The alkaloids of *Lycopodium cernuum* L. I. The structures of cernuine and lycocernuine[1]

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Structures IV and V are proposed for cernuine and lycocernuine, respectively, the major alkaloids present in *Lycopodium cernuum* L. The chemical and physical properties on which these structures are based are described. A possible mode of biogenesis of these alkaloids is discussed.

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The alkaloids of *Lycopodium cernuum* L. were first examined in 1948 by Marion and Manske (1). They reported the isolation of nicotine, a new alkaloid cernuine, $C_{16}H_{26}ON_2$, and a small amount of a crystalline base, m.p. 218°, which was designated alkaloid L. 33 but which was not further characterized. The fact that this species contained none of the common Lycopodium alkaloids (2) coupled with the fact that the spectral characteristics (see below) observed for cernuine[3] indicated that it did not possess the skeleton of any of the known alkaloids prompted us to reexamine the alkaloids of this plant.

The major alkaloid present in the *L. cernuum* examined, which was collected in Venezuela and in Mexico, proved to be identical with alkaloid L. 33, the minor alkaloid isolated by Marion and Manske (1). We have named this substance lycocernuine. The next most abundant alkaloid was cernuine. Examination, by thin-layer chromatography (t.l.c.), of the bases remaining after removal of most of the cernuine and lycocernuine revealed the presence of several other alkaloids. However, despite considerable efforts we have succeeded in isolating only one other alkaloid in a crystalline form, and it proved to be identical with dihydrodeoxycernuine, the product of the lithium aluminium hydride reduction of cernuine (see below).

Analytical and mass spectral data confirmed the molecular formula $C_{16}H_{26}ON_2$ for cernuine and established the formula $C_{16}H_{26}O_2N_2$ for lycocernuine. Both alkaloids contain two tertiary nitrogens, one basic and one non-basic, as revealed by the following observations. They both titrate as monoacidic bases (pK_a' values (50% MeOH): cernuine, 6.3; lycocernuine, 6.4), form C_{17} methiodides, and show no NH absorption in the infrared. The infrared spectra of both bases show strong absorption at 1 640 cm^{-1} (CCl_4 solution), suggesting that in each case the non-basic nitrogen is present as part of an amide or lactam grouping. The 1 640 cm^{-1} band is accompanied by a moderately intense band at 1 410 cm^{-1}, which disappears when the amide is reduced and which is displaced to lower frequency when the compound is treated with sodium methoxide in methanol-*O-d* (two deuterium atoms are incorporated, as revealed by mass spectrometry). This suggests the presence of the grouping $\rangle N—CO—CH_2—$ (3). Lithium aluminium hydride reduced the amide carbonyl of cernuine to give the diacidic base dihydrodeoxycernuine (pK_a' (80% Methyl Cellosolve) 6.90, 8.55), which shows no carbonyl

[1]A portion of this work was reported in a preliminary communication: W. A. Ayer, J. K. Jenkins, S. Valverde-Lopez, and R. H. Burnell, Tetrahedron Letters, 2201 (1964).

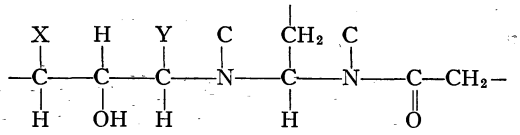
[2]Fellow of the Alfred P. Sloan Foundation.

[3]The initial spectra were determined on a sample of cernuine kindly provided by R. H. Manske, to whom we express our sincere thanks.

or NH absorption in the infrared. Similar reduction of lycocernuine gave dihydrodeoxylycocernuine. The dihydrodeoxy compounds show no absorption arising from an olefinic system in either their infrared or nuclear magnetic resonance (n.m.r.) spectra, and they are resistant to catalytic hydrogenation, indicating that the alkaloids are tetracyclic.

Besides the absorption bands mentioned previously, lycocernuine displays absorption at 3 620 cm^{-1} in the infrared, showing that the second oxygen is present in the form of a non-hydrogen bonded hydroxyl group. Acetylation of lycocernuine with acetic anhydride – pyridine yielded *O*-acetyllycocernuine. The appearance of a multiplet at τ 5.06 (1H, shifted down from τ 6.2 in lycocernuine itself) revealed the secondary nature of the hydroxyl group. This was confirmed by oxidation of lycocernuine with chromic acid in acetone to a ketone, dehydrolycocernuine, $C_{16}H_{24}O_2N_2$, which regenerated lycocernuine, together with a small amount of the epimeric alcohol,[4] when reduced with sodium borohydride in methanol. Absorption at 1 705 cm^{-1} in the infrared spectrum of dehydrolycocernuine indicates that the keto group is in an unstrained environment. Dehydrolycocernuine is very weakly basic, it is insoluble in dilute mineral acid, and its titration curve shows no inflection when it is titrated with 1 *N* sulfuric acid in 80% Methyl Cellosolve, but the fact that the newly introduced carbonyl group is an α-aminoketone rather than a γ-lactam is indicated by the $n \rightarrow \pi^*$ absorption at 318 mμ (ϵ 64) in the ultraviolet[5] and by the single Cotton effect (extrema at 339 mμ and 292 mμ) above 250 mμ in its optical rotatory dispersion spectrum. Further evidence for the α-aminoketone grouping is presented below. The ketone incorporated three deuterium atoms on acid-catalyzed deuterium exchange. Since lycocernuine did not incorporate deuterium (other than OH to OD) under these conditions, it may be concluded that there are three hydrogens alpha to the ketonic carbonyl. Wolff–Kishner reduction of dehydrolycocernuine gave cernuine in a good yield, showing that lycocernuine is simply a hydroxycernuine.

The n.m.r. spectra of cernuine and lycocernuine are very similar, except for the peaks associated with the hydroxyl group in lycocernuine. Both compounds show signals for a secondary *C*-methyl group at about τ 9.14 (splitting 6.5 c.p.s.) and both show a one-proton quartet at τ 4.54. This signal appears at τ 4.13 in the spectrum of cernuine methiodide and at τ 6.40 in the spectrum of dihydrodeoxycernuine, and may thus be assigned to a proton on a carbon which is bonded to both nitrogen atoms in the bases. The evidence presented thus far may be summarized in terms of partial structure A, where either X or Y, but not both, is a hydrogen.



