Tetrahedron, 1966, Vol. 22, pp. 3061 to 3083. Pergamon Press Ltd. Printed in Northern Ireland

HYDROGEN BONDING AND CONFORMATION OF GLUCOSE AND POLYGLUCOSES IN DIMETHYL-SULPHOXIDE SOLUTION*

B. CASU and M. REGGIANI
Istituto Scientifico di Chimica e Biochimica G.Ronzoni, Milano

G. G. GALLO and A. VIGEVANI
Laboratori Ricerche Lepetit S.p.A., Milano

(*Received* 7 *March* 1966)

Abstract—Hydrogen bonding and conformation of glucose and some di-, oligo- and polyglucoses in dimethylsulphoxide (DMSO) solution have been investigated by studying the NMR C_1H and OH and the IR νO—H absorptions. The chemical shift and the splitting of the C_1H proton signals are consistent with the chair "Cl" conformation of the glucopyranose units of all the products investigated.

The anomeric hydroxyl of glucose and related sugars gives a proton resonance at the lowest field, its chemical shift and splitting being characteristic of the configuration of the anomeric linkage [τ = 3·70–4·05 ppm, J = 4·0–4·5 c/s for α anomers (O_1H axial) and τ = 3·40–3·68 ppm, J = 6·0–7·0 c/s for β anomers (O_1H equatorial)]. Non-anomeric hydroxyls of glucose and most sugars related to glucose give NMR peaks in the range 5–6 τ, taken as characteristic of OH groups free to associate with DMSO by H-bonding. All the hydroxyls free to associate with DMSO give a symmetrical IR νO—H band in the region 3400–3320 cm^{-1}.

The "internal" O_2H and $O_3'H$ of maltose and maltosides show resonances at field lower than 5 τ, the shift down 5 τ increasing from maltose to amylose to cyclodextrins, and an IR νO—H band correspondingly broadened by an additional absorption on the low frequency side. These findings are consistent with the existence of an intramolecular H-bond between the hydroxyls at C_2 and C_3' of contiguous glucose units. The strongest H-bond found in cyclodextrins is explained in terms of a limited rotation of the glucose units of the macrocycle. Cellobiose and laminaran display one hydroxyl signal at field lower than 5 τ and a slight broadening of the IR νO—H band. An intramolecular H-bond between one hydroxyl of a unit and the ring oxygen of the contiguous unit is proposed for the two products.

THE use of dimethylsulphoxide (DMSO) as solvent for carbohydrates has been ever increasing in the past few years both for chemical reactions and for chemico-physical measurements. Hydroxylated molecules are usually strongly associated to DMSO through hydrogen bonding[1] and their conformation and reactivity can be affected by the solvent–solute interaction. In particular, this holds for flexible molecules, which can adapt their shape to give the strongest association with the solvent, balancing the "non-bonding" and the H-bonding energies. Since flexible pyranose units have been suggested in the past for maltose and maltosides (α-1,4-polyglucoses),[2–5] it appeared of interest to investigate the conformation of these products in DMSO and to compare it to that of glucose and polyglucoses with different positional linkages.

NMR spectroscopy is at present one of the most useful tools for studying the conformation of complex molecules in solution and it has been applied to glucose

* Presented in preliminary form at the 1965 Starch Round Table Conference, Pocono Manor, Pa., U.S.A.

[1] C. Agami, *Bull. Soc. Chim. Fr.* 1021 (1965) and refs therein.
[2] K. Freudenberg and F. Cramer, *Chem. Ber.* **83**, 296 (1950).
[3] R. E. Reeves, *J. Amer. Chem. Soc.* **76**, 4595 (1954).
[4] R. Bentley, *J. Amer. Chem. Soc.* **81**, 1952 (1959).
[5] J. Hollò, J. Szejtli and M. Tòth, *Die Stärke* **13**, 222 (1961).

