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PREPARATION OF ALLYL 2,3,4-TRI-*O*-ALLYL-D-GLUCOPYRANURONATE AND OF 1-*O*-ACYL-D-GLUCOPYRANURONIC ACIDS: ATTEMPTED SYNTHESIS OF 1-*O*-BILIRUBIN-D-GLUCURONIC ACIDS

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ABSTRACT

Allyl 2,3,4-tri-*O*-allyl-D-glucopyranuronate (7) has been prepared (overall yield, 37%) from methyl 1,2,3,4-tetra-*O*-acetyl-α- or -β-D-glucopyranuronate *via* a six-step reaction procedure. 1-*O*-Acetyl-, 1-*O*-methacryloyl-, and 1-*O*-phenylacetyl-αβ-D-glucopyranuronic acid are produced by esterification of 7 and removal of the allyl groups with palladium-on-carbon in an acidic medium. The reaction of 7 with the di-imidazole derivative of bilirubin yields the allyl-protected mono- and di-D-glucopyranosyluronic acid derivatives of bilirubin. Neither the bilirubin nor the corresponding biliverdin derivatives resisted the conditions required for de-allylation.

INTRODUCTION

Bilirubin, a tetrapyrrolic pigment produced by biological breakdown of haem, is excreted into bile as mono- and di-D-glucosyluronic acid derivatives having one or two propionic acid side-chains esterified with β-D-glucopyranuronic acid[1–3].

Esterification of the di-imidazole derivative of bilirubin with D-glucuronic acid yielded a mixture of 2-, 3-, and 4-*O*-acyl-D-glucosyluronic acid derivatives[4]. Clearly, HO-2,3,4 and the carboxyl group of D-glucuronic acid need to be protected if 1-esters are to be obtained. However, such protecting groups must be removable under very mild conditions, since migration of the bilirubin 1-*O*-acyl group occurs[1] at pH 7–9. Moreover, the asymmetric bilirubin-IXα tetrapyrrole readily undergoes[5] dipyrrole exchange, yielding a 2:1:1 mixture of bilirubin-IXα and the symmetric bilirubin-IIIα and -XIIIα. This reaction is catalysed by mineral acid for bilirubin and even occurs in neutral medium for the 1-*O*-bilirubin-D-glucuronic acids. Chain reactions are probably involved, with the formation of dipyrrylmethyl cations or radicals as the initiating species.

Although several 1-*O*-acyl-D-glucopyranuronic acids were prepared[6] from benzyl 2,3,4-tri-*O*-benzyl-D-glucopyranuronate[7,8], this method is not suitable for preparation of 1-*O*-bilirubin-D-glucuronic acids, since hydrogenolysis of benzyl groups also results in reduction of vinyl substituents. A possible solution of these

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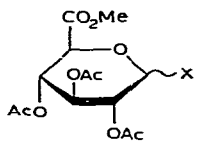
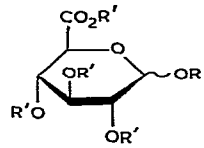
problems was indicated by the preparation of benzyl 2,3,4-tri-*O*-(chloroacetyl)-β-D-glucopyranosiduronic acid[9].

The preparation is now reported of allyl 2,3,4-tri-*O*-allyl-D-glucopyranuronate (**7**), a compound which appears to be useful for the synthesis of 1-*O*-acyl-D-glucopyranuronic acids.

RESULTS AND DISCUSSION

Methyl 1,2,3,4-tetra-*O*-acetyl-α- or -β-D-glucopyranuronate (**1**), which is readily prepared from D-glucurono-6,3-lactone, was converted[10] into the α-glycosyl bromide **2**. Hydrolysis of **2** in the presence of silver carbonate gave the 1-hydroxy derivative **3** which, with ethyl vinyl ether, gave the acetal **4**. Saponification of the ester groups in **4**, followed by allylation of the hydroxyl and carboxyl groups, and liberation of HO-1 afforded **7**, in an overall yield of 37%. Benzylation of **5** should give benzyl 2,3,4-tri-*O*-benzyl-D-glucopyranuronate, a compound that was recently prepared[8] by an eight-step procedure starting from methyl β-D-glucopyranoside (overall yield, ~4.3%).





