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23. Morizo Ishidate*¹, Yuji Imai, Yoshinobu Hirasaka, and Kenji Umemoto*¹: A New Action of Anion Exchange Resins on the Lactone Ring of Some Carbohydrates.

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Previously the authors reported that D-glucuronolactone (I) was converted to methyl D-glucopyranuronate (III) by reacting with dry methanol in the presence of anion exchange resin (A.R.).¹⁾ The present investigation describes a new action of A.R. on the lactone ring of some carbohydrates in aqueous solution.

As is well known, I is substantially a neutral compound and is not absorbed on A.R. of weak basic type, but it was found that I was quantitatively absorbed on A.R. of strong basic type such as Amberlite IRA-410 (OH form). Elution of the absorptive with dilute sulfuric acid resulted in quantitative liberation of D-glucuronic acid (II), 87 percent of which was free from the eluting agent. The result was given in Fig. 1.

Consequently the lactone ring seems to be catalytically hydrolyzed by the alkalinity of A.R. and converted to the acid group, which was then absorbed on A.R., and by the difference of affinity to the resin between II and sulfuric acid, a large amount of II was eluted alone from A.R. There has been no report regarding this fact.

From the eluted aqueous solution of II, the crystal of high quality was easily obtained. A small amount of II contaminated with sulfuric acid could be advantageously recovered by using its solution as an eluting agent of the resin in the next batch. By the repetition of this process over 20 cycles, about 98 percent of I had as a mean been converted to its free acid (II) (Fig. 2).

This is very interesting, because it proposes an improved preparative method of crystalline II, requiring no process to prepare any alkaline salt of II as an intermediate.*³

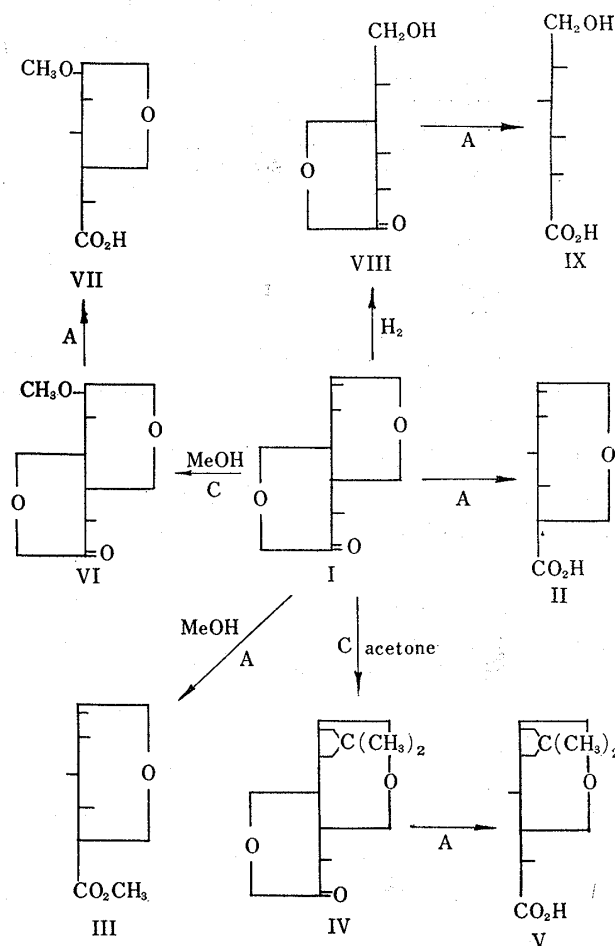


Chart 1.

C: Cation exchange resin.
A: Anion exchange resin.

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*³ The most recent method reported in Yakugaku Zasshi, 80, 1139 (1960) requires to prepare sodium D-glucuronate as an intermediate from D-glucuronolactone. Deionization is carried out by use of cation exchange resin.

1) Y. Hirasaka, K. Umemoto: Yakugaku Zasshi, 82, 1676 (1962).

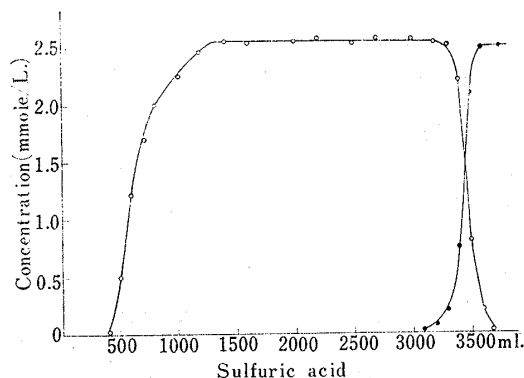


Fig. 1. Recovery of D-Glucuronic Acid from the Resin by the Elution with Sulfuric Acid

—○—○— glucuronic acid
—●—●— sulfuric acid
140 g. of D-glucuronolactone was absorbed on 600 ml. of Amberlite IRA-410 (OH) and the resin was eluted with 0.5N sulfuric acid. About 87% of glucuronic acid was recovered alone and the residual was further recovered in contamination with sulfuric acid.

