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Preliminary communication

Determination of the positions of glycosidic linkages from $^{13}C-^{13}C$ connectivity plots

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N.m.r. spectroscopy is used extensively in the characterization of di-, oligo-, and poly-saccharides. N.m.r.-spectral data (^1H and ^{13}C) are of great value for assigning the anomeric configuration of a glycosyl residue, on the basis of characteristic, chemical shifts for H-1 or C-1, and spin–spin coupling-parameters ($^3J_{H\text{-}1,H\text{-}2}$ or $^1J_{C\text{-}1,H\text{-}1}$).

Information as to the position of a glycosidic linkage may be deduced by ^{13}C-n.m.r. spectroscopy from the fact[1,2] that the carbon atom to which the anomeric C–O bond is attached is strongly deshielded relative to all other carbon atoms. Typically (C-4′ of β-cellobiose resonates ~9 p.p.m. downfield of C-4 of the (nonreducing) end group[1] Expressed somewhat differently, formation of one molecule of the disaccharide from two molecules of β-D-glucose results in marked deshielding of C-4 of one molecule, as well as of C-1 of the second; by contrast, the other carbon atoms exhibit much smaller, chemical-shift displacements ($< \pm 2$ p.p.m.). Generally, therefore, the resonance of a carbon atom engaged in a glycosidic linkage should be recognizable from its location far downfield of that of the corresponding carbon atom of the related monosaccharide.

Clearly, in order to employ ^{13}C chemical-shift correlations in this way, it is essential to identify the resonance signals of the appropriate carbon atoms unequivocally. A variety of techniques are used for assignment purposes, prominent among these being selective ^1H-decoupling, and heteronuclear-correlated 2-D spectroscopy[3,4]. However, the most generally satisfactory basis yet advanced[5,6] for a definitive analysis of ^{13}C-n.m.r. spectra is the two-dimensional version of the INADEQUATE experiment. This experiment makes use of the double quantum coherence generated in the AB spin systems formed by adjacent ^{13}C atoms in a molecule, at natural abundance. From the pattern of spin–spin coupling observed, which can be represented effectively as a "carbon–carbon connectivity plot (CCCP)", the entire sequence of carbon–carbon bonds in a molecule may be determined in a totally unambiguous manner.

A study[7] on β-D-glucose and related compounds by the CCCP technique furnished examples of its utility, as well as a detailed description of the experimental procedures that have been used in this work. Here, the technique is applied to β-cellobiose, in order to demonstrate its potential for locating the positions of glycosidic linkages in higher saccharides.

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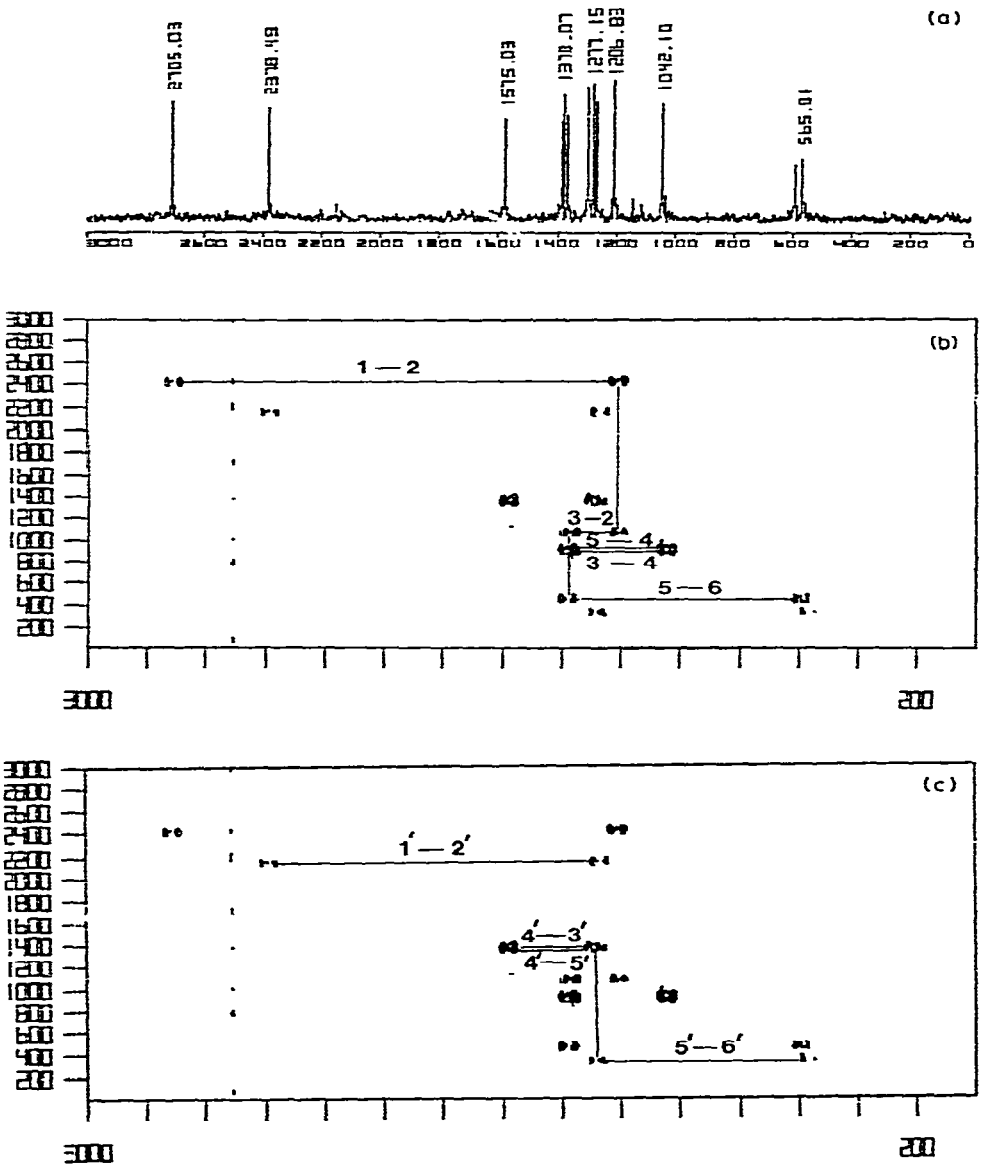

