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OBSERVATIONS ON N.M.R. SPECTRA OF STARCHES IN DIMETHYL SULFOXIDE, IODINE-COMPLEXING, AND SOLVATION IN WATER–DIMETHYL SULFOXIDE

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ABSTRACT

N.m.r. spectra (^{1}H- and ^{13}C-) in dimethyl sulfoxide-d_6, together with complementary ^{13}C-T_1 and -n.O.e. data, are described for a variety of starches, ranging in composition from high-amylopectin to high-amylose types. The spectra contain groups of minor ^{13}C, ^{1}H, or O^{1}H signals attributable to glycosyl end-groups and other residues associated with branching in amylopectin molecules. The relative intensities of these signals, and of the resonances due to the corresponding nuclei of main-chain residues, are evaluated in terms of the incidence of end groups, or branches, in the starches. They are also compared with spectrophotometric measurements on the iodine-complexing capacity of the starches, using a modified procedure. Complex-formation between amylose or amylopectin and iodine in water is suppressed by the introduction of dimethyl sulfoxide, due probably to competitive solvation of the starch molecules. This appears to be related to other observations, showing that the composition of water–dimethyl sulfoxide mixtures determines the rate of hydrolysis by beta amylase, optical activity, and ^{13}C-chemical shifts. All of these observations concur in suggesting that, in admixture, more than 4–5 moles of water per mole of dimethyl sulfoxide are necessary for amylose or amylopectin to be effectively solvated by the water molecules present.

INTRODUCTION

Amylose and amylopectin, the linear and branched components, respectively, of starches, are readily[1] differentiated by such chemical reactions as iodine-binding, methylation analysis, and periodate oxidation, as well as by enzymic means. Their n.m.r. spectra also differ notably, as evident[2] particularly in high-field, ^{13}C-n.m.r. spectra of solutions in dimethyl sulfoxide (Me_2SO).

Although the ratio of linear to branched molecules in starch is commonly ~1:3, it nevertheless varies widely: from waxy starches consisting entirely of amylopectin to specimens that contain at least 75% of amylose. Iodine-binding measurements provide[1] a ready estimate of the percentage of amylose in a starch and, by difference, its amylopectin content may then be assumed. Complementary

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information about the amylopectin content is directly accessible[2–8] from n.m.r. spectra, which can distinguish ^{13}C or ^{1}H nuclei of glycosyl end-groups and residues engaged in branching from those of main-chain residues in the molecule. Observations on the n.m.r. spectra of a variety of starches are reported here, and are assessed in the light of data obtained from spectrophotometric measurements on their iodine-binding properties.

RESULTS AND DISCUSSION

As noted previously, ^{13}C-n m.r. spectra of amylopectins in Me_2SO exhibit a number of resonances that may be ascribed[2] to D-glucosyl residues that are associated specifically with branching. These features are also found[8] among starches, being most pronounced in the spectra of waxy starches, which are essentially composed of amylopectin-type molecules. Hence, the 75-MHz ^{13}C-n.m.r. spectrum of waxy-rice starch in Me_2SO-d_6 (see Fig. 1A) contains signals at δ 70 1 and 61 0, which are analogous to the ones attributed[2] to C-4g and C-6g, respectively, of the (nonreducing) glycosyl group (*g* Fig. 2) of amylopectin. These relatively minor signals are well separated from those of the corresponding ^{13}C nuclei in (1→4)-linked residues (such as *e* and *f*) that constitute most of the remainder of the molecule. Although branch-point residue *b* is the complement of end-group *g*, its C-6 resonance (C-6g) is barely detectable (at δ 66.0), due to the broadening caused by a lessened rate of segmental motion in this region of the molecule.

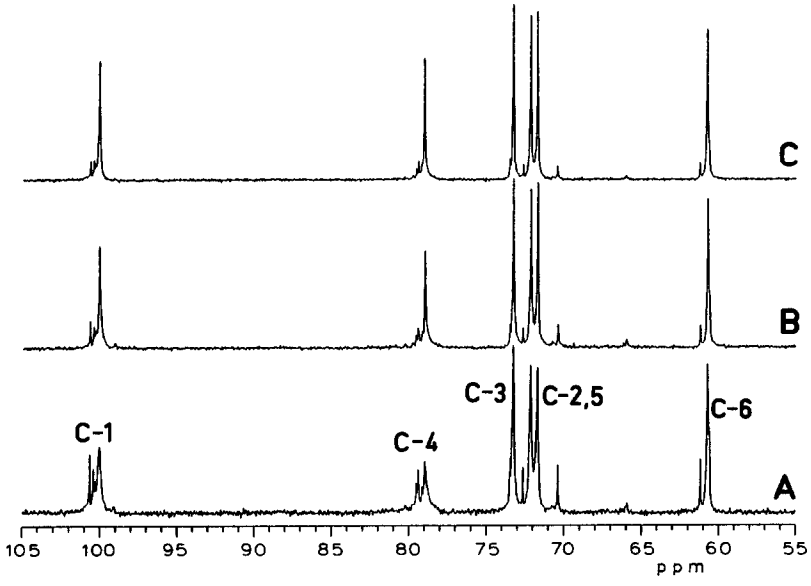

Fig 1 ^{13}C-N m r spectra (75 MHz) of starches in Me_2SO-d_6 at 80° A, waxy rice, B, wheat, C, high-amylose corn

