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Note

Preparation of some aryl α-L-arabinofuranosides as substrates for arabinofuranosidase

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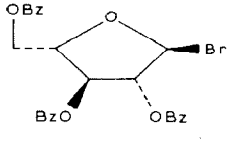
α-L-Arabinofuranosidase is widely distributed in Nature, mostly in the plant kingdom[1], but, despite the importance of the enzyme, the range of chromogenic and fluorogenic substrates is limited[2–4] and their tedious preparations continue to be subject to modification and improvement[5,6]. We now report that the 2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosides of the acidic phenols 2,4-, 3,4-, and 3,5-dinitrophenol, 4-nitrophenol, and 4-methylumbelliferone can be made by boiling under reflux the aglycon with crystalline 2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl bromide (**1**) (obtainable from arabinose without chromatography[7]) in anhydrous acetone in the presence of anhydrous potassium carbonate. As with the analogous reaction of acetylated glycopyranosyl bromides with phenols in acetone in the presence of potassium carbonate[8], the reaction of **1** with phenols of $pK > -8$ under these conditions is not useful preparatively. Presumably, the α-glycoside is formed by reaction of the phenolate anion with the cation **2**.



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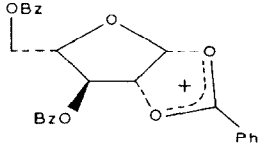

2

Three other routes to the α-L-arabinofuranoside of 4-phenylphenol, a weakly acidic phenol, were explored. Coupling of the phenol with **1**, either under S_N1 [$Hg(CN)_2$ in CH_3NO_2][9] or S_N2 conditions (anhydrous sodium phenolate in di-

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methoxyethane)[10] gave $\alpha\beta$-mixtures which had to be fractionated by chromatography, like the product of Helferich fusion of the phenol with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-L-arabinofuranoses[11].

The protected glycosides, with the exception of the 2,4-dinitrophenyl compound, were debenzoylated with methanolic ammonia, although these conditions opened the lactone ring of the 4-methylumbelliferyl fluorophore, which had to be re-closed by brief treatment with acid. The 4-phenylphenyl and nitrophenyl glycosides were debenzoylated also with methanolic barium methoxide.

EXPERIMENTAL

General procedure for reaction of **1** *with phenol in acetone in the presence of anhydrous potassium carbonate.* — A mixture of **1** (5 g) and dry phenol (0.18 mol) was heated under reflux in the presence of dry potassium carbonate (2.5 g) for 16 h, and then filtered. Insoluble material was washed with acetone, and the combined filtrate and washings were diluted with dichloromethane (100 mL) and poured into water (100 mL). The dichloromethane layer was washed with M sodium hydroxide until the washings were colourless, dried ($MgSO_4$), and concentrated. The following compounds were prepared in this manner.

2,4-Dinitrophenyl 2,3,5-tri-O-benzoyl-α-L-arabinofuranoside. — The residue crystrallised and the title compound (44%), m.p. 166–165.5°, $[\alpha]_D^{20}$ −73° (*c* 1, chloroform), was obtained after recrystallisation from acetone–methanol and acetone–ethanol. ^{1}H-N.m.r. data (60 MHz, $CDCl_3$): δ 4.8 (bs, 3 H, H-4,5,5), 5.7 (bs, 1 H, H-3), 5.8 (s, 1 H, H-2), 6.2 (s, 1 H, H-1), 6.9–8.3 (m, 15 H, 3 BzO), 8.4–8.7 (m, 3 H, nitrophenyl protons).

Anal. Calc. for $C_{32}H_{24}N_2O_{12}$: C, 61.15; H, 3.82; N, 4.46. Found: C, 61.50; H, 3.93; N, 4.26.

