$[\alpha]^{25}_{360}$ +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).

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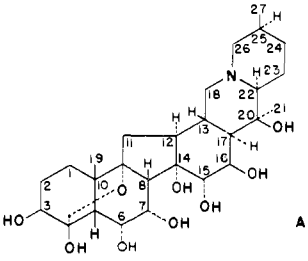
RECEIVED MAY 18, 1959

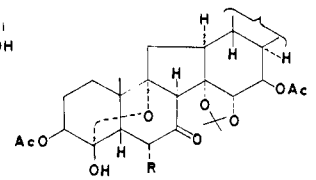
VERATRUM ALKALOIDS. XXXIV. THE CONFIGURATION OF PROTOVERINE[1]

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.

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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).

this technique is superior to established assay methods.

The carbon-14 form of the amino acid whose activation is being studied is incubated with adenosine triphosphate, hydroxylamine and the enzyme preparation in a total volume of as little as 100 μl. Aliquots are removed at appropriate time intervals, heated briefly to destroy the enzyme and evaporated onto a line one inch from the end of a $^3/_4$ by 5 inch strip of Amberlite IR-120 ion exchange paper (sulfonic acid resin, Na^+ form, courtesy of Rohm and Haas). A sodium phosphate buffer (*p*H 7.0, 0.05 *M*) is allowed to rise by capillarity through the strip which is then dried. Under these conditions all the free unreacted neutral amino acids move with the solvent front and the hydroxamates of the neutral amino acids remain at the origin. (Other conditions permit the separation of the acidic or basic amino acids from their hydroxamates.) Comparison of the radioactivity at the two sites indicates the fraction converted and hence the rate of activation. Although the paper absorbs some of the radiation, the use of high activity L-amino acids (20 mcuries/mmole) and a thin window Geiger counter (Nuclear Chicago D-47, 45% efficiency) permits us to recognize 10^{-12} mole of hydroxamate formation in the aliquot. A typical experiment is given:

INITIAL RATES (MμMOLES/ML./HR.) C-14 HYDROXAMATE FORMATION

	+ no C-12 amino acid	+11 mM. valine	+11 mM. isoleucine	+11 mM. alloisoleucine
Valine-C-14, mM.				
0.15	96		46	44
1.0	94			
Isoleucine-C-14, mM.				
0.15	85	34		30
1.0	82			
Alloisoleucine-C-14, mM.				
0.15	1.2	0.0	0.3	
1.0	9.0			

The incubation mixtures contained in addition to the amino acids, 10 mM. adenosine triphosphate, 12 mM. Mg^{++}, 25 mM. KCl, 50 mM. tris-(hydroxymethyl)-aminomethane, 0.2 *M* sucrose and 2.0 *M* hydroxylamine. The volume was 0.23 ml., the *p*H 7.4, and the temperature 25°. Each incubation flask contained 0.05 ml. of a dilute extract of alumina ground *E. coli*. Aliquots of 25 μl. were removed for analysis at zero, 30, 60 and 120 minutes. The rate of hydroxamate formation was linear except when the substrate was approaching exhaustion.

Similar assays can be devised for any system in which the starting material and product can be caused to differ markedly in charge; *e.g.*, the conversion of glucose into glucose phosphate, of acetate into acetohydroxamate, or the pyrophosphate exchange into adenosine triphosphate.

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RECEIVED JULY 6, 1959

VINCA ALKALOIDS. III.[1] CHARACTERIZATION OF LEUROSINE AND VINCALEUKOBLASTINE, NEW ALKALOIDS FROM *VINCA ROSEA LINN.*

Sir:

Leurosine,[2] a new alkaloid from *Vinca rosea Linn.*, was described recently, but no empirical formula was assigned.[2] Independently Noble, Beer and Cutts have reported the physical and biological properties of another new alkaloid, vincaleukoblastine.[3,4]

In view of the unusual properties of these two alkaloids,[2,3,4] we wish to present the analytical and physical data which led to the establishment of empirical formulas for vincaleukoblastine and leurosine and indicate their close structural relationship.

