Tumor Inhibitors. XV.[1] The Structure and Configuration of Cissampareine, a Novel Bisbenzylisoquinoline Alkaloid[2,3]

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Abstract: Evidence is presented for assignment of structure and configuration (I) to cissampareine. Elemental analysis and molecular weight determination by mass spectrometry supported a $C_{37}H_{38}N_2O_6$ molecular formula for cissampareine. Functional group analysis and spectral evidence showed the presence of three O-methyl groups and one N-methyl group, a hindered phenolic hydroxyl group, and a 3,4-dihydroisoquinoline partial structure. Sodium in liquid ammonia reduction of O-methylcissampareine (II) yielded a levorotatory nonphenolic base (III) and a dextrorotatory phenolic base (IV). The nonphenolic base (III) was characterized by its nmr and ORD spectral characteristics, by oxidation to V, and by direct comparison with a racemic sample prepared by total synthesis. The phenolic base (IV) was characterized by methylation to VI, by its negative Gibbs test, and by comparison with a sample prepared by total synthesis. Sodium in liquid ammonia reduction of O-ethylcissampareine (XX) gave III, indicating that the phenolic hydroxyl group is located in the benzyl-3,4-dihydroisoquinoline moiety of cissampareine. Assignment to the C-8 position in the 3,4-dihydroisoquinoline part was made on the basis of solubility, infrared, and nmr spectral characteristics of cissampareine and its derivatives.

Cissampareine is a cytotoxic alkaloid from *Cissampelos pareira* L., and its isolation and preliminary characterization have recently been reported.[4] It is the purpose of this paper to present, in detail, the elucidation of structure and configuration of cissampareine (I). Cissampareine represents a novel type of bisbenzylisoquinoline alkaloid; it appears to be the first recognized to contain a substituted *p*-xylyl moiety. In addition, cissampareine appears to be the first bisbenzylisoquinoline of the symmetrical type recognized to contain a 3,4-dihydroisoquinoline moiety.[5]

The molecular formula $C_{37}H_{38}N_2O_6$ was assigned for cissampareine on the basis of elemental analysis and molecular weight determination by mass spectrometry.[6] Analysis showed the presence of three O-methyl groups and one N-methyl group. The nmr spectrum in deuterated chloroform supports the analytical data, showing nine O-methyl protons (τ 6.08, 6.15, and 6.25) and three N-methyl protons (τ 8.01).[7] The infrared spectrum indicates the presence of aromatic rings, aromatic O-methyl groups, a free hydroxyl group, and a double bond.

Two derivatives were prepared to seek confirmation of the empirical formula as well as to yield information useful in characterizing functional groups.[4] Although cissampareine was found to be very sparingly soluble in 5% sodium hydroxide solution, its infrared spectrum shows a band at 2.88 μ, indicative of the presence of a free phenolic hydroxyl group. Methylation with diazomethane yielded O-methylcissampareine (II), which shows no hydroxyl absorption in the infrared. Analysis afforded results which support a $C_{38}H_{40}N_2O_6$ formula, with four O-methyl groups. Reduction of cissampareine (λ_{max} 6.22 μ (s), C=N) with sodium borohydride afforded dihydrocissampareine, $C_{37}H_{40}N_2O_6$, which shows ultraviolet absorption characteristic of bisbenzylisoquinoline alkaloids of the isochondrodendrine series, and greatly diminished absorption at 6.22 μ.[4] The sensitivity of cissampareine toward reduction with sodium borohydride was regarded as indicative of the presence of a C=N double bond, possibly in a 3,4-dihydroisoquinoline environment.[8] Strong support for the presence of a 3,4-dihydroisoquinoline system in O-methylcissampareine (λ_{max}^{EtOH} 279 mμ (ϵ 13,500)) was adduced from the observed bathochromic shift upon protonation ($\lambda_{max}^{alc\ HCl}$ 248 mμ (sh) (ϵ 13,000) and 325 mμ (ϵ 13,800).[9]

Perhaps the most useful reaction in structural studies of bisbenzylisoquinoline alkaloids has been cleavage by the action of metallic sodium in liquid ammonia.[10] The latter reduction was carried out on O-methylcissampareine (II) to cleave the suspected diphenyl ether linkage and split the molecule into two smaller moieties, III and IV. The ultraviolet absorption spectra of III and IV indicate that each is a benzylisoquinoline derivative.

The nonphenolic product (III) afforded analytical results in agreement with the empirical formula, $C_{20}H_{25}NO_2$, with one N-methyl and two O-methyl functions. The nmr spectrum of III supports the presence of two methoxy groups (τ 6.16, 6.47) and one

(1) Part XIV in the series: S. M. Kupchan, R. W. Doskotch, P. Bollinger, A. T. McPhail, G. A. Sim, and J. A. Saenz-Renauld, *J. Am. Chem. Soc.*, 87, 5805 (1965).

(2) This investigation was supported in part by Public Health Service Research Grants HE-02952 and CA-04500, from the National Institutes of Health. J. H. B. was a National Institutes of Health Predoctoral Fellow, 1963-1966.

(3) This work was presented, in part, at the Symposium on Selected Recent Advances in Natural Products Chemistry, 149th National Meeting of the American Chemical Society, Detroit, Mich., April 1965.

(4) S. M. Kupchan, A. C. Patel, and E. Fujita, *J. Pharm. Sci.*, 54, 580 (1965).

(5) For comprehensive reviews, see H.-G. Boit, "Ergebnisse der Alkaloid-Chemie bis 1960," Academie-Verlag, Berlin, 1961; and M. Kulka in "The Alkaloids," R. H. F. Manske and H. L. Holmes, Ed., Academic Press Inc., New York, N. Y.: Vol. IV, 1954; Vol. VII, 1960.

(6) The authors thank Professor K. Biemann and Dr. B. C. Das, Massachusetts Institute of Technology, for the mass spectral data.

