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Studies on L-Gulonic Acid Derivatives. II.[1] Benzoyl Migration in Derivatives of D-Glucitol

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Benzoyl migrations took place in the partical acid hydrolysis of 1,2,3,5-tetra-O-benzoyl-4,6-O-ethylidene-D-glucitol (III) to give 1,2,4,6-tetra-O-benzoyl-D-glucitol (IV). This finding is principally based on the experimental result that chromium trioxide oxidation of IV in acetone-sulfuric acid afforded the following five compounds: 1,3,5,6-tetra-O-benzoyl-L-sorbose (V), 1,2,4,6-tetra-O-benzoyl-D-*ribo*-3-hexulose (IX), 2,3,5,6-tetra-O-benzoyl-L-gulonolactone (VIII), di-O-benzoyl-L-glyceric acid (XI), and benzoyl-glycolic acid (XII). The formation of VIII from IV in this oxidation indicates that benzoyl migration occurred also during the oxidation.

As a result of enzymic studies on the formation of L-xylulose from L-gulonic acid (I) in the D-glucuronic acid metabolism in mammalian systems, evidence for the identification of keto-intermediate, β-keto-L-gulonic acid (II), was given.[3,4] Metabolism of D-glucuronic acid *via* I and L-xylulose to pentose phosphate cycle is considered to be the major pathway of D-glucuronic acid metabolism in mammals, especially in man, monkey and guinea pig which are known to be unable to synthesize L-ascorbic acid from D-glucuronic acid.[5] In order to clarify the mechanism of the formation of L-xylulose from I, sample of II has been required, although preparation of this compound seemed to be difficult because of its instability.

The present investigation was undertaken to examine if any convenient method could be worked out for synthesizing II. Thus, an attempt was made to prepare an adequately protected derivative of I having a single hydroxyl group at C-3, which may subsequently be oxidized to give the corresponding derivative of II. An experiment performed along this line is presented in this paper which deals with the structure determinations of tetra-O-benzoyl-D-glucitol (IV) derived from 1,2,3,5-tetra-O-benzoyl-4,6-O-ethylidene-D-glucitol (III)[6] and of the oxidation products of IV.

Selective removal of ethylidene group from III was effected by hydrolysis with ethanolic hydrochloric acid. The resulting product (IV) was readily purified by recrystallization and homogeneous on thin-layer chromatogram. It consumed no lead tetraacetate and was expected to be 1,2,3,5-tetra-O-benzoyl-D-glucitol. However, it did not react with trityl chloride and examination of the oxidation products of IV as described below revealed that IV was 1,2,4,6-tetra-O-benzoyl-D-glucitol, thus indicating benzoyl migration during the acid hydrolysis of III.

Oxidation of IV with chromium trioxide in acetone-sulfuric acid gave five products (P_1, P_2, P_3, P_4, and P_5) which were separated and characterized. They were extractable

1) Part I: *Yakugaku Zasshi*, **86**, 110 (1965).
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3) J.D. Smiley and G. Ashwell, *J. Biol. Chem.*, **236**, 357 (1961).
4) J. Winkelman and G. Ashwell, *Biochim. Biophys. Acta*, **52**, 170 (1961).
5) J.J. Burns and A.H. Conney, "Glucuronic Acid," ed. by G.J. Dutton, Academic Press, New York, 1966, p. 365; S. Hollmann, "Non-Glycolytic Pathways of Glucose Metabolism," translated and revised by O. Touster, Academic Press, New York, 1964, p. 83.
6) R.C. Hockett, D.V. Collins, and A. Scattergood, *J. Am. Chem. Soc.*, **73**, 599 (1951).

with chloroform and removal of the solvent afforded two crystalline products by fractional crystallization.

The first crystalline product (P_1) exhibited a hydroxyl group band at 3544 cm^{-1} and two carbonyl group bands at 1740 and 1720 cm^{-1} in the infrared spectrum. The elemental analysis indicated that P_1 is a monoketo-derivative of IV. Treatment of P_1 with sodium borohydride followed by debenzoylation gave iditol and glucitol, which were identified by gas chromatographic and paper electrophoretic analyses.[7] Furthermore, the reduction product was benzoylated to yield hexa-O-benzoyl-L-iditol (VI) and hexa-O-benzoyl-D-glucitol (VII) which were isolated and identified by direct comparison with the respective authentic samples prepared from L-sorbose.

