LYCOPODIUM ALKALOIDS

IV. THE CONSTITUTION AND STEREOCHEMISTRY OF LYCOCLAVINE, AN ALKALOID OF LYCOPODIUM CLAVATUM VAR. MEGASTACHYON[1]

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ABSTRACT

The alkaloids of *Lycopodium clavatum* var. *megastachyon* have been examined and two hitherto unreported alkaloids, lycoclavine and acetyllycoclavine, were isolated and their structures determined. The nuclear magnetic resonance spectra of these and related compounds are discussed in terms of the conformation of ring B in these alkaloids.

The alkaloids of *Lycopodium clavatum* Linn. have been examined extensively (1–4) and a total of 16 different alkaloids isolated. We have now investigated the alkaloidal content of *L. clavatum* var. *megastachyon* Fern. and Bissel (5), and two closely related, but hitherto unreported, alkaloids have been isolated and their structures established. In addition, four other alkaloids of known constitution were isolated, along with a small amount of a base of undetermined constitution.

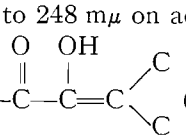
The total crude alkaloid of *L. clavatum* var. *megastachyon*, obtained by methanol extraction, amounted to approximately 0.12% of the dry weight of the plant. Separation of the alkaloids, achieved mainly by chromatographic techniques (see Experimental), yielded the following compounds: (i) lycopodine (30% of the total basic material); (ii) clavolonine (11%);[2] (iii) a compound, $C_{18}H_{29}O_3N$, m.p. 212–213° (7.5%), which appears to be new and which we have named lycoclavine; (iv) a compound, $C_{20}H_{31}O_4N$, m.p. 144–145° (11%), which proved to be the O-acetyl derivative of lycoclavine; (v) a substance, $C_{32}H_{52}O_3N_2$, m.p. 213–214° (1.5%), which was shown to be a 1:1 molecular complex of dihydrolycopodine ($C_{16}H_{27}ON$) and flabelliformine ($C_{16}H_{25}O_2N$).[3] In addition to the above-mentioned alkaloids, a small amount of an apparently new alkaloid, m.p. 261–263°, analyzing best for $C_{16}H_{25-27}O_2N$, was also isolated but has not yet been further characterized.

The structures of lycopodine (6), dihydrolycopodine (6), flabelliformine (7),[4] and clavolonine (8) are known. Furthermore, a comparison of the infrared spectrum of clavolonine with that of alkaloid L.34, first isolated by Manske in 1953 from *Lycopodium densum* Labill. (9), revealed their identity.[5] The composition of the molecular complex mentioned in (v) above was established in the following manner. Attempts to separate the complex by fractional crystallization and by chromatography were unsuccessful. However, treatment of the complex with chromium trioxide in pyridine oxidized the dihydrolycopodine to lycopodine and left the flabelliformine unchanged. The two components were then easily separable by chromatography. Finally, combination of equimolar quantities of dihydrolycopodine and flabelliformine gave a high yield of the C_{32} complex, identical in

[1]*Part III: W. A. Ayer and G. G. Iverach. Tetrahedron Letters, No. 3, 87 (1962).*
[2]*Identical in all respects with an authentic sample kindly furnished by Dr. R. H. Burnell, University of the West Indies, Jamaica.*
[3]*We wish to thank Dr. D. B. MacLean, McMaster University, for the comparison of our material with authentic flabelliformine.*
[4]*We wish to thank Dr. MacLean for a preprint of this paper.*
[5]*We wish to thank Dr. Manske for a sample of alkaloid L.34.*

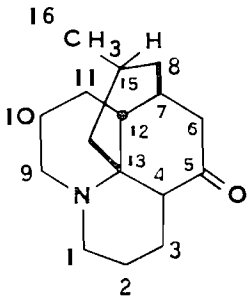
all respects with that obtained from the plant. It is interesting to note that flabelliformine also forms stable 1:1 molecular complexes with both lycopodine and lycodoline, although these latter are readily separable by chromatography (10).

Turning now to the determination of the structure of lycoclavine, $C_{18}H_{29}O_3N$, the presence of a hydroxyl group and an acetoxyl group was established by the following observations. The infrared spectrum (in dilute CCl_4) showed a concentration-independent peak at 3600 cm^{-1}, with a shoulder at 3620 cm^{-1}, indicative (11) of an intramolecularly hydrogen-bonded hydroxyl group. Peaks at 1736 cm^{-1} and 1240 cm^{-1} in the infrared, and at 7.93 τ (3 H) in the n.m.r. suggested the presence of an O-acetyl group and this was confirmed by hydrolysis to acetic acid and a diol, $C_{16}H_{27}O_2N$, henceforth called desacetyllycoclavine. The secondary nature of these functional groups was indicated by the n.m.r. spectra of lycoclavine, which showed one-proton peaks at 5.11 τ (doublet, splitting 6.9 c.p.s., $\rangle$*C*HOAc) and 6.40 τ (singlet, $\rangle$*C*HOH). Acetyllycoclavine, prepared by acetylation of lycoclavine with acetic anhydride – pyridine, showed similar peaks at 4.92 τ (doublet, splitting 6.8 c.p.s.) and 5.32 τ (singlet), the latter peak being attributed to the proton on the carbon bearing the hydroxyl group in the unacetylated compound. The n.m.r. spectra also revealed the presence of a secondary C-methyl group (doublets at 9.09 τ and 9.08 τ, splitting ca. 6 c.p.s., in lycoclavine and acetyllycoclavine, respectively). Lack of NH absorption in the infrared spectra of lycoclavine and its acetyl derivative indicated the tertiary nature of the amino nitrogen. Since lycoclavine could not be reduced catalytically and showed no olefinic protons in the n.m.r., it appeared to be tetracyclic.

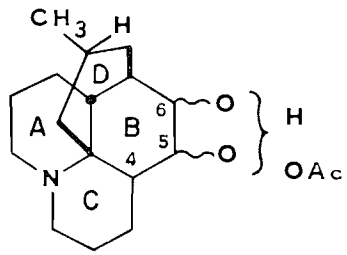
Oxidation of lycoclavine with chromic acid in acetic acid, followed by chromatography over alumina, yielded a basic compound, $C_{18}H_{27}O_2N$, m.p. 174–175°, which showed peaks in the infrared at 1751 cm^{-1} (O-acetyl) and 1724 cm^{-1} (cyclohexanone), and in the ultraviolet at 282 mμ (log ϵ, 2.56). Hydrolysis of this ketone, which we shall refer to as "lycoclavinone", with dilute sodium hydroxide yielded an amphoteric compound, $C_{16}H_{23}O_2N$, which had the properties of a diosphenol. In particular, the ultraviolet spectrum showed a maximum at 282 mμ (log ϵ, 3.99) in neutral solution, shifted to 327 mμ in dilute base, and to 248 mμ on acetylation. These values are indicative of the presence of the grouping  (12). The formation of the enolic α-diketone indicated that lycoclavinone was an α-acetoxy ketone, the α-diketone arising from aerial oxidation of the initially formed α-ketol. The susceptibility of α-ketols to aerial oxidation in alkaline solution is well authenticated (13) and it was found that the rate of formation of the diketone (as determined by the rate of increase of the ϵ value at 327 mμ) was substantially reduced when the reaction was carried out in a nitrogen atmosphere and accelerated when oxygen was bubbled through the hydrolysis solution. Hydrolysis of lycoclavinone with aqueous acid yielded the α-ketol, which underwent aerial oxidation to the diosphenol in alkaline solution. These results demonstrate the presence of the grouping —CHOH—CHOAc—, flanked on at least one side by a methine group, in lycoclavine.