A

Reaction of lycocernuine with methanesulfonyl chloride in pyridine followed by treatment of the resulting crude mesylate with hot alcoholic sodium hydroxide gave anhydrolycocernuine, $C_{16}H_{24}ON_2$. The n.m.r. spectrum revealed the presence of a single olefinic proton giving rise to a multiplet at τ 5.22. The high-field position of the olefinic proton suggested an enamine β-proton (5). The presence in the infrared spectrum of a medium-intensity band at 1 650 cm^{-1}, in addition to the lactam carbonyl at 1 630 cm^{-1}, is also indicative of the enamine system (5). The anhydro compound was very sensitive to acid, and attempts to prepare the immonium salt were unsuccessful. Further proof of the location of the double bond in anhydrolycocernuine is described below. This result

[4]In our preliminary communication we reported that the epimeric alcohol was the major product of the reduction. This report is in error. Lycocernuine exists in dimorphic forms of melting point 212–213° and 230–231°. The lower melting form was obtained for the first time from the borohydride reduction. The two forms give different infrared spectra in Nujol mull, but show identical solution spectra. The epimeric alcohol melts at 174–176° and exhibits different spectral properties and different chromatographic behavior.

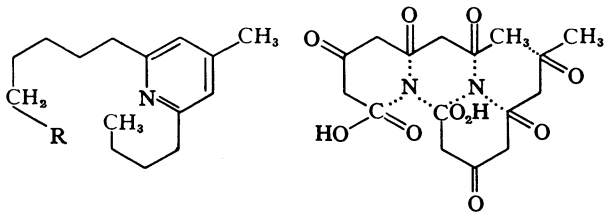
[5]Tropin-2-one, for example, shows λ_{max} 314 mμ (ϵ 68) (4).

allows us to define, at least tentatively, the nature of X (hydrogen) and Y (carbon) in partial structure A. This partial structure clearly indicates that these alkaloids differ from the known types (2, 6) of Lycopodium alkaloids.

To gain further insight into the overall carbon–nitrogen framework present in the bases, we subjected cernuine to vigorous catalytic dehydrogenation. The mixture of basic products obtained was separated by preparative t.l.c., and the major component, of which we obtained about 9 mg, was identified as 2-*n*-butyl-4-methyl-6-pentylpyridine (I) in the following manner. The mass spectrum indicated a molecular weight of 219 (consistent with the molecular formula $C_{15}H_{25}N$), the ultraviolet spectrum in both a neutral and an acidic medium was very similar to that of 2,4,6-collidine, and the n.m.r. spectrum showed the presence of only two hydrogens on the pyridine ring, which from their chemical shifts (τ 3.19) must both be in β positions (7). The presence of a methyl group in the 4 position was indicated by a three-proton signal at τ 7.69 which appeared as a triplet (J = 0.5 c.p.s.) and which collapsed to a singlet when the signal at τ 3.19 was simultaneously irradiated. A four-proton signal centered at τ 7.22 suggested the presence of two "benzylic" methylene groups, and a complex six-proton signal at τ 9.0–9.15 indicated that there were two methyl groups on saturated carbons. These data indicate that the alkyl substituents in the 2 and 6 positions are *either* *n*-butyl and *n*-pentyl *or* *n*-propyl and *n*-hexyl. An examination of the fragmentation pattern observed in the mass spectrum indicated that the former is correct, since the base peak occurred at *m/e* 163 (loss of butene from a pentyl side chain (8)) and the second most intense peak occurred at *m/e* 177 (loss of propene from a butyl side chain). An authentic sample of 2-*n*-butyl-4-methyl-6-*n*-pentylpyridine (I), prepared by alkylation of 2-*n*-butyl-4-methylpyridine (9) with *n*-pentyllithium, was identical with the dehydrogenation product.

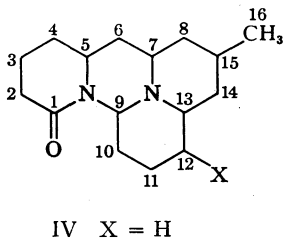
The pyridine I accounts for 15 of the 16 carbon atoms of cernuine. Since we felt that possibly the carbon atom lost during the dehydrogenation was the lactam carbonyl carbon, we subjected dihydrodeoxycernuine to dehydrogenation and obtained a homologue (II) of the pyridine I. The ultraviolet spectrum of II was almost identical with that of I, but the mass spectrum showed a parent peak at *m/e* 233 ($C_{16}H_{27}N$). The base peak, however, appeared again at *m/e* 163 (loss of a pentene from a hexyl side chain), whereas the peak associated with loss of the other side chain had shifted to *m/e* 191. Since insufficient material was available for an n.m.r. spectrum, it was not possible to show conclusively that the hexyl chain is unbranched. However, since the peak at M-15 in the mass spectrum of the C_{16} dehydrogenation product is no more intense than the M-15 peak in the C_{15} compound, and since the spectrum of the C_{16} compound shows an appreciable peak at M-57 (corresponding to loss of a butyl radical) which is not present in the spectrum of I, we feel that II correctly represents the C_{16} dehydrogenation product. A second product of this dehydrogenation has been identified tentatively as 2-(α-pyridylmethyl)-4-methylquinoline (see Experimental).

Provided that the dehydrogenation products I and II arise *without rearrangement* of the carbon skeleton, it is possible, on the basis of the above results, to arrive at a reasonable working hypothesis for the



I R = H
II R = CH_3

III



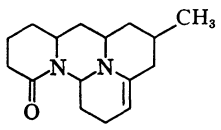
IV X = H
V X = OH
VI Carbonyl at C-12

structures of these alkaloids. A plausible biogenetic scheme for the Lycopodium alkaloids, involving the condensation of two 3,5,7-triketooctanoic acid equivalents (III), has been suggested by Conroy (10). If we assume that the C-4 methyl of the pyridines I and II represents the *C*-methyl group of cernuine and that this group is derived from the terminal methyl of one of the polyoctanoic acid chains, then aldol condensation of the C-7 carbonyl of this chain with the methyl group of the other chain and condensation with 2 equivalents of ammonia (dashed lines in III) followed by adjustment of the oxidation level lead to structures for cernuine (IV)[6] and lycocernuine (V) which account for the formation of pyridines I and II and which incorporate the features of partial structure A. On the basis of structures IV and V, dehydrolycocernuine is VI, anhydrolycocernuine is VII, dihydrodeoxycernuine is VIII, and dihydrodeoxylycocernuine is IX.

