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## The Synthesis of 5-D-Glucosido-D-arabinose

BY NORMAN S. MAC DONALD AND WM. LLOYD EVANS<sup>1</sup>

The disaccharide, 5-D-glucosido-D-arabinose, is of much scientific interest. In the first place, this compound is a possible intermediate in the alkaline degradation of the 6-hexosido-hexose type<sup>2</sup> of carbohydrate. Furthermore, the acetylated 5-D-glucosido-D-arabinose would possess both the pyranoid ring in the hexose section of the molecule and the furanoid ring in the pentose section. So far as the authors are aware, a carbohydrate of this type has not been synthesized hitherto.

The purpose of the present work was to prepare this disaccharide and characterize it. After trying to obtain the compound by several of the well known degradative and synthetic procedures, its preparation was finally achieved by the Wohl technique for the degradation of sugar oximes, as modified by Zemplén.<sup>3</sup>

The 5-D-glucosido-D-arabinose heptaacetate was characterized by an elementary analysis, determination of acetyl number, and by an estimation of the pentose content.

Deacetylation of the acetate gave a white amorphous hygroscopic powder whose aqueous solution exhibited mutarotatory power. The deacetylated sugar failed to give an aldehyde reaction with Schiff reagent, a fact which when coupled with that of mutarotation indicates the presence of a ring in the reducing section of this

new disaccharide. Since the point of biosidic linkage of the reducing section is carbon atom five, it therefore follows that the lactal ring of the arabinose section is of the furanoid type. Treatment of this water solution with phenylhydrazine led to a phenylosazone.

The acetylation of the free sugar was carried out with hot acetic anhydride-sodium acetate and, therefore, it seems likely that the crystalline material is the  $\beta$ -isomer.

Upon working up the mother liquors of the degradation, a small amount of a different crystalline substance was isolated. An insufficient quantity of this compound prevented complete characterization. It is suggested, however, that this lower melting, higher rotating compound is the  $\alpha$ -isomer.

### Experimental Part

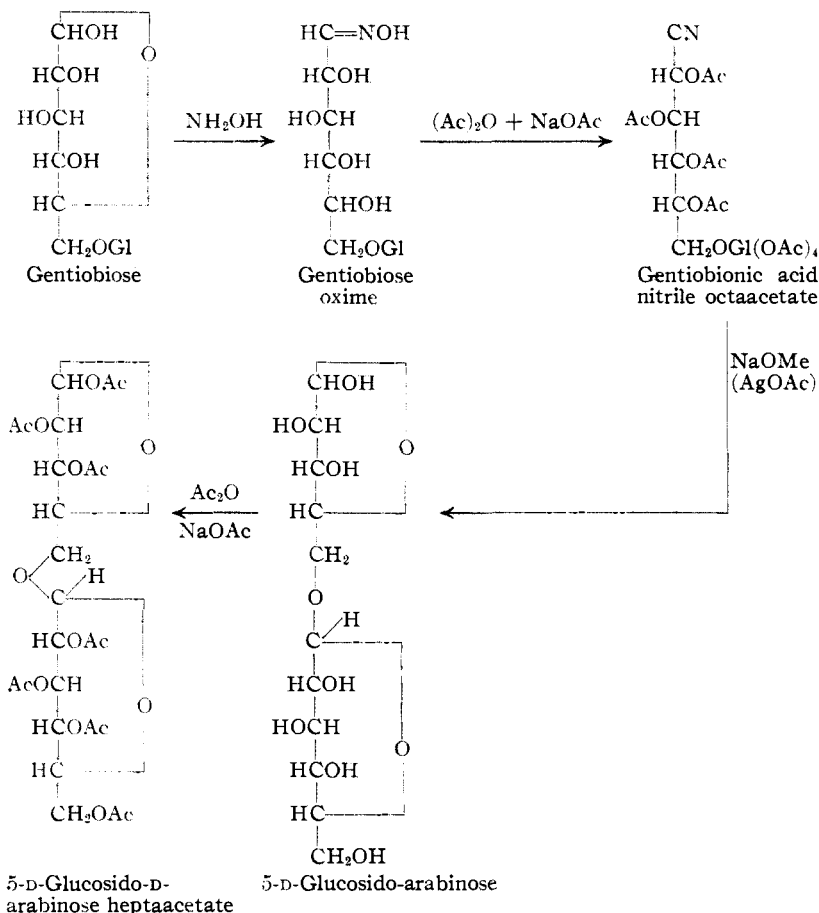
**Preparation of Gentiobiose Oxime.**—To a solution of 17.5 g. of gentiobiose, obtained by the deacetylation of  $\beta$ -gentiobiose octaacetate<sup>4</sup> with NaOMe, was added an alcoholic solution of hydroxylamine. The latter was made by adding 2.6 g. sodium in 65 cc. of absolute ethanol to 8.9 g. of hydroxylamine hydrochloride (98% pure) in 6 cc. of hot water, cooling, and filtering the precipitated sodium chloride. The hydroxylamine hydrochloride used is 150% in excess while the sodium is 90% of the amount required to neutralize the hydrochloric acid completely. After the dropwise addition of the hydroxylamine to the sugar solution, 10 g. of powdered calcium carbonate was added to neutralize any possible acidity which might hydrolyze the biosidic link. The temperature was then raised to 55° and kept at that point for two hours. Without removing the calcium carbonate, the solution was

