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Original Scientific Paper

Glucuronic Esters. II.* The Preparation of Anomeric Methyl 2,3,4-tri-O-acetyl-1-O-acyl-D-glucopyranuronates. Deacylation of D-Glucuronic Acid Derivatives in Alkaline Medium

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The α- and β-anomers of methyl 2,3,4-tri-O-acetyl-1-O-acyl--D-glucopyranuronates were prepared by the silver salt method, by fractional crystallization, and/or by β → α anomerization. It was established that the acid-catalyzed anomerization is specific for the anomeric centre. By applying Hudson's rules of isorotation, the *A* and *B* values for some methyl 2,3,4-tri-O-acetyl-1-O-acyl--D-glucopyranuronates were calculated.

Alkaline hydrolysis of acetylated D-glucopyranuronates showed that the splitting of the ester bond at C-1 always occurs before the complete deacetylation takes place.

1-O-Acyl derivatives of D-glucuronic acid, commonly referred to as »ester glucuronides« are naturally occurring compounds, and their formation in the animal body is considered mainly to be a result of detoxication metabolism. When isolated from biological material, they are identified in the form of their acetylated esterified derivatives. »Ester glucuronides« generally show a negative optical rotation and, consequently, the β-D-configuration has been ascribed to this class of compounds.[1] However, little is known about the chemical and physical properties of these substances.

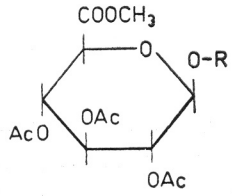
It has recently[2] been shown that, by the carbodi-imide method, aliphatic, aromatic, and indolic acids are easily esterified with the hemiacetal hydroxyl group of methyl 2,3,4-tri-O-acetyl-D-glucopyranuronate to give the corresponding 1-ester derivatives. In this way, with some exceptions due to steric hindrance, both anomers of methyl 2,3,4-tri-O-acetyl-1-O-acyl-D-glucopyranuronate were formed. They crystallized as anomeric mixtures, and only in the case of the 1-O-cinnamoyl derivatives, it was possible to separate the anomers instantly from the reaction product.

In the present work, we tried to prepare the pure anomers of methyl 2,3,4-tri-O-acetyl-1-O-acyl-D-glucopyranuronates. In addition, an atempt was made to get a better insight into the selective hydrolysis of these compounds.

As the first step, the synthesis of some methyl 2,3,4,-tri-O-acetyl-1-O-acyl--D-glucopyranuronates *via* the silver salt method[3] was performed. As expected, methyl 2,3,4-tri-O-acetyl-1-bromo-1-deoxy-α-D-glucopyranuronate[4] yielded, with the silver salt of propionic and phenylacetic acid, respectively, laevorotatory

* Part I.: N. Pravdić and D. Keglević, *J. Chem. Soc.* **1964**, in press.

products I and II. According to the generally accepted mechanism[5] of this reaction, the β-D-configuration could be ascribed to the products obtained.



I $R = CH_3CH_2CO$
II $R = C_6H_5CH_2CO$

I and II were also obtained in high yield when propionic and phenylacetic acid, respectively, and silver carbonate were used as reactants, instead of the corresponding silver carboxylate.

As the next step the resolution of anomeric mixtures of acetylated 1-O--acyl-glucopyranuronates[2], prepared by the carbodi-imide method, was tried. It is known[6] that, by fractional crystallization, methyl 1,2,3,4-tetra-O-acetyl--D-glucopyranuronate was resolved into both anomers. However, with other methyl 2,3,4-tri-O-acetyl-1-O-acyl-D-glucopyranuronates, we found this way to be tedious and unsatisfactory. Only the 1-O-(phenylacetyl) derivative was readily resolved into the pure β-D-anomer II; the purification of the corresponding α-D-anomer could not be brought to completion (see Table I).

