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91. Masakatsu Sakata, Masanobu Haga, Setsuzo Tejima, and Masuo Akagi : Thiosugars. VI.[*1] Reaction Products of Potassium Alkyl- and Benzylxanthates with Acetylated Glucosyl Halides.[*2]

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In earlier papers of this series, ethylxanthate derivatives of D-glucuronic acid,[1] D-glucosamine,[2] D-ribose and D-mannose[*1] were synthesized as an useful intermediate for the preparation of thiosugars. The antitumor activity of thiosugar derivatives obtained by us was tested, and among the samples tested, β-D-mannopyranosyl ethylxanthate[*1] showed a remarkable elongation of longevity of *dd* strain of mouse inoculated with Ehrlich ascites carcinoma cells intraperitoneally.[3]

Further, the increasing interests are directed to the sugar xanthates from the viewpoint of optical rotatory dispersion.[4]

In a continuation of the effort to seek more effective potential antitumor compound among thiosugar derivatives, and to investigate the optical rotatory dispersion of sugar xanthates with a view of studying the partial rotatory contribution of the functional groups, the present study was designed to confirm the best condition for the preparation of acetylated glucosyl xanthates. However, the authors found that unexpected products other than acetylated glucosyl xanthates were often formed during the xanthate formation when we carried out the methods which had hitherto been reported in literature. Eventually, it was clarified that the xanthate formation is dependent on choice of solvent, the reaction temperature, the potassium xanthate used, and the starting glucosyl halide.

First of all, corresponding acetylated glucosyl xanthates (Ⅱa, Ⅱb, Ⅱc) were obtained in good yield by treatment of 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide (I) with potassium ethyl-, propyl-, or butylxanthate in boiling acetone as well as in boiling ethanol. According to literatures, condensation of acetylated glycosyl halides with potassium xanthate had been carried out in ethanol,[5] methanol,[6] benzene,[7] or ethanol-acetone mixture.[8] In this study, acetone or ethanol was used as the solvent.

All of these glucosyl xanthates (Ⅱa, Ⅱb, Ⅱc) showed in ethanol a strong ultraviolet absorption at 274 mμ, which is a characteristic of acetylated glycosyl xanthates, and afforded sodium 1-thio-β-D-glucopyranose (Ⅲ) by treatment with sodium methoxide.

[*1] Part V. S. Tejima, T. Maki, M. Akagi : This Bulletin, **12**, 528 (1964).

[*2] This work was presented at the 145th national meeting of the American Chemical Society, Sept. 10, 1963.

[*3] Nishi-5-chome, Kita-12-jo, Sapporo (阪田正勝, 羽賀正信, 手島節三, 赤木満洲雄).

1) M. Akagi, S. Tejima, M. Haga : This Bulletin, 8, 1114 (1960).

2) *Idem* : *Ibid.*, **9**, 360 (1961).

3) M. Akagi, M. Haga, S. Tejima, D. Mizuno, S. Okamoto, K. Takano, Y. Hirokawa : Abstract of Papers presented at the XIVth Pharmaceutical Assembly, 81st Annual Meeting of the Pharmaceutical Society of Japan, 193 (1961).

4) D. Horton, M.L. Wolfrom : J. Org. Chem., **27**, 1794 (1962); Y. Tsuzuki, K. Tanabe, M. Akagi: Abstract of Papers presented as the XVIth Annual Meeting of the Chemical Society of Japan, 236 (1963).

5) W. Schneider, R. Gille, K. Eisfeld : Ber., **61**, 1244 (1928); H.G. Fletcher, Jr., C.S. Hudson : J. Am. Chem. Soc., **69**, 1672 (1947).

6) M. Gehrke, W. Kohler : Ber., **64**, 2696 (1931).

7) D. Horton, M.L. Wolfrom : J. Org. Chem., **27**, 1794 (1962).

8) W. Meyer zu Reckendorf, W.A. Bonner : Chem. Ber., **94**, 2431 (1961).

Successive acetylation of Ⅲ with acetic anhydride and pyridine gave a crystalline 1-S-acetyl-1-thio-2,3,4,6-tetra-O-acetyl-β-D-glucopyranose (Ⅳ).

On the other hand, expected glucosyl xanthate could not be obtained when potassium methyl- or benzylxanthate, in place of potassium alkylxanthate mentioned above, had been reacted with I in boiling acetone. The condensation product was confirmed to be a mixture of three parts of bis(2,2′,3,3′,4,4′,6,6′-octa-O-acetyl-β,β′-D-glucopyranosyl)sulfide (Ⅴ) and one part of methyl- or benzyl 1-thio-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (Ⅵa or Ⅵb). The physical constants of sulfide and thioglucoside obtained by us were in agreement with those reported by Schneider[9] and by Černý[10].

