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Ceanothine-B, a Naturally Occurring Oxazacyclononadiene

Sir:

The root bark of *Ceanothus americanus* (rhamnaceae) has a long medical history and the presence therein of alkaloids, reported to have hypotensive properties,[1] has been realized for almost a century.[2] Although there have been sporadic attempts to resolve the complex mixture of bases, up to the present these efforts have been unsuccessful.[3,4]

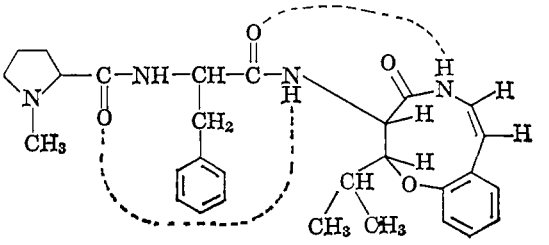
We wish to report evidence which establishes structure I for ceanothine-B, the major alkaloid of *C. americanus*. This structure includes the unusual feature of an oxazacyclononadiene ring common to all the ceanothus alkaloids we have examined.

Ceanothine-B, $C_{29}H_{36}O_4N_4$, m.p. 238.5–240.5°, $[\alpha]_D$ −293° (chloroform), is monobasic.[4] Quantitative comparison of the integrated carbonyl infrared absorption of the alkaloid, its derivatives, and model compounds disclosed the presence of three amide groups, accounting for the function of all four nitrogen atoms and three of the oxygen atoms. By elimination the remaining oxygen atom is ethereal since no hydroxyl or other carbonyl group could be detected. There are three amide NH peaks visible in the n.m.r. spectrum. Hydrogenation (Pd–C) saturated one *cis* double bond (–HC=CH–, δCD_3COOD = 6.01 and 6.82 p.p.m., J = 8 c.p.s.), whose vinyl protons are coupled only to each other in perdeuterioacetic acid, and produced dihydroceanothine-B, $C_{29}H_{38}O_4N_4$,[5] m.p. 272–278°, $[\alpha]_D$ −87° (methanol).

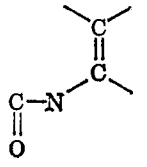
From the extremely intense base peak at *m/e* 84 in the mass spectra of the alkaloid and its dihydro derivative and the fact that the basic tertiary nitrogen atom bears one N-methyl group (n.m.r.), the N_{basic}-terminal group is N-methylproline.[4] The mass spectrum also contained peaks at *m/e* 91, 103, 120, and 131 characteristic of β-phenylalanine.[6] The presence of both these amino acids was verified by 6 *N* hydrochloric acid hydrolysis of dihydroceanothine-B and thin layer chromatographic comparison with authentic specimens. From the hydrolysate was isolated a compound, $C_{23}N_{29}O_3N_3$, m.p. 248–254°, $[\alpha]_D$ +63° (ethanol), dihydroceanothine-B minus the N-methylprolyl group.

(1) J. T. Groot, *J. Pharmacol. Exptl. Therap.*, **30**, 275 (1927); H. Wastl, *Federation Proc.*, 7, 131 (1948); A. A. Manian, Ph.D. Thesis, Purdue University, 1954.
(2) J. H. M. Clinch, *Am. J. Pharm.*, **56**, 131 (1884).
(3) A possible exception is the ceanothine of A. Bertho and W. S. Liang, *Arch. Pharm.*, 271, 273 (1933), which may be identical with the ceanothine-B described in this work.
(4) See E. W. Warnhoff, S. K. Pradhan, and J. C. N. Ma, *Can. J. Chem.*, **43**, 2594 (1965), for details of the isolation and purification.
(5) Satisfactory analytical data have been obtained for all new compounds reported.
(6) K. Heyns and H.-F. Grützmacher, *Ann.*, **669**, 191 (1963).

