PARTIAL BENZOYLATION OF SUCROSE

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ABSTRACT

Tribenzoylation of sucrose followed by column chromatography gave a bitter fraction containing four tribenzoates, namely, 6,1',3'-, 2,6,1'-, 2,6,6'-, and 6,3',6'- tri-O-benzoylsucrose. A slower moving and less bitter fraction consisted of the expected major product, 6,1',6'-tri-O-benzoylsucrose.

INTRODUCTION

Although it is well established that the acetylation of sugars frequently imparts bitter-taste characteristics¹, little attention has been paid to the effect of other acyl groups. The only taste data available for sucrose esters refer to the octa-, hepta-, and hexa-acetates². For other known sucrose esters, such as the higher benzoates, the aqueous solubility may be insufficient to elicit a taste response. Since the aqueous solubility would be expected to increase as the number of benzoate groups decreased, the effect of the degree and position of substitution on the taste characteristics is of interest³. We now report on the availability of sucrose tri- and tetra-benzoates by the direct benzoylation of sucrose.

RESULTS AND DISCUSSION

Partial benzoylation of sucrose with 3 mol. equiv. of benzoyl chloride in pyridine gave products ranging from mono- to octa-benzoate. Column chromatography of this mixture gave a major fraction consisting of tri-, tetra-, and penta-benzoates, and other fractions containing dibenzoates, and hexa-benzoates and higher benzoates. Further column chromatography of the major fraction gave the

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expected tribenzoate, 6,1',6'-tri-O-benzoylsucrose (1). The u.v. (ε 36,000, cf. 11,500 for a monobenzoate) and m.s. data established that 1 was a sucrose tribenzoate, and the ¹H-n.m.r. data indicated the absence of benzoyl groups at secondary positions. Acylation at a secondary position results in a downfield shift of the signal of the corresponding methine proton, normally to a position in the range δ 5–6. The splitting patterns of the five methine protons in sucrose can be anticipated on the basis of published data for acetylated and benzoylated derivatives⁴⁻⁷ as follows: H-2 (dd, $J \sim 10, 2$ Hz), H-3 (t, $J \sim 10$ Hz), H-4 (t, $J \sim 10$ Hz), H-3' (d, J 5–8 Hz), and H-4' (dd, both J 5–8 Hz).



1 $R^{1} = R^{3} = H, R = R^{2} = R^{4} = Bz$ **2** $R^{1} = H, R = R^{2} = R^{3} = R^{4} = Bz$ **3** $R^{3} = H, R = R^{1} = R^{2} = R^{4} = Bz$ **4** $R^{1} = R^{2} = H, R = R^{3} = R^{4} = Bz$ **5** $R^{2} = R^{3} = H, R = R^{1} = R^{4} = Bz$ **6** $R^{3} = R^{4} = H, R = R^{1} = R^{2} = Bz$ **7** $R^{1} = R^{4} = H, R = R^{2} = R^{3} = Bz$ **8** $R = R^{3} = H, R^{1} = R^{2} = R^{4} = Bz$

Two tetrabenzoates, namely, 6,1',3',6'- (2) and 2,6,1',6'-tetra-O-benzoylsucrose (3), were also isolated. The presence of four benzoate groups was indicated by the u.v. data (ε 44,000 and 46,000) and by the mass spectra of their tetra-acetates which also showed an asymmetric distribution of the benzoate groups in 2 but not in 3. Examination of the δ 5-6 region of the ¹H-n.m.r. spectra showed that a single secondary position was benzoylated in 2 (C-3') and 3 (C-2).

A further fraction, which was eluted between 1 and the tetrabenzoates and had greater bitterness than these materials, was subsequently shown by h.p.l.c. (Fig. 1) to contain four tribenzoates, three of which were isolated pure. Each tribenzoate had two primary positions benzoylated; two were also benzoylated at position 2 and another at position 3'. These three tribenzoates were identified, after synthesis of four of the six possible isomers by unambiguous routes^{8,9}, as 6,1',3'-tri-O-benzoylsucrose⁹ (4), 2,6,1'-tri-O-benzoylsucrose⁹ (5), and 2,6,6'-tri-O-benzoylsucrose (6) by elimination, since it was different from 2,1',6'-tri-O-benzoylsucrose⁹ (8).



Fig. 1. H.p.l.c. of the "bitter tribenzoate" region of the products formed on treatment of sucrose with 3 mol. equiv. of benzoyl chloride in pyridine. Conditions: isocratic, 44% methanol/water, 25 μ L of a 40 mg/mL solution injected.

6,3',6'-Tri-O-benzoylsucrose⁸ (7) was also synthesised, and shown by h.p.l.c. and ¹H-n.m.r. spectroscopy to correspond to the fourth component of the bitter tribenzoate mixture.