However, acetylated diglucosyl sulfide (Ⅴ) and corresponding acetylated glucosyl xanthate (Ⅱd or Ⅱe) were obtained in the ratio of 1:1, when the same method had been carried out in boiling ethanol instead of boiling acetone. Further, the authors found that the reaction temperature affects the condensation product; thus, diglucosyl sulfide (Ⅴ) could not be separated when the reaction had been carried out at a low temperature (30~40°), while the only crystalline material obtained was confirmed to be corresponding acetylated glucosyl xanthate (Ⅱd or Ⅱe).

Reaction of 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl chloride (Ⅶ) with potassium methyl- or benzylxanthate in boiling acetone afforded a yellow sirup (Ⅷa or Ⅷb) having in ethanol a strong ultraviolet absorption at 274 mμ, while diglucosyl sulfide (Ⅴ) could not be separated under this condition. The starting chloride (Ⅶ) was prepared according to the procedure similar to that reported by Lemieux,[11] and numerous attempts to obtain the sirup (Ⅷa or Ⅷb) in crystalline form failed.

Alkaline degradation of Ⅷa or Ⅷb by the method similar to that of 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl ethylxanthate described by Schneider[5] afforded sodium 1-thio-α-D-glucopyranose (Ⅸ). Acetylation of Ⅸ gave a crystalline 1-S-acetyl-1-thio-2,3,4,6-tetra-O-acetyl-α-D-glucopyranose (Ⅹ), which was also obtained by the condensation of 3,4,6-tri-O-acetyl-β-D-glucopyranosyl chloride[12] with potassium thiolacetate followed by acetylation. Schneider[4] already reported the preparation of Ⅹ, who started from sodium 1-thio-β-D-glucopyranose (Ⅲ) followed by anomerization and acetylation. The physical constants of Ⅹ obtained by us were in agreement with those described by Schneider. Therefore, the sirup (Ⅷa or Ⅷb) is presumed to be 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl methyl- or benzylxanthate, respectively, and this is the first reported example of the preparation of α-type glucosyl xanthate.

As mentioned in earlier part of this paper, the authors found a simple method for the preparation of acetylated di-D-glucopyranosyl sulfide (Ⅴ) (octa-O-acetyl-isothiotrehalose); it is easily obtainable by the reaction of potassium methyl- or benzylxanthate upon acetobromoglucose (I) in boiling acetone.

Thus, in the next step, the authors projected the synthesis of other two isomers of octa-O-acetyl-isothiotrehalose. Thiotrehalose should be an attractive compound as a substrate on glycosidase owing to its structural analogue with naturally occurring trehalose.

The second isomer, bis(2,2′,3,3′,4,4′,6,6′-octa-O-acetyl-α,α′-D-glucopyranosyl)sulfide (Ⅺ) was obtained in 40% yield by the condensation of 3,4,6-tri-O-acetyl-β-D-glucopyranosyl chloride[12] with potassium methyl- or benzylxanthate in boiling acetone followed by acetylation. The third isomer, bis(2,2′,3,3′,4,4′,6,6′-octa-O-acetyl-α,β′-D-glucopyranosyl)sulfide (Ⅻ) was prepared in 60% yield by treatment with sodium 1-thio-α-D-

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9) W. Schneider, F. Wrede: Ber., **50**, 793 (1917).

10) M. Černý, J. Pacák: Collection Czechoslov. Chem. Communs., **24**, 2566 (1961).

11) R.U. Lemieux, C. Brice: Can. J. Chem., **30**, 295 (1952).

12) R.U. Lemieux, G. Huber: *Ibid.*, **31**, 1040 (1953); P. Brigl: Z. physiol. Chem., Hopps-Seyler's **116**, 1 (1921).

glucopyranose (Ⅸ) with acetobromoglucose (I) in boiling methanol followed by acetylation.

These compounds showed in chloroform specific rotations of −38.0°, +259.2°, and +115.0°, respectively. The values were in accordance with their chemical configuration at carbon 1.

An investigation in D-xylose, D-galactose, or D-glucuronic acid series will be the subject of a future paper.

Experimental

2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl Ethylxanthate (IIa)——2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl ethylxanthate (Ⅱa) was prepared by the method similar to that described by Horton.13) The use of Me_2CO as the solvent was found satisfactory for this compound.

