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188. Masuo Akagi, Setsuzo Tejima, and Masanobu Haga : Biochemical Studies on Thiosugars.*1 I. Synthesis of 1-Thio-D-glucuronic Acid.

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A few sugars containing sulfur were found in nature and several thiosugars have been synthesized.[1] As for sulfur-containing glucuronic acid and its derivatives, Clapp[2] reported that thioglucuronide was isolated as a metabolite of benzothiazole-2-sulfonamide, and Helferich, *et al.*[3] synthesized some alkyl- and aryl-thioglucuronides.

This paper describes the synthesis and characterization of 1-thio-D-glucuronic acid. Hitherto, some thiosugars were synthesized by means of usual method for thiol synthesis. Wrede[4] synthesized 1-thio-D-glucose by reductive cleavage of octaacetyldiglucosyl 1,1-disulfide which was prepared from acetobromoglucose and potassium disulfide. Schneider, Gille, and Eisfild[5] synthesized it by hydrolysis of tetraacetylglucosyl ethylxanthate and tetraacetylglucosyl isothiouronium hydrobromide, prepared from acetobromoglucose and potassium ethylxanthate and thiourea, respectively, and Fraser and Owen[6] synthesized it by hydrolysis of pentaacetyl-1-thio-D-glucose prepared from acetobromoglucose and potassium thiolacetate.

In the present work, 1-thio-D-glucuronic acid was synthesized through xanthate. A mixture of methyl 2,3,4-tri-O-acetyl-1-bromo-1-deoxy-α-D-glucopyranuronate (I), prepared according to the method of Bollenback, *et al.*,[7] and potassium ethylxanthate was boiled in dehyd. ethanol and methyl 2,3,4-tri-O-acetyl-1-thio-β-D-glucopyranuronate 1-ethylthionocarbonate (II), m.p. 114~115°, was obtained in ca. 70% yield. (II) was treated with cold methanolic ammonia by which deacetylation and decomposition of xanthate occurred simultaneously and 1-thio-β-D-glucopyranurono-6,1-thiolactone (III) was obtained in one step as a highly hygroscopic amorphous powder which had no distinct melting point.

By treatment with methanolic ammonia, methyl uronate usually gives its amide, but (III) was obtained directly. Deacetylation of (II) with sodium methoxide was unsuccessful.

1-Thio-D-glucose gave color reaction with sodium nitroferricyanide, reduced cold Fehling solution, and showed mutarotation.[4]

On the other hand, (III) gave no color reaction of thiol, did not reduce cold Fehling solution, and did not show appreciable mutarotation, but reduced hot Fehling solution and gave color reaction of thiol after treatment with alkali.

The infrared absorption spectrum of (III) exhibited a characteristic absorption at 1674 cm^{-1} which corresponds to thiol ester,[8] but glucuronolactone and methyl uronate have no absorption in the range of 1600~1700 $cm.^{-1}$ Evans and Owen[9] reported that γ-thiolactone forms in the course of synthesis of 4,5-dimercaptovaleric acid and thiolactone ring is

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easily decomposed by alkali. Fry[10] reported that tri-O-acetyl-β-D-glucopyranurono-6,1-lactone was prepared from tetra-O-acetyl-β-D-glucopyranuronic acid. From these facts, it is concluded that (III) has 6,1-thiolactone structure. (III) is slightly soluble in water, methanol, and ethanol, and practically insoluble in other organic solvents. (III) is very unstable and decomposes gradually in a desiccator.

(III), suspended in methanol, reacted gradually with diazomethane and formed methyl (methyl 1-thio-β-D-glucopyranosid)uronate which, without isolation, was acetylated with acetic anhydride and pyridine to methyl (methyl 2,3,4 tri-O-acetyl-1-thio-β-D-glucopyranosid)uronate (IV), m.p. 118°, in ca. 25% yield. (IV) is identical with the substance prepared according to the method of Helferich, *et al.*,[3] from (I) and potassium methylmercaptide. (III) reacted with phenylhydrazine, evolving hydrogen sulfide, and gave the same osazone as from glucuronolactone. There are discrepancies in the past literature[11,12] regarding the osazone of glucuronic acid. Detailed reports on this subject will be published in the near future.

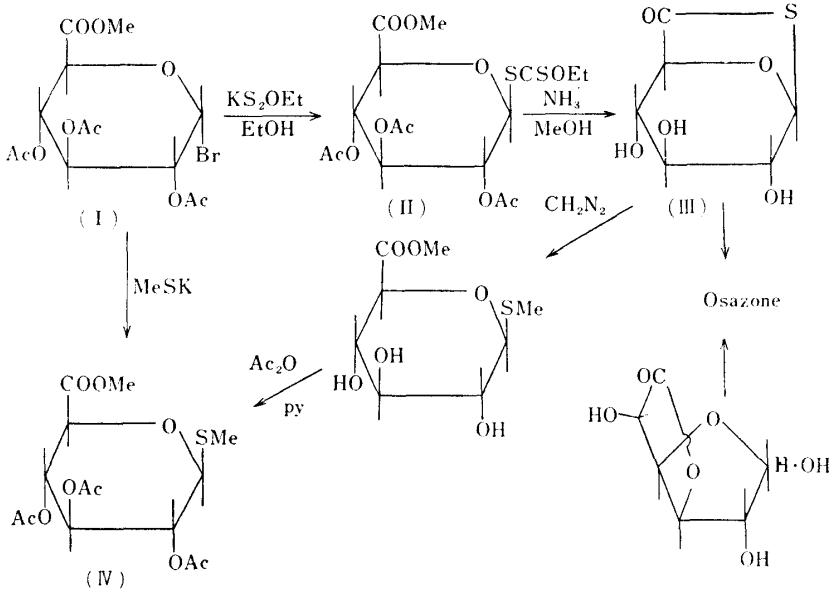

Chart 1.

