tained, the recorded spectra were consistent in appearance with their being due only to a summation of the spectra of the two products. The uv spectral values used in the calculations are as follows: for the nitrone III, ϵ_{308} 8100 and ϵ_{346} 23,500; for the O-methyl ether II, ϵ_{308} 11,260 and ϵ_{346} 450. These values were obtained from the slopes of the Beer's law plots for the nitrone and O-methyl ether.

Ceanothus Alkaloids. Americine

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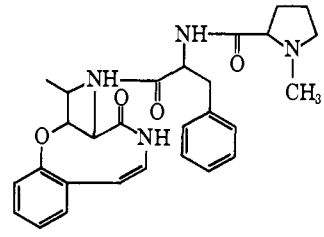
Contribution from the Department of Chemistry, University of California, Berkeley, California 94720. *Received November 15, 1967*

Abstract: Americine, a tryptophan-containing monobasic peptide alkaloid, has been isolated from dried, ground *Ceanothus americanus* root bark. Evidence from chemical degradation and high-resolution nmr and mass spectrometry established for americine a structure incorporating a *p*-alkoxystyrylamino residue in a 14-membered ring.

Peptide alkaloids[2] have thus far been isolated from several species of plants in five different botanical orders of the *Dicotyledonae*.[3-8] Stimulus for the pharmacognosic examination of these plants has largely been their utilization as folk medicines in North and South America, Africa, and Asia. Much attention has been devoted to plants of the *Rhamnaceae* (order *Rhamnales*), from three genera of which peptide alkaloids have been isolated and structurally characterized.[4,6,7] One of these genera, *Ceanothus*, is endemic to North America and consists of approximately 55 species, about 45 of which occur abundantly in California.

Ceanothus americanus Linn. ("New Jersey Tea") is native to the United States east of the Mississippi River. The Cherokee Indians made medicinal use of the plant as early as 1700, and infusions of various parts of this plant were used as panaceas for a wide variety of afflictions. Its proprietary use continued well into the 20th century, and root bark alkaloid mixtures were shown to have hypotensive effects.[9] A series of fruitless chemical investigations of *C. americanus* alkaloids spanning 76 years has been reviewed.[6]

Recently,[6a] five alkaloids have been isolated from dried, ground *Ceanothus americanus* root bark, and a structure was proposed for one of them, ceanothine-B[6b]. All alkaloidal materials reported in that investigation displayed uv spectra characteristically devoid of any absorption maxima or minima. Our studies dealing with alkaloids isolated from ostensibly identical plant material revealed that perhaps 40% of our total alkaloid was composed of materials displaying uv spectra with maxima strongly suggestive of an indole chromophore. This paper deals with one of these intriguing alkaloids.



We herein report the isolation and structure elucidation of americine, an optically active alkaloid constituting 0.02% of dried, ground *C. americanus* root bark. Ethanolic extracts obtained by exhaustive continuous percolation of solvent through the plant material, after removal of neutral and acidic material, yielded crude alkaloid subsequently purified by alumina chromatography and multiple recrystallizations. Accompanying this alkaloid and extremely difficult to remove by preparative thin layer chromatography was 4% of a second alkaloid, homoamericine, as estimated by thin layer chromatography and mass spectroscopy. Once the close, homologous relationship between these two substances was established early in the investigation, all structural work was conducted on this americine–homoamericine (4%) mixture. Elemental ultramicroanalysis of americine fitted the empirical formula $C_{31}H_{39}N_5O_4$, corroborated by observation in the mass spectra of M^+ and M^{2+} peaks at *m/e* 545 and 272.5, respectively. Homoamericine would therefore be $C_{32}H_{41}N_5O_4$, corresponding to the less abundant peak found at *m/e* 559. Although a high degree of unsaturation was implied in the empirical formula, americine reacted quantitatively (followed by tlc) with only 1 equiv of hydrogen under catalytic conditions to yield dihydroamericine (*m/e* 547; *m/e* 545 ion absent).

Extremely intense amide absorption in the infrared spectrum of americine (ν_{max} 3260 cm^{-1}, NH stretch;

(1) Woodrow Wilson Honorary Fellow; National Science Foundation Predoctoral Fellow, 1964–1966; National Institutes of Health Predoctoral Fellow, 1966–1968.

(2) Peptide alkaloids may represent a new class of photochemicals more widely distributed in the plant kingdom than might be suspected from the limited scope of existing investigations. On the basis of preliminary observations on other fractions and other species, we anticipate the discovery of considerably greater structural variety than has thus far been encountered.

(3) (a) M. Païs, J. Mainil, and R. Goutarel, *Ann. Pharm. Franc.*, **21**, 139 (1963); (b) O. Blanpin, M. Pais, and M. A. Quevauviller, *ibid.*, **21**, 147 (1963).

(4) (a) E. L. Menard, J. M. Müller, A. F. Thomas, S. S. Bhatnagar, and N. J. Dastoor, *Helv. Chim. Acta*, **46**, 1801 (1963); (b) E. Zbiral, E. L. Menard, and J. M. Müller, *ibid.*, **48**, 404 (1965).

(5) (a) M. Païs, X. Monseur, X. Lusinchi, and R. Goutarel, *Bull. Soc. Chim. France*, 817 (1964); (b) M. Païs, F.-X. Jarreau, X. Lusinchi, and R. Goutarel, *Ann. Chim.*, 83 (1966).

(6) (a) E. W. Warnhoff, S. K. Pradhan, and J. C. N. Ma, *Can. J. Chem.*, **43**, 2594 (1965); (b) E. W. Warnhoff, J. C. N. Ma, and P. Reynolds-Warnhoff, *J. Am. Chem. Soc.*, **87**, 4198 (1965).

(7) R. Tschesche, R. Welters, and H. W. Fehlhaber, *Chem. Ber.*, **100**, 323 (1967).

(8) M. Païs, J. Marchand, X. Monseur, F. X. Jarreau, and R. Goutarel, *Compt. Rend.*, **264**, 1409 (1967).

(9) (a) J. T. Groot, *J. Pharm. Exptl. Therap.*, **30**, 275 (1927); (b) H. Wastl, *Federation Proc.*, **7**, 131 (1948); (c) A. A. Manian, Ph.D. Thesis, Purdue University, 1954.

