action similar to that of choline. For example, an intravenous injection of 0.135 cc. (1 mg./kg.) in a rabbit was followed by a few spasmodic jerks and marked vagal stimulation. After the vagal slowing had been abolished by intravenous injection of 0.1 cc. 5% atropine sulfate—which produced no visible effect—doses of 0.27 cc. (2 mg./kg.) caused a sudden, sharp, epinephrin-like increase in blood pressure (180%) which returned to normal within two minutes.

Summary

(Tetraacetyl-β-*d*-glucosido)-ethyltriethylammonium bromide, (tetraacetyl-β-*d*-glucosido)-choline chloride, γ-(tetraacetyl-β-*d*-glucosido)-homocholine chloride and β-*d*-glucosidocholine chloride have been prepared. These are new compounds.

(Tetraacetyl-β-*d*-glucosido)-1-trimethylammonium bromide and (β-*d*-glucosido)-1-trimethylammonium bromide have also been prepared, and their effect on the vagus center of rabbits studied. They were inactive.

Glucosamine reacts with methyl iodide and potassium hydroxide to give a substance which does produce a marked stimulation of the vagus center of the rabbit.

PITTSBURGH, PENNA.
BROOKLYN, N. Y.

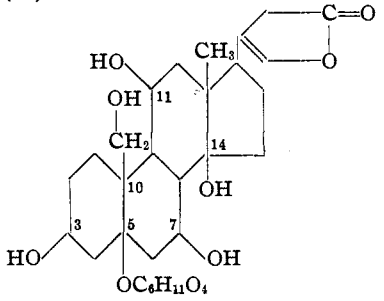
RECEIVED MARCH 19, 1938

[CONTRIBUTION FROM THE FRICK CHEMICAL LABORATORY, PRINCETON UNIVERSITY]

Studies in the Oxidation of the Trianhydrolactone of Ouabain, and of *epi*-Neoergosterol

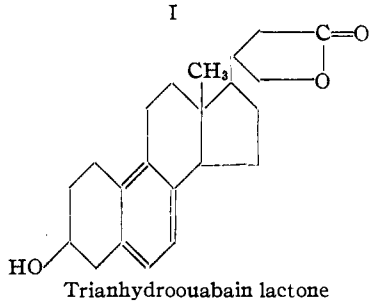
BY P. N. CHAKRAVORTY[1] AND EVERETT S. WALLIS

In order to explain certain results obtained by Arnaud,[2] and by Jacobs and Bigelow,[3] Fieser[4] recently has suggested the following structural formulas for ouabain, (I) and for its trianhydrolactone (II).



Ouabain

I



Trianhydroouabain lactone

II

Spectroscopic data in support of the assumption that an aromatic ring may be present in a lactone of this type has since been adduced by Fieser and Newman.[5]

On the basis of this tentative formula we started to study the oxidative degradation products of this lactone (II) in the hope of obtaining further evidence of its constitution, for it seemed to us that if either ring A or B were aromatic ketonic compounds would be obtained the structure of which easily could be elucidated. In the meantime in a paper published by Tschesche and Haupt[6] dehydrogenation experiments on this compound were described the results of which could not be reconciled with the above proposed formula. Oxidation experiments, therefore, have an added interest and we wish at this time to record certain observations which we have made in the course of our studies on compounds of this type. In our hands oxidation of trianhydroouabain lactone monoacetate with chromic acid failed to give any ketonic material with a benzenoid ring B, even though the reaction was studied under varying conditions of temperature, concentration and acidity. Since these results were contrary to our expectations, we were led to study the nature of the lactone more closely. Oxidations with nitric acid were made. In view of the apparent steroid nature of this molecule it was

(1) Fellow on Special Grant from the American Philosophical Society.

(2) Arnaud, *Compt. rend.*, **126**, 1280 (1898).

(3) Jacobs and Bigelow, *J. Biol. Chem.*, **96**, 647 (1932); **101**, 15 (1933).

(4) Fieser, "The Chemistry of Natural Products Related to Phenanthrene," Reinhold Publishing Corporation, New York, N. Y., 1936, p. 292.

(5) Fieser and Newman, *J. Biol. Chem.*, **114**, 705 (1936).

(6) Tschesche and Haupt, *Ber.*, **70**, 43 (1937).

hoped that either methylbenzene-tetracarboxylic acid or benzene-1,2,3,4-tetracarboxylic acid could be obtained as an oxidation product. No such acid was isolated, however, and the reaction products even failed to react with diazomethane. Apparently a complex mixture of nitrated compounds is produced during the oxidation with nitric acid. It would thus seem in consideration of these results together with those of Tschesche and Haupt that the formula for trianhydroouabain lactone tentatively proposed by Fieser is inadequate to explain its behavior.