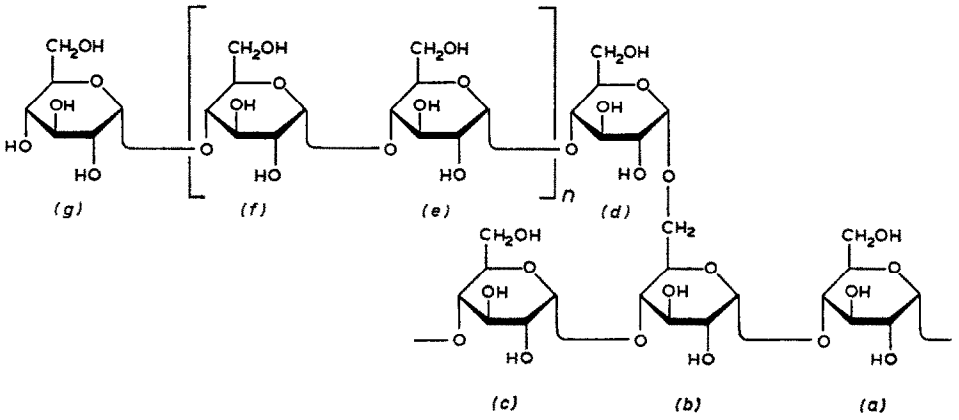

Fig 2 Representation of a portion of an amylopectin molecule; *n* corresponds to the number of repeating sequences of residues *e* and *f* commensurate with the length of the branch.

The spectra of such starches as those of wheat, corn, and potato have the same general appearance (see Fig. 1B), although the signals of the end-unit carbon atoms are noticeably less prominent than in Fig. 1A. This is due not only to their lower amylopectin content, but also to the fact[2] that resonances of C-1 to C-6 of the amylose present are virtually coincident in chemical shift with those of the *e* and *f* type, main-chain residues of the amylopectin. Hence, a "high amylose" corn starch produces (see Fig. 1C) a relatively weak group of signals due to the end units and, in fact, its spectrum is not too dissimilar from that[2] of an isolated amylose.

In addition to the minor signals attributed to end group *g*, others form clusters at the downfield edge of the main-chain $C\text{-}1_m$ and $C\text{-}4_m$ signals, and two additional ones appear at δ 72.5 and 70.6. As the latter is analogous in its extreme broadening to that of $C\text{-}6_g$, and is adjacent to the main C-5 signal ($C\text{-}5_m$, δ 71.8), it is most likely due to $C\text{-}5_g$ of the end group. A comparison of the spin–lattice relaxation characteristics of the various ^{13}C nuclei (see Table I) shows that the peaks at δ 100.4 and 72.5 can be grouped with $C\text{-}4_g$, because all three T_1 values* are uniformly longer than those of the other ^{13}C nuclei. Hence, they are ascribed to C-1g and C-2g, as suggested earlier[2], leaving $C\text{-}3_g$ and $C\text{-}5_g$ as the only ^{13}C nuclei of the end group that are not deshielded in Me_2SO relative to their main-chain counterparts. The T_1 values for the remainder of the minor signals (two C-1 and four C-4 atoms) are closer in magnitude to those of the C_m resonances (see Table I). Presumably, these signals originate in C-1 and C-4 nuclei of residues (*e.g.*, *a* and *c*) in the vicinity of the branch point or the end group, which experience solvation conditions slightly different from those of the C_m nuclei (see later).

The relative intensities of appropriate signals in the ^{13}C-n.m.r. spectra of starches should afford[2,8] an estimate of the proportion of branched molecules

*The spin–lattice relaxation time is denoted as T_1.

TABLE I

CARBON-13 SPIN–LATTICE RELAXATION TIMES (T_1, s) FOR STARCHES[a]

Starch (type)	*C-1*				*C-4*						*C-2*		*C-6*	
	100 4(g)	*100 2*	*100 1*	*99 9(m)*	*79 3*	*79 2*	*79 1*	*79 0*	*78 9(m)*	*70 4(g)*	*72 5(g)*	*72 0(m)*	*61 0(g)*	*60 6(m)*
Sorghum (waxy)	0 29	0 22	0 19	0 19	0 19	0 23	[b]	0 19	0 20	0 28	0 27	0 21	0 22	0 12
Wheat (modified II)	0 25	0 22	0 19	0 19	0 17	0 17	[b]	0 18	0 21	0.23	0 24	0 21	0 20	0 12
Wheat (modified III)	0 31	0 22	0 22	0 18	0 20	0 20	[b]	0 20	0 19	0 30	0 24	0 20	0 20	0 12
Wheat (whole)	0 30	0 19	0 18	0 18	0 18	0 18	0 20	0 20	0 20	0 23	0 27	0 20	0 16	0 12
Potato (whole)	0 28	0 20	0 18	0 18	0 19	0 22	0 21	0 17	0.20	0 30	0 25	0 19	0 18	0 12
Corn (high amylose)	0 25	0 22	0 15	0 19	[b]	0 15	[b]	0 17	0 20	0 23	0 26	0 20	0 18	0 12

