copolymer" character presumably is responsible for the variation in X-ray and physical property data observed with type III poly-(methyl methacrylate). The exact nature of the crystals themselves is under more intensive investigation.

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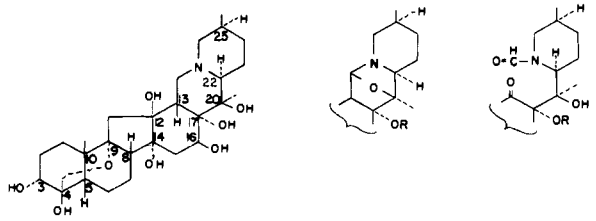
THE CONFIGURATION OF CEVINE

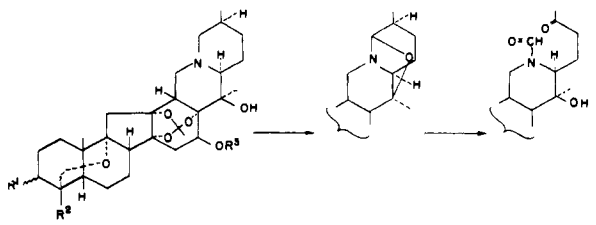
Sir:

The configurations of nine (C_3, C_4, C_5, C_9, C_{10}, C_{12}, C_{14}, C_{17}, C_{25}) of the fourteen asymmetric centers of cevine were established almost simultaneously with the climactic structure elucidation in 1954–55.[1–3] Recent work in our laboratory made possible assignment of configuration at C_{16} and C_{20} and provides support for previously considered[1] configurational assignments at C_8 and C_{13}.[4] Evidence is presented herewith for assignment of configuration at the remaining asymmetric center (C_{22}) of cevine which now can be represented completely by formula I. It is highly probable that closely related alkaloids, such as zygadenine and germine, also have this basic configuration.

Oxidation of veracevine D-orthoacetate triacetate (IIa)[5] (pK_a' 7.4) with N-bromosuccinimide[6] yielded a dehydro compound (m.p. 280–282° dec., $[\alpha]D$ +33° dioxane; found, C, 63.51; H, 7.35; Ac, 25.52), evidently the bridged oxide IIIa for the following reasons. (1) The pK_a' (found, 3.8) was in the lower range expected for a stable carbinolamine ether.[7] (2) The infrared spectrum showed no absorption in the hydroxyl region. (3) The 16-acetate group survived prolonged treatment with methanol and triethylamine, an indication of the absence of an axial hydroxyl at C_{20} to facilitate methanolysis.[4] (4) Chromic anhydride-pyridine oxidation of IIIa afforded a neutral product (m.p. 263–264° dec., $[\alpha]D$ +54° dioxane, λ_{max}^{chf} 6.07 μ, –NCO–, found, C, 60.59; H, 7.17), evidently the formamido ketone IVa. Acid hydrolysis afforded one mole equivalent of formic acid. Alkaline hydrolysis of the corresponding formamido ketone IVb (m.p. 288° dec., $[\alpha]D$ +52° py., λ_{max}^{chf} 6.07 μ; found, C, 60.98; H, 7.08) derived from cevine gave the desacetyl-formamido-ketone IVc (m.p. 259–260° dec., $[\alpha]D$ +22° py., found, C, 61.66; H, 7.24). In addition to the amide band, the infrared spectrum of this substance exhibited normal ketone absorption at 5.85 μ.



I V VI



II III IV

a (R^1 = β-OAc, R^2 = OAc; R^3 = Ac)
b (R^1 = α-OAc, R^2 = OAc; R^3 = Ac)
c (R^1 = α-OH, R^2 = OH; R^3 = H)

The alternative formulations V and VI for the oxide and formamido ketone were excluded on the basis of the following evidence. (1) The formamido ketone IVb derived from cevine readily formed a semicarbazone (m.p. 273–274° dec.; found, C, 57.91; H, 6.80, N, 6.93). (2) The formamido ketone IVa showed active methylene group reactivity in the Zimmermann test[8] and upon treatment in alkaline solution with furfural. (3) The corresponding formamido ketone (m.p. 253–255° dec., $[\alpha]D$ –83° dioxane; found, D, 60.96; H, 7.10) from cevagenine-C-orthoacetate diacetate[5] was, like the parent alkaloid, stable to lead tetraacetate. This behavior is characteristic of a rigid *trans* diaxial glycol system at C_{17}, C_{20}, a situation clearly not satisfied by VI.