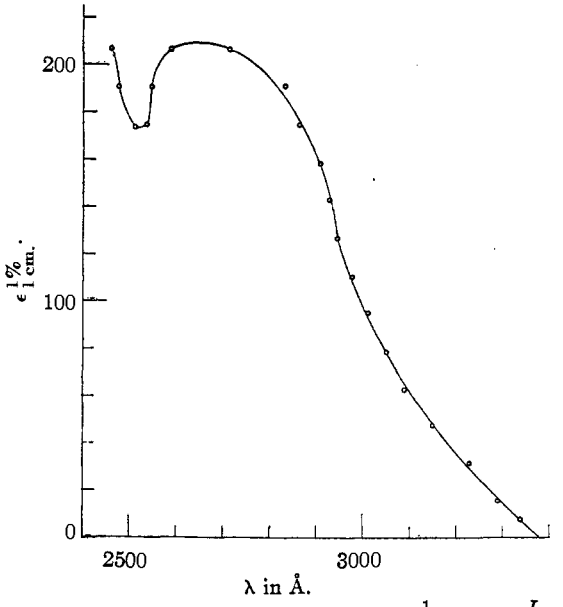


Fig. 1.—$I/I_0 = 10^{-\epsilon cl}$. $\epsilon^{1\%}_{1\ cm.} = \frac{1}{c \times l} \log_{10} \frac{I_0}{I}$.
c measured in grams/100 cc., l measured in cm.

Our next experiments were carried out on neoergosterol as the starting material. While this work was in progress a communication by Marker,[7] *et al.*, appeared which stated that they had been able to prepare oestrone. A serious objection to this work, however, was soon reported by Windaus and Deppe.[8]

In view of these differences of opinion, a description of results obtained in our work seems pertinent. While preparing *epi*-neoergosterol from neoergosterol, the crystals first obtained melted at 171–172°, a melting point recorded by Marker, *et al.*, for tetrahydrodehydroneoergosterol. Our compound, however, was not phenolic and in agreement with the observation of Windaus and Deppe did not form a sodium salt. It did contain small amounts of neoergosterol which could be removed by recrystallization. When so purified a melting point of 176° was obtained (*cf.* Windaus and Deppe).

This latter material, in the form of its acetate, m. p. 98° (*cf.* Windaus and Deppe), was oxidized with chromic acid in acetic acid solution. From the oxidation products a ketone was isolated in the form of semicarbazone, m. p. 255°. Hydrolysis of this material gave a ketone, m. p. 114–115°. Examination showed that either during the formation of the semicarbazone or during the hydrolysis the hydroxyl group at C_3 was lost and a double bond, presumably between C_3–C_4, was introduced. This behavior is not surprising. In fact, it is in accordance with the behavior of *epi*-neoergosterol which under certain circumstances has been shown by Windaus and Deppe to yield neoergopentaën. The absorption spectra of this ketone also show characteristics similar to that of neoergopentaën (Fig. 1).

From these experiments it is evident that the ketone formed by oxidation of *epi*-neoergosteryl acetate is as we would expect essentially different from oestrone in its physical properties. This fact is of special interest in the light of conflicting results obtained by Marker, *et al.*, and by Windaus and Deppe.

Experimental Part

Preparation of Trianhydroouabain Lactone Monoacetate.—This compound was prepared according to the method of Jacobs and Bigelow.[3] Difficulties were encountered in obtaining a crystalline product until seed crystals were kindly furnished us by Dr. Jacobs. Our compound melted at 172°, the melting point previously recorded by Jacobs and Bigelow.

Oxidation of Trianhydroouabain Lactone Monoacetate with Chromic Acid.—To a solution of 0.2 g. of trianhydroouabain lactone monoacetate in 10 cc. of glacial acetic acid there was added, drop by drop under constant stirring in the course of two and one-half hours, a solution of 0.2 g. of chromic acid dissolved in 2 cc. of acetic acid and 0.1 cc. of water. The oxidation was carried out on the water-bath, and after the addition of the oxidizing solution was complete, heating was continued for five and one-half hours. The reaction mixture was then cooled and worked up in the usual manner. The neutral products of oxidation were treated with an alcoholic solution of semicarbazide acetate, but no detectable amount of semicarbazone was produced.

Oxidation of Trianhydroouabain Lactone Monoacetate with Nitric Acid.—This lactone monoacetate (0.72 g.) was heated on the water-bath with 11 cc. of concentrated nitric acid (sp. gr. 1.4). The lactone dissolved with evolution of nitrous fumes and the solution turned red. Slow heating was continued for seven hours. No crystals appeared on cooling. When thrown out with water an amorphous

(7) Marker, Kamm, Oakwood and Laucius, THIS JOURNAL, **58**, 1504 (1936).

(8) Windaus and Deppe, *Ber.*, **70**, 76 (1937).

residue was obtained which could not be crystallized. When an acetone solution of this material was treated with an ethereal solution of diazomethane no evolution of nitrogen took place.

