SYNTHESIS OF 3-O-α-D-GLCOPYRANOSYL-D-GLUCOSE (SAKEBIOSE OR NIGEROSE) I. REACTION OF 1, 2-5, 6-DI-O-ISOPROPYLIDENE-D-GLUCOFURANOSE AND 2, 3, 4, 6-TETRA-O-ACETYL-α-D-GLUCOPYRANOSYL BROMIDE

SYNTHESIS OF 3-*O*-*α*-D-GLCOPYRANOSYL-D-GLUCOSE (SAKÉBIOSE OR NIGEROSE)*

I. REACTION OF 1, 2-5, 6-DI-*O*-ISOPROPYLIDENE-D-GLUCOFURANOSE AND 2, 3, 4, 6-TETRA-*O*-ACETYL-*α*-D-GLUCOPYRANOSYL BROMIDE

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(*Received October 25, 1958*)

A chemical synthesis of 1, 3-*α*-linked glucobiose was carried out by the condensation of 1, 2-5, 6-di-*O*-isopropylidene-D-glucofuranose and 2, 3, 4, 6-tetra-*O*-acetyl-*α*-D-glucopyranosyl bromide with mercuric cyanide. The reaction products, after removal of the protecting groups, were fractionated by carbon : Celite column chromatography. Besides 1, 3-linked sakébiose and laminaribiose, a considerable amount of 1, 6-linked isomaltose and gentiobiose was obtained.

One of the authors has previously isolated an unfermentable disaccharide from Koji extract as its crystalline octaacetate which was named sakébiose (1). Since this sugar had properties similar to nigerose, which had been obtained by Barker *et al* (2) from the partial acid hydrolysate of the fungal polysaccharide "nigeran", and also to *y*-sugar, an unidentified sugar isolated by Thompson *et al* (3) from the acid reversion product of D-glucose, it was suggested that these sugars might be identical. The identity of these sugars has been proved later (4) by direct comparison of the crystalline acetate of sakébiose with that of nigerose prepared by the method of Barker *et al* (2) and also with *y*-acetate which had been provided by Dr. Thompson. The structure of nigerose was established later by Barker *et al* (5) from the identity of its osazone with that of turanose (3-*O*-*α*-D-glucopyranosyl-D-fructose) and further structural evidence by means of the methylation study was recently provided by the same authors (6).

In 1946, Gakhokidze (7) obtained a new disaccharide by the condensation of 4, 6-*O*-benzylidene-1, 2-*O*-isopropylidene-D-glucose and 2, 3, 4, 6-tetra-*O*-acetyl-*α*-D-glucose with zinc chloride and phosphoric oxide followed by the re-

* The original report (in Japanese) is contributed to "Nippon Nogeikagaku Kaishi (Journal of the Agricultural Chemical Society of Japan)".

moval of the protecting groups. In the later report, the structure of this sugar was established as 3-*O*-α-D-glucopyranosyl-D-glucose by the same author (8). The properties of the free sugar and a crystalline acetyl derivative were recorded, but these values are different from those of sakébiose and nigerose. The present study was carried out to solve the question by chemical synthesis.

The desired disaccharide has α-glucosidic linkage. The synthesis of α-glucosidic linkage is difficult and with the exception of α, β-trehalose (9) it is only in the last few years that maltose (10), sucrose (11), α, α-trehalose (12) and kojibiose (13) has been chemically synthesized. As the synthetic methods of α-linkage, two types of reaction have been known. One is the additional reaction of Brigl's anhydride (14) and the other is the application of the Königs-Knorr reaction using mercuric salts as the condensing agent. By the former method, α, β-trehalose was at first synthesized by Haworth and Hickinbottom (9) and recently maltose (10), sucrose (11) and α, α-trehalose (12) were synthesized by Lemieux and his coworkers. Königs-Knorr reaction has been applied usually to the synthesis of β-linked disaccharides, and in this case silver salts were commonly used as the condensing agents.

In 1931, however, Zemplén (15) suggested that a considerable amount of α-linkage was formed in addition to β-linkage when mercuric salt, was used in this reaction instead of silver salts. This view was confirmed recently by the studies of Micheel *et al* (16) and Helferich *et al* (17) who have synthesized α, β-trehalose by using mercuric cyanide, and further by the fact that kojibiose has been synthesized by us (13) by the same method.

In the present work, 1, 2-5, 6-di-*O*-isopropylidene-D-glucofuranose (I) was used as the starting material of 3-*O*-substituent. This substance was used by Bächli and Percival (18) and also by Freudenberg and Oertzen (19) in their syntheses of laminaribiose.