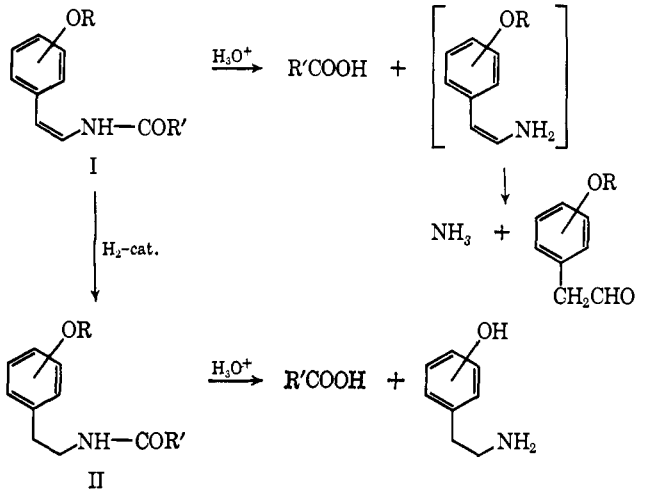
1625–1225 cm^{-1}, amide I, II, III bands) suggested a peptide. Acidic and alkaline "total" hydrolytic degradations were performed on both americine and dihydroamericine. Automatic amino acid analyses of the hydrolysates (Table I) detected tryptophan, leucine,

Table I. Analyses of Americine and Dihydroamericine Hydrolysates

Compound	Americine — Acid hydrolysis	Americine — Basic hydrolysis	Dihydroamericine — Acid hydrolysis	Dihydroamericine — Basic hydrolysis
Tryptophan	++	+++	++	+++
Leucine	−	+	+	+++
2-(Hydroxyphenyl)-ethylamine[a]	−	−	++++	+++
Ammonia	++++	++++	++	++
Unidentifiable fragments	+	+	+++	++++

[a] Amino acid autoanalysis proved incapable of differentiating 2-(4-hydroxyphenyl)ethylamine (tyramine) from authentic synthetic samples of the 2- and 3-hydroxy isomers; all three components emerged from the ion-exchange column just after ammonia.

ammonia, 2-(hydroxyphenyl)ethylamine, and minor amounts of several ninhydrin-positive fragments which had retention times unlike those of any common α-amino acids. Leucine, apparently a significant degradation product only from dihydroamericine, was most effectively released from the peptide chain under alkaline conditions; such a difference would seem to reflect some unusual bonding of this amino acid residue to the molecule and will be discussed in a later section. But most striking in the hydrolytic data was the reciprocal nature of the 2-(hydroxyphenyl)ethylamine and ammonia yields between americine (I) and dihydroamericine (II), strongly suggesting that saturation of a proto-2-(hydroxyphenyl)ethylamine moiety had changed the course of the hydrolysis.

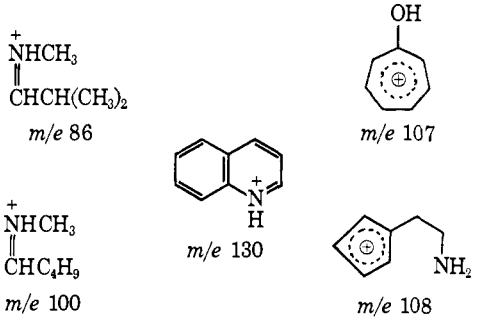


Thin layer chromatography of an alkaline hydrolysate of americine with visualization by the *p*-nitrobenzoyl chloride–pyridine reagent[10] (specific for N-methyl-α-amino acids) revealed the presence of N-methylvaline, characterized by chromatographic comparison with authentic synthetic material. Automatic amino acid analysis, employing ninhydrin-reaction monitoring of the column effluent, could not detect N-methyl-α-amino acids. N-Methylisoleucine, anticipated as a possible product from homoamericine, was undetectable in the hydrolysate by a similar method. N-Methylvaline and leucine together accounted for Herzig–Meyer and Kuhn–Roth determinations of one N-methyl and two C-methyl groups for americine. Observation of fragment ion diagnostic peaks in the mass spectrum of an acidic hydrolysate of dihydroamericine confirmed the presence of N-methylvaline (*m/e* 86), 2-(hydroxyphenyl)ethylamine (*m/e* 107, 108), tryptophan (*m/e* 130), and possibly a six-carbon amino acid (*m/e* 100). That the 2-(hydroxyphenyl)ethylamine was either the 2 or 4 isomer was suggested but not established by the great abundance of the *m/e* 107 and 108 ions and by the ratio of the relative abundances of the two fragments (Table II).

Table II. Mass Spectrometry of 2-(Hydroxyphenyl)ethylamines

Isomer	*m/e* 107, %	*m/e* 108, %	Ratio
2-Hydroxy[a]	5	7	1.4
3-Hydroxy[a]	12	10	0.8
4-Hydroxy[a]	72	93	1.3
Hydrolysate[b]	35	50	1.4

[a] Base peak at *m/e* 30 ($CH_2{=}NH_2^+$). [b] Base peak at *m/e* 86 (N-methylvaline diagnostic peak).



Confirmation of the presence of tryptophan in the peptide chain was supplied not only by the characteristic purple color reaction with *p*-dimethylaminobenzaldehyde (Ehrlich test)[11] but also by the distinctive uv spectrum of the compound. This absorption was practically coincident with the spectrum of indole in the region λ >270 mμ in position and intensity of absorption maxima and minima, assuming a molecular weight of 545 and one tryptophan residue per molecule. Continuously rising end absorption indicated by the difference between the two spectral curves was ascribed to the several amide chromophores present in the molecule.

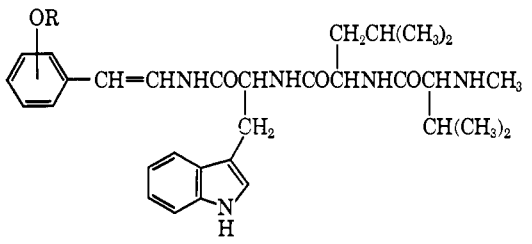
Thus tryptophan, leucine, N-methylvaline, and 2-(hydroxyphenyl)ethylamine, established from hydrolytic, uv, and mass spectral evidence, together accounted for all carbon, nitrogen, and oxygen atoms in americine.[12]

(10) (a) S. Edlbacher and F. Litvan, *Z. Physiol. Chem.*, **265**, 241 (1940); (b) P. A. Plattner and U. Nager, *Helv. Chim. Acta*, **31**, 665, 2192, 2203 (1948); (c) J. C. Sheehan, H. G. Zachau, and W. B. Lawson, *J. Am. Chem. Soc.*, **80**, 3349 (1958).

