Tetrahedron, 1965, Vol. 21, pp. 1897 to 1901. Pergamon Press Ltd. Printed in Northern Ireland

GLUCURONIC ESTERS—III*

THE SYNTHESIS OF THE FULLY BENZYLATED C-1 HYDROXYL FREE GLUCURONIC ACID

N. PRAVDIĆ and D. KEGLEVIĆ
Tracer Laboratory, Institute "Ruder Bošković", Zagreb, Yugoslavia

(*Received* 26 *February* 1965)

Abstract—The synthesis of crystalline benzyl 2,3,4-tri-O-benzyl-D-glucopyranuronate (IX) starting from tri-O-benzyllaevoglucosan through a number of reactions on C-1 and C-6 atoms is described. The optical data of IX suggest a β-configuration for this compound.

IN THE first paper,[1] it was reported that methyl 2,3,4-tri-O-acetyl-1-O-acyl-D-glucopyranuronates can be synthesized by the carbodi-imide method from methyl 2,3,4-tri-O-acetyl-D-glucopyranuronate and the corresponding organic acid. Furthermore, it was shown[2] that the acetylated glucopyranuronates can not be converted by alkaline hydrolysis into the free 1-O-acyl-glucopyranuronates because the splitting of the ester bond at C-1 always occurs before the complete deacetylation takes place. Consequently, an effort was made to obtain a glucuronic acid moiety masked by nonparticipating, easily removable groups in neutral medium. In the present paper a synthetic route to the fully benzylated C-1 hydroxyl free glucuronic acid is presented.

The benzyl group was chosen because both hydroxyl and carboxyl groups are easily converted into benzyl-ethers and -esters respectively, which can be cleaved easily by catalytic hydrogenation at room temperature. Tri-O-benzyl-laevoglucosan,[3] easily available from laevoglucosan *via* its tri-O-acetyl derivative,[3] was converted by Zemplen procedure[3] into 1,6-di-O-acetyl-2,3,4-tri-O-benzyl-α-D-glucopyranose (I) in high yield. When I was treated with hydrogen bromide in glacial acetic acid the corresponding bromide, described already by Zemplen[3] as a syrup having the odour of benzyl bromide, was obtained; in our hands this unstable compound gave low and erratic yields in the following methylation reaction step. However, when I was treated with a solution of hydrogen chloride in ether, the stable, crystalline 2,3,4-tri-O-benzyl-6-O-acetyl-1-chloro-1-deoxy-α-D-glucopyranose (II) resulted which could be readily transformed into the methyl glucoside (III). The acetyl group on the C-6 atom of III was removed quantitatively with sodium methoxide yielding methyl 2,3,4-tri-O-benzyl-β-D-glucopyranoside (IV).

In order to obtain high yields in the catalytic oxidation of IV into 1-O-methyl-2,3,4-tri-O-benzyl-β-D-glucopyranuronic acid (V) the reaction conditions should be strictly followed. A water suspension of IV, to which the catalyst is added, must be stirred at a high rate and oxygen bubbled through vigorously. The temperature of the reaction mixture must be kept below the melting of IV, at 76–78°; as even small

* Part II, *Croat. Chem. Acta* **36**, 73 (1964).

[1] N. Pravdić and D. Keglević, *J. Chem. Soc.* 4633 (1964).

[2] N. Pravdić and D. Keglević, *Croat. Chem. Acta* **36**, 73 (1964).

[3] G. Zemplén, Z. Csürös and S. Angyal, *Ber. Dtsch. Chem. Ges.* **70**, 1848 (1937).

deviations cause considerable decrease in yield. With commercially available 10% Pt—C (Fluka puriss.) good results were obtained. The free carboxylic group of V was then blocked with the benzyl group using carbodi-imide method whereupon benzyl 1-O-methyl-2,3,4-tri-O-benzyl-β-D-glucopyranuronate (VI) was obtained.