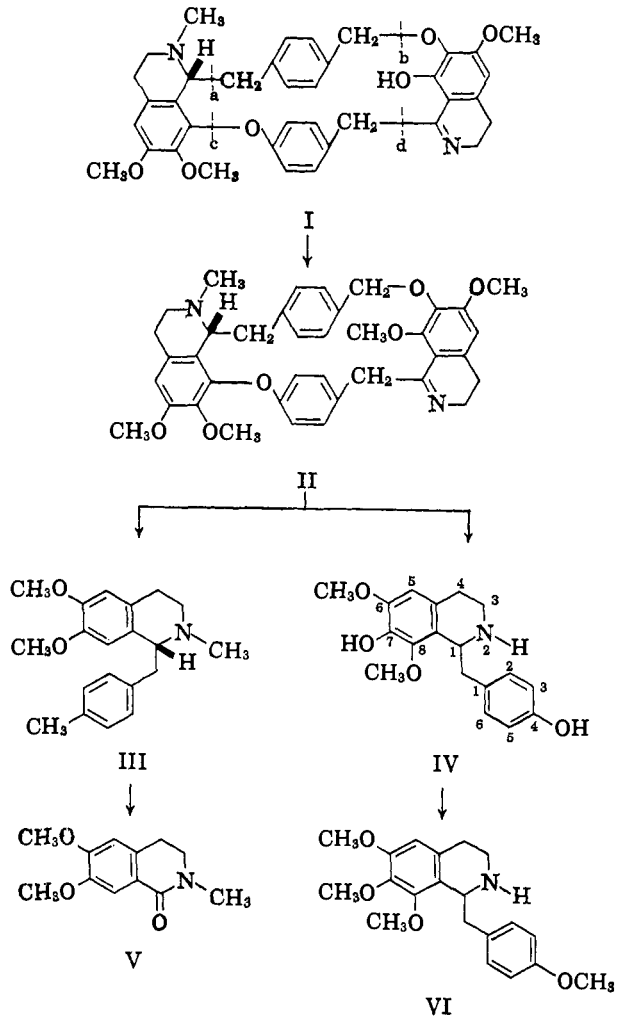
(7) The unusual high-field chemical shift of the N-methyl protons is noteworthy and is probably attributable to a large anisotropic effect of a neighboring aromatic ring; *cf.*, *e.g.*, the N-methyl signal of protopine, Spectrum No. 339 in "NMR Spectra Catalog," Vol. 1, Varian Associates, Palo Alto, Calif., 1962.

(8) *Cf.* Y. Kanaoka, E. Sato, O. Yonemitsu, and Y. Ban, *Tetrahedron Letters*, 2419 (1964).

(9) *Cf.* Y. Ban, O. Yonemitsu, and M. Terashima, *Chem. Pharm. Bull.* (Tokyo), 8, 194 (1960).

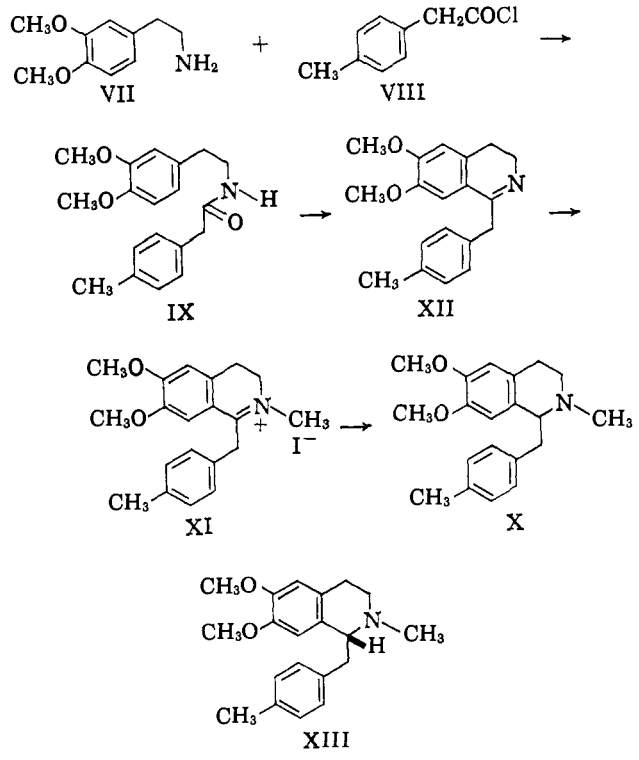
(10) M. Tomita, *Progr. Chem. Org. Nat. Prod.*, 9, 175 (1952).

N-methyl group (τ 7.47), and, in addition, an aryl C-methyl group (τ 7.69). Oxidation of III with 1% potassium permanganate yielded 1-oxo-2-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (V). The latter finding indicated that III possesses a 2-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline system, and



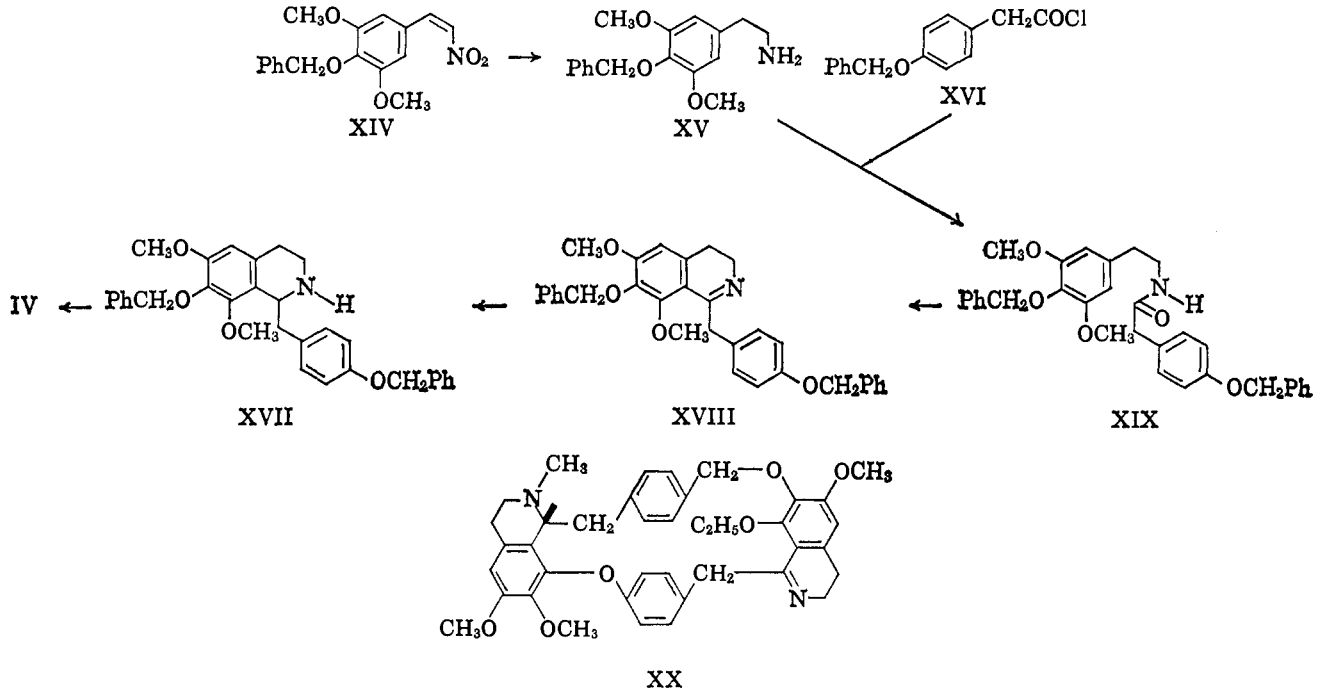
that the aryl C-methyl group consequently is located in the aromatic ring of the benzyl moiety. A tentative assignment of structure III to the nonphenolic product was made on the basis of the aryl proton region in the nmr, which shows four equivalent protons (τ 2.97, 4 H), one more shielded proton (τ 3.45), and one highly shielded proton (τ 4.00). The compound was positively identified as (−)-1-(4-methylbenzyl)-2-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (III) by direct comparison with a racemic sample prepared by total synthesis. Treatment of 2-(3,4-dimethoxyphenyl)-ethylamine (VII) with the acid chloride of *p*-tolylacetic acid (VIII) yielded N-(3,4-dimethoxyphenethyl)-2-(4-methylphenyl)acetamide (IX). Bischler–Napieralski ring closure afforded the 3,4-dihydroisoquinoline XII, and treatment with methyl iodide gave the methiodide XI. Sodium borohydride reduction of XI afforded (±)-1-(4-methylbenzyl)-2-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (X). Spectral and chromatographic comparison of X with III confirmed the structural identity (apart from configuration) of the respective samples. Tomita and Kunitomo have shown that levorotatory bases of coclaurine type (*e.g.*, (−)-O,O,N-trimethylcoclaurine, $[\alpha]_D$ −85.7°, $CHCl_3$) possess the D absolute configuration and may be represented as in XIII.[11] The levorotatory nonphenolic cissampareine cleavage product ($[\alpha]_D$ −96°, $CHCl_3$) shows an ORD curve of similar shape to those of D(−)-laudanidine and D(−)-armepavine[12] and is assigned the D configuration, as represented in III.