and some polyglucoses in D_2O solution.[6-8] The NMR approach was based on the determination of the orientation (axial or equatorial) of the "anomeric" C_1H proton by chemical shift and splitting correlations. Of the three ring conformations proposed as the most probable for glucose and polyglucoses,[5*] the so-called "Cl" has the C_1H bond equatorial, while "Bl" and "3B" have the C_1H bond axial. Moreover, the splitting of the C_1H resonance permits the dihedral angle between the $H—C_1—C_2$ and the $C_1—C_2—H$ planes to be evaluated by a modified Karplus equation.[6] Although this splitting depends on factors other than the dihedral angle,[11] the angles of the "Cl", "Bl" and "3B" forms (60°, 60° and 0°, respectively) are so different that the measured splitting allows an axial–equatorial (Cl and Bl) or a diaxial (3B) orientation of H_1 and H_2 protons to be differentiated. Glucose[6] and maltose[7,8] in D_2O solution were found by NMR to have their pyranose units substantially in the "Cl" conformation. The same "Cl" conformation has been suggested for the glucopyranose units of cyclodextrins[7,8] and of other polyglucoses (dextrans, laminarin and hydrolysed starch)[8] in D_2O. Glass[8] observed some overlapping of the range of equatorial and axial C_1H proton signals of polyglucoses and explained it in terms of electronegativity of the C_1 atom as determined by the adjacent substituents. The rather high value of the chemical shift of the C_1H proton of cyclodextrins was explained as due to ring current effects.

Our preliminary NMR investigations in DMSO solution[12] have given further information by hydroxyl proton resonances.† Contrary to common NMR solvents, DMSO displays, in fact, hydroxyl proton resonances as usually well resolved peaks.[13] Carbohydrates give OH signals often superimposing each other, but glucose and many related sugars show characteristic OH resonances,[12] whose chemical shifts are independent of concentration in the range 5–20% w/w. The C_1H proton gives the only non-hydroxyl resonance at fields below 6·0 τ, its signal being often hidden by those of hydroxyls. However, it has been better observed by removing the OH resonances by deuteration or by temperature variation. Maltodextrins and amylose in DMSO have shown the C_1H signal within the "equatorial range" of monosaccharides, their splittings being of the same order as those observed in D_2O.[12b]

A further account is here given of NMR investigations on the conformation of glucose and some polyglucoses in DMSO, with particular emphasis on solvent–solute interactions as deduced from NMR and IR hydroxyl absorptions.

* Conformations are named according to Reeves' convention.[9] An alternative nomenclature has been more recently proposed by Isbell and Tipson.[10] According to this latter convention, Reeves' "Cl" conformation is called "CA" when the anomeric substituent is axial and "CE" when it is equatorial.

† While this paper was in press, we became aware of a communication dealing with hydroxyl proton resonances of sugars in DMSO, presented by S. J. Angyal, V. A. Pickles and O. Rajendra at the IUPAC Symposium on the Chemistry of Natural Products, held in Kyoto, Japan, April 1964.

[6] R. W. Lenz and J. P. Heeschen, *J. Polym. Sci.* **51**, 247 (1961).

[7] V. S. R. Rao and J. F. Foster, *J. Phys. Chem.* **67**, 951 (1963).

[8] C. A. Glass, *Canad. J. Chem.* **43**, 2652 (1965).

[9] R. E. Reeves, *Adv. Carbohydrate Chem.* **6**, 107 (1951) and refs therein.

[10] H. S. Isbell and R. S. Tipson, *J. Research NBS*, **64A**, 171 (1960).

[11] M. Karplus, *J. Chem. Phys.* **30**, 11 (1959); *J. Amer. Chem. Soc.* **85**, 2870 (1963).

[12a] B. Casu, M. Reggiani, G. G. Gallo and A. Vigevani, *Tetrahedron Letters* 2839 (1964); [b] *ibid.*, 2253 (1965).

[13] O. L. Chapman and R. W. King, *J. Amer. Chem. Soc.* **86**, 1256 (1964).

RESULTS

C_1H *and* OH *resonances*

The 60 Mc/s spectra of α- and β-glucose and of the corresponding methylglucosides in DMSO are shown in Fig. 1. Only the OH and C_1H signals appear within the range accessible with DMSO as solvent (below 6·0 τ). The assignment of the resonances to O_1H, C_1H and O_6H protons was made as previously reported.[12b] In addition, a spin decoupling experiment was performed on α-glucose at 100 Mc/s, confirming the assignment of O_1H and C_1H: irradiation of the O_1H doublet near 3·85 τ reduces the multiplicity of the C_1H signal to a doublet with a 3·0 c/s splitting, while irradiation of the C_1H triplet-shaped signal near 5·08 τ cancels the splitting of the O_1H doublet.