In order to ascertain that no anomerization had taken place, the catalytic debenzylation of IV and VI was performed. The first compound gave methyl β-D-glucopyranoside[4] and the second the anticipated methyl-β-D-glucopyranosiduronic acid ($[\alpha]_D$ −60°, in water) in quantitative yield.

The acetolysis of the methyl glycosidic group of VI with the equimolar amount of sulphuric acid in acetic anhydride–acetic acid yielded the anomeric 1-O-acetyl derivative (VII) which could not be induced to crystallize. When VI was treated with dry hydrogen chloride in acetic acid, benzyl 2,3,4-tri-O-benzyl-1-chloro-1-deoxy-D-glucopyranuronate (VIIIa) resulted; the analytically pure oily product showed $[\alpha]_D$ +43° in chloroform. The corresponding 1-bromo derivative (VIIIb) was also prepared from VI by the standard method; the crude product showed $[\alpha]_D$ +59° in chloroform. However, all attempts to obtain VIIIb analytically pure failed; column chromatography over carbon–celite resulted in the decomposition of the compound. With regard to their optical rotation it may be assumed that in VIIIa and in VIIIb the α-anomer predominates. It is interesting that none of the fully benzylated glycosyl halides has as yet been obtained in the crystalline state,[5,6] although their optical rotations suggest a strong predominance of one anomer.

Although the fully benzylated C-1 hydroxyl free glucuronic acid (IX) may be produced by several alternative routes; only the pathway: VI–VII–VIIIb–IX proved to be satisfactory as a preparative method. All these reaction steps were performed without isolation and purification of the intermediates. The hydrolysis of VIIIb in the presence of silver carbonate as the acid acceptor yielded a semi-solid mixture which was fractionated by column chromatography on alumina whereupon the crystalline benzyl 2,3,4-tri-O-benzyl-D-glucopyranuronate (IX) was obtained in 40–43% yield, calculated on VI. Other routes leading to IX proved to be much less efficient: the one-step conversion of VI to VIIIb with hydrogen bromide in acetic acid resulted in a heavily contaminated bromo compound; on the other hand 1-chloro derivative (VIIIa) showed a considerable inertness in the hydrolysis step with silver carbonate.

The fact that IX has a sharp m.p. a negative rotation in chloroform, as well as that it shows a dextromutarotation in the presence of pyridine, suggests that the fully benzylated C-1 hydroxyl free glucuronic acid has a β-configuration or at least a strong predominance of this configuration.

EXPERIMENTAL

M.ps are uncorrected. Specific rotations were measured at 20–23° in $CHCl_3$ (*c*, 1 ± 2%) if not stated otherwise. IR spectra were recorded for Nujol mulls on a Perkin–Elmer model 137 instrument.