[a]Designations "g" and "m" refer to signals attributed to the glycosyl end-group and main-chain residues, respectively [b]Signal not adequately resolved

present, *i.e.*, the amylopectin content, as well as a measure of the degrees of branching in the amylopectin components. In this context, the C-1, C-4, and C-6 signals just described were selected for measurements of relative intensities. Allowance was made for differences between the motional characteristics of an end unit (*g*) and those of residues within the main chain, which could adversely affect a comparison of ^{13}C signal intensities. That is, as the end unit is relatively more mobile, its ^{13}C nuclei should not only have longer T_1 values, but also experience a stronger nuclear Overhauser enhancement (n.O.e.). These differences are evident in some representative data for starch samples (see Table II), in that the n.O.e.** values for C-1_g, C-4_g, or C-6_g are larger than those of the corresponding nuclei of main-chain residues C-1_m, C-4_m, or C-6_m (and are also larger than those of the other minor signals listed in Table II). For no apparent reason, the sole exception is the relatively low (but reproducible) n.O.e. for C-6_g of wheat starch. Otherwise, only minor variations are found among these data, as already apparent (see Table I) for the corresponding T_1 values for the same nuclei.

In accord with these observations, the C-1_g:C-1_m, C-4_g:C-4_m, and C-6_g:C-6_m ratios of signal intensities were appropriately smaller when the spectra were recorded without n.O.e. (gated ^1H-decoupling) than when full n.O.e. was permitted (conventional ^1H-decoupling). Expressed as the percentage of glycosyl end-groups (%*G*) in the starches (see Table II), the ratios have been so adjusted as to incorporate data for C-6 of the (1→6)-linked residue (*b*). Although its resonance (C-6_b) is barely detectable (at δ 67), it should be quantitatively equivalent to C-6_g. Consequently,

$$\%G = I_g/I_g + I_b + I_0,$$

where I_g, I_b, and I_0 are the intensities of the ^{13}C signals of the glycosyl group, residue *b*, and all other residues, respectively. In the measurements on the C-6 signals, $I_b = I_g$.

Corresponding information based on C-4 (see Table II) is less certain, because the C-4_b signal is obscured within the cluster of C-4 signals centered at δ 79.5. However, by accepting that the minor peak at δ 70.6 is due to C-5_b, and noting that it is more prominent than the C-6_b signal, we expect an even narrower line-width for C-4_b, and close to a full contribution to the C-4_0 integral; *i.e.*, when based on C-4, the measurement of I_0 is assumed to include I_b. This assumption is taken to apply in the C-1 measurements as well. Moreover, as the C-1_g, C-4_g, and C-6_g signals in the spectrum of each starch exhibited some degree of overlap with adjacent signals (see Fig. 1), base-line corrections were invariably required. Uncertainties inherent in this procedure probably account for the variability observed (see Table II) when different nuclei serve as the basis for estimating %*G*. There is

**As has been observed[9] with several high-molecular-weight polysaccharides, none of these n O e values approach the limiting value of 2 988 characteristic[10] of rapid molecular rotation

TABLE II

VALUES OF n O e FOR ^{13}C NUCLEI OF STARCHES, AND % END-GROUPS (%*G*)

Starch (type)	*n O e*						*%G[a], based on*					
	C-1$_g$	*C-1$_m$*	*C-4$_g$*	*C-4$_m$*	*C-6$_g$*	*C-6$_m$*	*C-1*	*C-4*	*C-6*	*C-1*	*C-4*	*C-6*
							(+ n O e)			*(− n O e)*		
Sorghum (waxy)	2 15	1 69	1 99	1 71	2 27	1 84	5 9	6 6	5 8	4 6	5 0	4 6
Wheat (modified II)	1 94	1 60	1 89	1 55	2 46	1 77	5 0	6 8	5 4	4 0	4 9	3 9
Wheat (modified III)	2 00	1 60	1 83	1 71	2 09	1 80	4 9	4 3	5 0	3 8	3.6	3 3
Wheat (whole)	2 22	1 76	1 91	1 74	1 76	1 92	4 6	4 3	3 9	3 2	3 5	3 5
Potato (whole)	2 58	1 66	2 11	1 71	2 52	1 82	5 0	4 2	3 8	2 7	3 1	2 7
Corn (high amylose)	1 99	1 65	2 35	1 71	2 37	1 87	3 8	3 1	2 5	2 3	2 2	1 8

