations to determine a measure of 'fit'. The closer the value is to 1000 the better the unknown matches the library spectrum.

The purity fit equation uses both the intensity of the masses in the unknown and the library.

$$\text{Purity fit} = \frac{1000(\Sigma \text{Ium} \times \text{ILm})^2}{\Sigma \text{Iu}^2 \times \Sigma \text{IL}^2}.$$

The mixture fit uses only the intensity of the masses in the unknown which also occur in the library.

$$\text{Mixture fit} = \frac{1000(\Sigma \text{Ium} \times \text{ILm})^2}{\Sigma \text{Ium}^2 \times \Sigma \text{IL}^2}.$$

The reverse fit uses only the intensity of the masses in the library which occur in the unknown.

$$\text{Reverse fit} = \frac{1000(\Sigma \text{Ium} \times \text{ILm})^2}{\Sigma \text{Iu}^2 \times \Sigma \text{ILm}^2}.$$

Iu = Intensity of a peak in the unknown; IL = Intensity of a peak in the library; Ium = Intensity of a peak in the unknown which matches a peak in the library; ILm = Intensity of a peak in the library which matches a peak in the unknown.

At run time it is possible to omit masses from the search (such as column bleed or background). Search files were created using the six largest peaks in the MS.

In the GC/MS runs a gas chromatograph was interfaced to the mass spectrometer. The detector end of the column described above was inserted through a heated (250°) glass lined stainless steel tube to within a few millimeters of the electron beam. Conditions: temp. prog. 50–300°, 10°/min; carrier gas He, 0.2 bar. An on-column injector was used with 1 $\mu$l of extract (1 mg/ml) and the mixture of even numbered *n*-alkanes $C_{16}$–$C_{36}$ was co-injected. Mass spectrometer operating conditions were as described above with the exception of scanning at one decade per second. The ion source was kept in the tripped position until the solvent eluted. Alkaloids of established structure were identified from their retention indices and by comparison of their mass spectra with those of authentic samples previously entered into the data base. Chemical ionization mass spectra were recorded on the same instrument using $CH_4$ or $NH_3$ at *ca* 1.0 torr as reagent gases.

*Isolation of fastigiatine and* N*-methylfastigiatine.* The extract from *L. fastigiatum* was chromatographed on an alumina column (50 cm × 2 cm). The column was developed with 250 ml each of $C_6H_6$, $C_6H_6$–$Et_2O$ (1:1), $Et_2O$, EtOAc and MeOH. Fractions (125 ml) were collected and examined by GC/MS. Fraction 10 contained fastigiatine and des-*N*-methylfastigiatine along with lycoflexine (traces) and dihydrolycopodine as determined by GC/MS. Fraction 10 was rechromatographed on a silica column 50 cm × 2 cm which was developed with $CHCl_3$–$Et_2NH$ (98:2) and the fractions collected were examined by GC/MS. Fractions 1 and 2 (first eluted) contained the two new alkaloids while fractions 3 and 4 contained dihydrolycopodine and small amounts of uncharacterized material. All fractions after and including fraction 5 contained only dihydrolycopodine.

In an attempt to separate fastigiatine and des-*N*-methylfastigiatine fraction 1 was applied to a preparative (1.5 mm) alumina plate 20 cm × 20 cm. The plate was developed half-way with EtOAc–MeOH (9:1), allowed to dry and then developed with EtOAc. Two bands were observed under UV light. The bands were cut off and extracted with MeOH and

