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Preliminary communication

Solid-state ^{13}C-c.p.–m.a.s. n.m.r. of starches

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Although the crystalline X-ray patterns of starch have been known for many years from the pioneering work of Katz and Van Italie[1], their interpretation has lagged behind that of cellulose because of hydration phenomena. Cereal starches give a pattern classed as "*A*" and the starches of tubers yield a "*B*" pattern. The presence of water favors starch crystallinity and indeed, the "*A*" and "*B*" structures have been interpreted in terms of hydrates, but dehydration does not destroy the crystalline organization as such[2]. Recent surveys of amylose crystal structures[3] and starch organization[4] have emphasized the fact that a double-helical organization is the most probable crystallization mode of starch molecules and that the uniquely shaped amylopectin molecule with its clusters of short branches is the predominant crystalline component. Indeed, starches consisting only of amylopectin crystallize as well or better than amylose-containing starches. In the present work, we show how ^{13}C cross-polarization and magic-angle spinning (c.p.–m.a.s.) nuclear magnetic resonance of water-saturated starches can add to our understanding of starch crystallization.

The importance of ^{13}C cross-polarization (c.p.) and magic-angle spinning (m.a.s.) n.m.r. spectroscopy as an analytical tool for detecting the presence of isomers in carbohydrate crystals has been demonstrated[5]. There is still debate, however, concerning the interpretation of multiplet signals from sugars[6] and such carbohydrate polymers as cellulose[7–9]. A simple rule would seem to be that the number of signals should relate directly to the number of carbon atoms in the asymmetric unit. This relationship is observed in the ^{13}C n.m.r. spectra of the crystalline methyl α- and β-xylosides[10]. In the case of the β anomer, the spectrum shows a single resonance for each carbon atom in the structure, whereas two lines are observed per carbon atom in the spectrum of the α anomer. This observation correlates well with diffraction data, which show that the asymmetric units in the crystalline structures of these sugars contain a single molecule in the β sugar[11] and two molecules in the case of the α anomer[12]. Experiments with cyclomaltohexaose hexahydrate and cyclomaltoheptaose heptahydrate also support the rule; their n.m.r. spectra show a number of overlapping signals which, for C-4, are resolved to exactly the number of C-4 atoms in the asymmetric unit[13].

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This rule for the interpretation of multiplets gives good agreement between the proposed space groups for "*A*" and "*B*" starches. The spectra for Amioca, a 100% amylopectin starch, giving an "*A*" X-ray pattern, and potato starch (which yields a "*B*" pattern), are shown in Fig. 1. The two spectra are typical of what has been found by the authors for "*A*" and "*B*" starches when these are carefully hydrated. This is performed by conditioning in a desiccator over a period of days to weeks at 100% relative humidity. A marked increase in the sharpness of the X-ray patterns was noted as conditioning proceeded to the limiting situation shown in Fig. 1.

Previous studies of the ^{13}C c.p.–m.a.s. spectra of carbohydrates have indicated that the ^{13}C resonances of the carbon atoms involved in the glycosidic linkage are particularly noteworthy. The well resolved resonances for C-1 and C-4 in cellulose and the β-(1→4)-glucan oligomers display multiplicities that are sensitive to the crystalline structures of these carbohydrates[7]. In the case of crystalline starches, the C-1 signal is clearly multiple: a triplet for the "*A*" form and a doublet for the "*B*". This result is in keeping with the recently proposed[3] space groups for "*A*" and "*B*" starches, which both crystallize