2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl Propylxanthate (IIb) and 2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl Butylxanthate (IIc)——2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl bromide (I) (10 g.) was added to a solution of potassium xanthate (4.3 g. of propyl; 4.6 g. of butyl) dissolved in 50 ml. of warm EtOH and refluxed for 5 min. After cooling to room temperature, the mixture was poured into 300 ml. of H_2O. The precipitates were filtered off, washed with H_2O, then air-dried. Yield, Ⅱb; 7.5 g., Ⅱc; 8 g. The crude product was recrystallized from EtOH to colorless needles. Ⅱb; m.p. 90~91°, $[\alpha]^{20}_{\mathrm{D}}$ +31.3° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{18}H_{26}O_{10}S_2$: C, 46.34; H, 5.62; S, 13.75. Found: C, 46.17; H, 5.63; S, 13.78. Ⅱc; m.p. 114~115°, $[\alpha]^{20}_{\mathrm{D}}$+28.8° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{19}H_{28}O_{10}S_2$: C, 48.32; H, 5.87; S, 13.35. Found: C, 48.39; H, 5.95; S, 13.52.

Sodium 1-Thio-β-D-glucopyranose (III) and 1-S-Acetyl-1-thio-2,3,4,6-tetra-O-acetyl-β-D-glucopyranose (IV)——Sodium 1-thio-β-D-glucopyranose (Ⅲ) was prepared by a slight modification of the procedure reported by Schneider.6) Solution of 20 g. of Ⅱb or Ⅱc dissolved in 40 ml. of $CHCl_3$ was cooled with ice and salt, then treated, under stirring and cooling, with 60 ml. of MeONa solution (3 g. Na in 100 ml. of MeOH). The mixture was allowed to stand in an ice-bath for 10 min. The resulting precipitates were filtered, washed with cold EtOH to remove excess MeONa, then immediately dried under reduced pressure. The material was highly hygroscopic, amorphous powder and used without further purification. Yield, 10 g. Na salt (Ⅲ) (2 g.) was added to an ice-cold mixture of pyridine (10 ml.) and Ac_2O (10 ml.). After 24 hr., the reaction mixture was poured into ice H_2O, and the resulting solid material was collected by filtration. Recrystallization from warm EtOH gave 2,3,4,6-tetra-O-acetyl-1-S-acetyl-1-thio-β-D-glucopyranose (Ⅳ), m.p. 121°, $[\alpha]^{20}_{\mathrm{D}}$ +9.5° (c=2.0, $CHCl_3$).

Bis(2,2′,3,3′,4,4′,6,6′-octa-O-acetyl-β,β′-D-glucopyranosyl)sulfide (V) and Methyl 1-Thio-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (VIa)——2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl bromide (I) (5 g.) was added to a solution of 1.8 g. of potassium methylxanthate dissolved in 25 ml. of warm Me_2CO, and refluxed for 5 min. After cooling, the mixture was poured into 200 ml. of H_2O, then extracted with 40 ml. of $CHCl_3$. The solvent was removed under vacuum to dryness, and the residue was triturated with 20 ml. of Et_2O. The insoluble crude V was collected by filtration. Recrystallization from warm EtOH gave pure material (2.4 g.), m.p. 175~176°, $[\alpha]^{20}_{\mathrm{D}}$ −35.5° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{28}H_{38}O_{18}S$: C, 48.41; H, 5.51; S, 4.62. Found: C, 48.24; H, 5.56; S, 4.46.

Petr. ether was added to persist a slight turbidity to the filtrate from which crude V had been filtered off, then kept overnight in a refrigerator to deposit a crystalline, crude Ⅵa. Recrystallization from EtOH gave pure material (0.8 g.), m.p. 91°, $[\alpha]^{20}_{\mathrm{D}}$ −12.0° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{15}H_{22}O_9S$: C, 47.61; H, 5.86. Found: C, 47.51; H, 5.94.

Ⅵa was also prepared by treatment of Ⅲ with MeI in hot MeOH according to the direction of Černý.10)

Benzyl 1-Thio-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (VIb) and V——Benzyl 1-thio-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (Ⅵb) and V were obtained by treatment of 5 g. of I with 2.8 g. of potassium benzylxanthate in boiling Me_2CO according to the procedure described above. Yield of V was 2.1 g., Ⅵb; 0.7 g. Ⅵb melted at 100~101° and rotated $[\alpha]^{20}_{\mathrm{D}}$ −92.8° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{21}H_{26}O_9S$: C, 55.50; H, 5.77; S, 7.06. Found: C, 55.32; H, 5.81; S, 6.93.