From these results it became apparent that P_1 has the L-sorbose configuration. Further concordant data were provided by the nuclear magnetic resonance spectrum of this compound. Thus it showed a typical AB quartet pattern centered at 4.90 τ (2H, δ=0.18 ppm, J=17.5 cps) which is ascribable to geminal coupling of methylene protons adjacent to ketone group.[8] IV and the other monoketo-derivative of IV (P_3) described below did not exhibit any absorption in this area. Moreover, it was evident that the hydroxyl and ketone groups of P_1 are not vicinal, since it did not consume any lead tetraacetate. This assumption was further supported by the fact that 1,2,5,6-tetra-O-benzoyl-D-mannitol (XIV)[9] and 1,2,5,6-tetra-O-benzoyl-D-glucitol (XV) both having vicinal diol grouping underwent ready oxidative fission of the carbon-carbon bond between two hydroxylated carbon atoms when they were subjected to oxidation with chromium trioxide in acetone-sulfuric acid, affording di-O-benzoyl-D-glyceric acid (XVI) in the case of XIV, which was characterized as the methyl ester (XVIa). Finally, the isolation and characterization of the other monoketo-derivative of IV (P_3) described below clearly demonstrated that P_1 has the hydroxyl group at C-4 in the L-sorbose configuration. On the basis of these results, the structure of P_1 has been established to be 1,3,5,6-tetra-O-benzoyl-L-sorbose (V).

The second crystalline product (P_2) did not exhibit any absorption band due to hydroxyl group but strong carbonyl group bands at 1810 and 1725 cm^{-1} in the infrared spectrum, indicating the presence of a γ-lactone and benzoate group respectively. It was found to be identical with 2,3,5,6-tetra-O-benzoyl-L-gulonolactone (VIII) by direct comparison with an authentic sample.[10]

The third oxidation product (P_3) obtained as a sirup was slightly more polar than V on thin-layer chromatogram. The combined mother liquor from crystallizations of V and VIII was subjected to column chromatography and then to preparative thin-layer chromatography on silica gel to afford P_3. It exhibited a hydroxyl group band at 3450 cm^{-1} and two carbonyl group bands at 1730 and 1700 cm^{-1} in the infrared spectrum. The elemental analysis indicated that P_3 is also a monoketo-derivative of IV. Treatment of P_3 with sodium borohydride followed by debenzoylation gave glucitol and allitol, which were identified by gas chromatographic and paper elelctrophoretic analyses.[7] Accordingly, it was evident that P_3 possesses the D-*ribo*-3-hexulose configuration. Position of the hydroxyl group of P_3 at C-5 in this configuration could reasonably be deduced from the position of the ketone group in V described above. Thus, the structure of P_3 has been established to be 1,2,4,6-tetra-O-benzoyl-D-*ribo*-3-hexulose (IX).

On the other hand, the remaining two products, P_4 and P_5, were separated from the acid fraction after oxidizing IV with chromium trioxide in acetone-sulfuric acid. The combined

7) Gas chromatographic and paper electrophoretic identifications of the hexitols are reported elsewhere.[17]
8) L.M. Jackman, "Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, Ltd., London, 1959; N. Bhacca and D.A. Williams, "Applications of NMR Spectroscopy in Organic Chemistry," Holden-Day, Inc., San Francisco, 1964.
9) N. Chü and C.E. Ballow, *J. Am. Chem. Soc.*, **83**, 1711 (1961).
10) P. Kohn, R.H. Samaritano, and L.M. Lerner, *J. Am. Chem. Soc.*, **87**, 5475 (1965).

mother liquor from crystallizations of V and VIII was extracted with cold sodium hydrogen carbonate solution. The acid fraction was obtained by acidification of the aqueous layer and re-extraction with benzene.

The fourth product (P_4) was obtained in crystalline form from this fraction and gave an intense blue naphthoresorcinal reaction which is characteristic of glyceric acid.[11] It was methylated with diazomethane and the resulting methyl ester was found to be identical with methyl di-O-benzoyl-L-glycerate (XIa)[12] by direct comparison with an authentic specimen. Therefore, P_4 has been identified as di-O-benzoyl-L-glyceric acid (XI).