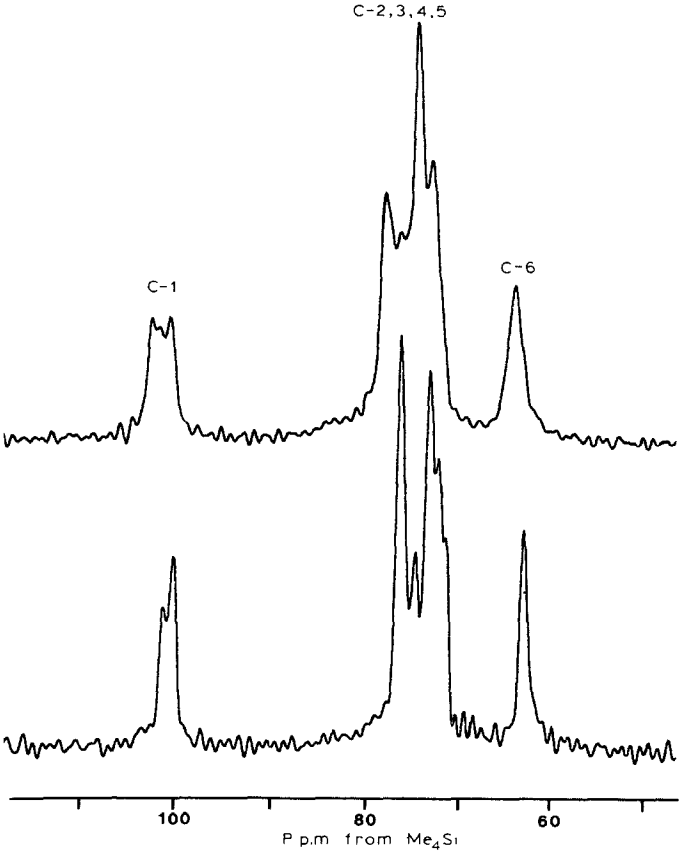

Fig. 1. Solid-state n.m.r. spectra of hydrated starches: top spectrum is for Amioca, a 100% amylopectin starch from corn; bottom spectrum is for potato starch.

as double helices with parallel strands, but which differ in unit-cell type, water disposition, and duplex packing[14,15]. The consequence of the assigned $P2_1$ space group for "*A*" starch, with the 2_1 axis perpendicular to the six-residues-per-turn strands of the double helix, is that maltotriose (*or half a turn*) must be taken as the asymmetric unit. This is corroborated by the three signals for C-1 of the Amioca starch. For the "*B*" starch, the trigonal space group $P3_121$ specifies an asymmetric unit of two glucose residues, i.e., one-third of a turn. This is in keeping with the doublet signal for C-1, which we have found characteristic of the "*B*" starches, all of which come from tubers.

Table I provides some quantitative chemical-shift data for the two starch polymorphs. Interesting are the differences in the breadths of the C-6 resonances for the two polymorphs. This difference may be related to a mixture of C-6 rotamers for the "*A*" starch compared to a single rotameric component for the "*B*" sample, as proposed in the X-ray structure refinement[14,15].

TABLE I

^{13}C CHEMICAL SHIFTS[a] FROM ^{13}C C.P.–M.A.S. SPECTRA OF HYDRATED, CRYSTALLINE STARCH POLYMORPHS

Resonance	*A* *(Amioca)*	*B* *(Potato)*
C-1	102.4, 101.5, 100.4	101.3, 100.2
C-6	62.9	62.1

[a] ^{13}C Chemical shifts in p.p.m. relative to tetramethylsilane.

Because starches of high amylose content have a tendency to be less crystalline than starches composed only of amylopectin, a "lintner starch" prepared from potato starch by removal of ~35% by weight of non-crystalline material through room-temperature hydrolysis with 4M HCl was examined. The X-ray diffraction spectrum was still characteristic of "*B*" starch and the ^{13}C c.p.–m.a.s. spectrum of the carefully hydrated starch was not significantly changed (Fig. 2). However, the ^{13}C c.p.–m.a.s. spectrum of the dry material is of interest because it shows the loss of peak resolution that accompanies drying (desiccation over P_2O_5), similar to the loss in intensity in the X-ray diffraction pattern[4]. The resonance that appears on drying at $\delta = 82$ p.p.m. is believed to be characteristic of the C-4 resonance of non-crystalline material. Thus, the dramatic narrowing of the ^{13}C resonances on hydration may be associated with an increase in the local short-range order. This effect has been previously observed in ^{13}C-n.m.r. studies of solid (1→3)-β-D-glucans[16], although the detailed mechanism is not known.

