LYCOPODIUM ALKALOIDS

V. THE BROMINATION OF LYCOPODINE AND THE STRUCTURE OF ALKALOID L.20[1]

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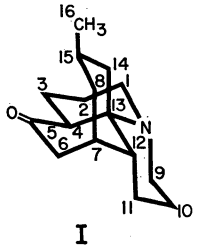
Received November 13, 1962

ABSTRACT

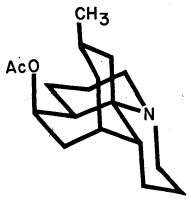
Monobromination of lycopodine hydrobromide in chloroform yields 6α-bromolycopodine hydrobromide, which can be epimerized to 6β-bromolycopodine hydrobromide. Hydrolysis of 6α-bromolycopodine proceeds with overall retention of configuration to give 6α-hydroxylycopodine, which is identical with the *Lycopodium lucidulum* alkaloid L.20. On the basis of some of the rotatory dispersion data presented it is suggested that positively charged nitrogen has a negative specific rotativity.

In 1956 Barclay and MacLean reported (1) that the bromination of lycopodine, now known (2, 3) to have structure I, in carbon tetrachloride yielded a monobrominated product, isolated as its hydrobromide, of melting point 290–295° (decomp.). Attempts to isolate the corresponding free base were unsuccessful. In connection with structural studies on lycoclavine (4) we have had occasion to repeat and extend this work and wish at this time to report the results of this investigation.

In our hands treatment of lycopodine with an equimolar amount of bromine in either carbon tetrachloride or chloroform without added hydrogen bromide led to the isolation of a crystalline product which proved to be a mixture of lycopodine hydrobromide and bromolycopodine hydrobromide. The melting point of this mixture (285–295°) corresponds to that reported by the earlier workers (1) and differs considerably from that of pure bromolycopodine hydrobromide (see below). The composition of the crystalline product was shown in the following manner. The analytical results obtained on an extensively recrystallized sample fitted best an approximately 1:1 mixture of bromolycopodine hydrobromide and lycopodine hydrobromide. Treatment of the mixture with aqueous sodium hydroxide followed by chromatography led to the isolation of lycopodine, in about 50% yield, as well as the enolic α-diketone III. Cocrystallization of equimolar quantities of lycopodine hydrobromide and bromolycopodine hydrobromide from a small volume of solvent yielded material the melting point and infrared spectrum



I



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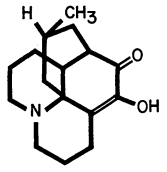


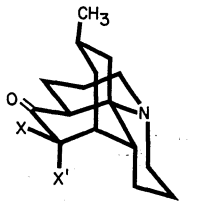
III

of which were similar to those of the recrystallized bromination product. The analytical results recorded by Barclay and MacLean suggest that their material contained approximately 80% of the bromo compound. Since bromolycopodine hydrobromide is some-

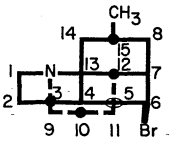
[1]*Part IV: W. A. Ayer and D. A. Law. Can. J. Chem.* **40**, *2088 (1962).*

what more soluble than lycopodine hydrobromide in the solvents used, the material obtained after several recrystallizations is actually richer in the non-brominated material. It was possible to obtain small quantities of pure bromolycopodine hydrobromide by fractional crystallization of the mother liquors. Since all of the bromine is consumed during the reaction, we attribute the incomplete bromination to competitive oxidative attack by bromine at the tertiary nitrogen. This view is supported by the observations that the preformed hydrobromide is cleanly monobrominated when treated with 1 mole of bromine (see below) and that acetyldihydrolycopodine (II) rapidly decolorizes 0.5 equivalent of bromine. In the latter reaction it was possible to recover 70% of the acetyldihydrolycopodine *as its hydrobromide*, indicating that reduction of the bromine had occurred.

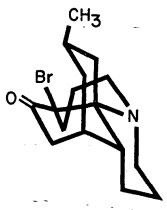
Bromination of lycopodine hydrobromide in chloroform containing a slight excess of hydrogen bromide gave, in better than 90% yield, 6α-bromolycopodine hydrobromide[2] (IV·HBr), m.p. 266–269°. The position and orientation of the bromine were shown in the following way. The nuclear magnetic resonance spectrum of IV showed a one-proton signal at 5.82 τ (broadened singlet), indicating that bromination had occurred at C-6 rather than C-4. The infrared spectrum of the hydrobromide in chloroform solution showed carbonyl absorption at 1711 cm^{-1}, little shifted from that of lycopodine hydrobromide (1707 cm^{-1}) and indicative of the axial nature of the C-6 bromine (6). The ultraviolet spectrum of the bromo compound (λ_{max} 306 mμ) showed the expected (7) bathochromic shift relative to lycopodine hydrobromide (λ_{max} 280 mμ).



