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Note

The ^{13}C-n.m.r. spectrum of (1→4)-β-D-mannans in intact endosperm tissue of the date (*Phoenix dactylifera*)

MICHAEL C. JARVIS
Department of Agricultural Chemistry, University of Glasgow, Glasgow G12 8QQ (Great Britain)

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Intact seeds were among the first biological materials to which c.p.-m.a.s. ^{13}C-n.m.r. spectroscopy was applied[1,2]. Recently, seeds and other living plant materials with grossly thickened cell walls have been examined by this technique in order to reveal the structure *in vivo* of their cell-wall polysaccharides[3]. The rigidity of the thickened cell walls and the low content of moisture in the seed tissues prevented their disintegration due to centrifugal forces in the m.a.s. rotor. The broad resonances of the highly branched galactomannans which dominated the spectra of carob and fenugreek seeds had chemical shifts similar to those obtained in solution, consistent with a non-crystalline or glassy structure[3].

Related, but linear, (1→4)-β-D-mannans are the major constituent of the endosperm of *Phoenix dactylifera* (date), which, like the endosperm of carob seeds, is a living tissue with greatly thickened cell walls[4,5]. These mannans are sufficiently crystalline to give clear X-ray and electron diffraction patterns[4,6–8]. The crystallographic data indicate that two crystal forms, mannans I and II, are present in endosperm of the date, as in the cell walls of the related ivory nut and of certain algae[4]. Mannan I is the more highly crystalline form, but, unlike cellulose I, it is granular rather than fibrous. It preponderates in the cell walls of the ivory nut, where it encases microfibrils of mannan II and cellulose. The c.p.-m.a.s. ^{13}C-n.m.r. spectrum of the date mannans *in vivo* is the subject of this note.

The spectrum of intact tissue from the endosperm of date stones, shown in Fig. 1, is dominated by resonances from the mannans. Each of the signals is sharp, although broadened at the base. The line-width at half height is only ~1 p.p.m. (*cf.* 3–5 p.p.m. for seed galactomannans and most other cell-wall polysaccharides[3] *in situ*). By analogy with cellulose[9], it is assumed that the sharp central resonance corresponds to the more crystalline mannan I. The C-4 resonance shows a distinct downfield shift and those for C-2, C-3, and C-5 show small upfield shifts, as compared with the solution spectrum (Table I). In these respects, the mannans closely resemble cellulose I, the native crystalline form of cellulose[9]. The spectrum

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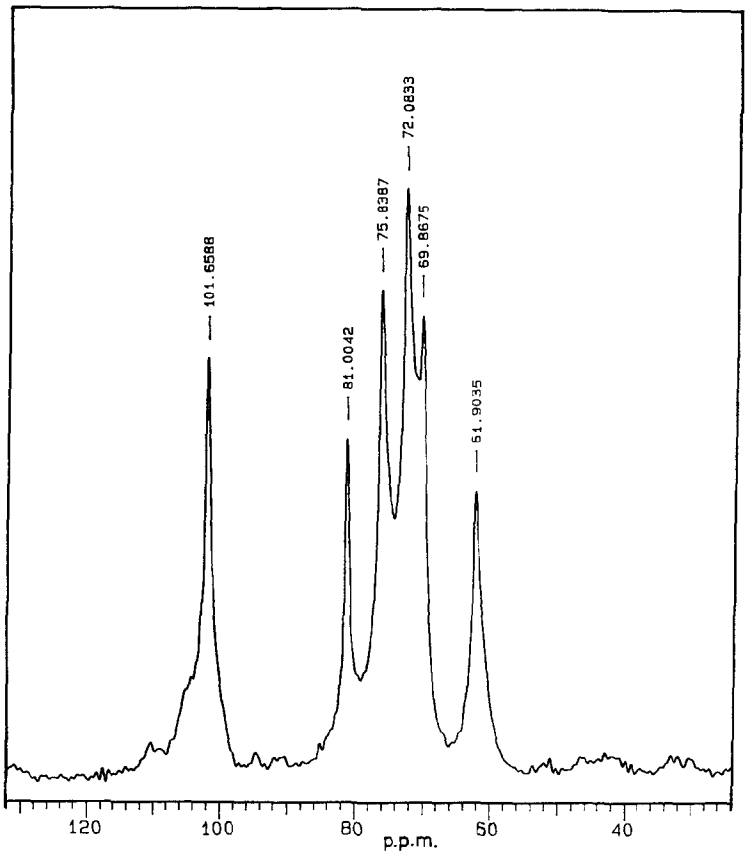

Fig. 1. C.p.-m.a.s. ^{13}C-n.m.r. spectrum of intact tissue from the endosperm of the date.