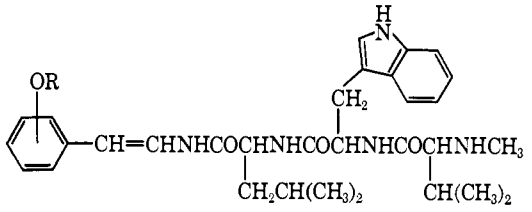
(11) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Vol. 3, John Wiley and Sons, Inc., New York, N. Y., 1961, p 2323.

(12) Any simple comparison of the molecular rotation of americine with that of the peptides composed of the constituent amino acids of americine revealed a large discrepancy. Preliminary ORD results indicate the presence of additional asymmetry in the americine molecule. These stereochemical aspects will be discussed in a future publication.

The base peaks of the mass spectra of most linear peptides as well as most of the peptide alkaloids of known structure corresponded to the N-terminal amino acid of the chain.[5b,6,7,13] Therefore, the fact that the N-methylvaline diagnostic peak at *m/e* 86 in the mass spectra of americine was the base peak implied that N-methylvaline was at a chain terminus. Alkaline properties of americine were attributed to the terminal secondary amine function. Logical working structures were thus III and IV, both of which had empirical

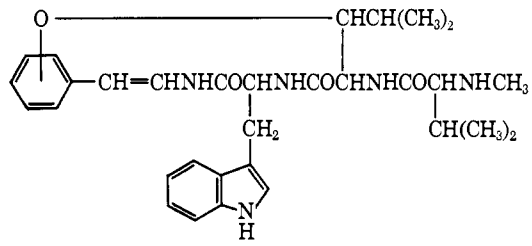


III

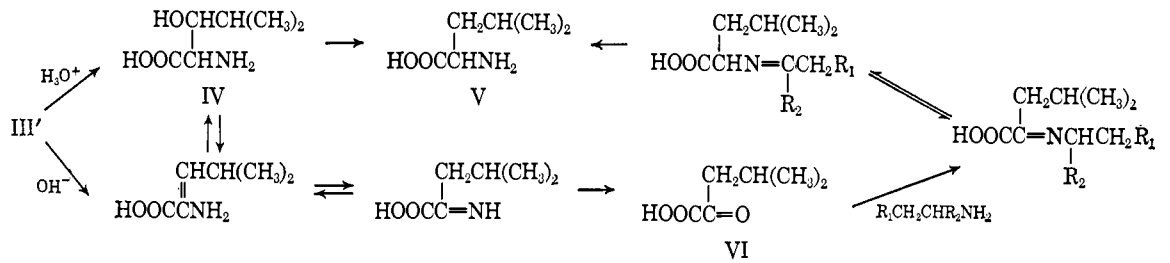


IV

formulas of $C_{31}H_{40}N_5O_4R$ and 14 units of unsaturation. To bring the unsaturation number to the required 15 for americine, introduction of another,



III′



VI

hydrogenation-resistant double bond seemed less reasonable than formation of a ring to the hydroxystyrylamino moiety. This would also permit reconciliation of the uv absorption by invoking a steric factor (see following) to explain the absence of any styrylamine chromophore. In order to account for the very low hydrolytic yields of leucine from the peptide chain, the ring was formed to the β-carbon of the leucyl residue to give working structure III′, $C_{31}H_{39}N_5O_4$.

To rationalize the enhancement of hydrolytic yields of leucine under alkaline conditions [in particular during the hydrolysis of dihydroamericine (Table I)] a general hydrolytic mechanism was proposed for this amino acid, incorporating two previous suggestions.[5,7] At least traces of leucine (V) would arise from 2-hydroxyleucine (IV) under any hydrolytic conditions. Alkaline hydrolysis of americine could produce additional leucine through the transamination of the α-keto acid VI (or α-imino acid) by tryptophan (R_1 = 3-indolyl, R_2 = COOH). Transamination during alkaline hydrolysis of dihydroamericine, in which large quantities of 2-(hydroxyphenyl)ethylamine are present (R_1 = hydroxyphenyl, R_2 = H), would proceed even more efficiently; a concomitant reduction in the yield of 2-(hydroxyphenyl)ethylamine (Table I) verified its partial consumption. Inasmuch as basic nitrogens would be protonated under acidic conditions, the transamination pathway would be inaccessible in acid hydrolysis.

Remaining was the substantiation of the *para* ring fusion of the alkoxystyrylamino grouping intimated by mass spectroscopy of the alkaline hydrolysate of dihydroamericine. Ozonolysis of the styryl double bond[7,14] could produce an alkoxybenzaldehyde, the uv spectrum of which would depend upon the ring substitution pattern. For example, whereas 2-methoxybenzaldehyde had absorption maxima at 253 and 319 mμ, and the 3-methoxy isomer had peaks at 252 and 310 mμ, 4-methoxybenzaldehyde had an absorption maximum at 277 mμ and distinctive shoulders at 282 and 290 mμ.[15] However, integrity of the indole nucleus in americine would be lost upon ozonolysis to yield an N-formylkynurenyl peptide,[16] which would introduce additional complexity to the uv spectrum of the total ozonolysis product of americine (VII). Microozonolysis of indole and subsequent decomposition of the ozonide presumably to 2-formylaminobenzaldehyde gave a product exhibiting an absorption maximum at 262, a minimum at 287, and a shoulder at 270 mμ.

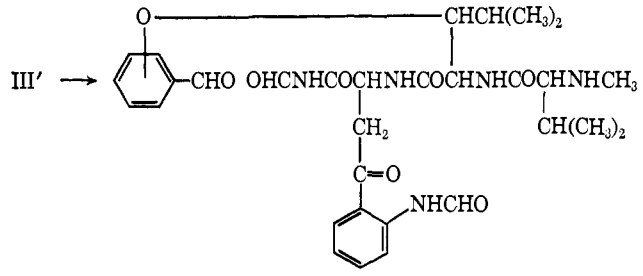
Upon microozonolysis and conversion of the ozonides to aldehydes with trimethyl phosphite, americine gave a product with a uv spectrum displaying a peak at 273 mμ and shoulders at 282 and 290 mμ. Moreover, a quite definite, strong shoulder at 262 mμ, characteristic of the ozonized tryptophan residue, was clearly visible in the spectrogram. Approximate calculations based on measured extinction coefficients indicated that the yield of perozonized product was ubout 75%. This unequivocal evidence for a *p*-alkoxystyrylamino chromophore permitted formulation of the proposed

(13) G. A. Junk and H. J. Svec, *Anal. Biochem.*, **6**, 199 (1963).

