which was crystallized from MeOH. The product, methyl penta-O-acetyl-L-gulonate, was melted at 68.0°. *Anal.* Calcd. for $C_{10}H_{15}O_5$: C, 48.57; H, 5.76. Found: C, 48.46; H, 5.75.

The authors are indebted to Mr. K. Yamamoto for his technical assistance and wishes to thank Miss M. Ishii for elementary analyses.

Summary

It was found that some carbohydrates with lactone group was quantitatively absorbed on anion exchange resins in aqueous solution, and by elution with suitably mineral acid they were recovered in high yield as the corresponding free acid.

Thus D-glucuronic acid (II), 1,2-O-isopropylidene-D-glucofuranosiduronic acid (V), methyl D-glucofuranosiduronic acid (VII) and L-gulonic acid (IX) were obtained advantageously from their lactone derivatives. It is noteworthy that some of them have not been obtained in success by the direct neutralization of lactone derivatives with alkaline medium.

(Received October 14, 1964)

[Chem. Pharm. Bull. 13(2) 176~179 (1965)]

UDC 547.458 : 547.483

24. Yoshinobu Hirasaka and Isao Matsunaga: Studies on the α-(1,4)linked Polysaccharides of D-Glucuronic Acid and D-Glucose. VII.*1 Synthesis of 4-O-(α-D-Glucopyranosyl)-D-Glucuronic Acid.

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As a part of the investigations on the nitric acid oxidized starch, synthesis of three types of the constituent disaccharides containing glucuronic acid residue became necessary and two of them were reported in the preceding papers.[1~3]

Namely, 4-O-(α-D-glucopyranosiduronyl)-D-glucose (UG) was selectively obtained by catalytic oxidation of benzyl maltoside,[1] and moreover tritylation of maltose resulted in 6′-tritylmaltose and 6,6′-ditritylmaltose, which afforded UG and 4-O-(α-D-glucopyranosiduronyl)-D-glucuronic acid (UU) respectively by acetylation followed by detritylation and potassium permanganate-chromic acid oxidation.

But 4-O-(α-D--glucopyranosyl)-D-glucuronic acid (GU) could not be obtained by the similar methods probably because of poor reactivity of the primary alcohol of the reducing glucose unit of maltose.

The present investigation was undertaken to synthesize GU by another way, as was illustrated in Chart 1.

By condensation of octa-O-acetyl-β-maltose (I) with phenol in the presence of catalytic amount of *p*-toluene sulfonic acid, phenyl hepta-O-acetyl-β-maltoside (II) was obtained in 55 percent yield. In this reaction Lindberg, *et al.* used some amounts of

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*1 Part VI. Y. Hirasaka, M. Sukegawa, I. Matsunaga: Yakugaku Zasshi, 83, 1073 (1963).

*2 Ukima-cho, Kita-ku, Tokyo (平坂義信, 松永 功).

1) Y. Hirasaka: Yakugaku Zasshi, 83, 960 (1963).
2) Y. Hirasaka, I. Matsunaga, K. Umemoto, M. Sukegawa: *Ibid.*, 83, 966 (1963).
3) Y. Hirasaka: *Ibid.*, 83, 971 (1963).