The phenolic cleavage product IV was assigned the empirical formula $C_{18}H_{21}NO_4$ on the basis of elemental analysis of the free base and its hydrochloride. Analysis showed also the presence of two methoxyl groups and absence of an N-methyl group. Methylation with diazomethane gave 1-(4-methoxybenzyl)-6,7,8-trimethoxy-1,2,3,4-tetrahydroisoquinoline (VI), characterized



by direct comparison of the amorphous base and its crystalline hydrochloride with samples synthesized by the procedure of Tomita and Okui.[13] Initial placement of the phenolic groups as in IV was based on the fact that the compound gave a negative Gibbs test. Extensive studies by Tomita[14] and Inouye[15] have shown that benzylisoquinolines bearing a free phenolic group at C-6 or C-8 give a characteristic color reaction with 3,5-dichlorobenzoquinonechloroimide, and a spectrophotometric modification of the procedure has been described.[16] The fact that the phenolic base gave a negative test (using the spectrophotometric procedure[16]) indicated the absence of phenolic groups from C-6 and C-8, and supported assignment of structure IV. The compound was positively identified as 1-(4-hydroxybenzyl)-7-hydroxy-6,8-dimethoxy-1,2,3,4-tetrahydroisoquinoline (IV) by direct comparison with

(11) M. Tomita and J. Kunitomo, *J. Pharm. Soc. Japan*, **82**, 734 (1962).
(12) J. C. Craig and S. K. Roy, *Tetrahedron*, **21**, 401 (1965).
(13) M. Tomita and K. Okui, *J. Pharm. Soc. Japan*, **76**, 632 (1956).
(14) M. Tomita and Y. Kondo, *ibid.*, 77, 1019 (1957).
(15) H. Inouye, Y. Kanaya, and Y. Murata, *Chem. Pharm. Bull.* (Tokyo), 7, 573 (1959).
(16) F. E. King, T. J. King, and L. C. Manning, *J. Chem. Soc.*, 563 (1957).



XX

a sample prepared by total synthesis. Reduction of 4-benzyloxy-3,5-dimethoxy-ω-nitrostyrene (XIV)[17] with lithium aluminum hydride yielded 2-(4-benzyloxy-3,5-dimethoxyphenyl)ethylamine (XV). Treatment of XV with *p*-benzyloxyphenylacetyl chloride (XVI)[18] gave N-(4-benzyloxy-3,5-dimethoxyphenethyl)-2-(4-benzyloxyphenyl)acetamide (XIX). The amide was cyclized with polyphosphate ester[8] to 1-(4-benzyloxybenzyl)-7-benzyloxy-6,8-dimethoxy-3,4-dihydroisoquinoline (XVIII), which was directly reduced with sodium borohydride to 1-(4-benzyloxybenzyl)-7-benzyloxy-6,8-dimethoxy-1,2,3,4-tetrahydroisoquinoline (XVII). Catalytic hydrogenolysis of XVII gave (±)-1-(4-hydroxybenzyl)-7-hydroxy-6,8-dimethoxy-1,2,3,4-tetrahydroisoquinoline, and direct comparison of the synthetic material with that (IV) derived from O-methylcissampareine confirmed the structural identity (apart from configuration) of the respective samples.

The nmr spectrum of cissampareine shows a singlet at τ 4.85 (2 H), attributable to ArCH_2–OR, and lacks the aryl C-methyl singlet of III. Furthermore, sodium–liquid ammonia cleavage of O-ethylcissampareine (XX) gave III, indicating that the phenolic hydroxyl group in cissampareine is located in the benzyl-3,4-dihydroisoquinoline moiety. These facts and the structures of cleavage products III and IV limited the possible structures for O-methylcissampareine to II, XXI, XXII, and XXIII. Hypothetical structures XXII and XXIII were deemed as unlikely because the nmr spectral characteristics of cissampareine and its alkyl ether derivatives far more closely resemble those of the symmetrical (*e.g.*, curine) bisbenzylisoquinoline alkaloids than those of the unsymmetrical (*e.g.*, berbamine) type.[19] Strong support for assignment of a symmetrical bisbenzylisoquinoline structure was obtained from the mass spectrum of cissampareine.[6] Major fragment ions appear at *m/e* 312 and 310, which correspond to the favored cleavage at a–d (see I) with hydrogen transfer.[20,21] This type of fragmentation has recently been shown to be characterisitic of alkaloids of the symmetrical bisbenzylisoquinoline type.[22] Further important fragments at *m/e* 502 and 500 can be explained by fission at a–b, and those at *m/e* 206 and 204 correspond to cleavage at a–c, again with hydrogen transfer. Hypothetical structure XXI may be discounted as a likely possibility on the basis of its unprecedented diaryl ether linkage type. The foregoing considerations led to consideration of II as the most probable structure for O-methylcissampareine.

The structural problem which remained at this point was the choice between C-6 and C-8 of the benzyl-3,4-dihydroisoquinoline moiety of cissampareine as the location of the free hydroxyl group. Assignment to the C-8 position (*cf.* I) was made on the basis of solubility, infrared spectral, and nmr spectral data. The limited solubility of cissampareine in 5% sodium hydroxide solution, noted earlier, is indicative that the phenolic group is located in a sterically hindered position. A molecular model indicates that the C-8 substituent is in an exceedingly hindered position, whereas the substituent at C-6 is relatively unhindered. Secondly, the sharp (unbonded) hydroxyl band (2.88 μ) in the infrared spectrum of cissampareine supports the view that the hydroxyl group is located at a hindered position. Thirdly, the nmr spectrum of O-methylcissampareine (Figure 1) shows but three three-proton signals, even though the integration curve shows a twelve-proton count for the methoxyl region. This observation is in good agreement with the view that the new methoxyl introduced during methylation of cis-

(17) K. Kratzl, T. Horejschi, and G. Billek, *Monatsh.*, **85**, 1154 (1954).

