Dehydration with Iodine.—Five grams of amino alcohol and a few crystals of iodine were heated at 200° for thirty minutes, and the mixture then distilled. The first fraction (2 g.) was much less viscous than the original alkamine, had a lower index of refraction ($n^{20}D$ 1.3650) and contained a slight aqueous layer. The second fraction was unreacted amino alcohol ($n^{20}D$ 1.4450).

Summary

The preparation and properties of four aliphatic tertiary amino alcohols, 2-methyl-2-hydroxy-1-dimethylaminopropane, 2-methyl-2-hydroxy-4-dimethylaminobutane, 2-methyl-2-hydroxy-5-dimethylaminopentane and 2-methyl-2-hydroxy-6-dimethylaminohexane, have been described.

Dehydration studies carried out with iodine, copper sulfate and potassium hydroxide on these amino alcohols indicate that when the amino group is adjacent to the hydroxyl group dehydration does not occur as easily as with ordinary tertiary alcohols. When the amino group is removed farther from the hydroxyl group, dehydration becomes easier.

The products formed by sulfuric acid dehydration, and by pyrolysis of the Grignard complex of 2-methyl-2-hydroxy-1-dimethylaminopropane, have been studied.

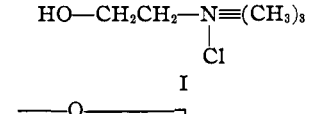
NOTRE DAME, INDIANA RECEIVED FEBRUARY 28, 1938

[CONTRIBUTION FROM MELLON INSTITUTE OF INDUSTRIAL RESEARCH, AND E. R. SQUIBB & SONS]

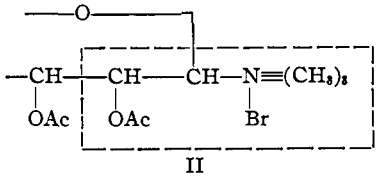
Halogeno-alkyl Glycosides. III. Quaternary Salts. Glucosamine Quaternary Derivative

BY HAROLD W. COLES[1] AND FRANK H. BERGEIM[2]

It is known that alterations in the structure of the alkanol group attached to the quaternary nitrogen in choline chloride (I)[3] or other changes such as acylation[4] of this group affect the activity and behavior of this compound. It therefore seemed of interest to us to determine how the choline action would be affected by replacing the alkanol group with sugar residues so as to have the latter attached directly to the quaternary nitrogen and by etherifying the alkanol group with sugar residues so as to have the latter attached to the quaternary nitrogen indirectly. Besides contributing to the solubility of the active principles



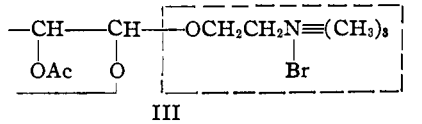
I



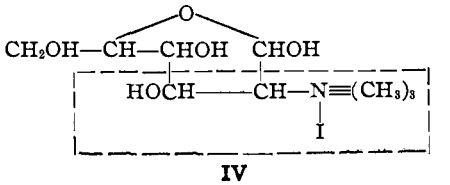
II

attached to the sugars the combinations, by gradual decomposition, would tend to give a milder and more general reaction instead of a more local toxic effect. It was therefore anticipated that the sugar residues might abolish or at least greatly diminish the toxicity of the quaternary group.

There are few literature references to pharmacological tests on choline derivatives of the carbohydrates. It was decided to prepare and test three different types. The first type is illustrated by (II). The acetylcholine part of the formula will be recognized within the broken line. It is at the same time a cyclic ether and an acetic ester, both linkages of which enhance the muscarine action of choline. It was considered also possible that the sugar part of the molecule would cause the product to be absorbed in parts of the body where choline is not ordinarily absorbed. Numerous quaternary derivatives of this type have been prepared by earlier workers. The two representatives of this first type prepared by the authors were (tetraacetyl-β-*d*-glucosido)-1-trimethylammonium bromide and (β-*d*-glucosido)-1-trimethylammonium bromide.



III



IV

(1) Senior Industrial Fellow, E. R. Squibb & Sons Industrial Fellowship, Mellon Institute.

