

80~100 mesh. The solid support was used after a washing with acid, silanized with dimethyldichlorosilane in toluene and then the packing was prepared by the solution coating technique. The standard operating conditions were listed in Tables I and II. The temperature of the detector and sample heater were adjusted at 240° and 270°, respectively. A sample of 0.5% solution of either hexane or acetone was injected with a Hamilton microsyring.

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Summary

Gas chromatographic behaviors of seventeen kinds of hopane-zeorinane group and of twelve kinds of onocerane group were investigated using NGS and SE-30 phases. The relationships between the position of double bond and the effect to the retention time were discussed.

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44. Yoshinobu Hirasaka and Kenji Umemoto : Structure of D-Glucosaccharodilactone.

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D-Glucosaccharodilactone (IV) was first isolated in crystalline state (m.p. 134~135°) by Rehorst and Scholz¹⁾ in 1936. Later in 1944, Smith²⁾ obtained the similar substance (m.p. 133°) by lactonization of D-glucosaccharo-3,6-lactone (III) and presumed that this dilactone possessed the 1,5-3,6-dilactone structure since it was different from that derived from D-glucosaccharo-1,4-lactone (II)*2 and indicated rapid mutarotation was shown in aqueous solution in contrast to D-mannosaccharo-1,4-3,6-dilactone (X). However, the authors have recently isolated a crystalline dilactone from either II or III respectively, and found that two dilactones were completely identical in mixed melting point, infrared spectrum and optical rotation, and therefore the presumption proposed by Smith has become doubtful. The structure of IV has not so far been substantiated certainly as such.

The present investigation was undertaken to elucidate the structure of this dilactone using its acetyl derivative, since methylation of this dilactone gave an unsatisfactory result in affording principally 2,5-di-O-methyl-4⁴-D-glucosaccharo-3,6-lactone 1-methyl ester (IX) instead of the desired 2,5-di-O-methyl-D-glucosaccharo-dilactone.

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*2 Smith assumed in his report that the syrupy dilactone derived from II would possess the 1,4-3,6-dilactone structure.

1) K. Rehorst, H. Scholz : Ber., 69, 524 (1936).

2) F. Smith : J. Chem. Soc., 1944, 633.

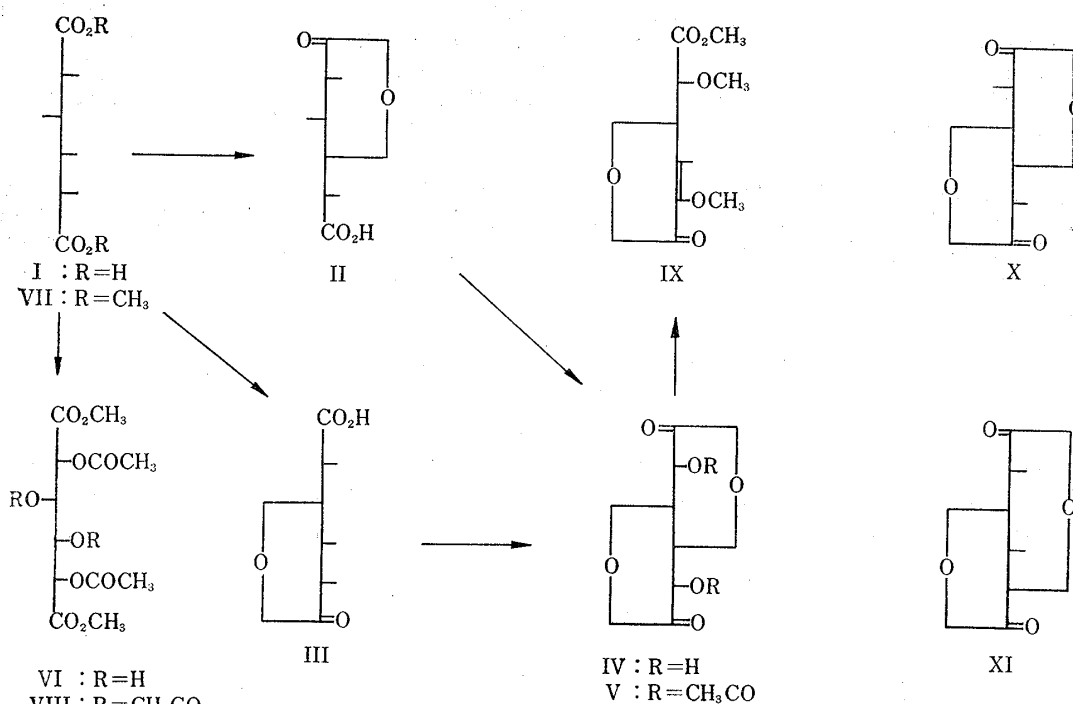


Chart 1.