The approximate percentages of 1–7 in the mixture of benzoates, determined by h.p.l.c. after allowing for their different u.v. absorption intensities, were 15, 9, 6, 5, 6, 4, and 2, indicating the order of benzoylation to be HO-6 > HO-1' = HO-6' > HO-2 = HO-3'. Since benzoylation of one hydroxyl group is likely to modify the reactivity of the remaining hydroxyl groups, this order is not necessarily the same as the relative reactivity of the hydroxyl groups. A minor tribenzoate, isolated but not fully characterised, had two primary positions benzoylated and also either position 3 or 4. There was no other evidence for the formation of tribenzoates involving positions 3, 4, or 4'. The proportion of products substituted at positions 1' and/or 3' was greater than expected since, for example, the reactivity of the hydroxyl groups in sucrose towards tosylation has been postulated⁷ to be HO-6 \approx HO-6' > HO-1' > HO-2.

The threshold of bitterness (p.p.m.) for the tri- and tetra-benzoates was as follows: 6,1',6'-tri (1), 20; 6,1',3',6'-tetra (2), 11; 2,6,1',6'-tetra (3), 17; 6,1',3'-tri (4), 3; 2,6,1'-tri (5), 8; 2,6,6'-tri (6), 4; and 6,3',6'-tri (7), 5. Compounds with a benzoate group at either position 2 or 3' show enhanced bitterness compared with the 6,1',6'-tribenzoate 1. The tetrabenzoates 2 and 3, however, are not as bitter as the tribenzoates 4–7.

EXPERIMENTAL

General methods. - An Optical Activity Automatic Polarimeter AA-10 (1-

dm tubes) was used to determined optical rotations. U.v. spectra were recorded with a Pye Unicam SP8-100 spectrometer. T.l.c. was performed on Kieselgel 60 F_{254} (Merck) with detection by u.v. light or by charring with ceric sulphate-sulphuric acid. Column chromatography was carried out with B.D.H. silica gel unless otherwise stated. Preparative t.l.c. was performed on Analtech silica gel GF. The h.p.l.c. system consisted of two Altex 110 A pumps, an Altex solvent programmer, a Cecil CE 2012 variable wavelength detector operating at 254 nm, a Hewlett-Packard 3390 reporting-integrator, and a Waters µ-Bondapak C-18 column, unless otherwise stated, with a flow rate of 1.5 mL/min. Analysis of total benzoylation mixtures was performed using gradient elution (pump A-46% methanol/water, pump B-methanol, 1→95%B during 35 min, exponent 3). Light petroleum refers to the fraction b.p. 40-60° and was distilled prior to use. Pyridine, dried by refluxing over potassium hydroxide, was distilled and stored over molecular sieves. ¹H-N.m.r. spectra (internal Me₄Si) were recorded with a Varian HA100, Jeol FX90Q, or P.C.M.U. Harwell 220-MHz spectrometer. E.i. mass spectra (70 eV) were obtained with a VG 70-70F spectrometer and a VG 2250 data system. C.i. mass spectra were obtained using ammonia as the reagent gas.

The threshold of bitterness was determined by diluting a solution of the compound (10 mg) in ethanol (2 mL) to 500 mL with mineral water. This solution was diluted to give solutions containing 10, 5, 2.5, 1.25, and 0.625 p.p.m. Panelists tasted the solutions, starting with mineral water, in order of increasing sample concentration, until bitterness was just perceived.

Benzoylation of sucrose. — To a solution of sucrose (20 g, 0.058 mol) in pyridine (1 L) at the freezing point of the solvent was added benzoyl chloride (22.4 g, 0.17 mol) dropwise with stirring during 1 h. The cooled solution was stirred for a further 1 h, crushed ice (20 g) was then added, and the solution was allowed to attain ambient temperature. The bulk of the solvent was then evaporated under diminished pressure followed by co-distillation with toluene. The residual syrup was partitioned between chloroform (300 mL) and water (300 mL), and the aqueous layer was extracted with chloroform (2×150 mL). The combined chloroform phase and extracts were washed successively with 0.5M hydrochloric acid, 0.5M sodium hydrogencarbonate, and water, dried (Na₂SO₄), and concentrated to dryness. The syrupy residue (30.2 g) was adsorbed on to silica gel (60 g) and then subjected to dry column chromatography on silica gel (560 mL) with chloroformmethanol (9:1, 25-mL fractions). Fractions 19-32 (12.8 g) were subjected to further column chromatography (25-mL fractions), using the same solvent but with preslurried silica gel (560 mL). Fractions 36-41 (0.6 g) contained mainly 2 and fractions 42-45 (1.7 g) contained a mixture of 2 and 3. A portion of the material from fractions 42-45 (160 mg) was subjected to preparative t.l.c. (chloroform-methanol, 9:1) to give 2 (40 mg) and 3 (80 mg).

Compound **2** had $[\alpha]_{D}^{25}$ +92° (*c* 0.5, chloroform). ¹H-N.m.r. data (220 MHz, CDCl₃): δ 2.8–4.8 (m, 16 H), 5.58 (d, 1 H, $J_{3',4'}$ 7.4 Hz, H-3'), 5.64 (d, 1 H, $J_{1,2}$ 3.0 Hz, H-1), 7.35 (m, 12 H, Ar-H), 7.95 (m, 8 H, Ar-H *ortho* to carbonyl). Mass spectrum (tetra-acetate): e.i. *m/z* 517, 393; c.i. *m/z* 944 (M + \hbar H₄).