The fifth product (P_5) was separated and characterized from the residual acid fraction not as its original form. Thus the mother liquor of XI was treated with diazomethane to yield a sirup which showed similar mobility to XIa on thin-layer chromatogram, while further treatment of which with ammonia in methanol gave a crystalline product that showed two spots on thin-layer chromatogram. Comparison with the authentic samples on thin-layer chromatogram suggested that the slower moving component is glyceric acid amide and the other one is hydroxyacetamide (XIII) respectively. Actually the latter was isolated in crystalline form by preparative thin-layer chromatography and found to be identical with XIII.[13] Therefore, although the presence of benzoyl group in P_5 was not characterized definitely, it seems reasonable to assign the structure of benzoyl-glycolic acid (XII) to P_5, in view of the separation process described above as well as its formation from IV.

Thus, chromium trioxide oxidation in acetone-sulfuric acid of the tetra-O-benzoyl-D-glucitol (IV) derived from 1,2,3,5-tetra-O-benzoyl-4,6-O-ethylidene-D-glucitol (III) by

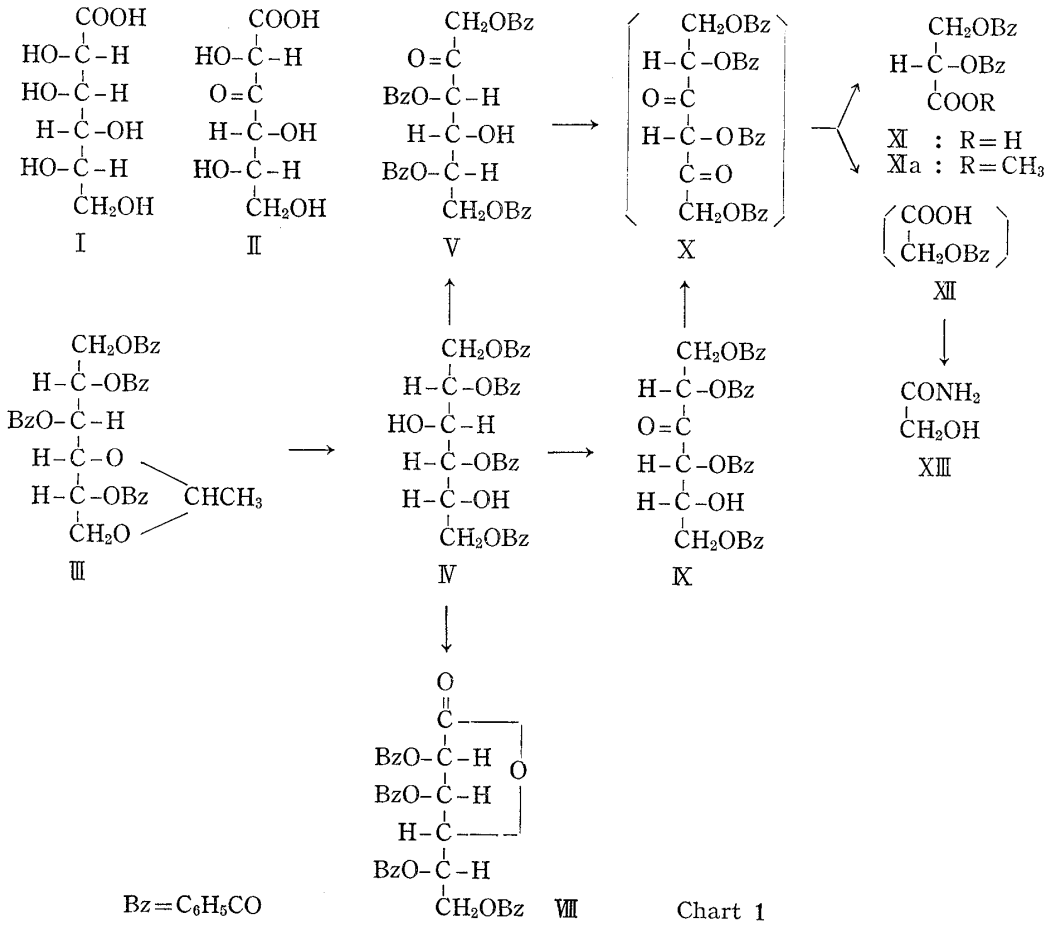

Chart 1

11) F. Feigl, "Spot Tests in Organic Analysis," 5th ed., Elsevier, New York, 1956, p. 349.
12) P. Frankland and J. MacGregor, *J. Chem. Soc.*, **69**, 104 (1896).
13) A. Darapsky, *Ber.*, **43**, 1112 (1910).

acid hydrolysis gave at least five compounds, V, VIII, IX, XI and XII, which were separated and characterized. This finding indicates that benzoyl migration took place during the acid hydrolysis of III or the oxidation of IV.

