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Sucrose octabenzoate: assignment of ^{13}C and ^{1}H resonances of the sucrose moiety and the ^{13}C resonances of the carbonyl carbons. Use of ^{13}C-n.m.r. spectroscopy for the study of selective deacylation*

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ABSTRACT

Assignment of the ^{1}H and ^{13}C signals arising from the carbohydrate portion of sucrose octabenzoate has been achieved using homonuclear shift correlation experiments (COSY) and one-bond ^{1}H–^{13}C heteronuclear shift correlation measurements, respectively. The ^{13}C resonances of the carbonyl carbon atoms of the eight benzoyl groups are readily distinguished for solutions in benzene-d_6–pyridine-d_5 (1:1), and have been assigned by means of three-bond ^{1}H–^{13}C shift correlation studies coupled with measurement of the ^{13}C-n.m.r. spectrum of a sucrose octabenzoate specifically labelled with ^{13}C in some of the carbonyl groups. With this assignment, products of partial deacylation of the octabenzoate may readily be identified by treatment with excess of benzoyl-*carbonyl*-^{13}C chloride followed by measurement of the ^{13}C-n.m.r. spectrum of the labelled sucrose octabenzoate, so prepared, in the carbonyl region.

INTRODUCTION

Partially *O*-substituted carbohydrates are of considerable importance in synthetic studies. Such compounds may be prepared by selective introduction of protecting groups[1] or by their selective removal[2] from fully substituted derivatives. In the case of oligosaccharides, determination of the positions of substituents may sometimes prove difficult or impossible by n.m.r. spectroscopic measurements on the partially protected compounds, and there is a need for the rapid identification of structure in such cases. The use of isotopically labelled protecting groups offers a relatively quick and unambiguous solution to this problem, and previous work by Horton and Lauterback[3] has shown how the products of partial acetylation of methyl α-D-glucopyranoside may be

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readily identified by means of ^{1}H-n.m.r. spectroscopy on the peracetylated derivatives obtained after treatment with acetic anhydride-d_6. A similar technique has recently been applied to the identification of sucrose acetates[4]. Fundamental to the success of this type of approach is the identification of characteristic separate resonances arising from each of the protecting groups on the various positions in the fully substituted derivative.

In connection with studies of the preparation of partially *O*-benzoylated sucroses, we have developed a rapid method for allocating substituent positions based on measurement of the ^{13}C chemical shifts of carbonyl carbon atoms in sucrose octabenzoate (**1**) and, in the process, have carried out an assignment of the ^{1}H and ^{13}C resonances of the carbohydrate moiety.

RESULTS AND DISCUSSION

In the ^{13}C-n.m.r. spectrum (100.4 MHz, $CDCl_3$) of sucrose octabenzoate[5] (**1**), only five signals are resolved in the carbonyl region, owing to overlap of three pairs of resonances. However, the spectrum of a ~15% solution of the ester in benzene-d_6–pyridine-d_5 (1:1) affords eight separate signals in this region, with δ 165.54, 165.63, 165.69, 165.75, 165.99, 166.05, 166.10, and 166.18*. Clearly, under these conditions, a three-bond ^{1}H–^{13}C shift correlation experiment allows an assignment to be made of the ^{13}C resonances to the individual carbonyl groups, if all of the protons in the carbohydrate moiety of the octabenzoate have been previously assigned.

The ^{1}H-n.m.r. spectrum (399.65 MHz) of a solution of **1** in benzene-d_6–pyridine-d_5 exhibits a low-field doublet at δ 6.59 (J 3.7 Hz), readily identified as the signal for H-1†, and a doublet at δ 6.42 (J 5.0 Hz) which arises from H-3′. A conventional homonuclear shift correlation experiment allowed assignment of the remaining ^{1}H-resonances arising from the carbohydrate portion of the molecule (Table I), although overlap of signals for H-1′a and H-1′b with those for H-6′a and H-6′b precluded determination of the precise chemical shifts of the latter pair of protons. Proton–proton coupling constants, with the exception of $J_{6'a,6'b}$, $J_{5',6'a}$, and $J_{5',6'b}$, could also be obtained directly from the spectrum; the sum $J_{5',6'a} + J_{5',6'b}$ (10 Hz) could be measured from the multiplet for H-5′ centred at δ 4.81.