(14) P. Kolsaker and P. S. Bailey, *Acta Chem. Scand.*, **21**, 537 (1967).

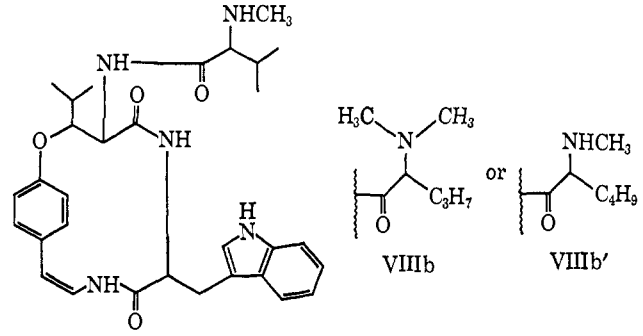
(15) M. J. Kamlet, Ed., "Organic Electronic Spectral Data," Vol. 1, Interscience Publishers, Inc., New York, N. Y., 1960, p 191. Also measured in these laboratories.

(16) (a) M. Morishita, F. Sakiyama, and K. Narita, *Bull. Chem. Soc. Japan*, **40**, 433 (1967); (b) A. Previero and M. A. Coletti-Previero, *Compt. Rend.*, **264**, 633 (1967).



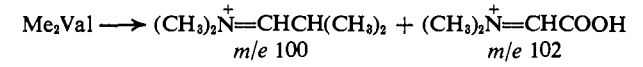
VII

structures of americine (VIIIa) and homoamericine (VIIIb,b′). Molecular models revealed that the styryl double bond is twisted out of the plane of the benzene ring and hence out of conjugation, thus making a negligible contribution to the uv spectrum of americine, as is observed.

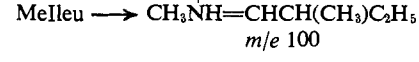


VIIIa VIIIb VIIIb′

Evidence suggesting but not establishing that homoamericine terminated in the N-methylhomoamino acid (either N-methylleucine, N-methylisoleucine, or N-methylnorleucine), rather than the N,N-dimethyl derivative, was supplied by the mass spectrum of the alkaline hydrolysate of dihydroamericine–dihydrohomoamericine. Authentic N,N-dimethylvaline displayed diagnostic peaks at *m/e* 100 (51% of base peak at *m/e* 44) and *m/e* 102 (56%); N-methylisoleucine, for example, had a peak only at *m/e* 100. No *m/e* 102



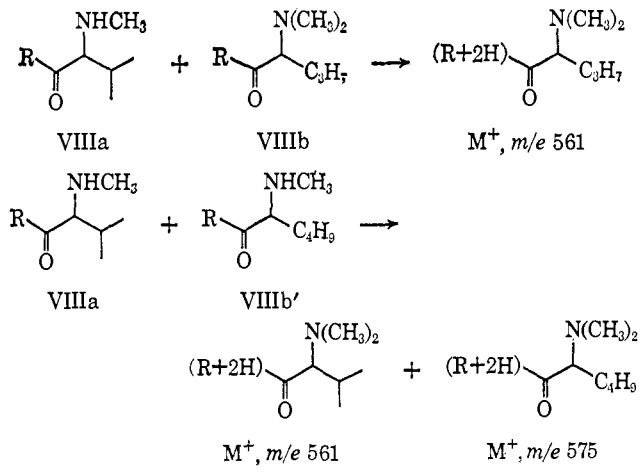
m/e 100 *m/e* 102



m/e 100

peak was present in the mass spectrum of the hydrolysate. Conclusive evidence was provided by the observation of *m/e* 561 and *m/e* 575 molecular ion peaks in the mass spectrum of dihydromethylamericine–dihydromethylhomoamericine, obtained by reductive methylation of the basic nitrogens. If homoamericine had terminated in a dimethylamino group, methylation would have transformed dihydroamericine into dihydrohomoamericine, and only a single molecular ion peak at *m/e* 561 would have been observed.

Straightforward fragmentations consistently rationalized formation of the principal ions in the mass spectra of americine and dihydroamericine (Figure 1). Moreover, the spectra furnished evidence against three additional possible structures, IX, X, and XI, originating from working structures III and IV. Beyond the fact that the 11-membered rings in IX and X, involving only seven atoms to bridge *para* positions, could not exist

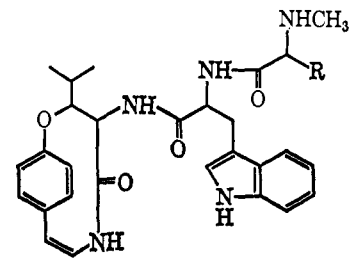


VIIIa VIIIb M+, *m/e* 561

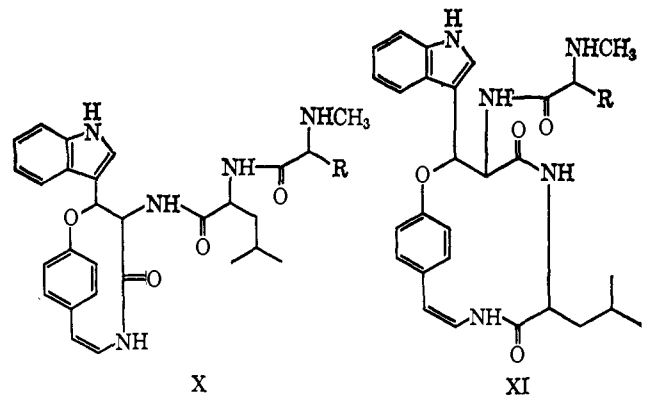
VIIIa VIIIb′

M+, *m/e* 561 M+, *m/e* 575

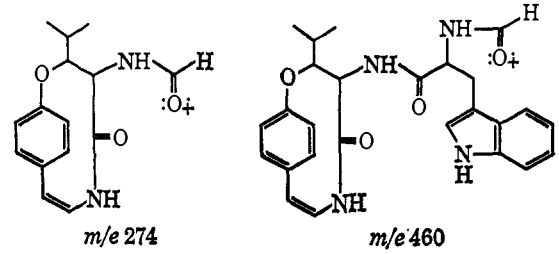
without incredible distortions of bond lengths and angles, structure IX should have led to a *m/e* 274 fragment resulting from cleavage of a dipeptide residue from the molecule in analogy to the observed *m/e* 460 ion. No *m/e* 274 ion was observed in the mass spectrum of americine. Structures X and XI could not



IX



X XI



m/e 274 *m/e* 460

rationalize the formation of a *m/e* 97 ion ($C_6H_9O^+$) or the abundant *m/e* 130 ion ($C_9H_8N^+$) as easily as could VIII. Finally structures X and XI would give relative yields of tryptophan and leucine exactly the reverse of those actually observed in the acidic and alkaline hydrolyses.