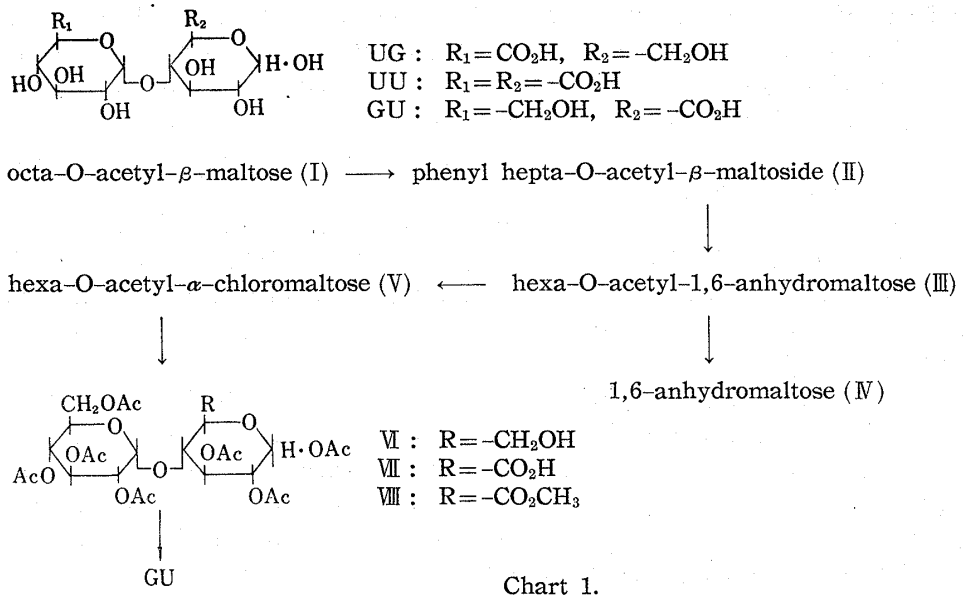

Chart 1.

acetic anhydride and tried to remove unreacted phenol only by extraction with sodium hydroxide (the reported yield of II was 45.6 percent).[4]

But acetic anhydride was found to be not always necessary in this condensation reaction, and since a large amount of remaining phenol which could not be removed by extraction with sodium hydroxide was found to obstruct crystallization of II, it was removed by concentration as far as possible and the yield of II was improved.

II was converted to 2,3,2′,3′,4′,6′-hexa-O-acetyl-1,6-anhydromaltose (III) in 87 percent yield by refluxing with potassium hydroxide for 40 hours, followed by usual acetylation.

Deacetylation of III by ordinary method afforded 1,6-anhydro-maltose (IV) in crystalline state.

III was chlorinated to 2,3,2′,3′,4′,6′-hexa-O-acetyl-α-chloromaltose (V) in chloroform according to the procedure reported by Johansson, *et al.*[5]

V was successively converted to 1,2,3,2′,3′,4′,6′-hepta-O-acetyl-β-maltose (VI) by treatment with mercuric acetate in acetic acid.

VI was then oxidized with potassium permanganate in acetic acid at a room temperature in the dark, and the reaction mixture was accompanied by thin-layer chromatography, which indicated that the desired oxidation took place rapidly and the oxidation reaction terminated after 2 days.

The reaction mixture was decolorized by addition of suitable amount of sodium bisulfite, and acidified with hydrochloric acid followed by extraction with chloroform.

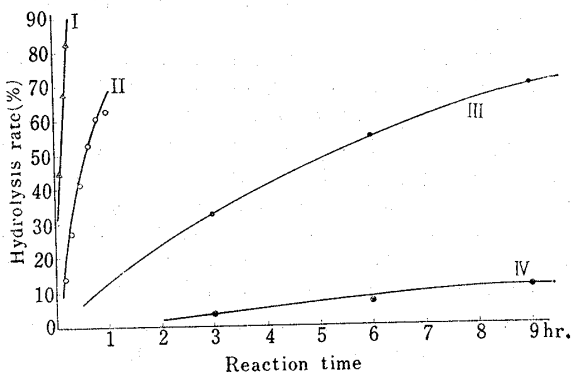

Fig. 1. Hydrolysis Rate of Some α-(1,4) linked Disaccharides

I : maltose
II : 4-O-(α-D-glucopyranosyl)-D-glucuronic acid (GU)
III : 4-O-(α-D-glucopyranosiduronyl)-D-glucose (UG)
IV : 4-O-(α-D-glucopyranosiduronyl)-D-glucuronic acid (UU)
Hydrolysis condition : under refluxing with 5% H_2SO_4

4) B. Lindberg, L. Asp : Acta Chem. Scand., **6**, 941 (1952).
5) I. Johansson, B. Lindberg, O. Theander : *Ibid.*, **17**, 2019 (1963).

Evaporation of the solvent resulted in easy crystallization of the oxidation product, hepta-O-acetyl-4-O-(α-D-glucopyranosyl)-D-glucuronic acid (Ⅶ) in 54 percent yield.