The presence of the above-mentioned grouping, a $\rangle$$CHCH_3$ group, and a tertiary nitrogen, together with the fact that lycoclavine occurs along with lycopodine (I) and several other alkaloids having a lycopodine skeleton, and that all *Lycopodium* alkaloids

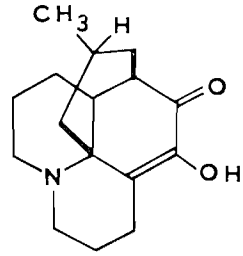
of known constitution bear an oxygen or its biogenetic equivalent (i.e. unsaturation or nitrogen) at C-5, suggested structure II (without stereochemical implications) as a possibility for lycoclavine, and hence III for the enolic α-diketone. Indeed, oxidation of lycopodine with 1 mole[6] of SeO_2 in refluxing dioxane gave, in addition to unchanged lycopodine, the enolic diketone III (25% yield), identical in all respects with that prepared from lycoclavine. Compound III has also been prepared by hydrolysis of bromolycopodine in the presence of air (14).



I



II



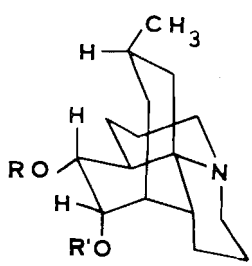
III

Since both the relative (15) and absolute (16) stereochemistry of lycopodine are known these results show that lycoclavine is represented by II, the only remaining points to be determined being the relative positions of the hydroxyl and acetoxyl groups and the stereochemistry at C-4, C-5, and C-6.[7] In order to simplify the discussion, the hydrogen at C-4 will now be placed trans to the C-7, C-13 bridge as in structures IV–VII. That this is indeed the correct orientation at C-4 will be demonstrated later.

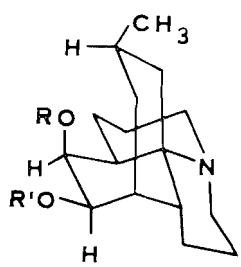
On the basis of the n.m.r. data presented earlier, structure IV (R = Ac, R′ = H) was first favored for lycoclavine, since in this structure the axial proton on C-5 is flanked by an axial proton on C-4 which could lead to the observed splitting (ca. 7 c.p.s.), whereas the equatorial proton on C-6 is flanked by gauche protons on C-5 and C-7 and might be expected to be weakly coupled, resulting in the broadened singlet (half-height width about 3.5 c.p.s.) actually observed (17). Several facts, however, were not in agreement with this formulation. Thus, although lycoclavine showed weak intramolecular hydrogen bonding in the infrared, the corresponding diol, desacetyllycoclavine, obtained by alkaline hydrolysis or, better, by $LiAlH_4$ reduction of lycoclavine, showed a single OH stretching vibration at 3620 cm^{-1}. The diol was also resistant to oxidation with periodic acid. Both these facts speak against the cis relationship of the two groups. Furthermore, hydrolysis of acetyllycoclavine with refluxing 10% aqueous HCl for 1½ hours gave lycoclavine in better than 90% yield. If IV (R = Ac, R′ = Ac) were indeed the correct formulation for the diacetate, the retention of the relatively unhindered equatorial acetoxyl at C-5 while the hindered axial acetoxyl group at C-6 was hydrolyzed would not be expected.

Structure V would also be expected to lead to a diol which would show intramolecular hydrogen bonding and react with periodic acid. Since it was felt that the oxygenated ring might be considerably distorted from the chair form (see below) structures VI and VII remained for consideration.

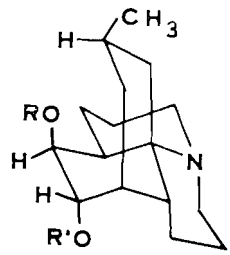
[6]*Use of excess* SeO_2 *led to the formation of compound(s) absorbing at 306, 340, and 380 mμ in the ultraviolet.*
[7]*The numbering system used (see structure I) is that suggested by K. Wiesner (16(b)).*



