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88. Yoshinobu Hirasaka, Kenji Umemoto, Mitsuo Sukegawa, and Isao Matsunaga : On Equilibrium among D-Glucosaccharic Acid and its Lactones in Aqueous Solution.

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Previously the authors observed that D-glucosaccharo-1,4:6,3-dilactone (Ⅳ) was rapidly converted to D-glucosaccharic acid (Ⅰ), D-glucosaccharo-1,4-lactone (Ⅱ) and -6,3-lactone (Ⅲ) in aqueous solution, and that the reciprocal transformation among these substances took place when one of them was dissolved. This suggests that Ⅰ and its lactones are in equilibrium in aqueous solution.[1]

The present investigation was undertaken to clarify the above equilibrium relation which had not been reported elsewhere in the literature.

Ⅰ, Ⅱ, Ⅲ, and Ⅳ prepared in pure state were paper-chromatographed under various conditions, and they were satisfactorily separated on paper by use of the upper layer of a mixture of *n*-buthanol-ethanol-formic acid-water (4:1:1:5) as a solvent. The Rf values of Ⅰ, Ⅱ, Ⅲ, and Ⅳ were 0.13, 0.32, 0.24, and 0.54, respectively.

Ⅰ was detected by spraying a periodic acid solution followed by a benzidine solution. Ⅱ, Ⅲ, and Ⅳ were detected by spraying an alkaline solution of hydroxylamine followed by an acidic ferric chloride solution.

These substances were attempted to determine after separation by paper. After a mixture of the above authentic samples was paper-chromatographed, the individual regions were cut out and extracted with cold water for 20 minutes.

Ⅰ was determined by alkaline titration.[*2] Ⅱ, Ⅲ, and Ⅳ were determined by the hydroxamic acid method. Although each lactone afforded different color-intensity, the absorbancy of produced color was found to be proportional to the concentration of each lactone within the range of 0.5 to 6 mmole per liter.

Table Ⅰ. Recovery Test of Lactones

Material	Weighed amount (γ)	Determined amount[a] (γ)	Recovered amount (%)
Ⅱ	121	119	98.3
〃	242	234	96.8
〃	484	480	99.2
Ⅲ	116	118	101.5
〃	232	229	98.7
〃	464	451	97.2
Ⅳ	105	103	97.6
〃	210	214	102.0
〃	420	415	98.8

a) The authentic material was paper-chromatographed, extracted with ice-water and determined by the hydroxamic acid method.

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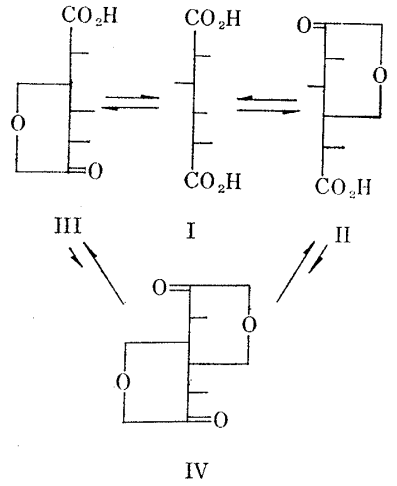
*2 M. Ishidate, M. Okada and M. Matsui proposed an improved method for the determination of Ⅰ in a small amount (Anal. Biochem., in press). Ⅰ is oxidized with periodic acid under the definite condition and the amount of produced glyoxylic acid was determined colorimetrically. But so far as a comparatively large amount of the samples was used, the alkaline titration method afforded approximate values with those obtained by this colorometrical method, and was found to be satisfactory for the present purpose.

1) Y. Hirasaka, K. Umemoto : This Bulletin, 13, 325 (1965).

The result of the recovery test by this procedure was illustrated in Table I, which showed that each substance could be recovered enough satisfactorily and that few unexpected transformation had happened during the process of paper chromatography and extraction.



I : D-glucosaccharic acid
II : D-glucosaccharo-1,4-lactone
III : D-glucosaccharo-6,3-lactone
IV : D-glucosaccharo-1,4:6,3-lactone

Chart 1.