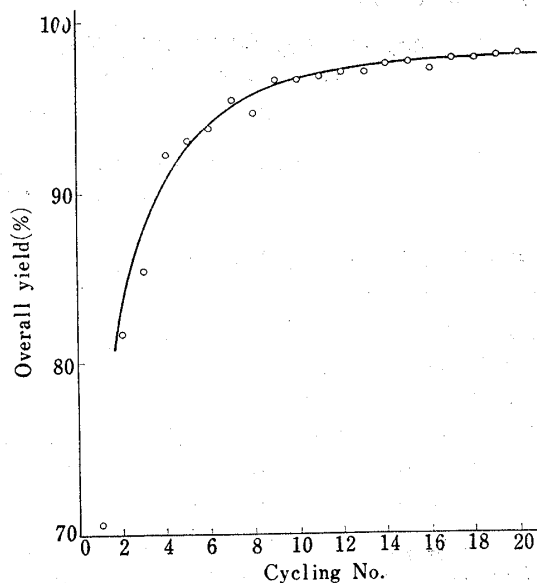


Fig. 2. Overall Yield of Crystalline D-Glucuronic Acid from D-Glucuronolactone by the Cycling Method

The amount of used D-glucuronolactone at each cycle was about 700 to 1000 g., and its total amount over 20 cycles was about 14.4 kg. The total amount of obtained crystalline D-glucuronic acid was about 15.6 kg.

As was illustrated in Chart 1, this method is applicable for preparation of some derivatives with free carboxylic acid group which can not be derived directly from their lactones.

1,2-O-Isopropylidene-D-glucofuranuronic acid (V) was previously prepared only by catalytic oxidation of 1,2-O-isopropylidene-D-glucose.²⁾ But by the present method V could be obtained more easily from 1,2-O-isopropylidene-D-glucofuranosidurono-3,6-lactone (IV).

Similarly methyl D-glucofuranosidurono-3,6-lactone (VI) was converted to its corresponding free acid (VII) by the present method. It is quite difficult to neutralize VI satisfactorily with alkaline solution directly since the reaction mixture became extremely colored, its decolorization with charcoal was unsuccessful and the obtained salt was always impure.

As VII was found to tend to lactonize rapidly in aqueous solution, VII was separated as its barium salt by use of barium carbonate.

The structure of VII to be furanoside was confirmed by periodate oxidation. As was shown in Fig. 3, VII consumed 1 mole/mole of periodic acid and formic acid could not be detected.

Moreover L-gulonolactone (VIII) was converted to L-gulonic acid (IX) by the same method. As IX could not be obtained in crystalline form, it was esterified with diazomethane followed by acetylation, when methyl penta-O-acetyl-L-gulonate was obtained.

The resin applicable to this method is only of strong basic type such as Amberlite IRA-400, 410, 411 in OH form. About 70 percent of the total exchange capacity of the resin is available in absorbing lactones.

2) C. L. Mehltretter, B. H. Alexander, *et al.*: J. Am. Chem. Soc., 73, 2424 (1951).

The new action of anion exchange resin reported here seems to be applicable widely, for example, to prepare some carbohydrates with free carboxylic acid group from their corresponding lactone which is resistant to hydrolysis by an ordinary method with alkaline medium.

Experimental

All evaporations were carried out under reduced pressure at the bath temperature below 40°. All melting points were not corrected.

D-Glucuronic Acid (II) from D-Glucuronolactone (I) by the Cycling Method—100 ml. of an aqueous solution containing 10 g. of I was passed through 60 ml. of Amberlite IRA-410 (OH) and the resin was washed with 100 ml. of H₂O. In the passed solution no sugar was detected. The resin was then eluted with 0.14N H₂SO₄.

The initially eluted solution of about 250 ml. contained II alone (9.6 g., colorimetrically determined, 87%). This solution was concentrated and crystallized. The product (II), 9.2 g. The melting point was 152~154°. It was quite identical with the authentic one in mixed melting point and IR spectrum. The later eluted H₂SO₄ solution containing 1.6 g. of II was used as eluting agent of the resin after 8.5 g. of I had been absorbed as described above. From the first eluted solution, 9.1 g. of crystalline II was obtained. The result of repetition of this process was given in Fig. 2.