IV X = H; X' = Br
VI X = Br; X' = H
X X = H; X' = OH



V



VII

With the structure of the bromo compound thus assigned, it was of interest to examine its optical rotatory dispersion curve in the light of the axial haloketone rule (8) and provide further evidence for the absolute configuration of lycopodine. The absolute configuration represented by structure I was first assigned by application of the octant rule to lycopodine itself (9). If structure IV represents the absolute configuration of 6α-bromolycopodine the axial haloketone rule predicts that it will show a positive Cotton effect whereas the enantiomer will show a negative Cotton effect. In fact, 6α-bromolycopodine hydrobromide does exhibit a *positive* Cotton effect with extrema at 332 and 280 mμ, amplitude[3] +10,650°. Lycopodine hydrobromide exhibits a negative Cotton effect with extrema at 305 and 268 mμ, amplitude −2770°. Since lycopodine itself shows a positive Cotton effect (9), amplitude +17,000°, it appears that positively charged nitrogen (which in the octant diagram (V)[4] for this system appears in the rear upper

[2]*The numbering system used is that suggested by Wiesner (5). Substituents in rings A, B, and C are referred to as α if trans to the C-7 to C-13 bridge and β if cis to the bridge.*

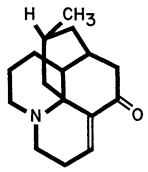
[3]*Amplitudes are given as the algebraic difference between molecular rotation for peak and trough.*

[4]*In the octant diagram V carbons 9, 10, and 11 lie directly behind carbons 3, 4, and 5, respectively, i.e., they they are in the horizontal plane.*

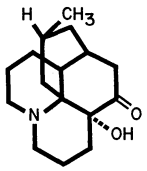
left octant) has, like fluorine, a negative specific rotativity (10). An alternate explanation, that the difference is due to a change in conformation on salt formation, seems, on the basis of the examination of molecular models (Dreiding), unlikely. Both lycopodine perchlorate and lycopodine methiodide show negative Cotton effects with amplitudes similar to that of the hydrobromide. A solution of lycopodine in acetic acid also exhibits a negative Cotton effect. In agreement with the octant rule (10) 6α-bromolycopodine shows a more strongly positive Cotton effect (amplitude +29,500°) than does lycopodine (see V).

When a solution of 6α-bromolycopodine hydrobromide (IV·HBr) in glacial acetic acid was heated for an hour on the steam bath the product, isolated in 75% yield after evaporation of the acetic acid and washing of the residue with acetone, showed carbonyl absorption (nujol) of approximately equal intensity at 1720 and 1703 cm^{-1}. By fractional crystallization from methanol it was possible to isolate the component responsible for the 1720 cm^{-1} band in about 10% overall yield. This has been assigned the structure 6β-bromolycopodine hydrobromide (VI·HBr) on the basis of its analysis and its infrared (ν_{max} 1728 cm^{-1} in $CHCl_3$) and ultraviolet (λ_{max} 276 mμ) spectra, both of which are indicative of an equatorial α-bromoketone (6, 7). The alternate formulation, 4β-bromolycopodine hydrobromide, although not excluded by this data, is considered highly unlikely, since it would force the molecule to adopt a conformation in which ring C is in a boat conformation involving a serious bowsprit–flagpole interaction (VII). The negative rotatory dispersion curve is almost plain, with a slight inflexion toward the negative side at 310 mμ. Attempts to effect the epimerization in higher yield by use of more strenuous reaction conditions resulted in extensive decomposition and much lower overall yields. When 6β-bromolycopodine hydrobromide (VI·HBr) was subjected to the epimerizing conditions noted above, the crude product did not show a detectable (by infrared) amount of the 6α-epimer, demonstrating that the product obtained from the 6α-compound is not an equilibrium mixture.