Fig. 1. ^{13}C-N.m.r. spectra of β-cellobiose in Me_2SO-d_6 (1 g/mL): (a) conventional, F.t.-n.m.r. spectrum; (b) full CCCP spectrum, showing connectivities between ^{13}C satellite pairs for the carbon atoms of one D-glucose moiety; (c) full CCCP spectrum, showing connectivities between ^{13}C satellite pairs for the carbon atoms of the second D-glucose moiety. Frequencies in Hz, unreferenced to Me_4Si (for chemical shifts, see Table I); the vertical axis in (b) and (c) lists double quantum frequencies. The series of peaks in (b) and (c) at ~2.500 kHz along the ^{13}C chemical-shift axis results from the aliasing from outside the spectral region of residual, non-suppressed intensity from Me_2SO-d_6.

TABLE I

^{13}C-CHEMICAL SHIFTS (δ) ASSIGNED

Carbohydrate	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
β-Cellobiose [a]						
D-glucopyranosyl	103.39	73.61	76.73	70.33	77.02	61.33
Δ [b]	(+6.49)	(−1.37)	(−0.06)	(−0.09)	(+0.38)	(−0.11)
D-glucopyranose	96.90	74.80	75.31	80.93	75.00	60.87
Δ [b]	(0)	(−0.18)	(−1.48)	(+10.51)	(−1.64)	(−0.67)
β-D-Glucose [a]	96.90	74.80	76.79	70.42	76.64	61.44

[a] In Me_2SO-d_6. [b] Difference in chemical shift relative to β-D-glucose.

The twelve ^{13}C resonance signals of β-cellobiose in dimethyl sulfoxide-d_6, shown in Fig. 1a and listed according to their chemical shifts in Table I, were assigned directly by the CCCP technique from the connectivity plots represented by Figs. 1b and 1c. By interconnecting the appropriate doublets with horizontal or vertical lines, or both, a pattern for the six carbon atoms of one of the D-glucose moieties was obtained (see Fig. 1b). It extends from the doublet, upfield, of one of the pair of methylenic carbon atoms (C-6; by off-resonance decoupling) through to the doublet of the least-shielded nucleus (δ 103.39) which, therefore, is that of an anomeric carbon atom (C-1). For the second D-glucose moiety, connectivity was traced (see Fig. 1c) from the second upfield doublet (C-6′) through to the doublet for C-4′ (δ 80.93), and then to C-3′, whereas the remaining two carbon atoms were accounted for by the connectivity between the doublets due to the other anomeric carbon atom (C-1′, at δ 96.90) and the carbon atom resonating at δ 74.80 which must, therefore, be C-2′.

The fact that a pair of doublets representing the C-2′–C3′ bond is not observed in Fig. 1c* results from the fact that these two nuclei form such a tightly coupled, AB pair ($\Delta\delta$ = 0.51 p.p.m.; see Table I) that their intensity in the CCCP experiment has fallen below the limit of detection. This is due[5–7] to the dependence of the absolute, as well as the relative, intensity of the doublets on the AB character of this spin system, a feature peculiar to the INADEQUATE (and CCCP) experiment. Hence, although only nine of the ten carbon–carbon bonds in the molecule are represented, the redundancy of information – with more than one carbon atom being coupled to both C-2′ and C-3′ – allows for the unambiguous assignment of both.

Definitive assignments for all of the resonances having been obtained, a comparison may now be made with the chemical-shift data[7] (see Table I) for a model monosaccharide, β-D-glucose, in the same solvent. As expected, this comparison shows that the resonances of only two carbon atoms of β-cellobiose are markedly downfield, relative to those of the corresponding carbon atoms in β-D-glucose, *i.e.*, C-1 in one series and C-4′ in the other.

Data from the connectivity plot may also be utilized in a more-abbreviated fashion, owing to the fact[1,2] that secondary carbon atoms engaged in glycosidic bonding

*This is more clearly evident from an expanded view of the C-2′,C-3′ region (not shown).

usually resonate farther downfield (near δ 80) than unsubstituted, secondary carbon atoms. Accordingly, the signal at δ 80.93 in Fig. 1a is characteristic of a linkage carbon atom. Furthermore, the signal must be due to a C-4 nucleus, because the corresponding doublet in Fig. 1c exhibits connectivity with the doublet of a methylenic carbon atom (C-6) through only two carbon–carbon bonds. Hence, by reference to this limited region of the total plot, connectivity is traced from C-6′, through C-5′, to C-4′, which is commensurate with a (1→4′) linkage.

By furnishing an independent proof that the glycosidic bond in β-cellobiose is located between C-1 and C-4′, the present findings demonstrate the potential of the CCCP technique for determining positions of glycosidic linkages. Also, it is evident that the ability to assign, unambiguously, ^{13}C signals enhances possibilities for the identification of intact glycosidic residues through correlations of observed chemical shifts with known, or predicted, values. Although applications of the CCCP technique are limited at present by the need for high spectrometer-sensitivity, new advances (*e.g.*, see ref. 8) should facilitate its broader use in the determination of the structure of carbohydrates of high molecular weight.

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