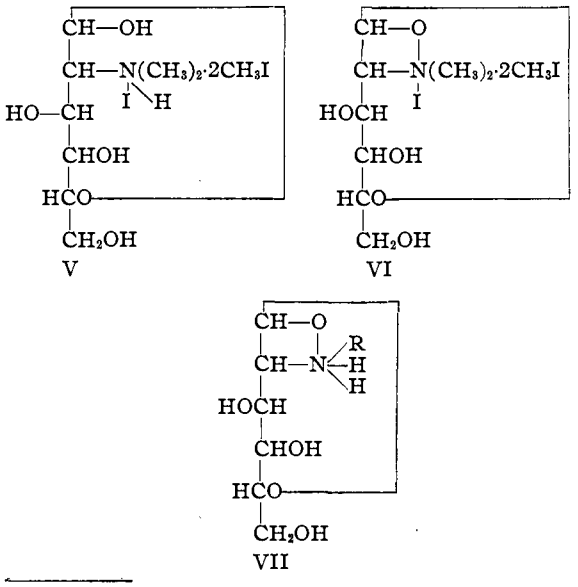
(2) Research Laboratories, E. R. Squibb & Sons, Brooklyn, N. Y.

(3) Dyson, "The Chemistry of Chemotherapy," Ernest Benn, Limited, London, 1928, pp. 105–120.

(4) Dale, *J. Physiol.*, **48**, III (1914).

The second type consisted of choline glucosides (III). These derivatives were of interest for several reasons. The active amines in ergot appear to be combined as glucosides. Again, the quaternary nitrogen is not joined directly to carbon 1 of the sugar chain as in (II). The choline group is indicated by the broken line. The acetylated derivatives were considered the most important.[5] These glucosidic choline substances were prepared from the tetraacetyl halogenoalkyl glucosides previously described.[6] The representatives of these types are: (tetraacetyl-β-*d*-glucosido)-choline chloride, (β-*d*-glucosido)-choline chloride, γ-(tetraacetyl-β-*d*-glucosido)-homocholine chloride and (tetraacetyl-β-*d*-glucosido)-ethyltriethylammonium bromide.

The third type, namely, a quaternary derivative of glucosamine is shown by figure (IV). There were several reasons for choosing the glucosamine derivative. The biological importance of glucosamine and the physiological activity of numerous glucosamine compounds made this type attractive. By alternate treatment of glucosamine in methyl alcohol with methyl iodide and potassium hydroxide on a boiling water-bath, a physiologically active, high-melting crystalline substance was obtained. The iodine analysis (61.71% I) and certain chemical properties described in the experimental part indicate that it is possibly the addition compound of the glucosamine salt with two molecules of methyl iodide (V) (61.51% I) and not the simple quaternary salt (IV) (36.35% I).



A lack of reducing power toward Fehling's solution supports the structure shown as VI (61.71% I) which is seen to bear a close resemblance to the compound (VII) described by Irvine and Hynd.[7] The study of this substance will be continued by the senior author.

The authors are grateful to Dr. George D. Beal, Assistant Director of Mellon Institute, for his advice during the progress of this work.

Experimental Part

Preparation of the Glucosido Quaternary Salts.—The (tetraacetyl-β-*d*-glucosido)-1-trimethylammonium bromide (m. p. 192°, $[\alpha]^{18}D$ +10.2° (water) was first prepared by Karrer and Smirnoff.[8] Their directions are rather incomplete and, in order to obtain good yields, it is necessary to observe certain precautions. The alcohol solvent must be absolutely anhydrous, the flask should be stored not longer than ten hours in the refrigerator after the acetobromoglucose has gone into solution and the ratio should be 120 g. of acetobromoglucose to 100 g. of 33% amine solution in ethyl alcohol.

The *d*-glucosido-1-trimethylammonium bromide was obtained from the foregoing acetylated salt according to the directions of Karrer and ter Kuile.[9] This deacetylated salt has a melting point of 161–162° and a rotation of $[\alpha]^{16}D$ +5°. It is hygroscopic, very soluble in water but very difficultly soluble in absolute alcohol.

Preparation of the Choline Glucosides.—These glucosides were prepared by the addition of the tertiary amines to the respective acetylated halogeno-alkyl glucosides.[6] The procedure of Gulewitsch[10] was employed first, but was soon abandoned as the alkalinity of the tertiary amine in alcohol was sufficient to remove the acetyl groups from the sugar residue with the production of the sirupy, hygroscopic deacetylated product. The treatment of the glucoside with trimethylamine and triethylamine in a sealed tube with alcohol or dioxane as solvent gave poor results. The same was true of simple refluxing of the glucoside and triethylamine. Successful results were obtained by following the suggestion of Major and Cline[11] who recommended the use of benzene as a non-polar solvent for this reaction.

