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.

The authors are deeply indebted to professor E. Ochiai for his encourgement and they also thank Mrs. N. Morisaki for her assistance in operating the gas chromatography and Mr. Y. Otake for the preparation of the samples. Part of the expenses of this work was financed from Grant-in-Aid for Scientific Research from the Ministry of Education and from the Hoansha Foundation, to which authors' thanks are due.

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.

(Received October 27, 1964)

[Chem. Pharm. Bull. 13(3) 325~329 (1965)]

UDC 547.474.6.02

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 Scholz[1] in 1936. Later in 1944, Smith[2] 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-Δ^4-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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*1 Ukima-cho, Kita-ku, Tokyo (平坂義信, 梅本賢次).

*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.

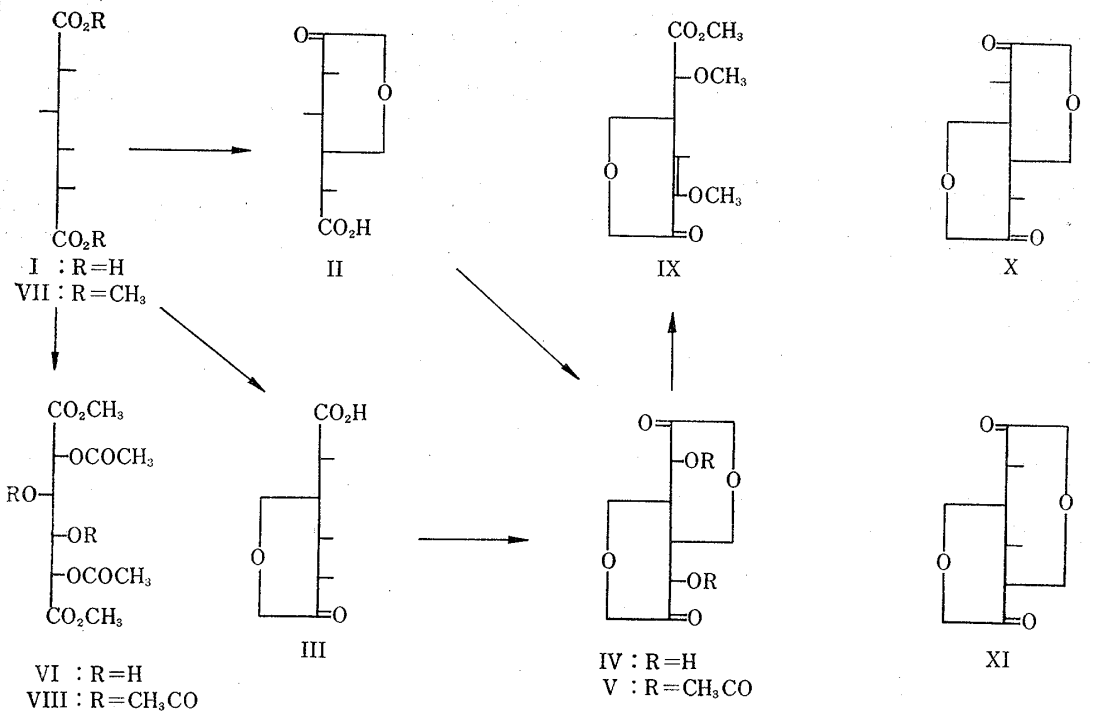

Chart 1.

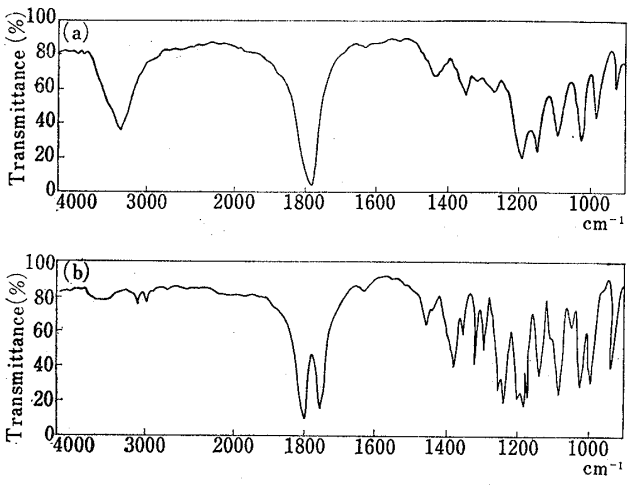

Fig. 1. Infrared Spectrum of D-Glucosaccharo-dilactone (a) and its Diacetate (b)

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 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.

*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.

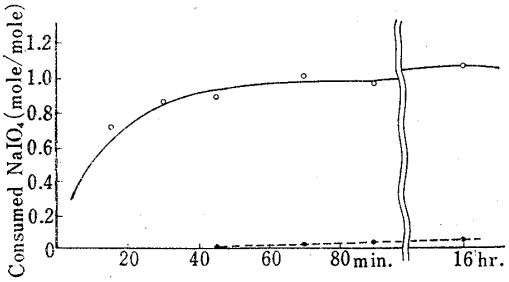

Fig. 2. Preiodic Acid Oxidation of 2,5-Di-O-acetyl D-Glucosaccharic Acid Dimethyl Ester (Ⅵ), (—○—○—)and 2,3,4,5-Tetra-O-Acetyl-D-Glucosaccharic Acid Dimethyl Ester (Ⅷ) (---•---•---)