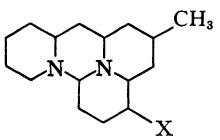
Further degradations have served to confirm structures IV and V. Platinum-catalyzed reduction of anhydrolycocernuine (VII) in ethyl acetate yielded a small amount of cernuine, but the major product was a compound isomeric with cernuine, which we have called allocernuine. Allocernuine showed no NH absorption in the infrared, but showed absorption for a lactam carbonyl at 1 625 cm^{-1}. The mass spectrum of allocernuine was similar to that of cernuine, supporting the view that the two are stereoisomers. When allocernuine was subjected to catalytic reduction in methanol, it took up 1 mole of hydrogen to give dihydroallocernuine (X). Catalytic hydrogenation of anhydrolycocernuine (VII) in methanol gave X directly. Dihydroallocernuine shows both lactam and NH absorption in the infrared, and was recovered unchanged from an attempted acetylation. Lithium aluminium hydride reduction of X yielded the dihydrodeoxy compound XI, which, on Eschweiler–Clarke methylation, gave the *N*-methyl derivative XII. The mass spectra of these substances (see Experimental), which differ markedly from those of the tetracyclic compounds, are particularly informative and are summarized in Scheme 1. Each shows a weak molecular ion at the expected mass and a base peak at *m/e* 152 (ion *a*) as well as a strong peak at *m/e* 110 (ion *b*). In addition, compound XI shows an intense peak at *m/e* 84 (ion *c*, R = R′ = R″ = H), and compound XII an intense peak at *m/e* 98 (ion *c*; R = CH_3, R′ = R″ = H). The peak at *m/e* 98 (ion *c*; R = H, R′,R″ = carbonyl) in dihydroallocernuine is not particularly strong, but this presumably reflects the decreased tendency of the amido nitrogen to bear the positive charge. The facile loss of the elements of propene from ion *a* to yield ion *b* (this fragmentation path is supported in all three cases by the appearance of a metastable peak at *m/e* 80.1; calculated for 152 → 110, 79.6) provides evidence in support of the positioning of the *C*-methyl group relative to the methylene group linking the two heterocyclic ring systems. It does not, of course, distinguish between C-14 and C-15 for the location of the methyl group.

The facile hydrogenolysis of allocernuine in methanol solution can be rationalized (11) in terms of the hexahydropyrimidine system present in the base. In the polar solvent, the zwitterion XIII, or its protonated form, must be formed to a certain extent and undergo reduction. In ethyl acetate, zwitterion XIII is not present to any appreciable extent. In agreement with

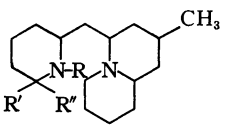
[6]The numbering system employed is an adaptation of the scheme suggested by Wiesner (6) for the Lycopodium alkaloids.



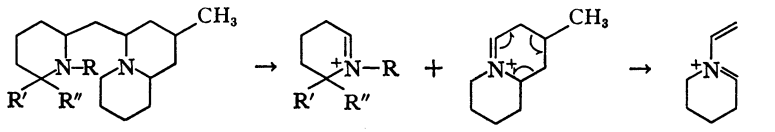
VII



VIII X = H
IX X = OH



X R = H; R′,R″ = carbonyl
XI R = R′ = R″ = H
XII R = CH_3, R′ = R″ = H



c *a* (*m/e* 152) *b* (*m/e* 110)

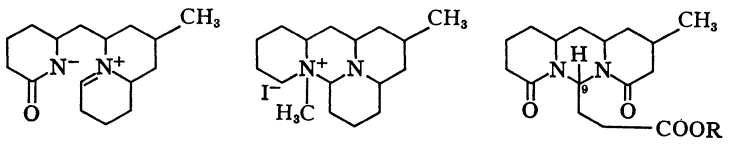
SCHEME 1.

this, sodium borohydride in methanol reduced allocernuine to dihydroallocernuine (X), but lithium aluminium hydride in ether gave a compound (VIII*a*) stereoisomeric with dihydrodeoxycernuine (VIII). Cernuine, however, is not reduced either by sodium borohydride in methanol or by catalytic hydrogenation in polar solvents, so that stereochemical factors must play an important role in the cleavage. To bring about the cleavage in the cernuine series, it was necessary to prepare the monomethiodide XIV from dihydrodeoxycernuine (VIII). This was achieved by treating VIII with a limited amount of methyl iodide and then separating the resulting mixture of methiodides by chromatography and fractional crystallization. When XIV was treated with sodium borohydride in methanol, reduction occurred and a compound isomeric with the *N*-methyl compound XII resulted. The infrared spectrum of this compound differed from that of XII, but the mass spectrum was almost identical, indicating that this cleavage product is stereoisomeric (presumably at C-13) with XII.

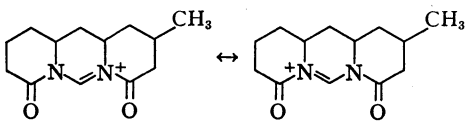
Further evidence for the position of the double bond in anhydrolycocernuine (VII) and for the position of the carbonyl group in dehydrolycocernuine (VI) was obtained in the following manner. Ozonolysis or permanganate–periodate oxidation (12) of VII gave, in a low yield, an acidic product XV which was characterized in the form of its methyl ester XVI. This same product was obtained in a much better yield by aerial oxidation[7] of dehydrolycocernuine (VI) under alkaline conditions.

In agreement with the assigned structure, compound XVI is non-basic and displays lactam and ester absorption in the infrared. The n.m.r. spectrum is also consistent with the assigned structure. In particular, a one-proton triplet at τ 2.68 may be assigned to the proton at C-9 which is coupled to the methylene group at C-10. The mass spectrum, which has a small peak for the molecular ion at *m/e* 322, shows a very intense peak at *m/e* 235 corresponding to the loss of the propionate side chain with formation of the resonance-stabilized ion *d*. The formation of XVI from both dehydrolycocernuine (VI) and anhydrolycocernuine (VII) locates the position of the keto group and the double bond in these substances, and thus the hydroxyl group in lycocernuine.

[7]Many α-aminoketones have been reported to be unstable to air (13, 14).

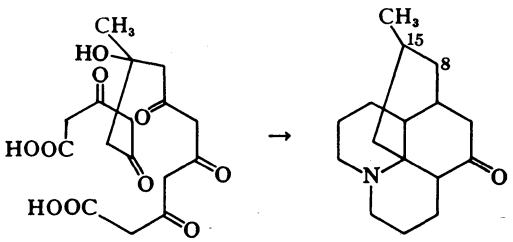


XIII XIV XV R = H XVI R = CH_3



d

To place the structures derived above on a more secure basis, we have synthesized the *dl* forms of compounds VIII*a* and XI. The synthesis of these compounds, as well as the derivation of the relative and absolute stereochemistry of the compounds discussed herein, is described in parts II and III of this series (15, 16). The mass spectra of cernuine and lycocernuine and the tetracyclic derivatives described here, along with various deuterated analogues, will be discussed in a separate communication.