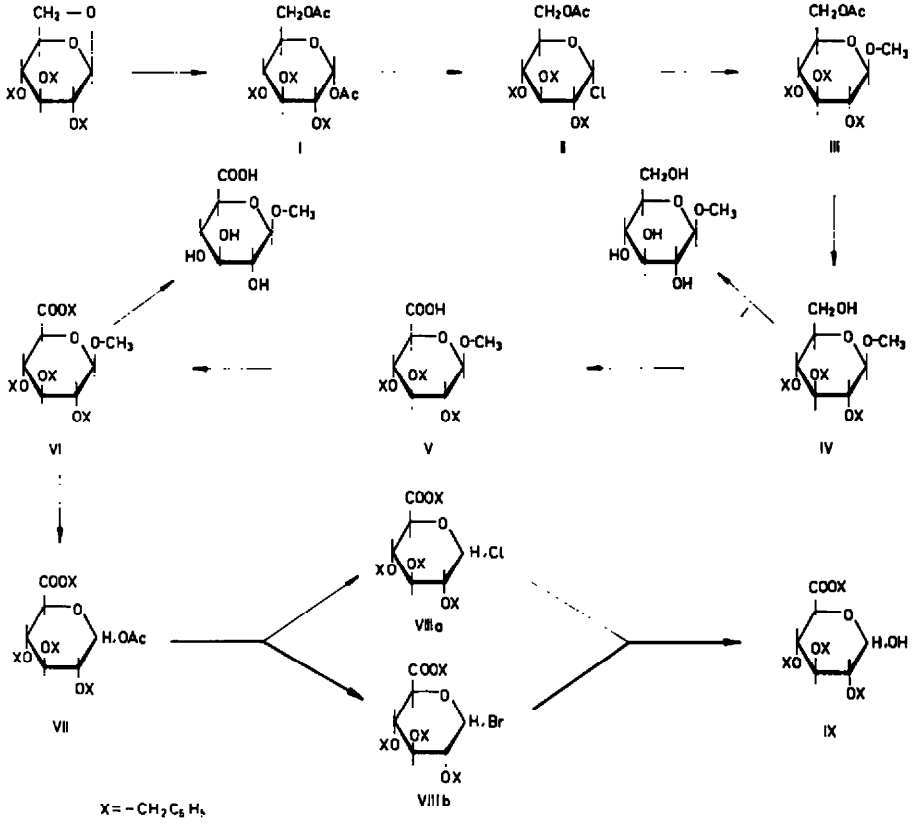
1,6-*Di*-O-*acetyl*-2,3,4-*tri*-O-*benzyl*-α-D-*glucopyranose* (I)

Tri-O-benzyl-laevoglucosan in 10 g batches was converted to I following the procedure given by Zemplen *et al.*[3] I crystallized from absolute EtOH in 67–70% yield; m.p. 60–62° and after one recrystallization, m.p. 64–65·5°, $[\alpha]_D$ +68°. Zemplen reports 56% yield, m.p. 66°, $[\alpha]_D$ +62·5° ($CHCl_3$). (Found: C, 69·54; H, 6·55. Calc. for $C_{31}H_{34}O_8$: C, 69·65; H, 6·41%.)

[4] A. L. Raymond and E. F. Schröder, *J. Amer. Chem. Soc.* **70**, 2785 (1948).
[5] S. Tejima and H. G. Fletcher, Jr., *J. Org. Chem.* **28**, 2999 (1963).
[6] C. P. J. Glaudemans and H. G. Fletcher, Jr., *J. Org. Chem.* **29**, 3286 (1964).

2,3,4-*Tri*-O-*benzyl*-6-O-*acetyl*-1-*chloro*-1-*deoxy*-α-D-*glucopyranose* (II)

Compound I (20·0 g, 37·5 mmoles) was dissolved in 100 ml absolute ether containing 21 g dry HCl. The solution was kept at 0° for 4 days, the solvent evaporated *in vacuo* and the remaining HCl removed by repeated evaporation with absolute benzene. The thick colourless oil solidified on seeding. (In this state the substance was pure enough for the preparation of III.) When crystallized from pet. ether (b.p. 60–80°) 15·3 g of II (80%), m.p. 62–63° was obtained. For analysis it was recrystallized from the same solvent; m.p. 63–64°, $[\alpha]_D$ +90°. (Found: C, 68·22; H, 6·05; Cl, 7·29. $C_{29}H_{31}ClO_6$ requires: C, 68·16; H, 6·11; Cl, 6·94%.) IR: ν_{max} 1730 (C=O), 1240 (C—O—C), and 840 cm^{-1} (assigned to α-anomer).[7]



Methyl 2,3,4-*tri*-O-*benzyl*-6-O-*acetyl*-β-D-*glucopyranoside* (III)

Crude II, obtained from 20 g of I, was dissolved in 20 ml absolute benzene and 40 ml absolute MeOH added. Freshly prepared dry Ag_2CO_3 (9 g) was added in small portions with occasional stirring during 1 hr. The suspension was shaken for 15 hr, the precipitate filtered off, washed with absolute benzene and the combined filtrates evaporated *in vacuo*. The remaining oil solidified on drying over H_2SO_4. It was crystallized from absolute MeOH with addition of charcoal; 12–13 g of III (64–69% calculated on I) with m.p. 59–61° (40° softening) was obtained. For analysis the substance was twice recrystallized from absolute MeOH; 61–63° (softening after 40°), $[\alpha]_D$ +26°. (Found: C, 71·01; H, 6·93. $C_{30}H_{34}O_7$ requires: C, 71·12; H, 6·77%.)