Table 5. Mass spectra entered into the data base

| Alkaloid* | Source† | $M_r$ | Reference |
|---|---|---|---|
| Luciduline | | 207 | 13 |
| Dihydroluciduline | | 209 | 13 |
| Anhydrodihydrolycopodine | | 231 | 14 |
| Lycodine | 1 | 242 | 14 |
| Selagine | | 242 | 14 |
| Anhydrolycodoline | 1 | 245 | 7 |
| Dehydrolycopercurine | 2 | 245 | 15 |
| Anhydrodeactylpaniculine | | 247 | 16 |
| Epidihydrofawcettidine | | 247 | 17 |
| Lycopecurine | 3 | 247 | 18 |
| Lycopodine | 1 | 247 | 14 |
| Dihydrodeoxycernuine | | 248 | 7 |
| Dihydrolycopodine | 1 | 249 | 14 |
| *N*-Methyllycodine | 3 | 256 | — |
| Alolycopine | | 259 | 19 |
| Anhydrolycocernuine | | 260 | 7 |
| Des-*N*-methyl-α-obscurine | | 260 | 20 |
| Acrifoline | | 261 | 14 |
| Anhydroaposerratinine | | 261 | 21 |
| 8-Deoxy-13 dehydroserratinine | | 261 | 17 |
| Gnidioidine | | 261 | 7 |
| Inundatine | | 261 | 15 |
| Isoinundatine | | 261 | 15 |
| Lycophlegmine | | 261 | 17 |
| Serratidine | 4 | 261 | — |
| Cernuine | 1 | 262 | 22 |
| Annofoline | | 263 | 14 |
| Clavolonine | 1 | 263 | 14 |
| 8-Deoxyserratinine | | 263 | 23 |
| Flabelliformine | 1 | 263 | 14 |
| L20 | 1 | 263 | — |
| L23 | | 263 | 24 |
| Lucidioline | 3 | 263 | 25 |
| Lycodoline | 1 | 263 | 14 |
| Dihydroxydeoxylycocernuine | | 264 | 7 |
| Deacetylfawcettiine | 1 | 265 | — |
| Deacetyllycoclavine | | 265 | 16 |
| Deacetylpaniculine | | 265 | 16 |
| β-Obscurine | 1 | 272 | — |
| 5-Dehydromagellanine | | 273 | 11 |
| α-Obscurine | 1 | 274 | 14 |
| Sauroxine | | 274 | 26 |
| Annotine | 1 | 275 | 27 |
| Annotinine | 1 | 275 | 14 |
| Lycoflexine | | 275 | 28 |
| Magellanine | 1 | 275 | 29 |
| Carolinianine | 2 | 276 | 30 |
| Hydroxy-des-*N*-methyl-α-obscurine | | 276 | 20 |
| Lycopaniculatine | 1 | 277 | 31 |
| Lycocernuine | 1 | 278 | 30 |
| *N,N*-Dimethylphlegmarine | | 278 | 8 |
| Alopecuridine | 3 | 279 | — |
| Serratinine | 4 | 279 | 23 |
| Flabellidine | 1 | 288 | 20 |
| Flabelline | 1 | 288 | 20 |
| Acetyldihydrolycopodine | 1 | 291 | 14 |
| Annopodine | 3 | 291 | — |
| Lyconnotine | | 291 | 32 |
| Saurudine | | 291 | 28 |
| Serratanidine | 4 | 295 | 23 |
| Lycoverticine | | 304 | 21 |
| Serratinidine | 4 | 304 | – |

Table 5 (*Contd.*)

| Alkaloid* | Source† | $M_r$ | Reference |
|---|---|---|---|
| Acetyldebenzoylalopecurine | | 305 | 33 |
| $N_a$-Acetyl-$N_\beta$-methylphlegmarine | 1 | 306 | 7 |
| α-Lofoline | 1 | 307 | 14 |
| Fawcettiine | 1 | 307 | — |
| Lycoclavine | 1 | 307 | 14 |
| Paniculine | | 307 | 16 |
| Lycofawcine | 5 | 323 | 34 |
| Megastachine | 2 | 331 | 35 |
| Acetyllofoline | | 349 | 36 |
| Alopecurine | 3 | 367 | 33 |
| Lycognidine | 2 | 457 | 21 |
| Lucidine B | | 467 | 37 |
| Spirolucidine | | 483 | 38 |

*Alkaloids listed in order of molecular weight.

†The numbers indicate the source of the alkaloid (see below) and that the spectrum was recorded in this investigation; other spectra were taken from the literature. 1, This laboratory; 2, J. C. Braekman; 3, W. A. Ayer; 4, Y. Inubushi; 5, R. H. Burnell.

filtered to remove alumina particles. The band with $R_f$ 0.67 was mainly fastigiatine while the band with $R_f$ 0.30 was mainly des-*N*-methylfastigiatine: fastigiatine: mp 143–146° ($Et_2O$); $[\alpha]_D^{23}$ 289.9 (*c* 1.36; $CHCl_3$); UV $\lambda_{max}^{MeOH}$ nm (log ε): 224 (2.78); IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 1620; EIMS *m/z* (%): 300 $[M]^{+\cdot}$ (38), 285 (20), 257 (28), 176 (18), 125 (28), 124 (100). Des-*N*-methylfastigiatine: oil; EIMS *m/z* (%): 286 $[M]^{+\cdot}$ (57), 271 (16), 243 (24), 176 (100), 111 (64), 110 (42); $^1H$ NMR ($CHCl_3$, 90 MHz): δ0.82 (3H, *d*, *J* = 8.0 Hz), 2.04 (2H, *s*), 5.15 (1H, *d*, *J* = 4.8 Hz).