XVIII XVII

The structures of the *L. cernuum* alkaloids differ markedly from those of the other known Lycopodium alkaloids, although, as mentioned previously, it is possible that all of the alkaloids have common biogenetic precursors. It is interesting to note that, if indeed these alkaloids do arise from the condensation of two polyketooctanoic acid chains (10), only one carbon–carbon bond (C-8 to C-15) is formed between these two chains in the biosynthesis of cernuine and lycocernuine. If, as suggested by Wiesner (6), lycopodine (XVII), which contains a C-8 to C-15 bond, is the central intermediate in the biosynthesis of the other Lycopodium alkaloids, the formation of cernuine and lycocernuine in *L. cernuum* perhaps indicates that in most cases the first carbon–carbon bond formed between the two C_8 precursors is the C-8 to C-15 bond to give an intermediate such as XVIII. *L. cernuum* is the first species of *Lycopodium* examined which does not appear to contain alkaloids closely related to lycopodine (XVII).

EXPERIMENTAL

Infrared spectra were measured either on a Perkin–Elmer model 441 or model 337 spectrophotometer, and ultraviolet spectra on a Cary spectrophotometer, model 14. Nuclear magnetic resonance spectra were measured in deuteriochloroform either on a Varian Associates model A-60 or model HR-100 spectrometer. The spectra determined at 60 Mc.p.s. are so indicated. Internal tetramethylsilane was used as a standard. The low-resolution mass spectra were determined on an AEI MS2-H mass spectrometer, and the high-resolution mass spectra were determined on an AEI MS-9 mass spectrometer. Unless pertinent to the discussion or otherwise specified, only those peaks of *m/e* greater than 100 and with an intensity of at least 5% of the most intense peak are recorded. Thin-layer chromatograms, unless otherwise specified, were carried out on microslides prepared from alumina G (Research Specialties Co., Richmond, California), and were developed in an iodine chamber or with Dragendorff's reagent. Skellysolve B refers to Skelly Oil Company light petroleum, b.p. 62–70°. Melting points were determined on a hot stage and are uncorrected. Microanalyses were performed by C. Daesslé, Montreal, and by F. Pascher, Bonn, Germany, who also carried out the pK_a determinations.

Isolation of the Alkaloids

Finely ground *L. cernuum* (11 kg, collected in Venezuela) was stirred at room temperature with methanol (ca. 10 l) for 24 h and filtered, and the methanolic extract was concentrated at reduced pressure. This process was repeated twice and the combined extracts were warmed with 3% aqueous tartaric acid (ca. 3 l). The tartaric acid insoluble portion was removed by filtration and then extracted again with tartaric acid (ca. 2 l). The combined filtrates were extracted with ether, made strongly basic with ammonium hydroxide, and extracted with chloroform. Evaporation of the chloroform and crystallization of the crude bases (12.0 g) from acetone gave *lycocernuine* (2.07 g), which, after recrystallization from acetone, melted at 230–231°, $[\alpha]_D$ −24.5° (*c*, 0.1 in methanol).

Anal. Calcd. for $C_{16}H_{26}O_2N_2$: C, 69.03; H, 9.41; N, 10.06. Found: C, 69.09; H, 9.32; N, 9.80.

Infrared spectrum: $\nu_{max}^{CHCl_3}$ 3 615, 1 620, and 1 410 cm^{-1}. Nuclear magnetic resonance spectrum (60 Mc.p.s.): τ 4.54 (1H, poorly resolved quartet, large splitting about 11 c.p.s.), 6.2 (1H, broad singlet), and 9.13 (3H, doublet, splitting 6 c.p.s.). Mass spectrum: *m/e* 278 (19, molecular ion), 277 (5), 250 (8), 249 (23), 233 (10), 221 (16), 220 (58), 219 (100), 205 (8), 191 (5), 166 (20), 165 (37), 152 (29), 151 (6), 138 (8), 124 (7), 122 (6), 110 (15), and 108 (5). The methiodide was prepared in methanol and recrystallized from acetone containing a few drops of methanol, m.p. 270–272°.

Anal. Calcd. for $C_{16}H_{26}O_2N_2 \cdot CH_3I \cdot \frac{1}{2}H_2O$: C, 47.55; H, 7.04. Found: C, 47.59, 47.89; H, 7.25, 7.54.

The lycocernuine was identical (infrared spectrum, mixture melting point) with an authentic sample of alkaloid L. 33 (1).

The mother liquors from the crystallization were dissolved in benzene and subjected to chromatography over basic alumina (200 g, activity III). Elution with benzene and benzene–ether (1:1) gave 0.2 g

of a mixture of bases (see below). Elution with ether gave *cernuine* (1.1 g), and elution with chloroform gave first a mixture of cernuine and lycocernuine (0.3 g) and then lycocernuine (0.7 g). Elution with chloroform–methanol (50:1 to 5:1) gave a mixture of bases (2.1 g) which has as yet not been resolved into pure components.

Cernuine crystallized with difficulty from Skellysolve B and was most conveniently purified by sublimation at 100° and 0.05 mm, m.p. 103–104°, $[\alpha]_D$ −20.5° (*c*, 0.1 in methanol).

Anal. Calcd. for $C_{16}H_{26}ON_2$: C, 73.24; H, 9.99. Found: C, 73.04, 73.19; H, 9.95, 9.88.

Infrared spectrum: $\nu_{max}^{CCl_4}$ 1 640 and 1 415 cm^{-1}. Nuclear magnetic resonance spectrum: τ 4.53 (quartet, splittings 11 and 2.5 c.p.s.), 6.3–7.1 (3H, complex series of peaks), 7.55–7.80 (2H, multiplet), and 9.14 (3H, doublet, splitting 6 c.p.s.). Mass spectrum (only the 14 most intense peaks are listed): *m/e* 262 (49, molecular ion), 261 (15), 247 (15), 234 (26), 233 (100), 221 (16), 220 (91), 219 (57), 205 (28), 191 (24), 178 (15), 164 (20), 151 (21), and 150 (20). The methiodide was prepared in methanol and, after recrystallization from methanol–acetone, melted at 237–238°.

Anal. Calcd. for $C_{16}H_{26}ON_2 \cdot CH_3I$: C, 50.50; H, 7.23; N, 6.93. Found: C, 50.27, 50.15; H, 7.56, 7.48; N, 6.55.