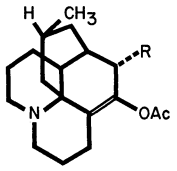
The free bromoketone IV was extremely sensitive to basic conditions. It was prepared by shaking a chloroform solution of the hydrobromide with cold dilute ammonium hydroxide or sodium bicarbonate. It decomposed on melting, with the elimination of hydrogen bromide and the formation of the unsaturated ketone VIII (see below), or on standing in methanol, as revealed by the decrease in amplitude of the optical rotatory dispersion curve with time and by the appearance of salt bands ($\overset{+}{N}H$) in the infrared spectrum of material recovered from the optical rotatory dispersion measurements. Treatment of 6α-bromolycopodine hydrobromide with aqueous sodium hydroxide at room temperature yielded a mixture of the enolic α-diketone III, previously prepared by selenium dioxide of lycopodine (4), and the unsaturated ketone VIII. The diketone III is presumably formed by aerial oxidation of the Δ^5-enolate form of the corresponding α-ketol (4), and the unsaturated ketone VIII by 1,4-elimination of HBr from the Δ^4-enol form of the bromoketone. The structure of the ketone VIII was shown in the following way. The ultraviolet spectrum showed a maximum at 244 mμ (log ε 3.9). The infrared spectrum showed peaks of almost equal intensity at 1680 and 1610 cm^{-1}, indicating (11) the cisoid nature of the α,β-unsaturated ketonic function. The n.m.r. spectrum revealed the presence of a single olefinic proton (poorly resolved triplet at 3.04 τ) located at the β-position of the unsaturated system (12). Reduction of the unsaturated ketone VIII with lithium and ammonia gave lycopodine (I), showing that no skeletal rearrangement had occurred during the dehydrobromination. The ketone VIII has recently been prepared by dehydration of flabelliformine (IX) (13).



VIII



IX



XI R = OAc
XII R = H

When sodium bicarbonate was substituted for sodium hydroxide in the hydrolysis of 6α-bromolycopodine hydrobromide a crystalline compound, $C_{16}H_{25}O_2N$, m.p. 258–259°, was obtained in high yield. The high melting point and the infrared spectrum, which showed (in nujol) an unusually broad OH stretching vibration (2400–3200 cm^{-1}) were reminiscent of alkaloid L.20, first isolated by Manske and Marion from *Lycopodium lucidulum* Michx. (14) and later in these laboratories from the same plant.[5] Direct comparison (mixed melting point, infrared spectra, optical rotatory dispersion curves) confirmed the identity of the compounds from the two sources. This finding, coupled with the evidence presented below, necessitates a revision of the molecular formula of alkaloid L.20 from $C_{17}H_{27}O_2N$ (14) to $C_{16}H_{25}O_2N$.

The infrared spectrum of alkaloid L.20 in alcohol-free chloroform showed absorption at 3620 cm^{-1} (free OH) and 1710 cm^{-1} (ketone), thus defining the nature of the two oxygen atoms. Reduction of L.20 with calcium and ammonia (15) gave lycopodine, defining the carbon–nitrogen skeleton and the position of the carbonyl. The fact that the hydroxyl group was eliminated during the reduction shows that it is α to the carbonyl and suggests that it is axially orientated (15). This orientation is confirmed by the ultraviolet spectrum, which shows carbonyl absorption at 296 mμ (16), and the infrared spectrum, which reveals a non-hydrogen-bonded hydroxyl. The secondary nature of the hydroxyl group follows from the observation that L.20 is transformed to the diketone III in aqueous sodium hydroxide in the presence of air, and from the fact that the nuclear magnetic resonance spectrum of its acetyl derivative shows a one-proton signal at 5.11 τ (C*H*OAc). Thus alkaloid L.20 is 6α-hydroxylycopodine (X) and the hydrolysis of 6α-bromolycopodine proceeds with overall retention of configuration. When alkaloid L.20 (X) was treated with sodium propoxide in *n*-propanol in an atmosphere of nitrogen it was epimerized to the known (4) 6β-hydroxylycopodine. The epimerization also occurred when L.20 was passed through a column of basic alumina. The unsaturated ketone VIII was obtained when L.20 was subjected to the action of hot 10% hydrochloric acid.

Displacement reactions of α-haloketones normally proceed with inversion of configuration (17); however, in this case attack from the backside is extremely hindered by the C-7 to C-13 bridge and displacement is likely preceded by epimerization. In agreement with this view, the 6β-bromoketone (VI) also yields alkaloid L.20 (X) when treated with aqueous bicarbonate.

Acetylation of alkaloid L.20 with pyridine – acetic anhydride at −10° yielded the expected acetyl-L.20 (X, OAc in place of OH). However, when the acetylation was carried out at room temperature a high yield of the enol acetate XI was obtained. Compound XI absorbed in the infrared at 1760 and 1680 cm^{-1} (enol acetate carbonyl and double bond, respectively) and at 1735 cm^{-1} (acetoxyl). The nuclear magnetic resonance

[5]*We wish to thank Dr. L. Marion for an authentic sample of alkaloid L.20.*

spectrum showed two acetoxyl methyls (7.98 and 8.06 τ) as well as a one-proton signal at 5.02 τ (C*H*OAc). Hydrolysis of XI with dilute acid at room temperature afforded acetyl-L.20 and hydrolysis with aqueous bicarbonate gave back alkaloid L.20 (X). The same enol acetate XI was obtained, together with the unsaturated ketone VIII, when 6α-bromolycopodine hydrobromide was refluxed with sodium acetate in acetic acid – acetic anhydride.