*Conversion of des-N-methylfastigiatine to fastigiatine.* Formaldehyde (28%, 1 ml) was added to a soln of des-*N*-methylfastigiatine (10 mg) in MeOH (1 ml). The soln was then treated with $NaBH_4$ until effervescence ceased whereupon the reaction mixture was poured into $H_2O$ and extracted with $CHCl_3$. The product, which crystallized from $Et_2O$, was identical with fastigiatine in mp and in spectroscopic properties.

*Isolation of unknown C from* L. australianum. Polar residues were removed from the crude extract using dry CC. A column 50 cm × 2 cm was packed with alumina for dry CC and developed with EtOAc. The column was then sectioned into four sections and the alumina extracted with MeOH. Only the fourth fraction (bottom of the column) contained alkaloids.

This purified extract was then chromatographed on an alumina column (20 cm × 1 cm). The column was eluted with 100 ml each of $C_6H_6$, $C_6H_6$-$Et_2O$ (1 : 1), $Et_2O$, EtOAc and finally MeOH. Five fractions were collected and examined by FSC/MS. Fractions 1 and 2 contained only dioctyl phthalate. Fraction 3 contained unknown C, while fraction 4 contained only cernuine. Fraction 5 contained many unidentified polar residues. Fraction 3 was taken to dryness yielding 0.8 mg of unknown C which was used for the MS examination.

*Mass spectral data on unknowns.* (a) *L. australianum*, unknown C: 344 $[M]^{+\cdot}$ (< 1%), 167 (15), 166 (100), 164 (13), 150 (3). (b) *L. fastigiatum*, unknown I: 273 $[M]^{+\cdot}$ (100), 272 (37), 258 (23), 110 (35), 96 (26), 84 (31), 71 (28), 70 (58), 58 (40), 57 (48). (c) *L. clavatum* var. *barbonicum*, unknown I: 279 (23), 263 (25), 262 (100), 208 (16), 206 (9), 150 (12). (d) *L. deuterodensum*, unknown M: 273 (18), 272 (100), 151 (20); unknown F: 272 (37), 271 (100), 243 (45), 190 (31), 163 (85); unknown K: 279 (25), 263 (20), 262 (100), 206 (21), 190 (21), 151 (25).

*Acknowledgements*— We thank Dr. J. C. Braekman, Chimie-Bioorganique, Universite libre de Bruxelles, Bruxelles, Belgique, Dr. W. A. Ayer, University of Alberta, Edmonton, Canada, Dr. Y. Inubushi, Kyoto University, Sakyo-Ku, Kyoto, Japan, and Dr. R. H. Burnell, Université Laval, Quebec, Canada, for their gifts of authentic *Lycopodium* alkaloids. We also thank Dr. L. Nyembo and Dr. J. C. Braekman for making available to us a generous sample of *L. clavatum* var. *borbonicum* and the Council of Scientific and Industrial Research, Christ Church, for the plant material collected in New Zealand. The financial support of the Natural Sciences and Engineering Research Council of Canada is gratefully acknowledged.

## REFERENCES

1. MacLean, D. B. (1967) in *The Alkaloids* (Manske, R. H. F., ed.) Vol. 10, p. 305. Academic Press, New York.
2. MacLean, D. B. (1973) in *The Alkaloids* (Manske, R. H. F., ed.) Vol. 14, p. 347. Academic Press, New York.
3. MacLean, D. B. (1985) in *The Alkaloids* (Brossi, A., ed.) Vol. 26, p. 241. Academic Press, New York (in press).
4. Braekman, J. C., Nyembo, L. and Symoens, J. J. (1980) *Phytochemistry* **19**, 803.
5. Braekman, J. C., Nyembo, L., Bourdoux, P., Kahindo, N. and Hootele, C. (1974) *Phytochemistry* **13**, 2519.
6. Wilce, J. H. (1972) *Am. Fern. J.* **62**, 65.
7. Nyembo, L. (1977) Ph.D. Thesis, U. L. B., Belgium.
8. Nyembo, L., Goffin, A., Hootele, C. and Braekman, J. C. (1978) *Can. J. Chem.* **56**, 851.
9. Manske, R. H. F. (1953) *Can. J. Chem.* **31**, 894.
10. Ayer, W. A. and Law, D. A. (1962) *Can. J. Chem.* **40**, 2088.
11. Loyola, L. A., Morales, G. and Castillo, M. (1979) *Phytochemistry* **18**, 1721.
12. Achmatowicz, O. and Werner-Zamojska, (1958) *Roczniki Chem.* **32**, 1127; *Chem. Abstr.* **53**, 5590.
13. Ayer, W. A., Masaki, N. and Nkunika, D. S. (1968) *Can. J. Chem.* **46**, 3631.
14. MacLean, D. B. (1963) *Can. J. Chem.* **41**, 2654.
15. Braekman, J. C., Hootele, C. and Ayer, W. A. (1971) *Bull. Soc. Chim. Belg.* **80**, 83.