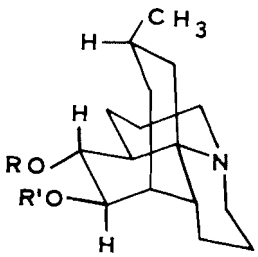
IV



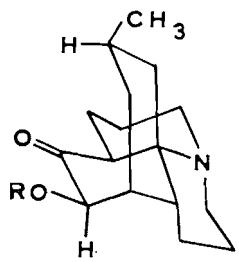
V



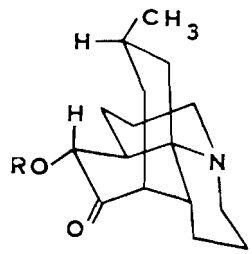
VI



VII



VIII

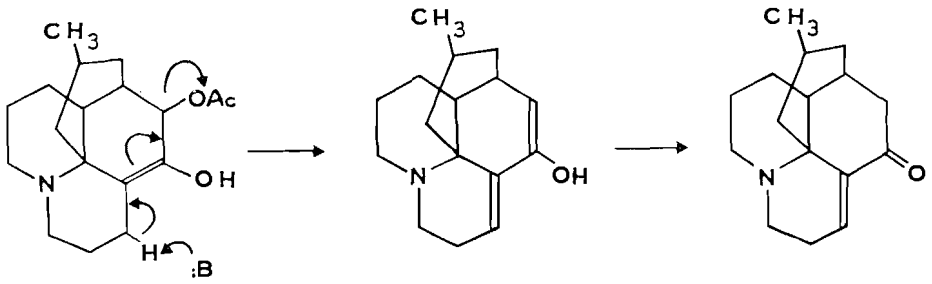


IX

The position of the acetoxyl group was determined in the following manner. Lycoclavinone, mentioned above, could be assigned structure VIII (R = Ac) on the following basis. The optical rotatory dispersion curve showed a positive Cotton effect with extrema at 308 mμ and 271 mμ and an amplitude of 23,000°. Lycopodine (VIII, RO replaced by H) under the same conditions also shows a positive Cotton effect with extrema at 306 mμ and 271 mμ, amplitude 17,000°. The octant rule (18) predicts a positive Cotton effect if the keto group is at C-5 (as in VIII) and a negative Cotton effect if it is at C-6 (as in IX). The equatorial nature of the acetoxyl group is indicated both by the position of the extrema in the optical rotatory dispersion spectrum (19) and by the position (282 mμ, lycopodine 285 mμ) of the ketonic maximum in the ultraviolet (20). The fact that the α-ketol obtained by acid hydrolysis of the acetoxy ketone was reconverted by acetic anhydride – pyridine to the same acetoxy ketone is also consistent with structure VIII, since under these equilibrating conditions ketol VIII (R = H), in which the serious non-bonded interaction between C-15 and C-5 is minimized, should be favored over ketol IX (R = H). The fact that the hydroxyl group in the ketol is intramolecularly hydrogen bonded (concentration-independent band at 3500 cm^{-1} in the infrared) is consistent with the assigned equatorial position.

Pyrolysis of VIII (R = Ac) at 240° for 8 minutes led to a good yield of the α,β-unsaturated ketone X, which had previously been prepared from bromolycopodine (14). Presumably this facile elimination of acetic acid proceeds via the enol form of the ketone, as formulated below, with the reacting material acting as its own base.

It was now important to determine whether lycoclavinone (VIII, R = Ac), which is



X

presumably the thermodynamically more stable of the four possible C-5, C-6 acetoxyketones, was actually the first product of the oxidation of lycoclavine. It was found that if the acetic acid oxidation solution was first diluted with $CHCl_3$ and then ice-cold ammonium hydroxide added, evaporation of the $CHCl_3$ extract and crystallization from *n*-hexane gave, instead of lycoclavinone, the α-acetoxyketone XI, m.p. 115–118°. If the oxidation solution was first made basic by addition of ammonium hydroxide, then extracted with $CHCl_3$ and filtered *rapidly* through alumina, the ketone IX (R = Ac), m.p. 143°, was obtained. Both IX (R = Ac) and XI were isomerized to VIII (R = Ac) when adsorbed on basic alumina for any extended length of time. Similar isomerizations of steroidal α-acetoxyketones have been reported (21). Acid hydrolysis of IX (R = Ac) gave the α-ketol VIII (R = H). The structural assignments are based mainly on optical rotatory dispersion and ultraviolet measurements. Both IX and XI showed *negative* Cotton effect curves, as predicted by the octant rule (see above). The extrema for IX (R = Ac) were at 312 mμ and 280 mμ (amplitude −2300°), for XI at 334 mμ and 297 mμ (amplitude −11,300°), indicative (19) of equatorial and axial α-acetoxy groups, respectively. The ultraviolet maxima were at 286 mμ for IX and 308 mμ for XI. The fact that XI shows a more negative Cotton effect than does IX no doubt reflects the distortion of the ketone-containing ring from an ideal chair (see below). Similar observations have been made with α-acetoxy 11- and 12-ketosteroids (22).

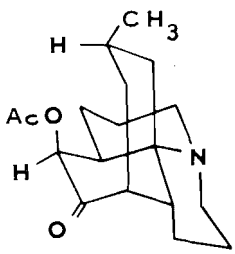
The formation of lycoclavinone I thus proceeds via the sequence

$$\text{lycoclavine} \xrightarrow{CrO_3\text{–HOAc}} \text{XI} \xrightarrow[Al_2O_3]{NH_4OH \text{ or}} \text{IX} \xrightarrow{Al_2O_3} \text{VIII}$$

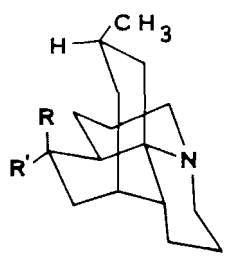
and hence the acetoxyl group in lycoclavine is located on C-5 cis to the C-7, C-13 bridge, in agreement with structure VI (R = Ac, R′ = H) but not with structure VII.

Further evidence for the diaxial orientation of the substituents at C-5 and C-6 and for the configuration at C-4 was obtained by a study of the reduction products obtained from the various isomeric α-ketols and α-acetoxylketones now available to us. It has been shown (8) that lithium aluminum hydride reduction of lycopodine (I) leads to the axial alcohol dihydrolycopodine (XII, R = OH, R′ = H), whereas dissolving metal reduction gives the equatorial alcohol α-dihydrolycopodine (XII, R = H, R′ = OH). We have independently arrived at this same conclusion,[8] and, furthermore, have shown that

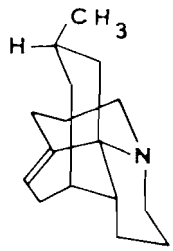
[8]*Since the previous workers (8) have not reported physical constants or experimental details for the preparation of α-dihydrolycopodine, this is included in the experimental section.*



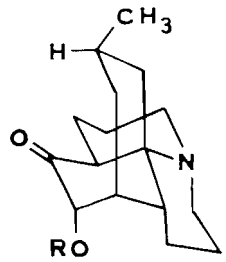
XI



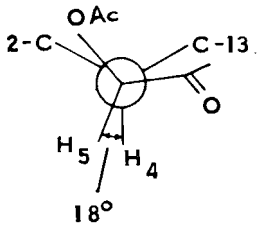
XII



XIII



XIV



XV

the methiodide of the xanthate (XII, R = H, R′ = OCS_2CH_3), derived from α-dihydrolycopodine, yields anhydrodihydrolycopodine (XIII) on pyrolysis, confirming the cis arrangement of the hydroxyl and the C-4 hydrogen.

Reduction of alkaloid L.20 (XIV, R = H), in which the configuration at C-4 is known (14), with lithium aluminum hydride in ether yielded the diol VI (R = R′ = H) identical in all respects with desacetyllycoclavine. The structure VI (R = R′ = H) was assigned on the assumption that the approach of the reducing agent would be on the side opposite to the C-7, C-13 bridge, as is the case with lycopodine. This assumption is supported by the results obtained on reduction of ketones VIII and IX. Reduction of the ketol VIII (R = H) with $LiAlH_4$ gave a new diol, m.p. 230–231°, assigned structure V (R = R′ = H) on the basis of its mode of formation. Lithium–ammonia–methanol reduction of ketol VIII yielded a third diol, m.p. 209–210°, assigned the diequatorial structure VII (R = R′ = H), again on the basis of its mode of formation. Attempts to prepare the fourth diol (IV, R = R′ = H) by dissolving metal reduction of alkaloid L.20 were unsuccessful, leading only to reductive removal of the C-6 hydroxyl (14). However, reduction of IX (R = Ac) with $LiAlH_4$ led to a diol, m.p. 234–235°, differing from the other three, and therefore assigned structure IV (R = R′ = H). All four diols showed different infrared spectra, single spots on paper chromatography, and depression of mixed melting points, where appropriate. It is interesting to note that hydride reduction of IX (R = Ac) involves approach of the reducing species from the same side as the C-7, C-13 bridge. Presumably in this case approach to the opposite side is hindered by the C-12 methylene group. Since each of the latter three diols must have at least one equatorial

hydroxyl group, the hydride reduction product of alkaloid L.20, and hence desacetyllycoclavine, is the diaxial diol VI (R = R′ = H), and lycoclavine itself is VI (R = Ac, R′ = H).