The product (Ⅵb) was also obtained by treatment of $PhCH_2Cl$ with Ⅲ according to the direction of Černý.10)

2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl Methylxanthate (IId)——A solution of 5 g. of I in 30 ml. of EtOH was added at 30~40° to a solution of 1.8 g. of potassium methylxanthate dissolved in 50 ml. of MeOH. Mechanical stirring was continued at the temperature for 1 hr., then the mixture was poured into 200 ml. of H_2O, and followed by extraction with $CHCl_3$ (40 ml.). The extract was washed with H_2O,

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13) R. L. Whistler, M. L. Wolfrom: "Methods in Carbohydrate Chemistry," Vol Ⅱ, 433 (1963), Academic Press Inc., New York and London.

then dried over Na_2SO_4. Evaporation of the solvent under vacuum afforded a yellow sirup which was dissolved in a small amount of warm EtOH. Colorless crystals began to deposit on cooling. Several recrystallizations from aq. EtOH gave pure material (3.3 g.), m.p. 99°, $[\alpha]_D^{20}$ +35.0° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{16}H_{22}O_{10}S_2$: C, 43.83; H, 5.07. Found : C, 43.90; H, 5.16.

When the reaction was performed in boiling EtOH for 5 min., 1.3 g. of IId and 1.5 g. of V were obtained.

2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl Benzylxanthate (IIe)——2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl benzylxanthate (IIe) was obtained by treatment of 5 g. of I with 2.8 g. of potassium benzylxanthate in 50 ml. of EtOH at 30~40° according to the procedure described above. Yield, 3.5 g., m.p. 129°, $[\alpha]_D^{20}$ +27.1° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{22}H_{26}O_{10}S_2$: C, 51.35; H, 5.09. Found : C, 51.02; H, 5.12. In boiling EtOH for 5 min. 1.2 g. of IIe and 1.5 g. of V were obtained.

Bis(2, 2′, 3, 3′, 4, 4′, 6, 6′-octa-O-acetyl-α, α′-D-glucopyranosyl)sulfide (XI)——3, 4, 6-Tri-O-acetyl-β-D-glucopyranosyl chloride[12] (3 g.) was added to a solution of potassium xanthate (1.4 g. of methyl- or 2.1 g. of benzyl xanthate) dissolved in 25 ml. of warm Me_2CO, then the mixture refluxed for 5 min. After removal of the precipitated KCl by filtration, the filtrate was concentrated under vacuum to a sirup. Acetylation of the sirup was carried out with 30 ml. of pyridine and 20 ml. of Ac_2O. After standing at room temperature for 18 hr., the mixture was poured into 200 ml. of ice H_2O. Precipitates were filtered, then air-dried. Several recrystallizations from EtOH gave pure material, m.p. 191~192°, $[\alpha]_D^{20}$ +259.2° (c=2.0, $CHCl_3$). Yield, 1.2 g. (methyl); 1 g. (benzyl). *Anal.* Calcd. for $C_{28}H_{38}O_{18}S$: C, 48.41; H, 5.51; S, 4.62. Found : C, 48.49; H, 5.48; S, 4.99.

Sodium 1-Thio-α-D-glucopyranose (IX)——2, 3, 4, 6-Tetra-O-acetyl-β-D-glucopyranosyl chloride (VII) (3 g.) prepared according to the procedure reported by Lemieux,[11] was added to a solution of potassium xanthate (1.2 g. of methyl- or 1.9 g. of benzylxanthate) dissolved in 25 ml. of Me_2CO, and the mixture was refluxed for 5 min. After cooling to room temperature, the mixture was poured into 300 ml. of H_2O. A yellow sirup was deposited and followed by extraction with $CHCl_3$. The extract was washed with H_2O, dried over Na_2SO_4, then evaporated the solvent under vacuum to a yellow sirup (VIIIa or VIIIb). The EtOH solution had a strong UV absorption at 274 mμ. A solution of 5 g. of VIIIa or VIIIb dissolved in 10 ml. of $CHCl_3$ was treated with 15 ml. of MeONa solution (3 g. of Na in 100 ml. of MeOH) according to the similar procedure described in the preparation of its β-anomer (III).[13] Sodium 1-thio-α-D-glucopyranose was obtained as a highly higroscopic, amorphous powder (2.5 g.), and used without further purification.

