preparation of glycol **16**. Hydrolysis of the intermediate osmate ester furnished 577 mg of crude material, which gave 243 mg of homogeneous, but amorphous, glycol on chromatography over Florisil. The glycol thus produced was dissolved in 5 ml of dry benzene and was treated directly with a solution of 384 mg of lead tetraacetate in 7 ml of anhydrous benzene. After 15 min at room temperature, the reaction mixture was worked up as previously described, yielding 214 mg of crystalline residue: $\lambda_{max}^{CS_2}$ 3.67, 5.80, and 5.94 μ.

Attempts to purify a small sample of the keto aldehyde **36** by chromatography (alumina) and subsequent crystallization from methylene chloride–petroleum ether gave an isomer, mp 215–216°, $\lambda_{max}^{CS_2}$ 2.81, 3.65, and 5.83 μ, for which structure **37** is suggested.

Anal. Calcd for $C_{24}H_{26}O_4$: C, 76.17; H, 6.92. Found: C, 76.23; H, 7.05.

In view of this difficulty the remainder of the crude keto aldehyde above was reduced directly with lithium aluminum hydride in ether as described for the reduction of keto ester **33**. Chromatography of the reduction product (Florisil) and crystallization from ethyl acetate–petroleum ether yielded a sample of glycol **34**, mp 210.5–211.5°, that was indistinguishable from the specimen derived from keto ester **33**.

Alkaloid Studies. LVI.[1] The Constitution of Vallesiachotamine[2]

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Abstract: Through a combination of chemical and spectroscopic techniques (notably nuclear magnetic resonance and mass spectrometry) it has been possible to show that vallesiachotamine, an alkaloid isolated from the Peruvian *Apocynaceae Vallesia dichotoma* Ruiz et Pav, possesses structure I. A likely biogenetic route to this unusual structure is discussed.

Extensive studies[3–6] in our laboratory on the constituents of the Peruvian plant *Vallesia dichotoma* Ruiz et Pav (family, *Apocynaceae*) have resulted in the isolation of 28 alkaloids, all but six of which have now been fully characterized. We should now like to report the structure elucidation of one of the remaining alkaloids, present to the extent of approximately 0.001%, which we have named vallesiachotamine.[7] As will be shown below, its structure (I) is of unusual biogenetic interest.

The very limited amount of material demanded great dependence upon physical measurements and establishment of empirical formulas principally through mass spectrometric measurements rather than combustion analyses. The alkaloid is relatively unstable to exposure to air and light, but this feature did not apply to some of its transformation products. The empirical formula $C_{21}H_{22}N_2O_3$ was established by high-resolution mass measurements and supported by the only combustion analysis[6] performed in this work. Its ultraviolet absorption spectrum (Figure 1), though strongly suggestive of an indole, exhibited abnormally high extinction in the 290-mμ region, which suggested the existence of a second chromophore absorbing in that region. The infrared spectrum exhibited bands at 2.89, 2.98, 6.02, and 6.25 μ typical of NH or OH and of α,β-unsaturated carbonyl groupings. The mass spectrum (Figure 2)[8] displayed a pattern, which was unlike that of any of the various indole alkaloids hitherto investigated,[9] but the high-resolution mass measurements did shed some light on the nature of the three oxygen atoms, which appeared to be incorporated in one carbonyl (see *m/e* 322 in Figure 2) and one methoxycarbonyl (see *m/e* 291 and 263 in Figure 2) group. These conclusions were verified by the 100-Mc nmr spectrum, whose most salient features are summarized in Table I. The spectrum was complicated by the fact that most of the signals appeared in pairs presumably due to restricted rotation of one or more groups,[5] but this did not preclude making the assignments summarized in Table I.

Table I. Nmr Spectrum of Vallesiachotamine (I)

Nmr signal,[a] δ (ppm)	No. of protons	Assignment
10.2/9.3	1	C(O)—*H*
8.6/8.55	1	N*H* (indole)
7.7/7.67	1	>C=C—*H*
7.6–7.1	4	Aromatic *H*
6.65/6.55 (two quartets, J = 7.5 cps)	1	CH_3CH=C<
2.18/2.07 (two doublets, J = 7.5 cps)	3	CH_3CH=C<
3.64	3	CO_2CH_3

[a] Doubling of signals presumed to be due to restricted rotation.

As will be shown below, these conclusions could be verified by nmr studies (see Figure 3) on derivatives, which did not exhibit these complicating features.

The analytical and spectral data cited so far can be summarized in terms of the following expression, which

(1) For paper LV see R. R. Arndt and C. Djerassi, *Experientia*, 21, 566 (1965).

(2) Financial support from the National Institutes of Health (Grant No. GM-11309) of the U. S. Public Health Service is gratefully acknowledged. The purchase of the Atlas CH-4 mass spectrometer used in this investigation was made possible through NASA Grant NsG 81–60.

(3) J. S. E. Holker, M. Cais, F. A. Hochstein, and C. Djerassi, *J. Org. Chem.*, 24, 314 (1959).

(4) K. S. Brown, Jr., H. Budzikiewicz, and C. Djerassi, *Tetrahedron Letters*, 1731 (1963).

(5) A. Walser and C. Djerassi, *Helv. Chim. Acta*, 47, 2073 (1964).

(6) A. Walser and C. Djerassi, *ibid.*, 48, 391 (1965).

(7) This was earlier[6] referred to as alkaloid number 20.

(8) Empirical formulas are marked for those peaks where the composition was established by high-resolution mass measurements.

(9) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. I, Holden-Day, Inc., San Francisco, Calif., 1964, Chapters 3–9.