The material eluted with benzene and benzene–ether was dissolved in pentane and chromatographed over alumina (11 g, activity I–II). After elution with pentane, benzene, and then ether, elution with ether–dichloromethane (1:1) yielded a fraction (14 mg) which solidified when allowed to stand. This material proved to be identical with *dihydrodeoxycernuine* (below), as evidenced by its infrared spectrum, mass spectrum, and t.l.c. behavior.

Further supplies of crude alkaloids from plant material collected in Mexico were obtained from Smith, Kline and French Laboratories, Philadelphia, and gave similar proportions of cernuine and lycocernuine.

Dihydrodeoxycernuine

Cernuine (54 mg) was added to a slurry of lithium aluminium hydride (0.11 g) in anhydrous ether (20 ml), and the resulting mixture was heated under reflux for 20 h and then worked up by the method of Mićović and Mahailović (17). The product, which showed a single spot on t.l.c., melted at 64–65° after molecular distillation.

Anal. Calcd. for $C_{16}H_{28}H_2$: C, 77.36; H, 11.38; N, 11.28; mol. wt. 248. Found: C, 76.95, 77.49; H, 11.18, 11.01; N, 11.09, 11.21; mol. wt. 248 (mass spectrometry).

Nuclear magnetic resonance spectrum: τ 6.40 (1H, quartet, J = 11 and 2.5 c.p.s.), 6.73–7.85 (5H, complex series of peaks), and 9.15 (3H, doublet, splitting 5.5 c.p.s.).

Dihydrodeoxylycocernuine

Lycocernuine (40 mg) was reduced with lithium aluminium hydride as described for cernuine. Dihydrodeoxylycocernuine, after recrystallization from acetone, melted at 193–194°. The compound was analyzed by high-resolution mass spectrometery.

Anal. Calcd. for $C_{16}H_{28}ON_2$: mol. wt. 264.2202. Found: mol. wt. 264.2207.

Infrared spectrum: ν_{max}^{Nujol} 3 160 cm^{-1} (broad). Nuclear magnetic resonance spectrum: τ 6.20 (1H, poorly resolved multiplet), 6.40 (1H, quartet, J = 11.5 and 2.5 c.p.s.), and 9.17 (3H, doublet, splitting 6.5 c.p.s.).

Deuterium Exchange with Lycocernuine

Lycocernuine (20 mg) was added to a solution of sodium (40 mg) in methanol-*O*-*d* (2 ml). The resulting solution was left at room temperature for 24 h and then was refluxed for 8 h. The solvent was evaporated and the residue distributed between deuterium oxide and chloroform. Evaporation of the chloroform and crystallization of the residue from wet acetone yielded deuterated lycocernuine, the mass spectrum of which indicated the presence of 75% dideuterated and 21% monodeuterated lycocernuine. Infrared spectrum: ν_{max}^{Nujol} 3 200 and 1 635 cm^{-1}. The band appearing at 1 410 cm^{-1} in the spectrum of lycocernuine is not present in the spectrum of the deuterated compound.

O-Acetyllycocernuine

Lycocernuine (28 mg) was dissolved in pyridine–acetic anhydride (3 ml, 1:1) and left overnight at room temperature. The solvents were removed *in vacuo* and the residue was filtered through a short column of alumina. *O*-Acetyllycocernuine could not be induced to crystallize and was purified by molecular distillation at 180° and 0.1 mm.

Anal. Calcd. for $C_{18}H_{28}O_3N_2$: mol. wt. 320. Found: mol. wt. 320 (mass spectrometry).

Infrared spectrum: $\nu_{max}^{CHCl_3}$ 1 730, 1 640, 1 410, and 1 230 cm^{-1}. Nuclear magnetic resonance spectrum: τ 4.45 (1H, quartet, splitting 11.5 and 3 c.p.s.), 5.06 (1H, poorly resolved triplet), 7.91 (3H, singlet), and 9.14 (3H, doublet, splitting 6.0 c.p.s.).

Dehydrolycocernuine

A solution of lycocernuine (84 mg) in acetone (25 ml) was cooled in an ice bath, and Jones' reagent (18) (0.08 ml) was added. After 20 min most of the solvent was removed at the pump, water (5 ml) added, and the resulting solution extracted with chloroform (6 × 5 ml). Evaporation of the chloroform yielded dehydrolycocernuine (48 mg). Basification of the aqueous solution and extraction with chloroform yielded 30 mg of a mixture of lycocernuine and dehydrolycocernuine (t.l.c.) which, on further oxidation, gave 20 mg of the ketone. Dehydrolycocernuine, purified by sublimation at 120° and 0.1 mm, melted at 161–164°.

Anal. Calcd. for $C_{16}H_{24}O_2N_2$: C, 69.53; H, 8.75; mol. wt. 276. Found: C, 69.74; H, 8.87; mol. wt. 276 (mass spectrometry).

Infrared spectrum: ν_{max}^{Nujol} 1 705, 1 635, and 1 410 cm^{-1}. Nuclear magnetic resonance spectrum: τ 3.91 (1H, poorly resolved quartet) and 9.10 (3H, doublet, splitting 6 c.p.s.). Rotatory dispersion in methanol

(c, 0.23): $[\alpha]_{589}$ +10 ± 5°, $[\alpha]_{400}$ +45°, $[\alpha]_{339}$ +3 140°, $[\alpha]_{292}$ −3 840°, and $[\alpha]_{275}$ −3 600°.

Sodium Borohydride Reduction of Dehydrolycocernuine

Sodium borohydride (45 mg) and sodium carbonate (100 mg) were added to a solution of dehydrolycocernuine (45 mg) in ethanol (10 ml), and the resulting mixture was stirred overnight at room temperature; then most of the solvent was removed at the pump and water was added. Extraction with $CHCl_3$ yielded a mixture (45 mg) of two products (t.l.c.). Crystallization of the mixture from acetone–ether gave lycocernuine (20 mg), m.p. 212–213°, identical (infrared spectrum in chloroform) with an authentic sample. Chromatography of the material obtained from the mother liquors over alumina (2 g, activity III) gave more lycocernuine (10 mg), eluted with ether–chloroform (9:1), and then isolycocernuine (10 mg), m.p. 174–176°. The mass spectrum of isolycocernuine shows a molecular ion at m/e 278 and is very similar to that of lycocernuine. The infrared spectrum (Nujol mull) shows hydroxyl absorption at 3 460 cm^{-1} and lactam carbonyl absorption at 1 635 cm^{-1}.