Methylation of Ⅶ with diazomethane in chloroform resulted in formation of the methyl ester of Ⅶ (Ⅷ) and deacetylation of Ⅷ with sodium methoxide followed by de-esterification with barium hydroxide afforded the desired GU in 72 percent yield as a slightly hygroscopic amorphous powder.

GU was hydrolyzed with 5 percent sulfuric acid under refluxing. The amount of liberated glucuronic acid in the hydrolyzate was determined by the naphthoresorcinol method after removal of polymerized carbohydrate, initial material and neutral monosaccharides such as glucose with charcoal and ion exchange resin chromatography, as was previously described.[6,7]

The amount of decomposed glucuronic acid during acid hydrolysis was determined by the furfural continuous extraction method previously reported.[8]

Fig. 1 illustrates the hydrolysis rate of GU calculated from the total amount of remaining and destructed glucuronic acid, according to the previous method.[7]

As expected, GU was found to be remarkably hydrolyzable in comparison with the other constituent disaccharides, UG and UU.

Experimental

All melting points are not corrected. All evaporations were carried out under reduced pressure, keeping the bath temperature below 40° to 45°.

Octa-O-acetyl-β-maltose (I) from Maltose——Maltose monohydrate (100 g.) was dissolved in pyridine (400 ml.) and Ac_2O (500 ml.) under stirring without cooling, and after 40 hr. at room temperature the solution was poured into ice-H_2O (6 L.) and the precipitate was collected. The crude product was dissolved in $CHCl_3$ (500 ml.), washed successively with H_2O, $NaHCO_3$ aq. solution, H_2O and then dried over Na_2SO_4. The $CHCl_3$ solution was concentrated to a sirup, which was crystallized by addition of EtOH. Ⅰ: Yield, 148.5 g. (79.0%). Recrystallization from EtOH. m.p. 155~158°. $[\alpha]_D^{25}$ +57.1° (c=5.75, $CHCl_3$). *Anal.* Calcd. for $C_{28}H_{38}O_{19}$: C, 49.56; H, 5.64. Found: C, 49.61; H, 5.66.

Phenyl Hepta-O-acetyl-β-maltoside (II) from I——A mixture of Ⅰ (100 g.), phenol (104 g.) and *p*-toluene sulfonic acid (1.5 g.) was heated at 100° for 1 hr. in an oil bath under vacuum (3 mm. Hg.). The mixture was then dissolved with $CHCl_3$ (200 ml.), washed with H_2O, 2*N* NaOH (150 ml.), H_2O and dried over Na_2SO_4.

The solution was concentrated to a sirup, which was crystallized by addition of EtOH. Ⅱ: Yield., 57.3 g. (54.5%). Recrystallization from EtOH. m.p. 154~155°. $[\alpha]_D^{25}$ +44.9° (c=4.97, $CHCl_3$). *Anal.* Calcd. for $C_{32}H_{40}O_{18}$: C, 53.93; H, 5.66. Found: C, 53.75; H, 5.68.

2,3,2′,3′,4′,6′-Hexa-O-acetyl-1,6-anhydromaltose (III) from II——A suspension of Ⅱ (50 g.) in 2.6*N* KOH (800 ml.) was refluxed for 40 hr. on the oil bath (115° to 120°), and neutralized with 4*N* H_2SO_4 (380 ml.). The filtrate was concentrated to a sirup, accompanied by removals of precipitates (K_2SO_4, C_6H_5OK) during the concentration and thereafter dried under vacuum. The product was dissolved in pyridine (200 ml.) and Ac_2O (250 ml.), and left for 40 hr. at room temperature. The solution was then poured into ice-H_2O (3 L.), extracted with $CHCl_3$ (500 ml.), washed with H_2O, $NaHCO_3$, H_2O and dried over Na_2SO_4. After decolorization by passing through a column of alumina, the solution was concentrated. Crystallization began easily. The crude product was collected by use of Et_2O. Ⅲ: Yield, 35 g. (86.6%). Recrystallization from EtOH. m.p. 181.5~183.5°. $[\alpha]_D^{25}$ +48.9° (c=5.44, $CHCl_3$). *Anal.* Calcd. for $C_{24}H_{32}O_{16}$: C, 50.00; H, 5.59. Found: C, 50.33; H, 5.82.