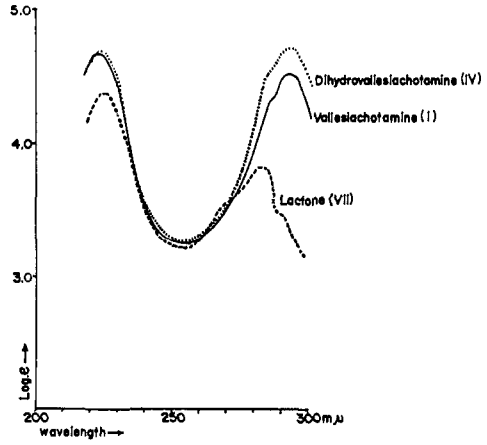
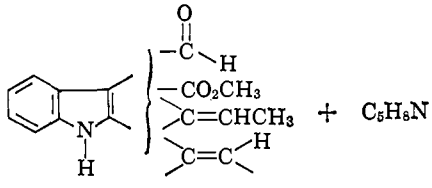


Figure 1. Ultraviolet absorption spectra of vallesiachotamine (I), dihydrovallesiachotamine (IV), and lactone (VII).

prompted chemical experiments involving one or more of the carbonyl groups or double bonds.



The first reaction attempted was lithium aluminum hydride reduction, which led to a complex mixture. By means of thin layer chromatography, there was isolated one homogeneous, amorphous product (subsequently assigned structure VI), whose empirical formula (established by mass spectrometry) corresponded to the reduction of the two carbonyl groups of vallesiachotamine, an assumption which was supported by the complete absence of carbonyl absorption in the infrared. Most significant was the observation that the ultraviolet absorption spectrum was now completely typical of a simple 2,3-disubstituted indole.[10] The abnormally high extinction of vallesiachotamine in the 290-mμ region (Figure 1) must, therefore, be associated with one of the carbonyl groups. Due to the small amount of relatively insoluble product, only a poorly resolved nmr spectrum could be obtained, but the absence of any signal associated with an N-methyl function demonstrated that the formyl group of vallesiachotamine could not be attached to nitrogen. The mass spectrum of this lithium aluminum hydride reduction product was grossly different from that (Figure 2) of the parent alkaloid. While the base peak was still due to the molecular ion (*m/e* 324), the second most intense peak was now at *m/e* 323, and, aside from a strong peak at *m/e* 306 (M − H_2O), the most diagnostic peaks were found at *m/e* 184, 169, 170, and 156. These four peaks, coupled with a strong M − 1 ion, have been shown earlier[9,11a] to be associated with a substituted tetrahydro-β-carboline system and have been attributed to structures a–e. The presence of these same peaks in the mass spectrum of the lithium aluminum hydride product thus points toward the existence of a tetrahydro-β-carboline moiety in vallesiachotamine.

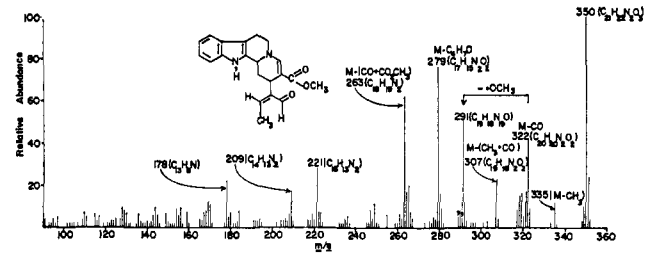
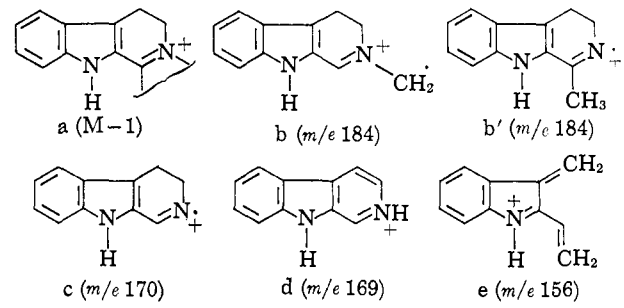


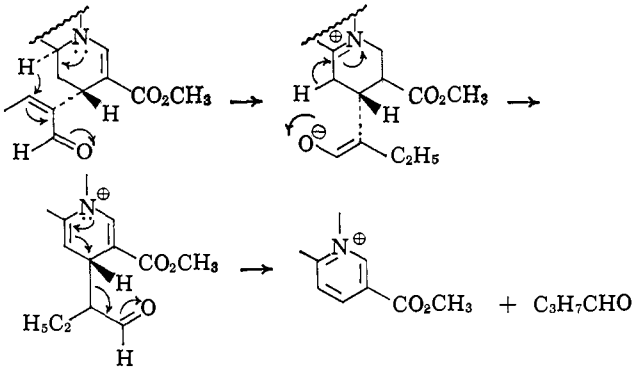
Figure 2. Mass spectrum of vallesiachotamine (I).



Experimentally, a much more satisfactory reduction of vallesiachotamine was achieved by means of sodium borohydride in ethanol solution, which proceeded in high yield to afford a crystalline dihydrovallesiachotamine (subsequently shown to be IV). The ultraviolet absorption spectrum (see Figure 1) was very similar to that of vallesiachotamine and the high extinction in the 290-mμ range demonstrated that the carbonyl chromophore responsible for that absorption was still intact. The nmr spectrum (Figure 3) exhibited the same signals as the parent alkaloid (Table I) except for the absence of the lowest field signal associated with the aldehydic proton of vallesiachotamine and the presence of a new two-proton signal at δ 4.03 due to the grouping $=CCH_2O$. Aside from the expected two mass unit shift of the molecular ion peak (as compared to Figure 2), the most significant feature of the mass spectrum of dihydrovallesiachotamine (IV) was the absence of an M − CO ion. This cumulative evidence, coupled with the diminished intensity of the infrared carbonyl absorption band, is only compatible with the presence of grouping A in vallesiachotamine (I), which has been transformed into B in dihydrovallesiachotamine (IV) upon reduction with sodium borohydride. Partial structure A is

(10) *Cf.* A. W. Sangster and K. L. Stuart, *Chem. Rev.*, **65**, 69 (1965).