Experimental

Methyl 2,3,4-Tri-O-acetyl-1-thio-β-D-glucopyranuronate 1-Ethylthionocarbonate (II)—A solution of 8.0 g. of potassium ethylxanthate dissolved in 150 cc. of dehyd. EtOH was heated to boiling. To this solution, 25.0 g. of (I) was added in small portions with continuous stirring until precipitation of KBr was complete. After cool, the precipitate was filtered off and washed with EtOH. The filtrate and washings were combined, poured into 500 cc. of H_2O, and allowed to stand in a refrigerator for 2 hr. Recrystallization from EtOH gave colorless crystals, m.p. 114~115°, $[\alpha]_D^{16}$ 42° (c=1.0, $CHCl_3$); yield, 17 g. (ca. 70%). *Anal.* Calcd. for $C_{16}H_{22}O_{10}S_2$: C, 48.53; H, 4.60. Found: C, 48.38; H, 4.68.

1-Thio-β-D-glucopyranurono-6,1-thiolactone (III)—A solution of 2.5 g. of (II) dissolved in 30 cc. of MeOH saturated with NH_3 at 0° was allowed to stand in a refrigerator overnight. The slightly colored solution was evaporated under a reduced pressure to a syrupy residue, which by trituration with dehyd. EtOH, gave white amorphous powder. This material was highly hygroscopic and had no distinct m.p., $[\alpha]_D^{16}$ −72° (c=1.4, H_2O). *Anal.* Calcd. for $C_6H_8O_5S$: C, 37.52; H, 4.20. Found: C, 38.02; H, 4.22.

Methyl (Methyl 2,3,4-Tri-O-acetyl-1-thio-β-D-glucopyranosid)uronate (IV)—To 2.5 g. of (III) suspended in 50 cc. of MeOH, an excess of CH_2N_2 in Et_2O was added and allowed to stand in a refrigerator for 1 week. The solvent and excess of CH_2N_2 were removed under a reduced pressure and the resulting residue was acetylated with 30 cc. of pyridine and 20 cc. of Ac_2O as usual. Colorless

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needles, m.p. 117~118° (reported[3] m.p. 118~119°), $[\alpha]_D^{16}$ −19.5° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{14}H_{20}O_9S$: C, 46.15; H, 5.53. Found: C, 46.20; H, 5.58. This substance was identical with the substance prepared from (I) and potassium methylmercaptide according to the method of Helferich, *et al.*[3]

Phenylosazone of (III) and Glucuronolactone—A solution of 0.5 g. of (III), 2 g. of phenylhydrazine, and 2 cc. of 50% AcOH dissolved in 20 cc. of H_2O was heated on a steam bath to a clear solution, and the osazone appeared as yellow needles following evolution of H_2S. After cool, the resulting crystals were collected and washed with cold H_2O and EtOH. Recrystallization from pyridine-H_2O gave pale yellow needles, m.p. 171~172°, $[\alpha]_D^{16}$ −15° (c=1.0, pyridine).

This substance was identical with the compound prepared similarly from glucuronolactone and apparently different from the osazone of m.p. 125°, prepared from D-glucopyranuronic acid.

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Summary

1-Thio-β-D-glucopyranurono-6,1-thiolactone was prepared by hydrolysis of methyl 2,3,4-tri-O-acetyl-1-thio-β-D-glucopyranuronate 1-ethylthionocarbonate which was prepared from methyl 2,3,4-tri-O-acetyl-1-bromo-1-deoxy-α-D-glucopyranuronate and potassium ethylxanthate. Methylation of this compound with diazomethane and subsequent acetylation afforded methyl (methyl 2,3,4-tri-O-acetyl-1-thio-β-D-glucopyranosid)uronate.

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189. Morizo Ishidate[*1] and Yasuhiro Yamane[*2]: Detection of β-Diketones with Uranyl Ion.

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In the previous papers, the authors reported that enolisable β-diketones are liable to form a stable chelate compound with uranyl ion, producing a yellow color.[1~3] Geyer[4] and Sakaguchi[5] also reported that 1-hydroxyanthraquinone and tetracycline derivatives which possess a phenolic β-diketone in the molecule similarly produce chelate compound with the same metal. It seemed possible to apply this color reaction for the detection of compounds having enolisable β-diketone or phenolic β-diketone group.

For the detection of β-diketones, the color reaction with iron(III) chloride[6,7] or titanium(III) chloride[8] has usually been employed. As these color reactions take place similarly

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