As noted above, lycoclavine shows intramolecular hydrogen bonding but the corresponding diol does not. To account for this, it must be assumed that the oxygenated ring is severely distorted toward the half-chair, i.e., the dihedral angle between the two groups must approach 120°. It is known, for instance, that *trans*-1,2-pentanediol does not show intramolecular hydrogen bonding, but that the corresponding monoacetyl derivative does (23). Measurements on Drieding models indicate an internuclear distance of ca. 1 Å between the C-5 oxygen and the C-15 hydrogen in lycoclavine. The sum of the van der Waals radii is 2.6 Å, so that a distortion of ring B (and presumably also ring D) is to be expected.

A more quantitative estimate of this distortion can be obtained by a consideration of the n.m.r. spectra (Table I) of some of the compounds mentioned earlier. It is well

TABLE I

Nuclear magnetic resonance data for lycoclavine and related compounds

Compound	Chemical shifts* (splitting)†			
	C-5	C-6	—OCOCH$_3$	C-16
VI (R = Ac, R′ = H)	5.11 (6.9)	6.40 (3.3)‡	7.93	9.09 (6.0)
VI (R = R′ = Ac)	4.92 (6.8)	5.32 (3.2)‡	7.92, 7.93	9.08 (6.0)
VIII (R = Ac)		4.60 (5.5)	7.83	9.17 (5.5)
IX (R = Ac)	4.73 (11.5)		7.83	8.96 (5.8)
XI	4.61 (9.0)		7.89	9.03 (6.0)
XIV (R = Ac)		5.11 (3.5)	7.94	9.16 (4.5)
I				9.14 (5.0)
XII (R = R′ = H)				9.13 (6.0)
XII (R = OAc, R′ = H)	4.90§		7.96	9.08 (6.0)
XII (R = H, R′ = Ac)	4.98§		7.95	9.09 (5.7)

*τ-Values.
†In c.p.s. units.
‡Width at half height.
§Approximate center of multiplet.

known (17) that the coupling constant between vicinal hydrogens is related to their dihedral angle. In the particular case of α-acetoxycyclohexanones in fused-ring systems, the following relationships have been found (24) to apply:

$$J_{HH'} \begin{cases} 10\cos^2\phi & 0° \leqslant \phi \leqslant 90° \\ 16\cos^2\phi & 90° \leqslant \phi \leqslant 180°. \end{cases}$$

In compound XI the coupling constant between the protons on C-4 and C-5 (J_{45}) is 9 c.p.s. Using the Johnson expression given above this indicates a dihedral angle of about 18°, indicating a considerable distortion from the normal 60° angle. This is illustrated in the Newman projection XV. Compound IX (R = Ac) shows a similar distortion (J_{45} = 11.5 c.p.s., ϕ = 142° instead of 180°). In the C-5 ketone VIII (R = Ac), where the C-5, C-15 interaction is minimized because of the trigonal nature of C-5, the calculated angle is 42° ($J_{67} \simeq 5.5$ c.p.s.). The acetyl derivative of XIV gave a broadened singlet (width at half height about 3.5 c.p.s.), indicative of an angle of about 60°. Thus it appears that in the C-6 ketone series the non-bonded interaction between C-5 and C-15 is relieved, at least in part, by a distortion of ring B from an ideal chair towards the half-chair. This

strain could also be relieved if ring D assumed the boat conformation, but as has been pointed out previously (15), this introduces a serious bowsprit–flagpole interaction between C-12 and C-16. The n.m.r. results obtained with the C-5 ketones indicate a similar, but smaller, distortion of ring B in this series. Such a flattening of the B ring would increase the angle between C-4 and C-6 and account for the unusually low (1700 cm^{-1}) (6) carbonyl stretching frequency of lycopodine (27).

The n.m.r. spectra of lycoclavine and its acetyl derivative (Table I) are in good agreement with the proposed structure if we make the reasonable assumption that ring B is distorted to at least the same extent as in the C-6 ketones. Applying the Karplus–Conroy correlation (17) (the Johnson expression applies only to six-membered rings containing a trigonal carbon atom adjacent to the bonds under consideration (24)) a H_4—C—C—H_5 dihedral angle of 20° would lead to a coupling constant J_{45} of 7 c.p.s. (observed, 6.8 and 6.9 c.p.s.). J_{56} and J_{67} would be expected to be small (both angles approach 90–100° in the distorted chair), explaining the fact that C—H_5 is not further split and C—H_6 is a somewhat broadened singlet.

Inspection of Table I suggests a possible relationship between the chemical shift of the C-16 methyl group and the presence or absence of a carbonyl group at C-5. Thus those compounds with a C-5 keto group show the C-methyl resonance at 9.14–9.17 τ, while, with one exception, those without a carbonyl group in ring B show this peak at 9.08–9.09 τ. The exception is compound XII (R = R′ = H), not previously mentioned but readily available by Wolff–Kishner reduction of lycopodine, which has the C-methyl doublet centered at 9.13. The differences noted are possibly due to the long-range shielding effect of the carbonyl group (25). Inspection of models reveals that in the C-6 ketones the C-methyl group no longer lies in the shielding region of the carbonyl and may in fact be deshielded by it. In agreement with this view, the two C-6 ketones absorb at 8.96 τ and 9.03 τ.

In view of the fact that acetyllycoclavine is hydrolyzed by hot dilute mineral acid to lycoclavine, the possibility arose that the lycoclavine isolated was actually formed during the isolation process. Although this possibility cannot be completely excluded, it seems unlikely, since lycoclavine can also be isolated in good yield by percolation of the ground plant with cold dilute acetic acid and immediate extraction of the alkaloids into chloroform.