(11) (a) L. D. Antonaccio, N. A. Pereira, B. Gilbert, H. Vorbrueggen, H. Budzikiewicz, J. M. Wilson L. J. Durham, and C. Djerassi, *J. Am. Chem. Soc.*, **84**, 2161 (1962); see also G. Spiteller and M. Spiteller-Friedmann, *Monatsh.*, **93**, 795 (1962); B. Gilbert, J. A. Brissolese, N. Finch, W. I. Taylor, H. Budzikiewicz, J. M. Wilson, and C. Djerassi, *J. Am. Chem. Soc.*, **85**, 1523 (1963). (b) One of the referees suggested the following mechanism for the generation of butyraldehyde.



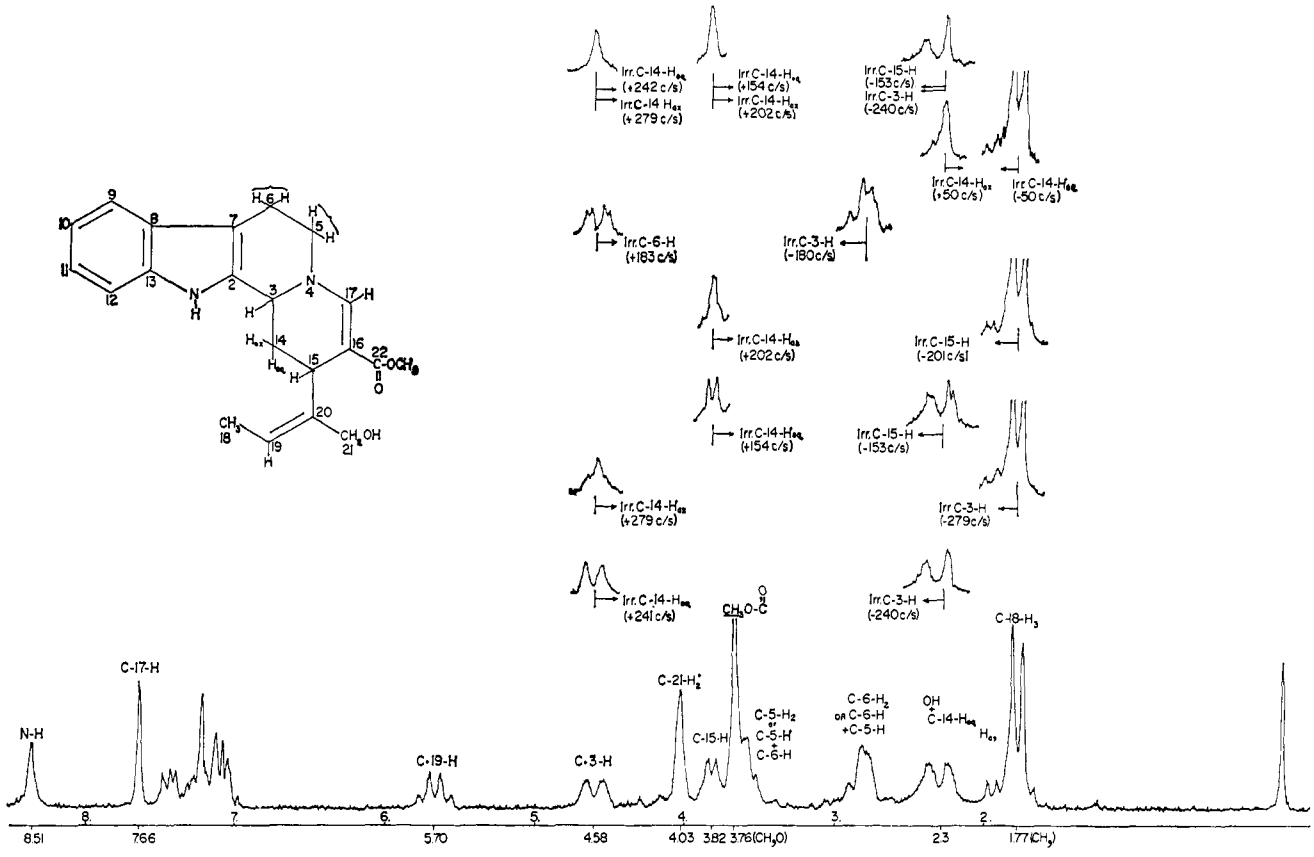
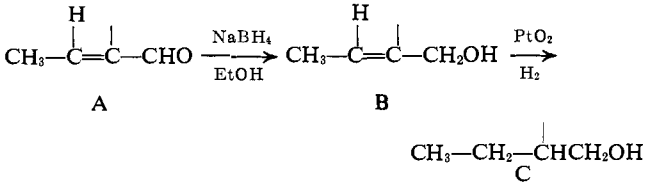


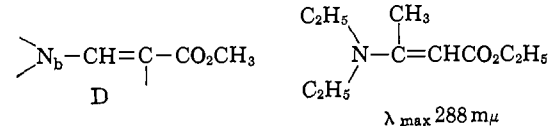
Figure 3. Nmr spectrum (100 Mc) of dihydrovallesiachotamine (IV).

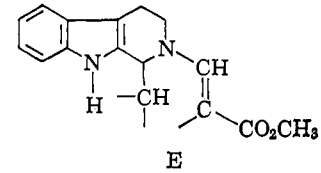
further supported by the experimentally verified, though mechanistically obscure,[11b] isolation of *n*-butyraldehyde upon high-temperature pyrolysis of vallesiachotamine.



Microhydrogenation of dihydrovallesiachotamine (partial structure B) resulted in the uptake of 1 molar equiv of hydrogen and formation of tetrahydrovallesiachotamine (partial structure C) as shown by the additional two mass unit shift of the molecular ion (*m/e* 354) and the unchanged character of the ultraviolet absorption spectrum.