1 X = OAc
2 X = Br
3 X = OH
4 X = OCH(Me)OEt

5 R = CH(Me)OEt, R' = H
6 R = CH(Me)OEt, R' = CH_2—CH=CH_2
7 R = H, R' = CH_2—CH=CH_2

Esterification of **7** variously with acetic anhydride, methacryloyl chloride, and 1-phenylacetylimidazole gave αβ-mixtures which were characterised by g.l.c.–m.s.

Allyl ethers are cleaved[11] by Rh(I)-catalysed isomerisation to 1-propenyl ethers and subsequent acid hydrolysis at pH 2. Apparently, in the first step, an equilibrium mixture is formed containing ~95% of the 1-propenyl ether[11,12]. Cleavage of the four allyl groups in 1-*O*-acyl esters of **7** was effected in the presence of dilute hydrogen chloride by using a palladium-on-carbon catalyst[13]. The 1-*O*-acyl-D-glucopyranuronic acids were shown to be the major products by t.l.c. and m.s.

Esterification of **7** with the di-imidazole derivative of bilirubin[4] yielded the mono- and mainly the di-D-glucosyluronic acid derivatives. Subsequent oxidation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone gave the corresponding biliverdin derivatives. The allyl-protected 1-*O*-bilirubin-D-glucuronic acids were characterised further as the dipyrrolic azopigments, prepared by using a diazonium reagent derived from ethyl anthranilate[14]. This reagent cleaves the asymmetrical bilirubin tetrapyrrole into dipyrrolic azopigments having either an *endo*- or an *exo*-vinyl substituent[15–18]. Four azopigment isomers, corresponding to the anomeric pairs of the *exo*- and

endo-vinyl compounds, were isolated by t.l.c. The *endo*-vinyl isomers exhibited intense molecular ions (M^+ at *m/z* 798, 20% relative abundance), whereas the less-stable, *exo*-vinyl isomers gave low-intensity molecular ions (M^+ at *m/z* 798, 0.2%). In the n.m.r. spectra of the *endo*-vinyl isomers, the signals for the pyrrolinone and pyrrole methyl groups coincided (δ 2.12), whereas they occurred at δ 2.19 and 2.11, respectively, for the corresponding methyl groups of the *exo*-vinyl isomers[16–18]. The α and β anomers could not be assigned, because of overlap of the signals for the anomeric and various vinylic protons.

Neither the bilirubin nor the biliverdin allyl-protected D-glucosyluronic acids resisted the conditions required for removal of the allyl groups. The solutions were decolorised gradually before all protecting groups had been removed. Degradation of the chromophoric systems and/or adsorption of the pigments to carbon could have occurred.

EXPERIMENTAL

General. — Column chromatography was performed on Merck silica gel (0.06–0.2 mm). T.l.c. was conducted on pre-coated silica gel plates (Merck aluminum sheets), and detection was effected by spraying with a 3:1 mixture of 0.2% naphthalene-1,3-diol in ethanol and 20% sulphuric acid, followed by heating at 120°. G.l.c. was performed with 3% of OV-101 (glass column, 2 m × 6.3 mm) on Gaschrom Q, with helium as the carrier gas (50 ml/min) and a 1:1 outlet split to the flame-ionisation detector and mass spectrometer. Mass spectra were recorded by using either the direct insertion probe of an AEI MS902 S mass spectrometer or a g.l.c.–m.s. combination (glass jet separator).

N.m.r. spectra ($CDCl_3$, internal Me_4Si) were recorded with a Jeol 100-MHz or a Varian XL 100 spectrometer.

*Allyl 2,3,4-tri-*O*-allyl-*D*-glucopyranuronate* (**7**). — Compound **1** (5 g) was converted[10] into the glycosyl bromide **2**, to a solution of which in acetone (50 ml) were added water (0.35 ml) and silver carbonate (6 g). The mixture was stirred for 24 h at 22°, and t.l.c. (9:1, chloroform–acetonitrile) then showed quantitative conversion of **2** (R_F ~0.7) into **3** (R_F ~0.3). The mixture was filtered, and concentrated *in vacuo* to yield crude, oily **3**.