(18) M. Tomita, K. Nakaguchi, and S. Takagi, *J. Pharm. Soc. Japan*, 71, 1046 (1951).

(19) *Cf.* I. R. C. Bick, J. Harley-Mason, N. Sheppard, and M. J. Vernengo, *J. Chem. Soc.*, 1896 (1961).

(20) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. 1, Holden-Day, Inc., San Francisco, Calif., 1964, pp 173–175.

(21) *Cf.* A. R. Battersby, R. B. Herbert, and F. Santavy, *Chem. Commun.*, 415 (1965).

(22) M. Tomita, T. Kikuchi, K. Fujitani, H. Kato, H. Furukawa, Y. Aoyagi, M. Kitano, and T. Ibuka, *Tetrahedron Letters*, 857 (1966); D. C. DeJongh, S. R. Shrader, and M. P. Cava, *J. Am. Chem. Soc.*, **88**, 1052 (1966).

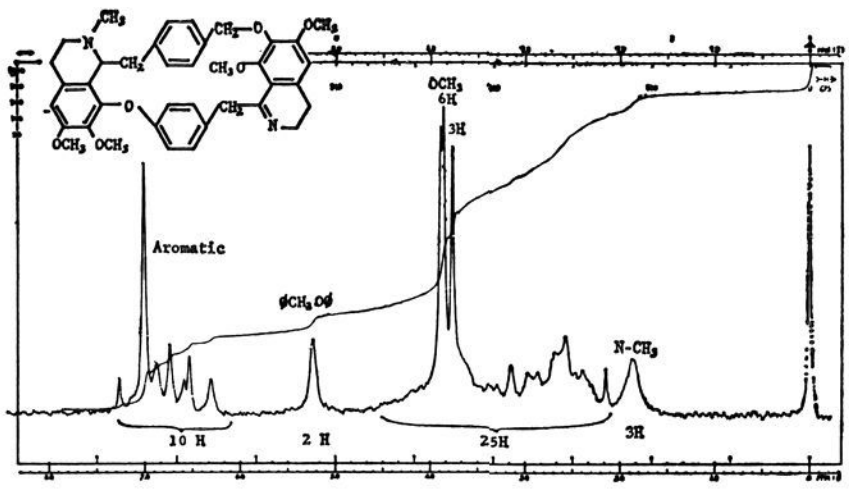


Figure 1. Nmr spectrum (60 Mc) of O-methylcissampareine (II).

sampareine is in a strongly hindered position, where the methyl group is not free to rotate (*cf.* Figure 2). Hence, each proton is in a different environment from the others, and the resultant signal for the methyl protons is a complex multiplet attributable to splitting of the peaks for the nonequivalent protons.[23] The nmr spectrum of O-ethylcissampareine (Figure 3) shows a triplet at both 60 and 100 Mc for the methyl protons of the ethoxy group, but the quartet expected for the methylene group is lacking. Irradiation of the triplet at τ 8.53 (J = 7 cps) changed the pattern in the τ 5.75 and 6.1 regions, indicating the nonequivalence of the methylene protons.[24] Irradiation of the proton "A" at τ 5.75 collapsed the triplet at τ 8.53 into a doublet (J = 7 cps). Irradiation within the methoxyl signals (τ 6.1–6.2) caused partial collapse of the triplet at τ 8.53. Although the exact location of the proton "B" signal was not found, it is presumed to be near τ 6.16, as shown by the extrapolated splitting diagram in Figure 4. Thus the ABX_3 splitting pattern shown by the spectrum of O-ethylcissampareine is attributable to the nonequivalence of the methylene protons of the ethyl group. It is noteworthy that the two protons of the benzyl ether also appear to be nonequivalent and an AB quartet (τ 4.67, J = 11 cps; τ 4.85, J = 11 cps) now appears in place of the singlet found in the spectra of O-methylcissampareine (Figure 1) and cissampareine. The latter fact supports the view that the methylene group is in a highly hindered position in XX. Finally, the aromatic region of the nmr spectrum of XX can be interpreted as follows: the benzene ring with the ether linkage shows an A_2B_2 quartet at τ 2.99 (J = 9 cps) and τ 3.32 (J = 9 cps), the symmetrical ring exhibits a large singlet at τ 2.99, and the two single aromatic protons on the isoquinoline rings show signals at τ 3.49 and 3.80.

The sole naturally occurring 3,4-dihydroisoquinolines described previously appear to be bisbenzylisoquinoline alkaloids of the unsymmetrical type: epistephanine and hypoepistephanine,[25a] thalmethine and O-methylthalmethine,[25b] and thalsimine.[25c] The sole

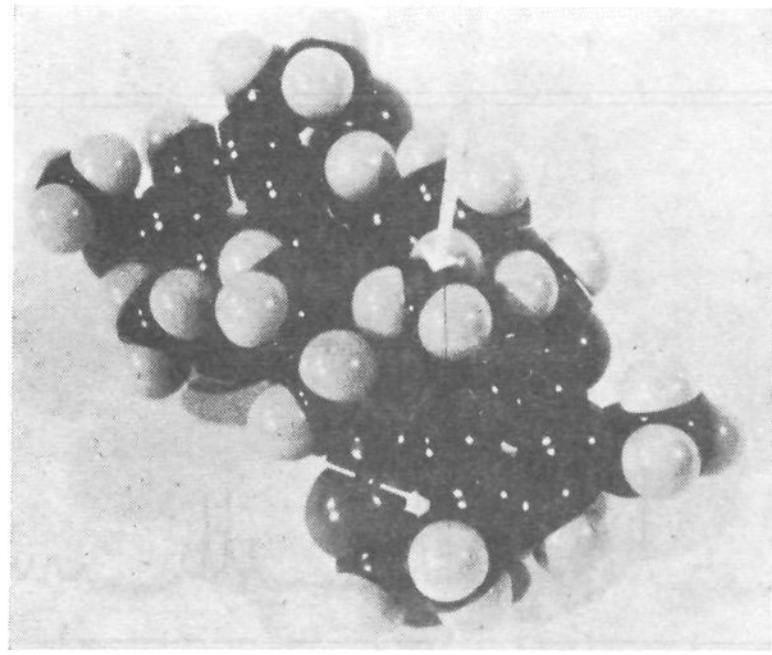
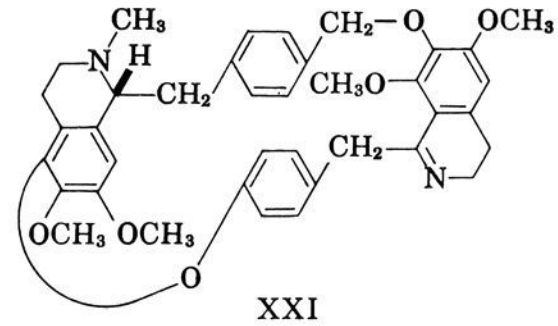
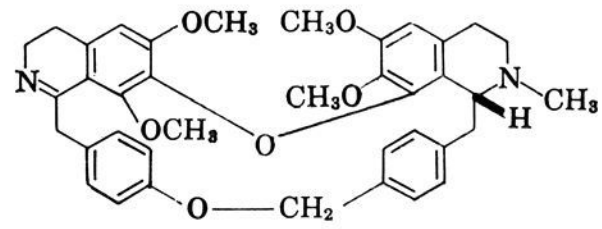


Figure 2. Catalin model of O-methylcissampareine (II), with arrow indicating methoxyl group introduced during methylation.