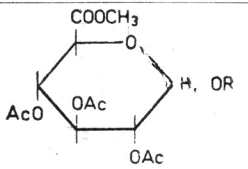
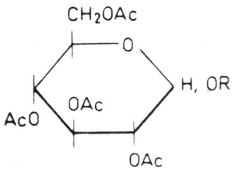
In the series of methyl 2,3,4-tri-O-acetyl-1-O-acyl-D-glucopyranuronates of α-configuration, only 1-O-acetyl[6] and 1-O-cinnamoyl[2] derivatives have been described so far. Therefore, the $\beta \rightarrow \alpha$ anomerization of the C-1 ester bond through the agency of a Lewis acid, used with success in the sugar acetate series[7], was attempted on the D-glucuronic acid derivatives. Indeed, methyl 1,2,3,4-tetra-O-acetyl-β-D-glucopyranuronate in absolute chloroform, with an equimolar amount of stannic chloride, rearranged to its α-D-anomer III. However, the anomerization proceeded slowly, and a considerably longer interconversion time was necessary than in the case of the $\beta \rightarrow \alpha$ transformation of penta-O-acetyl-D-glucopyranose. In the same way α-D-anomer of methyl 2,3,4-tri-O-acetyl-1-O-propionyl-D-glucopyranuronate (IV) was prepared in low yield; again, the conversion was much slower than with the corresponding D-glucose analogue[8]. The anomerization of the 1-O-benzoyl[2], 1-O--(phenylacetyl) (II), and 1-O-cinnamoyl[2] derivatives failed by this method.

The kinetics of anomerization of acetylated aldoses in acid anhydrides containing acid catalyst has been studied extensively by Bonner[9]. He has established that the reaction is specific for the anomeric centre only. We found this also to be true in the acetylated D-glucopyranuronate series. Methyl 1,2,3,4-tetra-O-acetyl-β-D-glucopyranuronate, in propionic anhydride with sulphuric acid as catalyst, gave methyl 2,3,4-tri-O-acetyl-1-O-propionyl-α-D--glucopyranuronate (IV) in high yield. This fact suggests this route as a general method to reach the α-D-anomers of acetylated 1-O-acyl-glucopyranuronates.

In Table I, the molecular rotations of methyl 2,3,4-tri-O-acetyl-1-O-acyl--D-glucopyranuronates and of the related D-glucopyranosides are listed. By applying Hudson's rules of isorotation,[10] the *A* and *B* values for both sets of anomers were calculated. There is a good agreement of the *A* values (effect

TABLE I

Rules of Isorotation Applied to D-*Glucuronic Acid and* D-*Glucose Derivatives*

							
R		$[M]_D$	$\frac{A}{(M_\alpha - M_\beta)/2}$	$\frac{B}{(M_\alpha + M_\beta)/2}$	$[M]_D$	$\frac{A}{(M_\alpha - M_\beta)/2}$	$\frac{B}{(M_\alpha + M_\beta)/2}$
acetyl	α-	+ 37,220	17,220	20,000	+ 39,620[11]	19,050	20,570
	β-	+ 2,780[4]			+ 1,520[11]		
propionyl	α-	+ 38,220	17,960	20,260	+ 41,210[8]	19,900	21,310
	β-	+ 2,300			+ 1,410[8]		
(phenylacetyl)	α-	+ 40,360	27,365	12,995	—		
	β-	— 14,370			— 13,280[12]		
cinnamoyl	α-	+ 60,740[2]	33,920	26,820	—		
	β-	— 7,100[2]			—		
phenyl	α-	+ 64,575[4]	39,585	24,990	+ 71,500[13]	40,520	30,980
	β-	— 14,595[4]			— 9,540[4]		

of the O-acyl group at C-1) in the D-glucuronic acid and D-glucoside series. The *B* values (rotatory contribution of the rest of the asymmetric carbon atoms) should be approximately the same for the derivatives possessing identical ring configuration. The *B* value for the 1-O-acetyl and 1-O-propionyl derivatives in the uronic acid series agree well. From the *B* value for the 1-O-(phenylacetyl) derivatives it is evident that, by fractional crystallization, the rotation value of the pure α-D-anomer had not been achieved. The values for the 1-O-cinnamoyl and 1-O-phenyl derivatives are considerably higher. It is interesting that the same effect can be seen for the 1-O-phenyl derivative in the D-glucose series. However, the significance of the *B* values in the uronic acid series must necessarily await syntheses of other 1-O-acyl-α-D-derivatives.