(I) and 2, 3, 4, 6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (II) were refluxed in dry benzene with mercuric cyanide for eight hours. The reaction products were deacetylated and after removal of the isopropylidene group by treating with dilute sulfuric acid, fractionated on carbon: Celite column by successive elution with water, 5, 10 and 15 percent ethanol. Both 1, 3-α-linked sakébiose and 1, 3-β-linked laminaribiose were obtained. Moreover, it was noticed that a considerable amount of 1, 6-linked glucobioses (isomaltose and gentiobiose) was obtained unexpectedly in addition to the desired disaccharide. This fact seems to be very interesting, but the mechanism of 1, 6-linkage formation was not ascertained. Bächli and Percival (18) have described in their report concerning the chemical synthesis of laminaribiose that an unidentified substance with R_G 0.4 was present in the reaction mixture of (I) and (II) followed by the removal of the protecting groups. The R_G value 0.4 seems to be very similar to that of 1, 6-linked glucobiose, but no further

explanation was given for this problem.

After this work was completed, a chemical synthesis of nigerose was reported by Haq and Whelan (20) who used 3, 4, 6-tri-*O*-acetyl-β-D-glucopyranosyl chloride and 3-*O*-sodio-1, 2-5, 6-di-*O*-isopropylidene-D-glucofuranose as the starting materials. They obtained kojibiose, α, α-trehalose and an unidentified disaccharide besides the main product nigerose. There is, therefore, little overlap between their work and ours, since their experimental methods and the starting materials they used are not the same as those reported in the present paper.

Experimental

〔1〕 *Preparation of the Starting Materials.*

1. *1, 2-5, 6-Di-O-isopropylidene-D-glucofuranose* (I). This was prepared from glucose by the method of Freudenberg and Smeykal (21). Yield, 43 g from 65 g of glucose. It had m. p. 110°C.

2. *2, 3, 4, 6-Tetra-O-acetyl-α-D-glucopyranosyl bromide* (II). This was prepared from penta-*O*-acetyl-β-D-glucose by the method of Scheurer and Smith (22). Yield, 51 g from 72 g of pentaacetate. It had m. p. 88°C.

〔2〕 *Condensation of* (I) *and* (II).

(I) 13 g, (II) 20.5 g and mercuric cyanide 6.1 g were refluxed in 200 *ml* of dry benzene on a boiling water bath for eight hours. The solution was washed with a saturated solution of sodium carbonate and then repeatedly with water, dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residue (28.5 g) was deacetylated with 150 *ml* of 0.05 *N* sodium methylate at 0°C overnight. The precipitate deposited was dissolved by adding a small amount of water and the solution was neutralized with a few drops of acetic acid. Removal of most of the methanol under reduced pressure was followed by the removal of the isopropylidene group by treatment of the residue with 180 *ml* of 0.1 *N* sulfuric acid at 70°C for two hours. After neutralization with barium carbonate and filtration, the remaining ions were removed by Amberlite IR-120 and IRA-410 ion exchange resins. Evaporation of water left a light yellow syrup, yield 17 g. Examination on the paper chromatogram with pyridine: butanol: water (4 : 6 : 3) showed the presence of glucose, 1, 3-linked glucobiose, 1, 6-linked glucobiose and an unknown substance with R_G 1.8.

〔3〕 *Fractionation of the Reaction Products by Carbon: Celite Column.*

Thirty two grams of the above reaction products (two runs) was dissolved in 500 *ml* of water and poured on a chromatographic column of carbon: Celite

(500 g : 500 g) and eluted with water and 5~15 per cent ethanol successively. The effluents were caught in two liters and evaporated under reduced pressure and examined by paper chromatography. The results are shown in Table 1.

Table 8. Fractionation of the Reaction Products.

Fract. No.	Solvent used for elution	Probable sugar component by PPC	Yield (g)
1-2	Water	—	
3-6	,,	Glucose	} 2.6
7-8	,,	—	
9-10	5 % Ethanol	Glucose	
11-12	,,	Isomaltose, Unknown sugar (R_G 1.8)	1.5
13	,,	Isomaltose, Sakébiose	0.5
14-16	,,	Sakébiose, Gentiobiose	4.4
17-20	,,	Gentiobiose	1.8
21	,,	Gentiobiose, Laminaribiose	0.2
22-39	,,	Laminaribiose	5.3
40-43	,,	—	
44-53	10 % Ethanol	Non-reducing substance	} 4.4
54-63	15 % Ethanol	,,	