16. Morales, G., Loyola, L. A. and Castillo, M. (1979) *Phytochemistry* **18** 1719.
17. Inubushi, Y. and Harayama, T. (1982) *Yakugaku Zasshi* **102**, 434.
18. Ayer, W. A. and Masaki, N. (1971) *Can. J. Chem.* **49**, 524.
19. Ayer, W. A. and Altenkirk, B. (1969) *Can. J. Chem.* **47**, 2457.
20. Alam, S. N., Adams, K. A. H. and MacLean, D. B. (1964) *Can. J. Chem.* **42**, 2456.
21. Nyembo, L., Kahindo, N., Braekman, J. C. and Hootele, C. (1976) *Bull. Soc. Chem. Belg.* **85**, 595.
22. Ayer, W. A., Jenkins, J. K., Valverde-Lopez, S. and Burnell, R. H. (1967) *Can. J. Chem.* **45**, 433.
23. Inubushi, Y., Ibuka, T., Harayama, T. and Ishii, H. (1968) *Tetrahedron* **24**, 3541.
24. Ayer, W. A., Altenkirk, B., Burnell, R. H. and Moinas, M. (1969) *Can. J. Chem.* **47**, 449.
25. Ayer, W. A. and Altenkirk, B. (1969) *Can. J. Chem.* **47**, 499.
26. Ayer, W. A., Habgood, T. E., Deulofeu, V. and Juliani, H. R. (1965) Tetrahedron **21**, 2169.
27. MacLean, D. B. and Curcumelli-Rodostamo, M. (1966) *Can. J. Chem.* **44**, 611.
28. Kahindo, B. M. (1976) Ph.D. Thesis, U. L. B., Belgium.
29. Castillo, M., Loyola, L. A., Morales, G., Singh, I., Calvo, C., Holland, H. L. and MacLean, D. B. (1976) *Can. J. Chem.* **54**, 2893.
30. Miller, N., Hootele, C., Braekman-Danheux, C., and Braekman, J. C. (1971) *Bull. Soc. Chim. Belg.* **80**, 629.
31. Castillo, M., Morales, G., Loyola, L. A., Singh, I., Calvo, C., Holland, H. L. and MacLean, D. B. (1976) *Can. J. Chem.* **54**, 2900.
32. Anet, F. A. L., Haq, M. Z., Khan, N. H., Ayer, W. A., Hayatsu, R., Valverde-Lopez, S., Deslongchamps, P., Riess, W., Ternbah, M., Valenta, Z. and Wiesner, K. (1964) *Tetrahedron Letters* 751.
33. Ayer, W. A., Altenkirk, B., Masaki, N. and Valverde-Lopez, S. (1969) *Can. J. Chem.* **47**, 2449.
34. Ayer, W. A., Bowman, W. R., Kebarbe, P. and Burnell, R. H. (1965) *Can. J. Chem.* **43**, 328.
35. Braekman, J. C., Hootele, C., Miller, N., Declercq, J. P., Germain, G. and Van Meerssche, M. (1979) *Can. J. Chem.* **57**, 1691.
36. Ayer, W. A., Hogg, A. N. and Soper, A. C. (1964) *Can. J. Chem.* **42**, 949.
37. Ayer, W. A., Browne, L. M., Nakahara, Y., Tori, M. and Delbaere, L. T. J. (1979) *Can. J. Chem.* **57**, 1105.
38. Ayer, W. A., Ball, L. F., Browne, L. M., Tori, M., Delbaere, L. T. J. and Silverberg, A. (1984) *Can. J. Chem.* **62**, 298.