It is interesting to note that the enol acetylation of lycopodine (I) with acetic anhydride – *p*-toluenesulphonic acid yields the Δ^4-enol acetate XII, as shown by the lack of olefinic absorption in the n.m.r. spectrum, whereas bromination occurs via the Δ^5-enol. Provided that the direction of acid-catalyzed enol acetylation is a measure of the preferred direction of acid-catalyzed enolization in this system, the position of bromination must be due to the greater degree of steric hindrance at C-4 than at C-6.

EXPERIMENTAL

Ultraviolet absorption spectra were determined in 95% ethanol. Optical rotatory dispersion spectra were measured on a Rudolph Automatic Recording Spectropolarimeter. Nuclear magnetic resonance spectra were measured on ca. 10% w/v solutions in deuteriochloroform using a Varian Associates Model A-60 spectrometer with tetramethylsilane as an internal standard. Melting points were determined on a hot stage and are uncorrected. Alumina, unless otherwise specified, means basic alumina of activity III–IV (Brockmann scale). Skellysolve B refers to Skelly Oil Company light petroleum, b.p. 62–70°.

Bromination of Lycopodine

A solution of lycopodine (0.45 g) in chloroform (20 ml) was treated with a solution of bromine (0.30 g) in chloroform (20 ml). After standing 2 hours at room temperature the faintly yellow solution was evaporated at the pump. The solid residue was washed with a small volume of cold acetone, leaving a colorless solid (0.57 g), m.p. 280–295° (decomp.). The analytical sample, prepared by recrystallizing three times from methanol, melted at 285–295° (decomp.). Calc. for $C_{16}H_{26}ONBr \cdot C_{16}H_{25}ONBr_2$: C, 52.26; H, 6.99; Br, 32.60%. Found: C, 51.95, 52.27; H, 6.93, 6.88; Br, 32.09%. Infrared spectrum: ν_{max}^{nujol} 2540 (+NH), 1700 cm^{-1} (C=O).

By fractional crystallization of the combined mother liquors of several such brominations from methanol–acetone it was possible to isolate small quantities of relatively pure 6α-bromolycopodine hydrobromide, m.p. 260–269° (decomp.).

The recrystallized bromination product (0.30 g) was stirred overnight with 3% aqueous sodium hydroxide, then the reaction mixture adjusted to pH 7.5 and extracted with ether to give a dark brown oil (0.21 g). Chromatography of the oil over alumina (5 g) gave lycopodine (91 mg), eluted with benzene, and the enolic α-diketone III (36 mg), eluted with chloroform–methanol (99:1).

Bromination of Lycopodine Hydrobromide

A solution of bromine (0.78 g) in chloroform (15 ml) was added dropwise with stirring to a solution of lycopodine hydrobromide (1.45 g) in chloroform (30 ml) to which had been added 0.1 ml of chloroform saturated with hydrogen bromide. The mixture (a small amount of precipitate formed shortly after bromine addition was completed) was kept at room temperature overnight, then evaporated at the pump, and cold acetone (20 ml) was added to the residual off-white solid. The solid was collected and crystallized once from methanol to give almost pure 6α-bromolycopodine hydrobromide (1.64 g, 91%), m.p. 261–265° (decomp.). Two further recrystallizations from methanol furnished the analytical sample, m.p. 266–269° (decomp.). Calc. for $C_{16}H_{25}ONBr_2$: C, 47.19; H, 6.19; N, 3.50; Br, 39.25%. Found: C, 47.25, 47.45; H, 6.32, 6.43; N, 3.44; Br, 39.27%. Infrared spectrum: $\nu_{max}^{CHCl_3}$ 2470 (+NH), 1711 cm^{-1} (C=O); ν_{max}^{nujol} 2550, 1703 cm^{-1}. Ultraviolet spectrum: λ_{max} 306 mμ (log ϵ 2.19). Rotatory dispersion in methanol (*c*, 0.10): $[\phi]_{589}$ +150°, $[\phi]_{400}$ +910°, $[\phi]_{332}$ +4550°, $[\phi]_{280}$ −6100°, $[\phi]_{250}$ −2300°.

Optical Rotatory Dispersion Spectra of Lycopodine Salts

The salts were prepared by the usual methods.

