Long-range Proton–Carbon Chemical Shift Correlation by 1D and 2D N.M.R. Spectroscopy: Structure of a Sucrose Ester

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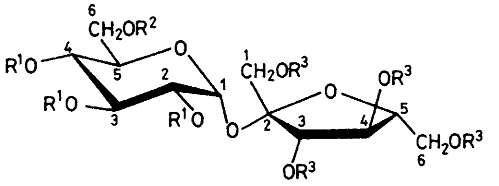
The structure of an octa-acylated derivative of a disaccharide isolated from oriental tobacco has been confirmed with the aid of long-range proton–carbon-13 shift correlation by 2D n.m.r. spectroscopy using a 10 mg sample.

Acylated sucrose derivatives constitute important polar constituents of the cuticular wax of oriental tobaccos; the most abundant component has been shown[1,2] by chemical means to be 6-*O*-acetyl-2,3,4-tri-*O*-[(+)-(3*S*)-methylvaleryl]-α-D-glucopyranosyl-β-D-fructofuranoside (**1**). Other derivatives possess acyl groups (*e.g.* acetyl, 2-methylbutyryl, or 3-methylvaleryl) at various sites on sucrose, and the determination of their structures would be facilitated by the development of more sophisticated spectroscopic methods for characterising the various acyl substituents. We have recently reported[3] the complete proton and carbon-13 spectral assignment of sucrose octa-acetate (**2**), using a two-dimensional (2D) n.m.r. experiment to identify long-range couplings between carbonyl carbons and sugar ring protons. In this communication we describe the use of 2D and 1D long-range heteronuclear chemical shift correlation to confirm the acylation sites of sucrose by acetyl and 3-methylvaleryl groups in (**1**).



(**1**) R^1=$COCH_2Bu^s$, R^2=COMe, R^3=H
(**2**) R^1=R^2=R^3=COMe
(**3**) R^1=$COCH_2Bu^s$, R^2=R^3=COMe

Given the proton and carbon-13 chemical shift assignments for (**2**), the confirmation of structure (**1**) is most readily obtained by studying its penta-acetate (**3**). Structural proof for (**3**) depends on detecting scalar couplings between carbonyl carbons and on the one hand acyl group protons, and on the other the sugar ring CH protons at the points of attachment. Two COLOC[4] experiments were performed on (**3**), using the pulse sequence:

$$90_H\text{–}t_1/2\text{–}180_H,\ 180_C\text{–}t_1/2\text{—}(\Delta_1 - t_1)\text{–}90_H,$$
$$90_C\text{–}\Delta_2\text{–decouple protons, acquire } {}^{13}C$$

The spectrum shown in Figure 1a was obtained using a delay Δ_1 of 0.071 s, chosen to give strongest signals for couplings $^nJ_{CH}$ around 7 Hz, while that of Figure 1b used Δ_1 = 0.104 s, optimised for couplings of about 4.8 Hz. Thus in Figure 1a the