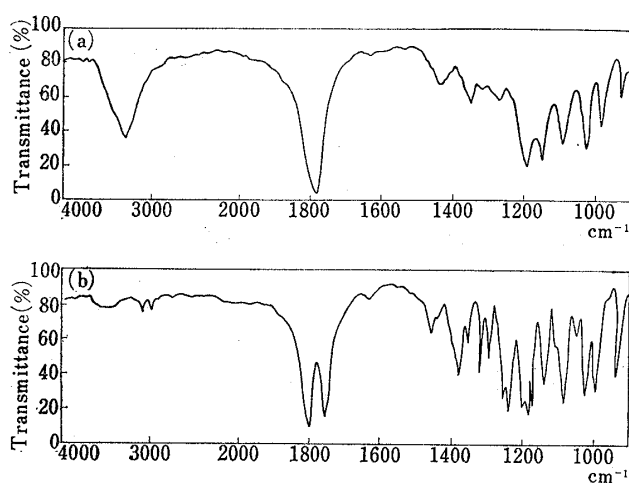


Fig. 1. Infrared Spectrum of D-Glucosaccharo-dilactone (a) and its Diacetate (b)

was found to be entirely identical with the authentic sample prepared by esterification of D-glucosaccharic acid (I) with diazomethane followed by acetylation, as is illustrated in Chart 1.

If IV possesses two γ -lactone rings, then VI would involve one vicinal hydroxyl group at the position of C_3 and C_4 , and this carbon chain should be cleaved by periodic acid oxidation.

IV was acetylated in the usual way to give 2,5-di-O-acetyl-D-glucosaccharo-dilactone (V), whose infrared spectrum (Fig. 1) exhibited a band at 1780 cm^{-1} indicating the presence of γ -lactone. V was then treated with methanol in the presence of anion exchange resin*³ to yield syrupy 2,5-di-O-acetyl-D-glucosaccharic acid dimethyl ester (VI).^{*4}

Presence of two carboxylic acid methyl ester groups and of two acetyl groups in VI was proved by determining its methoxy and acetyl contents respectively.

Acetylation of VI in the usual way afforded 2,3,4,5-tetra-O-acetyl-D-glucosaccharic acid dimethyl ester (VIII) which

*³ The similar catalytic reaction had already been reported by the authors in Yakugaku Zasshi, 82, 1676 (1962). D-Glucuronolactone was converted to methyl D-glucopyranuronate by the same method almost quantitatively.

*⁴ This syrupy substance could not be crystallized, but was found to afford only one spot by thin-layer chromatography.

On the contrary, if IV contains δ -lactone ring at the position of C_1 and C_5 ,^{*5} then the corresponding dimethyl ester of the dilactone diacetate should not react with the same oxidizing agent.

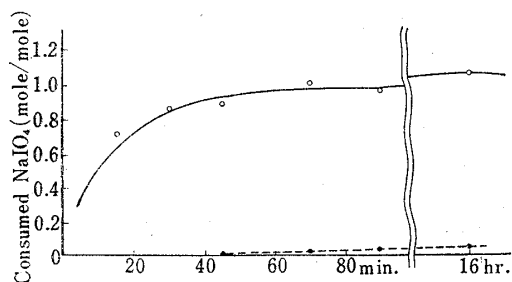


Fig. 2. Periodic Acid Oxidation of 2,5-Di-O-acetyl D-Glucosaccharic Acid Dimethyl Ester (VI), (—○—○—) and 2,3,4,5-Tetra-O-Acetyl-D-Glucosaccharic Acid Dimethyl Ester (VIII) (---●---)

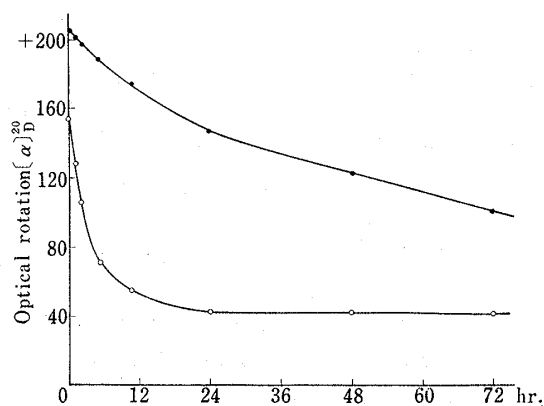


Fig. 3. Mutarotation of D-Glucosaccharo-dilactone (IV) (—○—○—) and D-Mannosaccharo-dilactone (X) (—●—●—) in Aqueous Solution