Attention should now be directed at the chromophoric system, which is responsible for the high ultraviolet absorption (Figure 1) of vallesiachotamine near 290 mμ. It must incorporate the methoxycarbonyl function (still present in dihydrovallesiachotamine (IV) and tetrahydrovallesiachotamine (V), but absent in the lithium aluminum hydride reduction product VI) as well as the strongly deshielded olefinic proton, which is responsible for the sharp downfield singlet at δ 7.66 in the nmr spectra of vallesiachotamine (Table I) and dihydrovallesiachotamine (Figure 3). These properties taken together with the associated infrared data (λ_{max} 6.02 and 6.25 μ) and the inability of vallesiachotamine to form a methiodide are uniquely accommodated in partial structure D, which gains powerful support from the observation that the related ethyl β-diethylaminocrotonate exhibits its principal ultraviolet absorption maximum at 288 mμ.[12]

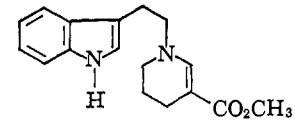




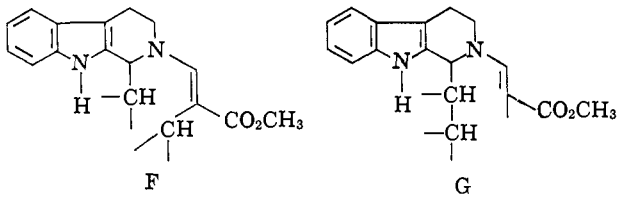
E

Combination of moiety D with the previously discussed tetrahydro-β-carboline fragment leads to partial structure E, which together with A accounts for all of the atoms in vallesiachotamine except for one carbon and two hydrogen atoms. This extra carbon atom can be attached to E in only two ways, leading to expressions F and G. Attachment of the crotonaldehyde grouping

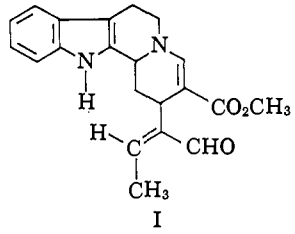
(12) K. Bowden, E. A. Braude, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, 45 (1946); K. Bowden, E. A. Braude, and E. R. H. Jones, *ibid.*, 948 (1946). Shortly after submission of our manuscript there appeared an article by E. Wenkert, K. G. Dave, and F. Haglid, *J. Am. Chem. Soc.*, 87, 5461 (1965), describing the synthesis of the following tetrahydropyridine. This substance represents an even closer model to vallesiachotamine and its spectral properties fully support our conclusions.



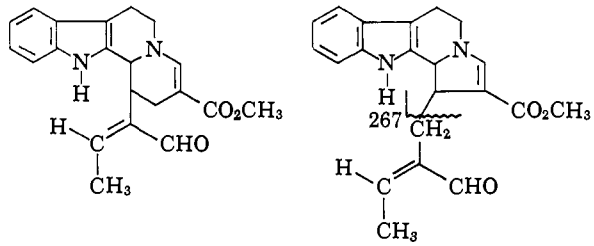
(A) and completion of the last ring (required by the molecular formula $C_{21}H_{22}N_2O_3$) can then lead to only three possible structural formulas (I, II, and III) for vallesiachotamine.



F G



I



II III

Aside from biogenetic likeliness, which clearly favors alternative I over II and III, mass spectrometric evidence can also be presented which tends to eliminate structures II and III. The peak at *m/e* 156 (e)[11a] present in the mass spectra of appropriate vallesiachotamine derivatives (*e.g.*, Figure 4) would be very difficult to rationalize on the basis of II or III. Furthermore, a substance with structure III would be expected to show an important peak at *m/e* 267 (see wavy line in III) and this is contrary to the experimental findings (see Figure 2).

Conclusive evidence in favor of expression I for vallesiachotamine can be presented from two sides. The first is a detailed nmr study together with spin decoupling using dihydrovallesiachotamine, which was selected because of its ease of preparation, stability, and absence of double signals (*cf.* Table I *vs.* Figure 3). The results are summarized below by cross reference to Figure 3 (100-Mc nmr spectrum) and establish unambiguously the sequence $—N_bCHCH_2CHC=$, which is only compatible with expression IV for dihydrovallesiachotamine and hence V for tetrahydrovallesiachotamine and I for the alkaloid itself. The amorphous lithium aluminum hydride reduction product can then be depicted in terms of VI.

The signals for N–H, C-17–H, methoxyl and C-21–H_2 as well as the peaks due to the ethylidene side chain are obvious and do not require any further comment. The broad doublet centered at *ca.* δ 4.6 is assigned to the C-3 proton and is made sharper by irradiation of the equatorial hydrogen at C-14 (241 cps upfield) while irradiation of the axial hydrogen (279 cps upfield) induces a merging into a broad singlet; appearance of fine structure results when the long-range coupling with the C-6 protons is removed by irradiation at 183 cps

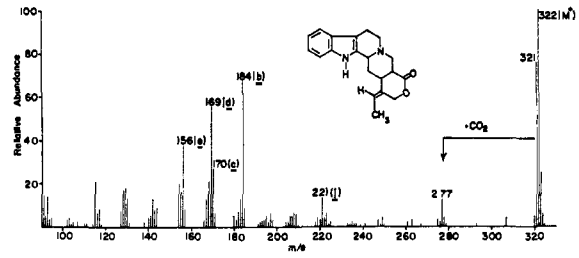
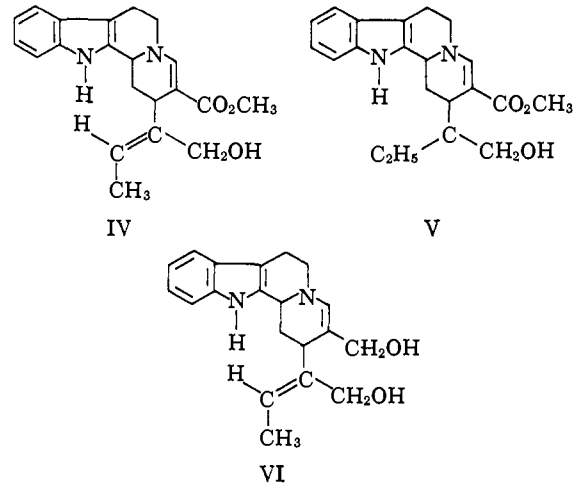


Figure 4. Mass spectrum of lactone VII.