1,2-O-Isopropylidene-D-glucofuranuronic Acid (V) from its Lactone (IV)—7 g. of 1,2-O-isopropylidene-D-glucofuranurono-3,6-lactone (IV), which was prepared from I and MeOH using cation exchange resin as a catalyst, was dissolved in 100 ml. of H₂O and passed through a column of 80 ml. of Amberlite IRA-410 (OH).

The passed solution was found entirely free from any sugar. The resin was then eluted with 0.5N H₂SO₄ and the first eluted solution (250 ml.), free from H₂SO₄, was concentrated to a sirup, which was crystallized. The product, V, melted at 142~143°. The yield was 3.4 g. *Anal.* Calcd. for C₉H₁₄O₇: C, 46.20; H, 5.99. Found: C, 45.98; H, 5.94.

Methyl D-Glucofuranosiduronic Acid (VII) from its Lactone (VI)—8 g. of Methyl D-glucofuranosidurono-3,6-lactone (VI) was dissolved in 100 ml. of H₂O and passed through a column containing 60 ml. of Amberlite IRA-410 (OH). The residue was washed with H₂O and thereafter eluted with 0.125N H₂SO₄ solution.

The eluted solution (600 ml.) was stirred with BaCO₃ and the precipitate was filtered off. The filtrate was concentrated to a sirup, which was crystallized by addition of acetone. Barium salt of VII, 5.2 g. $[\alpha]_D^{25} -23.4^\circ$ (c=1.0, H₂O). *Anal.* Calcd. for C₇H₁₁O₇·½Ba: C, 30.47; H, 3.99. Found: C, 29.82; H, 4.00.

Periodate Oxidation of VII—482.6 mg. of barium salt of VII was dissolved in 10 ml. of H₂O and then passed through 4 ml. of Amberlite IR-120 (H) which was thereafter washed with 15 ml. of H₂O. The combined solution was mixed with 10 ml. of NaIO₄ solution, containing 784.0 mg. of NaIO₄ under cooling in the dark and then messed up to 50 ml. The obtained result was given in Fig. 3. The determination of NaIO₄ was carried out by the usual method and formic acid was tried to detect as formaldehyde by the chromotropic acid test after reduction with magnesium.

L-Gulonolactone (VIII) from I by Catalytic Reduction—10 g. of I was dissolved in 100 ml. of H₂O and catalytically reduced at room temperature under atmospheric pressure, using Raney Ni as a catalyst. Consumed H₂ after about 5 hr. was about 630 ml. The catalyst was filtered off, and the filtrate was concentrated to a sirup, which was crystallized from dry EtOH. The product, VIII, was melted at 179°. $[\alpha]_D^{25} +58.0^\circ$ (c=2.0, H₂O). The yield was 8.2 g. The obtained crystal was quite identical with the authentic sample by mixed melting point and IR spectrum.

L-Gulonic Acid (IX) from VIII—8 g. of VIII was dissolved in 200 ml. of H₂O and absorbed on 50 ml. of Amberlite IRA-410 (OH) as described above. The resin was then eluted with 0.125N H₂SO₄ and the first eluted solution (300 ml.) indicated no presence of H₂SO₄ and also negative to the hydroxamic acid test. The solution was concentrated under reduced pressure at the bath temperature below 35° and the obtained sirup was dissolved in MeOH and methylated with ethereal solution of diazomethane. The concentrated sirup was acetylated with pyridine and Ac₂O as usual. The reaction mixture was concentrated to a sirup,

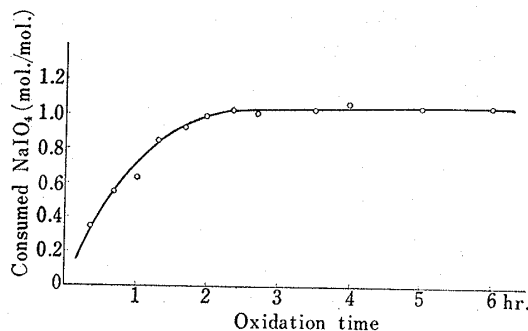


Fig. 3. Periodate Oxidation of Methyl D-glucofuranosiduronic Acid

Formic acid could not be detected by the chromotropic acid test after reduction with magnesium to convert to formaldehyde.

which was crystallized from MeOH. The product, methyl penta-O-acetyl-L-gulonate, was melted at 68.0°. *Anal.* Calcd. for $C_{10}H_{16}O_5$: 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.

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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

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

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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).