Deuterium Exchange with Dehydrolycocernuine

Dehydrolycocernuine (15 mg) was dissolved in a solution (1 ml) of deuterium chloride in acetic acid-*O-d* (stock solution prepared by adding a 38% solution of deuterium chloride in deuterium oxide (10 g) dropwise to cold (0°) acetic anhydride (31.6 g)), and kept at room temperature for 10 h, after which the solvent was evaporated. Since analysis of the deuterium content of the ketone by mass spectrometry is complicated by exchange in the inlet system, the crude product was dissolved in methanol-*O-d*, sodium carbonate (25 mg) and sodium borohydride (25 mg) were added, and the mixture was left overnight at room temperature. The product was isolated in the usual manner and crystallized from acetone to yield deuterated lycocernuine, m.p. 230–231°. Mass spectrometry indicated the presence of 74% trideuterated and 21% dideuterated species, accompanied by small amounts of mono- and tetradeuterated material.

Wolff–Kishner Reduction of Dehydrolycocernuine

Since dehydrolycocernuine is sensitive to air, especially in an alkaline solution, it is essential to conduct this reaction in an inert atmosphere.

Hydrazine hydrate (0.5 ml) was added to a solution of dehydrolycocernuine (50 mg) in diethylene glycol (5 ml) under a nitrogen atmosphere. The solution was heated at 100–130° for 1 h, a pellet of sodium hydroxide was then added, and the temperature was raised to 180–190° for 3 h. The cooled solution was diluted with water and extracted several times with chloroform. The chloroform solution was washed with water and evaporated at the pump, and the residue, which contained traces of diethylene glycol, was dissolved in $CHCl_3$ and filtered through alumina. Evaporation of the chloroform yielded cernuine (30 mg), m.p. (after sublimation) 103–104°, identical (infrared spectrum, mixture melting point, t.l.c. behavior) with an authentic sample.

Anhydrolycocernuine

Lycocernuine (40 mg) in pyridine (3 ml) containing methanesulfonyl chloride (0.5 ml) was kept at 5° for 3 days. The cold solution was then diluted with dilute sodium hydroxide and extracted several times with ether. The crude product obtained by evaporation of the ether was heated under reflux with methanolic sodium methoxide (60 mg sodium in 3 ml methanol) for 3 h; then the solution was concentrated at the pump, water added, and the resulting solution extracted several times with ether. The product, anhydrolycocernuine (26 mg), showed a single spot on t.l.c. and, after sublimation (120° at 0.1 mm), melted at 140–142°.

Anal. Calcd. for $C_{16}H_{24}ON_2$: mol. wt. 260. Found: mol. wt. 260 (mass spectrometry).

Infrared spectrum: ν_{max}^{Nujol} no OH absorption, 1 655 and 1 635 cm^{-1}. Nuclear magnetic resonance spectrum: τ 4.41 (1H, quartet, splittings 11 and 2 c.p.s.), 5.27 (1H, broad singlet), 6.49 (1H, multiplet), and 9.03 (3H, doublet, splitting 6.3 c.p.s.).

Allocernuine

Anhydrolycocernuine (20 mg) in ethyl acetate (5 ml) was hydrogenated at atmospheric pressure in the presence of Adams' catalyst (10 mg). After 5 h the catalyst was removed and the solvent evaporated. The product showed two spots on t.l.c., a minor one corresponding in R_f to cernuine, and a major component of higher R_f value. The minor component was removed by chromatography over alumina and the major component, allocernuine, which was obtained as a colorless oil that could not be induced to crystallize, was purified by molecular distillation at 120° and 0.5 mm.

Anal. Calcd. for $C_{16}H_{26}ON_2$: mol. wt. 262.2045. Found: mol. wt. 262.2041.

Infrared spectrum: $\nu_{max}^{CCl_4}$ 1 625 and 1 415 cm^{-1}, fingerprint region distinctly different from that of cernuine. Nuclear magnetic resonance spectrum: τ 5.01 (1H, quartet, splittings 10 and 2.5 c.p.s.) and 9.05 (3H, doublet, splitting 5.5 c.p.s.). Mass spectrum (only the 15 most intense peaks are listed): m/e 262 (26), 261 (15), 247 (7), 234 (18), 233 (100), 220 (15), 219 (33), 205 (10), 192 (7), 191 (11), 165 (9), 164 (13), 151 (14), 150 (20), and 136 (9).

Dihydroallocernuine

(a) From Anhydrolycocernuine

A solution of anhydrolycocernuine (70 mg) in methanol (6 ml) was hydrogenated at atmospheric pressure for 24 h in the presence of 5% palladium on charcoal (70 mg). The catalyst was filtered off and washed several times with warm methanol, and the methanol was removed at the pump. The product (65 mg), which showed three spots on t.l.c., was chromatographed over alumina (2.5 g, activity III). Elution with benzene–ether (1:1) provided allocernuine (13 mg), elution with ether gave trace amounts of a compound whose R_f value was identical with that of cernuine, and elution with chloroform yielded dihydroallocernuine (40 mg), m.p. 99–100°.

Anal. Calcd. for $C_{16}H_{28}ON_2$: mol. wt. 264. Found: mol. wt. 264 (mass spectrometry).

Infrared spectrum: $\nu_{max}^{CHCl_3}$ 3 400 and 1 645 cm^{-1}. Nuclear magnetic resonance spectrum: τ 3.70 (1H, broad, amide NH), 6.3–6.7 (3–4H, complex multiplet), and 9.07 (3H, doublet, splitting 6 c.p.s.). Mass spectrum: *m/e* 264 (3), 166 (4), 153 (19), 152 (100), and 110 (16).

(b) From Allocernuine

Allocernuine (16 mg) was added to a solution of $NaBH_4$ (20 mg) in methanol (5 ml) containing potassium carbonate (20 mg). The mixture was left overnight; then, since t.l.c. analysis of an aliquot indicated the presence of some starting material, more $NaBH_4$ (20 mg) was added and the mixture was refluxed for 2 h. Work-up in the usual manner yielded a mixture (10 mg) of starting material (identified by t.l.c., minor component) and dihydroallocernuine (major component). The dihydroallocernuine (6 mg) was isolated in a pure form by chromatography of the mixture over alumina, and was shown to be identical with that prepared by method *a*. Catalytic reduction of allocernuine in methanol also gave dihydroallocernuine.

$LiAlH_4$ Reduction of Dihydroallocernuine

Dihydroallocernuine (30 mg) was allowed to react with $LiAlH_4$ (40 mg) in refluxing ether for 10 h and then worked up in the usual manner. The crude product (23 mg) was chromatographed over alumina. Elution with chloroform yielded XI as an oil (17 mg) which showed a single spot on t.l.c. The analytical sample of XI was prepared by molecular distillation at 120° and 0.1 mm.