3,4-Dinitrophenyl α-L-arabinofuranoside. — The residue from the coupling reaction was subjected to chromatography on silica gel (180 g, 4-cm wide column), using dichloromethane–light petroleum (3:2). 3,4-Dinitrophenyl 2,3,5-tri-*O*-benzoyl-α-L-arabinofuranoside was eluted (between 600 and 1500 mL of eluant) and recrystallisation from methanol gave material with m.p. 71–73°, $[\alpha]_D^{20}$ −78.5° (c 5, chloroform), in 15% yield. ^{1}H-N.m.r. data ($CDCl_3$, 200 MHz): δ 4.79 (m, 3 H, H-4,5,5), 5.72 (m, 1 H, $J_{3,4}$ 3.4 Hz, H-3), 5.82 (d, 1 H, $J_{1,2}$ 1.2 Hz, H-2), 6.06 (d, 1 H, H-1), 7.45 (m, *m*- and *p*-H of BzO, aglycon H-5), 8.08 (*o*-H of BzO, aglycon H-2,6).

Anal. Calc. for $C_{32}H_{24}N_2O_{12}$: C, 61.15; H, 3.82; N, 4.46. Found: C, 61.38; H, 3.86; N, 4.19.

The benzoylated glycoside (0.62 g) was treated with saturated methanolic ammonia for 16 h at 4°. The methanol was evaporated and a solution of the residue in water (500 mL) was extracted with chloroform, then concentrated at 30° (bath), and lyophilised. Flash column chromatography of the residue (320 mg) on silica gel with light petroleum–ethyl acetate (1:6) afforded the title product (12%) as a gum,

$[\alpha]_D^{20}$ −170° (*c* 1, methanol). ^{1}H-N.m.r. data (CD_3OD, 90 MHz): δ 3.72 (m, 2 H, H-5,5), 4.04 (m, 2 H, H-3,4), 4.31 (m, 1 H, H-2), 5.73 (d, 1 H, $J_{1,2}$ 1.3 Hz, H-1), 7.61 (d, 1 H, $J_{2,6}$ 2.6 Hz, aglycon H-2), 8.15 (d, 1 H, $J_{5,6}$ 9.2 Hz, aglycon H-5), 7.44 (d, 1 H, aglycon H-6).

Anal. Calc. for $C_{11}H_{12}N_2O_9$: C, 41.78; H, 3.83; N, 8.86. Found: C, 41.12; H, 4.02; N, 8.31.

The material liberated 94% of the theoretical amount of 3,4-dinitrophenol on treatment with purified α-L-arabinofuranosidase[12].

3,5-Dinitrophenyl α-L-arabinofuranoside. — This compound was made as for the 3,4-isomer, but the purified benzoylated glycoside was characterised only by the ^{1}H-n.m.r. data (60 MHz, $CDCl_3$): δ 4.73 (m, 3 H, H-4,5,5), 5.67 (m, 1 H, H-3), 5.75 (s, 1 H, H-2), 5.95 (s, 1 H, H-1), 7.35 and 7.95 (10 and 8 H, aromatic protons).

The debenzoylated compound, obtained in 7% yield, had m.p. 93–96°, $[\alpha]_D^{20}$ −138° (c 1, methanol). ^{1}H-N.m.r. data (90 MHz, CD_3OD): δ 3.74 (m, 2 H, $J_{4,5a}$ 4.0, $J_{4,5}$ 2.6, $J_{5,5a}$ 9.0 Hz, H-5,5a), 4.04 (m, 2 H, H-3,4), 4.30 (m, 1 H, H-2), 5.64 (d, 1 H, $J_{1,2}$ 1.3 Hz,H-1). 7.51 (d, 2 H, $J_{2,4}$, 2.2 Hz, aglycon H-2,6), 7.82 (d, 1 H, aglycon H-4).

Anal. Calc. for $C_{11}H_{12}N_2O_9$: C, 41.78; H, 3.83; N, 8.86. Found: C, 42.09; H, 4.13; N, 8.63.

