[Chem. Pharm. Bull. 12(11)1302~1307(1964)] UDC 547.474.6.07

180. Hiroshi Nakamura, Setsuzo Tejima, and Masuo Akagi: Studies on the Bromine Addition Compounds derived from 3,4,6-Tri-O-acetyl-D-glucal.*1

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Recently, several papers[1] dealing with the addition products on the double bond in the glycals have been found in the literature, and the glycals have been an increased emphasis on experimentation aimed toward a valuable starting material for the synthesis of not easily obtainable sugars, such as halogeno-, deoxy-, or amino-sugars, and 2-deoxy-nucleosides.[2]

Since several years ago, as we have been synthesizing thiosugars,[3] in which one hydroxyl in sugar molecule is replaced by sulfhydryl group, the authors have been interested in the derivatives of the glycals, especially the bromine addition products of 3,4,6-tri-O-acetyl-D-glucal (I), as the starting material for thiosugar synthesis.

Concerning the bromine addition products, so far as we are aware of, only one systematic paper which was described by Fischer *et al.*[4] has been refered in the literature. Although they reported the separation of a crystalline dibromide directly from the bromination mixture of I, it is not so difficult to assume that the isolation of the pure compound must be rather difficult; hence the addition product must be a mixture of four different stereoisomers, producible in different amounts. Further, some of the derivatives obtained by them have not yet been assigned the configurations at C1 and C2.

From the viewpoint, the authors restudied their works, and demonstrated the structures of the "triacetyl-methylglucoside-2-bromohydrins I and II"*3 as methyl 2-bromo-2-deoxy-3,4,6-tri-O-acetyl-β-D-glucopyranoside (III) and methyl 2-bromo-2-deoxy-3,4,6-tri-O-acetyl-β-D-mannopyranoside (IV), respectively. Further, we prepared one crystalline, stable dibromide (XI) starting from X, and assigned as 2-bromo-2-deoxy-3,4,6-tri-O-acetyl-α-D-glucopyranosyl bromide. The physical constants of XI were not identical with that of the dibromide which had been separated by Fischer, *et al.*

The nuclear magnetic resonance spectra of the compounds obtained by us were measured, and the conclusion on the configurations at C1 and C2 was in agreement with that obtained by the chemical methods. The works will now be described.

Reaction of one molar portion of bromine upon triacetyl-D-glucal (I) in carbontetrachloride afforded a syrupy dibromide mixture (II) after the evaporation of the solvent under vacuum. The product was unstable, its chloroform solution mutarotated rather rapidly at room temperature. Various efforts to separate crystalline materials directly from II were failed, while we could separate two crystalline glycosides (III and IV) after

*1 A preliminary communication appeared in Yakugaku Zasshi, **82**, 1337 (1962). A part of the work was reported at the 20th Hokkaido Branch Meeting of the Pharmaceutical Society of Japan, Jan. 20, 1964.

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*3 Fischer, *et al.* designated the two crystalline isomeric glycosides obtained by bromination and methanolysis of I as such.

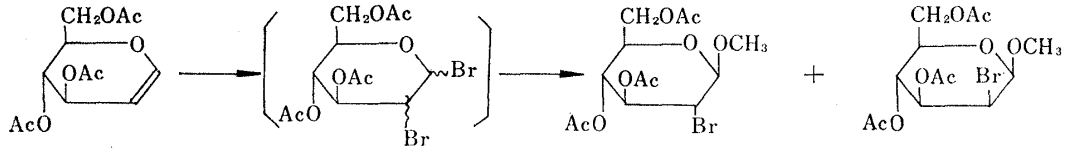
1) G. R. Inglis, J. C. P. Schwarz, L. MacLaren: J. Chem. Soc., **1962**, 1014; R. J. Ferrier, W. G. Overend, A. E. Ryan: *Ibid.*, **1962**, 3667; P. T. Manolopoulos, M. Mednick, N. N. Lichtin: J. Am. Chem. Soc., **84**, 2203 (1962); L. Vargha, J. Kuszmann: Chem. Ber., **96**, 411, 2016 (1963); W. J. Serfontin, J. H. Jordaan, J. White: Tetrahedron Letter, **1964**, 1069.