In the light of the fact that oxidation of the hydroxyl group was observed at C–3, C–5, and C–6 in the D-glucitol configuration, the experimental results presented in this paper could reasonably be elucidated according to Chart 1 as follows: 1) Benzoyl migrations from C–3 to C–4 and C–5 to C–6 in the D-glucitol configuration took place in the partial acid hydrolysis of III to give IV. 2) Oxidation of IV afforded two monoketones, V and IX. 3) V and IX were further oxidized to give identical β-diketone (X), whose instability prevented its isolation, affording XI and XII by oxidative fission of the carbon–carbon bonds. 4) On the other hand, partial benzoyl migration from C–6 to C–5 during the oxidation and subsequent oxidation of the resulting primary hydroxyl group gave VIII by lactonization.

Experimental[14]

1,2,4,6-Tetra-O-benzoyl-D-glucitol (IV)——A mixture of 1,2,3,5-tetra-O-benzoyl-4,6-O-ethylidene-D-glucitol (III)[6] (105 g) in 95% EtOH (1 liter) and 36% HCl (50 ml) was refluxed for 5 hr. The reaction mixture was concentrated *in vacuo* to give crystals (54 g) melting at 160—164°, which were recrystallized from 99% EtOH to afford fine needles (46 g) of IV, mp 164—166°, $[\alpha]_D^{18}$ +16.8° (c=1.08, dimethylformamide). *Anal.* Calcd. for $C_{34}H_{30}O_{10}$: C, 68.22; H, 5.05. Found: C, 68.13; H, 4.83. IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 1720, 1710 (CO). IV was tritylated in the usual way at room temperature for 3 days, but it was recovered from the reaction mixture in 77% yield, while triphenyl carbinol derived from trityl chloride during the isolation procedure was recovered in 94% yield. IV did not consume any lead tetraacetate in 104 hr.

Chromium Trioxide Oxidation of 1,2,4,6-Tetra-O-benzoyl-D-glucitol (IV)——To a solution of IV (10 g) in acetone (250 ml) was added chromium trioxide (5 g) in 7N H_2SO_4 (25 ml) dropwise over a period of 10 min. The reaction mixture was kept at room temperature for 3 hr and then filtered. The filtrate was concentrated under reduced pressure at 20° to a small volume and poured into cold water, and then extracted with $CHCl_3$. The extract was washed with water, dried over Na_2SO_4, and concentrated *in vacuo* at 30° to yield a sirup (9.5 g).

1,3,5,6-Tetra-O-benzoyl-L-sorbose (V)——The above sirup was crystallized from MeOH to give crystals (3.6 g) melting at 120—128°, which were recrystallized from MeOH to yield fine needles (2.0 g) of V, mp 127—128°, $[\alpha]_D^{18}$ +14.7° (c=1.36, dimethylformamide). *Anal.* Calcd. for $C_{34}H_{28}O_{10}$: C, 68.45; H, 4.73. Found: C, 68.28; H, 4.73. IR ν_{max}^{KBr} cm^{-1}: 3544 (OH), 1740, 1720 (CO). NMR τ: 4.90 (2H, δ=0.18 ppm, J=17.5 cps, $-COCH_2OCO-$). V consumed no lead tetraacetate in 104 hr.

Sodium Borohydride Reduction of 1,3,5,6-Tetra-O-benzoyl-L-sorbose (V); Identification of Iditol and Glucitol, and Preparation of Hexa-O-benzoyl-L-iditol (VI) and Hexa-O-benzoyl-D-glucitol (VII)——A solution of V (102 mg) in MeOH (10 ml) was cooled in an ice–water and $NaBH_4$ (43 mg) was added over a period of 5 min. The reaction mixture was kept in an ice–water for 2.5 hr and Amberlite IR–120 (H^+) resin was added to make pH of the solution at *ca.* 3.6, and then the reaction mixture was filtered. The filtrate was evaporated *in vacuo* at 40° to give a sirupy residue. Addition of MeOH (10 ml) and evaporation of the solvent were repeated seven times to eliminate boric acid as the volatile methyl ester. Examination of the resultant sirup