[a]For corresponding data based on ^1H data, see Table III and Fig. 6

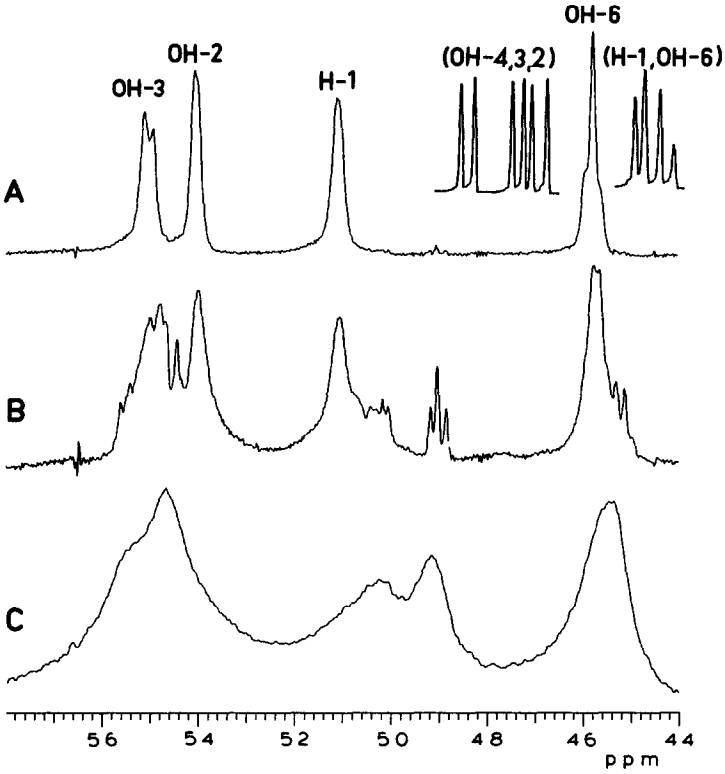

Fig 3 Partial ^{1}H-n.m r spectra (300 MHz) in Me_2SO-d_6 at 23° A, amylose, B, waxy-sorghum starch, C, glycogen, inset, methyl α-D-glucopyranoside

generally good agreement among these estimates, nevertheless, as well as with the corresponding data based on the ^{1}H-n.m.r. spectra of the starches.

^{1}H-N.m.r. spectra of starches in dimethyl sulfoxide. — Deuterium oxide has generally been used[4–8] as the solvent for detecting ^{1}H signals associated with (1→6)-linked branches in amylopectins, modified starches, dextrans, and related polysaccharides. For whole starches, however, advantages are offered[11] by Me_2SO, in that it gives less opaque, and more stable, solutions.

Due to a low rate of exchange, the hydroxyl-proton resonances are readily observed in Me_2SO solution, as seen[12,13] in the spectrum of amylose (see Fig. 3A). It appears that the signals for OH-2 and OH-3 are strongly deshielded by intramolecular H-bonding, involving[14] OH-3 as the principal donor. The prominent signals at δ 5.4–5.5 in the spectrum of waxy-sorghum starch (see Fig. 3B) indicate that analogous, intramolecular H-bonding largely persists in amylopectin, causing comparable deshielding of OH-2 and OH-3, which is made more apparent by the fact that the secondary-hydroxyl protons of methyl α-D-glucopyranoside (inset, Fig. 3A) are more shielded by ~0.8 p.p.m. Also evident, relative to the signals of the

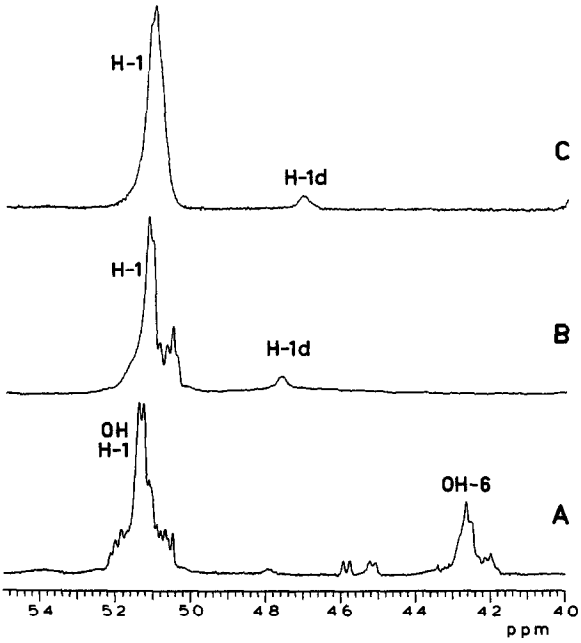

Fig 4 Partial ^{1}H-n.m.r. spectra (300 MHz) at 80°. A, waxy-sorghum starch in Me_2SO-d_6, B, sample A after exchange with D_2O, C, waxy-sorghum starch in D_2O

glycoside, is the extreme broadness of the polymer resonances, including that of H-1.