Vincaleukoblastine sulfate[5] melted at 284–285°, $[\alpha]^{26}D$ −28° (CH_3OH). Calcd. for $C_{46}H_{58}O_9N_4 \cdot H_2SO_4 \cdot H_2O$: C, 59.59; H, 6.74; O, 24.16; N, 6.04; S, 3.46. Found: C, 59.68; H, 6.72; O, 24.27; N, 6.19; S, 3.37. The free base, recrystallized from ether, formed a stable etherate, loss of solvent at 180–182°, m.p. 201–211°, $[\alpha]^{26}D$ +42° ($CHCl_3$). Calcd. for $C_{46}H_{58}O_9N_4 \cdot (C_2H_5)_2O$: C, 67.85; H, 7.74; O, 18.09; N, 6.32; mol. wt., 885. Found: C, 67.89, 67.93; H, 7.63, 7.76; O, 18.08; N, 6.38, 6.43; mol. wt., 887.8 (X-ray data). Ether of solvation was demonstrated by vapor phase chromatography and a band at 8.4 μ in the infrared disappearing on evaporation of a chloroform solution of the etherate. The base from methanol melted at 211–216°, calcd. for $C_{46}H_{58}O_9N_4 \cdot 2CH_3OH \cdot H_2O$: C, 64.55; H, 7.68; N, 6.27; mol. wt., 894. Found: C, 64.11; H, 7.49; N, 6.36; mol. wt., 889 ± 5 (electrometric titration, H_2O; pK'_a 5.4, 7.4). After drying at 180° (1 min.), calcd. for $C_{46}H_{58}O_9N_4$: C, 68.12; H, 7.21; O, 17.75; N, 6.90; weight loss, 9.19. Found: C, 68.15; H, 7.44; O, 18.05; N, 6.65; weight loss, 8.81. Vincaleukoblastine formed a dihydrochloride dihydrate, m.p. 244–246° (dec.). Calcd. for $C_{46}H_{58}O_9N_4 \cdot 2HCl \cdot 2H_2O$: C, 60.06; H, 7.01; O, 19.13; N, 6.09; Cl, 7.71. Found: C, 60.36, 59.95; H, 7.24, 7.18; O, 19.04; N, 5.94; Cl, 7.37.

Leurosine[2] was recrystallized from acetonitrile, m.p. 202–205° (dec.) (loss of solvent at 172–175°), $[\alpha]^{26}D$ +72° ($CHCl_3$). Calcd. for $C_{46}H_{58}O_9N_4 \cdot 8H_2O$: mol. wt., 955.09. Found: mol. wt., 955.3 ± 1% (X-ray data); 932 ± 10 (electrometric titration, pK'_a 5.5 and 7.5 in water). After drying at 130° *in vacuo*, weight loss calcd.: 15.09. Found: 15.60. Calcd. for $C_{46}H_{58}O_9N_4$: C, 68.12; H, 7.21; O, 17.75; N, 6.90. Found: C, 68.11, 67.88; H, 7.30, 7.45; O, 17.34, 18.05; N, 7.10, 6.93. The sulfate from ethanol, $[\alpha]^{26}D$ −8.3 (CH_3OH), m.p. 238–242° (dec.), was dried at 130° *in vacuo*. Calcd. for $C_{46}H_{58}O_9N_4 \cdot H_2SO_4$: C, 60.77;

(1) Vinca Alkaloids II, M. Gorman et al., *J. Am. Pharm. Assoc. Sci. Ed.*, **48**, 256 (1959).

(2) G. H. Svoboda, *J. Am. Pharm. Assoc. Sci. Ed.*, **47**, 834 (1959).

(3) R. L. Noble, C. T. Beer and J. H. Cutts, *Ann. N. Y. Acad. Sci.*, **76**, 882 (1958).

(4) R. L. Noble, C. T. Beer and J. H. Cutts, *Biochemical Pharmacology*, **1**, 347 (1958).

(5) The alkaloid sulfate was first tentatively formulated as a $C_{23}H_{25}N_2O_{7\frac{1}{2}}H_2SO_4$ compound.[3] The scarcity of the material did not allow at that time the corroboration of this formula. The alkaloid and its derivatives solvate readily and retain tenaciously solvents of crystallization.