To a solution of **3** in tetrahydrofuran (30 ml) at 0° were added ethyl vinyl ether (10 ml) and trifluoroacetic acid (1 ml). The formation of **4** was incomplete after 1 day at 0° and nearly complete after 2 more days at 22°, as shown by g.l.c. and t.l.c. of the neutralised reaction mixture. Dichloromethane (50 ml), triethylamine (5 ml), and ice-cold water (50 ml) were added, and the organic layer was washed four times with water, dried over molecular sieves, and concentrated, to yield crude **4**. T.l.c. (15:1 chloroform–acetonitrile) showed two closely moving spots with R_F ~0.7 (anomers or diastereoisomers of **4**). G.l.c.–m.s. (180°, isothermal) of an acetylated (2:1 pyridine–acetic anhydride) sample of **4** showed 98–99% conversion of **3** into **4** (1–2% of unreacted **3** was detected as **1**). The retention time of **4** relative to that of **1** was 1.55.

The mass spectrum of **4** (mol. wt. 406) showed peaks at *m/z* 347, 304, and 317, corresponding to losses of CO_2CH_3, HOAc + CH_2CO, and OCHMeOEt, respectively.

Hydrolysis of **4** with methanol–triethylamine–water (2:1:1, 60 ml), first at 0° for 3 h and then at 22° for 2 days, gave a major product with R_F ~0.3 (t.l.c., 9:2 1-propanol–water). Concentration of the hydrolysate and drying of the residue *in vacuo* yielded the crude triethylammonium salt of **5**, $[\alpha]_D$ +38° (*c* 1, water).

A solution of the salt in dry *N*,*N*-dimethylformamide (30 ml) was stirred at 22° under nitrogen with sodium hydride (3 g) added in portions. Allyl bromide (20 g) was added dropwise during ~30 min and the reaction was monitored by g.l.c.–m.s. (150° →, 4°/min). Samples of the reaction mixture (0.2 ml) were dissolved in dichloromethane (1 ml), internal standard (0.2 ml of a 1% solution of **1**) was added, and the solution was washed with water. An optimum yield of the allyl ether **6** (eluted at 188°) was observed after a reaction time of 2.5–3 h. Minor side-products were eluted at 180° (probably the methyl ester of **6**) and 183° (fully allylated glucuronic acid). Dichloromethane (50 ml) was then added to the reaction mixture, and the organic layer was washed four times with water and concentrated, to give crude **6**, $[\alpha]_D$ +50° (*c* 1, chloroform).

A solution of crude **6** in acetic acid (30 ml) and 0.1M hydrochloric acid (10 ml) was kept at 22° for 2 h and then at 90° for 30 min. T.l.c. (9:1 chloroform–acetonitrile) showed quantitative conversion of **6** (R_F ~0.6) into **7** (R_F ~0.3). The reaction mixture was cooled, diluted with water, and extracted with chloroform. Column chromatography (200 g of silica gel) with 15:1 chloroform–acetonitrile yielded **7** (1.75 g) as a colorless liquid, $[\alpha]_D$ +42° (*c* 1, chloroform). The purity of **7** was checked by t.l.c., and by g.l.c. of the α,β-acetate (see below).

The mass spectrum of **7** (mol. wt. 354) showed peaks at *m/z* 336, 313, 295, 251, and 239, corresponding to losses of H_2O, allyl, H_2O + allyl, H_2O + allyl-CO_2, and allyl-OH + allyl-O, respectively. Intense peaks were observed at *m/z* 153 (allyl-O^+=CH-CH=CH-O-allyl) and 41 (allyl$^+$). The n.m.r. spectrum showed signals at δ 3.20–4.10 (m, 3 H, H-2,3,4), 4.10–4.40 (m, 6 H, CH_2-O ether groups), 4.43 (d, $J_{4,5}$ 9 Hz, H-5), 4.68 (d, 2 H, *J* 6 Hz, CH_2-O ester group), 4.80 (d, 0.2 H, $J_{1,2}$ 7 Hz, H-1β), 5.00–5.50 (m, 8 H, CH_2=C), and 5.60–6.20 (m, 4 H, CH=C).

Anal. Calc. for $C_{18}H_{26}O_7$: C, 61.0; H, 7.4. Found: C, 60.7; H, 7.4.

Allyl 1-O-acyl-2,3,4-tri-O-allyl-D-glucopyranuronates. — The 1-*O*-acetyl derivatives were prepared by the reaction of **7** with an excess of pyridine–acetic anhydride (2:1) at 22° for 2 h, followed by evaporation of reagents *in vacuo* and column chromatography of the residue (chloroform–acetonitrile, 49:1). The product had $[\alpha]_D$ +53° (*c* 1, chloroform).