EXPERIMENTAL

Ultraviolet spectra were measured in 95% ethanol and, unless otherwise specified, infrared spectra in carbon tetrachloride. For hydrogen-bonding studies, spectra were determined on 0.1, 0.05, and 0.025 *M* solutions in CCl_4 using a Perkin–Elmer Model 221 spectrophotometer. Nuclear magnetic resonance spectra were measured on ca. 10% w/v solutions in chloroform using a Varian Associates Model A-60 spectrometer. Internal tetramethylsilane was used as a standard. Some of the optical rotatory dispersion curves were obtained by Dr. M. M. Marsh, Eli Lilly, Indianapolis, to whom we extend our best thanks; the others were determined by Mr. R. N. Swindlehurst of these laboratories using a Rudolph Automatic Recording Spectropolarimeter. Melting points were determined on a hot stage and are uncorrected. Alumina, unless otherwise specified, means basic alumina of activity III. Skellysolve B refers to Skelly Oil Company light petroleum, b.p. 62–70°. Microanalyses are by Pascher Mikroanalytisches Laboratorium, Bonn, West Germany, and C. Daesslé, Montreal, Quebec.

Isolation of the Alkaloids

Dried, powdered *L. Clavatum* var. *megastachyon* (15.5 kg) was Soxhlet-extracted for 24 hours with methanol. Most of the methanol was removed by distillation and the residue treated with ice-cold 1% hydrochloric acid (5 l.). The acid solution was filtered (celite) from insoluble material. The insoluble material was then vigorously stirred with cold 1% HCl (5 l.) for several hours, and the mixture again filtered. The combined acid solutions were basified (NH_4OH, ice) and extracted five times with chloroform. The chloroform was removed by distillation and the oily residue dissolved in ice-cold 1% HCl (5 l.) and washed three

times with ether to remove neutral material. The aqueous solution was then basified (ice-cold NH_4OH) and extracted four times with chloroform. Evaporation of the chloroform left 18.2 g of basic material as a dark brown gum.

Purification of the Alkaloids

The crude alkaloid was placed on a column of alumina (1 lb) in ether and eluted in four fractions: A, 9.01 g, 3 l. ether; B, 1.88 g, 6 l. ether; C, 8.50 g, 6 l. $CHCl_3$; D, 3.32 g, 1 l. MeOH.

Fraction B was crystallized from acetone to give the dihydrolycopodine–flabelliformine complex, m.p. 213–214° (0.3 g). Calc. for $C_{32}H_{52}O_3N_2$: C, 75.00; H, 10.16; O, 9.37; N, 5.47%. Found: C, 74.78; H, 10.16; O, 9.82; N, 5.44%. Infrared spectrum: ν_{max} (nujol) 3300–2600 (broad OH superimposed on C—H), 1710 cm^{-1} (C=O).

Fraction C crystallized from acetone to given clavolonine, m.p. 233–234° (1.53 g), identical with an authentic sample furnished by Dr. R. H. Burnell.

Fraction D was chromatographed over alumina and yielded further clavolonine (0.50 g) when eluted with $CHCl_3$. Elution with $CHCl_3$–MeOH (99:1) and crystallization from methanol–ether yielded 70 mg colorless needles, m.p. 261–263°. Found: C, 72.36, 72.49; H, 9.36, 9.58; N, 5.60%. The infrared spectrum (nujol) showed strong —OH absorption (3400 cm^{-1}) but no carbonyl absorption. This compound has not been further investigated.

Fraction A and the residues from fractions B and C were combined and carefully chromatographed over alumina (1 lb). Elution with benzene (5 l.) yielded lycopodine (5.36 g), identical with an authentic sample. Elution with ether (5 l.) and crystallization from Skellysolve B gave *acetyllycoclavine*, m.p. 144–145° (2.03 g). Calc. for $C_{20}H_{31}O_4N$: C, 68.74; H, 8.94; O, 18.31; N, 4.01%. Found: C, 68.74, 68.54; H, 8.69, 9.15; O, 19.03; N, 3.55%. Infrared spectrum: ν_{max} 1745, 1225 cm^{-1} ($OCOCH_3$).

Elution with chloroform and crystallization of the eluates from acetone yielded *lycoclavine* (1.32 g). The analytical sample, m.p. 212–213°, $[\alpha]_D$ −9° (95% ethanol), was prepared by several recrystallizations from acetone. Calc. for $C_{18}H_{29}O_3N$: C, 70.32; H, 9.51; N, 4.54; O, 15.57%. Found: C, 70.11, 70.19; H, 9.43, 9.48; N, 4.57; O, 15.71%. pK_a (50% MeOH) 9.6. Infrared spectrum: ν_{max} 3620 (sh), 3600, 1736, 1240 cm^{-1}. The methiodide melted at 314°, the perchlorate at 283–286°, and the hydrochloride at 281–285°.

Further small quantities of crystalline material could be obtained by chromatography of the mother liquors from the above operations.

Proof of Composition of Molecular Complex M.p. 213–214°

Dihydrolycopodine (10.6 mg) and flabelliformine (11.2 mg) were combined in hot acetone. The solution deposited colorless crystals (15.2 mg), m.p. 204–206°. The infrared spectrum (nujol) was identical with that of the material isolated from the plant, and the mixed melting point (206–210°) showed no depression.

Oxidation of the complex with CrO_3–pyridine and alumina chromatography of the resulting product yielded lycopodine (eluted with benzene) and flabelliformine (eluted with ether).

Desacetyllycoclavine (VI, R = R′ = H)

(a) By Alkaline Hydrolysis of Lycoclavine

Lycoclavine (0.109 g) was refluxed for 1 hour with 2% NaOH in 80% methanol. Removal of most of the methanol, dilution with water, and extraction with chloroform gave, after removal of solvents, a colorless foam (0.09 g). Crystallization from a small volume of acetone yielded elongated prisms (40 mg), m.p. 207–208°. The compound was difficult to crystallize in good yield and was sublimed for analysis. Calc. for $C_{16}H_{27}O_2N$: C, 72.41; H, 10.25; N, 5.28%. Found: C, 72.28, 72.48; H, 10.30, 10.34; N, 5.45%. The infrared spectrum showed a concentration-independent band at 3620 cm^{-1} and no carbonyl absorption.

The perchlorate, prepared by neutralization of an acetone solution of the base with 70% perchloric acid and crystallization from either acetone–ether or methanol–ether, exists in two crystalline forms, one melting at 230–238° and the other at 276–278°. The two forms have different infrared spectra (nujol mulls) but are readily interconverted by seeding.

(b) By $LiAlH_4$ Reduction of Acetyllycoclavine

Acetyllycoclavine (VI, R = R′ = Ac) (0.20 g) in ether (50 ml) was refluxed for 2½ hours with lithium aluminum hydride (0.25 g), then worked up using the method of Mićović and Mihailović (26). Sublimation of the crude product gave the diol (0.148 g), m.p. 203–206°, identical with that obtained above. Similar reduction of lycoclavine also gave the diol in good yield.

(c) By Acid Hydrolysis of Acetyllycoclavine

Acetyllycoclavine (60 mg) was refluxed for 16 hours in 20% aqueous HCl (30 ml), then the solution neutralized with sodium bicarbonate and continuously extracted with ether. Evaporation of the dried ether extract gave a colorless solid which on sublimation affords desacetyllycoclavine (32 mg), m.p. 201–206°.