Branching in the waxy starch does, however, give rise to several features distinct from those of the amylose spectrum. In Fig. 3B, there is a broad dispersion of resonances centered at δ 5.5, and a prominent group of signals at δ 5.0 overlapping that of H-1, in addition to other signals (see later) at δ 4.9. As glycogen, in which there is a higher incidence of (1→6) linkages, produces a spectrum (see Fig. 3C) that contains a more-prominent counterpart of the resonances in the δ 4.9–5.0 region, it is apparent that the pattern of intramolecular H-bonding, and competitive solvation by the Me_2SO, in the linear D-glucan is substantially altered by the introduction of branching. There are no effects having this magnitude on the primary hydroxyl groups, as the OH-6 resonance at δ 4.6 of amylose and the waxy starch is almost coincident with the OH-6 triplet of the methyl glycoside (see Figs. 3A and B).

Although the signal at δ 4.9 in Fig. 3B appears to be a triplet, it is displaced upfield by increasing the temperature and, at 80°, it becomes two doublets (δ 4.58 and 4.52; see Fig. 4A). As expected, the resonances of OH-6, OH-3, and OH-2 are shifted upfield as well, with the latter two now coincident with that of H-1. Upon proton-exchange with D_2O, only peaks attributable to anomeric protons remain in this region (see Fig. 4B). It is also apparent that the group of relatively sharp signals comprising a shoulder on the upfield side of the H-1 peak is not due to hydroxyl protons, but to other (anomeric) protons. Possibly, the latter are related to the two

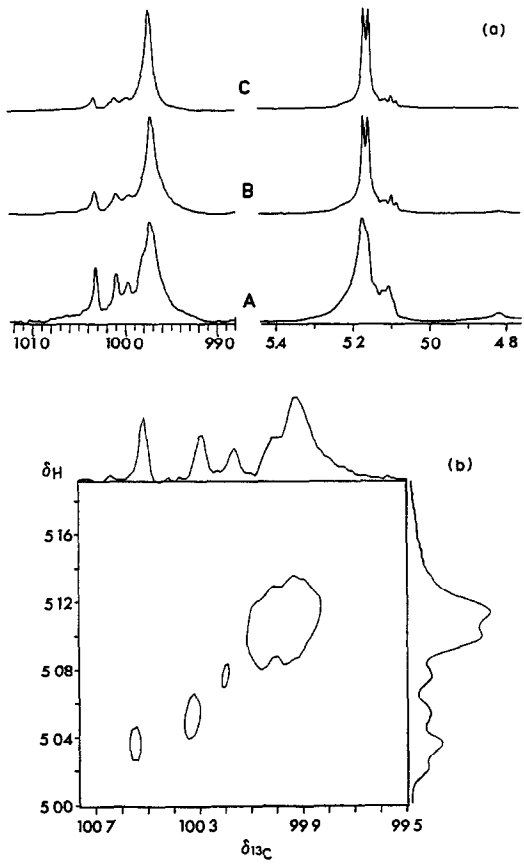

Fig 5. (a) A comparison of the signals of ^{13}C-1 (left, 75 MHz, 80°) and ^{1}H-1 (right, 300 MHz, 80°, after D_2O-exchange) in Me_2SO-d_6: A, waxy-sorghum starch; B, wheat starch, C, high-amylose corn starch (b) Partial 2D-(^{13}C, ^{1}H) heterocorrelation spectrum corresponding to the signals of waxy-sorghum starch (A) in Fig 5a

OH-doublets as well as the multiplets bordering the upfield side of the OH-6 signal in Fig. 4A (δ 4.58, 4.52, and 4.20, respectively), and represent residues that are solvated, or H-bonded intramolecularly, or both, in a distinctive fashion. The absence of a third OH-doublet (as for the methyl glycoside; see Fig. 3A) precludes the glycosyl end-group (g) as a source of these signals.

It is noteworthy that the corresponding spectral region for these compounds in D_2O solution (see refs. 7 and 8, and Fig. 4C) shows little of such differences, because a similar pattern is found among the ^{13}C spectra. When the solvent is Me_2SO, but not when it is D_2O, there is a cluster of minor peaks at the downfield edge of the main C-1 signal (and C-4 signal). This analogy between ^{1}H and ^{13}C chemical shifts is emphasized in Fig. 5A by the H-1 and C-1 signals for a group of starches having differing ratios of amylose and amylopectin. Moreover, it is appa-

TABLE III

PERCENT END GROUPS[a] (%G) OF STARCHES, BASED ON H-1_g AND H-1_m RATIOS AND CORRESPONDING "BLUE VALUES" (B V)

Starch (type)	%G	*B V*	*Starch (type)*	%G	*B V*
Rice (waxy)	4 8	0 07	Potato (whole)	2 8	0 52
Wheat (modified III)	3 8	0 40	Corn (high amylose I)	2 7	0 56
Wheat (whole)	3 1	0 51	Corn (high amylose II)	2 3	0 67

[a]For the corresponding data for these and/or other starches, see Table II and Fig 6

rent from a 2D-heterocorrelation spectrum (see Fig. 5B) that the minor ^{13}C and ^{1}H signals are produced by the same residues; having already identified the signal of C-1_g, that of the appended proton, H-1_g, is readily recognized in Fig. 5B. Likely sources of the other minor C-1 resonances are such residues in the vicinity of branch points, as *a* and *c*, which become more populous as the incidence of branching increases, and may be expected to have solvation patterns distinct from those of the other main-chain residues (*e*, *f*)*.