Lycopodine hydrobromide, in methanol (*c* 0.12): $[\phi]_{589}$ −330°, $[\phi]_{500}$ −430°, $[\phi]_{400}$ −600°, $[\phi]_{305}$ −2340°, $[\phi]_{268}$ +430°, $[\phi]_{253}$ ±0°.

Lycopodine, in acetic acid (*c* 0.10): $[\phi]_{589}$ −230°, $[\phi]_{500}$ −345°, $[\phi]_{400}$ −545°, $[\phi]_{300}$ −2270°, $[\phi]_{263}$ +540°, $[\phi]_{255}$ +400°.

Lycopodine methiodide, in methanol (*c* 0.12): $[\phi]_{589}$ −110°, $[\phi]_{500}$ −160°, $[\phi]_{400}$ −560°, $[\phi]_{304}$ −2450°, $[\phi]_{268}$ +260°, $[\phi]_{255}$ ±0°.

Lycopodine perchlorate, in methanol (*c* 0.096): $[\phi]_{589}$ +80°, $[\phi]_{500}$ ±0°, $[\phi]_{400}$ −360°, $[\phi]_{302}$ −1600°, $[\phi]_{260}$ +280°, $[\phi]_{250}$ ±0°.

Reaction of Acetyldihydrolycopodine with Bromine

A solution of bromine (14 mg, 0.09 mmole) in chloroform (10 ml) was added all at once to a solution of acetyldihydrolycopodine (II) (55 mg, 0.18 mmole). The solution decolorized immediately and after 15 minutes was evaporated to dryness to give a light brown foam.

Crystallization from acetone–ether yielded acetyldihydrolycopodine hydrobromide (49 mg, 71%), identical with an authentic sample. The mother liquors yielded a light brown oil which rapidly darkened on standing and failed to yield further crystalline material.

6α-Bromolycopodine (IV)

A solution of 6α-bromolycopodine hydrobromide (0.156 g) in chloroform (60 ml) was shaken with cold dil. ammonium hydroxide (aqueous sodium bicarbonate gave similar results). The chloroform layer was separated, washed with water, dried ($MgSO_4$), and evaporated to give a colorless solid (0.12 g). Crystallization from ether or acetone yielded colorless spars which melted at 163–165° with partial decomposition, then resolidified and melted with decomposition at 238–240°. Calc. for $C_{16}H_{24}ONBr$: C, 58.90; H, 7.41; N, 4.29; Br, 24.49%. Found: C, 59.63; H, 7.42; N, 4.46; Br, 24.80%. Infrared spectrum: ν_{max}^{nujol} 1702 cm^{-1}; $\nu_{max}^{CCl_4}$ 1711 cm^{-1}. Ultraviolet spectrum: λ_{max} 305 mμ (log ε 2.22). Nuclear magnetic resonance spectrum: 5.82 τ (C*H*Br, broadened singlet, half-height width 3.2 c.p.s.), 9.16 τ (CH—C*H*$_3$, doublet, splitting 3.8 c.p.s.). Rotatory dispersion in methanol (*c* 0.064, freshly prepared solution): $[\phi]_{400}$ +1,070°, $[\phi]_{335}$ +8,950°, $[\phi]_{294}$ −20,550°, $[\phi]_{250}$ −4,500°. After 2½ hours the amplitude had fallen to +27,250°, and after 24 hours, to +13,900°. The material recovered from the optical rotatory dispersion measurements (24 hours) showed distinct +NH bands in the infrared. When a sample of the 6α-bromoketone was heated to 170° for 1 minute the infrared spectrum of the dark solid remaining showed peaks (1690, 1620, 2520 cm^{-1}) characteristic of the unsaturated ketone VIII as well as typical +NH stretching vibrations.

6β-Bromolycopodine Hydrobromide (VI·HBr)

A solution of 6α-bromolycopodine hydrobromide (IV·HBr, 2.24 g) in glacial acetic acid (50 ml) was heated on the steam bath for 1 hour, then cooled to room temperature and evaporated to dryness under reduced pressure. The residue was washed with cold acetone (50 ml) and filtered to give a colorless solid (1.71 g). Infrared spectrum: ν_{max}^{nujol} 2450, 1720, 1703 cm^{-1}.

