$[\alpha]^{25}_{360}$ +220°; $[\alpha]^{25}_{340}$ +258° [H_2O, *c* 0.54). *Anal.* Calcd. for $C_{10}H_{13}N_5O_3$: C, 47.80; H, 5.22; N, 27.88. Found: C, 48.01; H, 5.49; N, 27.74. Like its anomer, the substance shows an absorption peak at 260 mμ characteristic of a 9-substituted adenine,[7] the molar absorbancy (A_M) being 15,900.

Hydrolysis of a sample with 1% aqueous acetic acid, and then paper chromatography in four different solvent systems, revealed the presence of adenine, 2-deoxy-D-ribose and unchanged nucleoside.

(7) J. M. Gulland and L. F. Story, *J. Chem. Soc.*, 259 (1938).

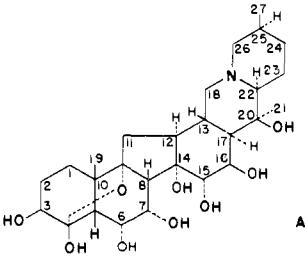
NATIONAL INSTITUTES OF ARTHRITIS AND METABOLIC DISEASES
NATIONAL INSTITUTES OF HEALTH ROBERT K. NESS
BETHESDA 14, MARYLAND HEWITT G. FLETCHER, JR.

RECEIVED MAY 18, 1959

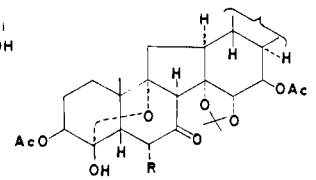
VERATRUM ALKALOIDS. XXXIV. THE CONFIGURATION OF PROTOVERINE[1]

Sir:

Protoverine[2-4] is the alkamine obtained by alkaline hydrolysis of the clinically useful[5] hypotensive ester alkaloids protoveratrine A[6] and protoveratrine B.[6] Evidence is advanced herewith for assignment of configuration at each of the seventeen asymmetric centers of protoverine which now can be represented completely by formula I.



I



II, R = OAc
III, H = H

The orientations at fourteen of the asymmetric carbon atoms of protoverine have been established by a single degradation. Treatment of 7-dehydroprotoverine 14,15-acetonide 3,6,16-triacetate (II)[4] in tetrahydrofuran with calcium in liquid ammonia[7] afforded the known[8] 7-dehydrogermine 14,15-acetonide 3,16-diacetate (III). The configurations at C_3, C_4, C_5, C_9, C_{10}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, C_{20}, C_{22}, and C_{25} are therefore the same as those at the corresponding asymmetric carbon atoms in germine.[8]

The steric hindrance to acetylation of the C_7-hydroxyl group of protoverine by the α-oriented 14,15-acetonide grouping[4] is explicable uniquely on the basis of a C_8-β-hydrogen (as in all other naturally occurring steroids), C_7-α-hydroxyl configuration. Support for assignment of α-orientation to the C_7-hydroxyl is presented: (a) sodium borohydride reduction of II proceeded stereoselectively to give protoverine 14,15-acetonide 6,16-diacetate, m.p. 229–230° dec.; $[\alpha]^{25}_D$ +4° (*c* 0.95, pyr.). The latter compound consumed one mole equivalent of sodium periodate and yielded an amorphous product showing infrared absorption at 3.65 and 5.62 μ characteristic of an aldehydo-γ-lactone resulting from cleavage of the Ring A glycol.[4] Upon acetylation, the 14,15-acetonide 6,16-diacetate gave the known protoverine 14,15-acetonide 3,6,16-triacetate.[4] The molecular model of the ketone (II) shows that the β- is much less hindered than the α-face for approach to the borohydride, which suggests that reaction would proceed to give an α-oriented hydroxyl.[9] (b) Acetylation of protoverine with acetic anhydride-pyridine, reagents known to acetylate the C_4-hemiketal hydroxyl in veracevine,[10] afforded protoverine 3,6,7,15,16-pentaacetate,[4] consistent with rapid acetylation of the α-hydroxyl group at C_7 and resultant hindrance to reaction of the C_4-hydroxyl group by the 7-α-acetoxy group, (as in germine[8]).

Formation of the 6,7-acetonide derivative[4] of isoprotoverine requires that the C_6 hydroxyl group be oriented *cis* to the C_7-hydroxyl; hence protoverine possesses the 6-α-hydroxygermine structure and configuration (I).[11,12]

(1) Part XXXIII in the series: S. M. Kupchan and T. Masamune, *Chemistry and Industry*, 632 (1959).

(2) W. Poethke, *Arch. Pharm.*, **275**, 357, 571 (1937).

(3) L. C. Craig and W. A. Jacobs, *J. Biol. Chem.*, **149**, 271 (1943).

(4) S. M. Kupchan, M. Neeman, C. I. Ayres, R. H. Hensler and S. Rajagopalan, *Chemistry and Industry*, 1626 (1958).

(5) O. Krayer in V. A. Drill, "Pharmacology in Medicine," McGraw-Hill Book Co., Inc., New York, N. Y., Second Edition, 1958, pp. 515–524.

(6) S. M. Kupchan and C. I. Ayres, THIS JOURNAL, **81**, 1009 (1959), and references therein.

(7) *Cf.* J. H. Chapman, J. Elks, G. H. Phillips and L. H. Wyman, *J. Chem. Soc.*, 4344 (1956).

(8) S. M. Kupchan and C. R. Narayanan, THIS JOURNAL, **81**, 1913 (1959).

(9) *Cf.* W. G. Dauben, G. J. Fonken and D. S. Noyce, *ibid.*, **78**, 2579 (1956).

(10) S. M. Kupchan, D. Lavie, C. V. Deliwala and B. Y. A. Andoh, *ibid.*, **75**, 5519 (1953).

(11) Satisfactory analytical and spectral data were obtained for the new compound reported herein.

(12) This investigation was supported by grants from The National Institutes of Health (H-2275(C3)) and the Wisconsin Alumni Research Foundation.

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY
UNIVERSITY OF WISCONSIN S. MORRIS KUPCHAN
MADISON 6, WISCONSIN TADASHI MASAMUNE
C. IAN AYRES

RECEIVED JULY 16, 1959

A NEW ASSAY METHOD FOR AMINO ACID ACTIVATING ENZYMES[1]

Sir:

We wish to report a new technique for the estimation of amino acid activating enzymes.[2] It is extremely simple to carry out, rapid, sensitive and conservative of all materials. This method permits assay of a specific amino acid activating enzyme in the presence of all other amino acids and activating enzymes (plus other hydroxamate forming or adenosine triphosphate-pyrophosphate exchanging systems). The method also permits the detailed study of competition between two or more amino acids both of which are activated by a single enzyme. Particularly in these latter two respects,

(1) This Publication No. 968 of the Cancer Commission of Harvard University; the work was supported by United States Public Health Grant No. C-2387 and by United States Atomic Energy Commission contract AT(30-1)609.

(2) M. B. Hoagland, *Biochim. et Biophys. Acta*, **16**, 288 (1955).