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Note

Synthesis of 1-*O*-(2-Ethylbutyryl)-, 1-*O*-(3,4-Dimethoxyphenyl)-, and 1-*O*-(3,4-Dimethoxybenzyl)-β-D-glucopyranuronic Acids

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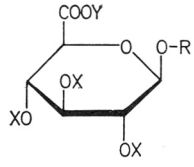
In our studies[1] on the enzymic hydrolysis of glucuronic esters and glucuronic ethers, the influence of the type of linkage as well as of the aglycone substituents on the rate of hydrolysis of ester- and ether-type conjugates by mammalian and bacterial β-glucuronidase was investigated. For purposes of comparison, the title compounds were required as substrates, and herein we report on their synthesis.

1-*O*-(2-Ethylbutyryl)-β-D-glucopyranuronic acid (II), a member of the glucuronic ester class possessing the aliphatic aglycone structure, was isolated by Kamil, Smith, and Williams[2] from rabbits urine as the main detoxication product of diethylethanol. The authors obtained the compound as the potassium salt and characterized it in the form of the tri-*O*-acetyl methyl ester derivative. To reach II synthetically, we have chosen the Koenigs-Knorr condensation of benzyl 2,3,4-tri-*O*-benzyl-1-bromo-1-deoxy-α-D-glucopyranuronate[3] with the silver salt of 2-ethylbutyric acid; the reaction proceeded with a net Walden inversion, and the fully benzylated β-1-*O*-ethylbutyryl derivative I was obtained as a crystalline compound in 44% yield. Debenzylation of I was effected by hydrogenation using palladium-on-charcoal as the catalyst; purification of the product over silicagel followed by crystallisation afforded the unprotected glucuronic ester II as an amorphous solid.



I-II R = $(MeCH_2)_2CHCO-$

III - IV R = MeO- (OMe)

V - VI. R = MeO- (OMe) $-CH_2-$

I X = Y = $PhCH_2$

II X = Y = H

III X = Ac Y = Me

IV X = Y = H

V X = Ac Y = Me

VI X = Y = H

Kinetic studies with 1-*O*-(3,4-dimethoxybenzoyl)-β-D-glucopyranuronic acid[4] necessitated comparative experiments with the structurally related glucuronic

ethers, and therefore the synthesis of 1-*O*-(3,4-dimethoxyphenyl)-β-D-glucopyranuronic acid (IV) and of its homologue 1-*O*-(3,4-dimethoxybenzyl)-β-D--glucopyranuronic acid (VI) was undertaken. The glucuronic ether IV was prepared *via* the tri-*O*-acetyl methyl ester derivative III; the latter compound was obtained in very poor yields (10—15%) by two ways: *a)* by condensation of methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucopyranuronate[5] with 3,4--dimethoxyphenol in the presence of silver carbonate, and *b)* by fusing 3,4--dimethoxyphenol with methyl tetra-*O*-acetyl-β-D-glucopyranuronate[5] in the presence of *p*-toluenesulphonic acid. The failure of both procedures to give better yields of III could be ascribed mainly to the high lability of the phenolic component which decomposes spontaneously already at room temperature. Deprotection of III was achieved by alkaline hydrolysis to give the desired glucuronide IV in 64% yield. In contrast to 3,4-dimethoxyphenol, its conjugates III and IV proved to be stable compounds.

For the preparation of the homologous glucuronic ether VI, the Koenigs--Knorr condensation of methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucopyranuronate with 3,4-dimethoxybenzyl alcohol was used; after purification of the reaction product, the tri-*O*-acetyl methyl ester derivative V was obtained in a 43% yield. Alkaline hydrolysis of the acetyl and methyl ester bonds was performed in the same manner as with III to give the crystalline unprotected glucuronide VI in the form of semi-hydrate.

EXPERIMENTAL*

Melting points are uncorrected. Evaporations were performed in a rotatory evaporator *in vacuo* the bath temperature being kept below 45°. Column chromatography was performed on silicagel (Merck, 0.05—0.2 mm.). Solvent systems were: *A,* petroleum ether-benzene-methanol (20 : 15 : 2); *B,* ethyl acetate-*iso*propanol-water (3 : 3 : 2); *C,* petroleum ether-benzene-methanol (15 : 60 : 4); *D,* benzene-ethyl acetate--petroleum ether (8 : 4 : 1). Thin-layer chromatography (TLC) was carried out on chromatoplates of Silicagel G (Merck); spots were located with 10% sulphuric acid and heating.