The bridged *β-oriented* oxide structure of IIIa and b requires that the hydrogen at C_{22} be α-oriented. That the stereochemical integrity of the molecule was preserved during the oxide formation was demonstrated by catalytic hydrogenation of IIIa over platinum in acetic acid. Two mole-equivalents of hydrogen was absorbed to give a substance identical with the product of hydrogenation (one mole-equivalent uptake) of IIa, namely veracevine-D-dihydroorthoacetate triacetate (m.p. 299–300° dec., $[\alpha]D$ +21° dioxane; found, C, 63.53; H, 7.85; Ac, 19.98).[1,9]

(1) D. H. R. Barton, C. J. W. Brooks and P. De Mayo, *J. Chem. Soc.*, 3950 (1954).
(2) F. Gautschi, O. Jeger, V. Prelog and R. B. Woodward, *Helv. Chim. Acta*, **38**, 296 (1955).
(3) S. M. Kupchan, THIS JOURNAL, **77**, 686 (1955).
(4) S. M. Kupchan and W. S. Johnson, *ibid.*, **78**, 3864 (1956).
(5) S. M. Kupchan, D. Lavie, C. V. Deliwala and B. Y. A. Andoh, *ibid.*, **75**, 5519 (1953).
(6) *Cf.* O. E. Edwards, F. H. Clarke and B. Douglas, *Can. J. Chem.*, **32**, 235 (1954).
(7) H. Bloom and L. H. Briggs, *J. Chem. Soc.*, 3591 (1952).
(8) W. Zimmermann, *Z. physiol. Chem.*, **233**, 257 (1935); D. H. R. Barton and P. de Mayo, *J. Chem. Soc.*, 887 (1954).
(9) This work was supported in part by a grant (H-2275 (C2)) from the National Heart Institute of the National Institutes of Health.

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16α-CHLORO- AND 16α-IODOESTRONE METHYL ETHER, NEW AND POTENT LIPID-SHIFTING AGENTS

Sir:

The problem of finding estrogen-like substances capable of altering blood lipid composition, and which at the same time are not feminizing, is important in clinical treatment of atherosclerosis. A

striking separation of properties has now been demonstrated in certain C-16 halogenated estrone derivatives. Some of these show lipid-shifting to feminizing ratios about one hundred times that of estrone as, for example, 16α-chloroestrone methyl ether (I) and 16α-iodoestrone methyl ether (II).

The enol acetate of estrone methyl ether[1] in carbon tetrachloride was treated with chlorine and potassium carbonate to give I, m.p. 177–179°; $[\alpha]_D$ +161°;[2] (*Anal.* Calcd. for $C_{19}H_{23}ClO_2$: C, 71.57; H, 7.27; Cl, 11.12. Found: C, 71.53; H, 7.59; Cl, 11.17). To establish the configuration of the chlorine atom I was reduced with lithium aluminum hydride,[3] yielding 16α-chloroestradiol 3-methyl ether (III), m.p. 112–114°; $[\alpha]_D$ +72.5°; (*Anal.* Calcd. for $C_{19}H_{25}ClO_2$: C, 71.12; H, 7.85. Found: C, 71.40; H, 7.71) and 16α-chloroepiestradiol 3-methyl ether (IV), m.p. 162–164°; $[\alpha]_D$ +68.4°; (*Anal.* Found: C, 71.39; H, 7.80). Chromic acid oxidation converted both III and IV again into the ketone I. Treatment with alcoholic 3-methoxy-potassium hydroxide rearranged the *cis* chlorohydrin, IV, to estrone 3-methyl ether; similar treatment converted III into 16β,17β-epoxy-1,3,5-(10)-estratriene, m.p. 116–117°, $[\alpha]_D$ +115° (*Anal.* Calcd. for $C_{19}H_{24}O_2$: C, 80.24; H, 8.51. Found: C, 80.04; H, 8.75) whose structure was confirmed by reduction with lithium aluminum hydride to 3-methoxy-16β-hydroxy-1,3,5(10)-estratriene, m.p. 105–107°, identical with an anthentic sample.[4]

Iodination[5] of the enol acetate yielded II, m.p. 161–166°; $[\alpha]_D$ +91°; (*Anal.* Calcd. for $C_{19}H_{23}IO_2$: C, 55.62; H, 5.65; I, 30.93. Found: C, 55.71; H, 6.04; I, 30.27) and 16β-iodoestrone 3-methyl ether (VI), m.p. 163–166°; $[\alpha]_D$ +178°; (Found: C, 55.43; H, 5.73; I, 30.48). Configurational assignments for II and IV were made relative to molecular rotational differences given for the 16-bromoandrostan-17-ones.[3]

TABLE I

LIPID-SHIFTING AND ESTROGENIC POTENCIES

Compound	N[a]	Lipid potency (A)	N[b]	Estrogen potency (B)	Ratio (A/B)
Estrone	95	100 (standard)	28	100 (standard)	1
I	39	90	17	0.79	114 (86–151)[c]
VII	11	22	6	1.3	20 (12.5–32.0)
II	24	143	10	1.8	95 (65–139)

[a] Total number of animals used at a minimum of three dose levels employed in calculation of lipid-shifting potency. [b] Number of groups of 8–10 mice employed in calculation of estrogenic potency. [c] Numbers in parentheses represent limits within which the true value has a 95% probability of being found.