1,6-Anhydromaltose (IV) from III——Ⅲ (3 g.) was suspended in dry MeOH (90 ml.) and 0.56*N* MeONa methanolic solution (22.4 ml.) was added under stirring. After left stand overnight in a refrigerator the solusion was neutralized with Amberlite IR-120 (H: 7.5 g.) and concentrated to a sirup, which was crystallized by addition of acetone. Recrystallization from EtOH. Ⅳ: Yield quantitative. m.p. 154~156°. $[\alpha]_D^{25}$ +92.0° (c=1.71, H_2O). *Anal.* Calcd. for $C_{12}H_{20}O_{10}\cdot\frac{1}{2}H_2O$: C, 43.24; H, 6.35. Found: C, 43.50; H, 6.33.

1,2,3,2′,3′,4′,6′-Hepta-O-acetyl-β-maltose (VI) from III——Ⅲ (25 g.) dissolved in $CHCl_3$ (300 ml.) was mixed with EtOH (5 ml.) and $TiCl_4$ (40 g.) and refluxed for 3 hr. at 70°. The reaction mixture was poured

6) Y. Imai: Yakugaku Zasshi, **81**, 1109 (1961).
7) Y. Hirasaka, M. Sukegawa, I. Matsunaga: *Ibid.*, **83**, 1078 (1963).
8) Y. Hirasaka, M. Sukegawa: *Ibid.*, **83**, 1073 (1963).

into ice-H_2O, extracted with $CHCl_3$, washed with H_2O, $NaHCO_3$, H_2O, dried over Na_2SO_4 and then concentrated to a sirup at the bath temperature below 30°. The obtained sirup was dissolved in AcOH (250 ml.) and stirred with $Hg(OAc)_2$ (25 g.) at room temperature overnight. The reaction mixture was poured into ice-H_2O and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O, $NaHCO_3$, H_2O, dried over Na_2SO_4 followed by decolorization with charcoal, and concentrated to a sirup, which was dissolved in EtOH (125 ml.) and left in a refrigerator overnight. The precipitate (unreacted material) was removed and the filtrate was concentrated to 50 ml. The first crystal was then obtained. The filtrate was dissolved in H_2O (450 ml.) and left in a refrigerator, when the second crystal was obtained. Ⅵ: Yield, 7.4 g. (26.8%). Recrystallization from EtOH : H_2O (1:9), m.p. 140~141°. $[\alpha]_D^{25}$ +64.2° (c=5.10, $CHCl_3$). *Anal.* Calcd. for $C_{26}H_{36}O_{18}$: C, 49.06; H, 5.70. Found: C, 48.74; H, 5.86.

10.8 g. (43.2%) of the unreacted Ⅲ was recovered.

Hepta-O-acetyl-4-O-(α-D-glucopyranosyl)-D-glucuronic Acid (Ⅶ) from Ⅵ——Ⅵ (6.0 g.) was dissolved in AcOH (60 ml.) and powdered $KMnO_4$ (2.0 g.) was added all at once. The reaction mixture was stirred at room temperature in the dark. After 6 and 24 hr. $KMnO_4$ (each 2.0 g.) were added respectively. Thin-layer chromatography of the reaction mixture indicated that the material had disappeared after 2 days. The reaction mixture was concentrated moderately, decolorized with suitable amount of $NaHSO_3$ aq. solution, acidified to pH 1.5 with HCl and extracted with $CHCl_3$ (100 ml.). The $CHCl_3$ layer was washed with H_2O, dried over $MgSO_4$ and concentrated to a sirup, which was dissolved in EtOH. Crystallization began spontaneously. Ⅶ: Yield, 3.3 g. (53.9%). Recrystallization from EtOH. m.p. 186~186.5°. $[\alpha]_D^{25}$ +64.2° (c=5.015, $CHCl_3$). *Anal.* Calcd. for $C_{26}H_{34}O_{19}$: C, 48.00; H, 5.27. Found: C, 48.22; H, 5.25.