Oxidation of *epi*-Neoergosteryl Acetate.—*epi*-Neoergosterol was prepared from neoergosterol according to the method of Windaus and Deppe.[8] Crystals were first obtained which melted at 171–172°. Further recrystallizations from an ether–acetone solution gave a product which melted sharply at 175–176°.

The acetate, m. p. 98°, prepared from 4.0 g. of *epi*-neoergosterol was dissolved in 240 cc. of glacial acetic acid. To this solution there was added drop by drop in the course of three hours a solution of 8 g. of chromic acid anhydride in 1 cc. of water and 30 cc. of glacial acetic acid. The solution was stirred mechanically and during the oxidation the temperature was kept at 60–65°. After the addition of the chromic acid the heating was continued for four more hours. The solution was then cooled and worked up in the usual manner. The ether residue which contained the neutral products of oxidation was dissolved in alcohol, and to it was added an aqueous alcoholic solution of 1.7 g. of semicarbazide hydrochloride and 1.7 g. of sodium acetate. The mixture was refluxed for two hours, and then worked up in the usual manner. The semicarbazone thus prepared melted at 255° with decomposition; yield 47 mg.

The above semicarbazone was taken up in alcohol and hydrolyzed in the usual manner with a small amount of sulfuric acid. Crystals of the free ketone were obtained which melted at 114–115°. Its absorption spectrum in alcohol was measured by Dr. T. J. Webb of the Research Laboratories of Merck and Company, Inc., Rahway, N. J. A portion of this ketone was reconverted into its semicarbazone. The compound so obtained again melted at 255° with decomposition. Analysis of the semicarbazone: Calcd. for $C_{19}H_{23}ON_3$: N, 13.58. Found: N, 13.64.

We wish to take this opportunity to express our thanks to the American Philosophical Society for a grant-in-aid for this work and to Dr. T. J. Webb, Research Laboratories, Merck and Company, Inc., Rahway, N. J., for the absorption spectrum curve published in this article.

Summary

Trianhydroouabain lactone monoacetate has been oxidized with chromic acid in acetic acid solution. No ketonic material could be isolated from the reaction products. Attempts to obtain either benzene-tetracarboxylic acid-(1,2,3,4) or methylbenzene-tetracarboxylic acid from the products of oxidation of the above lactone with concentrated nitric acid have been described. Certain observations on the structure of this lactone are made in the light of these results.

A study of the nature of the oxidation products of *epi*-neoergosteryl acetate with chromic acid in acetic acid has been made. A ketone in the form of its semicarbazone (m. p. 255° with decomposition) has been isolated. Hydrolysis of this compound gives a ketone which melts at 114–115°. Its absorption spectrum is recorded. A discussion of the significance of these experiments in connection with certain observations of Marker and his co-workers and of Windaus and Deppe is given.

PRINCETON, N. J. RECEIVED MARCH 24, 1938

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE OHIO STATE UNIVERSITY]

The Transformation of Dihydroxyacetone Derivatives into Pyruvic Aldehyde Derivatives[1]

BY CHARLES LLEWELLYN BERNIER AND WM. LLOYD EVANS

The conversion of the ketotriose, dihydroxyacetone, into pyruvic aldehyde has long been known. Pinkus[2] distilled a mixture of the former compound with dilute sulfuric acid and obtained, in the distillate, pyruvic aldehyde which he identified as the phenylosazone. Neuberg and Rewald[3] obtained pyruvic aldehyde phenylosazone by treating dihydroxyacetone with phenylhydrazine in either dilute ammonium hydroxide or sodium carbonate solution. More recently it has been found that the extent of the conversion in potassium hydroxide solution is influenced by the concentration of the alkali and the temperature.[4]

The transformation of the ketotriose is paralleled by a similar reaction of the isomeric aldotriose, glyceric aldehyde, from which Wohl[5] obtained pyruvic aldehyde phenylosazone by reaction with phenylhydrazine in weakly alkaline solution. It has been shown[6] that the experi-

(1) Abstracted from a thesis which was offered by Charles Llewellyn Bernier to the Graduate School of The Ohio State University in partial fulfilment of the requirements for the degree of Master of Science. This paper was presented at the Denver Meeting of The American Chemical Society, 1932.

(2) G. Pinkus, *Ber.*, **31**, 36 (1898).

(3) C. Neuberg and B. Rewald, *Biochem. Z.*, **71**, 144 (1915).

(4) W. L. Evans and W. R. Cornthwaite, THIS JOURNAL, **50**, 486 (1928).

(5) Wohl in V. Lippmann, "Chemie der Kohlenhydrate," 1891; *cf. Biochem. Z.*, **5**, 56 (1907).

(6) W. L. Evans and H. B. Hass, THIS JOURNAL, **48**, 2703 (1926)