(1) Abstracted from a Thesis presented by N. S. MacDonald to the Graduate School of The Ohio State University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

(2) (a) W. L. Evans and R. C. Hockett, *THIS JOURNAL*, **53**, 4384 (1931); (b) W. L. Evans, *Chem. Revs.*, **6**, 281 (1929).

(3) G. Zemplén, *Ber.*, **59**, 1254 (1926).

(4) D. D. Reynolds and Wm. Lloyd Evans, *THIS JOURNAL*, **60**, 2559 (1938).



(cor.); yield 14.5 g. (35%);  $[\alpha]^{25}_D +8.60^\circ$ , ( $\text{CHCl}_3$ ,  $c = 2.5$ ). It may be noted here that the sign of the optical rotation is in accord with that predicted by the Hudson-Levene hydrazide rule, as extended to sugar acid nitriles.<sup>5</sup> This empirical generalization states that if the hydroxyl group on carbon two lies on the right side in the straight chain structural formula, the acetylated nitrile of the sugar acid will be dextrorotatory.

*Anal.* Calcd. for  $\text{C}_{27}\text{H}_{37}\text{O}_{18}\text{CN}$ : C, 49.78; H, 5.48; N, 2.07. Found: C, 49.73, 49.77; H, 5.51, 5.57; N, 2.38, 2.57.

The compound gives a positive qualitative test for  $-\text{CN}$  (addition of sodium hydroxide and ferrous sulfate followed by acidification gives the characteristic prussian blue precipitate).

**Quantitative Determination of  $-\text{CN}$  Group.**—The weighed sample (0.1156 g.) was dissolved in 25 cc. of absolute methanol and a solution of 0.3 g. of silver nitrate in 4 cc. of water added. After adding 10 cc. of methanol saturated with ammonia, the flask was stoppered and allowed to stand at room temperature for ten hours.

The clear solution was then acidified with dilute nitric acid and allowed to stand for three hours in a dark place. The precipitated silver cyanide was filtered into a weighed Gooch crucible, washed, dried at  $100^\circ$  and weighed. Calcd.:  $-\text{CN}$ , 3.85. Found:  $-\text{CN}$ , 3.60.

**Degradation of Gentiobionic Acid Nitrile Octaacetate to 5-D-Glucosido-D-arabinose Heptaacetates.**—Four grams of the nitrile was dissolved in 10 cc. of dry chloroform and a solution of 0.2 g. of sodium in 15 cc. of absolute methanol added, after cooling both in ice-water. The mixture gelatinized in one minute, but was allowed to stand in the bath for an additional five minutes. The gel was shaken with 15 cc. of water and immediately acidified with 5 cc. of acetic acid. After diluting with 15 cc. of water, the solution was extracted with 25 cc. of chloroform. A suspension of 5 g. of silver acetate in 10 cc. of glacial acetic acid was added to the water layer, with shaking. After ten minutes, the silver cyanide and excess silver acetate were removed by filtration, the filtrate giving no precipitate with a drop of fresh silver acetate solution. The filtrate was then warmed and dilute hydrochloric acid added dropwise to precipitate the  $\text{Ag}^+$ . After filtration, the colorless solution was treated with a small amount of sodium bicarbonate to reduce the acidity, then concentrated to a sirup *in vacuo*. This sirup was dried by repeatedly evaporating to dryness with absolute ethanol. To the dried sirup was added 20 cc. of acetic anhydride and 3 g. of freshly fused