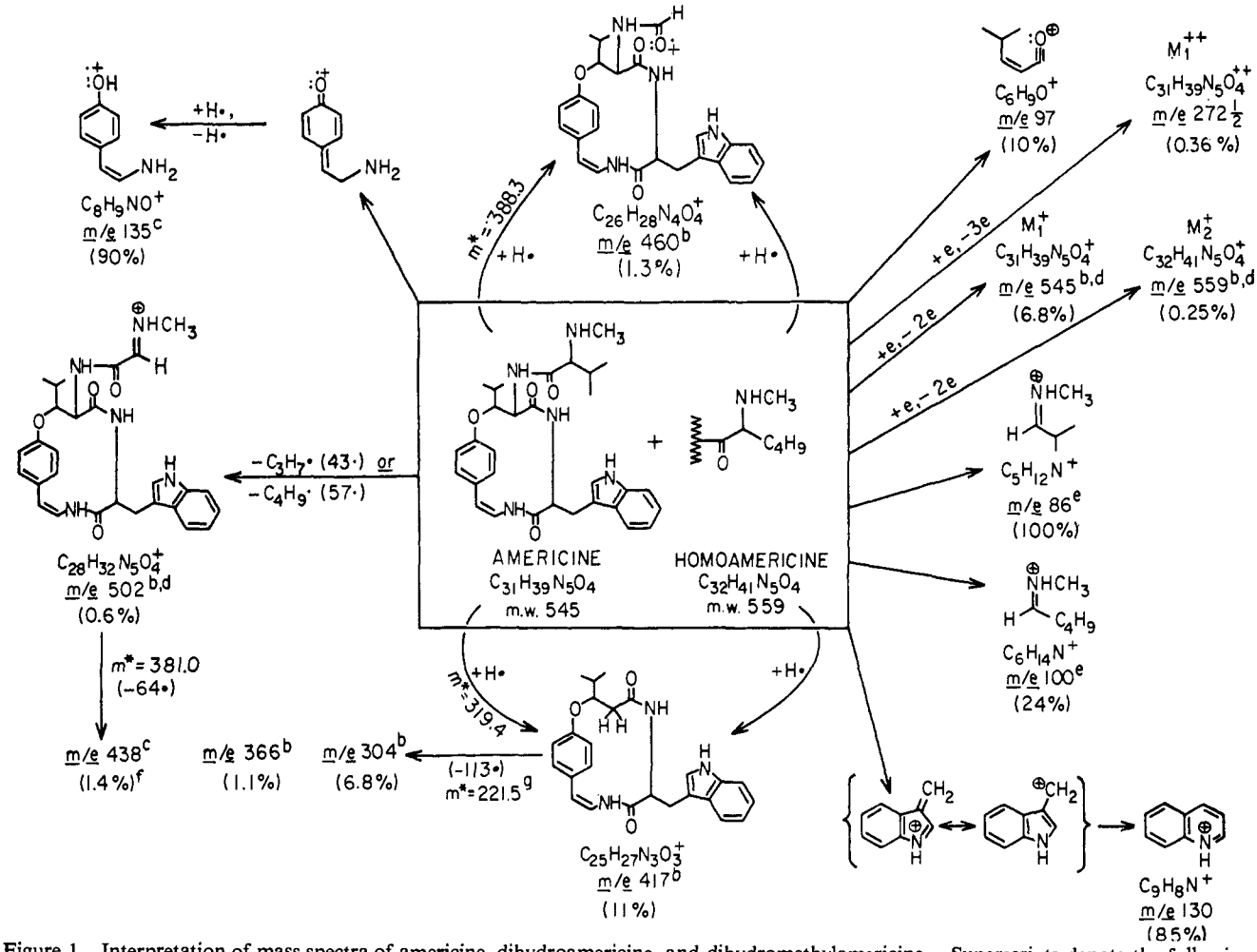
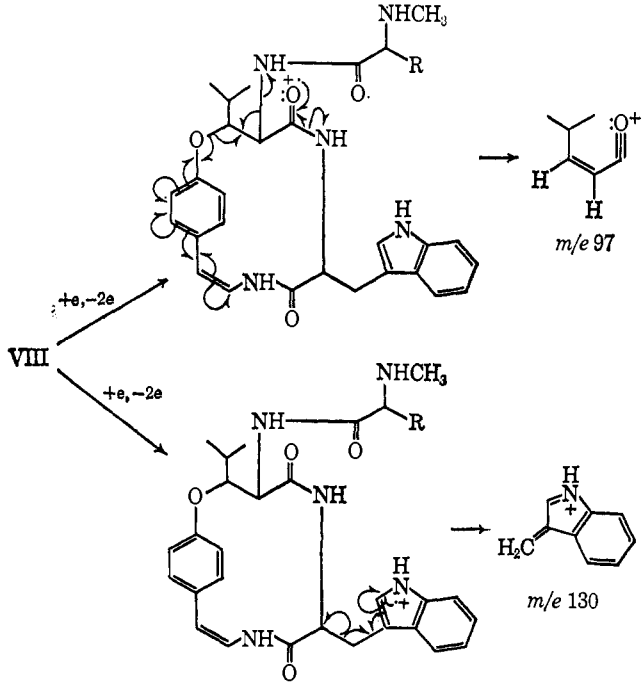


Figure 1. Interpretation of mass spectra of americine, dihydroamericine, and dihydromethylamericine. Superscripts denote the following. [a] Relative abundances measured on a C.E.C. 21-103c. [b] In mass spectrum of dihydroamericine, peak appears at $m/e + 2$ mass units. [c] Absent in mass spectrum of dihydroamericine. [d] In mass spectrum of dihydromethylamericine, peak appears at $m/e + 16$ mass units. [e] In mass spectrum of dihydromethylamericine, peak appears at $m/e + 14$ mass units. [f] Relative abundance increases with length of heating time in the spectrometer. [g] Metastable peaks are indicated by m^* (A.E.I. MS-9).