Seven recrystallizations from methanol yielded pure 6β-bromolycopodine hydrobromide (VI·HBr, 0.21 g), m.p. 257–262° (decomp.). Calc. for $C_{16}H_{25}ONBr_2$: C, 47.19; H, 6.19; N, 3.44; Br, 39.25%. Found: C, 47.21, 47.22; H, 6.28, 6.32; N, 3.71; Br, 39.62, 39.94%. Infrared spectrum: $\nu_{max}^{CHCl_3}$ 2440 (+NH), 1728 cm^{-1} (>C=O); ν_{max}^{nujol} 2590, 2550 (+NH), 1718 cm^{-1}. Ultraviolet spectrum: λ_{max} 276 mμ (log ε 2.07). Rotatory dispersion in methanol (*c* 0.10): $[\phi]_{589}$ −265°, $[\phi]_{500}$ −400°, $[\phi]_{400}$ −660°, $[\phi]_{350}$ −880°, $[\phi]_{310}$ −1000°, $[\phi]_{300}$ −1290°.

Because of the low yields obtained in the epimerization no attempt was made to prepare the free base. When a solution of 6β-bromolycopodine hydrobromide in glacial acetic acid was heated on the steam bath for an hour, the infrared spectrum of the solid recovered after evaporation of the acetic acid was almost identical with that of the starting material.

Reaction of 6α-Bromolycopodine Hydrobromide with Sodium Hydroxide

6β-Bromolycopodine (0.35 g) was suspended in 3% aq. sodium hydroxide (40 ml) and stirred at room temperature for 18 hours. The dark brown solution thus obtained was adjusted to pH 7.5 with acetic acid and extracted four times with chloroform to give a brown semisolid (0.15 g). The semisolid was chromatographed over alumina (5 g). Elution with ether yielded the unsaturated ketone VIII as a pale yellow oil (0.05 g). The unsaturated ketone did not crystallize readily and was characterized as the methiodide, which after recrystallization from acetone, melted at 271–273°. Calc. for $C_{16}H_{23}ON\cdot CH_3I$: C, 52.72; H, 6.77%. Found: C, 52.71, 52.47; H, 6.74; 6,82%. Infrared spectra: methiodide: ν_{max}^{nujol} 1698, 1629 cm^{-1}; free base: $\nu_{max}^{CCl_4}$ 1686, 1614 cm^{-1}. Ultraviolet spectrum: λ_{max} 244 mμ (log ε 3.90). Nuclear magnetic resonance spectrum: 3.04 τ (olefinic H), 9.18 τ (CHC*H*$_3$, doublet, splitting 3.5 c.p.s.).

Elution of the chromatogram with chloroform–methanol (49:1) yielded a light brown solid (0.057 g) which on sublimation afforded the pure diosphenol III (41 mg), m.p. 185–186°, identical with that prepared previously (4) from lycopodine.

Lithium–Ammonia Reduction of the Unsaturated Ketone VIII

3,4-Dehydrolycopodine (VIII, 0.103 g) in ether (25 ml) was added to a stirred solution of lithium (0.2 g) in ammonia (150 ml). After 30 minutes solid ammonium chloride was added until the blue color disappeared, and the ammonia was allowed to evaporate. The residue was distributed between chloroform and water and the layers separated. The aqueous layer was washed with chloroform and the combined organic extracts washed with water. Evaporation of the chloroform left a colorless oil (0.105 g) which solidified on scratching. The solid material was dissolved in acetone and neutralized with 70% perchloric acid. The perchlorate which separated (0.115 g) was identical with authentic lycopodine perchlorate.

Alkaloid L.20

(a) From 6α-Bromolycopodine Hydrobromide

6α-Bromolycopodine hydrobromide (IV·HBr, 1.32 g) was added to a stirred solution of 5% aq. sodium bicarbonate (80 ml) at room temperature. The bromo compound slowly dissolved but a second crystalline phase began to separate before solution was complete. After 24 hours the reaction mixture was transferred to a liquid–liquid extractor and continuously extracted with ether. The ethereal extract was dried ($MgSO_4$) and evaporated to yield an off-white solid (0.78 g, 92%) whose infrared spectrum was identical with that of an authentic sample of alkaloid L.20 (see Section (*b*)). Crystallization from methanol yielded colorless needles, m.p. 258–259°, both pure and mixed with an authentic sample of alkaloid L.20. Both samples had identical rotatory dispersion curves.

(b) From L. lucidulum Michx.