[7] S. Barker, E. Bourne, M. Stacey and D. Whiffen, *J. Chem. Soc.* 171 (1954).

Methyl 2,3,4-*tri*-O-*benzyl*-β-D-*glucopyranoside* (IV)

Into a suspension of III (11·2 g; 22 mmoles) in 80 ml absolute MeOH, 50 ml 1·2 N MeONa was added. The solution was left to stand at room temp overnight and then was passed, followed by MeOH, through a column of Dowex 50-X8(H^+). The eluate was evaporated to dryness, giving 9·8 g (97%) of crude IV, m.p. 78–80°. Two recrystallizations from 90% MeOH gave the analytical sample; m.p. 90–91°, $[\alpha]_D$ +10°. (Found: C, 72·21; H, 6·98. $C_{35}H_{38}O_6$ requires: C, 72·39; H, 6·94%.) IR: ν_{max} 3700 (OH), 1220 (C—O—C) and 889 cm^{-1} (assigned to β-anomer).[7]

Catalytic debenzylation of IV. Compound IV (1·16 g; 2·5 mmoles) was hydrogenated in 10 ml ethyl acetate over 100 mg 10% Pd—C catalyst. After theoretical consumption of H_2, the catalyst was removed by centrifugation, dried and eluted with water. The water eluate was evaporated to dryness leaving 440 mg (91%) of crystalline product, m.p. 103–107°. After two recrystallizations from EtOH analytically pure methyl-β-D-glucopyranoside as the hemihydrate was obtained; m.p. 104–105°, $[\alpha]_D$ −32° (*c* 2·04, in water). [Lit.[4] m.p. 104–106°, $[\alpha]_D$ −32° (water)]. IR: ν_{max} 3500 (OH), 1220 (C—O—C) and 887 cm^{-1} (assigned to β-anomer).

1-O-*Methyl*-2,3,4-*tri*-O-*benzyl*-β-D-*glucopyranuronic acid* (V)

In a 2·5 l. three-necked flask equipped with a vibro-stirrer, a gas inlet tube and a thermometer, 1·1 l. water, 1·7 g $NaHCO_3$ and 1·2 g Na_2CO_3 were placed. Methyl-2,3,4-tri-O-benzyl-β-D-glucopyranoside (IV; 9·6 g; 10·8 mmoles) and 9·0 g of 10% Pt—C catalyst (Fluka, puriss.) were added whereupon a strong stream of O_2 was bubbled through the aqueous suspension. The mixture was vigorously stirred and kept at 76–78° for 9 hr. The catalyst was removed by suction, the filtrate concentrated *in vacuo* to about 300 ml and acidified with conc. HCl. The precipitated uronic acid (V) was filtered off, washed with water and dried; yield 6·5 g (65%), m.p. 118–119°. For analysis the substance was crystallized from 50% EtOH, m.p. 121–121·5°, $[\alpha]_D$ −8°. (Found: C, 70·47; H, 6·27. $C_{35}H_{36}O_7$ requires: C, 70·28; H, 6·32%.) IR: ν_{max} 3200 (OH), 1740 (C=O), and 1210 cm^{-1} (C—O—C). The unreacted IV was recovered in almost quantitative yield by extracting the dried catalyst with chloroform. The catalyst could be used repeatedly (8–15 times) with undiminished activity.

