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Note

N.m.r. method for the identification of sucrose acetates*

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Chemical and enzymic modifications of sucrose have frequently generated sucrose acetates as synthetic intermediates. As interest in the use of this disaccharide as a chemical feedstock increases the need for a convenient method of identification of sucrose acetates likewise becomes more apparent.

As part of a research programme on the generation and characterisation of sucrose esters, the present article describes a study of sucrose acetates by 250-MHz ^{1}H-n.m.r. spectroscopy. Unambiguous identification of any sucrose acetate is made possible by comparison of its ^{1}H-n.m.r. acetate signal pattern, after per-*O*-deuterioacetylation, with that of assigned sucrose octaacetate. In addition, application to mixtures of sucrose acetates, produced either by selective *O*-acetylation of sucrose or *O*-deacetylation of sucrose octaacetate, would provide valuable information on reaction selectivity and rate. An earlier investigation[1] on the ^{1}H-n.m.r. spectroscopy of sucrose octaacetate reported incomplete separation of the acetate signals (at 220 MHz) when using benzene-d_6 (7 signals), acetone-d_6 (5 signals), or chloroform-*d* (4 signals) as solvent. Suami *et al.*[2] subsequently used a perdeuterioacetylation approach in a study of sucrose pentaacetates. The investigation was carried out at 100 MHz and $CDCl_3$ was used as solvent, under which conditions six signals were obtained for the eight acetate groups of sucrose octaacetate. More recently, the six acetate signals observed in $CDCl_3$ were assigned by using heteronuclear 2D n.m.r. techniques[3].

RESULTS AND DISCUSSION

None of the individual solvents evaluated produced adequate separation of the acetate signals of sucrose octaacetate (**1**); the best solvents for this purpose were bromobenzene-d_5 (the best individual solvent), pyridine-d_5 (insufficient separation of high-field signals) and benzene-d_6 (insufficient separation of low-field signals). Benzene

* Dedicated to Professor Leslie Hough in the year of his 65th birthday.

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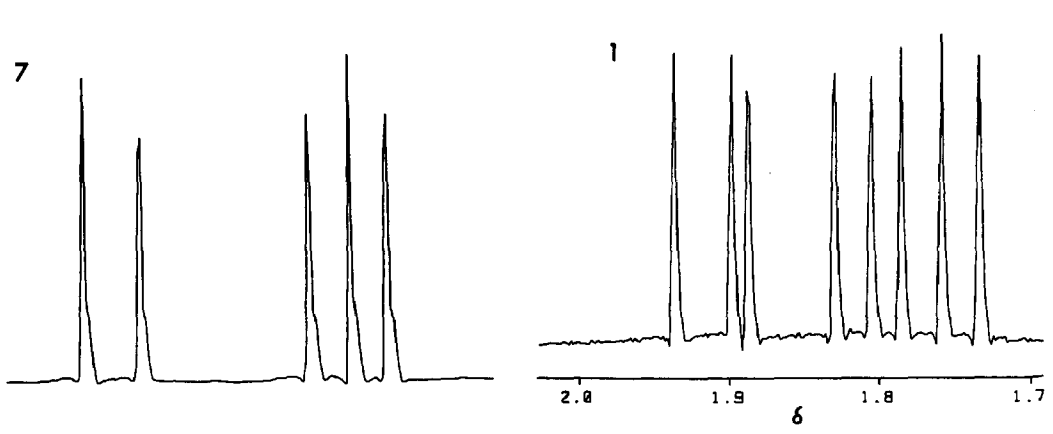
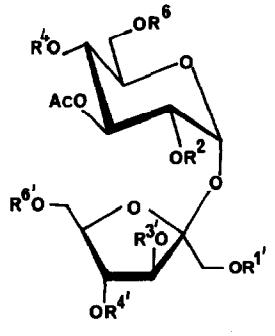


Fig. 1. Comparison of the ^{1}H-n.m.r. spectra of **1** and **7** in the acetate-resonance region. Solvent: 1:1 benzene-d_6–pyridine-d_5.



Ac = $COCH_3$
Ac-d_3 = $COCD_3$

Compound Number	R^2	R^4	R^6	$R^{1'}$	$R^{3'}$	$R^{4'}$	$R^{6'}$
1	Ac	Ac	Ac	Ac	Ac	Ac	Ac
2	Ac	Ac	H	H	Ac	Ac	H
3	Ac	H	Ac	H	Ac	Ac	H
4	H	Ac	Ac	H	Ac	Ac	Ac
5	Ac	Ac	Ac	Ac	Ac	H	Ac
6	H	Ac	Ac	H	H	Ac	Ac
7	Ac	Ac	Ac-d_3	Ac-d_3	Ac	Ac	Ac-d_3
8	Ac	Ac-d_3	Ac	Ac-d_3	Ac	Ac	Ac-d_3
9	Ac-d_3	Ac	Ac	Ac-d_3	Ac	Ac	Ac
10	Ac	Ac	Ac	Ac	Ac	Ac-d_3	Ac
11	Ac-d_3	Ac	Ac	Ac-d_3	Ac-d_3	Ac	Ac

and pyridine produce the largest spread of signals (0.27 p.p.m., compared with 0.13–0.19 p.p.m. for the other six solvents).

Mixtures of benzene-d_6 and pyridine-d_5 in varying proportions were evaluated to obtain improved separation of the acetate signals of **1**. A ratio of 1:1 gave baseline separation of all signals (spread = 0.2 p.p.m.) and was selected for further work.