Anal. Calc. for $C_{20}H_{28}O_8$: C, 60.6; H, 7.1. Found: C, 60.4; H, 7.0.

The 1-*O*-methacryloyl derivatives were prepared by dissolving **7** (5 mg) in dichloromethane (2 ml) and pyridine (0.2 ml) and treatment with methacryloyl chloride (0.1 ml) at 0° for 3 h. The solution was washed thoroughly (aqueous sodium hydrogencarbonate, 0.3M hydrochloric acid, and water) and concentrated *in vacuo*.

Column chromatography (chloroform–acetonitrile, 49:1) yielded an $\alpha\beta$-mixture, $[\alpha]_D$ +49° (*c* 1, chloroform).

Anal. Calc. for $C_{22}H_{30}O_8$: C, 62.55; H, 7.2. Found: C, 62.4; H, 7.1.

The 1-*O*-phenylacetyl derivatives were prepared by the reaction of 7 (50 mg) with 1-phenylacetylimidazole (100 mg) in acetonitrile (2 ml) in the presence of triethylamine (0.2 ml) at 22° for 24 h. Alternatively, a solution of 7 and 1-phenylacetylimidazole in chloroform was concentrated *in vacuo* and the residue was heated under argon at 100° for 30 min. The 1-phenylacetylimidazole reagent was prepared by the reaction of phenylacetic acid (70 mg) with carbonyldi-imidazole (100 mg) in ethanol-free chloroform (10 ml) at 22° for 1 h. The chloroform solution was washed four times with water and dried with molecular sieves. Column chromatography of the product prepared at 100° gave an $\alpha\beta$-mixture, $[\alpha]_D$ +35° (*c* 1, chloroform).

Anal. Calc. for $C_{26}H_{32}O_8$: C, 66.1; H, 6.8. Found: C, 66.1; H, 6.9.

G.l.c.–m.s. of the esters generally revealed a 2:1 $\alpha\beta$-mixture. For the 1-*O*-phenylacetyl ester prepared at 100°, a 1:1 $\alpha\beta$-mixture was obtained. G.l.c. was performed from 150° at 4°/min. The elution temperatures were 175 and 177° (α- and β-1-*O*-acetyl esters, respectively), 180 and 184° (α- and β-1-*O*-methacryloyl esters), and 209 and 216° (α- and β-1-*O*-phenylacetyl esters). The mass spectra of the 1-*O*-acyl esters were very similar to the spectrum for 7. A series of weak-intensity peaks in the higher mass region were due to the molecular ions and associated fragment ions corresponding to loss of allyl, allyl-O, allyl-OH, allyl-O + allene, allyl-OH + allene, and allyl-CO_2.

The n.m.r. spectrum of the 1-*O*-phenylacetyl ester showed signals at δ 3.2–3.8 (m, 3 H, H-2,3,4), 3.70 and 3.72 (2 H, Ph-CH_2 for α and β anomers), 3.8–4.4 (m, 6 H, CH_2-O ether), 4.28 (d, $J_{4,5}$ 7 Hz, H-5), 4.66 (d, J 6 Hz, CH_2-O ester), 5.0–5.48 (m, 8 H, CH_2=C), 5.55 (d, $J_{1,2}$ 7 Hz, H-1β), 5.6–6.2 (m, 4 H, CH=C), 6.30 (d, $J_{1,2}$ 3 Hz, H-1α), and 7.28 (s, 5 H, Ph).