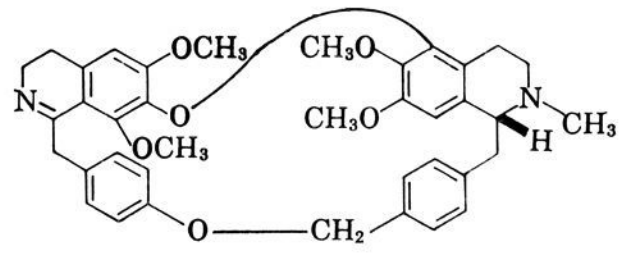
bisbenzylisoquinoline alkaloids recognized previously to contain xylyl residues are insulanoline (XXIV, R = H) and its methyl ether, insularine (XXIV, R = CH_3).[26] The xylyl substitution pattern is of the *meta* type in the latter alkaloids, rather than *para*, as in cissampareine.



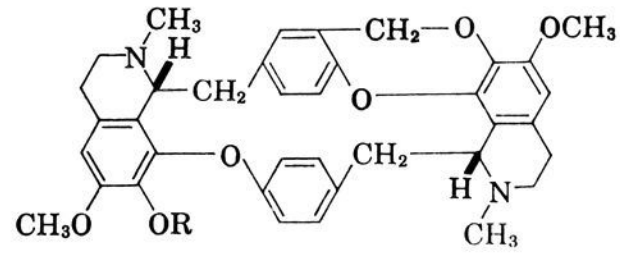
XXI



XXII



XXIII



XXIV

(23) *Cf.*, *e.g.*, the doublet for the dioxymethylene protons of bulbocapnine, Spectrum No. 333, ref 7.

(24) The authors thank Dr. R. Pitcher (Varian Associates) for the spin-decoupling and time-averaging measurements on the Varian HA-100 spectrometer, and Dr. R. Pitcher and Dr. T. Shingu (Kyoto University) for helpful discussions.

(25) (a) M. Tomita and J. Kunitomo, *J. Pharm. Soc. Japan*, **82**, 741 (1962); M. Tomita and E. Fujita, *Chem. Pharm. Bull.* (Tokyo), **2**, 378 (1954); H. Kondo and T. Nozoe, *J. Pharm. Soc. Japan*, **63**, 333 (1943); (b) N. M. Mollov, H. B. Dutschewska, and H. G. Kirjakov, *Chem. Ind.* (London), 1595 (1965); (c) M. Shamma, *et al.*, *Chem. Commun.*, 7 (1966).

(26) J. Kunitomo, *J. Pharm. Soc. Japan*, **82**, 1152 (1962); H. Kondo, M. Tomita, and S. Uyeo, *ibid.*, **62**, 534 (1942); M. Tomita and S. Uyeo, *J. Chem. Soc. Japan*, **64**, 64, 70, 77, 147 (1943); M. Tomita and T. Kikuchi, *J. Pharm. Soc. Japan*, 77, 997 (1957); T. Kikuchi and K. Bessho, *ibid.*, **78**, 1408, 1413 (1958).

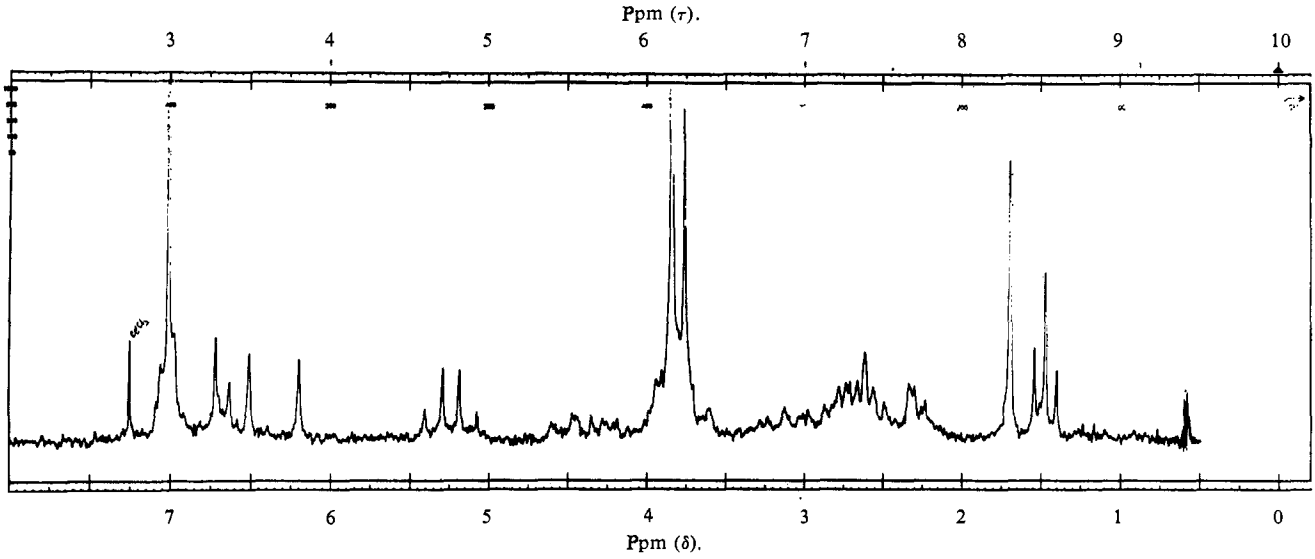


Figure 3. Nmr spectrum (100 Mc) of O-ethylcissampareine (XX).

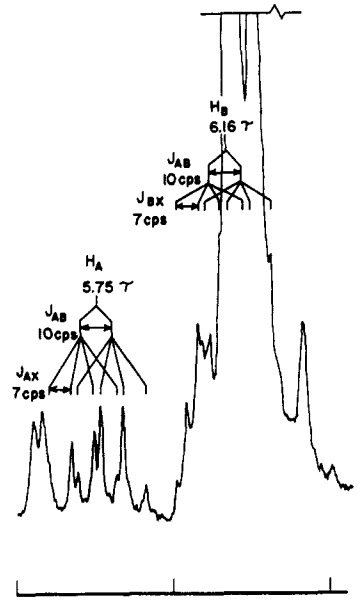


Figure 4. Nmr spectrum (100 Mc) of O-ethylcissampareine in the τ 5.5–6.5 region with increased signal amplitude.