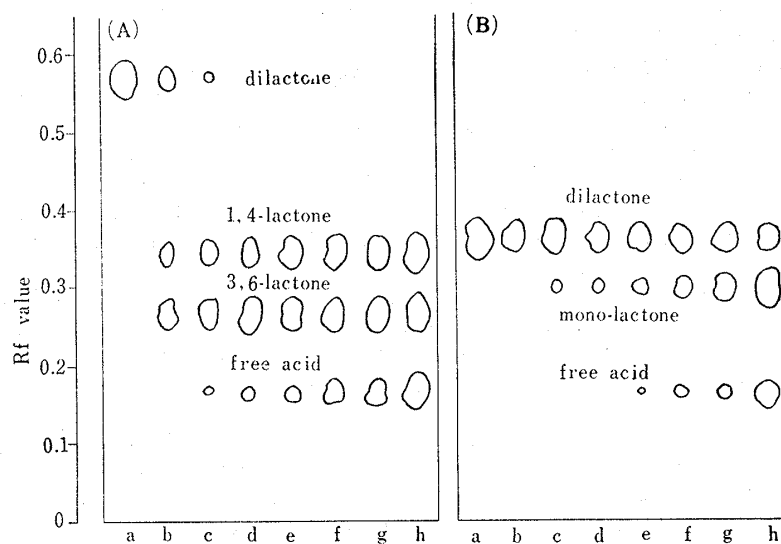


Fig. 4. Paperchromatography of the Aqueous Solution of D-Glucosaccharo-dilactone (A) and D-Mannosaccharodilactone (B)
a, b, c, d, e, f, g and h indicate the time 0, 1, 2, 5, 10, 24, 48 and 72 (hr.) respectively.

Fig. 2 illustrates the result obtained by periodic acid oxidation of VI at pH 3.50 under ice-cooling in the dark.

VI consumed rapidly the calculated amount (1 mole/mole) of the oxidant. In order to ascertain the stability of the acetyl groups under the oxidation condition, VIII was subjected to the same oxidation and it was found that no oxidation took place even after prolonged reaction time.

Moreover, the oxidation product of VI was treated with sodium hydroxide (de-esterification) followed by sodium borohydride reduction to afford methyl glycerate which was identified by paperchromatography.

*5 The presence of 3,6- γ -lactone ring in IV seems to be probable since IV is rapidly converted to III in water.

From these results it was established that D-glucosaccharodilactone possessed the 1,4-3,6-dilactone structure.

IV was fairly stable in crystalline state, but in aqueous solution it underwent spontaneous ring opening to yield mixture of I, II, and III. On the other hand, X prepared by nitric acid oxidation of D-mannose was found to resist considerably to the ring opening under the same condition.

Results of paperchromatography, which were carried out using the upper layer of a mixture of BuOH-EtOH-HCO₂H-H₂O (4:1:1:5) as solvent, were shown in Fig. 4.

Each lactone was detected by spraying alkaline hydroxylamine solution followed by acidic ferric chloride solution.

Mutarotation of IV and X in aqueous solution is shown in Fig. 3. The two γ -lactone rings in X are entirely indistinguishable stereochemically with each other, and they appear to behave similar to the 3,6-lactone ring of IV. It is well known that the 1,4-lactone ring of II undergoes ring opening more rapidly than the 3,6-lactone ring of III in aqueous solution.

Eventually the rapid mutarotation of IV in comparison with X would not be attributable to the presence of the labile δ -lactone ring, but to the instability of the 1,4-lactone ring in aqueous solution.

Experimental

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

D-Glucosaccharo-1,4-3,6-dilactone (IV) from D-Glucosaccharo-1,4-lactone (II)—The monohydrate of II (m.p. 91~93°) (30 g.) was heated at 80° to 100° in vacuum over P₂O₅. After 15 min., it melted gradually and was converted to colorless, stiff syrup, under the gentle evolution of bubbles. After 2 hr. the reaction temperature was gradually raised to 100° to 120° and the heating was continued for further 3.5 hr. Crystals of II disappeared entirely and IV began to appear. When bubbling ceased, the product was dissolved in 60 ml. of acetone and concentrated moderately. When the solution was left in a refrigerator, IV crystallized spontaneously. It was recrystallized from acetone. Yield; 20.5 g. or 82.5%. m.p. 132~133°. $[\alpha]_D^{20} +168^\circ$ (3 min., c=1.0, H₂O). *Anal.* Calcd. for C₆H₈O₆: C, 41.38; H, 3.45. Found: C, 41.13; H, 3.25.