With the aim to remove the protecting groups from methyl 2,3,4-tri-O-acetyl-1-O-acyl-D-glucopyranuronates, the conditions of deacylation were studied. Several authors[14,15] have already pointed out the alkaline lability of the C-1 ester bond. We tried to remove the protecting groups of methyl 2,3,4-tri-O-acetyl-1-O-(*m*-nitrobenzoyl)-D-glucopyranuronate[2] (V) and of methyl 2,3,4-tri-O-acetyl-1-O-benzoyl-D-glucopyranuronate[2] (VI) in anhydrous alkaline medium under very mild conditions. When V was subjected to hydrolysis with methanol saturated with ammonia at 0°, *m*-nitrobenzamide was isolated in quantitative yield.

The hydrolysis of V or VI with various molar ratios of sodium methoxide was then tried; the splitting of the ester bond at C-1 always occurred before the complete deacetylation took place. However, with catalytic amounts of sodium methoxide, VI remained unchanged.

It follows, therefore, that in order to obtain free 1-O-acyl-glucopyranuronates, the sugar moiety should be blocked by groups easily removable in neutral medium. The synthesis of a fully benzylated D-glucuronic acid having the hydroxyl group at C-1 free will be the subject of another paper.

EXPERIMENTAL

Melting points are uncorrected. All specific rotations were measured at 20—23° in chloroform (*c*, 1±2%) if not stated otherwise. Evaporations were carried out *in vacuo* at 40—45°.

Methyl 2,3,4-tri-O-acetyl-1-O-propionyl-β-D-glucopyranuronate (I)

A. Into a solution of methyl 2,3,4-tri-O-acetyl-1-bromo-1-deoxy-α-D-glucopyranuronate[4] (1.6 g., 4 mmoles) in absolute chloroform (25 ml.) powdered silver propionate[16] (0.9 g., 5 mmoles) was added and the mixture refluxed for 4 hr. After cooling the precipitate was filtered off, the filtrate evaporated to dryness, and the residual oil crystallized from *iso*-propanol. The crude product (1.1 g., 70%, m.p. 130—145°) was twice recrystallized from the same solvent; m.p. 146—147°, $[\alpha]_D + 4.5°$.

Anal. $C_{16}H_{22}O_{11}$ (390.34) calc'd.: C 49.23; H 5.68%
found: C 49.37; H 5.59%

B. Methyl 2,3,4-tri-O-acetyl-1-bromo-1-deoxy-α-D-glucopyranuronate (6.0 g., 15 mmoles) was dissolved in dry benzene (30 ml.) and, to the solution, propionic acid (1.1 g., 15 mmoles) was added, followed by freshly prepared silver carbonate (5.0 g., 18 mmoles). The mixture was shaken for 15 hr., and the precipitate filtered off and washed with warm benzene. The filtrate and washings were combined and evaporated to dryness; the residue crystallized from *iso*-propanol giving 3.5 g. (60%) of a product with m.p. 148—149°, $[\alpha]_D + 5.9°$.

found: C 49.49; H 5.78%

Methyl 2,3,4-tri-O-acetyl-1-O-(phenylacetyl)-β-D-glucopyranuronate (II)

A. Into a solution of methyl 2,3,4-tri-O-acetyl-1-bromo-1-deoxy-α-D-glucopyranuronate (1.2 g., 3 mmoles) in absolute chloroform (30 ml.), powdered silver phenylacetate[16] (2.0 g., 8.2 mmoles) was added, and the reaction was carried out as described for I. Yield: 1.0 g. (74%). Crystallization from methanol gave the analytical sample, m.p. 137—138°, $[\alpha]_D$ — 31.8°.

Anal $C_{21}H_{24}O_{11}$ (452.42) calc'd.: C 55.75; H 5.35%
found: C 55.67; H 5.25%

B. Into a solution of methyl 2,3,4-tri-O-acetyl-1-bromo-1-deoxy-α-D-glucopyranuronate (1.0 g., 2.5 mmoles) in dry benzene (15 ml.), was added phenylacetic acid 0.34 g., 2.5 mmoles) followed by freshly prepared silver carbonate (1.5 g., 5.5 mmoles), the mixture was shaken for 15 hr. and worked up as described for I. Crystallization from methanol gave 0.55 g. (48%) of a product with $[\alpha]_D$ — 29.1°, m.p. 138—139°, undepressed on admixture with the sample obtained above.