2) W. A. Bowles, P. K. Robins: J. Am. Chem. Soc., **86**, 1252 (1964).

3) M. Sakata, M. Haga, S. Tejima, M. Akagi: This Bulletin, **12**, 652 (1964).

4) E. Fischer, M. Bergmann, H. Schotte: Ber., **53**, 509 (1920).

methanolysis of II in the presence of silver carbonate. One of which (III), m.p. 138～139°, $[\alpha]_D^{20}$ +39.0°, was easily crystallizable, while the other one (IV), m.p. 115～116°, $[\alpha]_D^{23}$ −91.0°, quite difficult to separate; the product crystallized only after the storage under −20° for several days. The physical constants of III and IV were identical with that of the "triacetylmethylglucoside-2-bromohydrins I and II."

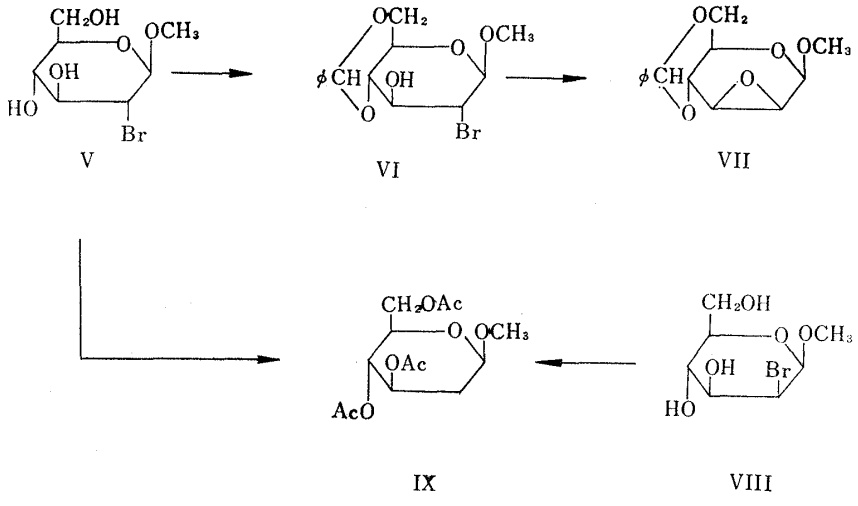


I II III IV

Chart 1.

The structure of III was demonstrated in the following manner. Deacetylation of III afforded a crytalline methyl 2-bromo-2-deoxy-β-D-glucopyranoside (V), m.p. 181～182°, $[\alpha]_D^{20}$ +2.7°, which was condensed with benzaldehyde in the presence of anhydrous zinc chloride to give a monobenzylidene compound (VI), m.p. 208～209°, $[\alpha]_D^{20}$ −20.0°. Successive treatment of VI with sodium methoxide in methanol afforded epoxide (VII), m.p. 183°, $[\alpha]_D^{16}$ −28.0°. The physical constants of VII were identical with that of methyl 4,6-O-benzylidene-β-D-mannopyranoside 2,3-anhydride which had been reported by Peat and Wiggins.[5] Therefore, the configurations of bromine at C2 and hydroxyl at C3 in VI must be *trans*, and the benzylidene group at C4 and C6.

The structure of the other crystalline glycoside (IV) was demonstrated as methyl 2-bromo-2-deoxy-3,4,6-tri-O-acetyl-β-D-mannopyranoside, a C2-diastereoisomer of III, hence the identical methyl 2-deoxy-3,4,6-tri-O-acetyl-β-D-glucopyranoside (IX) was obtained when the respective deacetylated product (V or VIII) had been reduced with sodium amalgam, followed by acetylation. This finding was in agreement with that of Fischer, *et al.*



V VI VII

IX VIII

Chart 2.