TABLE I

C.P.-M.A.S. ^{13}C-N.M.R. ASSIGNMENTS FOR DATE MANNAN, AND COMPARISON WITH PREDICTED SOLUTION-STATE CHEMICAL SHIFTS

	Chemical shifts (p.p.m.)					
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
C.p.-m.a.s. spectrum[a]	101.7	69.9	72.1	81.0	75.9	61.9
Solution state[b]	101.6	71.4	73.0	77.9	76.5	62.0

[a]Assignments based on solution-state spectra. The resonances for C-2 and C-3 could possibly be transposed. Chemical shifts are downfield from the signal for Me_4Si. [b]From ref. 24: the data in ref. 25 are closely in agreement when allowance is made for the fact that chemical shifts are reported with respect to external 4,4-dimethylsilapentane sulphonate instead of Me_4Si.

assumed to be from mannan I differed from that of cellulose I[10–12] in having sharp singlets for the C-1 and C-4 resonances.

Several crystal structures for mannan I, each having the mannan chain in a two-fold helix, have been suggested on the basis of X-ray diffraction data[6,13]. Conformational calculations and polarized i.r. spectra support a structure with antiparallel chains and an approximate $P2_12_12_1$ space group[6,14]. In this structure, each mannose residue would be in the same environment and singlet ^{13}C resonances would be expected, as observed. Small deviations[6] from $P2_12_12_1$ symmetry due to statistical variation in the conformation at O-6 would, if reflected in the chemical shifts as suggested for cellulose, be obscured by the broad line-bases attributed to mannan II. The slight regular variation in the conformation at C-6 reported by Atkins *et al.*[14] might be expected to cause some splitting, but if so it was too small to be observed.

Mannan I resembles cellulose I in having its chains in a two-fold helix, but the chains are packed quite differently. In cellulose I, they are believed to be parallel[15] and have different inter-chain hydrogen bonds[10–12]. This interpretation suggests that the qualitatively identical pattern of chemical shift displacements, on going from mannan and cellulose in solution in various solvents to mannan I and cellulose I, results from the adoption of the two-fold helix in the solid state. The same situation may be true of the other crystalline forms of cellulose. Since the glucan chains in cellulose I show no evidence of significant deviations from an exact two-fold helix[15], the small quantitative effects on the chemical shifts that cause multiplicity in the spectra of cellulose are more likely to be due to intermolecular hydrogen bonding[16,17] or other packing effects than to the helical conformation of the individual chains.

The lack of multiplicity in the spectrum of mannan I makes comparison with the solution state simpler and more reliable than for cellulose or other polysaccharides. Although there are differences in notation and interpretation, it has been suggested[16–20] that the chemical shifts for the resonances of C-1 and C-4′ depend on the torsion angles ψ (C-1–O-4′–C-4′–C-3′) and ϕ (O-5–C-1–O-4′–C-4′), respectively, of the C-4′–O-4′ and C-1–O-4′ bonds defining the helical form of a (1→4)-linked polysaccharide, and are displaced furthest downfield where H-1 and H-4′ are eclipsed (ψ 120°), or where C-4′ is *trans* to C-2 (ϕ −65°), using the notation of Mackie *et al.*[8]. Their map of conformational energies for mannan shows five linked wells at around (ϕ, ψ) values of (−90°, 90°), (−15°, 100°), (30°, 135°), (−95°, 140°), and (180°, 100°), on which it may be assumed, to a first approximation, that the preferred solution conformations are centred. This assumption does not take into account the influence of solvation energies, which has been evaluated only for mannobiose[21], but the preferred conformations of mannobiose were changed little by solvation although their relative energies were altered. The mannan I chain has (ϕ, ψ) (−92°, 96°). Thus, the deviation of (ϕ, ψ) from (−65°, 120°) is an unweighted average of (63°, 21°) for the conformations preferred in solution and (27°, 24°) for the conformation of mannan I, so that a downfield dis-

placement of the C-4 resonance in the solid state, with little change in the chemical shift of the C-1 resonance between solid and solution, would be predicted on this basis, as observed. The less-detailed conformational data of Sundararajan and Rao[22] lead to a similar prediction when allowance is made for the difference in notation.

The data for mannan I therefore support the relationship suggested[16–20] between the helical form of the polysaccharide and the chemical shifts of its C-1 and C-4 resonances, but the origin of this relationship is not clear. The γ-effect suggested as an approximation[16,19] is difficult to reconcile with the lack of obvious effects on the chemical shift of the C-2 resonance corresponding to the effects on C-4. Accurate quantitative prediction of the effect of conformation on the solid-state chemical shifts of the C-1 and C-4 resonances, and its extension to other ring carbon atoms, await further theoretical developments[16], such as the application of molecular orbital theory as has been attempted with polypeptides[23].

EXPERIMENTAL

Date seeds were washed, dried, and peeled with a sharp knife to a depth of ~1 mm. The embryos were removed. The endosperm was split into 1–2-mm pieces with a hammer and chisel, and packed into the n.m.r. rotor with talc to fill the voids. Talc alone gave no visible signal in the range 0–200 p.p.m.

The spectra were recorded at ambient temperature with a Varian VXR-300 spectrometer operating at 75.4 MHz for ^{13}C, with the following parameters: contact time, 1 ms; pulse width, 90°; acquisition time, 19.2 ms; relaxation delay, 1 s. A rather low spin-rate of 2.1 kHz was used, but there was no sign of interference from spinning side-bands. The spectrum was not artifically resolution-enhanced.

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