Silver salt of 2-ethylbutyric acid was prepared according to the general procedure[6] given for substituted silver benzoates. 3,4-Dimethoxyphenol was prepared from 3,4-dimethoxyaniline (5.5 g.) following essentially the procedure of Birch *et al.*[7]. The crude product was put immediately on a column of silicagel and eluted with chloroform-ether (5 : 1) to give 2.2 g. (44%) of chromatographically homogeneous material; a sample distilled at 115—120°/0.1 mm. Hg had m. p. 79—81° (lit.[8] : 81°). The compound was stored as a benzene solution under nitrogen at 0°; under these conditions no serious decomposition took place for up to two weeks.

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

Benzyl 2,3,4-tri-*O*-benzyl-1-bromo-1-deoxy-α-D-glucopyranuronate was prepared[3] from benzyl 2,3,4-tri-*O*-benzyl-1-*O*-methyl-β-D-glucopyranuronate (4.0 g., 7 mmoles) *via* the 1-*O*-acetyl derivative and used immediately in the reaction; it was dissolved in anhydrous benzene (20 ml.), and to the solution finely powdered Ag-salt of 2--ethylbutyric acid (1.56 g., 7 mmoles) and Drierite (0.75 g.) were added. The mixture was shaken at room temperature in the dark for 3—4 days (monitoring by TLC in solvent *A*). The precipitate was centrifuged off, the supernatant was evaporated *in vacuo,* and the residue was crystallised from ethanol: crystals of I (1.1 g., 43% calcd. on the fully benzylated 1-*O*-methyl derivative), m. p. 91—92° separated. A second crystallisation gave the analytically pure sample, m. p. 94—95.5°, $[\alpha]_D$ — 1.4° (c 1.5, chloroform) and $[\alpha]_D$ + 16.2° (c 1.2, benzene). NMR spectrum ($CDCl_3$): τ 4.22 (doublet, $J_{1,2}$ 7.5 Hz, H-1).

Anal. $C_{40}H_{44}O_8$ (652.75) calc'd.: C 73.60; H 6.79%
found: C 73.41; H 6.92%

* The synthesis of compound I was carried out by V. Šunjić, B. Sc.

1-O-(2-Ethylbutyryl)-β-D-glucopyranuronic Acid (II)

To a solution of compound I (650 mg., 1 mmole) in methoxyethanol (20 ml.), palladium-on-charcoal (Fluka, 10%, 350 mg.) was added, and the mixture was shaken in the presence of hydrogen at room temperature until the consumption of hydrogen was complete. The catalyst was removed by centrifugation, washed with methoxyethanol, and the combined supernatants were evaporated. The residue was dissolved in water (5 ml.), the solution was shaken repeatedly with small portions of ether to remove the unreacted material, and the aqueous solution was evaporated to dryness. The remaining solid (250 mg.) was dissolved in a minimum amount of water and passed through a silicagel column (50 × 1 cm.) with solvent *B*; chromatographically homogenous fractions (monitoring by TLC) were pooled and evaporated to dryness to give 160 mg. (55%) of the glucuronic ester II in the form of a solid foam. Crystallisation from *iso*propanol afforded II as an amorphous solid, m. p. 164° (softening at 150°), $[\alpha]_D$ — 28.0° (c 0.5, water). Kamil *et al.*[2] give for the potassium salt of II $[\alpha]_D$ — 27.0°.

Anal. $C_{12}H_{20}O_8$ (292.28) calc'd.: C 49.31; H 6.90%
found: C 49.03; H 7.04%

Methyl 2,3,4-tri-O-acetyl-1-O-(3,4-dimethoxyphenyl)-β-D-glucopyranuronate (III)

(*a*) To a solution of methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucopyranuronate[5] (400 mg., 1 mmole) and 3,4-dimethoxyphenol (616 mg., 4 mmoles) in anhydrous benzene (20 ml.), freshly prepared silver carbonate (274 mg., 1 mmole) and Drierite (200 mg.) were added, and the mixture was shaken in the dark at room temperature for 24 h. After removal of the precipitate, the filtrate was evaporated to dryness, and the residue was passed through a silicagel column with solvent *C*. Chromatographically homogeneous fractions were pooled and evaporated to dryness to give 80 mg. (10.6%) of crystalline III; after recrystallisation from ethanol the compound had m. p. 154—156° and $[\alpha]_D$ — 32.0° (c 1.0, chloroform).

Anal. $C_{21}H_{26}O_{12}$ (470.42) calc'd.: C 53.61; H 5.57%
found: C 53.81; H 5.51%

(*b*) A mixture of methyl tetra-*O*-acetyl-β-D-glucopyranuronate[5] (700 mg., 1.9 mmoles), 3,4-dimethoxyphenol (500 mg., 3.24 mmoles and *p*-toluenesulphonic acid (17 mg.) was kept at 100—120°/18 mm. Hg for 30 min. whereupon an additional amount of *p*-toluenesulphonic acid (5 mg.) was added to the melt, and the fusion was continued for 30 min. The cooled melt was extracted with benzene, the extract was washed with 2 *N* KOH and water, dried (Na_2SO_4) and evaporated. The residue was chromatographed over silicagel with solvent *C* to give 150 mg. (15%) of chromatographically homogenous III; after recrystallisation from ethanol the substance was analytically pure and identical with III described above.