Methyl Hepta-O-acetyl-4-O-(α-D-glucopyranosyl)-D-glucuronate (Ⅷ) from Ⅶ——Ⅶ (2.5 g.) was dissolved in $CHCl_3$ and neutralized with ethereal solution of diazomethane under cooling. The solvent was evaporated and the residue was crystallized with H_2O. Ⅷ: Yield, 2.1 g. (82.4%). It began to melt at 85° and decomposed entirely at 120°. $[\alpha]_D^{25}$ +65.9° (c=2.81, $CHCl_3$). *Anal.* Calcd. for $C_{27}H_{36}O_{19}$: C, 48.80; H, 5.46. Found: C, 48.41; H, 5.46.

4-O-(α-D-Glucopyranosyl)-D-glucuronic Acid (GU) from Ⅷ——Ⅷ (2.0 g.) was dissolved in dry MeOH (25 ml.) and 0.56*N* MeONa methanolic solution (8.4 ml.) was added under stirring at room temperature. After standing in a refrigerator, the solution was neutralized by addition of Amberlite IR-120 (H) under stirring, decolorized and concentrated to a sirup. The obtained sirup (0.8 g.) was dissolved in H_2O (15 ml.) and 0.25*N* $Ba(OH)_2$ aq. solution (14 ml.) was added under stirring. After standing for 1 hr. at room temperature the solution was deionized with Amberlite IR-120 (H), concentrated to a sirup and solidified by addition of acetone. GU: Yield, 0.7 g. (72%). $[\alpha]_D^{25}$ +73.5° (c=0.34, H_2O).

GU was neutralized with 0.1*N* NaOH aq. solution and the solution was concentrated to a sirup, which was crystallized by addition of EtOH. m.p. 126~129° (decomp). $[\alpha]_D^{25}$ +112.6° (c=0.71, H_2O), *Anal.* Calcd. for $C_{12}H_{19}O_{12}Na$: C, 38.10; H, 5.06. Found: C, 38.34; H, 5.51.

The Rf value by paper chromatography using the upper layer of a mixture of BuOH-EtOH-H_2O (2:1:1) as a solvent was 0.11.

The barium salt of GU was prepared by the similar procedure mentioned above. m.p. 149~150° (decomp.). $[\alpha]_D^{25}$ +90.9° (c=1.155, H_2O). *Anal.* Calcd. for $C_{12}H_{19}O_{12}Ba/2$: C, 34.00; H, 4.52. Found: C, 34.50; H, 5.19.

Acid-hydrolysis of GU——GU (about 20 mg.) was dissolved in 5% H_2SO_4 (2 ml.) and refluxed on the oil bath in the presence of xylene (25 ml.). The amount of remaining glucuronic acid in the acid-hydrolyzate was determined by the naphthoresorcinol method after removal of the unreacted disaccharide and glucose with a column of charcoal-celite and Amberlite IRA-410 (Cl).[6,7] The amount of decomposed glucuronic acid during acid hydrolysis was calculated from the amount of formed furfural extracted in a xylene layer, according to the previously described method.[8]

The authors express their gratitude to Prof. Emeritus M. Ishidate of the University of Tokyo for his valuable advices, and to Dr. S. Hayashi, a Managing Director of the Company, for his encouragements during the work. The authors are also grateful to Miss M. Ishii of the Central Analysis Room for the elementary analysis.

Summary

Hepta-O-acetyl-4-O-(α-D-glucopyranosyl)-D-glucuronic acid (Ⅶ) was obtained in crystalline state by potassium permanganate oxidation of 1,2,3,2′,3′,4′,6′-hapta-O-acetyl-D-maltose (Ⅵ), and by de-acetylation of Ⅶ, 4-O-(α-D-glucopyranosyl)-D-glucuronic acid (GU) was obtained.

The acid hydrolysis rate of GU was also decribed.

(Received October 14, 1964)