Regioselective enzymic deacetylation of octa-\(O\)-acetyl-sucrose: preparation of hepta-\(O\)-acetyl-sucroses

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ABSTRACT

Deacetylation of octa-\(O\)-acetyl-sucrose (1) with Alcalase or protease N gave the 2,3,4,6,3',4',6'-hepta-acetate (2) as the initial major product with the 2,3,4,6,3',4'-hexa-acetate (3) as the subsequent main product. The 2,3,4,1',3',4',6'-hepta-acetate (5) was obtained from 1 by the action of lipase OF or lipase AP6, and the 2,3,4,6,1',3',6'- (4) and 2,3,4,6,1',3',4'-hepta-acetate (7) by the action of Candida lipase and chymotrypsin, respectively. The 2,3,6,1',3',4',6'-hepta-acetate (6) was formed from 5 by acyl migration.

INTRODUCTION

Sucrose esters have potential applications in cosmetics, denaturants, plasticisers, food preservatives, and agriculture\(^1\)-\(^4\). Hence, the preparation of partially acetylated derivatives of sucrose has been investigated widely\(^5\). Partially acetylated mono- and di-saccharides, including sucrose, have been prepared by selective deacetylation or acetylation by chemical and enzymic methods\(^5\)-\(^20\). Thus, the 2,3,4,6,3',4',6'- (2), the 2,3,4,6,1',3',4'- (7), and the 2,3,4,6,1',3',6'-hepta-acetate (4) of sucrose were isolated after limited \(O\)-deacetylation of octa-\(O\)-acetyl-sucrose (1) on a column of aluminium oxide\(^18\)-\(^20\), and 4, the 2,3,4,6,1',3'-hexa-acetate, and the 2,3,4,6,3'-penta-acetate after the treatment of 1 with lipases\(^12\).

We now report the preparation of sucrose hepta-acetates by the regioselective hydrolysis of 1 with various hydrolytic enzymes.

RESULTS AND DISCUSSION

It was found that seven enzymes could deacetylate 1 in phosphate buffer or phosphate buffer–organic co-solvent to give hepta-acetates. The results are shown in Table I and Scheme 1. Alcalase and protease N cleaved \(AcO-1'\) and gave the 2,3,4,6,3',4',6'-hepta-acetate (2), and prolonged hydrolysis with alcalase gave the 2,3,4,6,3',4'-hexa-acetate (3).

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TABLE I

Regioselective hydrolysis of 1 by enzymic catalysis

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Reaction solution</th>
<th>Reaction time</th>
<th>Products (yield)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcalase</td>
<td>H₂O-HCONMe₂ (3:1)</td>
<td>24</td>
<td>2 (65%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>72</td>
<td>3 (60%)</td>
</tr>
<tr>
<td>Protease N</td>
<td>H₂O-HCONMe₂ (4:1)</td>
<td>96</td>
<td>2 (74%)</td>
</tr>
<tr>
<td>Lipase MY</td>
<td>H₂O-HCONMe₂ (4:1)</td>
<td>96</td>
<td>2 (10%) and 4 (40%)</td>
</tr>
<tr>
<td><em>Candida</em> lipase</td>
<td>H₂O-HCONMe₂ (4:1)</td>
<td>96</td>
<td>2 (6%) and 4 (54%)</td>
</tr>
<tr>
<td>Lipase AP6</td>
<td>H₂O-EtOH (4:1)</td>
<td>20</td>
<td>6 (40%)</td>
</tr>
<tr>
<td></td>
<td>Phosphate buffer</td>
<td>20</td>
<td>5 (40%)</td>
</tr>
<tr>
<td>Lipase OF</td>
<td>Phosphate buffer</td>
<td>24</td>
<td>5 (30%)</td>
</tr>
<tr>
<td>Chymotrypsin</td>
<td>Phosphate buffer</td>
<td>72</td>
<td>7 (30%)</td>
</tr>
</tbody>
</table>

* Location of acetyl groups: 2, 2,3,4,6,3',4',6'; 3, 2,3,4,6,3',4'; 4, 2,3,4,6,1',3',6'; 5, 2,3,4,1',3',4',6'; 6, 2,3,6,1',3',4',6'; 7, 2,3,4,6,1',3',4'.