14) Lead tetraacetate consumption was measured by iodimetry.[15] Thin-layer chromatography (TLC) was carried out on 5×20 cm glass plates coated with a 0.25 mm layer of silica gel G (Merck) with detection by ammonium metavanadate–sulfuric acid.[16] Preparative TLC was done on 20×20 cm glass plates coated with a 0.5 mm layer of silica gel G (Merck), and column chromatography on silicic acid (Mallinckrodt, 100 mesh). Gas chromatography (GC) of the trifluoroacetyl derivatives of hexitols was carried out according to the procedure reported elsewhere.[17] Paper electrophoresis (PE) of hexitols was performed on Toyo Roshi No. 51 using basic lead acetate as solvent.[17,18] Nuclear magnetic resonance (NMR) spectra were obtained using solutions in deuteriochloroform with tetramethylsilane as internal standard and Japan Electron Optics Laboratory 4H–100 spectrometer. Infrared (IR) spectra were recorded for potassium bromide disks and carbon tetrachloride solutions on Hitachi EPI–S2 spectrophotometer. Optical rotations were determined on Hitachi PO–B polarimeter in 10 cm tubes of 1 ml capacity. Melting points were determined on a Kofler block and are uncorrected.

15) A.S. Perlin, "Methods in Carbohydrate Chemistry," Vol. I, ed. by R.L. Whistler and M.L. Wolfrom, Academic Press, New York, 1962, p. 427.
16) M. Ishidate, M. Matsui, and M. Okada, *Anal. Biochem.*, **11**, 176 (1965).
17) M. Matsui, M. Okada, T. Imanari, and Z. Tamura, *Chem. Pharm. Bull.* (Tokyo), **16**, 1367 (1968).
18) J.L. Frahn and J.A. Mills, *Australian J. Chem.*, **12**, 65 (1959).

(103 mg) by TLC using $CHCl_3$–MeOH (40:1) as solvent indicated that V was completely reduced and the reduced product had the same mobility as IV (*Rf*: V, 0.52; IV and reduced product, 0.22). The sirup (5 mg) was treated with NH_3–saturated MeOH (5 ml) at room temperature overnight and concentrated *in vacuo* to afford a sirup. Analysis of the sirup by GC and PE revealed the presence of iditol and glucitol. A solution of the sirup (96 mg) in pyridine (1.0 ml) was treated with benzoyl chloride (0.20 ml) at room temperature overnight. The reaction mixture was poured into cold water and extracted with $CHCl_3$. The extract was washed with water, 5% $NaHCO_3$, and water ,then dried over Na_2SO_4. Evaporation of the solvent *in vacuo* gave a sirup, which afforded crude crystals (102 mg) melting at 115—118° on addition of EtOH. The crude crystals (75 mg) were recrystallized from EtOH to give plates of VI (38 mg), mp 143—144°, $[\alpha]_D^{18}$ +14.4° (c=1.05, $CHCl_3$). The mother liquor was concentrated *in vacuo* to afford needles of VII (30 mg), mp 128—130°, $[\alpha]_D^{18}$ +28.0° (c=1.10, $CHCl_3$). Treatment of L-sorbose with $NaBH_4$ followed by benzoylation with benzoyl chloride as decribed above gave VI,[19] mp 143—144°, $[\alpha]_D^{18}$ +16.7° (c=1.20, $CHCl_3$), and VII,[20] mp 128—130°, $[\alpha]_D^{18}$ +27.6° (c=1.10, $CHCl_3$). These compounds were identical with those obtained from V by mixed melting point and IR spectrum respectively.

2,3,5,6-Tetra-O-benzoyl-L-gulonolactone (VIII)——The mother liquor from recrystallization of V was concentrated *in vacuo* to give crystals (772 mg) melting at 152—156°. Recrystallization from MeOH gave needles (493 mg) of VIII, mp 154—156°, $[\alpha]_D^{18}$ +89.1° (c=1.21, $CHCl_3$). *Anal.* Calcd. for $C_{34}H_{26}O_{10}$: C, 68.68; H, 4.41. Found: C, 68.96; H, 4.60. IR ν_{max}^{KBr} cm^{-1}: 1810, 1725 (CO). The melting point of the mixture with an authentic sample[10] (mp 154—156°, $[\alpha]_D^{18}$ +88.0° (c=1.16, $CHCl_3$)) prepared from L-gulonolactone by benzoylation in the usual way showed no depression, and IR spectra of the two samples were identical in all respects.