Dried, finely ground *L. lucidulum* Michx. (11 kg) was Soxhlet-extracted with methanol and the extract concentrated to a thick, dark residue. The residue was digested for 24 hours with cold, dilute hydrochloric acid, filtered, and the filtrate washed with ether. The acid solution was adjusted to ca. pH 8 with ammonium hydroxide and extracted with chloroform (extract A), then the aqueous layer was made strongly basic with ammonium hydroxide and again extracted with chloroform (extract B). Extract B was concentrated at the pump and the residue (19.1 g) dissolved in methanol. After standing several days in the refrigerator the solution deposited crystals (0.31 g) which, after recrystallization from methanol or acetone, melted at 258–259°. This substance was shown to be alkaloid L.20 by comparison (infrared spectrum, mixed melting point, rotatory dispersion curve) with an authentic sample kindly furnished by Dr. L. Marion. Calc. for $C_{16}H_{25}O_2N$: C, 72.96; H, 9.57; N, 5.32%. Found: C, 72.43, 72.57; H, 9.76, 9.81; N, 5.24%. Infrared spectrum: $\nu_{max}^{CHCl_3}$ 3620 (OH), 1710 cm^{-1} (>C=O). Ultraviolet spectrum: λ_{max} 296 mμ (log ε 1.85). Rotatory dispersion in methanol (*c* 0.137): $[\phi]_{500}$ +105°, $[\phi]_{400}$ +400°, $[\phi]_{330}$ +4,300°, $[\phi]_{285}$ −10,500°, $[\phi]_{270}$ −3,000°.

Chromatography of the mother liquors obtained from extract B yielded lycopodine and lycodoline (alkaloid L.8) as the only crystalline material. Extract A was reserved for future examination.

(c) From 6β-Bromolycopodine Hydrobromide

6β-Bromolycopodine hydrobromide (VI·HBr, 84 mg) was stirred for 24 hours with 5% aq. sodium bicarbonate (20 ml). The resulting brown solution was extracted six times with chloroform. The product obtained was crystallized from acetone to give alkaloid L.20 (19 mg, 35%). Although the yield is lower than that quoted in section (*a*) we believe that this is largely due to the change in reaction conditions (w/v ratio), since the 6α-bromo compound, when treated under conditions identical with those described here, gave only a 30% yield of alkaloid L.20.

Calcium–Ammonia Reduction of Alkaloid L.20

A solution of alkaloid L.20 (X, 97 mg) in tetrahydrofuran (7 ml) was added to a blue solution of calcium (0.10 g) in liquid ammonia (150 ml). The reaction mixture was stirred for 15 minutes, then the excess calcium destroyed by addition of bromobenzene followed by water (5 ml). After most of the ammonia had evaporated more water was added and the aqueous layer was extracted several times with chloroform. Evaporation of the chloroform left a light brown oil (0.07 g) which was dissolved in dichloromethane and chromatographed over alumina (3 g). Dichloromethane eluted lycopodine (43 mg), chloroform eluted epidihydrolycopodine (20 mg) (4), and chloroform–methanol (24:1) eluted the diosphenol III (3 mg). All three products were identified by comparison with authentic samples.

When lithium–ammonia–methanol was used for the reduction only epidihydrolycopodine was obtained.

6β-Hydroxylycopodine from Alkaloid L.20

(*a*) A solution of alkaloid L.20 (X, 45 mg) in *n*-propanol (10 ml) containing sodium propoxide (80 mg) was shaken in an atmosphere of nitrogen for 40 hours, then chloroform (50 ml) and water (50 ml) were added. The chloroform layer was separated, washed with water, and evaporated. The residue was crystallized from acetone, yielding 6β-hydroxylycopodine (30 mg), m.p. 121–122°, both pure and mixed with an authentic sample (4). Both samples had identical infrared spectra ($CHCl_3$).

(*b*) A solution of alkaloid L.20 (0.13 g) was absorbed on a column of basic alumina (10 g) in ether–benzene (1:1). After 12 hours the column was eluted with ether–methanol, affording a colorless semisolid (0.12 g) which on sublimation furnished 6β-hydroxylycopodine (0.10 g), identical with an authentic sample.

Dehydration of Alkaloid L.20

A solution of alkaloid L.20 (X, 0.19 g), in 10% aq. hydrochloric acid (30 ml) was heated under reflux for 3 hours. After being cooled, the solution was neutralized with sodium bicarbonate and continuously extracted with ether. The residue (0.18 g) was triturated with Skellysolve B (in which the starting material is insoluble). The Skellysolve solution was concentrated, the residual oil dissolved in cold acetone (5 ml), and methyl iodide (1 ml) added. On standing the solution overnight in the refrigerator crystals (0.23 g) of 3,4-dehydrolycopodine methiodide (VIII·CH_3I), m.p. 270–273°, separated.