A one bond ^{1}H–^{13}C heteronuclear shift correlation experiment allowed assignment of all of the resonances in the ^{13}C spectrum of the ester arising from the carbohydrate moiety with the exception of those for C-1′ and C-6′. Owing to the very similar ^{1}H chemical shifts of H-1′a, H-1′b, H-6′a, and H-6′b, which all lie in the range 5.00–5.03 p.p.m., the ^{13}C resonances at 64.8 and 65.3 p.p.m. can be correlated either with the resonances of protons on C-1′ and C-6′, respectively, or *vice versa*.

A long-range ^{1}H–^{13}C shift correlation study (Fig. 1) for resonances of the carbohydrate protons and those of the carbonyl carbons led to an unequivocal assignment of

*The differences between the chemical shifts of the carbonyl carbon resonances are relatively invariant ($< \pm$ 0.005 p.p.m.) and the sequence of peaks is unaltered within the concentration range 5–25% (w/v); all spectroscopic measurements were conducted within this range.

† Unprimed and primed numbers refer to the glucose and fructose moieties of sucrose, respectively.

TABLE I

^1H and ^{13}C chemical shifts (p.p.m.) and ^1H–^1H coupling constants (Hz, first-order analysis) for the D-glucopyranose and D-fructofuranose moieties of sucrose octabenzoate measured in C_6D_6–C_5D_5N (1:1)

Atom[a]	^1H	$^3J_{H,H}$		^{13}C	*CO*
1	6.59	$J_{1,2}$	3.7	91.7	—
2	5.90	$J_{2,3}$	10.4	72.0	165.99
3	6.72	$J_{3,4}$	10.0	71.1	166.05
4	6.25	$J_{4,5}$	9.9	69.9	165.54
5	5.17	$J_{5,6a}$	3.8	69.9	—
		$J_{5,6b}$	3.1		
6a	4.71	$J_{6a,6b}$	−12.6	63.0	166.10
6b	4.85				
1'a	5.01	$J_{1'a,1'b}$	−12.1	64.8 or 65.3	165.75
1'b	5.03				
2'	—	—		105.6	—
3'	6.42	$J_{3',4'}$	5.0	73.8	165.63
4'	6.30	$J_{4',5'}$	5.9	77.6	165.69
5'	4.81	$J_{5',6'a} + J_{5',6'b}$	10.0	80.2	—
6'a, 6'b	5.00–5.03	[b]		65.3 or 64.8	166.18

[a] Unprimed and primed numbers refer to the D-glucose and D-fructose moieties of sucrose, respectively.
[b] $J_{6'a,6'b}$ could not be determined.

the six carbon resonances at δ 165.99, 166.05, 165.54, 166.10, 165.63, and 165.69 to carbonyl carbons attached to O-2, O-3, O-4, O-6, O-3', and O-4', respectively. However, because of the close similarity of the ^1H chemical shifts of H-1'a, H-1'b, H-6'a, and H-6'b, a decision could not be made regarding allocation of the remaining two carbon resonances at 165.75 and 166.18 p.p.m. to the carbonyl groups at O-1' and O-6'.

In order to complete the assignment of carbonyl resonances in sucrose octabenzoate, 3,3',4',6'-tetra-*O*-benzoylsucrose[6] (**2**) was acylated with 10 atom % benzoyl-*carbonyl*-^{13}C chloride in pyridine, to afford the octabenzoate containing benzoyl groups at O-2, O-4, O-6, and O-1' enriched with ^{13}C in the carbonyl carbon atom. Comparison of the carbonyl region of the ^{13}C-n.m.r. spectrum of this labelled compound with that of the unenriched ester allowed unequivocal assignment of the resonance at 165.75 p.p.m. to the carbonyl carbon attached to O-1' and, hence, that at 166.18 p.p.m. to the carbonyl group at O-6'.