The broad, minor signal at δ 4.85 in Fig. 4B is attributable[3,4] to H-1_6 of the (1→6)-linked residue, *d*. Although it is also detectable at the same position in Figs. 3B and 4A, the D_2O-exchange treatment allows for an unobstructed examination of this signal and the other H-1 signals (H-1_4) centered at δ 5.2. By analogy with measurements on D_2O solutions[7,8] (represented by Fig. 4C), a comparison of the relative intensities of H-1_6 and H-1_4 gave the percent of glycosyl end-groups ($\%G_H = I_6/I_6 + I_4$) listed in Table III. Some comparisons were also made with methyl α-D-glucopyranoside as an internal standard, at a concentration at which its H-1 signal (δ 4.55) was of approximately the same intensity as the H-1_6 signal. This was more facile than the alternative of comparing H-1_6 with the far-stronger H-1_4 group of signals, and inherently more accurate. However, there was no clear difference between the results obtained by these two sets of measurements.

Correlating the degree of branching in starch with its amylose content. — It is apparent that the reliability of these ^{1}H and ^{13}C measurements of end groups (%G) is dependent upon such limiting factors as overlapping peaks, excessive line-broadening, and uncertainty as to a signal's quantitative response, as well as instrumental reproducibility The data of Tables II and III (and also data to be

*All patterns of the minor signals in Fig 5, as well as those in Fig 1 and in other analogous spectra that we have examined or that have been presented elsewhere, show a striking uniformity in terms of the chemical shifts, relative intensities, and relaxation characteristics of the component signals This implies that there is also a high degree of uniformity in the fine structure of the amylopectin molecules present in all of these starches

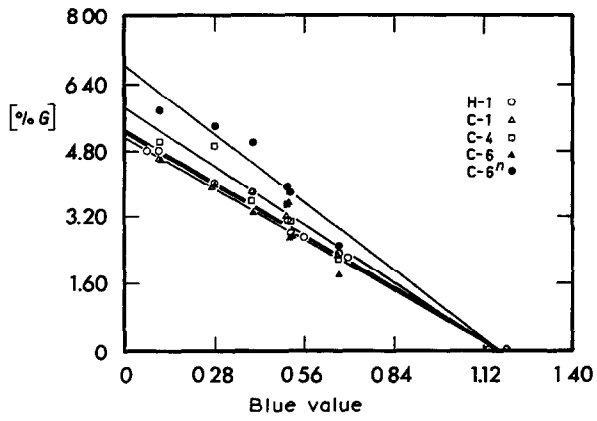

Fig. 6. Relationship between the percentages of glycosyl end-groups (%*G*, see Tables II and III) and the iodine-binding capacities ("blue values", see Table III) of starches [The starches show an increase in blue value (from left to right), and a corresponding decrease in %*G*, in the following order waxy rice, waxy sorghum, wheat (modified I), wheat (modified II), wheat (modified III), wheat (unmodified), potato (unmodified), corn (high amylose I), corn (high amylose II), corn (high amylose III); the two samples having blue values of 1 14 and 1.19, respectively, are amylose I and amylose II (~0% amylopectin, based on ^{13}C-n m r evidence) The least-squares correlation coefficients for the data based on H-1, and C-1, C-4, and C-6 (without n.O e) are 0 9925, 0.9874, 0.9803, and 0 9727, respectively. For the data based on C-6^n (with n.O e) the correlation coefficient is 0 9817, the corresponding values for C-1^n and C-4^n (data not shown) are 0 9416 and 0.9641, respectively]

cited later for additional starches) show that there is satisfactory agreement among the different values for each starch sample. Also, other n.m.r. studies reported[7,8] good correlations with different kinds of methods for the assay of branching. Nevertheless, we have attempted to evaluate the general validity of our findings by comparing them with measurements of amylose content, which should be inversely related to the incidence of branching, or amylopectin content, of the starch.

The iodine-binding capacity of a starch, which may be determined spectrophotometrically from the blue value[15–17] (see later), furnishes an estimate of the amylose present. Blue values for a variety of starches—ranging from high amylose to high amylopectin types—are compared in Fig. 6 (see also, Table III) with each set of the n m.r. data obtained for percent branching.

Clearly, there is a strong inverse correlation, for widely different kinds of starch, between the amylose content and percent of end groups, or branching*, when the latter is estimated from the ^{1}H spectra, or the ^{13}C spectra recorded without n.O.e. Although the value obtained from the ^{13}C spectra recorded with n.O.e. (represented in Fig. 6 by C-6 measurements) are unrealistically high, the degree of

*As three of the starches had been lightly acid-modified (reducing-end resonances were of negligable intensity), a correspondingly small proportion of the end groups in these materials does not arise from branching

overestimation by this technique is relatively constant, in accord with the general consistency observed among the n.O.e. values (see Table II).