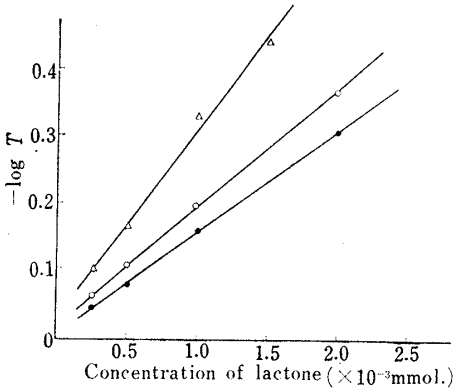
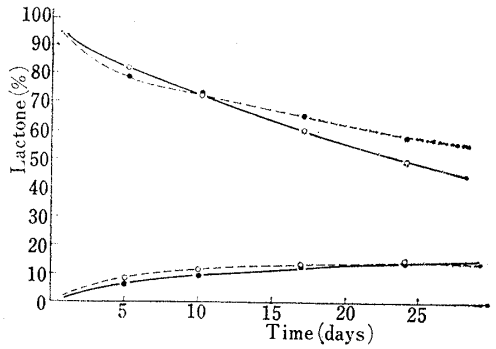
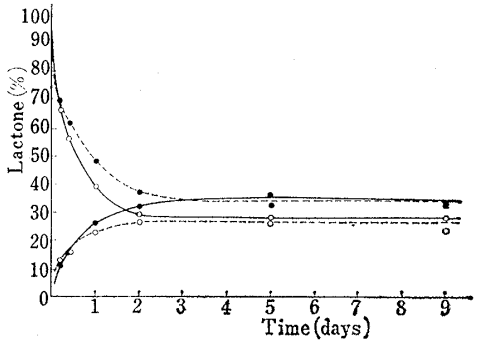


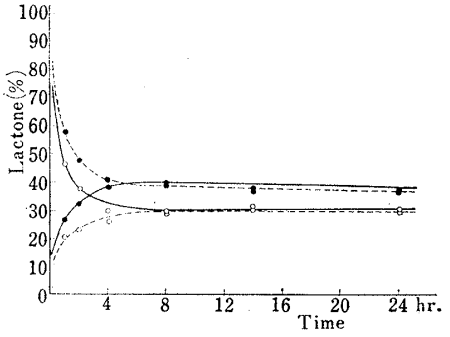
Fig. 1. The Caliburation Curves of D-Glucosaccharo-1,4-lactone (-○-), -6,3-lactone (-•-) and -1,4:6,3-dilactone (-△-) by the Hydroxamic Acid Method



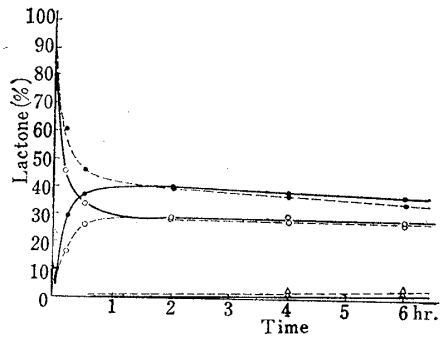
2a. (at 0° to 5°)



2b. (at room temperature)



2c. (at 70°)



2d. (under refluxing : —△— indicates the dilactone)

Fig. 2. Transformation of D-Glucosaccharo-1,4-lactone (-○-) and -6,3-lactone (-•-) in Aqueous Solution at Various Temperatures

—— and ------ indicate that the used starting materials were 1,4-lactone and 6,3-lactone, respectively.

II or III was dissolved in water to about 10 percent concentration at various temperatures and each substance was determined after separation by paper. The obtained results were given in Fig. 2.

The higher temperature causes the more increased amount of lactone content and this result can be conceivable from the relationship between D-glucuronic acid and its lactone.[2] IV was only detectable in a small amount at elevated temperature. It appears that the reciprocal transformation between II and III was caused *via* the free dicarboxylic acid pathway, and not the dilactone one, since the large part of intermediate under transformation detectable by paper chromatography was confirmed to be I, and not IV.