Treatmennt of dibromide mixture (II) with silver acetate in glacial acetic acid, followed by silica gel chromatography, gave 2-bromo-2-deoxy-1,3,4,6-tetra-O-acetyl-β-D-glucopyranose (X), m.p. 95～96°, $[\alpha]_D^{21}$ +63.0°, in 25～30% yield. Reaction of hydrogen bromide on X in glacial acetic acid afforded 2-bromo-2-deoxy-3,4,6-tri-O-acetyl-α-D-glucopyranosyl bromide (XI), m.p. 92～93°, $[\alpha]_D^{21}$ +260.0°, in 80% yield, which was rather

5) S. Peat, L. F. Wiggins: J. Chem. Soc., 1938, 1088.

stable and did not show mutarotation in chloroform. Treatment of the bromide (XI) with methanol in the presence of silver carbonate, or with silver acetate, regenerated III or X in good yield, during which Walden inversion involved at C1. This finding, along with the large dextrorotatory value, presents a proof on the α-configuration of the bromine in the bromide (XI), further the β-configuration of the methoxyl or the acetoxyl in III or X.

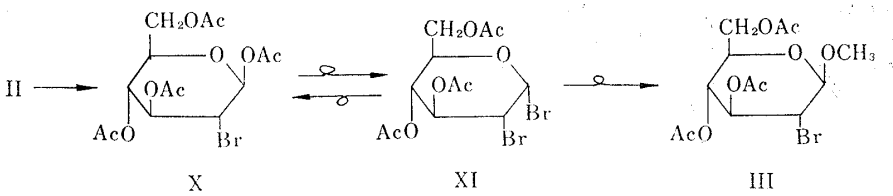


Chart 3.