(Tetraacetyl-β-*d*-glucosido)-ethyltriethylammonium Bromide, R—O—$CH_2CH_2N(C_2H_5)_3$·Br.—Three grams of tetraacetyl-β-*d*-(β-bromoethyl) glucoside (m. p. 117.3°) was placed in a Pyrex tube with about 10 cc. of benzene and about 3 g. of triethylamine and the tube sealed. The

(5) Quoting Goebel and co-workers [*J. Expll. Med.*, **60**, 85 (1934)]: "The specificity of carbohydrates is determined by the stereochemical configuration . . . and the introduction of a simple chemical group, such as acetyl, endows a carbohydrate with a new and distinct specificity which is determined by the chemical nature of the group thus introduced."

(6) Coles, Dodds and Bergeim, THIS JOURNAL, **60**, 1020 (1938). For dihalogenated glycosides, see Coles, Dodds and Bergeim, *ibid.*, 1167 (1938).

(7) Irvine and Hynd, *J. Chem. Soc.*, **103**, 41 (1913).

(8) Karrer and Smirnoff, *Helv. Chim. Acta*, **4**, 817 (1921).

(9) Karrer and ter Kuile, *ibid.*, **5**, 870 (1922).

(10) Gulewitsch, *Z. physiol. Chem.*, **24**, 515 (1898).

(11) Major and Cline, THIS JOURNAL, **54**, 242 (1932).

tube was heated for five hours on a boiling water-bath, chilled and opened. The contents were dissolved in hot alcohol, and the quaternary salt was precipitated out as a sirup by the addition of ether. The supernatant liquid was decanted and the sirup was washed several times with anhydrous ether. The sirup was taken up in a large volume of alcohol and ether added cautiously. The colorless needles, after recrystallization from the same solvents, melted at 67°.[12] They are very soluble in water, alcohol and chloroform; $[\alpha]^{20}D$ −33° (water).

Anal. (micro-Dumas). Calcd. for $C_{22}H_{38}O_{10}NBr$: N, 2.51. Found: N, 2.58.[13]

β-d-Glucosidocholine Chloride.—The reactants in aqueous alcohol solution were heated in a sealed tube for five hours on a water-bath. The Pyrex tube was strongly chilled and opened. The odor of acetic acid was noticeable. The tube contents were worked up in a manner similar to the foregoing example. The β-d-glucosidocholine chloride is a slightly yellow sirup, quite hygroscopic. It can be handled by chilling with Dry-Ice, when it hardens to a brittle glass.

(Tetraacetyl-β-d-glucosido)-choline Chloride, R—O—$CH_2CH_2N(CH_3)_3Cl$.[14]—This derivative was prepared in two ways.

(a) From β-d-Glucosidocholine Chloride.—Three grams of the sirup was heated with 20 g. of acetic anhydride on a water-bath for two hours. The addition of anhydrous ether caused the formation of very long needles. These were removed to a Büchner funnel by filtration, washed well with ether and, after recrystallization from absolute alcohol by the addition of ether, melted at 216–217°. The yield was 1.5 g.; $[\alpha]^{20}D$ −25° (water).

Anal. Calcd. for $C_{19}H_{32}O_{10}NCl$: N, 2.98. Found: N, 2.74.

(b) From Tetraacetyl-β-d-(β-chloroethyl) Glucoside.—Two grams of tetraacetyl-β-d-(β-chloroethyl)-glucoside was added to a chilled benzene solution of trimethylamine in a Pyrex tube and the tube was sealed. After heating the tube in a boiling water-bath for eight hours, the tube was chilled, opened and the contents dissolved in hot absolute alcohol. The addition of anhydrous ether caused the immediate formation of very fine needles. These needles were filtered off, washed well with ether and dried in the open air. They melted at 217–218° after recrystallization.

Anal. Calcd. for $C_{19}H_{32}O_{10}NCl$: N, 2.98. Found: N, 2.80.

γ-(Tetraacetyl-β-d-glucosido)-homocholine Chloride, R—O—$CH_2CH_2CH_2$—$N(CH_3)_3Cl$.—Three grams of the tetraacetyl-β-d-(γ-chloropropyl)-glucoside (m. p. 74°) was weighed out in a Pyrex glass tube with 7–8 cc. of benzene and an excess of trimethylamine. The tube was sealed, and heated for five hours in a boiling water-bath. After chilling the tube and opening, the contents were dissolved in hot alcohol. Anhydrous ether threw out a sirup. The supernatant liquid was decanted, the sirup washed several times with ether, and taken up in a large volume of absolute alcohol. The quaternary salt separated as long needles on the addition of anhydrous ether. The recrystallized salt melted at 165–167.5°. The crystals were very soluble in water and chloroform, moderately soluble in acetone and dioxane and insoluble in benzene and petroleum ether.