Scheme 1. Location of acetyl groups cleaved by the various enzymes.
Of the three lipases (Candida lipase, lipase MY, and lipase OF) isolated from Candida cylindracea, Candida lipase (Sigma) acted on 1 to give 4 (54%) and 2 (6%). Whereas the Candida lipase cleaved AcO-4' preferentially in 1, it selectively hydrolysed AcO-6' in glucose penta-acetate. Lipase MY cleaved AcO-1' and AcO-4' of 1. Kloosterman et al. showed 4 to be the main product of the hydrolysis of 1 by a lipase isolated from Candida cylindracea and suspected that it was formed from 2 through a 4' → 1' acyl migration. However, this migration seems unlikely because 2 was the sole product from the alcalase-catalysed hydrolysis of 1, and 4 was not detected. Lipase OF hydrolysed AcO-6 of 1 to produce the 2,3,4,1',3',4',6'-hepta-acetate. Lipase AP6 acted on 1 to give the 2,3,6,1',3',4',6'- (6) or 2,3,4,1',3',4',6'-hepta-acetate (5), depending on the reaction solution used. In phosphate buffer containing 20% ethanol, the major product was 6, whereas 5 was the main product if the reaction was carried out in phosphate buffer. H.p.l.c. showed that 5 was produced first and that 6 was formed gradually as the major product from 5 via a 4 → 6 acyl migration in the ethanol-containing phosphate buffer. This acyl migration did not require the enzyme since it occurred in the ethanol-containing phosphate buffer in the absence of enzyme. Similar 4 → 6 acyl migration has been observed in glucose and sucrose derivatives.

The main product obtained from the hydrolysis of 1 by chymotrypsin was the 2,3,4,6,1',3',4'-hepta-acetate (7).

The structures of the products 2–7 were assigned mainly on the basis of the 1H-n.m.r. data in comparison with those for 1 and other partially acetylated sucrases which have been published. The signals of α-protons in primary (C₆H₂OAc) and secondary (C₆HOAc) esters and β-protons (C₆H–CHOAc) are shifted upfield by 1.15, 0.65, and 0.25 p.p.m., respectively, after deacetylation. For 2, the H-1' signal of 2 was shifted upfield from δ 4.04–4.33, where it usually occurs in 1, to δ 3.48–3.62, and the splittings (q dd) (Fig. 1) strongly indicated deacetylation in the 1'-position. The 1H-n.m.r. spectrum of 4 (Fig. 2A) was the same as that of the 2,3,4,6,1',3',4'-hepta-acetate. Compounds 5 and 7, which have been prepared by chemical methods, had similar 1H-n.m.r. spectra, but they could be differentiated on the basis of the 2D 1H–1H COSY spectra (Figs. 2B and 3) and the formation of 6 from 5 by acyl migration but not from 7. The H-4 signal of 6 was shifted upfield from δ 5.25–5.35 in 1 to δ 4.36 in 6 and indicated deacetylation in the 4-position (Fig. 2C). In the 1H-n.m.r. spectrum of 3, the region δ 4.04–4.33, which was crowded in 1, was better resolved and the assignments were determined from the 2D 1H–1H COSY spectra (Fig. 4). The H-1' and H-6' resonances of 3 were shifted upfield and indicated deacetylation in the 1'- and 6'-positions.

The hepta-acetates 5 and 7 have been prepared by selective tritylation, full acetylation, and detritylation. However, these methods are tedious. An alternative approach involves the selective deacetylation of 1 on a column of alumina or silica gel, which gave 2, 4, 7, and the 2,3,4,6,1',4',6'-hepta-acetate. However, the products were not well separated and the yields were < 10%. Compared with these methods, enzymic deacetylations have several advantages: (a) due to the regioselectivity of the enzymes, the mixtures of products are less complex, (b) the products are more readily purified and obtained in better yields, and (c) the procedures are simpler and more efficient.
Fig. 1. A, Partial $^1$H-n.m.r. spectrum of 2. B, 2D $^1$H-$^1$H COSY spectrum of 2.
Fig. 2. Partial $^1$H-n.m.r. spectra: A, 4; B, 5; and C, 6.
Fig. 3. A, Partial $^1$H-n.m.r. spectrum of 7. B, 2D $^1$H-$^1$H COSY spectrum of 7.
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Fig. 4. A, Partial \(^1\)H-n.m.r. spectrum of 3; B, 2D \(^1\)H–\(^1\)H COSY spectrum of 3.

EXPERIMENTAL

_Candida_ lipase (Candida _cylindracea_) and chymotrypsin were purchased from Sigma, Lipase AP6 (_Aspergillus niger_) and protease N from Amano (Japan), alcalase from NOVO (Denmark), and lipase OF and MY (_Candida cylindracea_) from Meito Sangyo (Japan). T.l.c. was performed on Silica Gel 60 G (Merck), using MeOH–ether (1:100). Optical rotations were measured with a polartronic universal polarimeter.
1H-N.m.r. spectra (internal Me,Si) were recorded with a 300-MHz Bruker instrument. Octa-O-acetylsucrose (1) was synthesised by the established method, and its 1H- and 13C-n.m.r. spectra agreed with those published.