1-O-Acyl-D-glucopyranuronic acids. — A solution of the allyl 1-*O*-acyl-2,3,4-tri-*O*-allyl-D-glucopyranuronate (20 mg) in methanol (2 ml) and 0.1M hydrochloric acid (0.5 ml) was stirred under argon with 10% palladium-on-carbon (40 mg). The reaction was monitored by t.l.c. (9:2 1-propanol–water) and usually was complete within 48 h (sometimes catalyst poisoning[13] was observed); R_F values: ~0.35, 0.40, and 0.45 for the 1-*O*-acetyl, 1-*O*-methacryloyl, and 1-*O*-phenylacetyl-D-glucopyranuronic acids, respectively. Some less polar impurities were also observed. The reaction mixtures were filtered, and concentrated *in vacuo*, and the residues {1-*O*-acetyl, $[\alpha]_D$ +30° (*c* 1, water); 1-*O*-methacryloyl, $[\alpha]_D$ +50° (*c* 1, water); and 1-*O*-phenylacetyl, $[\alpha]_D$ +46° (*c* 1, water)} were treated with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (50 μl) and pyridine (100 μl). G.l.c.–m.s. (from 150°, 4°/min) of the silylated 1-*O*-acetyl- and 1-*O*-methacryloyl-D-glucopyranuronic acids revealed 2:1 $\alpha\beta$-mixtures; elution temperatures were 176.5° and 178° for the α- and β-1-*O*-acetyl-D-glucopyranuronic acids, and 179° and 180° for the α- and β-1-*O*-methacryloyl-D-glucopyranuronic acids. The 1-*O*-phenylacetyl-D-glucopyranuronic acids were not eluted from the column, and were analysed directly by mass spectrometry.

The mass spectra of the α and β anomers of the silylated 1-*O*-acyl-D-glucopyranuronic acids showed weak-intensity molecular ions at *m/z* 524, 550, and 600 for the 1-*O*-acetyl, 1-*O*-methacryloyl, and 1-*O*-phenylacetyl esters, respectively. Characteristic ions were due to loss of methyl, methyl + Me_3SiOH, RCO_2 (*m/z* 465), RCO_2H (*m/z* 464), RCO_2H + Me (*m/z* 449), and RCO_2 + Me_3SiOH (*m/z* 375). Commonly observed fragment ions were found at *m/z* 305, 217, 204, 147, and 73.

*Bilirubin and biliverdin 1-*O*-acyl derivatives of* 7. — The di-imidazole derivative of bilirubin was prepared[4] from bilirubin (100 mg) and carbonyldi-imidazole (200 mg) in dry *N,N*-dimethylformamide (5 ml). The solution was stirred at 22° for 24 h in the dark and the excess of reagent was decomposed with water (20 μl). After 15 min, a solution of 7 (200 mg) in dichloromethane (1 ml) was added and the solution was concentrated *in vacuo* at 50°. The residue was heated under argon at 85° in the dark for 40 min, cooled, and dissolved in dichloromethane. T.l.c. (97:2:1 chloroform–methanol–acetic acid) showed the absence of bilirubin (R_F ~0.7), and formation of the di-D-glucosyluronic acid derivative (R_F ~0.4) together with a small proportion of mono-D-glucosyluronic acid derivative (R_F ~0.6).

Allyl-protected 1-*O*-bilirubin-D-glucuronic acids were oxidised to the corresponding biliverdin compounds by shaking a dichloromethane solution (5 ml, containing the equivalent of 10 mg of bilirubin) with a fresh solution of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (10 mg) in water (5 ml) for ~3 min. The blue solution was washed thoroughly with water. T.l.c. (9:1 chloroform–methanol) showed the allyl-protected biliverdin di-D-glucosyluronic acid derivative as the major compound (R_F 0.77) and the mono-D-glucosyluronic acid derivative as the minor compound (R_F 0.45). Free biliverdin (R_F 0.03) was not observed.

Ethyl anthranilate azopigments were prepared[14] from the bilirubin derivatives by using *N,N*-dimethylformamide instead of ethanol as an accelerator of the diazonium reaction (to prevent formation of ethyl ester from possibly unreacted bilirubin-imidazole). After reaction at pH 2.7 for 30 min, water was added and the azopigments were extracted with dichloromethane. *N,N*-Dimethylformamide was removed by washing with water. T.l.c. revealed azodipyrrole free acid (more-polar azopigment derived from bilirubin mono-D-glucosyluronic acid derivatives) and, mainly, allyl-protected 1-*O*-acyl-D-glucopyranuronic acids (less-polar azopigments, derived from mono- and di-D-glucosyluronic acid derivatives). A first development (19:1 benzene–ethyl acetate) fractionated the 1:1 mixture of *exo*-vinyl (more labile) and *endo*-vinyl (less mobile) ester isomers. A second development (17:3 benzene–ethyl acetate) gave a further fractionation of the anomeric pairs. For each pair, the slower-moving azopigment was the major compound. Isolation of the *exo*- and *endo*-vinyl isomers and partial separation of the anomers was also effected by column chromatography (19:1 benzene-ethyl acetate).

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