Selective deacylation of sucrose octabenzoate was studied in isopropylamine. Storage of a 20% solution of the ester in the amine for 21 h at room temperature led, as indicated by t.l.c., to gradual disappearance of the octabenzoate with formation of a preponderant component, judged by its chromatographic mobility to be a heptabenzoate. Elemental analysis of the major component, which was isolated by column chromatography, was in agreement with its being a heptabenzoate and the material was then acylated with benzoyl-*carbonyl*-^{13}C chloride in pyridine. The ^{13}C-n.m.r. spectrum

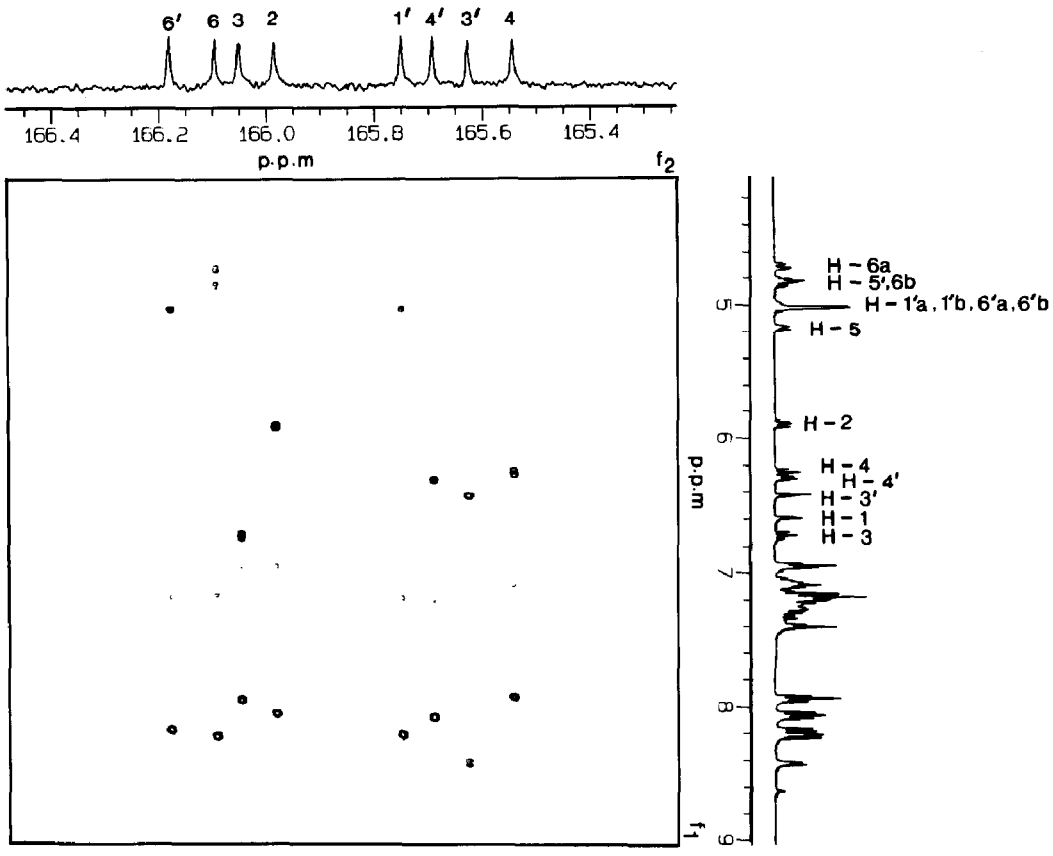
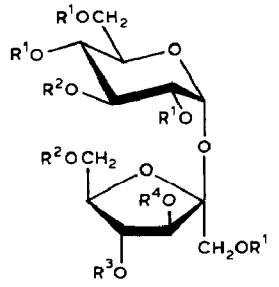


Fig. 1. Long-range ^1H–^{13}C correlation spectrum for the aliphatic hydrogen atoms and the carbonyl carbon atoms of sucrose octabenzoate in C_6D_6–C_5D_5N (1:1). The ^1H- and ^{13}C-n.m.r. spectra are shown along the f_1 and f_2 axes, respectively, and atoms of the D-glucose and D-fructose moieties are numbered 1–6 and 1'–6', respectively. Correlation with the aromatic *m*-hydrogens (6.90–7.50 p.p.m.) and *o*-hydrogens (7.90–8.50 p.p.m.) can also be observed.