〔4〕 *Characterization of the Reaction Products.*

1. *Isomaltose*

Fractions 11~12 was combined and evaporated to a syrup, which upon treatment with hot methanol gave 1.5 g of white amorphous powder. This was acetylated as usual to give 2.1 g of crude acetate. Direct crystallization from ethanol was not succeeded. This crude acetate was dissolved in 5 *ml* of benzene and poured on a 185×35 (diam.)mm. column of Magnesol : Celite (5 : 1) and developed with 1000 *ml* of benzene : t-butanol (100 : 1, by volume). A zone appeared at 80~150 mm from the top of the column by means of $KMnO_4$ streak indicator was sectioned from the column and eluted with acetone. Removal of the solvent left 0.4 g of syrup, which was crystallized very slowly from ethanol. Yield, 70 mg. After recrystallization from the same solvent, the products showed m. p. 144~145°C undepressed on admixture with the known β-isomaltose octaacetate ; $[\alpha]_D^{25}$ +96.6 (*c*, 1.2 ; chloroform). Evaporation of the effluent from the column gave 0.8 g of syrup. All attempts to crystallize this syrup was not successful.

2. *Gentiobiose*

Fractions 17~20 was treated as above to give 1.8 g of amorphous powder. This was acetylated as usual to give 3.2 g of crude acetate, which was crystallized from 50 *ml* of hot ethanol. Yield, 2.5 g. The twice recrystallized product showed m. p. 190~191°C, undepressed on admixture with the known β-gentiobiose octaacetate ; $[\alpha]_D^{25}$ −5.2 (*c*, 5.8 ; chloroform).

3. *Sakébiose*

Acetylation of fractions 14~16 treated as above (4.4 g) gave 8.1 g of crude acetate. 5.8 g of crystalline product was obtained by crystallization from ethanol. Twice recrystallized product showed m. p. 190~191°C, undepressed on admixture with the known β-gentiobiose octaacetate. The original and the first recrystallization's mother liquor was combined, from which 1 g of fine gelatinous crystal was obtained. From the concentrated filtrate of the crystal, 0.4 g of further crops were obtained. The combined crystal was twice recrystallized from ethanol. The recrystallized product was a fine prism, and showed m. p. 149~150°C, undepressed on admixture with the known β-sakébiose octaacetate isolated from koji extract; $[\alpha]_D^{23}+76.8$ (*c*, 3.0; chloroform). ***Anal.*** Calc. for $C_{28}H_{38}O_{19}$: C, 49.55; H, 5.64 percent. Found: C, 49.44; H, 5.70 percent.

4. *Laminaribiose*

Fractions 22~39 gave 5.3 g of white amorphous powder. This was acetylated as usual to give 8.6 g of crude acetate. Attempts to crystallize from ethanol was not successful. This crude acetate was deacetylated by treating with 85 *ml* of 0.05 *N* sodium methylate in methanol. The solution was diluted with water and deionized by ion exchange resin (Amberlite IR-120 and IRA-410) and finally concentrated under reduced pressure to a syrup. 0.5 g of crystalline free suger was obtained when the methanol solution was slowly evaporated at room temperature. From the mother liquor, 0.2 g of further crops were obtained. It had m. p. 196~200°C, $[\alpha]_D^{0}+24.3$ (15 min.)→+18.2 (24 hrs.) (*c*, 2.0; water). These values are in good agreement with the published values of α-laminaribiose. On the paper chrotogram, only one spot was detected.

0.5 g of crystalline laminaribiose was acetylated as usual to give 0.9 g of crude acetate, which was crystallized from ethanol. Yield, 280 mg. Twice recrystallized product showed m. p. 158~159°C, undepressed on admixture with the known β-laminaribiose octaacetate isolated from hydrol (23); $[\alpha]_D^{25}-26.2$ (*c*. 2.3; chloroform).

5. *Non-reducing Fraction*

Fractions 40–63 gave 4.4 g of amorphous powder, which is unreactive by aniline hydrogen phthalate on the paper chromatogram. It is presumed that this unreactivity may be occurred by the residual isopropyliden group due to the uncomplete deisopropylidenation. Treatment with 0.1 *N* H_2SO_4 followed by neutralisation, deionisation and concentration left 2.4 g of syrup. Paper chromatographic examination showed the presence of glucose, 1, 3- and 1, 6-linked glucobiose.

Acknowledgment

The authors wish to acknowledge their indebtedness to Prof. K. Aso for

his guidance and encouragement. Thanks are also due to the Laboratory of Biological Chemistry, Faculty of Science for microanalysis and measurement of optical rotation.

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