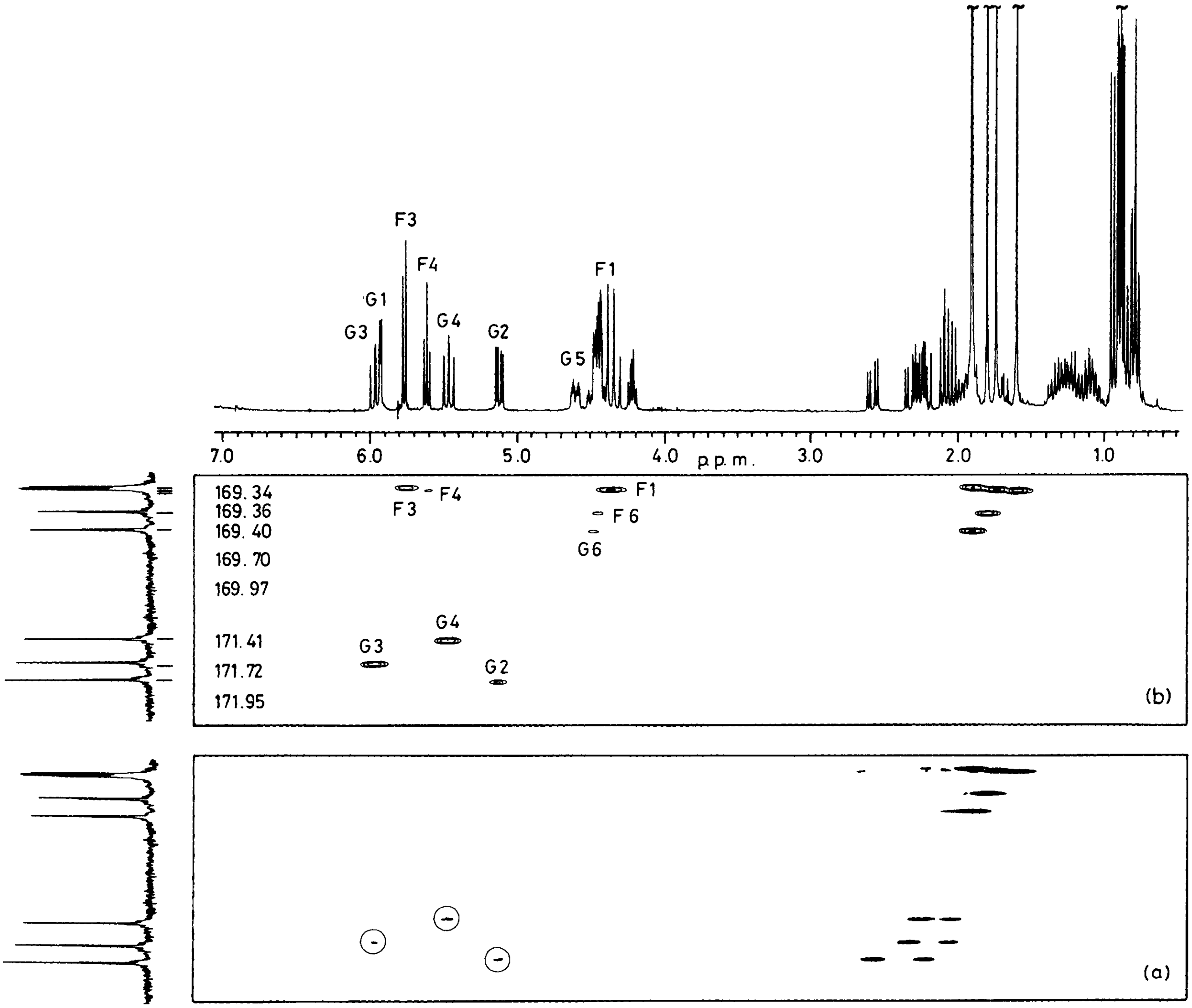


Figure 1. (a) Long-range 2D heteronuclear chemical shift correlation spectrum of a solution of the penta-acetate (**3**) (10 mg) in hexadeuteriobenzene (0.5 ml) obtained on a Varian XL300 spectrometer using the COLOC pulse sequence with delays Δ_1 and Δ_2 of 71 and 48 ms respectively. 512 Transients were measured for each of 128 t_1 increments, using spectral widths of 512 Hz in f_2 and 2028 Hz in f_1; total experimental time was 71 h. (b) As (a) but with Δ_1 = Δ_2 = 104 ms, and 1024 transients for each of 128 t_1 increments in a total time of 100 h. G = glucopyranosyl, F = fructofuranoside.