upfield; simultaneous irradiation of the nonequivalent protons at C-14 causes the collapse to a singlet. The C-15 proton signal occurs as a doublet at δ 3.82 which sharpens on saturation of the equatorial hydrogen at C-14 (154 cps upfield); irradiation of the axial hydrogen (202 cps upfield) results in a singlet further sharpened by the simultaneous irradiation of both protons. The precise origin of the broad signal at *ca.* δ 2.8 was not settled, but it is certain that this peak contains at least one of the C-6 protons as is evident from the sharpening caused by irradiation of the C-3 hydrogen at 180 cps downfield. The absorption of the hydroxylic proton (demonstrated by exchange with deuterium oxide) coincides with the left peak of the doublet centered at *ca.* δ 2.3 which is assigned to the equatorial hydrogen at C-14, while the pattern caused by the axial proton is partially hidden under the doublet arising from the methyl group; these coincidences complicate the analysis of the spectrum but it is still possible to observe the reverse of the results described above by irradiation of the appropriate protons, the loss of the small peak on the right side of the methyl doublet on saturation of the C-3 proton being worth noticing.



IV V

VI

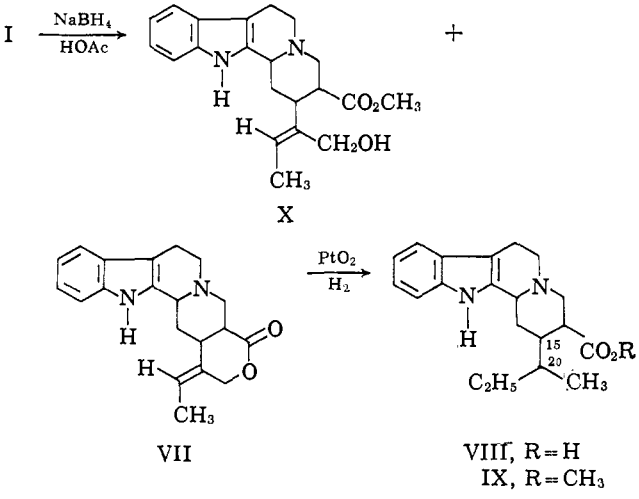
The other evidence in support of structure I for vallesiachotamine rests on chemical grounds. Marshall and Johnson[13] have shown that conjugated enamines can be reduced with sodium borohydride in acetic acid solution and it seemed to us that such a procedure should also be extendable to the vinylogous urethan counterpart embodied by partial structure D. Indeed, while this double bond had proved resistant to catalytic hydrogenation (see IV → V) as well as to lithium aluminum hydride (VI) or sodium borohydride in ethanolic solution (IV), vallesiachotamine (I) was readily attacked

(13) J. A. Marshall and W. S. Johnson, *J. Org. Chem.*, 28, 421 (1963).

by this latter reagent in acetic acid solution to afford two products, which could be separated easily by preparative thin layer chromatography.

The less polar reduction product was nicely crystalline and exhibited (Figure 1) a typical indole ultraviolet absorption spectrum, thus showing that the 290-mμ carbonyl chromophoric system (D) had indeed been destroyed. Aside from establishing the empirical formula $C_{20}H_{22}N_2O_2$, the mass spectrum (Figure 4) exhibited all of the typical features (intense M − 1 ion and peaks at *m/e* 184 (b), *m/e* 170 (c), *m/e* 169 (d), and *m/e* 156 (e)) of a tetrahydro-β-carboline.[11a] In addition, there was present a peak at *m/e* 277 corresponding to the loss of carbon dioxide from the M − 1 species. These data together with the lack of hydroxyl absorption in the infrared, the presence of a carbonyl band at 5.80 μ, the absence of any nmr signals (in d_5-pyridine) due to methoxyl or highly deshielded olefinic protons (compare with Table I), and the presence of a two-proton signal at δ 4.75 associated with the grouping $=CCH_2O-$ as well as the unchanged signals (δ 1.50 and 5.32) of the ethylidene grouping are uniquely accommodated by expression VII.

Further reduction of the lactone VII could be effected by catalytic hydrogenation in the presence of platinum oxide which led to a highly polar substance, which on the basis of its mass spectrometrically determined empirical formula ($C_{20}H_{26}N_2O_2$) and infrared spectrum (λ_{max} 2.90, 3.17, and 6.25 μ) was attributed the amino acid structure VIII. This assignment was confirmed by methylation with diazomethane to a methyl ester IX, whose mass spectrum displayed the expected molecular ion peak at *m/e* 340 ($C_{21}H_{28}N_2O_2$), all of the typical[11a] tetrahydro-β-carboline peaks (a–e) as well as a peak at *m/e* 283 due to the loss of the C_4H_9 side chain (fission of 15–20 bond).



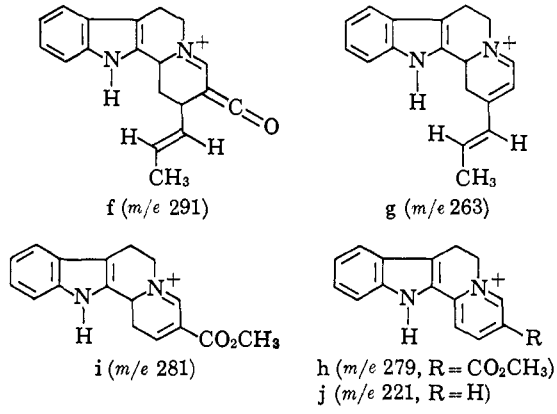
The more polar substance produced in the sodium borohydride–acetic acid reduction of vallesiachotamine (I) was amorphous and unstable. Expression X is attributed to it on the basis of the following spectral information. (i) Except for a molecular ion peak at *m/e* 354, its mass spectrum was very similar to that of the lactone VII, presumably due to lactonization in the mass spectrometer inlet system. (ii) Its infrared spectrum exhibited bands at 2.98 and 5.80 μ attributable to hydroxyl and methoxycarbonyl groupings. (iii) The nmr spectrum contained signals which are unambiguously assignable to the protons of the methoxyl (δ 3.51) and ethylidene (δ 5.62 and 1.53) moieties.