concentrated to dryness *in vacuo*. Twenty-five cc. of absolute ethanol was then added, and the solution again evaporated to dryness. The residue was dissolved in 20 cc. of water, decolorized with carboraffin, and evaporated several times with absolute alcohol in order to dry it completely. The powder so obtained could not be crystallized and hence it was used directly in the next step.

**Preparation of Gentiobionic Acid Nitrile Octaacetate.**—Twenty grams of freshly fused sodium acetate was added to 145 cc. of acetic anhydride in a 250-cc. Erlenmeyer flask and the mixture heated to  $110^\circ$  in an oil-bath. To this was added 17.9 g. of dry, powdered gentiobiose oxime in such small portions that the temperature neither rose above  $120^\circ$  nor fell below  $105^\circ$ . This operation required about one-half hour. After the temperature was kept at  $110$ – $115^\circ$  for an hour longer the deep brown solution was allowed to cool to  $80^\circ$ . It was then poured into 750 cc. of cold water and stirred for one hour. The crumbly material which appeared was stirred with fresh water, filtered, dissolved in ethanol and decolorized with carboraffin. Dilution of the resulting solution with water led to the separation of sirups. These sirups were each subjected to fractional precipitation from ethanol-water and, after some time, nuclei were obtained. Upon inoculating the sirups with these nuclei, crystals became readily available. The long needles are very soluble in chloroform and ethyl acetate, moderately soluble in methanol and ethanol, very slightly soluble in ligroin and water; m. p.  $108$ – $109^\circ$

(5) V. Deulofeu, *Nature*, **131**, 548 (1933).

sodium acetate, the mixture placed in a boiling water-bath and kept at that temperature for one hour. The dark solution was then poured into 125 cc. of cold water and stirred for one hour. The granular material was filtered off and dissolved in chloroform, dried over anhydrous sodium sulfate and treated with carboraffin. Upon concentrating *in vacuo*, a yellow sirup was obtained, which was subjected to fractional precipitation from ethanol-water. Two products were finally obtained in the crystalline state: I, (less alcohol-soluble fraction), rhombohedra, m. p. 161-162° (cor.);  $[\alpha]^{25D} - 14.4^\circ$  ( $\text{CHCl}_3$ ,  $c = 4.2$ ); yield 1.2 g. (32%).

*Anal.* Calcd. for  $\text{C}_{25}\text{H}_{34}\text{O}_{17}$ : C, 49.50; H, 5.61; acetyl, 11.6.<sup>6</sup> Found: C, 49.51, 49.25; H, 5.56, 5.67; acetyl, 11.7, 11.7.

II. (More alcohol-soluble fraction), needles; m. p. 132-133° (cor.);  $[\alpha]^{25D} + 23.1^\circ$  ( $\text{CHCl}_3$ ,  $c = 3.8$ .)

*Anal.* Calcd. for  $\text{C}_{25}\text{H}_{34}\text{O}_{17}$ : C, 49.50; H, 5.61. Found: C, 49.51, 49.40; H, 5.88, 5.82.

Such a small amount of this material was obtained that a satisfactory acetyl number could not be determined.

**Preparation of 5-D-Glucosido-D-arabinose.**—The free sugar was prepared in an amorphous condition by deacetylation of the heptaacetate I. To a cooled solution of 0.78 g. of the acetate in 30 cc. of absolute methanol was added 0.05 g. of sodium in 5 cc. of absolute methanol. After standing in the ice-box for two hours, and concentrating to a volume of 15 cc., a precipitate appeared. This material was filtered rapidly and washed with cold absolute methanol. The amorphous powder reduces Fehling's solution and is very hygroscopic; yield 0.25 g.; equilibrium rotation  $[\alpha]^{30D} - 3.14^\circ$  ( $\text{H}_2\text{O}$ ,  $c = 4.1$ ).