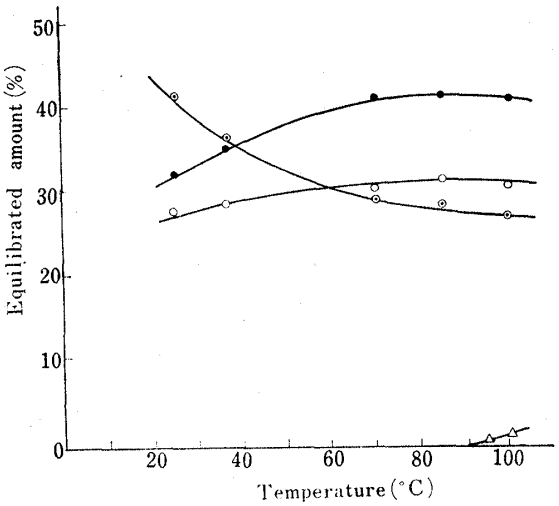


Fig. 3. The Equilibrated Amounts of D-Glucosaccharic Acid (-⊙-), 1,4-Lactone (-○-), 6,3-Lactone (-•-) and Dilactone (-△-) at Various Temperatures

The equilibrated amount of each substance at various temperatures was given in Fig. 3, which indicated an interesting fact that the amount of II in equilibrated solution was almost constant, being indifferent to temperature, although the amount of total lactones increased at higher temperature.

It is noteworthy that a large amount of IV disappeared during about 12 hours, as was shown in Fig. 4. This result is in good agreement with the observed mutarotation of the solution of IV, previously reported.[1]

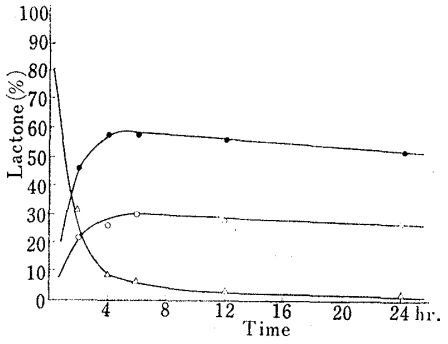


Fig. 4. Transformation of Dilactone (-△-) to 1,4-Lactone (-○-) and 6,3-Lactone (-•-) at Room Temperature

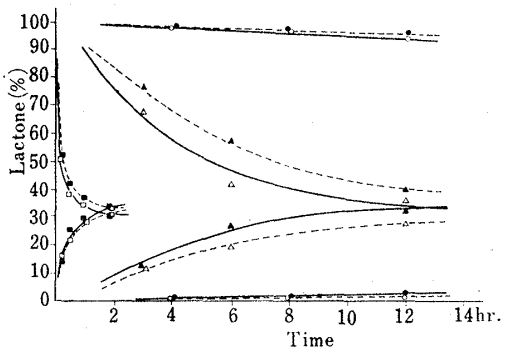


Fig. 5. Effect of Acidic Substances on Transformation Rates at Room Temperature

—○— and —●— indicate 1,4-lactone (II) and 6,3-lactone (III), respectively, in aqueous solution. —△— and —▲— indicate II and III, respectively, in the presence of excess cation exchange resin. —□— and —■— indicate II and III, respectively, in 2*N* sulfuric acid.

—— and ------ indicate that the used starting materials were II and III, respectively.

Fig. 5 shows the effect of addition of acidic substances such as mineral acid and cation exchange resin on the transformation rate, and it indicates that these acidic substances are good accerelating agents to bring the equilibrated state.

Experimental

Preparation of Materials——D-Glucosaccharic acid (I) was prepared by deionization of its monopotassium salt with cation exchange resin, as was previously reported. I: m.p. 117~118°, $[\alpha]_D^{20}$ +6.1° (c=1.0, H_2O, 5 min.). *Anal.* Calcd. for $C_6H_{10}O_8$: C, 34.29; H, 4.80. Found: C, 34.52; H, 4.87.