Hydrated starches of high amylose content show broad resonances for C-1 and C-4 possibly associated with less-crystalline material, in addition to the characteristic resonances of the "*A*" or "*B*" form. This is noteworthy because it tends to support the

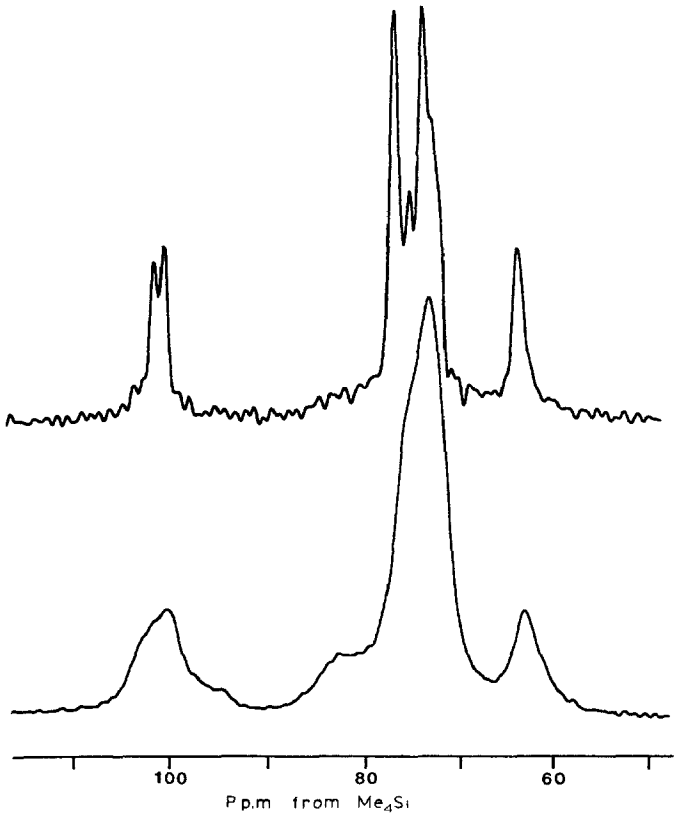

Fig. 2. Solid-state n.m.r. spectrum of "lintner" starch prepared from potato starch: top spectrum is for fully hydrated material; bottom spectrum is for the same material dried over P_2O_5.

concept that amylose, the linear component of starch, is less responsive to induced crystallization by water treatment than is the (highly branched) amylopectin. This surprising fact is probably due to the unique "*racemose*" architecture of branching[4] in the latter, which enables crystallization, whereas branching in the usual dendritic molecular architecture is a hindrance to crystallization.

Because X-ray patterns of starch contain such limited information, the c.p.–m.a.s. technique promises to provide the long-needed complementary tool in the field of starch structures.

The ^{13}C c.p.–m.a.s. spectra were acquired at 22.6 MHz with a Bruker CXP 100 spectrometer. ^{1}H Decoupling fields ~10 gauss were employed with a single 1 ms contact and 1 s recycle time. Typically, between 10,000 and 40,000 transients were accumulated and spectra were resolution-enhanced. Chemical shifts were determined by using hexamethyldisiloxane (δ = +2.1 p.p.m. *vs.* tetramethylsilane) as a substitution reference.

Starch samples were obtained from a variety of commercial sources. Hydrated materials were obtained by maintaining the starches at 100% relative humidity for several

days, during which time spectra were obtained at intervals. The "lintner" starch was kindly provided by Dr. Alain Buleon of the Institut National de la Recherche Agronomique, Nantes, France.

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