High-resolution mass spectrometry[17] located metastable peaks and supplied accurate mass measurements which verified the proposed fragmentation of americine (Table III).

High-resolution nmr spectroscopy accounted for virtually all of the protons in the proposed structure of americine (Table IV). Peaks in the region $0.70 < \delta < 4.0$ ppm accounted for 21–23 protons. Peaks in the region $6.01 < \delta < 8.11$ ppm accounted for eight aromatic protons, three amide protons, and two vinyl protons. Thus, a total of at least 34–36 protons had visible absorptions in the spectrum. Particularly interesting were the two different chemical shifts of the ring isopropyl methyl protons (δ 0.88 and 1.08), presumably a phenomenon of diamagnetic anisotropy already observed with other peptide alkaloids.[6b,7]

Americine shows greater structural similarity to peptide alkaloids isolated from other plants[4b,5,7,8] than to ceanothine-B,[6b] for which an *o*-alkoxystyrylamino residue in a nine-membered ring has been proposed. Perhaps both macrocyclic systems are produced by *Ceanothus americanus;* further investigations of this alkaloid-rich plant will be of considerable interest.

Experimental Section

General Experimental Procedures. Corrected melting points were determined on a Kofler hot stage. Optical rotations were

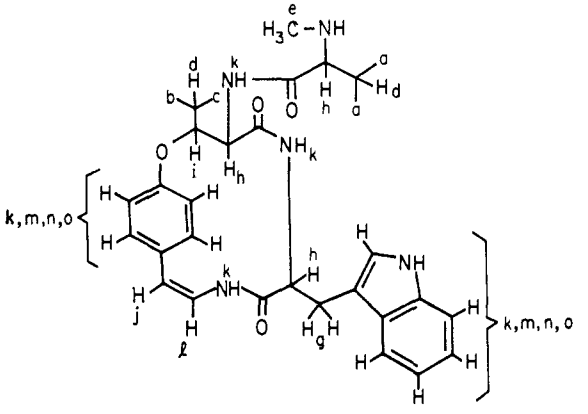
(17) Courtesy of Dr. Emilio Gallegos and Dr. J. B. Lavigne, Chevron Research Corporation, Richmond, Calif.

Table III. High-Resolution Mass Spectrum of Americine

Measured peak (C.E.C. 21-110)	Measured peak (A.E.I. MS-9)	Measured peak (A.E.I. MS-902B)	Possible empirical formulas[a]	Theoretical *m/e*[b]
	86.09791	86.09791	$C_5H_{12}N^+$	86.09697
	97.06572		$C_6H_9O^+$	97.06534
	100.11250	100.11260	$C_6H_{14}N^+$	100.11262
130.0657			$C_9H_8N^+$	130.06567
417.2033			$C_{25}H_{27}N_3O_3^+$	417.20523
438.2503	438.25612	438.24779	$C_{25}H_{32}N_3O_4^+$	438.23927
			$C_{24}H_{32}N_5O_3^+$	438.25050
		502.24520	$C_{28}H_{32}N_5O_4^+$	502.24541
		545.33033	$C_{36}H_{39}N_3O_2^+$ [c]	545.30521
			$C_{31}H_{39}N_5O_4^+$	545.30019
559.3184			$C_{32}H_{41}N_5O_4^+$	559.31584
561.3330[d]			$C_{32}H_{43}N_5O_4^+$	561.33149

[a] Measured values of peak positions were used as raw data to calculate all appropriate empirical formulas by the algorithm of J. Lederberg in "Structural Elucidation of Natural Products by Mass Spectrometry," Vol. 2, H. Budzikiewicz, C. Djerassi, and D. H. Williams, Ed., Holden-Day, Inc., San Francisco, Calif., 1964. [b] Atomic weight *C* = 12,000. [c] Elemental ultramicroanalytical data invalidate this formula as a possible molecular ion of americine. [d] Dihydromethylamericine.

Table IV. High-Resolution Nmr Spectroscopy of Americine



amercine ($C_{31}H_{39}N_5O_4$) (VIII)

Absorption, δ	Multiplicity	*J*, cps	Integration	Assignment
0.70	d (with extra splitting)	3.7	6	a
0.88	d	6.2	3	b
1.08	d	6.2	3	c
1.48	Quint or sept	1.5	2–3	d
1.80	s	...	3	e
[2.30	s	...	2–3	f][a]
2.68	m	...	2–3	g
3.50	Quint or sept	1.5	2–3	h
6.01	m	...	1	i
6.42	d	9.0	1	j
6.86	Broad	...	6	k
7.18	d	9.0	1	l
7.35	m	...	3	m
8.03	m	...	1	n
8.11	s	...	1	o

[a] The peak is probably due to an impurity and is visible only in a perdeuteriomethanol solution of americine. Perdeuteriodimethyl sulfoxide has a multiplet at around δ 2.3, which obscures the peak; perdeuteriomethanol has its multiplet at around δ 3.

measured with a Zeiss polarimeter. Ultraviolet spectra were taken in methanol on a Cary 14 spectrophotometer, infrared spectra as KBr disks at three different concentrations on a Perkin-Elmer 337 grating spectrometer, and nmr spectra in perdeuteriodimethyl sulfoxide on a Varian HA-100 spectrometer (reported as δ values, TMS, δ = 0). Mass spectra were determined on a Varian M-66 or on a Consolidated Electrodynamics Corporation 21-103c spectrometer at electron energies of 70 eV. High-resolution mass spectra were measured on an Associated Electronics Industries MS-9 or MS-902B or a Consolidated Electrodynamics Corporation 21-110 spectrometer at 70 eV. The method of direct insertion into the ion source was consistently employed. Peptide hydrolyses were performed in sealed tubes with constant-boiling HCl or with $Ba(OH)_2 \cdot 8H_2O$ for 24 hr at 105 ± 2° in the manner standard to biochemical practice. Thin layer chromatography was carried out on 200 × 50 × 0.25 mm layers of Kieselgel G D-5 (Camag) prepared in the standard manner, activated at 105° for 30 min, and protectively stored under conditions of normal laboratory humidity. Automatic amino acid analyses were run on a Beckman/Spinco Model 120B analyzer.[18] Elemental ultramicroanalyses were performed by the Microanalytical Laboratory, operated by the Department of Chemistry, University of California, Berkeley, Calif.