IV from D-Glucosaccharo-3,6-lactone (III)—Finely powdered III (m.p. 138°) (20 g.) was heated similar to the case of II. After about 2 hr. it turned to slightly colored syrup and after 5 hr. IV began to appear.

After further 9 hr. the product was dissolved in acetone and crystallized. Yield; 14.7 g. or 78.7%. The product was completely identical with one derived from II in melting point, IR spectrum and optical rotation.

2,5-Di-O-acetyl-D-glucosaccharo-1,4-3,6-dilactone (V) from IV—IV (2 g.) was dissolved in a mixture of pyridine (20 ml.) and Ac₂O (10 ml.) at 20°. After about 2 hr. the reaction mixture was poured into ice H₂O and the precipitate was filtered on. Recrystallization from the mixture of EtOH and ethyl acetate (2:1 by volume) to give V. Yield; 2.3 g. or 77.0%. m.p. 185~186°. *Anal.* Calcd. for C₁₀H₁₀O₈: C, 46.60; H, 3.88. Found: C, 46.36; H, 3.92.

2,5-Di-O-acetyl-D-glucosaccharic Acid Dimethyl Ester (VI) from V—A mixture of V (5 g.), dry Amberlite IR-4B (OH) (15 g.) and dry MeOH (100 ml.) was vigorously stirred at room temperature. The reaction was followed by thin-layer chromatography using CHCl₃-MeOH (3:1) as solvent. After about 120 hr. V disappeared almost entirely and only a new spot (R_f: 0.7) was detectable. The anion exchange resin was then filtered off and the filtrate was concentrated to give VI as syrup. Yield; 4.3 g. $[\alpha]_D^{20} +32^\circ$ (c=1.0, MeOH). *Anal.* Calcd.: -OCH₃ 19.2%, -OCOCH₃ 36.6%. Found: -OCH₃ 18.9%, -OCOCH₃ 35.7%.

D-Glucosaccharic Acid Dimethyl Ester (VII) from I—A mixture of monopotassium salt of I (8 g.), Amberlite IR-120 (H) (50 g.) and dry MeOH (150 ml.) was refluxed under stirring for about 3 hr., and then the filtrate was decolorized and concentrated to a syrup. After about 2 weeks the crystallized VII was filtered on. It was recrystallized from ethyl acetate. Yield; 3.2 g. or 39%. m.p. 162~163°. $[\alpha]_D^{20} +53.0^\circ$ (c=1.0, MeOH). *Anal.* Calcd. for C₈H₁₄O₈: C, 40.38; H, 5.88. Found: C, 40.66; H, 5.08.

2,3,4,5-Tetra-O-acetyl-D-glucosaccharic Acid Dimethyl Ester (VIII) from VII and VI—a) VII (2 g.) was acetylated with pyridine (20 ml.) and Ac₂O (20 ml.) at room temperature for 2 hr. and the reaction mixture was poured into ice H₂O. The crude crystalline VIII was recrystallized from EtOH. Yield; 2.5 g. m.p. 112~114°. $[\alpha]_D^{20} +30^\circ$ (c=1.0, MeOH). *Anal.* Calcd. for C₁₆H₂₂O₁₂: C, 47.40; H, 5.42. Found: C, 47.37; H, 5.57.

b) VI (2 g.) was acetylated by the above-described procedure to give crystal of m.p. 112~113°. It was completely identical with the sample derived from VII in mixed melting point, IR spectrum and optical rotation. Yield, 2.1 g.

D-Mannosaccharo-dilactone (X) from D-Mannose—D-Mannose (50 g.) was oxidized with 62% HNO_3 (75 ml.) containing NaNO_2 (0.05 g.) at 25° to 50° for 1 hr. and further at 55° to 60° for 4 hr. The reaction mixture was then heated at 85° for 1 hr. and concentrated under reduced pressure with the occasional addition of H_2O for the removal of remaining HNO_3 to yield a syrup which was left in a refrigerator for 3 days. The separated crystals were recrystallized from hot EtOH to give X. Yield; 11.2 g. m.p. 170~175°. $[\alpha]_D^{20} + 206.5^\circ$ (initially, $c=1.0$, H_2O). *Anal.* Calcd. for $\text{C}_6\text{H}_6\text{O}_6$: C, 41.39; H, 3.47. Found: C, 41.45; H, 3.51.