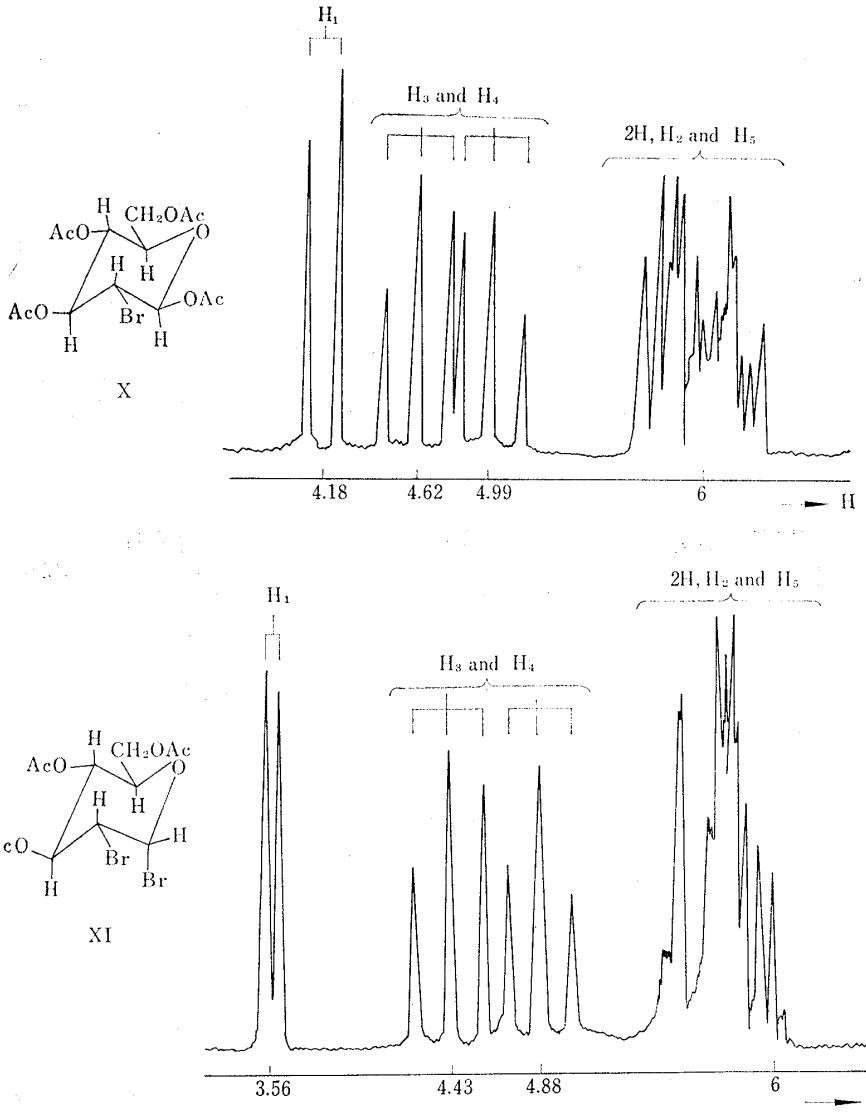


Fig. 1. Nuclear Magnetic Resonance Spectra of 2-Bromo-2-deoxy-1,3,4,6-tetra-O-acetyl-β-D-glucopyranose (X) and 2-Bromo-2-deoxy-3,4,6-tri-O-acetyl-α-D-glucopyranosyl Bromide (XI)

These spectra were measured by JNM-3H-60-spectrometer (Japan Electron Optics Laboratory Co., Ltd.) in $CDCl_3$ at 60 Mc. The chemical shifts are expressed as τ values referred to tetramethylsilane used as internal reference.

Fischer, *et al.* separated a crystalline dibromide, m.p. 117~118°, $[\alpha]_D^{21}$ +17.4°, directly from the reaction mixture of the bromination on triacetyl-D-glucal (I). They reported the bromide showed the correct composition, while it was quite unstable. The specific rotation was variable according to the kind of solvent used in recrystallization. They also described the formation of a syrupy 2-bromo-2-deoxy-1,3,4,6-tetra-O-acetyl-D-hexose. However, the acetate should be a mixture of isomers, thus they did not demonstrate the configurations at C1 and C2.

It is worthy of note our bromide (XI) was quite stable, contrary to the expectation that it must be reactive owing to the proximity of the bromine atom at C2; the pure material was able to storage for several months without decomposition.

We measured the nuclear magnetic resonance spectra in order to obtain further information on the configurations at C1 and C2 in X and XI, since X had a comparatively large dextrorotatory value for the β-series. They indicated, as shown in Fig. 1, the characteristic doublets[6] assigned to the proton at C1 in acetylated sugars. The large value (9.0 c.p.s.) of the coupling constant obtained from X was a reasonable order of magnitude for an axial-axial orientation at C1 and C2, while the low value (3.5 c.p.s.) in XI indicated an equatorial-axial situation.

Shortly before, Lemieux and Fraser-Reid[7] have reported on the nuclear magnetic resonance analyses of the sirupy dibromide mixture and "triacetylmethylglucoside-2-bromohydrins I and II" of Fischer, *et al.* Concerning the structure of the latter, they also have introduced the identical conclusion with us.

Experimental

Methyl 2-Bromo-2-deoxy-3,4,6-tri-O-acetyl-β-D-glucopyranoside (III) and Methyl 2-Bromo-2-deoxy-3,4,6-tri-O-acetyl-β-D-mannopyranoside (IV)——These compounds were prepared by a slight modification of the procedure described by Fischer, *et al.*[4] A solution of Br_2 (9 g.) in 45 ml. of CCl_4 was added to a solution of 3,4,6-tri-O-acetyl-D-glucal (I)[8] (15 g.) in an equal amount of the same solvent at such a rate as to keep the reaction temperature below 10°. The reaction mixture was protected from the moisture, and kept overnight in a refrigerator. The colorless solution was evaporated to dryness *in vacuo* below 30°, and the residual sirup (II) was dissolved in 170 ml. of warm, abs. MeOH. Freshly prepared Ag_2CO_3 (30 g.) was added to the solution, stirred for 1.5 hr. at room temperature, then filtered. The filtrate was concentrated to about 1/5 of its original volume *in vacuo*. Crude III was crystallized after standing overnight at 0°, and separated by filtration (7.2 g., 34%). Twice recrystallizations from abs. EtOH gave pure material as needles, m.p. 138~139°, $[\alpha]_D^{23}$ +39.0° (c=1.0, Me_2CO). Fischer, *et al.* reported m.p. 138° (corr. 139°), $[\alpha]_D^{18}$ +50.2° (acetylenetetrachloride) and yield 10~15%, for triacetyl-methylglucoside-2-bromohydrin I.