1 $R^1 = R^2 = R^3 = R^4 = PhCO$
2 $R^1 = H, R^2 = R^3 = R^4 = PhCO$
3 $R^1 = R^2 = R^4 = PhCO, R^3 = H$
4 $R^1 = R^2 = PhCO, R^3 = R^4 = H$

of the resulting sucrose octabenzoate indicated that a labelled benzoyl group was present at O-4′ and, therefore, that the compound originally isolated was 2,3,4,6,1′,3′,6′-hepta-*O*-benzoylsucrose (**3**).

Treatment of the octabenzoate with the amine for 80 h led to preponderant formation of a new compound having a chromatographic mobility lower than that of the heptabenzoate, and elemental analysis of the chromatographically isolated component indicated it to be a hexabenzoate. ^{13}C-N.m.r. analysis of the octabenzoate obtained after acylation of this compound with labelled benzoyl chloride showed it to be the 2,3,4,6,1′,6′-hexabenzoate (**4**). These experiments indicate that the 3′- and 4′-positions in the octabenzoate are the most susceptible to aminolysis, and a similar result has been obtained in related studies on the selective deacetylation of sucrose octa-acetate[7].

EXPERIMENTAL

The ^{1}H- and ^{13}C-n.m.r. spectra were recorded at 399.65 and 100.4 MHz, respectively, with a JEOL GX400 instrument, using tetramethylsilane as the internal standard.

The ^{1}H COSY spectrum was obtained using a 2048 (f_2) × 512 (f_1) data matrix with a spectral width of 2000 Hz in both dimensions. The one-bond ^{1}H–^{13}C shift correlation spectrum was acquired into a 2048 (^{13}C) × 256 (^{1}H) data matrix with spectral widths of 2000 Hz (^{1}H) and 8000 Hz (^{13}C: 60–140 p.p.m. region). A 1024 (^{13}C) × 512 (^{1}H) point data matrix was employed for the long-range ^{1}H–^{13}C shift correlation experiment with spectral widths of 250 Hz (^{13}C: carbonyl region only) and 2000 Hz (^{1}H). The standard shift correlation sequence was used[8] with refocusing delays, D_1 and D_2, of 3.5 and 1.8 ms, respectively, for the one-bond experiment, and 125 and 62.5 ms for the long-range correlation spectrum. All spectra were examined in the absolute value mode.

T.l.c. was performed on glass plates pre-coated with silica gel (Machery–Nagel SIL G-25UV$_{254}$) with detection by u.v. light at 254 nm. Column chromatography was carried out on Merck Silica Gel 60 (0.063–0.2mm). Diethyl ether was dried by distillation from lithium aluminium hydride and was then stored over sodium wire. Light petroleum refers to the fraction b.p. 40–60°.

Sucrose octabenzoate (**1**). — The ester was prepared by conventional acylation of sucrose with benzoyl chloride (8.8 molar equivalents) in pyridine and isolated in 87% yield as an analytically pure and chromatographically homogeneous foam*, $[\alpha]_D$ +39.1° (*c* 0.6, chloroform); lit.[5] $[\alpha]_D$ +40.6° (chloroform). N.m.r. data ($CDCl_3$): ^{1}H, δ 4.34 (dd, 1 H, $J_{6a,6b}$ −12.4, $J_{5,6a}$ 3.35 Hz, H-6a), 4.43 (dd, 1 H, $J_{5,6b}$ 2.9 Hz, H-6b), 4.57–4.75 (complex, 6 H, H-5,1′a,1′b,5′,6′a,6′b), 5.42 (dd, 1 H, $J_{1,2}$ 3.5, $J_{2,3}$ 10 Hz, H-2), 5.77 (t, 1 H, $J_{3,4} = J_{4,5}$ = 10 Hz, H-4), 5.98 (t, 1 H, $J_{3',4'} = J_{4',5'}$ = 5.5 Hz, H-4′), 6.00 (d, 1 H, H-3′), 6.20 (d, 1 H, H-1), 6.24 (t, 1 H, H-3), 7.00–8.30 (complex, 40 H, 8 Ph); ^{13}C, δ 62.4, 64.2, 65.0, 69.0, 69.3, 70.1, 71.4, 76.6, 77.7, 79.2, 90.9, 104.8 (C-1–C-6 and C-1′–C-6′), 128.2–130.2