To ensure consistency in solvent composition from sample to sample, a stock solution of benzene-d_6–pyridine-d_5 was used when studying a series of sucrose acetates. The acetyl chemical shifts of the sucrose octaacetate reference was re-measured for each new batch of solvent mixture and whenever the spectrum of an unknown sucrose acetate was determined.

Compounds **2–6** were *O*-deuterioacetylated and the ^{1}H-n.m.r. spectra of the products (**7–11**) recorded for each solvent used. Comparison of the patterns of acetate signals in the n.m.r. spectra of **7–11** with those of **1** allowed the identification of the acetate signals of the latter. The results are produced in Table I and illustrated in Fig. 1 for compound **7**.

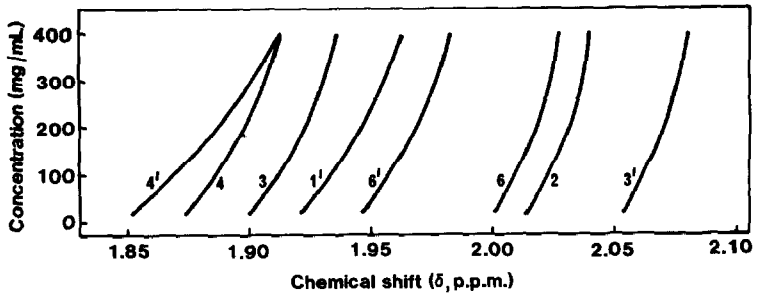


Fig. 2. Variation of acetyl-group chemical shifts with sample concentration. Solvent: 1:1 benzene-d_6–pyridine-d_5. The numbers of the curves denote acetyl-group positions.

The chemical shifts of the acetate groups of **1** showed significant variation with sample concentration when using benzene-d_6–pyridine-d_5 (1:1) as solvent (Fig. 2), dilute solutions (<15% w/v) being required for adequate separation between the 4'-and 4-*O*-acetyl signals.

TABLE I

^{1}H-n.m.r. chemical shifts for the acetate signals of sucrose octaacetate in various solvents

Solvent	*Chemical shifts (δ) of acetate signals*[a]							
	2	*3*	*4*	*6*	*1'*	*3'*	*4'*	*6'*
Chloroform-*d*	2.099	2.018	2.046	2.102	2.118	2.177	2.111	2.118
Acetone-d_6	2.076	1.979	2.017	2.043	2.080	2.162	2.080	2.078
Dimethyl sulphoxide-d_6	2.023	1.970	2.002	2.021	2.057	2.099	2.064	2.051
Acetonitrile-d_3	2.028	1.966	1.987	2.014	2.051	2.101	2.040	2.051
Pyridine-d_5	2.151	2.065	2.047	2.136	2.087	2.211	2.047	2.095
Benzene-d_6	1.867	1.700	1.682	1.875	1.740	1.875	1.602	1.780
Toluene-d_8	1.886	1.706	1.691	1.886	1.768	1.912	1.644	1.799
Bromobenzene-d_5	1.984	1.860	1.820	1.970	1.889	2.010	1.830	1.906
Benzene-d_6–pyridine-d_5 (1:1)	2.013	1.899	1.873	2.000	1.920	2.053	1.851	1.945

[a] Chemical shifts (p.p.m.) are relative to internal tetramethylsilane.

It has been demonstrated that, by using a mixed solvent system, it is possible to obtain eight well-resolved signals (at 250 MHz) for the acetyl group resonances of sucrose octaacetate. This n.m.r. method has been used successfully in the identification of a number of new sucrose acetates and applied to the analysis of reaction mixtures to obtain information on reaction selectivity and rate. It is particularly informative when combined with g.l.c. or h.p.l.c. results on a mixture.

EXPERIMENTAL

Spectra. — ^{1}H-N.m.r. spectra were obtained on a Bruker WM 250 spectrometer, using sample concentrations of 2% (w/v) unless otherwise stated.

Materials. — Sucrose octaacetate (**1**) was obtained from Sigma Chemical Co. Ltd. Acetic anhydride-d_6 (99+ atom% D) and deuterated n.m.r. solvents were purchased from Aldrich Chemical Co. Ltd. 2,3,4,3′,4′-Penta-*O*-acetylsucrose[4] (**2**), 2,3,6,3′,4′-penta-*O*-acetylsucrose[5,6] (**3**), 3,4,6,3′,4′,6′-hexa-*O*-acetylsucrose[7] (**4**), and 2,3,4,6,1′,3′,6′-hepta-*O*-acetylsucrose[8,9] (**5**), were synthesised according to published procedures and had physical constants in agreement with literature values.

3,4,6,4′,6′-Penta-*O*-acetylsucrose (**6**) was prepared by partial *O*-deacetylation of sucrose octaacetate using sodium isopropoxide in tetrahydrofuran[10].

Per-O-deuterioacetylation. — A sample of sucrose acetate **2–6** (10 mg) was dissolved in pyridine-d_5 (0.2 mL) in a sample vial and acetic anhydride-d_6 (0.2 mL) added. Deuterated pyridine was used to minimise signals in the n.m.r. spectrum due to traces of pyridine remaining in the sample after work-up. The solution was heated for 4 h at 50° and evaporated to dryness under vacuum. The residue in the vial was washed with water (2 × 1 mL) by decantation and dried at 50° under vacuum. The residue was analysed by t.l.c. (Silica Gel 60; eluant 2:1 EtOAc–petroleum spirit 40–60°) to confirm completion of reaction, using **1** as standard (R_F 0.65). The perdeuterioacetylated products (**7–11**) were dissolved in the n.m.r. solvent (0.5 mL) and analysed by ^{1}H-n.m.r. spectroscopy.

ACKNOWLEDGMENTS

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