Benzyl 1-O-*methyl*-2,3,4-*tri*-O-*benzyl*-β-D-*glucopyranuronate* (VI)

The uronic acid (V; 6·7 g, 14 mmoles) was dissolved in a mixture of 1·5 g (14 mmoles) benzyl alcohol in 60 ml dichloromethane and 0·8 ml dry pyridine. Dicyclohexylcarbo-di-imide (2·9 g, 14 mmoles) was gradually added and the solution was kept at room temp overnight. The precipitated dicyclohexylurea was filtered off, washed with dichloromethane and the combined filtrates evaporated *in vacuo* to dryness. The residue was treated with 25 ml absolute ether and a second crop of dicyclohexylurea was removed. The ethereal filtrate was evaporated *in vacuo*, the residual oil dried over H_2SO_4 and crystallized from EtOH; 5·4 g (67%) of VI, m.p. 68–69° was obtained. For analysis it was recrystallized from the same solvent; m.p. 69·5–70°, $[\alpha]_D$ −19°. (Found: C, 74·08; H, 6·38. $C_{35}H_{36}O_7$ requires: C, 73·92; H, 6·38%.)

Catalytic debenzylation of VI. To a solution of VI (568 mg; 1 mmol) in 15 ml ethyl acetate, 400 mg Pd—C catalyst was added and the mixture shaken until the consumption of H_2 was complete. After centrifugation, the catalyst was washed with water, and the eluates evaporated *in vacuo* leaving 198 mg (95%) of a thick oil. After thoroughly drying in high *vacuo* over P_2O_5 at 70°, methyl-β-D-glucopyranosiduronic acid was obtained as a very hygroscopic foam; $[\alpha]_D$ −60° (*c*, 0·92, in H_2O). (Found: C, 40·70; H, 5·96. $C_7H_{12}O_7$ requires: C, 40·39; H, 5·81%.) After longer standing the foam solidified to an amorphous mass, m.p. 77–80°, which showed to be the corresponding monohydrate. (Found: C, 37·44; H, 6·06. $C_7H_{14}O_8$ requires: C, 37·17; H, 6·24%.) Hardeger and Spitz[8] reported m.p. 78–82°, without giving further data.

Benzyl 1-O-*acetyl*-2,3,4-*tri*-O-*benzyl*-D-*glucopyranuronate* (VII)

Into a solution of VI (1·0 g; 1·76 mmoles) in 10 ml acetic acid and 1·6 ml acetic anhydride, a mixture of 0·10 ml conc H_2SO_4 (1·87 mmoles) and 1 ml acetic acid was dropped in under shaking at 0°. After standing for 24 hr at room temp the solution was poured on ice-water and extracted with ether. The combined extracts were washed with water, $NaHCO_3$ aq and water, dried over

[8] E. Hardeger and D. Spitz, *Helv. Chim. Acta* 33, 337 (1950).

Na_2SO_4 and evaporated *in vacuo* to a yellow oil; in this state the substance was used for the preparation of IX. The crude VII (780 mg) was dissolved in 1 ml pet. ether–benzene (1:1), and put on a carbon–celite (2:1 w/w) column (18·5 × 1·5 cm) prepared with the same solvent. By elution with pet. ether–benzene (15 ml fractions) VII emerged in fractions No 4–8 as a colourless thick oil; yield 596 mg, 60%. Fraction No 7, $[\alpha]_D$ +18° (*c*, 0·78) was submitted to analysis. (Found: C, 72·51; H, 6·34. $C_{36}H_{36}O_8$ requires: C, 72·47; H, 6·08%.)

Benzyl 2,3,4-*tri*-O-*benzyl*-1-*chloro*-1-*deoxy*-D-*glucopyranuronate* (VIIIa)