No monobenzylisoquinoline alkaloid bearing an aryl methyl group appears to have been reported to date, and this fact lends support to the view that the biosynthesis of cissampareine does *not* involve a *p*-xylylisoquinoline precursor. It is suggested that the *p*-xylyl residue in cissampareine may arise by attack *para* to the benzyl methylene group by a methoxyl group at C-7 in the 3,4-dihydroisoquinoline moiety of a bisbenzylisoquinoline precursor of cissampareine. The recently reported photooxidative cyclization of an alkoxy group with an aromatic nucleus in quercetin methyl ether[27] may well constitute a model for the cyclization step in the biosynthesis of cissampareine.

Experimental Section

Melting points were determined on a Fisher-Johns melting point apparatus. Values of [α]D have been approximated to the nearest degree. Optical rotatory dispersion was determined with a Cary Model 60 spectropolarimeter in 95% ethanol using 1-cm cells at 25°. Ultraviolet spectra were determined on a Beckman Model DK-2A recording spectrophotometer. Infrared spectra were determined on a Beckman Model IR-5A infrared recording spectrophotometer. Nmr spectra were determined on a Varian A-60 spectrometer in deuteriochloroform solution with tetramethylsilane as the internal standard unless otherwise noted. Paper chromatography was conducted by the descending technique on Whatman No. 4 paper pretreated with buffer at pH 3.5. Microanalyses were carried out by Mr. J. Alicino, Metuchen, N. J., and Spang Microanalytical Laboratory, Ann Arbor, Mich.

Sodium–Liquid Ammonia Reduction of O-Methylcissampareine (II). A three-necked, 1-l. flask equipped with a mechanical stirrer, a dropping funnel with a nitrogen gas inlet, and a nitrogen gas outlet was placed in an acetone–Dry Ice bath (bath temperature, −50 to −55°). Dried ammonia gas was induced into the flask through the nitrogen gas inlet to a volume of 450 ml of liquid ammonia. A small amount of metallic sodium sufficient to color the solution blue was added. O-Methylcissampareine[4] (1.176 g) was dissolved in 20 ml of dried toluene and placed in the dropping funnel. The reaction was executed under a nitrogen atmosphere by adding small portions of the toluene solution and metallic sodium to the reaction vessel alternately so that the blue color of the reaction mixture was maintained. The reaction was stopped 30 min after all the toluene solution had been added to the mixture; the mixture maintained its blue color. About 1 g of metallic sodium had been consumed in the reaction. The reaction mixture was allowed to stand overnight in a hood to evaporate the solvent. The residual toluene solution was extracted with water (50 ml) and with 5% sodium hydroxide solution (two 50-ml portions), and the aqueous extracts were combined. The toluene solution was next extracted with 2% sulfuric acid (two 50-ml portions). The acid extract was washed with ether, made alkaline with 5% sodium hydroxide solution, and extracted again with ether. The ethereal extract was washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness to yield 0.518 g of pale orange residue (the nonphenolic reaction product). The alkaline extract was acidified with 2% sulfuric acid, made alkaline with concentrated ammonium hydroxide, and extracted with ether (300 ml). The ether extract was extracted with 5% sodium hydroxide solution. The alkaline extract was acidified with 2% sulfuric acid, basified with concentrated ammonium hydroxide, and extracted with ether (300 ml). The ether extract was washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness to leave 0.451 g of residue (the phenolic reaction product).

The Nonphenolic Sodium–Liquid Ammonia Reduction Product (III). The crude residue (0.518 g) was crystallized from petroleum ether (Skellysolve B) to give colorless needles (0.32 g): mp 88.5–89.5°; $[\alpha]^{24}D$ −96° (*c* 0.78, chloroform); ORD (*c* 0.01): $[\alpha]_{350}$ −800°, $[\alpha]_{300}$ −2250°, $[\alpha]_{290}$ −3600° (trough), $[\alpha]_{275}$ +800° (peak), $[\alpha]_{237}$ −9400° (trough), $[\alpha]_{226}$ −3400° (peak), $[\alpha]_{223}$ −4400° (trough), $[\alpha]_{220}$ −1600° (peak), $[\alpha]_{215}$ −4600°; $\lambda_{max}^{CH_3OH}$ 286 mμ (ε 2800);

(27) A. C. Waiss, Jr., and J. Corse, *J. Am. Chem. Soc.*, 87, 2068 (1965).

nmr spectrum: τ 2.97 (4 H, aryl C–H), 3.45 (H, aryl C–H), 4.00 (H, aryl C–H), 6.16, 6.47 (6 H, O-methyl), 7.47 (3 H, N-methyl), 7.69 (3 H, aryl C-methyl).

Anal. Calcd for $C_{20}H_{25}NO_2$: C, 77.13; H, 8.09; N, 4.50; 2(OCH_3), 19.91; (NCH_3), 9.05. Found: C, 76.91; H, 7.95; N, 4.66; (OCH_3), 20.33; (NCH_3), 7.37.

The Phenolic Sodium–Liquid Ammonia Reduction Product (IV). The crude material (0.451 g) was washed with a small amount of methanol and crystallized from methanol–ether to yield colorless prisms (0.168 g): mp 212–214°, $[\alpha]^{24}D$ +36° (*c* 0.14, methanol); $\lambda_{max}^{CH_3OH}$ 225 mμ (sh) (ε 24,400) and 280 mμ (ε 4000).

Anal. Calcd for $C_{18}H_{21}NO_4$: C, 68.55; H, 6.71; N, 4.44; 2(OCH_3), 19.61. Found: C, 69.19; H, 7.04; N, 4.35; (OCH_3), 19.11.

The hydrochloride showed mp 242–246°; $\lambda_{max}^{CH_3OH}$ 225 mμ (sh) (ε 21,000) and 278 mμ (ε 3450).

Anal. Calcd for $C_{18}H_{22}ClNO_4$: C, 61.45; H, 6.30; N, 3.98; Cl, 10.01. Found: C, 61.09; H, 6.31; N, 3.95; Cl, 10.17.

Oxidation of the Nonphenolic Reduction Product (III) to 1-Oxo-2-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (V). A solution of III (50 mg) in acetone (10 ml) was treated with 1% potassium permanganate in acetone (5 ml), and the solution was stirred at room temperature for 4 hr. Methanol was added dropwise to decompose excess potassium permanganate, and the mixture was filtered and evaporated to dryness under a stream of nitrogen gas. The residue was extracted with ether, and the extract was washed with 5% potassium carbonate, 2% sulfuric acid, and water, and dried over anhydrous sodium sulfate. The solvent was evaporated to yield a residue which was crystallized from petroleum ether to yield colorless prisms (12 mg), mp 125–126°. The melting point was not depressed by admixture of a sample of 1-oxo-2-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline prepared by the procedure of Späth and Epstein.[28] The infrared spectra and thin layer chromatographic properties of the respective samples confirmed their identity.