Isolation of Americine (VIII).[19] Dried, ground *C. americanus* root bark (23.4 kg) supplied by Flint, Eaton and Co., Decatur, Ill. (now Baxter Laboratories, Morton Grove, Ill.), was soaked overnight in excess 95% ethanol. The suspension of bark was added to a large continuous extractor constructed from a 10-gal. Pyrex jar assembled with a steam-heated 24-l. solvent reservoir. After continuous extraction of the bark with 95% ethanol for 48 hr, the 22 l. of deep red extract was brought to pH 8.3 with 4 l. of 4.5 *N* potassium hydroxide and filtered. The filtrate, combined with 2 l. of ethanol washings, was neutralized with tartaric acid and filtered through Celite after standing overnight. This filtrate (28 l.) was evaporated to a viscous residue under reduced pressure and shaken with a mixture of 750 ml of ethyl acetate plus 2250 ml of 0.5 *N* tartaric acid. The system was filtered to separate a gelatinous organic mass, and the filtrate was concentrated under reduced pressure to remove additional ethyl acetate. Treatment of the resultant alkaloidal solution with 15 *N* ammonium hydroxide produced a brown precipitate which was filtered and washed with 2 l. of water. Succeeding basifications of the mother liquors resulted in three more crops of crude alkaloid totalling 52.3 g.

Reextraction of the marc from the initial solvent treatment was continued in the same apparatus for 4 days, until the extracts were colorless. The extract, processed as described above, yielded additional product for a combined yield of 194 g of dried crude alkaloid (0.83% of root bark).

A suspension of the crude alkaloid was refluxed 30 min in 440 ml of absolute ethanol plus 350 ml of benzene, diluted to 4.6 l. with benzene, refluxed 1.5 hr, and diluted to 5.5 l. with benzene. Cooling and filtration left a dark brown residue of 26.0 g. The filtrate was chromatographed on 1 kg of alkali-free alumina and eluted with 4 l. of benzene, 1 l. each of 14, 24, 36, 54, and 54% absolute ethanol in benzene, followed by 5 l. of absolute ethanol. (Alkali-free alumina was prepared by slurrying 3 lb of Merck Reagent alumina with 1 l. of ethyl formate for 17 hr, filtering, washing with 4 lb of petroleum ether (bp 30–60°), air drying, and finally drying at 140° for 18 hr.) Three fractions were collected. Fraction I, combined with a 24-hr continuous benzene extract of the 26.0 g of dark brown residue obtained above, yielded 141.6 g of a white solid upon evaporation. Fractions II and III contained 12.9 and 6.3 g of material, respectively.

The material from fraction II was dissolved in refluxing benzene. On being cooled to room temperature the solution deposited a gelatinous precipitate which gave 9.76 g of an orange-white solid upon filtration and air drying. Five crystallizations of this material from benzene–absolute ethanol yielded 3.9 g of americine (2.0% of the crude alkaloid and 0.017% of the root bark). All filtrates obtained in the work-up of fraction II were combined with fraction III.

The isolated alkaloid exhibited the following properties: mp 135.5–137.0° and 142–182° (two crystal habits microscopically visible); two contiguous spots upon tlc (4% minor, less polar component); $[\alpha]^{20}D$ −198° (*c* 0.51, methanol); ν_{max} 3260 (intense and broad), 1625 (intense and broad), 1600, 1575, 1480, 1440, 1325, 1225, 1130, 1060, 960, 850, and 733 cm^{-1}; λ_{max} 221 mμ (ε 40,130), 273 (6280), 280 (6279), 290 (5057); λ_{min} 258 (5966), 288 (4000), no

(18) Automatic amino acid analyses were very generously conducted by Dr. Jonathan Dixon of the Hormone Research Laboratory, University of California Medical Center, San Francisco, Calif.

(19) We are deeply indebted to Dr. J. B. Lavigne, now of the Chevron Research Corporation, Richmond, Calif., for the large-scale extraction of *C. americanus* root bark which he conducted while in these laboratories.

acid or base shifts; δ 0.70 (6 H, d, J = 3.7 cps), 0.88 (3 H, d, J = 6.2 cps), 1.08 (3 H, d, J = 6,2 cps), 1.80 (3 H, s), 6.42 (1 H, d, J = 9.0 cps), and 7.18 ppm (1 H, d, J = 9.0 cps); *m/e* 86 (base peak; 100%), 97, 100, 130, 135, 141, 155, 170, 181, 186, 197, 304, 366, 417, 438, 460, 502, 545 (M^+; 6.8%), and 559 (M^+; 0.25%).

Anal. Calcd for $C_{31}H_{39}N_5O_4$ (mol wt, 545): C, 68.2; H, 7.2; N, 12.8; O, 11.7; $2CCH_3$, 5.5; NCH_3, 2.8. Found: C, 68.2; H, 7.3; N, 12.9; O, 11.6; CCH_3, 5.2; NCH_3, 2.8.

Dihydroamericine (II). Americine (15 mg) was dissolved in 3 ml of dioxane. Hydrogen was bubbled through the solution in the presence of 5 mg of PtO_2 at room temperature for 30 min. The supernatant was evaporated to dryness to yield thin layer chromatographically homogeneous (R_f values smaller than those for americine) dihydroamericine, purely the dihydro derivatives as estimated by tlc and mass spectroscopy.

Dihydromethylamericine. After hydrogen was bubbled through a suspension of 5 mg of PtO_2 in a solution of 15 mg of americine in 3 ml of dioxane for 30 min, 0.25 ml of 37% aqueous formaldehyde was added to the reaction. After 30 min of additional hydrogen treatment, the supernatant of the reaction mixture was evaporated to dryness to yield thin layer chromatographically homogeneous (R_f values larger than those for dihydroamericine) dihydromethylamericine, purely the dihydromethyl derivatives as estimated by tlc and mass spectroscopy.