1-O-(3,4-Dimethoxyphenyl)-β-D-glucopyranuronic Acid (IV)

To a solution of III (200 mg., 0.425 mmole) in anhydrous methanol (20 ml.) 0.1 *N* sodium methoxide in methanol (5 ml.) was added, and the progress of the reaction was monitored by TLC in solvent *B*; after 1 h. the spot associated with the starting compound disappeared completely. Water (10 ml.) was added, and the solution was kept at *p*H 8 by successive addition of 0.1 *N* sodium methylate until complete deacetylation was achieved (~ 5 h., monitoring by TLC in solvent *B*). The solution was passed through a column of Amberlite IR 120 H^+, the resin was washed with water until the eluent was neutral, the effluent was evaporated to dryness, and the residue was crystallised from *iso*propanol-petroleum ether to give 90 mg. (64%) of IV. After a second crystallisation the substance had m. p. 148—150° (softening at 110°) and $[\alpha]_D$ — 56.0° (c 0.5, water).

Anal. $C_{14}H_{18}O_9$ (330.28) calc'd.: C 50.91; H 5.49%
found: C 51.09; H 5.63%

Methyl 2,3,4-tri-O-acetyl-1-O-(3,4-dimethoxybenzyl)-β-D-glucopyranuronate (V)

A solution of methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucopyranuronate (4.0 g., 10 mmoles) and 3,4-dimethoxybenzyl alcohol (freshly redistilled, 8.0 g., 4.7

mmoles) was shaken with silver carbonate as described for III; the progress of the reaction was monitored by TLC in solvent *D*. After working up, the crude oily product was dissolved in ethanol; on standing at 0° crystals of V (2.1 g., 43%) separated. Two further crystallisations afforded the analytically pure sample: m. p. 122—123°, $[\alpha]_D$ — 58.0° (c. 1.0, chloroform).

Anal. $C_{22}H_{28}O_{12}$ (484.44) calc'd.: C 54.54; H 5.83%
found: C 54.41; H 6.09%

1-O-(3,4-Dimethoxybenzyl)-β-D-glucopyranuronic Acid (VI)

Hydrolysis of compound V (500 mg., 1.03 mmole) with 0.1 *N* sodium methoxide in methanol was performed as described for IV; after deionization and evaporation of the effluent, the residue was dried over phosphorus pentoxide. The resulting solid foam was dissolved in tetrahydrofurane at room temperature, and to the solution petroleum ether was added successively at 0°; white crystals of VI (320 mg., 90%) separated. A second crystallisation from ethanol afforded the analytical sample, m. p. 115° (softening at 110°) $[\alpha]_D$ — 60.0° (c. 1.0, water).

Anal. $C_{15}H_{20}O_9 \times 0.5\ H_2O$ (353.32) calc'd.: C 50.99; H 5.99%
found: C 50.86; H 6.21%

Thermograv. analysis: calc'd.: H_2O 2.55%
found: H_2O 2.5 (± 0.3)%.

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IZVOD

Sinteza 1-*O*-(2-etilbutiril)-, 1-*O*-(3,4-dimetoksifenil)-, i 1-*O*-(3,4-dimetoksibenzil)-β-D-glukopiranuronskih kiselina

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Kondenzacijom benzil 2,3,4-tri-*O*-benzil-1-bromo-1-deoksi-α-D-glukopiranuronata sa srebrnom soli 2-etilmaslačne kiseline dobiven je benzil 2,3,4-tri-*O*-benzil-1-*O*-(2-etilbutiril)-β-D-glukopiranuronat (I) koji je katalitičkim hidriranjem preveden u slobodni glukuronski ester II. Metil 2,3,4-tri-*O*-acetil-1-*O*-(3,4-dimetoksifenil)-β-D-glukopiranuronat (III) i metil 2,3,4-tri-*O*-acetil-1-*O*-(3,4-dimetoksibenzil)-β-D-glukopiranuronat (V) priređeni su iz metil 2,3,4-tri-*O*-acetil-1-bromo-1-deoksi-α-D-glukopiranuronata i 3,4-dimetoksifenola, odnosno 3,4-dimetoksibenzilnog alkohola; protektivne grupe na šećernoj komponenti uklonjene su alkalnom hidrolizom, i slobodni glukuronski eteri IV i VI izolirani su i karakterizirani.

Spojevi II, IV i VI bili su potrebni kao supstrati u ispitivanjima kinetike hidrolize glukuronskih estera i glukuronskih etera s β-glukuronidazom.

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