The crude VII, obtained from VI (1·5 g), was dissolved in 2 ml acetic acid and added to 20 ml of an acetic acid–dry HCl (3·8 g) solution. The mixture was set aside at 0° for 4 days, whereupon 15 ml $CHCl_3$ was added and the whole content poured on ice–water. The water layer was extracted several times with $CHCl_3$, the combined extracts washed with water, $NaHCO_3$ aq, and water and dried over Na_2SO_4. After the removal of the solvent the oily product was chromatographed on carbon–celite (2:1, w/w) column (45 × 2 cm) by successive elution with pet. ether, pet. ether–benzene (1:1), and benzene. With the last solvent a viscous colourless oil, showing a strong halogen reaction emerged; yield 570 mg, 38% (calc. on VI). The main fractions (280 mg) were pooled and subjected to a second chromatography on carbon–celite under identical conditions, whereupon analytically pure VIIIa was obtained; thick oil, $[\alpha]_D$ +43°. (Found: C, 70·88; H, 5·70; Cl, 6·60. $C_{34}H_{33}ClO_6$ requires: C, 71·26; H, 5·80; Cl, 6·19%.)

Benzyl 2,3,4-*tri*-O-*benzyl*-D-*glucopyranuronate* (IX)

(a) *Preparative method.* The crude 1-O-acetyl derivative VII prepared from VI (3·0 g; 5·27 mmoles) was dissolved in 20 ml $CHCl_3$ and 1·2 ml 28% HBr in glacial acetic acid was added. After standing 4 hr at room temp the mixture was poured on ice–water, extracted with $CHCl_3$, the extracts washed with water, $NaHCO_3$ aq, and water and dried over $CaCl_2$. On evaporation of the solvent the crude VIIIb was obtained as a yellow oil, $[\alpha]_D$ +59°. It was dissolved in 10 ml $CHCl_3$ and 1·5 g of freshly prepared Ag_2CO_3 and 1·2 ml of water were added to the solution. The mixture was shaken at room temp for 24 hr, whereupon the silver salts were removed by filtration, washed with $CHCl_3$, and the combined filtrates evaporated *in vacuo*. An oil was left which on standing in a desiccator gradually solidified. The semi solid mass was dissolved in 3 ml pet. ether–benzene (1:1) and chromatographed on an alumina (BDH, basic, 60 g) column (40 × 1·5 cm) by successive elution with pet. ether–benzene (1:1), benzene and $CHCl_3$. The last solvent eluted 1·235 g of IX as a colourless viscous oil which crystallized completely on standing; m.p. 118–123°, yield 42% (calc. on VI). One crystallization from EtOH gave analytically pure IX, m.p. 124–125°, $[\alpha]_D$ −12°; +11° (*c*, 0·26, in EtOH); +27° (*c*, 1·48, in C_6H_6). (Found: C, 73·61; H, 6·03. $C_{34}H_{34}O_7$ requires: C, 73·63; H, 6·18%.) IR: ν_{max} 3500 (OH), 1730 (C=O), and 1210 cm^{-1} (C—O—C). On addition of a trace of dry pyridine IX mutarotated: in $CHCl_3$ from −12° → 0° (5 hr), in benzene from +27° → +37° (30 min).

(b) *By direct conversion of* VI *to* VIIIb. Into a solution of VI (1·0 g) in 5 ml glacial acetic acid, 2 ml 28% HBr–acetic acid was dropped in under shaking, and the solution left to stand 2 hr at room temp. $CHCl_3$ was added, and the brown solution poured on ice–water, extracted with $CHCl_3$, the extracts washed with water, $NaHCO_3$ aq, and water and dried over $CaCl_2$. After the removal of the solvent a dark brown oil remained, which was treated in the same manner as the crude VIIIb. A 10% yield (calc. on VI) of impure IX was obtained.

(c) *Via the chloro-derivative* VIIIa. The crude VIIIa, $[\alpha]_D$ +37° (from 1·0 g VI), was treated in the same way as VIIIb, except that the reaction mixture was shaken with Ag_2CO_3 for 3 days, and then refluxed under stirring for 1 hr. After the removal of the solvent and chromatography over alumina, 230 mg, 23% (calc. on VI) of IX, m.p. 117–122° was obtained.

Acknowledgement—The authors are indebted to Mrs. O. Hadžija, M.Sc., and Miss N. Horvatić for microanalyses, and to Miss Š. Valenteković and Mr. Z. Lončarić for technical assistance.