Anal. Calcd. for $C_{16}H_{30}N_2$: mol. wt. 250.2409. Found: mol. wt. 250.2411.

The infrared spectrum showed very weak NH absorption at 3 300 cm^{-1} and no carbonyl absorption. Mass spectrum: *m/e* 250 (5), 205 (8), 166 (9), 153 (11), 152 (100), 124 (11), 110 (18), 97 (16), and 84 (25).

Preparation of Compound XII

Compound XI (above, 30 mg) was refluxed with formic acid – formaldehyde (1 ml, 1:1) for 12 h. The solution was then diluted with water, made basic with dilute sodium hydroxide, and extracted several times with ether. The product was an oil which showed a single spot on t.l.c. (R_f greater than that of XI), and was further purified by molecular distillation at 120° and 0.1 mm.

Anal. Calcd. for $C_{17}H_{32}N_2$: mol. wt. 264. Found: mol. wt. 264.

The infrared spectrum showed no NH or carbonyl absorption, but showed an N—CH_3 band at 2 790 cm^{-1}. Nuclear magnetic resonance spectrum: τ 7.70 (3H, singlet) and 9.10 (3H, doublet, splitting 4.5 c.p.s.). Mass spectrum: *m/e* 264 (9), 249 (5), 178 (8), 166 (10), 153 (11), 152 (100), 124 (6), 112 (6), 111 (11), 110 (18), and 98 (50).

$LiAlH_4$ Reduction of Allocernuine

Allocernuine (40 mg) was reduced with $LiAlH_4$ (40 mg) in refluxing ether for 10 h and then worked up in the usual manner to give the dihydrodeoxy compound VIII*a* (32 mg), m.p. 61–63° (after sublimation), $[\alpha]_D$ −43° (*c*, 0.25 in methanol).

Anal. Calcd. for $C_{16}H_{28}N_2$: mol. wt. 248.2253. Found: mol. wt. 248.2249.

Infrared spectrum: $\nu_{max}^{CCl_4}$ 2 790, 2 706, and 2 710 cm^{-1} (Bohlmann bands), no NH or carbonyl absorption. Nuclear magnetic resonance spectrum: τ 6.83 (1H, multiplet) and 9.15 (3H, splitting 5.5 c.p.s.).

Dehydrogenation of Cernuine

Cernuine (100 mg), 5% palladium on charcoal (300 mg), and freshly distilled tetralin (3 ml) were heated in a sealed tube at 300° for 7 days. The cooled reaction mixture was diluted with ether, the catalyst filtered off and washed well with ether and then a little methanol, and the filtrate extracted several times with dilute hydrochloric acid. The acid extract was made basic with aqueous ammonia and extracted with chloroform. The residue (0.05 g) remaining after removal of the chloroform showed two main spots on t.l.c. and was separated by preparative t.l.c. on alumina plates (0.75 mm layers, developed with benzene – Skellysolve B (3:2)). The more polar component (12 mg) was eluted from the absorbent with chloroform–methanol and subjected to pot-to-pot distillation to yield 2-*n*-butyl-4-methyl-6-*n*-pentylpyridine (9 mg), identical with an authentic sample, the preparation of which is described below. The less-polar component of the dehydrogenation (mol. wt. 231 (mass spectrometry), λ_{max}^{EtOH} 250–268 (broad shoulder) and 272 mμ, $\lambda_{max}^{EtOH,HCl}$ 273 and 296 mμ) has not been identified.

Synthesis of 2-n-Butyl-4-methyl-6-n-pentylpyridine

2-*n*-Butyl-4-methylpyridine (2 g, prepared according to ref. 9) in dry toluene (50 ml) was added dropwise, with stirring, to a solution of 2 equivalents of pentyllithium in ether (150 ml). The ether was distilled from the dark-red solution, the resulting solution kept at 110° for 9 h, the solution cooled in an ice bath, and water added cautiously, followed by dilute hydrochloric acid. The aqueous acidic layer was then separated, the organic layer was extracted again with dilute hydrochloric acid, and the combined aqueous extracts were made alkaline with aqueous ammonia and extracted with chloroform. Evaporation of the chloroform yielded a mixture (2.0 g) of starting material and product, which was separated by chromatography over basic alumina (75 g, activity III). Elution with benzene provided the desired product (1.1 g) and elution with ether gave starting material (0.7 g). Distillation of the benzene eluate yielded 2-*n*-butyl-4-methyl-6-*n*-pentylpyridine, b.p. 140–145° (bath temperature) at 1.5 mm, analyzed as the chloroplatinate (below). Infrared spectrum: ν_{max}^{film} 3 040, 2 960, 2 925, 2 860, 1 605, 1 570, 1 460, 1 380, and 855 cm^{-1}. Ultraviolet spectrum: λ_{max}^{EtOH} 264 (ϵ 4 000), 268 (ϵ 3 800), and 271.5 mμ (ϵ 3 500); $\lambda_{max}^{EtOH,HCl}$ 268 mμ (ϵ 9 000). Nuclear magnetic resonance spectrum: τ 3.19 (2H, slightly broadened singlet), 7.22 (4H), 7.69 (3H, triplet, J = 0.5 c.p.s.), 8.10–8.85 (10H, complex series of peaks), and 8.95–9.20 (6H, complex multiplet). Mass spectrum: *m/e* 219 (3), 218 (6), 204 (19), 191 (7), 190 (38), 178 (10), 177 (71), 176 (18), 164

(13), 163 (100), 148 (6), 147 (9), 146 (7), 135 (10), 134 (30), 133 (12), 121 (33), 120 (21), 107 (21), 91 (6), 79 (7), and 77 (11).

The *chloroplatinate*, prepared in the usual manner and recrystallized from ethanol containing a drop of hydrochloric acid, formed orange crystals, m.p. 189–190°.

Anal. Calcd. for $(C_{15}H_{25}N)_2 \cdot H_2PtCl_6$: C, 42.46; H, 6.18. Found: C, 42.35, 42.16; H, 6.19, 5.91.