*We were unable to obtain the crystalline adduct with carbon tetrachloride which had been formed[5] on crystallisation of the octabenzoate from carbon tetrachloride–methanol.

(aromatic C), 165.1, 165.4 (×2), 165.5 (×2), 165.8, 166.0 (×2). N.m.r. data measured for a solution in C_6D_6–C_5D_5N (1:1) are collected in Table I; data for carbonyl resonances were obtained from spectra measured at 250-Hz sweep width.

Anal. Calc. for $C_{68}H_{54}O_{19}$: C, 69.5; H, 4.6. Found: C, 69.55, H, 4.85.

Benzoyl-carbonyl-^{13}C *chloride (10 atom % ^{13}C).* — Benzoic acid and benzoic-*carboxy*-^{13}*C* acid were combined in the weight ratio of 9:1. A portion (0.261 g, 2.14 mmol) of this mixture was dissolved in dry ether (5 mL) containing *N*,*N*-dimethylformamide (5 μL), and oxalyl chloride (0.196 mL, 2.25 mmol) was added. After storage at room temperature for 1 h, the solvent was removed under reduced pressure (oil pump) to afford the crude benzoyl chloride, which was used without further purification.

3,3′,4′,6′-Tetra-O-*benzoyl-2,4,6,1′-tetra*-O-*benzoyl*-carbonyl-^{13}C-*sucrose.* — A solution of 3,3′,4′,6′-tetra-*O*-benzoylsucrose[6] (**2**, 0.09 g) in pyridine (1 mL) was added to benzoyl-*carbonyl*-^{13}*C* chloride (10 atom % ^{13}C) prepared from labelled benzoic acid (0.261 g, 2.13 mmol). After storage of the mixture for 12 h at room temperature, water (0.30 mL) was added and, after 15 min, the solution was poured into ice-cold, saturated aqueous sodium hydrogencarbonate (25 mL) with stirring. The mixture was extracted with dichloromethane (2 × 25 mL), and the combined organic extracts were back-extracted with aqueous sodium hydrogen carbonate, then water, and dried (Na_2SO_4). Concentration of the solution and removal of pyridine from the residue by co-evaporation with toluene (3 × 2 mL) afforded a syrup (0.131 g), which in t.l.c. (light petroleum–ethyl acetate, 2:1) was shown to consist of a component running identically with sucrose octabenzoate and a small amount of faster-running material. The latter contaminant was removed by chromatography on silica gel with light petroleum–ethyl acetate (2:1), to give the chromatographically homogeneous ester (0.089 g). The ^{13}C-n.m.r. spectrum of a solution of this material in C_6D_6–C_5D_5N (1:1) (0.55 mL) showed enhanced peaks at 165.57, 165.78, 166.01, and 166.13 p.p.m., corresponding to carbonyl carbons at O-4, O-1′, O-2, and O-6, respectively, and natural abundance peaks at 165.66, 165.73, 166.08, and 166.21 p.p.m., corresponding to carbonyl carbons at O-3′, O-4′, O-3, and O-6′, respectively.