**Phenylosazone of 5-D-Glucosido-D-arabinose.**—To the water solution of the free sugar, used for the determination of the optical rotation, was added a mixture of 0.3 g. of phenylhydrazine and 8 cc. of glacial acetic acid. After warming on the water-bath for forty-five minutes, the dark solution was diluted with 20 cc. of water and allowed to stand overnight. The crude material so precipitated was recrystallized by dissolving in the smallest possible amount of pyridine, diluting with a ten-fold volume of acetone, and adding ligroin to turbidity. A crop of yellow needles was obtained; m. p. 209-210° (cor.); mixed m. p. with glucose phenylosazone 198-200°.

*Anal.* Calcd. for  $\text{C}_{28}\text{H}_{30}\text{O}_8\text{N}_4$ : N, 11.43. Found: N, 11.17, 11.02.

**Estimation of Pentose in 5-D-Glucosido-D-arabinose Heptaacetate.**—The method used was a modification of the standard procedure for pentose determination<sup>7</sup> as developed by Tollens. Sixty-eight milligrams of the material (I) was transferred to a 20-cc. distilling flask equipped with a 25-cc. dropping funnel in the neck and a short water jacket for the arm. The acetate was dissolved in 8 cc. of absolute methanol and 1.0 cc. of a sodium methylate solution (1%) added. It was found that unless the material was deacetylated and thus rendered water soluble, before the acid distillation, results were not reproducible. After three minutes, 10 cc. of 12% hydro-

chloric acid was added, boiling stones dropped in and the distillation begun gently. After 10 cc. had come over, the distillate gave a red color to paper moistened with aniline acetate, a positive test for the presence of furfural. The distillate was allowed to drop through a filter into a graduated cylinder and 100 cc. was collected, fresh 12% hydrochloric acid being added to maintain a constant level in the distilling flask. To the colorless distillate was added a solution of 0.05 g. of phloroglucinol in 10 cc. of warm 12% hydrochloric acid. A yellow color developed immediately, which darkened rapidly. Within ten minutes, a blue-black precipitate appeared. The mixture was diluted to 150 cc. with water and allowed to stand twelve hours at room temperature, after which it was filtered into a weighed Gooch crucible, dried and weighed. By applying empirical correction factors,<sup>7</sup> the weight of pentose is calculated from the weight of furfural-phloroglucide obtained: weight of arabinose calcd., 0.017 g.; weight of pentose found, 0.016 g.

**Control.**—A mixture of 0.036 g. of D-arabinose tetraacetate and 0.052 g. of D-glucose pentaacetate was treated in exactly the same manner: weight of arabinose calcd., 0.017 g.; weight of pentose found, 0.016 g. In order to make sure that hydroxymethylfurfural, obtainable from the glucose section, did not interfere, 0.05 g. of gentiobiose octaacetate was subjected to exactly the same treatment. No coloration of aniline acetate and no precipitate with phloroglucinol were obtained under these conditions.

## Summary

1. Crystalline gentiobionic acid nitrile octaacetate was prepared by the removal of the elements of water from amorphous gentiobiose oxime. The sign of its optical rotation agrees with that predicted from the Hudson-Levene hydrazide rule, as extended to sugar acid nitriles by Deulofeu.

2. 5-D-Glucosido-D-arabinose heptaacetate was prepared by the degradation of gentiobionic acid nitrile octaacetate with sodium methoxide and the acetylation of the resulting compound.

3. Since the free sugar is mutarotatory in aqueous solution and shows no reaction with Schiff reagent, it is concluded that the arabinose section of this compound contains the furan ring.

4. The presence of arabinose was established by the detection of furfural in the products of the acid hydrolysis of the disaccharide.

5. The phenylosazone of 5-D-glucosido-D-arabinose was prepared from the amorphous free sugar.

6. It is suggested that the two acetylated compounds obtained from the degradation constitute an  $\alpha,\beta$ -isomeric pair.

(6) A. Kunz and C. S. Hudson, *THIS JOURNAL*, **48**, 1982 (1926).

(7) "Allen's Commercial Organic Analysis," 5th ed., Vol. 1, p. 498.