With the constitution of vallesiachotamine (I) being settled, it is now possible to consider in more detail the mass spectrum (Figure 2) of this alkaloid, especially since high-resolution data[8] were available for most of the prominent peaks in the higher mass range.

The loss of carbon monoxide (*m/e* 322) originates from the aldehyde function, since no such M − CO ion is observed in the mass spectrum of dihydrovallesiachotamine (IV). In terms of empirical formula, the *m/e* 291 peak appears to be due to simple loss of the methoxycarbonyl group, but the occurrence of a metastable peak at *m/e* 263.1 ($291^2/322 = 263.0$) shows that at least a portion of this ion should be depicted in terms of f arising by sequential loss of carbon monoxide from the aldehyde and of methoxyl from the ester function. The ion of mass 263 may be represented by g, and a metastable peak at *m/e* 215.0 ($263^2/322 = 214.8$) shows that it originates by expulsion of the methoxycarbonyl substituent from the M − CO species (*m/e* 322).

The ion of mass 279 evidently arises by loss of the C_4H_7O side chain and further elimination of two hydrogen atoms to give the highly stable aromatic structure h. This conclusion is supported by the observation that the base peak in the mass spectrum of dihydrovallesiachotamine (IV) also occurs at *m/e* 279 ($C_{17}H_{15}N_2O$ by high resolution), while it is shifted to *m/e* 281 ($C_{17}H_{17}N_2O_2$ base peak) in the spectrum of tetrahydrovallesiachotamine (V). Apparently, the loss of the additional two hydrogens in the genesis of ion h is intimately associated with the fission of the vinylic 15–20 bond of vallesiachotamine (I) and its dihydro derivative (IV), while, in the tetrahydro analog V, simple allylic cleavage results in the direct production of ion i (*m/e* 281).

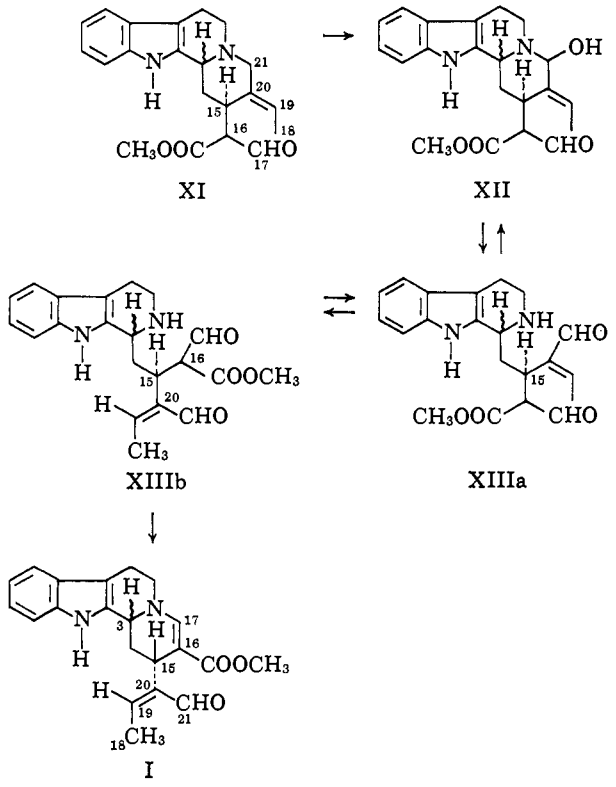
While no appropriate metastable ion could be observed to indicate the nature of its precursor, the ion of mass *m/e* 221 can be assigned the plausible representation j, since it was also observed in the mass spectra of the dihydro (IV) and tetrahydro (V) derivatives as well as of the lactone VII (Figure 4).



Biogenetically, vallesiachotamine (I) possesses an intriguing structure. Of the various hypothetical possibilities, the following seems most attractive and amenable to some experimental verification. Geissoschizine (XI), or a related base, could undergo hydroxylation of C-21 similar to the conversion[14] of

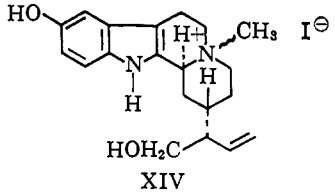
(14) D. H. R. Barton, G. W. Kirby, R. H. Prager, and E. M. Wilson, *J. Chem. Soc.*, 3990 (1965).

deoxyajmaline to ajmaline. The aldehyde ammonia form XIIIa of the resulting carbinol amine XII can now undergo reclosure *via* either one of the aldehyde groups. Thus rotation around the 14–15 bond followed by cyclization of XIIIb with N_b and β elimination of the hydroxyl group would lead directly to vallesiachotamine (I). The actual progenitor, of course, cannot be predicted and the ring opening and recyclization could also occur at the level of corynantheine (XI) or one of its relatives followed by double bond migration.



XI XII XIIIb XIIIa I

Implicit in this scheme is that C-17 of the corynantheine-like precursor (*e.g.*, XII) becomes attached to N_b[15] and that the absolute configuration at C-15 be opposite (β) to that (15α) encountered[16] in all indole alkaloids related to corynantheine (XI). Consequently, determination of the absolute configuration of vallesiachotamine (I) at C-15 would afford *prima facie* evidence whether the above outlined biogenetic scheme merits serious consideration and eventual biochemical verification. Chemical proof of absolute configuration is precluded by the present unavailability of the alkaloid, but it is hoped that this might be accomplished through X-ray analysis[17] of the crystalline methiodide of X. In this connection, it is pertinent to mention that the only other known indole alkaloids that may be derived by such a biogenetic route[18] are the two hunterburnine methiodides (N_b isomeric), whose structure (XIV) and *relative* configuration was established by X-ray analysis.[19]



XIV

Experimental Section[20]

Vallesiachotamine (I). The isolation and physical constants (except for rotation) have already been reported,[6] $[\alpha]^{27}D$ +160° (*c* 1.0, chloroform). The ultraviolet and mass spectra are reproduced in Figures 1 and 2, while the nmr data are collected in Table I.