(d) By $LiAlH_4$ Reduction of Alkaloid L.20

Alkaloid L.20 (XIV, R = H) (38 mg) was added to a slurry of $LiAlH_4$ (0.102 g) in ether (50 ml), and the mixture refluxed for 12 hours, then worked up as in part (*b*). Sublimation of the colorless semisolid remaining after removal of the solvents gave a colorless solid (32 mg), m.p. 191–196°, whose infrared

spectrum was virtually identical with that of authentic desacetyllycoclavine. The perchlorate, prepared from the sublimed material, was identical in all respects (melting point, mixed melting point, infrared spectrum) with the perchlorate of desacetyllycoclavine.

Acetylation of Lycoclavine

Lycoclavine (87 mg) was kept for 18 hours in acetic anhydride (4 ml) – pyridine (2 ml). The solution was then diluted with chloroform and shaken with ice-cold dilute ammonium hydroxide. The chloroform layer was separated, washed with water, dried, and evaporated. The off-white solid obtained was chromatographed on alumina (5 g). Elution with ether yielded a colorless solid (86 mg). Crystallization from Skellysolve B gave *actyllycoclavine*, m.p. 144–145°, identical in all respects with the naturally occurring compound described above.

Similar treatment of desacetyllycoclavine also gave acetyllycoclavine in high yield.

Lycoclavine from Acetyllycoclavine

Acetyllycoclavine (0.87 g) was refluxed for 1½ hours with 10% aqueous HCl, then the solution was neutralized with sodium bicarbonate and continuously extracted with ether. Evaporation of the ether and crystallization of the residue from acetone gave lycoclavine (0.71 g), m.p. 211–213°, identical with the natural material.

"Lycoclavinone" (VIII, R = Ac)

Lycoclavine (68 mg) was dissolved in 98% acetic acid (20 ml) containing chromium trioxide (70 mg) and the resulting solution kept at room temperature for 18 hours, then made basic with cold dilute ammonium hydroxide and extracted six times with chloroform. Evaporation of the chloroform gave a colorless semisolid (69 mg), which was chromatographed over alumina (5 g). Elution with ether gave "lycoclavinone" (40 mg). Elution with chloroform yielded unreacted lycoclavine (15 mg). Lycoclavinone, after recrystallization from Skellysolve B, melted at 174–175°. Calc. for $C_{18}H_{27}O_3N$: C, 70.79; H, 8.92; O, 15.72%. Found: C, 70.39, 70.64; H, 9.11, 9.06; O, 15.73%. Infrared spectrum: ν_{max} 1751, 1245 ($OCOCH_3$), 1724 cm^{-1} (C=O). Ultraviolet spectrum: λ_{max} 282 mμ (log ϵ 2.56). Rotatory dispersion in methanol (*c* 0.023): $[\phi]_{400}$ +290°, $[\phi]_{308}$ +8,300°, $[\phi]_{271}$ −15,400°, $[\phi]_{260}$ −7,500°.

Ketol VIII (R = H)

Lycoclavinone (VIII, R = Ac) (45 mg) was dissolved in 10% aqueous HCl and the solution refluxed for 2 hours, then cooled, basified with sodium bicarbonate, and extracted four times with chloroform. Evaporation of the chloroform and sublimation of the residue gave a colorless solid (23 mg), m.p. 127–132°. Four recrystallizations from Skellysolve-B gave analytically pure material, m.p. 135–136°. Occasionally, a second crystalline form, m.p. 122–123°, was obtained. The two forms had different infrared spectra in nujol mull, but identical solution spectra. Calc. for $C_{16}H_{25}O_2N$: C, 72.96; H, 9.57; N, 5.32%. Found: C, 72.88, 73.00; H, 9.62, 9.76; N, 5.26%. Infrared spectrum: ν_{max} 3510 (bonded OH), 1710 cm^{-1} (C=O). Ultraviolet spectrum: λ_{max} 280 mμ (log ϵ 1.95). Rotatory dispersion in methanol (*c* 0.11): $[\phi]_{400}$ 0∓20°, $[\phi]_{304}$ +6,400°, $[\phi]_{253}$ −19,100°, $[\phi]_{250}$ −19,000°.

Acetylation of the ketol VIII with pyridine – acetic anhydride at room temperature gave lycoclavinone in high yield.

Diosphenol III

(a) From Lycoclavinone

Lycoclavinone (VIII, R = Ac) (53 mg) was stirred at room temperature for 12 hours with 2% NaOH in 20% methanol (30 ml). The solution was then adjusted to pH 7.5 with dilute acetic acid and extracted with four 30-ml portions of chloroform. Evaporation of the chloroform gave a colorless solid which did not crystallize readily and was purified by sublimation. The analytical sample melted at 185–186°, $[\alpha]_D$ −45° (0.5 in ethanol). Calc. for $C_{16}H_{23}O_2N$: C, 73.53; H, 8.87; O, 12.24; N, 5.36%. Found: C, 73.41; H, 8.95; O, 12.12; N, 5.33%. Infrared spectrum: ν_{max} 3440 (bonded OH), 1672 (C=O), 1648 cm^{-1} (C=C). Ultraviolet spectrum: λ_{max} 282 mμ (log ϵ 3.99) shifted to 327 mμ in ethanolic NaOH.

When the reaction was carried out in an atmosphere of nitrogen the peak at 327 mμ developed at about one seventh the rate in air. When oxygen was bubbled through the reaction solution the rate of formation of diketone was about 1½ times that in air.

Acetylation of the diosphenol III with acetic anhydride – pyridine gave an oily product which showed a maximum at 248 mμ in the ultraviolet.

(b) From Lycopodine

A solution of lycopodine (I) (0.83 g) in dioxane (70 ml) was refluxed for 18 hours with selenium dioxide (0.37 g). The dark brown solution was then filtered from selenium and the dioxane removed at the pump. The resulting red-brown resin was dissolved in water, the pH adjusted to 7.5 with bicarbonate, and the solution extracted six times with chloroform. The reddish-brown oil (0.63 g) obtained by removal of the chloroform was chromatographed over alumina (12 g). Elution with ether gave a pale yellow solid (0.30 g) which proved to be mainly lycopodine. Elution with chloroform–methanol (49:1) yielded a dark brown

semisolid (0.22 g) which on sublimation furnished fairly pure diosphenol III, m.p. 183–185°, identical (infrared spectrum, ultraviolet spectrum, mixed melting point, rotation) with that prepared from lycoclavinone.