1,2,4,6-Tetra-O-benzoyl-D-*ribo*-3-hexulose (IX)——The combined mother liquor from crystallizations of V and VIII was concentrated under reduced pressure at 30° to give a sirup (5.3 g). The sirup (800 mg) was chromatographed on a column of silicic acid (40 g) using $CHCl_3$ as solvent. The appropriate fractions containing the component which was slightly more polar than V on TLC were combined and concentrated *in vacuo* to afford a sirup (298 mg). An analytical sample was obtained by preparative TLC using $CHCl_3$–MeOH (20:1) as solvent, $[\alpha]_D^{20}$ −51.5° (c=1.32, dimetylformamide). *Anal.* Calcd. for $C_{34}H_{28}O_{10}$: C, 68.45; H, 4.73. Found: C, 68.43; H, 5.19. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3450 (OH), 1730, 1700 (CO).

Sodium Borohydride Reduction of 1,2,4,6-Tetra-O-benzoyl-D-*ribo*-3-hexulose (IX); Identification of Glucitol and Allitol——A solution of IX (34 mg) in MeOH (1.0 ml) was cooled in an ice–water and treated with $NaBH_4$ (20 mg). The reaction mixture was kept in an ice–water for 2 hr and worked up in the similar way as described in the reduction of V. The sirup obtained was treated with NH_3–saturated MeOH (5ml) as described above to give glucitol and allitol, which were identified by GC and PE.

Methyl Di-O-benzoyl-L-glycerate (XIa)——The sirup (4.4 g) obtained from the combined mother liquor from crystallizations of V and VIII was dissolved in benzene and extracted with a cold 5% $NaHCO_3$ solution. The aqueous layer was acidified with 2N HCl and re-extracted with benzene. The extract was washed with water, dried over Na_2SO_4, and concentrated *in vacuo* to afford a sirup (2.1 g). The sirup was kept at room temperature for several days to yield crystals which were filtered with petroleum ether–toluene to give XI (675 mg) melting at 84—88°. A solution of XI (64 mg) in ether (2.0 ml) was treated with an excess of ethereal diazomethane. The reaction mixture was concentrated *in vacuo* to afford a sirup, which was crystallized from petroleum ether. The crude crystals (58 mg) melting at 52—57° were recrystallized from MeOH to give needles of XIa (41 mg), mp 57—59°, $[\alpha]_D^{20}$ +28.9 (c=0.90, MeOH). *Anal.* Calcd. for $C_{18}H_{16}O_6$: C, 65.85; H, 4.91. Found: C, 66.03; H, 4.89. IR ν_{max}^{KBr} cm^{-1}: 1740, 1725 (CO). XIa gave an intense blue naphthoresorcinol reaction which is characteristic of glyceric acid.[11] The melting point of the mixture with an authentic sample prepared as described below showed no depression, and IR spectra of the two samples were identical in all respects. An authentic sample of XIa[12] was synthesized from L-glyceric acid derivable from L-glyceraldehyde.[21] L-Glyceric acid was treated with dry HCl–MeOH and then with benzoyl chloride in the usual way to give XIa, mp 57.5—59°, $[\alpha]_D^{20}$ +29.4° (c=1.02, MeOH).

Hydroxyacetamide (XIII)——The mother liquor of XI was concentrated *in vacuo* to give a sirup (1.3 g). A solution of the sirup (200 mg) in ether (1.0 ml) was treated with an excess of ethereal diazomethane. The reaction mixture was concentrated *in vacuo* to afford a sirup. It was treated with NH_3–saturated MeOH (2.0 ml) overnight at room temperature. The reaction mixture was concentrated *in vacuo* to give a sirup, which was crystallized from acetone to afford needles (28 mg) melting at 70—75°. Examination of this substance by TLC using $CHCl_3$–MeOH (5:2) as solvent disclosed two components (*Rf*:0.36, 0.51) with similar mobility to glyceric acid amide (*Rf*:0.36) and XIII (*Rf*:0.51). The latter component was purified by preparative TLC using $CHCl_3$–MeOH (5:2) as solvent. Appropriate portions were extracted with MeOH and the combined extract was concentrated *in vacuo* to give crystals (8 mg) melting at 113—116°, which were re-