Dehydrogenation of Dihydrodeoxycernuine

An intimate mixture of dihydrodeoxycernuine (0.10 g) and powdered selenium (0.30 g) was heated to 300° in a sealed tube for 24 h; then the contents of the tube were extracted in a Soxhlet apparatus with benzene. The benzene extract was shaken with several portions of dilute hydrochloric acid, and the combined aqueous extracts were made alkaline with aqueous ammonia and extracted several times with ether. The residue (45 mg) contained two major components (t.l.c.), which were separated by preparative t.l.c. (0.75 mm alumina layers, developed with benzene–ether (9:1)). The component of higher R_f value (6 mg) was identified as 2-*n*-butyl-4-methyl-6-*n*-hexylpyridine on the basis of its ultraviolet spectrum (λ_{max}^{EtOH} 264 (ϵ 4 100), 268 (ϵ 3 850), and 271.5 mμ (ϵ 3 550); $\lambda_{max}^{EtOH,HCl}$ 269 mμ (ϵ 9 500)) and mass spectrum (*m/e* 233 (3), 232 (5), 218 (15), 204 (25), 192 (6), 191 (45), 190 (26), 177 (9), 176 (24), 164 (13), 163 (100), 148 (12), 146 (8), 135 (15), 134 (50), 133 (14), 121 (34), 120 (16), 107 (7), 91 (5), 79 (6), and 77 (10)). The component of lower R_f value was not obtained in a pure form, but the ultraviolet spectrum (λ_{max}^{EtOH} 227, 262, 268 (sh), 303, and 316 mμ; $\lambda_{max}^{EtOH,HCl}$ 238, 266, 305 (sh), and 316 mμ) was very similar to that of an equimolar mixture of 2,4-dimethylquinoline and 2-picoline, and the compound is tentatively identified as 2-(α-pyridylmethyl)-4-methylquinoline. In agreement with this view, the most intense peaks in the mass spectrum, which did not show a peak corresponding to the molecular ion, appeared at *m/e* 156 (100) and 92 (70).

Preparation and Cleavage of Dihydrodeoxycernuine Monomethiodide

Dihydrodeoxycernuine (60 mg) was dissolved in ether (5 ml), and methyl iodide (0.5 ml) was added. A gummy solid formed on the walls of the flask after a few minutes. After 6 h the solvent was decanted. The residue crystallized on addition of acetone and was recrystallized twice from acetone containing a small amount of methanol. The monomethiodide thus obtained melted at 236–238°.

Anal. Calcd. for $C_{16}H_{28}N_2 \cdot CH_3I$: C, 52.30; H, 8.00. Found: C, 51.70; H, 7.75.

The monomethiodide (18 mg) was refluxed for 24 h in ethanol (5 ml) containing sodium borohydride (30 mg). The reaction was worked up in the usual manner and the product purified by preparative t.l.c. (alumina) followed by molecular distillation at 125° and 0.1 mm. The mass spectrum of the product (below) was very similar to that of compound XII, but the infrared spectra and t.l.c. behavior were different. The n.m.r. spectrum showed an *N*-methyl singlet at τ 7.80 and a *C*-methyl doublet at τ 9.11. Mass spectrum: *m/e* 264 (14), 192 (7), 178 (14), 166 (23), 153 (14), 152 (100), 138 (10), 136 (9), 124 (13), 112 (12), 111 (17), 110 (35), and 98 (87).

Aerial Oxidation of Dehydrolycocernuine

Dehydrolycocernuine (50 mg) was suspended in 10% aqueous sodium hydroxide (10 ml), and enough methanol was added to dissolve the ketone. A stream of air was bubbled through the stirred solution for 20 h, at which time it was diluted with water and extracted with chloroform to remove unreacted starting material. The aqueous layer was then acidified with sulfuric acid and extracted with chloroform to give the crude acid XV as a solid, which showed a single spot on t.l.c. (silica gel plate). The acid was converted into the methyl ester XVI with ethereal diazomethane and the ester crystallized from ether, m.p. 103–105°.

Anal. Calcd. for $C_{17}H_{26}O_4N_2$: mol. wt. 322. Found: mol. wt. 322.

Infrared spectrum: ν_{max}^{Nujol} 1 725, 1 635, and 1 410 cm^{-1}. Nuclear magnetic resonance spectrum: τ 2.68 (1H, triplet, splitting 7 c.p.s.), 6.35 (3H, singlet), and 9.02 (3H, doublet, splitting 5.5 c.p.s.). Mass spectrum: *m/e* 322 (less than 1% of base peak), 291 (6), 236 (15), 235 (100), and 207 (7).

The same acid was obtained by permanganate–periodate oxidation (12) and by ozonolysis of anhydrolycocernuine.

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REFERENCES

1. L. Marion and R. H. F. Manske. Can. J. Res., B, **26**, 1 (1948).
2. H. G. Boit. Ergebnisse der Alkaloid-Chemie bis 1960. Akademie-Verlag, Berlin. 1961. pp. 916 and 1011.
3. R. N. Jones and C. Sandorfy. Technique of organic chemistry. Vol. IX. Interscience Publishers, Inc., New York. 1956. p. 498.
4. R. C. Cookson. Private communication.
5. G. Stork, A. Brizzolara, H. Landesman, J. Szmuszkovicz, and R. Terrell. J. Am. Chem. Soc. **85**, 207 (1963).
6. K. Wiesner. Fortschr. Chem. Org. Naturstoffe, **20**, 271 (1962).
7. L. M. Jackman. Applications of NMR spec-

troscopy in organic chemistry. Pergamon Press, New York. 1959. p. 64.
8. K. BIEMANN. Mass spectrometry. McGraw-Hill Book Co., Inc., New York. 1962. p. 130.
9. H. GILMAN and H. S. BROADBENT. J. Am. Chem. Soc. **70**, 2809 (1948).
10. H. CONROY. Tetrahedron Letters, 34 (1960).
11. E. M. WILSON. Tetrahedron, **21**, 2561 (1965).
12. R. U. LEMIEUX and E. VON RUDLOFF. Can. J. Chem. **33**, 1701 (1955).
13. N. J. LEONARD, G. FULLER, and H. L. DRYDEN. J. Am. Chem. Soc. **75**, 3727 (1953).
14. C. P. RADER, G. E. WICKS, JR., R. L. YOUNG, JR., and H. S. AARON. J. Org. Chem. **29**, 2252 (1964).
15. W. A. AYER, J. K. JENKINS, K. PIERS, and S. VALVERDE-LOPEZ. Can. J. Chem. **45**, 445 (1967).
16. W. A. AYER and K. PIERS. Can. J. Chem. **45**, 451 (1967).
17. V. M. MIĆOVIĆ and M. L. MAHAILOVIĆ. J. Org. Chem. **18**, 1190 (1953).
18. R. G. CURTIS, I. HEILBRON, E. R. H. JONES, and G. F. WOODS. J. Chem. Soc. 457 (1953).