Pyrolysis of Lycoclavinone

Lycoclavinone (28 mg) was heated at 240° in a nitrogen atmosphere for 8 minutes, then cooled to 120° and distilled (0.1 mm). The distillate, a pale yellow oil, was chromatographed over alumina. Elution with ether gave a colorless oil (13 mg) which showed a maximum in the ultraviolet at 244 mμ (log ε, 3.9) and bands in the infrared at 1680 and 1610 cm^{-1}. Treatment of an acetone solution of the oil with methyl iodide yielded a solid methiodide which melted, after recrystallization from methanol–ether, at 273–274°. The methiodide was identical with an authentic sample (14) of the methiodide of X.

Acetoxyketone XI

A solution of lycoclavine (0.47 g) and chromium trioxide (0.47 g) in 98% acetic acid (20 ml) was kept at room temperature for 18 hours, then diluted with chloroform (50 ml), and the resulting solution washed with enough dilute ice-cold ammonium hydroxide to neutralize the acetic acid. The chloroform layer was separated, washed with water, and evaporated to leave a colorless oil (0.41 g). Crystallization from Skellysolve B gave compound XI (234 mg), m.p. 115–118°. Calc. for $C_{18}H_{27}O_3N$: C, 70.79; H, 8.92; N, 4.59%. Found: C, 71.03, 70.91; H, 8.88, 8.94; N, 4.63%. Infrared spectrum (nujol): ν_{max} 1747, 1260 ($OCOCH_3$), 1712 cm^{-1} (C=O). Ultraviolet spectrum: λ_{max} 308 mμ (log ε 1.85). Rotatory dispersion in methanol (*c* 0.11): $[\phi]_{589}$ −200°, $[\phi]_{500}$ −500°, $[\phi]_{333}$ −5060°, $[\phi]_{298}$ +6100°, $[\phi]_{280}$ +1100°.

Isomerization of XI to VIII (R = Ac)

The acetoxyketone XI (50 mg) was adsorbed on alumina (5 g) in ether and eluted (ether) after 2 hours. Crystallization of the product from Skellysolve-B gave lycoclavinone (32 mg), m.p. 172–174°, identical with an authentic sample. The infrared spectrum of the mother liquors from the crystallization indicated a mixture of acetoxyketones VIII and IX.

Acetoxyketone IX (R = Ac)

A solution of lycoclavine (0.39 g) and chromium trioxide (0.39 g) in 98% acetic acid (40 ml) was kept at room temperature for 20 hours, then made basic with cold dilute ammonium hydroxide and extracted four times with chloroform. Evaporation of the chloroform left a tan-colored oil (0.4 g), which was dissolved in ether and filtered rapidly through alumina (5 g) to give a colorless oil (0.30 g) which crystallized (0.22 g) from Skellysolve-B. The analytical sample, prepared by two recrystallizations from Skellysolve-B followed by sublimation, melted at 143°. Calc. for $C_{18}H_{27}O_3N$: C, 70.79; H, 8.93; N, 4.59%. Found: C, 70.96; H, 8.77; N, 4.36%. Infrared spectrum (nujol): 1752, 1238 (OAc), 1720 cm^{-1} (C=O). Ultraviolet spectrum: λ_{max} 286 mμ (log ε 1.79). Rotatory dispersion in methanol (*c* 0.1): $[\phi]_{589}$ −80°, $[\phi]_{400}$ −315°, $[\phi]_{312}$ −2135°, $[\phi]_{280}$ +190°, $[\phi]_{265}$ −1000°.

Isomerization to VIII (R = Ac) was carried out on an alumina column as described above for ketone XI. Hydrolysis of IX (R = Ac) with 10% HCl (as described above for VIII (R = Ac)) gave ketol VIII in 85% yield.

α-Dihydrolycopodine (XII, R = H, R′ = OH)

Lithium metal (0.9 g) was added in small portions over a period of 30 minutes to a solution of lycopodine (0.76 g) in methanol (40 ml) – liquid ammonia (200 ml). After most of the ammonia had evaporated, water (200 ml) was added and the mixture extracted four times with chloroform. Evaporation of the chloroform left a colorless glass (0.77 g) which crystallized from ether in colorless needles (0.63 g). The analytically pure material, obtained by recrystallization from ether, melted at 133–134°. Calc. for $C_{16}H_{27}ON$: C, 77.06; H, 10.91; N, 5.62%. Found: C, 76.93; H, 11.00; N, 4.69%. Infrared spectrum: ν_{max} 3620 cm^{-1}.

The perchlorate, after recrystallization from acetone–ether, melted at 245–246°. Calc. for $C_{16}H_{27}ON \cdot HClO_4$: C, 54.94; H, 8.01; N, 4.01%. Found: C, 55.31; H, 8.24; N, 4.44%.

Acetylation with pyridine – acetic anhydride at room temperature gave acetyl-α-dihydrolycopodine as a colorless oil (no OH in infrared) in almost quantitative yield. The perchlorate, after recrystallization from acetone–ether, melted at 276–278° (decomp.). Calc. for $C_{18}H_{29}O_2N \cdot HClO_4$: C, 55.24; H, 7.67; N, 3.58%. Found: C, 55.17; H, 7.68; N, 4.15%. Infrared spectrum (nujol): 3120 (+NH), 1730, and 1250 cm^{-1} (OAc). The free base, liberated from the perchlorate, solidified, and after distillation melted at 74–76°.

Anhydrodihydrolycopodine (XIII) from α-Dihydrolycopodine

Treatment of α-dihydrolycopodine with phosphorus oxychloride in pyridine gave the chloro compound XII (R or R′ = Cl), characterized as the perchlorate, m.p. 230–231°, in good yield. However, heating the xanthate (XII, R = H, R′ = OCS_2CH_3) methiodide (0.12 g) at 265° for 20 minutes followed by distillation (0.1 mm, 140°) gave anhydrodihydrolycopodine (XIII) (0.04 g), identified as the perchlorate, m.p. 238–239°. The xanthate was prepared by refluxing an ethereal solution of α-dihydrolycopodine (0.48 g) with sodium (0.06 g) for 60 hours, then adding carbon disulphide (1 ml) and refluxing for a further 24 hours. Addition of methyl iodide and further heating caused a white precipitate to separate. The precipitate

was filtered and recrystallized from ethanol to give the xanthate methiodide (0.54 g), m.p. 280–282° (decomp.). Calc. for $C_{18}H_{29}OS_2N \cdot CH_3I$: C, 47.39; H, 6.70; S, 13.32; N, 2.91; I, 26.36%. Found: C, 47.49, 47.69; H, 6.85, 6.66; S, 13.22; N, 3.07; I, 26.41%.

Diol V (R = R′ = H)

The ketol VIII (R = H) (86 mg) was refluxed for 3 hours with a slurry of $LiAlH_4$ (0.15 g) in ether (50 ml). Working up in the usual manner gave a colorless solid (84 mg), m.p. 217–222°. The analytical sample, m.p. 230–231°, was prepared by recrystallization from acetone followed by sublimation. Calc. for $C_{16}H_{27}O_2N$: C, 72.41; H, 10.25; N, 5.28%. Found: C, 71.78; H, 10.29; N, 5.44%. The infrared spectrum (nujol) showed a sharp band at 3500 cm^{-1} and broad —OH absorption at 2600–3300 cm^{-1}.