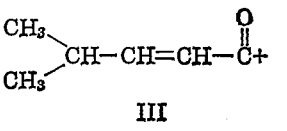
Subtraction of the N-methylprolyl and phenylalanyl residues from the molecular formula of ceanothine-B leaves $C_{14}H_{18}O_2N_2$. A C-methyl determination on the alkaloid or its dihydro derivative gave, as one of the volatile acids, isobutyric acid which could only have come from the C_{14} unit. In the n.m.r. spectrum of ceanothine-B the isobutyl *gem*-dimethyl group is the only C-methyl absorption present and appears as a pair of doublets at δ_{CDCl_3} 0.93 and 1.22 p.p.m. (J = 6.5 c.p.s.). The presence of an *ortho*-disubstituted benzene ring in addition to the phenyl group was shown by the n.m.r. (9 aromatic H) and infrared (strong peak at 757 cm.$^{-1}$) spectra. The ultraviolet chromophore, λ_{max} ~250 mμ (ε 4000), removed on hydrogenation is that of an enamide group (II). The C_{14} unit must therefore include isobutyl, *ortho*-substituted phenyl, and enamide fragments, further elaboration of which is provided by the following experiments.



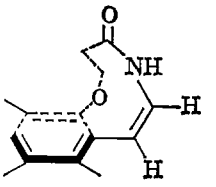
I



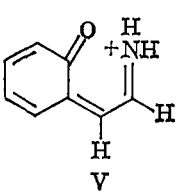
II



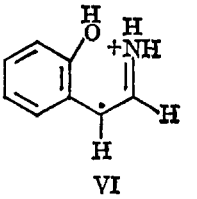
III



IV



V



VI

Since lithium aluminum hydride reduction of all three amide groups to amino functions did not change the ultraviolet chromophore (λ_{max} 230 mμ (ε 9000) and 280 mμ (ε 1000)) of dihydroceanothine-B, whether the polyamine was in ethanol or ethanolic hydrochloric acid solution, the aromatic moiety in the C_{14} unit is present as an *o*-alkyl phenol ether. In the mass spectra of all five pure ceanothus alkaloids and of dihydroceanothine-B is an intense peak at *m/e* 97. This peak must arise from some common unit and not from the N-methylprolyl or phenylalanyl groups which are known to be absent from most of the alkaloids. Nor can the peak result from the enamide or *o*-alkyl phenol ether,[7] but must, by exclusion, involve the isobutyl group. The most reasonable formula for the *m/e* 97 ion is $C_6H_9O^+$ for which the structure III follows. In support of a substituted isocaproic acid

(7) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Interpretation of Mass Spectra of Organic Compounds," Holden-Day, Inc., San Francisco, Calif., 1964, pp. 174–183.

formulation the n.m.r. spectrum of each of the five alkaloids has peaks at δ = ~4.4 (1 H, m) and 4.93 p.p.m. (1 H, d of d, J = 1.5, 7 c.p.s.) which are not removed by hydrogenation. These signals are assigned, respectively, to protons on the α- and β-carbon atoms of the isocaproic chain bearing, respectively, a nitrogen and an oxygen atom, the nitrogen atom being assigned the α-position on biogenetic grounds. Chemical confirmation of an α,β-disubstituted isocaproic acid fragment was provided by sulfuric acid hydrolysis of ceanothine-B to yield α-ketoisocaproic acid isolated as the *syn*- and *anti*-2,4-dinitrophenylhydrazones, identical with authentic specimens.

Joining of these expanded fragments of the C_{14} unit leads to a unique structure containing the nine-membered ring in I. Although the enamide double bond is formally conjugated with the aromatic ether and would be expected to have λ_{max} 319 mμ (ϵ ~8000),[8] ceanothine-B has no ultraviolet absorption beyond 300 mμ. The explanation is clear from a Dreiding model which shows that the plane of the double bond is held almost perpendicular to the plane of the aromatic ring, as in IV, and hence the ultraviolet spectrum is merely the superposition of the enamide and *o*-alkyl phenol ether chromophores. In agreement with this formulation for the C_{14} unit, there are strong peaks at *m/e* 134 and 135 attributed to the ions V and VI (or equivalents) in the mass spectra of each of the alkaloids but not of dihydroceanothine-B. Attachment of the two amino acid residues to the C_{14} unit being possible in only one sense, ceanothine-B, therefore, has the complete structure I.

Of the four asymmetric carbon atoms present, the three α-amino carbon atoms are assumed to have the L configuration. The coupling constants of the δ 4.93 p.p.m. doublet of doublets indicates the *threo* configuration for the β-carbon of the aryloxyleucine. The shift in position of the N-methyl group from δ_{CDCl_3} 1.97 to 2.15 p.p.m. on hydrogenation of ceanothine-B is understandable if this apparently distant group is actually held close to one face of the double bond by intramolecular hydrogen bonding of the amide groups (see dotted lines in I).