4-Nitrophenyl α-L-arabinofuranoside. — The residue from the coupling reaction was dissolved in dry methanol (200 mL), and methanolic M barium methoxide (4.5 mL, made by heating barium oxide in methanol under reflux, cooling, filtering, and diluting with 2 vol. of dry methanol) was added. After 6 h at 4°, barium ions were removed with Amberlite IRA-120 (H^+) resin, the solution was concentrated, and the methyl benzoate was evaporated with compressed air. Chromatography (either conventional or flash) on silica gel with ethyl acetate afforded the title glycoside (39%), m.p. 155–157° (from ethyl acetate); lit.[3] m.p. 154–157°.

4-Methylumbelliferyl α-L-arabinofuranoside. — The crude product from the coupling reaction was subjected to flash chromatography on a column (15 × 5 cm) of silica gel with light petroleum–ethyl acetate (3:2) and 40-mL fractions were collected. 4-Methylumbelliferyl 2,3,5-tri-*O*-benzoyl-α-L-arabinofuranoside (12%), m.p. 124–127°, $[\alpha]_D^{20}$ −50.5° (*c* 1, chloroform), was obtained by concentration of fractions 19–29. ^{1}H-N.m.r. data (200 MHz, $CDCl_3$): δ 2.41 (d, 3 H, $J_{3,9}$ 1.2 Hz, aglycon CH_3), 4.78 (m, 3 H, H-4,5,5), 5.71 (d, 1 H, $J_{3,4}$ 3.7 Hz, H-3), 5.82 (d, 1 H, $J_{2,3}$ 1.2 Hz, H-2), 6.05 (s, 1 H, H-1), 7.48 (s, 1 H, aglycon H-3), 7.5 and 8.1 (2 m, other aromatic protons).

Anal. Calc. for $C_{38}H_{30}O_8$: C, 69.67; H, 4.54. Found: C, 69.74; H, 4.63..

The purified tribenzoate (1.7 g) was treated with ammonia-saturated methanol for 3 days at 4°. The mixture was concentrated, a solution of the residue in water (450 mL) was extracted with chloroform, and the aqueous phase was acidified to pH 3 with acetic acid to reclose the coumarin ring.

Evaporation of the water gave the title compound as a monohydrate, m.p. 83–84° (from aqueous ethanol), or anhydrous, m.p. 125–128° (from anhydrous

methanol; in 10% yield after two recrystallisations), $[\alpha]_D^{20}$ −204° (*c* 1, methanol). ^{1}H-N.m.r. data (200 MHz, CD_3OD): δ 2.44 (d, 3 H, $J_{3,9}$ 1.5 Hz, aglycon CH_3), 3.68 and 3.79 (2 m, 2 H, $J_{4,5}$ 2.8, $J_{5,5a}$ 11.7 Hz, H-5,5a), 4.04 (m, 2 H, H-3,4), 4.29 (m, 1 H, $J_{2,3}$ 3.7 $J_{1,2}$ 1.7 Hz, H-2), 5.64 (d, 1 H, H-1), 6.18 (s, 1 H, H-3), 7.04 (s, 1 H, aglycon H-8), 7.06 (d, 1 H, $J_{5,6}$ 8.3 Hz, aglycon H-6), 7.68 (d, 1 H, aglycon H-5).

Anal. Calc. for $C_{15}H_{16}O_7$: C, 58.44; H, 5.23. Found: C, 57.89; H, 5.20. Calc. for $C_{15}H_{16}O_7 \cdot H_2O$: C, 55.21; H, 5.56. Found: C, 54.95; H, 5.34.

4-Phenylphenyl α-L-arabinofuranoside. — In the following methods, the anomeric composition of the tribenzoate was estimated after t.l.c. on silica gel GF_{254}, using benzene–chloroform (3:2) and detection by fluorescence on u.v. irradiation; R_F values: α anomer, 0.42; β anomer, 0.36.

Mercuric cyanide (0.5 g) was dissolved in boiling dry nitromethane (100 mL), and a solution of 4-phenylphenol (1.0 g) in warm, dry nitromethane (100 mL) was added. The solution was cooled to 22°, **1** (1.0 g) was added, and the mixture was stored thereat for 48 h. The nitromethane was evaporated, and a solution of the residue in chloroform was filtered, washed with water and 0.5M KBr, dried, and concentrated. The glycosylated product was largely α. Debenzoylation as described above for the nitrophenyl glycoside, followed by chromatography on silica gel with ethyl acetate, gave a ~4:1 αβ-mixture.