Compound **3** had $[\alpha]_D^{25} + 86^\circ$ (c 0.5, chloroform). ¹H-N.m.r. data (100 MHz. CDCl₃): δ 3.4-4.8 (m, 16 H), 5.05 (dd, 1 H, $J_{1,2}$ 3.0, $J_{2,3}$ 10.0 Hz, H-2), 5.86 (d, 1 H, $J_{1,2}$ 3.0 Hz, H-1), 7.35 (m, 12 H, Ar-H), 7.95 (m, 8 H, Ar-H ortho to carbonyl). Mass spectrum (tetra-acetate): e.i. m/z 455; c.i. m/z 944 (M + $\dot{N}H_4$).

Fractions 49–67 (2.2 g) comprised the "bitter tribenzoate" fraction and h.p.l.c. (Lichrosorb 10RP8, 60% methanol/water) of the individual fractions indicated partial separation. Fractions 49–53 (0.81 g) and 60–67 (0.62 g) were subjected to column chromatography (Kieselgel G, 7.5% methanol in chloroform) to give 4 and 5, respectively, which, together with their respective penta-acetates, were identical with the authentic compounds⁹.

Fractions intermediate between those containing only 4 or 5 were combined with fractions 54–58 and subjected to further column chromatography. Although 7 had a mobility intermediate between those of 4 and 5, overlapping of the bands prevented its isolation and an equimolar mixture of 4 and 7 was obtained.

Fractions 73-76 (250-mL fractions) were combined to give 1 (1.5 g) as a foam, $[\alpha]_D^{25}$ +30° (c 1, methanol). ¹H-N.m.r. data (90 MHz, CDCl₃): δ 3.13 (bs, 2 H, 2 OH), 3.3-4.8 (m, 13 H), 5.08 (bs, 2 H, 2 OH), 5.37 (bs, 1 H, OH), 5.43 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 6.9-8.0 (m, 15 H, 3 Ph). The ¹³C-n.m.r. data have been published⁹.

Anal. Calc. for C₃₃H₃₄O₁₄: C, 60.55; H, 5.20. Found: C, 60.26; H, 5.11.

Treatment of 1 with pyridine-acetic anhydride gave the penta-acetate as a foam, $[\alpha]_D^{25} + 56^\circ$ (c 4.1, chloroform). ¹H-N.m.r. data (90 MHz, CDCl₃): δ 1.92, 1.95, 2.01, 2.08, 2.16 (5 s, each 3 H, 5 OAc), 4.10–4.75 (m, 13 H), 4.87 (dd, 1 H, $J_{2,3}$ 10.2 Hz, H-2), 5.21 (t, 1 H, $J_{3,4} = J_{4,5} = 9.6$ Hz, H-4), 5.36–5.71 (m, 3 H, H-3,3',4'), 5.78 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 7.1–8.2 (m, 15 H, 3 Ph). The ¹³C-n.m.r. data have been published⁹.

2,6,6'-Tri-O-benzoylsucrose (6). — The crude product (8.6 g) from the benzoylation of sucrose (5 g) was subjected to column chromatography (silica gel, Merck 9385; dichloromethane-ethyl acetate-ethanol, 60:36:4). H.p.l.c. (conditions as in Fig. 1) of the fractions in the tribenzoate region were examined and fractions 42–56 (0.3 g), which contained the highest percentage of an unknown tribenzoate, were subjected to further column chromatography (dichloromethanemethanol, 100:8). Fractions 7 and 8 gave a single component in t.l.c. (dichloromethane-methanol, 100:8) and were combined to give **6** (40 mg), $[\alpha]_D^{25}$ +34° (c 2.6, methanol). ¹H-N.m.r. data (90 MHz, CD₃OD): δ 4.93 (d, 0.5 H, half of H-2), 5.78 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), 7.2–8.2 (m, 15 H, 3 Ph). The ¹³C-n.m.r. data have been published⁹.

Anal. Calc. for C₃₃H₃₄O₁₄: C, 60.55; H, 5.20. Found: C, 60.18; H, 5.33.

Treatment of **6** with pyridine–acetic anhydride gave the penta-acetate as an amorphous solid, $[\alpha]_D^{25}$ +73° (c 3.6, chloroform). ¹H-N.m.r. data (90 MHz, C₆D₆/CD₃OD): δ 1.62, 1.65, 1.65, 1.68, 1.96 (5 s, each 3 H, 5 OAc), 4.0–4.9 (m, 8 H), 5.24 (dd, 1 H, J_{2,3} 10.0 Hz, H-2), 5.53 (dd, 1 H, J_{4',5'} 5.0 Hz, H-4'), 5.54 (t, 1 H, J_{4,5} 10.0 Hz, H-4), 5.57 (d, 1 H, J_{3',4'} 6.2 Hz, H-3'), 6.03 (t, 1 H, J_{3,4} 10.0 Hz, H-3),

6.12 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 7.00–8.35 (m, 15 H, 3 Ph). E.i. mass spectrum: m/z 455, 393. The ¹³C-n.m.r. data have been published⁹.

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