Lithium Aluminum Hydride Reduction of Vallesiachotamine (I). A mixture of 25 mg of the alkaloid, 4 ml of freshly distilled tetrahydrofuran, and 100 mg of lithium aluminum hydride was heated under reflux for 4 hr and the excess reagent was destroyed by the addition of a saturated sodium sulfate solution. Extraction with ether and purification by tlc (benzene–ethyl acetate–ethanol, 2:2:1) afforded as the major component 6 mg of an amorphous substance (VI) (decomposition point 210–214°), which was sparingly soluble in methanol and did not exhibit any carbonyl absorption in the infrared. The ultraviolet absorption spectrum (λ_{max}^{EtOH} 225 mμ (log ϵ 4.53) 281 (3.91), and 290 (3.89)) was typical of a 2,3-disubstituted indole[10] and the most diagnostic peaks in its mass spectrum occurred at *m/e* 324 (100%) (M^+ calcd for $C_{20}H_{24}N_2O_2$: 324), 323 (62%), 306 (52%), 184 (20%), 170 (6%), 169 (8%), and 156 (8%).

Sodium Borohydride Reduction of Vallesiachotamine (I). A. In Ethanolic Solution. Sodium borohydride (50 mg) was added with stirring at room temperature to a solution of 15 mg of vallesiachotamine in 3 ml of ethanol, and after 15 min the mixture was diluted with water and the product was extracted with methylene dichloride. Purification by tlc (ether) afforded 13 mg of dihydrovallesiachotamine (IV), mp 172–174° (from ether–methanol), whose ultraviolet and nmr spectra (100 Mc) are reproduced in Figures 1 and 3. The infrared spectrum (KBr) exhibited bands at 3.0, 6.0, and 6.21 μ, while the most significant peaks in the higher mass range of its mass spectrum were found at *m/e* 352 (22%) (M^+ calcd for $C_{21}H_{24}N_2O_3$: 352), 335 (11%), 334 (13%), 321 (13%), 293 (16%), 281 (24%), 280 (37%), 279 (100%), 256 (51%), 255 (25%), and 221 (22%).

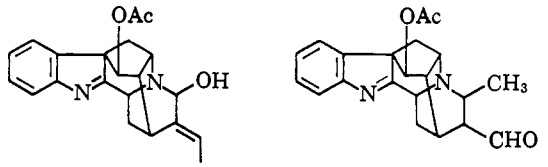
Approximately 0.5 mg of dihydrovallesiachotamine (IV) in ethanolic solution was shaken for 30 min in an atmosphere of

(15) It is for this reason that we are employing a numbering system for vallesiachotamine (I) which reflects this possible biosynthetic origin: see J. LeMen and W. I. Taylor, *Experientia*, 21, 508 (1965).

(16) E. Wenkert and N. V. Bringi, *J. Am. Chem. Soc.*, 81, 1474 (1959).

(17) Such an X-ray investigation is also required to settle the other stereochemical problems, which remain unanswered on the basis of our present studies, namely the stereochemistry at C-3 and the precise geometrical relationship around the 19–20 double bond.

(18) In view of the acid-catalyzed conversion (W. I. Taylor, A. J. Frey, and A. Hofmann, *Helv. Chim. Acta*, 45, 611 (1962)) of vomilenine (i) into perakine (ii), it may be argued that the proposed biosynthesis is in fact a description of a chemical process and that vallesiachotamine is an artifact produced during the isolation from a precursor of structure XII. While there is no presently available experimental test to rigorously exclude such a possibility, the mild isolation and separation conditions[4–6] as compared to the relatively drastic acid treatment required to convert into ii make it unlikely that vallesiachotamine is an artifact.



i ii

(19) J. D. M. Asher, J. M. Robertson, G. A. Sim, M. F. Bartlett, R. Sklar, and W. I. Taylor, *Proc. Chem. Soc.*, 72 (1962); C. C. Scott, G. A. Sim, and J. M. Robertson, *ibid.*, 355 (1962); J. D. Asher, J. M. Robertson, and G. A. Sim, *J. Chem. Soc.*, 6355 (1965); Z. M. Khan, M. Hesse, and H. Schmid, *Helv. Chim. Acta*, 48, 1957 (1965).

(20) All melting points were determined on the Kofler block and are uncorrected. Unless noted otherwise, ultraviolet absorption spectra were measured in ethanol and nmr spectra in deuteriochloroform (tetramethylsilane as internal standard) solution. The nmr spectra were obtained on Varian A-60 and HR-100 (60 and 100 Mc) nmr spectrometers. Double-resonance experiments were performed with the latter instrument. The mass spectra were obtained by direct insertion into the ion source of either an Atlas CH-4 mass spectrometer or an A.E.I. MS-9 double-focussing mass spectrometer through the courtesy of Drs. H. Budzikiewicz, A. Duffield, and D. Becher. In those instances where insufficient material was available for normal handling, the substance was mixed with powdered graphite and the resulting mixture was introduced into the mass spectrometer inlet system. Thin layer chromatography (tlc) was performed on silica gel HF_{254} (E. Merck, Darmstadt) and the spots were detected by spraying with ceric sulfate solution or by exposure to iodine vapor or simply by examination in ultraviolet light.

hydrogen in the presence of 5 mg of platinum oxide catalyst. Purification of the product by tlc afforded tetrahydrovallesiachotamine (V) with an unchanged ultraviolet absorption spectrum and a mass spectrum, which was characterized by only six peaks of relative intensity over 15% in the range *m/e* 150–355: *m/e* 354 (17%) (M^+ calcd for $C_{21}H_{26}N_2O_3$: 354), 322 (24%), 282 (21%), 281 (100%), 221 (17%), and 156 (22%).