Anal. Calcd. for $C_{20}H_{34}O_{10}NCl$: N, 2.89. Found: N, 3.01.

Action of Methyl Iodide and Potassium Hydroxide upon Glucosamine.—Ten grams of glucosamine hydrochloride was suspended in 50 cc. of methyl alcohol and, while refluxing, potassium hydroxide (13.3 g.) and methyl iodide (30 g.) were added in small amounts alternately, starting with the potassium hydroxide, in the course of six hours. The liquid became quite dark. Ten cc. of methyl alcohol was added and the refluxing was continued for several hours longer. The alcohol was evaporated and the residue stirred three times with ether to remove any methyl iodide. The residue was dissolved in 100 cc. of water, decolorized as much as possible with charcoal and the volume of the liquid reduced to 55 cc. with an air-stream. Thirty cc. of absolute methyl alcohol was added.

The crystalline material which separated out was recrystallized from a large volume of hot absolute alcohol and formed tufts of needles on crystallization. These crystals gave a Beilstein test for halogen, gave no test for potassium (either by the chloroplatinate test or spectroscopically) and did not melt below 280°. They were very soluble in water. The crystals melted readily on a spatula, vaporized without very much of a flame, giving an odor of methyl iodide. Apparently all the halogen is ionic, since all of the iodine is determinable by simple precipitation.

Anal. Calcd. for a simple quaternary salt of glucosamine ($C_9H_{20}O_5NI$): I, 36.35. Found: I, 61.71, 61.39 (grav.), 61.71 (Parr bomb).

The compound does not form compounds with silver iodide as indicated by the check in analyses by two different methods. Fehling's solution, cold or warm, is not reduced, but immediate reduction takes place on the addition of a few drops of acid, which fact is support for formula VI. The crystals do not form an insoluble picrate, which would indicate that it is already a molecular complex with methyl iodide (V or VI). Quaternary ammonium compounds form additive derivatives with iodoform and chloroform, and it might be expected that they would do the same with methyl iodide.[15]

Pharmacological Action[16]

(Tetraacetyl-β-d-glucosido)-1-trimethylammonium bromide and (β-d-glucosido)-1-trimethylammonium bromide produced no observable effect on the blood pressure of rabbits when given in doses of 5 mg. per kilogram weight of animal. β-d-Glucosidocholine chloride was likewise inactive. However, the glucosamine derivative had an

(12) All melting points are corrected for stem exposure.

(13) Micro-analyses by Mr. Saul Gottlieb, Columbia University.

(14) Schroeter and Strassberger [*Biochem. Z.*, **232**, 454 (1931)] report the preparation of an alpha-form of this choline glucoside which was inactive on mice, frogs and guinea pigs. While this article was in process of preparation, a note by Jackson appeared [THIS JOURNAL, **60**, 722 (1938)] which described this compound. He reported a melting point of 230° (uncorr.) and a rotation in water of −25.6°.

(15) See Steinkopf and others, *J. prakt. Chem.*, **109**, 230 (1925); **113**, 159 (1926), and *Ber.*, **54**, 2969 (1921).

(16) The authors are indebted to the E. R. Squibb & Sons Biological Laboratories, New Brunswick, N. J., for these tests.

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.

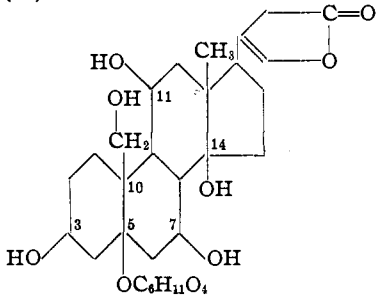
RECEIVED MARCH 19, 1938

[CONTRIBUTION FROM THE FRICK CHEMICAL LABORATORY, PRINCETON UNIVERSITY]

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

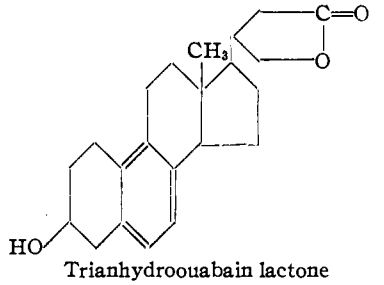
BY P. N. CHAKRAVORTY[1] AND EVERETT S. WALLIS

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).