NMR data in DMSO solution are reported in Table 1 for monosaccharides of the gluco-, galacto- and xylo-series and in Table 3 for some di-, oligo- and polysaccharides, mainly of the amylose series. In Table 2 a comparison is given of data of C_1H resonances of monosaccharides in DMSO and in D_2O, when available. From the tables some regularities in the spectra of α- and β- anomers of sugars of the gluco-, galacto- and xylo-series are apparent:

(1) O_1H resonances are the most downfield signals and are characteristic of α- and β-anomers, $\tau = 3{\cdot}70$–$4{\cdot}05$ ppm ($J = 4{\cdot}0$–$4{\cdot}5$ c/s) and $\tau = 3{\cdot}40$–$3{\cdot}68$ ppm ($J = 6{\cdot}0$–$7{\cdot}0$ c/s), respectively.

(2) C_1H signals are displayed, usually as triplet-shaped peaks,* between 4·83 and 5·45 τ for α-anomers and between 5·36 and 5·99 τ for β-anomers. These signals are displaced some tenths ppm upfield with respect to the values in D_2O solution. As reported in our previous paper,[12b] the splitting of the C_1H peaks of the investigated sugars is substantially the same in the two solvents.†

(3) O_2H, O_3H and O_4H peaks of glucoses and xyloses are displayed for α-anomers as well resolved doublets in the 5·24–5·60 τ range and for β-anomers as closed-up doublets near 5·25 τ. The splitting of the O_2H, O_3H, O_4H peaks ranges from 3·5 to 6·5 c/s the largest one being shown by α-anomers. The largest splitting has been tentatively attributed to the O_2H on the assumption that this hydroxyl should be the most affected by a change in the stereochemistry at C_1, while for the assignment of the O_3H and O_4H we have taken advantage of the suggestion of A. S. Perlin,‡ based on the comparison of a large number of sugars. The assignments of the O_2H, O_3H and O_4H peaks of α-D-glucose are shown in Fig. 1.

Concerning disaccharides, both α-α′-trehalose and α-melibiose display the absorptions from non-anomeric hydroxyls at fields between 5 and 6 τ, like all the pyranosidic monosaccharides so far examined. Maltose and cellobiose do not follow this behavior and show non-reducing hydroxyl absorption below 5 τ, the corresponding intensities being two and one proton, respectively. Concerning triglucoses, maltotriose shows non-anomeric hydroxyl absorptions below 5 τ (the main peak centered at 4·60 τ), while isomaltotriose does not.

In Fig. 2 the 100 Mc/s spectra of maltose and of the polyglucoses of the α-1,4-series, amylose, α- and β-cyclodextrin are shown. A further downfield displacement of the non-anomeric hydroxyl signal below 5 τ is apparent in the order given above going

* In at least one case (α-D-xylose) the C_1H resonance is resolved and clearly displayed as a pair of doublets.

† Similar results were independently obtained by C. T. Greenwood (private communication).

‡ Private communication. The assignments were later confirmed by spin-decoupling [(A. S. Perlin, *Canad. J. Chem.* **44**, 539 (1966)].

