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.; $[\alpha]^{22}D$ −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.; $[\alpha]^{22}D$ −60° (*c* 2.00, pyr.); $[\alpha]^{22}D$ −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.

This work was supported (in part) by grants from the National Institutes of Health and Eli Lilly and Company. The assistance of Eli Lilly and Company in gathering and extracting *Zygadenus venenosus* is gratefully acknowledged.

DEPARTMENT OF CHEMISTRY
HARVARD UNIVERSITY S. MORRIS KUPCHAN
CAMBRIDGE 38, MASSACHUSETTS C. V. DELIWALA

RECEIVED MAY 22, 1952

(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

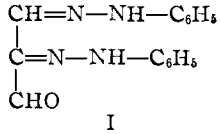
Sir:

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°; $[\alpha]^{25}D$ −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 ($[\alpha]^{25}D$ +52°) and D-glucose phenylosotriazole (m.p. 197°; $[\alpha]^{25}D$ −81°).

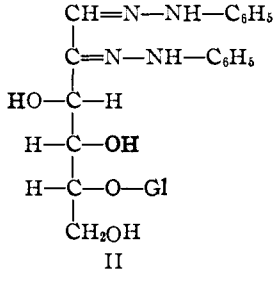
From this preliminary work it would appear that the sugar is a D-glucosyl-D-fructose with the glucosyl unit probably in the pyranose form since

only 1% hydrolysis is observed in 24 hours at room temperature in 0.2 *N* HCl.

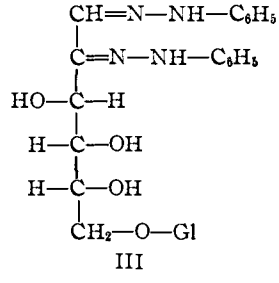
A tentative conclusion as to the point of attachment of the glucosyl group has been based on the following observations: Chargaff and Magasanik[1] noted that glucose phenylosazone was rapidly cleaved by sodium periodate to give a precipitate of compound I in 85% yield. We have extended this technique to the disaccharides and find it useful in the determination of the point of union of the component parts, since glycosidic linkages in the 3- and 4-positions block the reaction. The method should be applicable to any oligosaccharide giving an osazone.



I



II



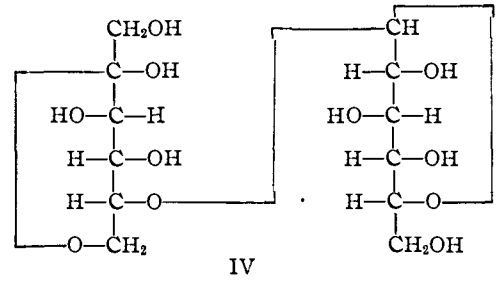
III

Under the conditions used by those workers we found that the phenylosazone of the new sugar as well as that of isomaltose (6-(α-D-glucopyranosyl)-D-glucose) and of gentiobiose (6-(β-D-glucopyranosyl)-D-glucose) gave rapid precipitation of compound I in yields of 70–80%. On the other hand, the phenylosazones of turanose (3-(α-D-glucopyranosyl)-D-fructose), laminaribiose (3-(β-D-glucopyranosyl)-D-glucose), maltose (4-(α-D-glucopyranosyl)-D-glucose), and cellobiose (4-(β-D-glucopyranosyl)-D-glucose) gave no precipitates (other than colorless inorganic salts) even on standing overnight. Such behavior is compatible only with structures II and III having the glucosidic linkage on positions 5 or 6, respectively. This conclusion is further supported by the fact that the phenylosazone of the new sugar gives an X-ray diffraction pattern readily distinguishable from that obtained with the phenylosazones of turanose, laminaribiose, maltose, or cellobiose.

The possibility of a 1,6 linkage was also rendered unlikely by the following considerations: The X-ray pattern of the phenylosazone of the new sugar was found to differ from that of gentiobiose phenylosazone; the pattern given by isomaltose phenylosazone, however, was so similar to that of the new phenylosazone that conclusive differentiation was not possible. Fortunately, identity could be ruled out by the melting point of isomaltose phenylosazone (205–207°) and the fact that it forms a crystalline phenylosotriazole (m.p. 179–180°).

Preliminary evidence, then, suggests that the new sugar may be a 5-(D-glucopyranosyl)-D-fructopyranose, a possible form of which is shown in formula IV.

(1) E. Chargaff and B. Magasanik, THIS JOURNAL, **69**, 1459 (1947).



IV

Apparently no 1,5-phenylosazones of the proper configuration are available for comparison. Freudenberg and v. Oertzen[2] recently synthesized 5-(β-glucosido)-glucose but were unable to obtain pure osazones.

Our work on the mechanism of dextran formation indicates that the new sugar plays a role in the polymerization process; we believe it advisable, therefore, to assign it the common name of "leucrose" which is suggested by its particular microbial origin.

Further structure studies are in progress.

We are indebted to Prof. E. L. Hirst and Dr. V. C. Barry for laminaribiose samples, to Dr. Allene Jeanes for the isomaltose, to Drs. N. K. Richtmyer and C. S. Hudson for the turanose, and to Dr. N. Hellman and Mr. H. F. Zobel for the X-ray determinations.

FERMENTATION DIVISION FRANK H. STODOLA
NORTHERN REGIONAL RESEARCH LABORATORY
HAROLD J. KOEPSELL
PEORIA, ILLINOIS[3] EUGENE S. SHARPE

RECEIVED MAY 2, 1952

(2) K. Freudenberg and K. v. Oertzen, *Ann.*, **574**, 37 (1951).

(3) One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

THE MOLECULAR WEIGHT AND SHAPE OF DESOXYPENTOSE NUCLEIC ACID

Sir:

The uncertainty which persists with regard to the molecular weight and shape of desoxypentose nucleic acid, DNA, from calf thymus appears to be due to the inadequacy of most macromolecular techniques in characterizing very large, charged polyelectrolytes and to the varying degrees of degradation inherent in the different methods of preparation. In a current paper[1] it is shown that the measurement of the angular distribution of scattered light from DNA solutions in the concentration range of 1 to 10 mg./100 cc. leads to a determination of the molecular weight as well as to some definite conclusions about the size and shape of several different samples. The sample having the highest molecular weight was that prepared by Schwander and Signer.[2] We wish to report measurements on a sample we have prepared by the Signer method which indicate the reproducibility of this preparative method and provide new information on the structure of the DNA molecule.

Light scattering measurements on the new preparation in 0.2 *M* NaCl show the molecular weight to be 7,700,000 in comparison with 6,700,000 found[1]

(1) P. Doty and B. H. Bunce, THIS JOURNAL, in press.

(2) H. Schwander and R. Signer, *Helv. Chim. Acta*, **33**, 1521 (1950).