Resolution of Methyl 2,3,4-tri-O-acetyl-1-O-(phenylacetyl)-D-glucopyranuronate into its Anomers by Fractional Crystallization

The title compound[2] (0.65 g., $[\alpha]_D$ + 24.5°, m.p. 99—101°) was twice recrystallized from absolute ethanol (40 ml. portions), yielding 0.2 g. of pure β-D-anomer (II) with m.p. 138—139°, $[\alpha]_D$ — 31.3°. The mother liquor, after evaporation, gave 0.27 g. of colourless crystals, m.p. 98—100°, $[\alpha]_D$ + 62.5°, which were submitted to further fractionations in absolute ethanol. After six recrystallizations, 25 mg. of a product with m.p. 113—114° and $[\alpha]_D$ + 89.3° was obtained.

Methyl 1,2,3,4-tetra-O-acetyl-α-D-glucopyranuronate (III)

To the β-D-anomer of III[4] (1.88 g., 5 mmoles, $[\alpha]_D$ + 10.9°) in absolute chloroform (15 ml.), anhydrous stannic chloride (0.60 ml., 5 mmoles) in absolute chloroform (15 ml.) was added, and the solution was refluxed for 24 hr. After cooling the dark solution was washed with ice-water, sodium bicarbonate solution, and water, and dried over anhydrous sodium sulphate. Evaporation of the solvent left a yellow syrup which was dissolved in hot ethanol (15 ml.), treated with charcoal, and filtered. On cooling, 431 mg. (23%) of III (m.p. 104—106°, $[\alpha]_D$ +90.7°) separated; a mixed m.p. with an authentic sample[6] gave no depression. From the filtrate, after standing overnight at 0°, an additional crop of III (342 mg., 18%) with m.p. 109—113°, $[\alpha]_D$ + 99.0° was obtained. Total yield of III : 41%.

Methyl 2,3,4-tri-O-acetyl-1-O-propionyl-α-D-glucopyranuronate (IV)

A. To I (2.34 g., 6 mmoles, $[\alpha]_D$ + 5.9°) in absolute chloroform (20 ml.), anhydrous stannic chloride (0.71 ml., 6 mmoles) in absolute chloroform (20 ml.) was added, and the solution was refluxed for 24 hr. The solution was worked up as described for III; evaporation of the solvent left a brown oil which was dissolved in absolute benzene and triturated cautiously with petroleum ether until a dark resin deposited. The straw-coloured supernatant was transfered to a second flask and kept at 0° for 5 days with occasional addition of petroleum ether; 503 mg. (22%) of material (m.p. 89—99°, $[\alpha]_D$ + 60.0°), identified as IV, crystallized. The mother liquor was evaporated and the remaining yellow syrup was submitted to a second benzene-petroleum ether crystallization; 120 mg. (6%) of α-D-anomer IV (m.p. 103—113°, $[\alpha]_D$ + 95.5°) was obtained.

Anal. $C_{16}H_{22}O_{11}$ (390.34) calc'd : C 49.23; H 5.68%
found : C 49.47; H 5.73%

B. Methyl 1,2,3,4-tetra-O-acetyl- β-D-glucopyranuronate[4] (2.5 g., 6.6 mmoles, $[\alpha]_D$ + 10.9°) was placed in a 50 ml. measuring flask, 40 ml. of propionic anhydride was added, and the mixture was shaken until the bulk of the material had dissolved. Concentrated sulphuric acid (1.5 ml.) was added, and the clear solution was filled up with propionic anhydride to the mark. The rotation of the solution increased from + 3.5 at 25 minutes to a constant + 4.8 after 3 hr. The solution was poured into ice-water, the mixture stirred for 5 hr. at 0°, left overnight in the ice-box, and extracted several times with ether. The ether extracts were washed repeatedly with

water, sodium bicarbonate solution, and water, and dried with sodium sulphate. The solvent was removed and the remaining colourless powder was crystallized from *iso*-propanol: 1.43 g., (55%) of IV, m. p. 106—117°, $[\alpha]_D$ + 88.0°, was obtained. On addition of petroleum-ether to the mother liquor, an additional crop of 0.24 g., (m.p. 101—111°, $[\alpha]_D$ + 85.5°) was obtained. Total yield: 64%. One recrystallization from *iso*-propanol gave the pure α-D-anomer, m.p. 118—119°, $[\alpha]_D$ + 98.0°.