N-Tosyl-N-methylvaline was prepared from 0.04 mol of amino acid by the method of Fischer.[20]

N-Methylvaline Hydrochloride. The N-tosylamino acid was hydrolyzed in a sealed tube with a 20-fold molar excess of HCl at 105° for 24 hr.[20] The cooled reaction mixture was brought to pH 5.5 with 12 *N* NaOH and filtered, and the filtrate was chromatographed on 200–400 mesh BioRad AG-X4 ion-exchange resin with elution by 1500 ml of water and 300 ml each of 1, 2, 4, and 6 *N* HCl. Evaporation to dryness under reduced pressure at 80° of the intermediate fractions yielded a residue which was purified by sublimation at 200°, 10–50 μ. Mass spectrometry and tlc indicated that the sublimate consisted of both amino acid hydrochloride and N-methylamino acid hydrochloride. Visible in the mass spectrum were peaks at *m/e* 72 (71%; $H_2N^+{=}CHCH(CH_3)_2$), 74 (54%; $H_2N^+{=}CHCOOH$), 86 (100%; $CH_3N^+H{=}CHCH(CH_3)_2$), and 88 (93%; $CH_3N^+H{=}CHCOOH$).

N-Tosyl-N-methylisoleucine was prepared exactly in the manner described above for valine.

N-Methylisoleucine Hydrochloride. A mixture of isoleucine hydrochloride and N-methylisoleucine hydrochloride (as determined by tlc and mass spectroscopy) was prepared in the manner described above for valine. Visible in the mass spectrum were peaks at *m/e* 74 (42%; $H_2N^+{=}CHCOOH$), 86 (75%; $H_2N^+{=}CHCH(CH_3)CH_2CH_3$), 88 (93%; $CH_3N^+H{=}CHCOOH$), 100 (100%; $CH_3N^+H{=}CH(CH_3)CH_2CH_3$).

Tlc Detection of N-Methylvaline from Americine. Americine (15 mg) was hydrolyzed with base in the standard manner. The desiccated residual hydrolysate was dissolved in pH 2.2 buffer, and a 1-μl aliquot was analyzed by tlc using water as a most effective eluent. Upon development of the eluted chromatograms with a spray of 1% *p*-nitrobenzoyl chloride in pyridine, a spot was observed consistently corresponding in R_f to authentic N-methylvaline. No N-methylisoleucine was detected in a parallel tlc analysis of a 25-μl aliquot of the hydrolysate solution.

2-(2-Hydroxyphenyl)ethylamine Hydrochloride. The method of Hahn and Rumpf[21] gave 20.0 g (50% yield from 2-hydroxybenzaldehyde) of 2-hydroxy-β-nitrostyrene, mp 133–134°. The procedure of Ramirez and Burger[22] for the preparation of 2-(3-methoxy-4-hydroxyphenyl)ethylamine hydrochloride was adapted to the synthesis of 0.5 g (43% yield from the nitrostyrene) of 2-(2-hydroxyphenyl)ethylamine hydrochloride, purified by sublimation at 180°, 5 μ. Chromatographically homogeneous in 16 tlc eluent systems, the product had the following properties: mp 156.0–156.5° (lit.[23] mp 155°); *m/e* 30 (100%), 39, 51, 77, 91, 107 (5%), 108 (7%), 137 (M^+) (Varian M-66, 40–60° probe).

2-(3-Hydroxyphenyl)ethylamine Hydrochloride. By using only a 15-min condensation time, the yield in the synthesis of Hahn and Rumpf[21] was increased 30% to give 15.5 g of 3-hydroxy-β-nitrostyrene, mp 135–136°. The procedure of Ramirez and Burger[22] was adapted to the synthesis of 8.5 g (52% yield from the nitrostyrene) of 2-(3-hydroxyphenyl)ethylamine hydrochloride, purified by sublimation at 120°, 60 μ. Chromatographically homogeneous in 16 tlc systems, the product had the following properties: mp 142–143° (lit.[24] mp 145°); *m/e* 30 (100%), 39, 51, 77, 91, 107 (12%), 108 (10%), and 137 (M^+) (same instrumental conditions as for the mass spectrometry of the 2-hydroxy isomer).

2-(4-Hydroxyphenyl)ethylamine Hydrochloride. Tyramine hydrochloride (Eastman 2028) was purified by sublimation at 110°, 60 μ. Chromatographically homogeneous in 16 tlc systems, the compound exhibited the following peaks: *m/e* 30 (100%), 39, 51, 77, 91, 107 (72%), 108 (93%), and 137 (M^+) (same instrumental conditions as for the mass spectrometry of the 2-hydroxy isomer).

Mass Spectrum of the Acidic Hydrolysate of Americine. Dihydroamericine (10 mg) was hydrolyzed with acid in the standard manner. After desiccation, the residual hydrolysate was taken up in 1 ml of methanol. An aliquot was evaporated to dryness in a capillary tube and subjected to mass spectrometry (Varian M-66). Spectral scans were taken at sample temperatures of 50–125°. Optimum spectra were obtained at 115°: *m/e* 28, 30, 36 (HCl^+), 38 (HCl^+), 44, 50, 86 (100%), 100 (10%), 107 (35%), 108 (50%), 130 (25%), 153, 181, and 224.

Ozonolysis of Americine. An excess of ozone-enriched oxygen was bubbled through a solution of 1.45 mg of americine in 0.2 ml of methanol at −65° for 15 min using a flow-through microozonizer.[25] The solution was then swept while cold with nitrogen for 5 min, treated with 0.3–7 μl of trimethyl phosphite, permitted to attain room temperature, diluted to 50 ml with methanol, and subjected to uv spectrophotometry. (In a separate experiment, trimethyl phosphite was found to make no contribution to the uv spectrum at the concentrations employed.)

Ozonolysis of Indole. Ozone-enriched oxygen was bubbled through a solution of 0.31 mg of indole in 0.2 ml of methanol at −65° (Dry Ice–acetone) for 15 min using a flow-through microozonizer.[25] The reaction mixture, permitted to attain room temperature, was treated with 0.2 ml of 50% formic acid, warmed to 70° under a stream of nitrogen and evaporated to 0.2 ml, diluted to 0.5 ml with water, basified with 0.2 ml of 4.5 *N* KOH, and extracted exhaustively with 0.3-ml portions of chloroform. The chloroform extracts, diluted to 50 ml with chloroform, were subjected to uv spectrophotometry.

N,N-Dimethylvaline Hydrochloride. Valine hydrochloride was reductively alkylated to N,N-dimethylvaline hydrochloride by the procedure of Bowman and Stroud.[26] Further purification was accomplished by sublimation at 140°, 100 μ. The mass spectrum showed important peaks at *m/e* 100 [51%; $(CH_3)_2N^+{=}CHCH(CH_3)_2$], 102 [56%; $(CH_3)_2N^+{=}CHCOOH$], and 145 (4%; M^+).

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