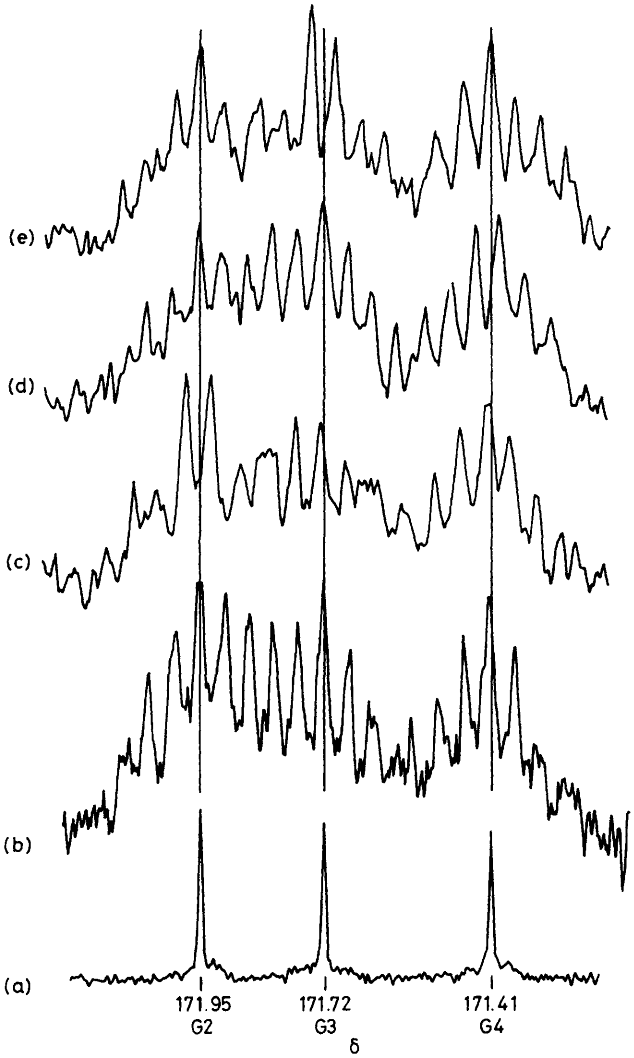


Figure 2. Carbon-13 spectra of the valeryl carbonyl carbons of (**3**), using the same sample as Figure 1 (only the three deshielded carbons are shown). (a) Broadband decoupled spectrum; (b) nuclear Overhauser enhanced proton coupled spectrum; (c)—(e) selectively decoupled spectra using a proton decoupler field strength of 28 Hz, irradiating the G2, G4, and G3 protons respectively. For each of the selectively decoupled spectra, 17 920 transients were acquired in 20 h.

CO–CH_2 cross-peaks are strong while the CH–O–CO peaks are only just visible, while in Figure 1b only the 3-bond CH–O–CO peaks appear. As expected the three valeryl carbonyls, identifiable from their couplings to methylene protons in Figure 1a, appear to low field (171.95, 171.72, and 171.41 p.p.m.). Taken together the two spectra of Figure 1 demonstrate conclusively the presence of 3-methylvaleryl substituents at sites G2, G3, and G4 in (**3**).

It is instructive to compare the possible alternatives to the COLOC experiment. The classical method for identifying long-range proton–carbon couplings is by selective proton decoupling. Figure 2 shows a set of spectra for the three valeryl carbonyls, illustrating the effects of irradiating the glucose protons G2, G3, and G4. The three decoupled spectra demonstrate reasonably clearly the presence of the 3-bond CH–O–CO couplings between acyl groups and ring protons. The total experimental time required was comparable to that for Figures 1a and 1b, but the 2D experiments also provided information on the acetyl group correlations. Given that the signal-to-noise ratio and resolution obtained in the 2D spectra is well in excess of the minimum necessary, the 2D approach is clearly preferable where more than one or two correlations are needed. The COLOC technique is preferable to the simple use of heteronuclear shift correlation with extended Δ_1 and Δ_2 delays[3] where high f_1 resolution is not required and well resolved couplings are expected.

2D heteronuclear chemical shift correlation *via* long range couplings is generally assumed to demand large amounts of sample. As the spectra reported here illustrate, under favourable circumstances useful results are obtainable with quite small quantities of material.

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