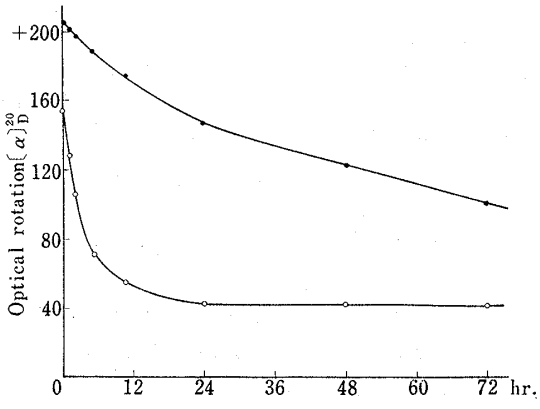

Fig. 3. Mutarotation of D-Glucosaccharo-dilactone (Ⅳ) (—○—○—) and D-Mannosaccharo-dilactone (X) (—•—•—) in Aqueous Solution

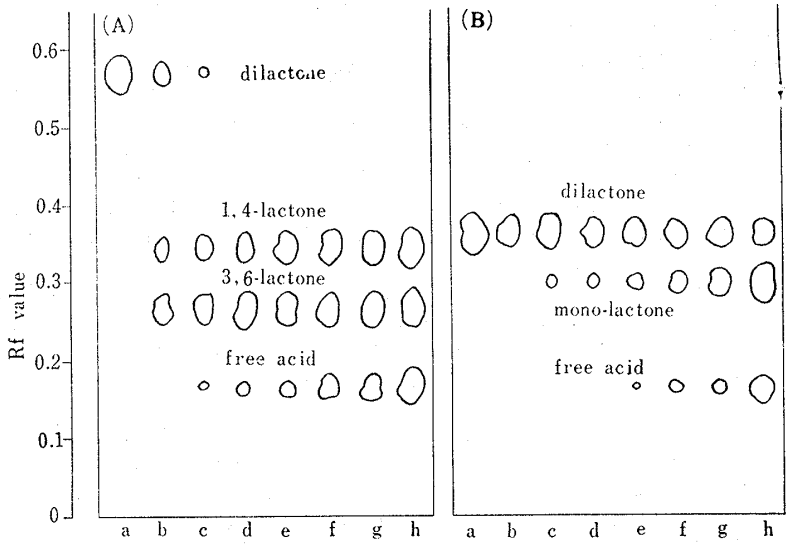

Fig. 4. Paperchromatography of the Aqueous Solution of D-Glucosaccharo-dilactone (A) and D-Mannosaccharodilactone (B)

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-HCO_2H-H_2O (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 mutarotion of Ⅳ in comparison with X would not be attributable to the presence of the labil 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 vaccum over P_2O_5. 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° (3 min., c=1.0, H_2O). *Anal.* Calcd. for $C_6H_6O_6$: 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 AC_2O (10 ml.) at 20°. After about 2 hr. the reaction mixture was poured into ice H_2O 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 $C_{10}H_{10}O_8$: 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 $CHCl_3$-MeOH (3:1) as solvent. After about 120 hr. V disappeared almost entirely and only a new spot (Rf : 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° (c=1.0, MeOH). *Anal.* Calcd. : $-OCH_3$ 19.2%, $-OCOCH_3$ 36.6%. Found : $-OCH_3$ 18.9%, $-OCOCH_3$ 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° (c=1.0, MeOH). *Anal.* Calcd. for $C_8H_{14}O_8$: 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 Ac_2O (20 ml.) at room temperature for 2 hr. and the reaction mixture was poured into ice H_2O. The crude crystalline Ⅷ was recrystallized from EtOH. Yield; 2.5 g. m.p. 112～114° $[\alpha]_D^{20}$ +30° (c=1.0, MeOH). *Anal.* Calcd. for $C_{16}H_{22}O_{12}$: 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° (initially, c=1.0, H_2O). *Anal.* Calcd. for $C_6H_6O_6$: 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 0.5*N* 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 an 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° (c=1.0, H_2O). *Anal.* Calcd. for $C_9H_{12}O_6$: 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 methylated under the same reaction condition for 30 hr.

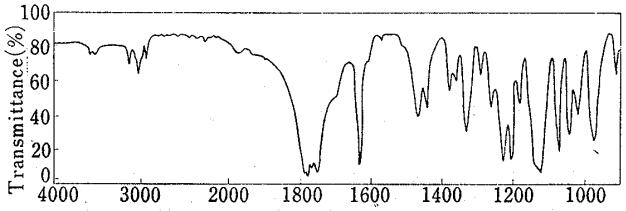

Fig. 5. Infrared Spectrum of 2,5-Di-O-methyl-Δ^4-D-glucosaccharo-3,6-lactone 1-Methyl Ester

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.

The authors are grateful to Prof. Emeritus M. Ishidate of the University of Tokyo for his kind encouragement during the course of this work, and to Dr. Y. Nitta and Y. Nakajima of the Central Research Institute for their valuable advices.

The authors are also indebted to Miss Ishii of the Central Analysis Room for the elementary analysis. The authors express their gratitude to Mr. M. Matsui of the Tokyo Biochemical Research Institute for his supply of authentic D- and L-glyceric acids.

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.

(Received November 19, 1964)