Since all five of the ceanothus alkaloids examined have the same basic ultraviolet spectrum which undergoes the same change on hydrogenation, and since all five contain the discernible n.m.r. peaks of the C_{14} unit, this unit is probably present in all of these bases. The ceanothus alkaloids have certain features relating them to the other recently discovered peptide alkaloids in which there is considerable current interest.[8-10]

Acknowledgment. This work was supported by the National Heart Institute under Grant HE-07394 (formerly H-5762).

(8) E. Zbiral, E. L. Ménard, and J. M. Müller, *Helv. Chim. Acta*, **48**, 404 (1965).
(9) M. Païs, X. Monseur, X. Lusinchi, and R. Goutarel, *Bull. soc. chim. France*, 817 (1964).
(10) M. Païs, J. Mainil, and R. Goutarel, *Ann. pharm. françs.*, **21**, 139 (1963).
(11) Fellow of the Alfred P. Sloan Foundation.

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Nucleophilic Assistance in the Acid-Catalyzed Reactions of Acetals and Glycosides[1]

Sir:

The possibility that in certain enzymically catalyzed hydrolyses of glycosides the enzyme provides nucleophilic assistance to the rupture of the glycosidic bond has frequently been considered,[2] but there has been little evidence to substantiate or refute the occurrence of such a process. One obvious argument that can be used against the proposal that the enzyme provides nucleophilic assistance is based on the mechanism of the acid-catalyzed hydrolysis of acetals and glycosides being invariably A1 without nucleophilic participation by water in the rate-determining step.[3] It would therefore be of interest if any simple acid-catalyzed reactions of acetals and glycosides which did not proceed by an A1 mechanism could be found. To this end the reactions of the acyclic dimethyl acetals of glucose and galactose in dilute aqueous hydrochloric acid were studied. Under these conditions, besides undergoing hydrolysis to glucose and galactose, these acetals also yield a mixture of the α- and β-furanosides by a concurrent ring closure.[4] The products and rates of these reactions are shown in Table I. Two mechanisms

Table I. Kinetically Controlled Products and Rate Constants for the Reactions of Some Acyclic Aldose Acetals in 0.05 *M* HCl at 35°

Dimethyl acetal of	Products, % Aldose	Furanosides	Pyranosides	$10^4 k_{total}$, sec.$^{-1}$
D-Glucose	<2	>98	<0.5	17
D-Galactose	29	71	<0.5	1.58

are possible for the ring closure to furanosides. In mechanism 1 a carbonium ion (I), of the type normally postulated to intervene in the hydrolysis of acetals,[3] is competed for by water and the internal hydroxyl group. In mechanism 2 the ring closure is synchronous with the rupture of the acetal bond. With mechanism 1 the total rate of reaction is the rate of ionization (step 1), and this should be independent of the configuration of carbon 4, but with mechanism 2 the rate of ring closure, and hence the total rate, could well depend on this configuration. The observation that k_{total} for the glucose acetal is about ten times greater than for the galactose acetal and that the product from the glucose acetal contains a much higher proportion of furanosides therefore supports mechanism 2. The anchimeric assistance associated with the ring closures is indicated by the results given in Table II. The rate of

(1) This work was supported by the Department of Scientific and Industrial Research and the Royal Society.
(2) D. E. Koshland in "The Mechanism of Enzyme Action," W. D. McElroy and B. Glass, Ed., The Johns Hopkins Press, Baltimore, Md., 1954, p. 608; E. H. Fisher and E. A. Stein, *Enzymes*, 4, 313 (1960); M. L. Bender and R. Breslow in "Comprehensive Biochemistry," Vol. 2, M. Florkin and E. H. Stotz, Ed., Elsevier Publishing Company, Amsterdam, 1962, p. 38; K. Wallenfels and O.P. Malhotra, *Advan. Carbohydrate Chem.*, **16**, 239 (1961); F. C. Mayer and J. Larner, *J. Am. Chem. Soc.*, **81**, 188 (1959).
(3) See L. L. Schaleger and F. A. Long, *Advan. Phys. Org. Chem.*, **1**, 27 (1963).
(4) The hydrolysis of these acetals was first investigated by M. L. Wolfrom and S. W. Waisbrot, *J. Am. Chem. Soc.*, **61**, 1410 (1939), who concluded that the products were the free aldoses and pyranosides. However, we have been unable to detect any pyranosides by paper chromatography.