Overall, as the ^1H spectra require shorter acquisition times and smaller quantities of sample, they offer advantages that are not compensated for by comparable measurements performed on ^{13}C spectra. In this regard, we are in agreement with the evaluation recently advanced by Gidley[8], although, in examining whole starches, we have preferred Me_2SO as the solvent, rather than D_2O.

Spectrophotometric measurement of iodine complexing. Influence of dimethyl sulfoxide. — Early methods employed[15,16] sodium hydroxide in order to solubilize the starch, followed by neutralization with hydrochloric acid prior to introduction of the iodine–iodide reagent. Dimethyl sulfoxide was used in the present study, as it gave starch solutions of greater clarity and stability, characteristics that were subsequently retained upon dilution with water for spectrophotometric measurements. It was also found that the introduction of sodium chloride, corresponding to the use of a combination of NaOH–HCl in the other procedures, enhances the intensity of the chromophore by ~5%, as well as its stability.

The concentration of Me_2SO in the starch–iodine solution assayed was limited to 0.5% by appropriate adjustment of volumes, because, at higher concentrations, this solvent interferes with the starch–iodine reaction. This is seen in Table IV, which shows that the absorbance (λ_{max} 600 nm) decreases in a fashion close to linear. No blue color is observed when the concentration of dimethyl sulfoxide is ~40%, as had already been noted by Ono *et al.*[18]. They attributed this behavior to a less-ordered, helical conformation of the amylose in dimethyl sulfoxide than in water (see also, ref. 19). However, as other studies[5,6,20–24] indicate that the converse is true, *i.e.*, that the organic solvent accommodates the helix more readily, it is uncertain that this interference with the formation of the chromophore is due to a solvent effect on the conformation of amylose.

It is noteworthy that, although amylopectin and iodine produce a chromophore (λ_{max} 530 nm) different from that from amylose, its absorbance is also progressively diminished by an increase in the concentration of Me_2SO (see Table IV). In this respect, the effect of solvent on the complexing ability of amylopectin is analogous to that of the more-linear starch component.

TABLE IV

EFFECT OF Me_2SO ON THE COMPLEXING OF I_2 (BLUE VALUE) WITH STARCHES IN H_2O

Starch type	*Me_2SO (%)*				
	0 2	*2 0*	*10*	*20*	*30*
	Blue value				
High-amylose corn	0 69	0 69	0 61	0 42	0 20
Waxy sorghum	0 13	0 11	0 07	0.03	—

Solvation of amylose and amylopectin in water–dimethyl sulfoxide. — The relative effectiveness of H_2O and Me_2SO in solvating[25] amylose and amylopectin may be a factor contributing to the effects observed. Depending on the ratio of these two solvents, the polymer can be exposed to environments ranging from H_2O-like through Me_2SO-like. Accompanying changes in solvation are thought[24] to be the source of a discontinuity in the optical rotations of amylose and amylopectin in ~2:1 H_2O–Me_2SO. According to this concept, the Me_2SO is the effective solvent when its content in the mixture exceeds one-third, which is also close to the region in which the iodine reaction is inhibited. Consequently, it appears that the blue, or red, chromophore can be generated only when the H_2O competes sufficiently with the Me_2SO to solvate the starch–I_2/I^- system* effectively, *i.e.*, at a minimum concentration of ~60% water.

If these considerations have some validity, it may be expected that other properties of starch molecules normally associated with an aqueous medium will be similarly affected in H_2O–Me_2SO mixtures. In this context, we find that, in Me_2SO, there is no reaction between amylose and the enzyme, beta amylase. The latter is inhibited, rather than deactivated†, by this solvent, presumably because H_2O is required in the hydrolysis. Only when the water content is at least 50% (see Fig. 7) does the reaction proceed at an appreciable rate. Although this minimum level is slightly lower than that (~60%) required for iodine-binding, its magnitude is such as to imply, once again, that the hydration of amylose is a relatively inefficient process in the presence of Me_2SO. In equivalent terms, at least 4–5 moles of water must be present, per mole of Me_2SO, for amylose to be even marginally solvated by water molecules.

Differences between the ^{13}C-chemical shifts of starches in H_2O and Me_2SO may also be cited. Recently, it was noted that[26], although most ^{13}C nuclei in a highly linear amylodextrin are less shielded in the latter of these two solvents, the signals of C-1 and C-4 exhibit 2–3-fold larger downfield shifts than do those of the other carbon atoms. Local conformational changes about the inter-residue linkages, in accord with the general view that Me_2SO promotes a more highly ordered helix, were regarded[26] as the origin of the specific deshielding. Analogous changes in chemical shift are exhibited by the main-chain residues of amylopectin and the waxy starches, because their C-1 and C-4 signals coincide with those of amylose in H_2O, or Me_2SO, or both[2,5,6]. Even larger downfield-shifts characterize the cluster of minor C-1 and C-4 signals adjacent to the main C-1 and C-4 signals (see Fig. 1) and, moreover, there is a concomitant, upfield shift for the H-1 resonances (see Fig. 5). As these converse effects on ^{13}C and ^1H chemical shifts are analogous to those[27,28] that accompany the introduction, or removal, of a steric interaction, they imply that, in the Me_2SO-rich environment, the glycosidic bonds