Petr. ether was added to the mother liquor of III, then allowed to stand in a deepfreezer at −20° for several days, whereupon crude IV crystallized gradually, and separated by filtration (1.8 g., 8.5%). After twice recrystallizations from abs. EtOH the material showed m.p. 115~116°, $[\alpha]_D^{16}$ −92.0° (c=1.0, Me_2CO). Fischer, *et al.* reported m.p. 115~116°, $[\alpha]_D^{16}$ −92.0° (acetylentetrachloride) and yield 6~8%, for triacetyl-methylglucoside-2-bromohydrin II.

Methyl 2-Bromo-2-deoxy-β-D-glucopyranoside (V)——The material, m.p. 181~182°, $[\alpha]_D^{20}$ +2.7° (c=0.75, H_2O), was prepared using the method described by Fischer, *et al.* for the preparation of methyl-glucoside-2-bromohydrin I. They reported m.p. 179~180° (corr. 182°), $[\alpha]_D^{14}$ +0.59° (H_2O).

Methyl 2-Bromo-2-deoxy-4,6-O-benzylidene-β-D-glucopyranoside (VI)——A mixture of anhyd. $ZnCl_2$ (4 g.), V (6.5 g.) and PhCHO (15 ml.) was shaken for 1 hr. at room temperature. The reaction mixture was diluted with a small amount of H_2O, followed by addition of a large amount of petr. ether. Crystallization was induced spontaneously, filtered and washed with H_2O, then with petr. ether. Twice recrystallizations from a large amount of MeOH gave colorless crystals (8 g.), m.p. 208~209°, $[\alpha]_D^{20}$ −20.0° (c=0.6, $CHCl_3$). *Anal.* Calcd. for $C_{14}H_{17}O_5Br$: C, 48.71; H, 4.96; Br, 23.15. Found : C, 48.88; H, 4.96; Br, 23.41.

6) R. U. Lemieux, R. K. Kullnig, H. J. Bernstein, W. A. Schneider : J. Am. Chem. Soc., **80**, 6098 (1958).
7) R. U. Lemieux, B. Fraser-Reid : Can. J. Chem., **42**, 532 (1964).
8) R. L. Whistler, M. L. Wolfrom : "Methods in Carbohydrate Chemistry" Vol II, 405 (1963), Academic Press Inc., New York and London.

Kent, *et al.*[9] reported m.p. 174°, $[\alpha]_D^{20}$ +1.0° (c=2.0, $CHCl_3$) for methyl 2-bromo-2-deoxy-4,6-O-benzylidene-β-D-glucopyranoside, while the product has less satisfactory composition than the product obtained by us.

Methyl 4,6-O-Benzylidene-β-D-mannopyranoside 2,3-anhydride (VII)——A mixture of Ⅵ (500 mg.) and MeONa in abs. MeOH (50 mg. of Na was dissolved in 16 ml. of MeOH) was refluxed for 5 hr. on a steam bath. After standing overnight in a refrigerator the resulting crystalline mass was separated by filtration, and washed thoroughly with H_2O. Twice recrystallizations from MeOH gave pure material (350 mg.), m.p. 183°, $[\alpha]_D^{16}$ −28.0° (c=0.82, $CHCl_3$). *Anal.* Calcd. for $C_{14}H_{16}O_5$: C, 63.62; H, 6.10. Found: C, 63.90; H, 6.19.