This dilactone did not contain any crystalline water probably because of its recrystallization from dry EtOH.

Periodate Oxidation of VI—VI (904.7 mg.) was dissolved in 50 ml. of H_2O containing 784.0 mg. of NaIO_4 and kept at 2° to 4° under cooling in the dark. The initial pH of the solution was 3.50. The result is shown in Fig. 2. The residual amount of NaIO_4 was determined by the usual way. Formation of formic acid was not detectable by alkaline titration nor by the chromotropic acid test after reduction with magnesium. In order to ascertain whether deacetylation would occur or not, the acetate (VIII) was oxidized under the same reaction condition mentioned above and the result obtained is also shown in Fig. 2.

The oxidation mixture of VI was deionized with Amberlite IR-120 (H, 10 ml.) and IR-4B (OH, 10 ml.) and the resins were filtered off and the filtrate was concentrated to give a syrup (583.4 mg.) which was de-esterified with 0.5N NaOH (12 ml.) for 20 min. at room temperature, and then reduced with NaBH_4 (150 mg.) under cooling overnight. Presence of glyceric acid in the solution was indicated by paperchromatography.

Its Rf value (0.22, using the upper layer of a mixture of BuOH-EtOH- HCO_2H - H_2O (4:1:1:5) as solvent and NaIO_4 -benzidine solution as spray reagent) was identical with the authentic sample. Moreover, the solution was deionized with Amberlite IR-120 (H), concentrated to a syrup and esterified with diazomethane in dry MeOH. This solution indicated the presence of methyl glycerate by paperchromatography (Rf, 0.44 using the same solvent described above).

Methylation of IV—a) IV (2 g.) was dissolved in dry MeOH (10 ml.), cooled to -5°, and an excess of an ice-cold ethereal solution of diazomethane added. There was a vigorous evolution of nitrogen and the solution persisted in a yellowish colour throughout the reaction.

After being kept for overnight at -5°, the solvent and the excess diazomethane were removed by distillation. The syrupy product was treated by repeating with above mentioned way.

The syrup obtained was gradually crystallized by rubbing glass wall and adhering syrup was removed

from the crystals by trituration with BuOH followed by crystallization from iso-PrOH. The IR spectrum of the crystals was shown in Fig. 5. Yield; 600 mg. m.p. 88~89°. $[\alpha]_D^{20} + 96^\circ$ ($c=1.0$, H_2O). *Anal.* Calcd. for $\text{C}_9\text{H}_{12}\text{O}_6$: C, 50.00; H, 5.56. Found: C, 50.08; H, 5.67. b) IV (2 g.) was dissolved in dimethyl formamide (30 ml.) and then methylated with silver oxide (8 g.) and methyl iodide (6.5 ml.) at the temperature below 40°. After 10 hr., the precipitates were filtered off and the filtrate was again

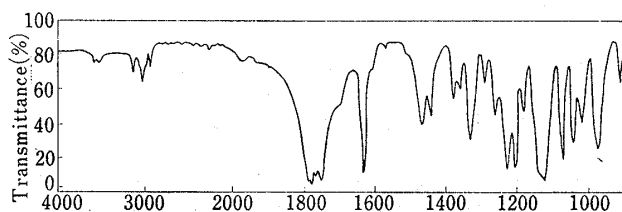


Fig. 5. Infrared Spectrum of 2,5-Di-O-methyl-4-D-glucosaccharo-3,6-lactone 1-Methyl Ester

methyated under the same reaction condition for 30 hr.

Then the solvent was evaporated, the precipitates were removed and the filtrate was concentrated to a syrup, which crystallized spontaneously. It was completely identical with the crystals from (a) in mixed melting point, IR spectrum and optical rotation. Yield; 700 mg.

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Summary

The crystalline dilactone (IV) of D-glucosaccharic acid (I) derived from both 1,4-lactone (II) and 3,6-lactone (III) was found to be identical, and its structure was established as 1,4-3,6-dilactone. It was confirmed that the rapid mutarotation of IV in aqueous solution was attributable to the instability of its 1,4-γ-lactone ring.

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