Partial O-*debenzoylation of sucrose octabenzoate with isopropylamine. (a) For 21 h.* — A solution of sucrose octabenzoate (**1**, 1 g) in isopropylamine (5 mL) was stored at room temperature for 21 h. T.l.c. (light petroleum–ethyl acetate, 2:1) indicated the formation of a major component with R_F 0.26, shown to be the 2,3,4,6,1′,3′,6′-heptabenzoate, which, after removal of the excess of amine under reduced pressure and column chromatography of the residue on silica gel with light petroleum–ethyl acetate (7:2), was isolated as an oil (0.22 g), $[\alpha]_D$ +67° (*c* 1.1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 4.25–4.80 (complex, 10 H, H-5,6a,6b,1′a,1′b,4′,5′,6′a,6′b, and OH), 5.41 (dd, 1 H, $J_{1,2}$ 3.7, $J_{2,3}$ 10 Hz, H-2), 5.57 (d, 1 H, $J_{3',4'}$ 7.8 Hz, H-3′), 5.64 (t, 1 H, $J_{3,4} = J_{4,5} = 10$ Hz, H-4), 6.13 (d, 1 H, H-1), 6.25 (t, 1 H, H-3), 7.15–8.19 (complex, 35 H, 7 Ph).

Anal. Calc. for $C_{61}H_{50}O_{18}$: C, 68.4; H, 4.7. Found: C, 68.3; H, 4.75.

A solution of this syrup (0.1 g, ~0.1 mmol) in pyridine (1 mL) was added to benzoyl-*carbonyl*-^{13}*C* chloride (10 atom % ^{13}C) prepared from labelled benzoic acid (0.61 g, 0.5 mmol). After storage for 12 h at room temperature, the mixture was

processed in the manner described for the related preparation from **2**. Trace contaminants in the labelled sucrose octabenzoate were removed by column chromatography on silica gel, using light petroleum–ethyl acetate (2:1) as eluent. The ^{13}C-n.m.r. spectrum of a solution of the octabenzoate in C_6D_6–C_5D_5N (1:1, 0.55 mL) showed an enhanced peak at δ 165.69 (C=O carbon at O-4′) and natural abundance peaks at δ 165.54, 165.63, 165.75, 165.99, 166.05, 166.10, and 166.18 (C=O carbons at O-4,3′,1′,2,3,6, and 6′, respectively).

(*b*) For 80 h. A solution of sucrose octabenzoate (**1**. 1.5 g) in isopropylamine (10 mL) was stored for 80 h at room temperature, after which time t.l.c. [light petroleum–ethyl acetate (2:1)] showed the formation of a major product with R_F 0.07. Excess of amine was removed under reduced pressure and the residue was chromatographed on a column of silica gel with light petroleum–ethyl acetate (7:2 changing gradually to 3:2), to give the major product, shown to be the 2,3,4,6,1′,6′-hexabenzoate **4**, as a syrup (0.314 g), $[\alpha]_D$ +49.3° (*c* 0.57, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 4.00–4.90 (complex, 12 H, H-5,6a,6b,1′a,1′b, 3′,4′,5′,6′a,6′b, 2 OH), 5.34 (dd, 1 H, $J_{1,2}$ 3.7, $J_{2,3}$ 10 Hz, H-2), 5.67 (t, 1 H, $J_{3,4} = J_{4,5}$ = 10 Hz, H-4), 6.09 (d, 1 H, H-1), 6.24 (t, 1 H, H-3), 7.10–8.20 (complex, 30 H, 6 Ph).

Anal. Calc. for $C_{54}H_{46}O_{17}$: C, 67.1; H, 4.8. Found: C, 66.7; H 4.8.

A solution of **4** (0.093 g, ~0.1 mmol) in pyridine (1 mL) was added to benzoyl-*carbonyl-^{13}C* chloride (10 atom % ^{13}C) prepared from labelled benzoic acid (0.104 g, 0.85 mmol). After storage of the mixture for 12 h at room temperature, the labelled sucrose octabenzoate was isolated as described for the analogous preparation from the product of the shorter reaction time. The ^{13}C-n.m.r. spectrum of a solution of this material in C_6D_6–C_5D_5N (1:1, 0.55 mL) showed enhanced peaks at 165.64 and 165.70 p.p.m. (C=O carbon at O-3′ and O-4′, respectively), and natural abundance peaks at 166.55, 165.76, 166.00, 166.06, 166.11, and 166.20 p.p.m. (C=O carbons at O-4, O-1′, O-2, O-3, O-6, and O-6′, respectively).

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