Peat, *et al.*[5] reported m.p. 183°, $[\alpha]_D^{18}$ −30.7° (c=0.82, $CHCl_3$) for methyl 4,6-O-benzylidene-β-D-mannopyranoside-2,3-anhydride.

Methyl 2-Bromo-2-deoxy-β-D-mannopyranoside (VIII)——The material, m.p. 183°, $[\alpha]_D^{20}$ −68.0° (c=1.0, H_2O), was prepared using the method described by Fischer, *et al.* for the preparation of methylglucoside-2-bromohydrin Ⅱ. They reported m.p. 180~181°, $[\alpha]_D^{16}$ −63.8° (H_2O).

Methyl 2-Deoxy-3,4,6-tri-O-acetyl-β-D-glucopyranoside (IX)——The material, m.p. 98°, $[\alpha]_D^{20}$ −25.6°, (C=2.0, $CHCl_3$) was prepared starting from Ⅴ or Ⅷ as described by Fischer, *et al.*

2-Bromo-2-deoxy-1,3,4,6-tetra-O-acetyl-β-D-glucopyranose (X)——A mixture of AcOAg (20 g.) and dibromide mixture (Ⅱ) in 150 ml. of glacial AcOH was stirred for 2 hr. at 80~90° on a steam bath. The starting dibromide (Ⅱ) was prepared as described in the preparation of Ⅲ and Ⅳ. The reaction mixture was filtered, the filtrate was concentrated *in vacuo* to afford a crystalline mass which was taken up in $CHCl_3$. The $CHCl_3$-layer washed with H_2O, aq. $NaHCO_3$ and H_2O, respectively. After drying over Na_2SO_4, the filtrate was concentrated *in vacuo* to a light yellow sirup which was dissolved in 50 ml. of benzene and put on a column (2.5×35 cm.) containing 80 g. of silica gel. Elution was carried out with 800 ml. of benzene. After the evaporation of the solvent *in vacuo* the residual sirup was dissolved in 50 ml. of warm abs. EtOH, and left at 10° for several days. Crystallization was induced by scratching with a glass rod, and the precipitates were collected by filtration. Several recrystallizations from abs. EtOH gave pure material (6 g.) m.p. 95~96°, $[\alpha]_D^{21}$ +63.0° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{14}H_{19}O_9Br$: C, 40.88; H, 4.62. Found : C, 40.89; H, 4.81.

2-Bromo-2-deoxy-3,4,6-tri-O-acetyl-α-D-glucopyranosyl Bromide (XI)——A solution of X (2.5 g.) in 15 ml. of glacial AcOH, containing 35% HBr and 2 drops of Ac_2O, was stirred for 2.5 hr. at room temperature, then the solution was poured into ice-water (100 ml.), followed by addition of $CHCl_3$ (50 ml.), and aq.-layer was extracted with $CHCl_3$. The $CHCl_3$-layer was washed with aq. $NaHCO_3$ and H_2O. After drying over Na_2SO_4, the filtrate was evaporated to give a sirup which was dissolved in abs. Et_2O. Petr. ether was added to give a slight turbidity, and left in a refrigerator. Deposited crystalline mass (2.1 g.) was separated by filtration and recrystallized from the same solvent to give pure material, m.p. 92~93°, $[\alpha]_D^{21}$ + 260.0° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{12}H_{16}O_7Br_2$: C, 33.36; H, 3.72. Found : C, 33.54; H, 3.72.

Synthesis of Methyl 2-Bromo-2-deoxy-3,4,6-tri-O-acetyl-β-D-glucopyranoside (III) from Bromide (XI)——Freshly prepared Ag_2CO_3 (0.5 g.) was added to a solution of bromide (Ⅺ) (0.5 g.) dissolved in abs. MeOH (10 ml.), and the mixture stirred for 1.5 hr. at room temperature. Insoluble materials were removed by filtration, and washed thoroughly with abs. MeOH. The filtrate and washings were combined, evaporated *in vacuo* to give a white crystalline mass (0.36 g., 81%). Twice recrystallizations from abs. EtOH gave pure material, m.p. 138°. The IR spectrum of this material was identical with that of methyl 2-bromo-2-deoxy-3,4,6-tri-O-acetyl-β-D-glucopyranoside (Ⅲ), and did not show mixed melting point depression with Ⅲ.

