ZYGADENUS ALKALOIDS. II. THE OCCURRENCE OF HYPOTENSIVE GERMINE ESTERS IN ZYGADENUS VENENOSUS

Sir:

A recent study[1] of the alkaloidal constituents of *Zygadenus venenosus* revealed the occurrence in the plant of the esters veratroylzygadenine and vanilloylzygadenine as well as the alkamines zygadenine and germine. We wish to report now the isolation from a batch of this plant collected in June, 1951 of the germine esters neogermitrine,[2] germidine,[3] protoveratridine,[4] and a new diester isomeric with germidine, for which we propose the name neogermidine.

Fractionation of the chloroform-extractable alkaloids of *Zygadenus venenosus* (WATS.)[5] by 8-plate countercurrent distribution using benzene and phosphate buffer at *p*H 7.1 yielded, in addition to the alkaloids obtained previously,[1] the germine monoester protoveratridine (from the O-plate fraction). Protoveratridine crystallized as rectangular plates upon reprecipitation from alcoholic acetic acid solution with aqueous ammonia; m.p. 272–273° dec.; $[\alpha]^{22}\mathrm{D}\ -9°$ (c 0.76, pyr.). *Anal.* Calcd. $C_{32}H_{51}O_9N$: C, 64.73; H, 8.66; N, 2.36. Found: C, 64.79; H, 8.62; N, 2.62.

The filtrates after removal of the crystalline alkaloids from the plate-8 fraction were combined with the material from plates 4 to 7, and the amorphous mixture obtained upon lyophilization was designated the "organophilic" fraction. The filtrates after removal of the crystalline alkaloids from the plate-O fraction were combined with the material from plates 1 to 3, and the residue obtained upon lyophilization was designated the "hydrophilic" fraction.

Neogermitrine and germidine were obtained by 24-plate countercurrent distribution of the organophilic fraction using benzene and 2*M* acetate buffer at *p*H 5.5.[2] The identity of these substances was confirmed by mixed melting point and infrared spectral comparisons with authentic specimens from *Veratrum viride* kindly provided by Dr. J. Fried.

The hydrophilic fraction was subjected to 8-plate countercurrent distribution using chloroform and 2*M* acetate buffer at *p*H 5.5. Neogermidine was obtained by chromatography on alumina of the material recovered from plates 5 to 8. Neogermidine crystallized from benzene as heavy prisms; m.p. 221–223° dec.; $[\alpha]^{22}\mathrm{D}\ -60°$ (c 2.00, pyr.); $[\alpha]^{22}\mathrm{D}\ -25°$ (c 2.00, chf.). *Anal.* Calcd. $C_{34}H_{53}O_{10}N$: C, 64.22; H, 8.38. Found: C, 64.15; H, 8.70. Neogermidine thiocyanate crystallized from acetone as needles, m.p. 247–249° dec. *Anal.* Calcd. $C_{34}H_{53}O_{10}N \cdot HNCS$: C, 60.50; H, 7.82; S, 4.61. Found: C, 60.16; H, 7.87; S, 4.65. In a volatile acid determination, 17.52 mg. of neogermidine was equivalent to 5.77 ml. of 0.009126 *N* sodium thiosulfate; calcd. for germine monoacetate mono-α-methylbutyrate, 6.05 ml. Alkaline hydrolysis of neogermidine afforded germine, acetic acid and α-methylbutyric acid. The acids were identified by conversion to their *p*-phenylphenacyl esters which were characterized after chromatographic separation. Methanolysis of neogermidine afforded protoveratridine. The large change in rotation attending the methanolysis of neogermidine to protoveratridine suggests that the site of attachment of the acetyl group on the alkamine germine is the same as that of the labile acetyl group in both neogermitrine and germitrine.

Acetylation of neogermidine with acetic anhydride and pyridine yielded acetylneogermitrine, m.p. 248–249° dec., identical with a sample prepared by acetylation of germidine.[2] Acetylation of protoveratridine under the same conditions also gave acetylneogermitrine. These facts show that the site of attachment of the α-methylbutyryl group is the same in each of the four germine esters isolated from *Zygadenus venenosus.*

Pharmacological experiments carried out with neogermidine at the laboratory of Professor O. Krayer at Harvard Medical School indicate that the circulatory action in the cat and the veratrinic effect on the frog muscle are similar to those of germidine.

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(1) S. M. Kupchan and C. V. Deliwala, THIS JOURNAL, **74**, 2382 (1952).

(2) J. Fried, P. Numerof and N. M. Coy, *ibid.*, **74**, 3041 (1952). We wish to thank Dr. J. Fried for private communication of these results prior to publication.

(3) J. Fried, H. L. White and O. Wintersteiner, *ibid.*, **72**, 4621 (1950).

(4) G. Salzberger, *Arch. Pharm.*, **228**, 462 (1890); W. Poethke, *ibid.*, **275**, 571 (1937).

(5) Plant gathered in northeastern Oregon in June, 1951. We are grateful to Dr. Reed Rollins, Gray Herbarium, Harvard University, for confirming the identity of the plant.

A NEW DISACCHARIDE PRODUCED BY LEUCONOSTOC MESENTEROIDES

Sir:

In studies on the enzymatic synthesis of dextran from sucrose by *Leuconostoc mesenteroides* we have noted that under certain conditions as much as 3% of the sucrose is diverted to the production of a new disaccharide which has the following properties: Crystallizes in the form of bars from methanol–ethyl acetate; m.p. 161–163°; $[\alpha]^{25}\mathrm{D}\ -8.8°$ after 3 minutes, $-6.8°$ after 24 hours (c, 4; H_2O); analyzes for $C_{12}H_{22}O_{11}$; reducing power by Somogyi method 46% of that of fructose; low order of reaction with hypoiodite (9% by Willstätter–Schudel method; turanose gave 11%); positive Seliwanoff test for fructose; hydrolyzes to glucose and fructose as shown by paper chromatography; phenylosazone (analyzing for $C_{24}H_{32}N_4O_9$) as needles from wet ethyl acetate (m.p. 186–188°); yields an amorphous phenylosotriazole which hydrolyzes to D-glucose ($[\alpha]^{25}\mathrm{D}\ +52°$) and D-glucose phenylosotriazole (m.p. 197°; $[\alpha]^{25}\mathrm{D}\ -81°$).

From this preliminary work it would appear that the sugar is a D-glucosyl-D-fructose with the glucosyl unit probably in the pyranose form since