**General procedure for the enzymic hydrolysis of 1.** — To a solution of 1 (1 g, 1.47 mmol) in either 0.1M phosphate buffer (50 mL, pH 7.0) containing 0.2M NaCl and 3 mM CaCl₂ or the same buffer (40 mL) containing organic solvent (10 mL, see Table I) was added the desired enzyme (2.5 g). The mixture was stirred at 37°C and the reaction was monitored by t.l.c. with detection by charring with sulfuric acid. The following R_f values were recorded: 10.85, 20.53, 30.31, 40.50, 50.51, 60.53, 70.51. The reaction was stopped by extracting the products with ethyl acetate. The extract was concentrated under reduced pressure and the residue was subjected to column chromatography on silica gel with elution by MeOH–ether (1:100). The purity of products was confirmed by h.p.l.c. with a C₁₈ reverse-phase column, using 25% acetonitrile in water at 2 mL/min and u.v. detection (214 nm). The retention times were as follows: 2 17.0, 3 7.1, 4 17.7, 5 17.8, 6 19.0, 7 17.5 min. The yields are given in Table I and the following compounds were obtained in this manner.

**3,4,6-Tri-O-acetyl-β-D-fructofuranosyl 2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside (2).** — Compound 2 (syrup) had [α]_D^{25} + 43° (c 2, chloroform). N.m.r. data (CDCl₃): 
- 1H, δ 1.99–2.19 (m, 21 H, 7 AC), 3.48 (d, 1 H, J₂₁,₁₂ 12.6 Hz, H-1'ₐ), 3.62 (d, 1 H, H-1'b), 4.04–4.33 (m, 6 H, H-5,6,5',6',6'), 4.96 (dd, 1 H, J₁₂,₂ 3.7, J₂₂,₂ 10.4 Hz, H-2), 5.06 (dd, 1 H, J₃₄ = J₄₅ = 9.8 Hz, H-4), 5.30–5.53 (m, 3 H, H-3,3',4'), 5.64 (d, 1 H, H-1); 13C, δ 20.45 (7 C), 61.61, 63.28, 63.50, 68.06, 68.20, 69.56, 70.00, 74.57, 76.06, 78.49, 89.60, 105.01, 169.36–170.53 (7 C).

**Anal.** Calc for C₆₆H₃₆O₁₈: C, 49.06; H, 5.66. Found: C, 48.95; H, 5.65.

**3,4-Di-O-acetyl-β-D-fructofuranosyl 2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside (3).** — Compound 3 had m.p. 125–128° (from ether), [α]_D^{25} + 45° (c 2, chloroform). N.m.r. data (CDCl₃): 
- 1H, δ 1.99–2.19 (m, 18 H, 6 AC), 3.53–3.83 (m, 4 H, H-1',1',6',6'), 4.02–4.27 (m, 4 H, H-5,6,5',6'), 4.88 (dd, 1 H, J₁₂,₂ 3.6, J₂₂,₂ 10.4 Hz, H-2), 5.06 (dd, 1 H, J₃₄ = J₄₅ = 9.5 Hz, H-4), 5.38–5.49 (m, 3 H, H-3,3',4'), 5.64 (d, 1 H, H-1); 13C, δ 20.55–20.70 (6 C), 61.39, 61.54, 64.01, 68.03, 68.65, 69.46, 70.22, 73.98, 76.68, 81.66, 90.01, 104.83, 168.99–170.65 (6 C).

**Anal.** Calc for C₆₆H₃₆O₁₈: C, 48.32; H, 5.70. Found: C, 48.15; H, 5.65.

**1,3,6-Tri-O-acetyl-β-D-fructofuranosyl 2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside (4).** — Compound 4 (syrup) had [α]_D^{25} + 52° (c 2, chloroform); lit. [α]_D^{25} + 53.8° (chloroform). N.m.r. data (CDCl₃): 
- 1H, δ 1.99–2.19 (m, 21 H, 7 AC), 4.00–4.38 (m, 9 H, H-5,6,6',5',6'), 4.85 (dd, 1 H, J₁₂,₂ 3.7, J₂₂,₂ 10.3 Hz, H-2), 5.00 (dd, 1 H, J₃₄ = J₄₅ = 9.7 Hz, H-4), 5.21 (d, 1 H, J₃₂₄ 7.8 Hz, H-3'), 5.39–5.42 (m, 2 H, H-3, H-3'), 5.63 (d, 1 H, J₂₂₁₂ 3.7 Hz, H-1); 13C, δ 20.55–20.65 (7 C), 62.13, 63.42, 63.99, 68.25, 68.53, 69.70, 70.03, 73.00, 78.33, 80.31, 88.89, 102.72, 169.51–171.17 (7 C).