N-(3,4-Dimethoxyphenethyl)-2-(4-methylphenyl)acetamide (IX). A mixture of *p*-tolylacetic acid (5.0 g) and thionyl chloride (12 ml) was heated on a steam bath for 1 hr. The excess thionyl chloride was evaporated under reduced pressure, and the residue was dissolved in dry ether (20 ml). The ethereal solution was transferred to a dropping funnel and added dropwise to a stirred mixture of 2-(3,4-dimethoxyphenyl)ethylamine (6.5 g) and 10% aqueous potassium hydroxide (90 ml). After addition had been completed, stirring at room temperature was continued for 2 hr. The precipitate was filtered and washed with 5% hydrochloric acid, and then with water. Crystallization from ethyl acetate yielded colorless needles (8.3 g), mp 120–121°.

Anal. Calcd for $C_{19}H_{23}NO_3$: C, 72.82; H, 7.40; N, 4.47. Found: C, 73.04; H, 7.67; N, 4.57.

1-(4-Methylbenzyl)-6,7-dimethoxy-3,4-dihydroisoquinoline (XII). A mixture of IX (3.0 g) and phosphoryl chloride (3 ml) in dry toluene (12 ml) was heated in an oil bath at 125–135° for 2.5 hr. Excess phosphoryl chloride and toluene were evaporated under reduced pressure, and the residue was washed with warm petroleum ether. Crystallization from acetone–methanol gave the colorless hydrochloride salt of XII (2.5 g), mp 200–207°.

Anal. Calcd for $C_{19}H_{22}ClNO_2$: C, 68.76; H, 6.63; N, 4.22. Found: C, 68.30; H, 6.87; N, 4.41.

The free base (XII) was prepared by treatment of an aqueous solution of the hydrochloride salt with dilute sodium hydroxide, extraction with ether, drying the ether over anhydrous potassium carbonate, and evaporation to dryness. The crystalline residue showed mp 83–86°.

Anal. Calcd for $C_{19}H_{21}NO_2$: C, 77.26; H, 7.17; N, 4.74. Found: C, 77.07; H, 7.15; N, 4.73.

1-(4-Methylbenzyl)-6,7-dimethoxy-3,4-dihydroisoquinoline Methiodide (XI). A solution of XII (0.75 g) in methanol (15 ml) was treated with methyl iodide (5 ml) and heated on the steam bath under reflux for 4 hr. Evaporation to dryness under reduced pressure left a yellow solid residue. The residue was washed with water and recrystallized from benzene–acetone to yield yellow prisms (0.95 g), mp 103–106°.

Anal. Calcd for $C_{20}H_{24}INO_2$: C, 54.93; H, 5.49; N, 3.20. Found: C, 55.07; H, 5.39; N, 3.37.

(±)-1-(4-Methylbenzyl)-2-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (X). A stirred solution of XI (0.90 g) in methanol (15 ml) was treated portionwise with sodium borohydride (0.50 g). Stirring at room temperature was continued for 2 hr after addition had been completed. The solvent was evaporated and the residue was treated with 5% sodium hydroxide and ether. The ethereal layer was washed with water, dried over anhydrous potassium carbonate, and evaporated to leave a colorless residue. Recrystallization from isopropyl ether yielded colorless prisms (0.45 g), mp 96–97°.

Anal. Calcd for $C_{20}H_{25}NO_2$: C, 77.13; H, 8.09; N, 4.50. Found: C, 77.41; H, 8.12; N, 4.57.

The infrared spectrum in chloroform and the nmr spectrum were superimposable upon those of the levorotatory sample (III) obtained from O-methylcissampareine, and the mobilities of the respective samples upon paper and thin layer chromatography were the same.

Methylation of the Phenolic Reduction Product (IV) to 1-(4-Methoxybenzyl)-6,7,8-trimethoxy-1,2,3,4-tetrahydroisoquinoline (VI). A solution of IV (18 mg) in methanol (4 ml) was treated with excess of an ethereal solution of diazomethane, and the solution was allowed to stand at room temperature for 48 hr. The solution was partially concentrated, treated with a second charge of the ethereal diazomethane solution, and allowed to stand for 24 hr. The solvent was evaporated, and the residue was dissolved in benzene and chromatographed on Woelm neutral alumina (2 g). Elution with benzene–chloroform (1:1) gave a homogeneous product (8 mg). The semisolid material was treated with 1 drop of concentrated hydrchloric acid and ether (1 ml) and rubbed to induce crystallization. Recrystallization from acetone gave colorless needles, mp 186–190°. The regenerated free base showed an infrared spectrum in chloroform which was superimposable upon that of the racemic sample synthesized by the procedure of Tomita and Okui,[13] and the mobilities of the respective samples upon paper and thin layer chromatography were the same.

2-(4-Benzyloxy-3,5-dimethoxyphenyl)ethylamine (XV). A solution of 4-benzyloxy-3,5-dimethoxy-ω-nitrostyrene[17] (XIV, 3.3 g) in purified dioxane (60 ml) was added to a stirred solution of lithium aluminum hydride (2.30 g) in ether (250 ml) over a period of 1 hr at room temperature. The reaction was stirred for an additional hour and was completed by warming on the steam bath for 5 min. Water (5 ml) was added slowly with stirring and cooling to decompose excess reagent, and the complex was treated with 20% sodium hydroxide (5 ml) and water (5 ml). The ether–dioxane layer was decanted, and the precipitate from the lower layer was collected and extracted repeatedly with ether. The combined ether–dioxane solution was dried over anhydrous potassium carbonate and evaporated to yield an oily residue. The residue was dissolved in ether (5 ml) and treated with a solution of oxalic acid (2.0 g) in methanol (4 ml). The precipitated oxalate was recrystallized from methanol as colorless needles (2.1 g), mp 199–200°.

Anal. Calcd for $C_{19}H_{23}NO_7$: C, 60.49; H, 6.14; N, 3.71. Found: C, 60.63; H, 6.13; N, 3.66.

N-(4-Benzyloxy-3,5-dimethoxyphenethyl)-2-(4-benzyloxyphenyl)acetamide (XIX). The oxalate salt of XV (1.9 g) was treated with 5% sodium hydroxide solution (15 ml) and extracted with ether to liberate free amine. To the stirred ether solution of free amine and 5% sodium hydroxide (50 ml) at 5° was added dropwise a solution of *p*-benzyloxyphenylacetyl chloride[18] (XVI, 1.5 g). The precipitated amide was collected by filtration and dissolved in chloroform. The chloroform solution was washed with water, 4% hydrochloric acid, and again with water, and dried over anhydrous potassium carbonate. Evaporation of the chloroform left an oily residue which was crystallized from methanol as colorless needles (1.3 g), mp 105–106°.

Anal. Calcd for $C_{32}H_{33}NO_5$: C, 75.13; H, 6.50; N, 2.74. Found: C, 75.23; H, 6.52; N, 2.91.