This series of halides, including 16α-bromoestrone 3-methyl ether (VII),[1,6] was tested for lipid-shifting and feminizing effects. An index of the former was the reduction in cholesterol-phospholipid ratio observed in a 3-day test with cholesterol-fed cockerels.[7] Uterine growth in intact, immature mice was used to estimate feminizing activity.[8] Potencies in these tests relative to the estrone standard are shown in the table. The ratio A/B is a measure of the separation of the two kinds of activity[9] and presumably will give some indication of therapeutic efficiency. The more potent members of this series are active on oral administration.

(1) W. S. Johnson and W. F. Johns, THIS JOURNAL, **79**, 2005 (1957).

(2) Analytical data was obtained by Dr. R. T. Dillon and the Analytical Department. All rotations were measured in chloroform; melting points were taken on the Kofler hot stage.

(3) *Cf.* J. Fajkos, *Collection Czechoslov. Chem. Communs.*, **20**, 312 (1955).

(4) M. N. Huffman and M. H. Lott, *J. Biol. Chem.*, **213**, 343 (1955). We wish to thank Dr. D. A. Tyner for kindly providing this material.

(5) *Cf.* C. Djerassi and C. T. Lenk, THIS JOURNAL, **76**, 1724 (1954).

(6) The 16α-configuration is apparent from molecular rotational data (*cf.* reference 3).

(7) D. L. Cook, R. A. Edgren and F. J. Saunders, *Endocrinology*, in press.

(8) R. A. Edgren, *Proc. Soc. Exptl. Biol. Med.*, **92**, 569 (1956).

(9) We wish to thank David W. Calhoun for statistical consultations.

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THE STEPWISE DEGRADATION OF THYMIDINE OLIGONUCLEOTIDES BY SNAKE VENOM AND SPLEEN PHOSPHODIESTERASES

Sir:

The chemical polymerization[1] of thymidine 5'-phosphate (TP) yields a number of 5' → 3' linked oligonucleotides[2] (T_nP_n), which bear 5'-phosphate end-groups. Using these compounds and the corresponding series (T_nP_{n-1}) obtained by dephosphorylation with prostate phosphomonoesterase, the mode of action of snake venom and spleen phosphodiesterases has been studied.

The snake venom (*Crotalus adamanteus*) diesterase preparation was obtained by acetone precipitation[3] followed by chromatography.[4] Incubation of pentathymidine tetraphosphate (T_5P_4) with this preparation gave the results shown in Table I. Thus, each of the lower homologs (T_4P_3, etc.) is formed successively and thymidine (T) appears last. The mononucleotide which accumulates is, as expected, thymidine 5'-phosphate. The data show that *degradation proceeds stepwise from the end bearing the 3'-hydroxyl group*. The degradation of oligonucleotides with 5'-phosphate end groups (T_nP_n), although much faster, also occurs stepwise. Further, in the hydrolysis of 3'-acetylated T_4P_4 the mononucleotide first released was 3'-acetyl-TP. This shows that the mode of degradation of these compounds is the same as found above and that hydrolysis does not begin from the end of the chain bearing the 5'-phosphate group.[5] Recent degradative experiments performed by Singer, Hilmoe and Heppel[6] on enzymatically synthesized polyribonucleotides confirm the present conclusions.

A phosphodiesterase from calf spleen has recently

(1) H. G. Khorana, W. E. Razzell, P. T. Gilham, G. M. Tener and E. H. Pol, THIS JOURNAL, **79**, 1002 (1957).

(2) H. G. Khorana, G. M. Tener, W. E. Razzell and R. Markham, *Fed. Proc.*, in press (1958).

(3) J. F. Koerner and R. L. Sinsheimer, *J. Biol. Chem.*, **228**, 1049 (1957).

(4) H. G. Boman and U. Kaletta, *Biochim. Biophys. Acta*, **24**, 619 (1957).

(5) M. P. de Garilhe and M. Laskowski, *J. Biol. Chem.*, **223**, 661 (1956).

(6) Personal communication from Dr. Leon Heppel, National Institutes of Health, Bethesda.