*As the u v absorption spectrum of iodine in Me_2SO is essentially the same as in water[18], it is unlikely that these observations are related to the solvation of the iodine *per se*

†Beta-amylase dissolved in Me_2SO was found to retain its activity during the duration of the measurements

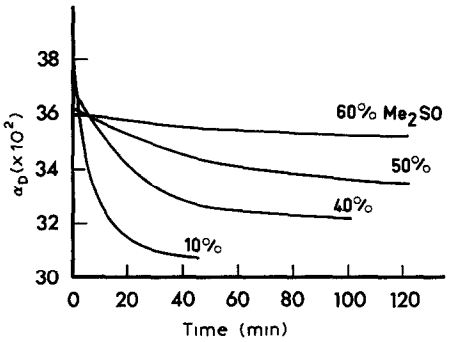

Fig 7 Influence of changes in the composition of H_2O–Me_2SO mixtures on the rate of hydrolysis of high-amylose corn starch by beta amylase (*i e* , starch→α,β-maltose), as monitored by changes in optical rotation [The observed decrease in the initial (0 min) rotatory values with a decrease in % H_2O, is in accord with data reported in ref 24]

of a number of residues associated with branching, including the glycosyl end-groups, experience less strain (^{13}C shift downfield, ^1H shift upfield)[27,28] than in water. Consequently, the fact that these chemical-shift differencers reflect changes in contributions to shielding as the solvation pattern is altered is related to the foregoing discussion. That is, the clusters of minor C-1 and C-4 signals are detectable only when the content of Me_2SO in the solvent exceeds 40 percent. In this region, solvation of the residues by Me_2SO may be expected to predominate over that by water

EXPERIMENTAL

The starches were obtained from various industrial and commercial sources. Before use, the starches were dried *in vacuo* for 3 h at 80°.

N m.r. spectroscopy. — The starch (50–100 mg) was dissolved in Me_2SO-d_6 (0.7 mL), by heating a stirred suspension of it at 80–100° until a clear solution was obtained (usually 10 min). Concentrations were so chosen that all of the solution viscosities were (visually) approximately the same. ^{13}C-N.m.r. spectra were recorded with a Varian XL-300 spectrometer (5-mm probe) operated at 75 MHz. In the ^1H-decoupled mode, a flip angle of 45° was used, and 10,000–40,000 scans were accumulated; in the gated-decoupled mode, the flip angle was 90°, the delay time (D_1) was $\geq 10\ T_1$ max, and 1000–3000 scans were accumulated. Chemical shifts are referenced with respect to Me_4Si, by use of the solvent Me_2SO-d_6 signal at δ = 39.6. Spin–lattice relaxation times (T_1) were measured by the inversion-recovery method; the standard deviation was ±2.5% for the main signals, and from ±4% to ±10% for the minor signals. Values of n.O.e. were calculated from the expression.

$$\text{n.O.e} = 1 + \frac{I_1 - I_0}{I_0},$$

where I_1 = intensity of the peak with n.O.e., and I_0 = intensity without n.O.e.

For recording of the ^{1}H-n.m.r. spectra, the Me_2SO solution (0.7 mL) of the starch was "exchanged" with D_2O (3 × 0.5 mL) by evaporation of the HOD–D_2O *in vacuo* at 50°; finally, a drop of D_2O was added to the solution.

Quantitative measurements on the ^{13}C and ^{1}H spectra were carried out by integration, or by excision of the peaks and weighing, using the baseline method.

Measurement of iodine complexing ("blue values"). — The dried starch (50 mg) was dissolved by heating in 5 mL of Me_2SO, and the volume was adjusted to 100 mL with water. An aliquot (2–5 mL) of the solution was transferred to a 50-mL flask, followed, in succession, by 1.0M sodium chloride (1 mL), water (40 mL), iodine solution (1 mL, containing 2.0 mg of I_2 and 20 mg of potassium iodide), and additional water to make a total volume of 50 mL (The concentration of starch was ~2 mg/dL for the high-amylose type, and ~5 mg/dL for the waxy type). After 30 min, the absorbance at λ = 600 nm was measured with a Pye Unicam SP8-150 u.v./vis spectrometer, using a 10-mm cell; the blue value[15,16] was expressed as 4 A/c (mg/mL).

Measurement of hydrolysis by beta amylase. — Aliquots (50 μL) of a solution of beta amylase powder (Henley Co., Inc., New York) in water (10 mg/mL) were added to aqueous Me_2SO solutions (5 mL) of high-amylose starch (25 mg) that respectively contained 60, 50, 40, and 10% (v/v) of Me_2SO. Optical rotations were measured (see Fig. 7) at 589 nm and r.t. with a Jasco DIP-140 digital polarimeter. After storage of the enzyme in pure Me_2SO, at the same concentration (opaque solution), for 2 d, the activity was tested by adding the enzyme solution (50 μL) to a solution (5 mL) of the starch in 9:1 water–Me_2SO, as before.

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