B. In Acetic Acid Solution. Vallesiachotamine (40 mg) was dissolved in 4 ml of glacial acetic acid by gentle warming and 300 mg of sodium borohydride was added in portions with stirring while cooling with tap water. After 15 min at room temperature, water was added, the solution was neutralized with sodium bicarbonate, and the product was extracted with methylene dichloride. Tlc purification (benzene–ethyl acetate–ethanol, 1:3:1) gave two products. The less polar one (8 mg, mp 261–264° after recrystallization from methanol) was the lactone VII, whose ultraviolet and mass spectra (M^+ calcd for $C_{20}H_{22}N_2O_2$: 322) are reproduced in Figures 1 and 4. The infrared spectrum (KBr) exhibited bands at 2.90 (indole NH) and 5.80 μ, while the nmr spectrum (d_5-pyridine) displayed the following diagnostically significant signals: δ 1.50 (doublets, J = 7 cps, CH_3CH=), 5.32 (quartet, J = 7 cps, CH_3CH=), 4.75 (singlet, =CCH_2O—), and 4.28 (multiplet, C-3 *H*).

Elution of the more polar band provided 25 mg of the unstable hydroxy ester X as a pale pink resin, which formed a methiodide, (mp 165–170° dec, after recrystallization from ethanol) upon standing overnight at room temperature in ether solution with methyl iodide. The base itself possessed the following spectral characteristics: λ_{max}^{EtOH} 226 mμ (log ϵ 4.45), 283 (3.76), and 290 (3.68); $\lambda_{max}^{CHCl_3}$ 2.85, 2.96, 5.80, and 5.99 μ; nmr signals at δ 1.53 (doublet, J = 7 cps, CH_3CH=), 5.62 (quartet, J = 7 cps, CH_3CH=), 4.11 (singlet, =CCH_2O—), 3.51 (singlet, CO_2CH_3), 4.43 (multiplet, C-3 *H*); mass spectral peaks at *m/e* 354 (10%) (M^+ calcd for $C_{21}H_{26}N_2O_3$: 354), 353 (8%), 322 (56%), 321 (47%), 184 (67%), 170 (20%), 169 (22%), and 156 (27%).

Reactions of the Lactone VII. A. Lithium Aluminum Hydride Reduction. Approximately 0.5 mg of the lactone was reduced with an excess of lithium aluminum hydride in ether solution (25° for 15 min) and after decomposition with aqueous sodium sulfate solution, the product was purified by tlc and subjected to mass spectral examination which confirmed that reduction of the lactone to the corresponding glycol had been effected: *m/e* 326 (57%) (M^+ calcd for $C_{20}H_{26}N_2O_2$: 326), 325 (46%), 295 (13%), 277 (6%), 253 (7%), 241 (9%), 235 (8%), 209 (16%), 184 (100%), 171 (17%), 170 (22%), 169 (20%), and 156 (28%).

B. Catalytic Hydrogenation. The lactone IV (3.5 mg) in ethyl acetate solution was shaken for 5 hr with 10 mg of prereduced platinum oxide catalyst in an atmosphere of hydrogen, whereupon the uptake of 2 molar equiv of hydrogen was complete. Filtration of the catalyst and evaporation of the filtrate left an amorphous solid, which was washed with chloroform to leave 2 mg of the amino acid VIII. The acid was homogeneous by tlc (methanol) and exhibited λ_{max}^{KBr} 2.90, 3.17, and 6.25 μ as well as mass spectral peaks at *m/e* 326 (100%) (M^+ calcd for $C_{20}H_{26}N_2O_2$: 326), 325 (82%), 282 (8%), 281 (7%), 269 (29%), 242 (16%), 241 (27%), 225 (17%), 223 (25%), 184 (39%), 171 (69%), 170 (72%), 169 (34%), and 156 (27%).

Methylation of 0.5 mg of the acid VIII in methanol solution with diazomethane followed by tlc (ethyl acetate) purification afforded the methyl ester (IX) as demonstrated by the mass spectrum: *m/e* 340 (100%)(M^+ calcd for $C_{21}H_{28}N_2O_2$: 340), 339 (78%), 283 (31%), 281 (13%), 256 (18%), 255 (15%), 253 (25%), 184 (15%), 170 (16%), 169 (7%), and 156 (4%).

Pyrolysis of Vallesiachotamine (I). A capillary tube containing 2 mg of vallesiachotamine was inserted into a sublimation furnace preheated to 250° and the temperature was raised to 290° while passing all effluent vapors into a saturated solution of 2,4-dinitrophenylhydrazine in 2 *N* hydrochloric acid. The precipitated hydrazone was centrifuged, washed with water, and then purified by tlc (ether–hexane 1:1). Identity of the principal spot with *n*-butyraldehyde 2,4-dinitrophenylhydrazone was demonstrated by the correspondence in tlc mobility and the complete identity of the respective mass spectra.

Studies on Polynucleotides. LIV.[1] A Further Study of the Reaction of Nucleotide Pyrophosphates with Carboxylic Acid Anhydrides[2]

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Contribution from the Institute for Enzyme Research, University of Wisconsin, Madison, Wisconsin. *Received November 13, 1965*

Abstract: In a further study of the previously observed degradation of disubstituted pyrophosphates (I) with an excess of acetic anhydride,[3] the reaction of P^1,P^2-di(3′-O-acetylthymidine-5′) pyrophosphate with varying amounts of acetic anhydride, benzoic anhydride, and benzoyl chloride in anhydrous pyridine has been investigated. The rate of the degradation reaction and amount of pyrophosphate surviving when equilibrium was attained were both determined and the influence of carboxylate anions and water on the reaction was also studied. Investigation of the reaction intermediates showed the rapid initial formation of polyphosphate species (*e.g.*, VII) followed by a slow step resulting in the predominant formation of the mixed anhydride between the mononucleotide and carboxylic acid (*e.g.*, VI).

The reaction of a disubstituted pyrophosphate (*e.g.*, I) with an excess of acetic anhydride in pyridine followed by an aqueous treatment was shown earlier[3] to lead to the degradation of the pyrophosphate linkage (eq 1). This procedure for the cleavage of pyrophosphate bonds has been used extensively in work in the polynucleotide field[4] where pyrophosphates linking

(1) Paper LIII in this series: M. W. Moon, S. Nishimura, and H. G. Khorana, *Biochemistry*, 4, 937 (1966).

(2) This work has been supported by grants from the National Science Foundation (Grant No. GB-3342), the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service (Grant No. CA-05178), and the Life Insurance Medical Research Fund (Grant No. G-62-54).

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