1-(4-Benzyloxybenzyl)-7-benzyloxy-6,8-dimethoxy-1,2,3,4-tetrahydroisoquinoline (XVII). A mixture of XIX (0.40 g), polyphosphate ester[29] (2.0 g), and chloroform (2 ml) was heated on a steam bath for 1 hr. Evaporation under reduced pressure gave an oil, which was treated with water, made alkaline with 5% sodium hydroxide, and extracted with chloroform. The chloroform solution was dried over anhydrous potassium carbonate, filtered, and evaporated to dryness to yield an oily residue (0.36 g). Thin layer chromatography on silica gel G with 5% methanol–chloroform indicated that the product was essentially homogeneous, but the material resisted attempts at crystallization.

A solution of the oily cyclization product (XVIII, 0.36 g) in methanol (4 ml) was treated portionwise with sodium borohydride (0.20 g) over a period of 0.5 hr at room temperature. The mixture

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(29) G. Schramm, H. Groetsch, and W. Pollmann, *Angew. Chem. Intern. Ed. Engl.*, 1, 1 (1962).

was then allowed to stand at room temperature for 3 hr. Evaporation of the methanol yielded a brownish residue which was suspended in water and extracted with ether. The ethereal solution was dried over anhydrous potassium carbonate, filtered, and concentrated to about 5 ml. Saturation of the ethereal solution with dry hydrogen chloride led to separation of a yellowish gummy solid. Crystallization from methanol–ether gave colorless needles (0.29 g), mp 143–145°.

Anal. Calcd for $C_{32}H_{34}ClNO_4 \cdot 0.5H_2O$: C, 71.03; H, 6.52. Found: C, 71.06; H, 6.73.

(±)-1-(4-Hydroxybenzyl)-7-hydroxy-6,8-dimethoxy-1,2,3,4-tetrahydroisoquinoline (IV). A suspension of 10% palladium on charcoal (0.03 g) in ethanol (10 ml) was saturated with hydrogen and XVII (0.255 g of hydrochloride salt) was added. After consumption of 2 molar equiv of hydrogen, the reaction mixture was filtered and evaporated to dryness. Crystallization from methanol–ether yielded colorless prisms (0.14 g), mp 240–244°.

Anal. Calcd for $C_{18}H_{22}ClNO_4$: C, 61.45; H, 6.30. Found: C, 61.61; H, 6.32.

The ultraviolet absorption spectrum of the sample was identical with that of the hydrochloride of the cleavage product IV.

Liberation of the free base was effected by treatment of the hydrochloride salt (0.12 g) in water (4 ml) with ammonium hydroxide and extraction with ether. Evaporation of the ether solution left a colorless residue which was crystallized from methanol–ether as colorless elongated prisms (0.05 g), mp 204–205°.

Anal. Calcd for $C_{18}H_{21}NO_4$: C, 68.57; H, 6.71; N, 4.44. Found: C, 68.33; H, 6.62; N, 4.46.

The ultraviolet absorption spectrum, nmr spectrum at 100 Mc in $CDCl_3$–DMSO-d_6 mixture,[21] and mobility upon paper and thin layer chromatography were the same as those of IV obtained from O-methylcissampareine.

O-Ethylcissampareine (XX). Cissampareine (1.178 g) in methanol was treated with an excess of an ethereal solution of diazoethane, and the solution was allowed to stand at room temperature for 48 hr. Partial concentration of the solution yielded needles (0.34 g), mp 148–149°. The mother liquor was evaporated to dryness, and the residue was dissolved in 2% sulfuric acid and washed with ether. The acid solution was made alkaline with 5% sodium hydroxide and extracted with ether. The ether extract was dried over anhydrous sodium sulfate and evaporated to a solid residue. Crystallization from methanol gave needles (79 mg), mp 148–150°. Recrystallization from methanol gave colorless needles, mp 152–153°, $[\alpha]^{27}D$ −157° (*c* 1.02, chloroform).

Anal. Calcd for $C_{39}H_{42}N_2O_6 \cdot H_2O$: C, 71.76; H, 6.79; N, 4.79. Found: C, 71.27; H, 6.84; N, 4.33.

Sodium–Liquid Ammonia Reduction of O-Ethylcissampareine (XX). O-Ethylcissampareine (XX, 0.50 g) was reductively cleaved by the procedure described for II. The nonphenolic reaction product was crystallized from petroleum ether to yield needles (212 mg), mp 86–88°. The melting point was undepressed by admixture of III, and the infrared spectra in chloroform and mobilities upon paper and thin layer chromatography were identical. Attempts to isolate the phenolic cleavage product were unsuccessful.

Biosynthesis of the Peyote Alkaloids. The Incorporation of Tyrosine-2-C^{14} into Mescaline and Anhalonidine[1]

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Abstract: Radioactive mescaline and anhalonidine were isolated from the cactus *Anhalonium lewinii* (peyote) which had been fed DL-tyrosine-2-C^{14}. Systematic degradation of the mescaline indicated that it was labeled solely at C-1 of the side chain. The anhalonidine was shown to have all its activity at C-3. These results substantiate the generally accepted hypotheses for the biosynthesis of these alkaloids. The administration of DL-phenylalanine-3-C^{14} to the cactus did not yield radioactive mescaline, indicating that phenylalanine is not converted to tyrosine in this species.

Mescaline (**3**) and anhalonidine (**7**) are two of the main alkaloids found in the peyote cactus[3] (*Anhalonium lewinii* Hennings = *Lophophora williamsii*). Mescaline is a simple phenylethylamine, and it has been generally accepted[4] that it is formed from tyrosine or an hydroxylated phenylalanine by decarboxylation and O-methylation. Anhalonidine is a tetrahydroisoquinoline, and it has been suggested, on the basis of *in vitro* experiments,[5] that alkaloids of this type are formed in nature by a Mannich reaction between a hydroxylated phenylethylamine (**1**) and an aldehyde or α-keto acid (Z = COOH) as illustrated in Figure 1. In the latter case, the alkaloid (**2**, Z = H) would be formed by a subsequent decarboxylation.

We have now tested these hypotheses by feeding DL-tyrosine-2-C^{14} to a single 4 year old peyote cactus (see Experimental Section for details of the method of administering the tracer to the plant). The plant was harvested 3 weeks after feeding the tracer. Since we anticipated that it would be difficult to separate and isolate the small amount of alkaloid present in one cactus, inactive mescaline was added as a carrier during the work-up of the plant. Radioactive mescaline was isolated and purified by crystallization of its hydrochloride. Inactive DL-anhalonidine hydrochloride was added to the mother liquor of the initial crystallization of the mescaline hydrochloride. After several crystallizations, anhalonidine hydrochloride was obtained having a constant specific activity. The absolute incorporations of tracer into mescaline and anhalonidine were 0.03 and 0.01%, respectively. In our preliminary work,[1] it was deduced that all the activity of the mescaline was located at C-1 of the side chain by oxidation with potassium permanganate to 3,4,5-trimethoxy-

(1) This investigation was supported by a Research Grant GM-13246 from the U. S. Public Health Service. A preliminary account of part of this work has appeared as a communication: E. Leete, *Chem. Ind.* (London), 604 (1959).

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