2) Y. Imai, Y. Hirasaka: Yakugaku Zasshi, **80**, 1139 (1960).

The 1,4-lactone (II) and 6,3-lactone (III) of I were prepared according to the method proposed by Smith.[3] II : m.p. 91~93°, $[\alpha]_D^{20}$ +56.8°(c=1.0, H_2O, 5 min.). *Anal.* Calcd. for $C_6H_8O_7 \cdot H_2O$: C, 34.29; H, 4.80. Found : C, 34.42; H, 4.73.

III : m.p. 138~140°, $[\alpha]_D^{20}$ +41.8°(c=1.0, H_2O, 5 min.). *Anal.* Calcd. for $C_6H_8O_7$: C, 37.50; H, 4.17. Found : C, 37.74; H, 4.33.

The 1,4 : 6,3-dilactone (IV) of I was prepared by lactonization of II, as was previously reported. IV : m.p. 132~133°, $[\alpha]_D^{20}$ +168°(c=1.0, H_2O, 3 min.). *Anal.* Calcd. for $C_6H_6O_6$: C, 41.38; H, 3.45. Found : C, 41.13; H, 3.25.

Separation of I and its Lactones by Paper Chromatography——The Toyo Roshi No. 50 filter paper, 40×40 cm., was used. II or III was dissolved in H_2O to about 10% concentration and kept at 0° to 5°, room temperature, 70° and 100°. 200 mg. of the solution were weighed and dropped along one edge of the paper. After simultaneous development by ascending method with the upper layer of a mixture of *n*-BuOH–EtOH–HCO_2H–H_2O (4:1:1:5), the paper was dried for at least 4 hr.

The regions of the spots were identified by spraying the detectable agents ($NaIO_4$–benzidine reagent and hydroxamic acid reagent) to a strip of the paper, and also by radiation of the fluorescence lamp. The individual regions of the paper were cut out and extracted with 30 ml. of cold H_2O for 20 min. under stirring.

Determination of I by Alkali-titration Method——The extracted solution corresponding to I was titrated with 0.01*N* NaOH under ice-cooling, using phenolphthalein as an indicator.

0.01*N* NaOH 1 ml.≡1.05 mg. of I.

Determination of II, III and IV by the Hydroxamic Acid Method——

Reagent A : $NH_2OH \cdot HCl$ (97.0%, 143.3 g.) was dissolved in H_2O to make 1 L.
Reagent B : JIS special class NaOH (147.2 g.) was dissolved in H_2O to make 1 L.
Reagent C : JIS special class HCl (1 vol.) was mixed with H_2O (2 vol.).
Reagent D : $FeCl_3$ (100 g.) was dissolved in 0.1*N* HCl to make 1 L.

2 ml. of the extracted solution corresponding to II, III or IV, after adjustment of the concentration of the lactone in the range of 0.5 to 6 mmol./L. if necessary, were mixed with 2 ml. of reagent A and B succesively and after 4 mln., 2 ml. of reagent C and D were added succesively. The density of the purple brown color was promptly measured at 505 mμ (at least during 10 min. after development of the color). The same procedure was applied to H_2O as a blanc test.

The Recovery Test by the Above Procedure——The reproducibility of individual measurement and the variation introduced by tne eluting procedure were given in Table I.

The variability appeared in Table I to be somewhat greater, but the present procedure for determination of each lactone appears satisfactory to the present purpose.

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The authors express their gratitude to Miss M. Ishii of the Central Analysis Room for elemental analysis.

Summary

The equilibrium relation among D-glucosaccharic acid and its lactones (namely 1,4- and 6,3-monolactones and 1,4:6,3-dilactone) in aqueous solution was investigated. It was found that the dilactone was detectable only at elevated temperature, and that the amount of the 1,4-lactone in equilibrium was almost indifferent to temperature.

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3) R. J. Bose, F. Smith, *et al.* : J. Org. Chem., **26**, 1300 (1961).