Anal. Calc. for $C_{17}H_{18}O_5$: C, 67.54; H, 5.96. Found: C, 67.92; H, 6.13.

4-Phenylphenol (1.0 g) was suspended in 1,2-dimethoxyethane (4 mL) previously dried over sodium wire. Sodium (0.11 g) was added, the mixture was stored for 2 h, **1** (2.0 g) was added, and the mixture was kept for 1 h at 50°, then cooled, and diluted with benzene (100 mL). The precipitate was removed, the filtrate was washed with water, M sodium hydroxide, and water, dried ($MgSO_4$), and concentrated. The residue was crystallised from methanol to give 4-phenylphenyl 2,3,5-tri-*O*-benzoyl-β-L-arabinofuranoside (20 mg), m.p. 108–110°. ^{1}H-N.m.r. data (60 MHz, $CDCl_3$): δ 4.9 (m, 3 H, H-4,5,5), 5.7 (dd, 1 H, $J_{1,2}$ 4.5, $J_{2,3}$ 2.5 Hz, H-2), 6.16 (d, 1 H, H-1), 6.24 (dd, 1 H, $J_{3,4}$ 4.9 Hz, H-3′), 7.0–7.7 (m, 18 H, aromatic protons), 8.0–8.2 (m, 6 H, aromatic protons).

Anal. Calc. for $C_{38}H_{30}O_8$: C, 74.26; H, 4.88. Found: C, 74.18; H, 5.08.

The remainder of the crystalline product was a mixture containing mainly the α anomer. Chromatography on silica gel with chloroform–benzene (3:2) at a substrate–adsorbent ratio of 200, so that only a portion of the total product was used, gave 4-phenylphenyl 2,3,5-tri-*O*-benzoyl-α-L-arabinofuranoside, m.p. 50–54°. ^{1}H-N.m.r. data (60 MHz, $CDCl_3$): δ 4.8 (s, 3 H, H-4,5,5), 5.7 (s, 1 H, H-3), 5.8 (s, 1 H, H-2), 6.0 (s, 1 H, H-1), 7.2–7.6 and 8.0–8.2 (2 m, 18 and 6 H, aromatic protons).

Anal. Found: C, 74.09; H, 5.28.

The 1-*O*-acetyl-2,3-5-tri-*O*-benzoyl-αβ-L-arabinofuranose, produced from methyl 2,3,5-tri-*O*-benzoyl-α-L-arabinofuranoside (5.0 g) according to the method of Tolman and Baker[11], was heated with 4-phenylphenol (1.8 g) and *p*-toluenesulphonic acid (30 mg) until a homogeneous melt was obtained. The heating was continued at 115–120°/0.1 mmHg for 1.5 h. T.l.c. (chloroform) of the cooled melt

revealed a ~10:1 $\alpha\beta$-mixture.

The crude mixture was stirred for 48 h at 4° with dry methanol (300 mL) to which saturated methanolic barium methoxide (3 mL) had been added. Barium was precipitated by the addition of solid carbon dioxide, and the mixture was filtered and concentrated. The residue (2.5 g) was extracted with ethyl acetate (0.5 g of insoluble material remained), and the extract was concentrated to give 4-phenylphenyl α-L-arabinofuranoside (130 mg), m.p. 98–100°, $[\alpha]_D$ −133° (*c* 1, chloroform), together with an $\alpha\beta$-mixture. This mixture required chromatography, in two portions, on silica gel (300 g), using ethyl acetate followed by decolourisation with charcoal and recrystallisation from aqueous ethanol. Only the α anomer was a substrate for α-L-arabinofuranosidase.

Anal. Calc. for $C_{17}H_{18}O_5$: C, 67.54; H, 5.96. Found: C, 67.32; H, 6.21.

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