19) E.J. Bourne, G.P. McSeeney, and L.F. Wiggins, *J. Chem. Soc.*, **1952**, 2524.
20) Y. Asahina and H. Shinoda, *Yakugaku Zasshi*, **50**, 1 (1930).
21) A.S. Perlin, "Methods in Carbohydrate Chemistry," Vol. I, ed. by R.L. Whistler and M.L. Wolfrom, Academic Press, New York, 1962, p. 61.

crystallized from acetone to afford needles (3 mg), mp 115—117°. IR ν_{max}^{KBr} cm^{-1}: 3350, 3200 (OH and NH_2), 1660, 1580 ($CONH_2$). The melting point of the mixture with an authentic sample[13] (mp 116—118°) prepared from glycolic acid showed no depression, and IR spectra of the two samples were identical in all respects.

Chromium Trioxide Oxidation of 1,2,5,6-Tetra-O-benzoyl-D-mannitol (XIV): Formation of Di-O-benzoyl-D-glyceric Acid (XVI) and Its Characterization as the Methyl Ester (XVIa)——A solution of XVI[9] (1.17 g) in acetone (20 ml) was treated with chromium trioxide (1.0 g) in 7NH_2SO_4 (2.5 ml) using the procedure described in the foregoing experiment with IV. The resultant crystals were filtered with petroleum ether to give needles (1.0 g) melting at 84.5—86°. They were recrystallized from toluene–petroleum ether to afford needles of XVI (809 mg), mp 86—88°. A solution of XVI (160 mg) in ether (2.0 ml) was treated with ethereal diazomethane and concentrated *in vacuo* to give needles (128 mg) of methyl di-O-benzoyl-D-glycerate (XVIa), mp 58—59°, $[\alpha]_D^{22}$ −29.0 (c=1.00, MeOH). *Anal.* Calcd. for $C_{18}H_{16}O_6$: C, 65.85; H, 4.91. Found: C, 65.66; H, 5.08. XVIa and XIa were identical in melting point and IR spectrum, but they exhibited specific rotation to the same extent in the opposite direction. Melting point of XVIa was depressed to 40—50° on admixture with XIa. XVI and XVIa gave an intense blue naphthoresorcinol reaction.

1,2,5,6-Tetra-O-benzoyl-D-glucitol (XV)——D-Glucitol (6.0 g) was dissolved in warm pyridine (14 ml) and cooled in an ice–water. Benzoyl chloride (7.0 ml) was added dropwise to the solution, and the reaction mixture was kept in a refrigerator overnight. Subsequent evaporation of the solvent *in vacuo* gave a sirup, which was crystallized from EtOH to afford plates of 1,6-di-O-benzoyl-D-glucitol (857 mg),[22] mp 143—144°. The mother liquor gave another crystals (140 mg) melting 167—171°, which were recrystallized from 99% EtOH to give needles of XV (110 mg), mp 169—171°, $[\alpha]_D^{18}$ −27.6° (c=0.87, dimethylformamide). *Anal.* Calcd. for $C_{34}H_{30}O_{10}$: C, 68.22; H, 5.05. Found: C, 68.10; H, 5.32. XV consumed one mole of lead tetraacetate in 3 hr and no more consumption of the oxidant was observed in 70 hr. XV showed the same mobility as IV and XIV on TLC using $CHCl_3$–MeOH (20:1) as solvent.

Chromium Trioxide Oxidation of 1,2,5,6-Tetra-O-benzoyl-D-glucitol (XV)——XV (40 mg) in acetone (1.5 ml) was oxidized with chromium trioxide (40 mg) in 7NH_2SO_4 (0.9 ml) using the procedure described for the oxidation of IV. The sirup (37 mg) was treated with ethereal diazomethane and subsequent evaporation of the solvent *in vacuo* gave a sirup, which is considered to be methyl di-O-benzoyl-DL-glycerate. It gave an intense blue naphthoresorcinol reaction which is characteristic of glyceric acid. The sirup (3.0 mg) was treated with NH_3–saturated MeOH (1.0 ml) at room temperature overnight and concentrated *in vacuo* to afford a sirup. Examination of the sirup by TLC using $CHCl_3$–MeOH (5:2) as solvent revealed only one spot with the same mobility as glyceric acid amide.

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22) R.C. Hockett and H.G. Fletcher, Jr., *J. Am. Chem. Soc.*, **66**, 469 (1944).