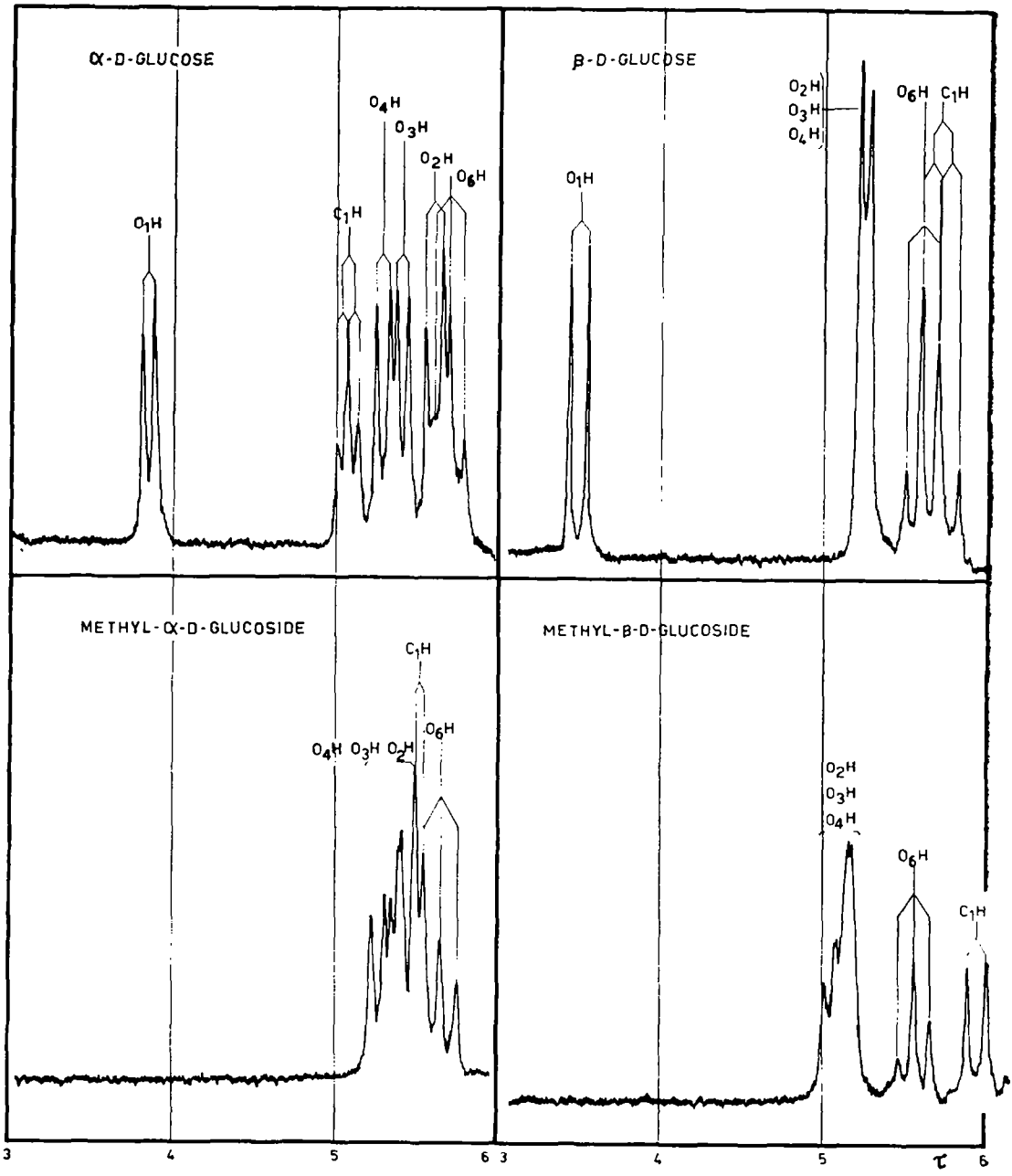


FIG. 1. NMR spectra of α-D- and β-D-Glucose and the corresponding methylglucosides in DMSO at 60 Mc/s.

TABLE 1. CHEMICAL SHIFTS, τ (ppm) AND COUPLING CONSTANTS (c/s, GIVEN IN PARENTHESES) OF GLUCOSE AND SOME RELATED MONOSACCHARIDES IN DMSO

Monosaccharides	O_1H doublet equat.	O_1H doublet axial	C_1H pair of doublets equat.	C_1H pair of doublets axial	O_2H, O_3H, O_4H doublets	O_6H triplet
α-D-Glucose		3·85(4·5)	5·08(4·5; 3·0[a])		5·28(5·0); 5·40(4·5); 5·60(6·5)	5·70(5·0)
β-D-Glucose	3·50(6·5)			5·70(6·5; 6·5[a])	5·25(3·5) three OH's	5·58(5·5)
α-D-Galactose		3·95(4·5)	5·05(4·5; ~2·0[a])		5·57(5·0); 5·74(7·0); 5·78(4·0)	5·54(5·0)
2-Deoxy-α-D-Glucose[b]		3·96(4·0)	4·83(4·0; 2·5[a]; 2·5[a]) multiplet		[c]	[c]
2-Deoxy-β-D-Glucose	3·58(6·5)			5·36(6·5; 9·5[a]; 2·0[a]) multiplet	5·30(4·0) two OH's	5·60(5·0)
6-Deoxy-α-L-Galactose(α-L-Fucose)		4·05(4·5)	5·11(4·5; 2·5[a])		[5·62; 5·71; 5·74; 5·80; 5·88; 5·95;][d]	
6-Deoxy-β-L-Galactose(β-L-Fucose)[b]	3·68(6·0)			[c]	[c]	
α-D-Xylose		3·90(4·5)	5·15(4·5; 3·5[a])		5·24(4·0); 5·36(3·5); 5·60(6·5)	
β-D-Xylose[b]	3·50(6·0)			[c]	[c]	
Methyl-α-D-Glucoside			5·45(3·0[a]) doublet		5·22(5·0); 5·32(3·5); 5·40(6·5)	5·62(5·5)
Methyl-β-D-Glucoside				5·95(7·0[a]) doublet	[5·01; 5·09; 5·18][d]	5·56(6·0)
Methyl-β-D-Xyloside				5·99(7·0) doublet	5·05(7·0); 5·11(4·0) two OH's	