found: C 49.32; H 5.57%

Hydrolysis of Protecting Groups from Methyl 2,3,4-tri-O-acetyl-1-O-(m-nitrobenzoyl)-D-glucopyranuronate (V) *and Methyl 2,3,4,-tri-O-acetyl-1-O-benzoyl--D-glucopyranuronate* (VI)

All experiments were performed on a one-mmole scale. Methanol dried after Lund and Bjerrum was used throughout.

Methanol Saturated with Ammonia

V was dissolved in methanol saturated with ammonia (4 ml.) and the solution left to stand at 0° for 3 hr. After evaporation to dryness, the residue was crystallized from water. The obtained product, (0.15 g.), m. p. 130—131°, was identified as *m*-nitrobenzamide (yield 90%).

Anal. $C_7H_6N_2O_3$ (166.17) calc'd.: N 16.86%
found : N 17.03%

Sodium Methoxide

a) V was dissolved in 25 ml. of methanol containing 1.7 mmoles of sodium methoxide and left to stand for 2 hours at room temperature. The solution was passed through a column of Dowex 50-X8 (H^+), the eluates were evaporated to dryness and to the residue, ethyl acetate (1 ml.) was added. The undissolved material (60 mg.) was identified as the starting compound (recovery 12%). From the solution, on standing at 0°, 248 mg. (68%) of methyl *m*-nitrobenzoate, m.p. 70—72°, crystallized. Mixed m.p. with an authentic sample gave no depression.

b) VI was dissolved in 15 ml. of methanol containing 0.36 mmole of sodium methoxide, and kept for 24 hr. at room temperature. The solution was evaporated to dryness; after addition of water, 118 mg. of undissolved material, identified as the starting compound (27%), was filtered off. The filtrate was passed through a column of Dowex 50-X8 (H^+). Evaporation of the eluate gave a residue identified as D-glucuronolactone (23 mg., 13%), m.p. 158—160°, mixed m.p. without depression.

c) V was dissolved in 20 ml. of methanol containing 0.2 mmole of sodium methoxide and left to stand for 72 hr. at room temperature. The solution was worked up as described in *b*): 163 mg. (90%) of methyl *m*-nitrobenzoate, m.p. 72—73°, and mixed m.p. 72°, was isolated. After evaporation of the eluate, a yellow oil (0.25 g.) remained; by analysis it was established that it contained 20% of acetyl and no nitrogen.

d) VI was dissolved in 10 ml. of methanol containing 0.03 mmole of sodium methoxide and left to stand for 18 hr. at 0°. Evaporation of the solution left a residue which was insoluble in water; 310 mg. (71%) of the starting material was recovered.

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IZVOD

Ester glukuronidi. II. Priprava anomera metil 2,3,4-tri-O-acetil-1-O-acil-D-glukopiranuronata. Deaciliranje derivata glukuronske kiseline u alkalnom mediju

N. Pravdić i D. Keglević

α- i β-Anomeri metil 2,3,4-tri-O-acetil-1-O-acil-D-glukopiranuronata pripravljeni su, pomoću metode srebrne soli, frakcionom kristalizacijom ili β → α anomerizacijom. Utvrđeno je da je anomerizacija u prisutnosti katalitičkih količina kiseline specifična za anomerni centar. Primjenjujući Hudsonova pravila izorotacije, izračunate su *A* i *B* vrijednosti za neke metil 2,3,4-tri-O-acetil-1-O-acil-D-glukopiranuronate.

Alkalna hidroliza acetiliranih 1-O-acil-D-glukopiranuronata dovodi uvijek do pucanja esterskog veza na C-1 atomu prije nego nastupi potpuna deacetilacija.

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ZAGREB

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