Diol VII (R = R′ = H)

A solution of the ketol VIII (R = H) (85 mg) in methanol (10 ml) was added to liquid ammonia (150 ml). The solution was stirred vigorously and lithium (0.2 g) added in small pieces over 10 minutes, then the ammonia was evaporated and water (100 ml) added. Continuous ether extraction yielded a yellow oil which, on distillation (0.1 mm, 150°), afforded a colorless oil that solidified on scratching. Four recrystallizations from acetone gave small, colorless needles of diol VII, m.p. 209–210°. Calc. for $C_{16}H_{27}O_2N$: C, 72.41; H, 10.25; N, 5.28%. Found (sublimed sample): C, 72.38, 72.21; 10.52, 10.55; N, 4.94%. Analysis of unsublimed material suggested that it was a monohydrate (found: C, 67.84; H, 9.31%). The infrared spectrum (nujol) showed OH stretching vibrations at 3580 (sharp) and 3080 (broad) cm^{-1}. The perchlorate melted at 243–245°. The mixed melting point with diol VI (m.p. 207–208°) was 176–184°.

Diol IV (R = R′ = H)

Acetoxyketone IX (27 mg) was refluxed with $LiAlH_4$ (0.1 g) in ether (30 ml) for 6 hours, then worked up in the usual manner to give a colorless solid (22 mg), m.p. 219–226°. Recrystallization from acetone raised the melting point to 234–235°. The compound, even after sublimation, analyzed as a hemihydrate. Calc. for $C_{16}H_{27}O_2N \cdot \frac{1}{2}H_2O$: C, 70.03; H, 10.28; N, 5.10%. Found: C, 70.38, 70.04; H, 10.49, 10.32; N, 5.79%. The mixed melting point with diol V (m.p. 230–231°) was 223–230°.

Wolff–Kishner Reduction of Lycopodine

Sodium (0.10 g) in diethylene glycol (40 ml) was heated to 180° and anhydrous hydrazine added until the solution refluxed freely at 180°. The solution was then cooled and lycopodine (0.63 g) added and refluxed for 17 hours. The temperature was then raised to 210° by distilling off excess hydrazine and refluxing was continued at this temperature for 24 hours. The cooled solution was diluted with water, acidified with hydrochloric acid, and continuously extracted with ether for 24 hours to remove diethylene glycol. Basification of the aqueous layer and further extraction with ether yield a colorless semisolid (0.5 g) which could not be recrystallized, but which was purified as the perchlorate, m.p. 224–225° (from ethanol–ether). Calc. for $C_{16}H_{27}N \cdot HClO_4$: C, 57.56; H, 8.45; N, 4.20%. Found: C, 57.38, 57.32; H, 8.73, 8.40; N, 4.71%. The free base, regenerated from the perchlorate, showed no absorption in the 3000–3600 cm^{-1} or 1500–1800 cm^{-1} regions in the infrared. The methiodide melted at 288–289° after recrystallization from ethanol–ether.

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REFERENCES

1. O. ACHMATOWICZ and W. UZIEBLO. Roczniki Chem. **18**, 88 (1938).
2. L. MARION and R. H. F. MANSKE. Can. J. Res. B, **22**, 137 (1944).
3. R. H. BURNELL and B. S. MOOTOO. Can. J. Chem. **39**, 1090 (1961).
4. W. A. AYER, D. A. LAW, and J. A. BEREZOWSKY. Unpublished.
5. H. A. GLEASON. New Britton and Brown illustrated flora of the northeastern United States and adjacent Canada. Vol. 1. Lancaster Press. 1952. p. 4.
6. W. A. HARRISON, M. CURCUMELLI-RODOSTAMO, D. F. CARSON, L. R. C. BARCLAY, and D. B. MACLEAN. Can. J. Chem. **39**, 2086 (1961).
7. M. CURCUMELLI-RODOSTAMO and D. B. MACLEAN. In press.
8. R. H. BURNELL and D. R. TAYLOR. Tetrahedron, **15**, 173 (1961).
9. R. H. F. MANSKE. Can. J. Chem. **31**, 894 (1953).
10. W. A. AYER and J. A. BEREZOWSKY. Unpublished.
11. A. R. H. COLE. Fortschr. Chem. Org. Naturstoffe, **13**, 30 (1956).
12. L. F. FIESER and M. FIESER. Natural products related to phenanthrene. 3rd ed. Reinhold Publishing

Corp. 1949. p. 195. D. H. R. BARTON, S. K. PRADHAN, S. STERNHELL, and J. F. TEMPLETON. J. Chem. Soc. 255 (1961).
13. S. J. ANGYAL and R. J. YOUNG. J. Am. Chem. Soc. **81**, 5251 (1959).
14. W. A. AYER, J. A. BEREZOWSKY, and D. A. LAW. In preparation.
15. F. A. L. ANET. Tetrahedron Letters, No. 20, 13 (1960).
16. (*a*) K. WIESNER, J. E. FRANCIS, J. A. FINDLAY, and Z. VALENTA. Tetrahedron Letters, No. 5, 187 (1961).
(*b*) K. WIESNER. Fortschr. Chem. Organ. Naturstoffe. In press.
17. H. CONROY. Advan. in Org. Chem. **2**, 308 (1960).
18. W. MOFFITT, R. B. WOODWARD, A. MOSCOWITZ, W. KLYNE, and C. DJERASSI. J. Am. Chem. Soc. **83**, 4013 (1961).
19. C. DJERASSI. Optical rotatory dispersion. McGraw-Hill. 1960. p. 113.
20. R. C. COOKSON and S. H. DANDEGAENKER. J. Chem. Soc. 352 (1955).
21. H. B. HENBEST, D. N. JONES, and G. P. SLATER. J. Chem. Soc. 4472 (1961).
22. W. KLYNE. Tetrahedron, **13**, 29 (1961).
23. T. C. BRUICE and T. H. FIFE. Tetrahedron Letters, No. 8, 263 (1961).
24. K. L. WILLIAMSON and W. S. JOHNSON. J. Am. Chem. Soc. **83**, 4623 (1961).
25. L. M. JACKMAN. Applications of nuclear magnetic resonance spectroscopy in organic chemistry. Pergamon Press. 1959. pp. 121–125.
26. V. M. MIĆOVIĆ and M. L. MIHAILOVIĆ. J. Org. Chem. **18**, 1198 (1953).
27. P. VON SCHLEYER and R. D. NICHOLAS. J. Am. Chem. Soc. **83**, 182 (1961).