**1,3,4,6-Tetra-O-acetyl-β-D-fructofuranosyl 2,3,4-tri-O-acetyl-α-D-glucopyranoside (5).** — Compound 5 (syrup) had [α]_D^{25} + 44.5° (c 2, chloroform); lit. [α]_D^{25} + 43.4° and +48.1° (chloroform). N.m.r. data (CDCl₃ + 10% of C₆D₆): 
- 1H, δ 1.68–2.00 (m, 21 H, 7 AC), 3.58 (dd, 1 H, Jₘₘₙₙ 5.2, Jₘₘₙₙ 12.4 Hz, H-6a), 3.70 (dd, 1 H, J₂₆₅ 2.1 Hz, H-6b),
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4.10–4.40 (m, 6 H, H-5,1',1',5',6',6'), 4.83 (dd, 1 H, J_{1,2} 3.6, J_{2,3} 10.4 Hz, H-2), 4.98 (dd, 1 H, J_{3,4} = J_{4,5} = 10 Hz, H-4), 5.37–5.50 (m, 3 H, H-3,3',4'), 5.70 (d, 1 H, H-1); ^{13} C, \delta 20.17–20.57 (7 C), 62.75, 63.44, 63.90, 69.60, 71.18, 71.60, 72.34, 75.91, 76.26, 80.02, 90.96, 104.93, 169.72–171.14 (7 C).

1,3,4,6-Tetra-O-acetyl-\beta-D-fructofuranosyl 2,3,6-tri-O-acetyl-\alpha-D-glucopyranoside (6). — Compound 6 (syrup) had [\alpha]^{25}_{D} +42^\circ (c 2, chloroform); lit. ^{19} [\alpha]^{25}_{D} +48^\circ (chloroform). N.m.r. data (CDCl_3): ^{1} H, \delta 1.68–2.00 (m, 21 H, 7 AC), 3.50–3.64 (m, 1 H, H-4), 4.2M.26 (m, 1 H, H-5), 4.31-4.47 (m, 5 H, H-6,6,1',5',5'), 4.57-4.63 (m, 2 H, H-6',6'), 4.97 (dd, 1 H, J_{2,3} 3.6, J_{2,3} 10.3 Hz, H-2), 5.53 (dd, 1 H, J_{3,4} 5.4 Hz, H-3), 5.69–5.75 (m, 2 H, H-3',4'), 5.83 (d, 1 H, H-1); ^{13} C, \delta 20.15–20.57 (7 C), 62.75, 63.44, 63.90, 69.60, 71.18, 71.60, 72.34, 75.91, 76.26, 80.02, 90.96, 104.93, 169.72–171.14 (7 C).

1,3,4,6-Tetra-O-acetyl-\beta-D-fructofuranosyl 2,3,4,6-tetra-O-acetyl-\alpha-D-glucopyranoside (7). — Compound 7 had m.p. 158–160°, [\alpha]^{25}_{D} +51^\circ (c 2, chloroform); lit. ^{15} [\alpha]^{25}_{D} +52.5^\circ and +53.5^\circ (chloroform). N.m.r. data (CDCl_3): ^{1} H, \delta 1.99–2.19 (m, 21 H, 7 AC), 3.64 (dd, 1 H, J_{a,6a} 3 Hz, H-6'a), 3.82 (dd, 1 H, J_{a,6b} 12.7 Hz, H-6'b), 4.05 (m, 1 H, H-5'), 4.08-4.35 (m, 5 H, H-5,6,6,1',1'), 4.85 (dd, 1 H, J_{1,2} 3.5, J_{2,3} 10.3 Hz, H-2), 5.06 (dd, 1 H, J_{3,4} 9.6 Hz, H-4), 5.40–5.50 (m, 3 H, J_{X,Y} 7.8 Hz, H-3,3',4'), 5.65 (d, 1 H, H-1); ^{13} C, \delta 20.15–20.31 (7 C), 61.19, 61.45, 63.92, 68.01, 68.76, 69.42, 70.28, 73.72, 76.15, 81.66, 90.10, 103.30, 169.70–170.36 (7 C).

REFERENCES