Synthesis of 2-Bromo-2-deoxy-1,3,4,6-tetra-O-acetyl-β-D-glucopyranose (X) from Bromide (XI)——A mixture of bromide (Ⅺ) (2.1 g.) and AcOAg (1 g.) in glacial AcOH (20 ml.) was stirred for 2 hr. at room temperature. Ag-salt was removed by filtration, the filtrate evaporated to dryness *in vacuo*. The residual sirup was taken up in $CHCl_3$ (30 ml.×3), and $CHCl_3$-layer washed with aq. $NaHCO_3$ and H_2O, then dried over $NaSO_4$. The filtrate was evaporated to dryness *in vacuo*, and a small amount of abs. EtOH was added to give crystals which was collected by filtration. Twice recrystallizations from abs. EtOH gave pure material, m.p. 96~97°, $[\alpha]_D^{23}$ +61.0° (c=1.0, $CHCl_3$). The product showed no depression of melting point on admixture with the product prepared from Ⅱ.

The NMR spectra were determined by Mr. S. Shimokawa of School of Technology, Hokkaido University, and a part of the elementary analyses was carried out by the Shimotakaido Laboratory, Kowa Co., Ltd. to all of whom the authors' thanks are due.

Summary

The works on the bromination of 3,4,6-tri-O-acetyl-D-glucal and methanolysis of the

9) P. W. Kent, F. O. Robson, V. A. Welch : J. Chem. Soc., **1963**, 3273.

product which had been reported by Fischer, Bergmann, and Schotte (Ber., **53**, 509 (1920)) were restudied. The structures of the two crystalline compounds termed the "triacetylmethylglucoside-2-bromohydrins I and II" were demonstrated as methyl 2-bromo-2-deoxy-3,4,6-tri-O-acetyl-β-D-glucopyranoside and methyl 2-bromo-2-deoxy-3,4,6-tri-O-acetyl-β-D-mannopyranoside, respectively.

2-Bromo-2-deoxy-1,3,4,6-tetra-O-acetyl-β-D-glucopyranose (X) was prepared as a crystalline form. Crystalline, stable bromide (XI) was prepared starting from X, and assigned as 2-bromo-2-deoxy-3,4,6-tri-O-acetyl-α-D-glucopyranosyl bromide which was not identical with the labile bromide by Fischer, *et al.*

The nuclear magnetic resonance spectra presented a further proof for the configurations at C1 and C2 in X and XI.

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181. Tsutomu Momose, Yo Ueda, and Chiharu Nakakura : Organic Analysis. LVI.*1 Determination of Free Cholesterol in Blood Serum. An Application of Perchloric Acid–Phosphoric Acid–Ferric Chloride Reagent to Cholesteryl Digitonide.

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The determination of free cholesterol in blood serum is usually carried out according to the following procedures : extraction of lipid fraction from serum with organic solvent, precipitation of free cholesterol with digitonin, and colorimetry of the precipitate by the Liebermann-Burchard reaction or Kiliani reaction.

The quantity of the digitonide may also be estimated by a turbidimetry,[1] a colorimetry with anthrone reagent,[2,3] or by other methods. Digitonin can be substituted either by tomatine[4~7] or by another saponin separated from the leaves of *Digitalis Purpurea* L.,[8] which are claimed to give more sensitive precipitation with cholesterol.

Column chromatography is also shown to be effective for the separation of free cholesterol from lipid fraction,[9~11] and gas chromatography might be a new technique.[12,13]

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4) J. J. Kabara, J. T. McLaughlin, C. A. Riegel : *Ibid.*, **33**, 305 (1961).
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