Acetylation of Alkaloid L.20

(a) At Room Temperature, Formation of Enol Acetate XI

A solution of alkaloid L.20 (X, 0.31 g) in acetic anhydride – pyridine (9 ml of 1:1) was kept at room temperature for 12 hours, then diluted with chloroform and ice-cold dil. ammonium hydroxide. The chloroform layer was separated and the aqueous layer extracted twice more with chloroform. The combined chloroform extracts were washed with water, dried ($MgSO_4$), and evaporated to give a colorless oil (0.39 g). The oil could not be induced to crystallize and was purified and characterized as the perchlorate, formed in acetone and recrystallized from acetone–ether, which melted at 258–259° (decomp.). Calc. for $C_{20}H_{29}O_4N \cdot HClO_4$: C, 53.63; H, 6.75; N, 3.11%. Found: C, 53.78; H, 6.82; N, 3.03%. Infrared spectra: perchlorate: ν_{max}^{nujol} 3060 (+NH), 1740 (2 OAc), 1690 cm^{-1} (C=C); free base: $\nu_{max}^{CCl_4}$ 1760, 1735 (2 OAc), 1680 cm^{-1} (C=C). The nuclear magnetic resonance spectrum of the free base showed signals at 5.02 τ (1H, broadened singlet, half-height width 4 c.p.s.), 7.98 and 8.06 τ (total of 6H), and 9.17 τ (3H, doublet, splitting 5.5 c.p.s.).

Hydrolysis of the enol acetate XI (0.39 g) with 8% aq. hydrochloric acid (20 ml) at room temperature for 3 hours, followed by working up in the usual manner, afforded a yellowish oil which after filtration through alumina and crystallization from Skellysolve B gave acetyl-L.20 (0.12 g), m.p. 143–144° (see below). The mother liquors (0.15 g) contained mainly starting enol acetate as judged by the infrared spectrum.

Hydrolysis of the enol acetate XI with aqueous methanolic sodium bicarbonate at room temperature afforded alkaloid L.20 in about 50% yield.

(b) At −10°, Formation of Acetyl-L.20

A solution of alkaloid L.20 (0.12 g) in acetic anhydride (5 ml) – pyridine (2 ml) was kept at −10° for 4 hours, then poured into chloroform and dil. ammonium hydroxide. The aqueous layer was separated and washed twice more with chloroform. The combined chloroform extracts, after washing with water and drying, were evaporated to give a colorless solid (0.11 g), m.p. 126–134°. Three recrystallizations from Skellysolve B gave analytically pure needles of acetyl-L.20 (58 mg), m.p. 143–144°. Calc. for $C_{18}H_{27}O_3N$: C, 70.79; H, 8.92; N, 4.59%. Found: C, 70.97, 70.66; H, 8.92, 8.82; N, 4.72. Infrared spectrum: $\nu_{max}^{CCl_4}$ 1755 (OAc), 1723 (ketone), 1230 cm^{-1} (OAc). Ultraviolet spectrum: λ_{max} 300 mμ (log ϵ 1.95). The rotatory dispersion spectrum showed a positive Cotton effect with peak at 327 mμ (+1,800°) and trough at 282 mμ (−10,900°). Details of the nuclear magnetic resonance spectrum have been published (4).

Lycopodine Enol Acetate (XII)

The method used was that of Barton *et al.* (18). A solution of lycopodine (0.5 g) and *p*-toluenesulphonic acid (0.45 g) in acetic anhydride (25 ml) was heated until the acetic anhydride slowly distilled. After 4 hours most of the acetic anhydride had been removed. The residue was diluted with ether and dil. aq. sodium hydroxide and shaken. The aqueous layer was separated and washed twice more with ether. The combined ethereal extracts were washed with water, dried ($MgSO_4$), and evaporated, leaving an off-white solid which was crystallized from acetone to give lycopodine enol acetate (0.4 g), m.p. 98–100°. The analytical sample was prepared by recrystallization from acetone, then from Skellysolve B, and melted at 100–101°. Calc. for $C_{18}H_{27}O_2N$: C, 74.70; H, 9.41; O, 11.06. Found: C, 74.30, 74.49; H, 9.24, 9.40; O, 11.49%. Infrared spectrum: $\nu_{max}^{CCl_4}$ 1755 (OAc), 1696 cm^{-1} (C=C). Nuclear magnetic resonance spectrum: 7.86 τ (3H, $OCOCH_3$), 9.12 τ (3H, $CHCH_3$, doublet, splitting 6 c.p.s.).

ACKNOWLEDGMENTS

We wish to thank Drs. K. Wiesner and Z. Valenta for valuable correspondence in connection with the rotatory dispersion data, Mr. M. A. Stillwell for the collection and identification of the plant material, Mr. R. N. Swindlehurst and Mrs. G. B. Conway for most of the infrared, nuclear magnetic resonance, and optical rotatory dispersion measurements, and the National Research Council of Canada for financial support.

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