1-S-Acetyl-1-thio-2,3,4,6-tetra-O-acetyl-α-D-glucopyranose (X)——A solution of 3,4,6-tri-O-acetyl-β-D-glucopyranosyl chloride[12] (5 g.) and AcSK (2 g.) in 50 ml. of Me_2CO was heated under reflux for 5 min. After removal of the precipitated KCl by filtration, the filtrate was concentrated under vacuum to a sirup. The residue was acetylated with 10 ml. of pyridine and 10 ml. of Ac_2O, then kept overnight at room temperature. The mixture was poured into 500 ml. of ice H_2O, followed by extraction with 40 ml. of $CHCl_3$. The extract was washed with 3*N* HCl, $NaHCO_3$-solution, and H_2O, respectively, dried over Na_2SO_4 then removed the solvent under vacuum to a thick sirup. The residue was dissolved in a small amount of EtOH, from which X crystallized gradually on cooling. Yield, 3.2 g., m.p. 125°, $[\alpha]_D^{20}$ +135° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{16}H_{22}O_{10}S$: C, 47.27; H, 5.46; S, 7.89. Found : C, 47.02; H, 5.51; S, 7.88.

The product was also obtained by acetylation of sodium 1-thio-α-D-glucopyranose (IX) with Ac_2O and pyridine.

Bis(2,2′,3,3′,4,4′,6,6′-octa-O-acetyl-α,β′-D-glucopyranosyl)sulfide (XII)——A solution of sodium 1-thio-α-D-glucopyranose (IX) (0.5 g.) and acetobromoglucose (I) (1 g.) in 50 ml. of MeOH was heated on a steam bath for 30 min. The mixture was concentrated under vacuum to a mixture of sirup and KBr followed by acetylation with 10 ml. of pyridine and 10 ml. of Ac_2O. After standing overnight at room temperature, the mixture was poured into 200 ml. of ice H_2O. The precipitates were filtered off, washed with H_2O, and air-dried. Recrystallization from EtOH gave colorless needles (1 g.), m.p. 170°, $[\alpha]_D^{20}$ +115° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{28}H_{38}O_{18}S$: C, 48.41; H, 5.51; S, 4.62. Found : C, 48.49; H, 5.51; S, 4.56.

A part of elementary analyses was carried out by the Microanalytical Center, Shimotakaido Laboratory, Kowa Co., Ltd. to all of whom the authors' thanks are due.

Summary

When potassium alkyl- or benzylxanthate was reacted on acetylated D-glucopyranosyl halides in ethanol or in acetone, acetylated D-glucopyranosyl xanthates, acetylated di-D-glucopyranosyl sulfides, and acetylated β-D-thioglucosides formed. The yield of

each condensation products was affected by choice of solvent, the reaction temperature, the potassium xanthate, and starting halide used.

A simple method for the preparation of bis(2,2′,3,3′,4,4′,6,6′-octa-O-acetyl-β,β′-D-glucopyranosyl)sulfide (octa-O-acetyl-isothiotrehalose) was confirmed by the reaction of potassium methyl- or benzylxanthate upon 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide. Two other isomers of octa-O-acetyl-isothiotrehalose were synthesized as crystalline forms.

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92. Kazuo Yoshida, Keitaro Kato, and Hisao Tsukamoto : Metabolism of Drugs. XLIV.[*1] Glucuronyl Transfer Reaction catalyzed by β-Glucuronidase by the Use of Ester Glucuronides as Substrates.

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Glucuronide conjugation is essentially a group transfer reaction in which the donor molecule donates its glucuronyl group to a suitable acceptor molecule. Dutton and Storey have reported that microsomal UDP-transglucuronylase catalyzes the transfer of the glucuronyl group from uridine diphosphate glucuronic acid to acceptor.[1] Another enzyme which is capable of transferring the glucuronyl group is β-glucuronidase. Fishman and Green have demonstrated that, under certain conditions, β-glucuronidase can transfer the glucuronic acid residue *in vitro* from ethereal glucuronides, such as aryl and alicyclic β-glucuronides, to simple alcohols and glycols.[2]

In their experiments, however, phenolic glucuronides could not be formed by this system. Until lately, there has been no information on a pathway of glucuronyl transfer with existing glucuronides acting as donors *in vivo.* In order to establish the glucuronyl transfer reaction of β-glucuronidase *in vivo,* it is necessary to identify the native endogenous glucuronyl donors and same time to prove phenolic glucuronides formation by this system.

The present investigation has been undertaken in order to clarify whether or not ester glucuronides, such as benzoyl β-D-glucuronide (B-GA), *p*-aminobenzoyl β-D-glucuronide (PABA-GA), and 2-hydroxy-4-aminobenzoyl β-D-glucuronide (PAS-GA), whose β-glucosiduronic linkage are labil could transfer their glucuronyl group to alcohols and phenols.

Materials and Methods

Donor Substrate and Reference Compounds——B-GA,[3] PABA-GA,[4] and PAS-GA[5] were isolated

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