[a] Value obtained from the deuterated product (see Experimental). [b] Product obtained by mutarotation and examined in mixture with the anomer. [c] Values not obtainable from the mixture of the anomers. [d] Prominent peaks from complex resonances.

from maltose to β-cyclodextrin. The peak assignments given in figure were made by identifying the C_1H signal as the only absorption unaffected by deuteration and the O_6H signal by its triplet shape, as expected from a primary hydroxyl. The fact that the intensity of the non-anomeric hydroxyl absorption of maltose below 5 τ corresponds to two hydroxyls per molecule originates the hypothesis that these "low-field" signals are due to the protons of the "internal" O_2H and $O_3'H$ groups, i.e. the hydro-

TABLE 2. CHEMICAL SHIFTS, τ VALUES (ppm) AND COUPLING CONSTANTS, $J_{H_1H_2}$ (c/s) OF THE C_1H RESONANCES OF SOME DEUTERATED MONOSACCHARIDES IN DMSO AND IN D_2O.

	Equatorial				Axial			
	DMSO		D_2O		DMSO		D_2O	
Monosaccharides	τ	J	τ	J	τ	J	τ	J
α-D-Glucose	5·06	3·0	4·78	2·4[a]				
			4·78	3·0[b]				
			4·80	3·2[c]				
			4·84	3·0[d]				
β-D-Glucose					5·68	6·5	5·36	7·5[a]
							5·37	7·4[b]
							5·42	6·8[c]
							5·42	7·4[d]
α-D-Galactose	5·05	~2·0	4·77	2·7[b]				
			4·74	1·8[d]				
2-Deoxy-α-D-glucose	4·83	~2·5; ~2·5	4·67	3·8; 0·7[a]				
2-Deoxy-β-D-glucose					5·36	9·5; 2·0	5·07	9·7; 2·0[a]
α-D-Xylose	5·14	3·5	4·82	2·2[a]				
			4·83	2·6[d]				
Methyl-α-D-glucoside	5·45	3·0	5·21	3·0[b]				
			5·25	3·3[c]				
Methyl-β-D-glucoside					5·95	7·0	5·54	7·7[a]
							5·62	7·4[b]
							5·65	7·4[c]

[a] From Ref. 6. [b] From J. M. Van der Veen, *J. Org. Chem.* **28**, 564 (1963). [c] From Ref. 8. [d] From M. Rudrum and D. F. Shaw, *J. Chem. Soc.* 52 (1965). [e] From Ref. 7.

xyls facing each other in adjacent α-1,4-linked glucose units. This hypothesis is also supported by the fact that the intensity of the low-field absorption of non-anomeric hydroxyls of maltotriose corresponds to four hydroxyls per molecule. Because of ill-defined NMR patterns, intensity measurements on maltotetraose, maltopentaose and maltohexaose were not as accurate as it would be required to evaluate exactly the number of the hydroxyls corresponding to the low-field absorption. Anyhow, the intensity of this absorption going from the tetramer to the polymer shows the trend of approaching the hydroxyl ratio 2/3.*

Linear dextran, like the 1,6-linked saccharides, gentiobiose, melibiose and isomaltotriose, does not absorb at field below 5 τ. Branched dextran, whose spectrum appears more complex than that of linear dextran, also does not absorb below 5 τ. Laminaran, on the contrary, displays at 4·75 τ a hydroxyl signal corresponding to one proton.

In order to investigate the effect of the molecular environment on hydroxyl

* Recent spin-decoupling experiments on β-maltose have confirmed the assignment of the "low-field OH signals" to the internal O_2H (doublet at 4·66 τ) and $O_3'H$ (singlet at 4·63 τ). In amylose and cyclodextrins the O_2H doublet is displayed at field lower than the O_3H signal.

TABLE 3. CHEMICAL SHIFTS, τ (ppm) AND COUPLING CONSTANTS (c/s, GIVEN IN PARENTHESES) OF SOME DI-, OLIGO- AND POLYSACCHARIDES IN DMSO

Di-, oligo- and polysaccharides	O_1H doublet equat.	O_1H doublet axial	C_1H doublet equat.	C_1H doublet axial	OH's under 5 τ doublets	Other OH's
α,α'-Trehalose[1-O(α-D-glucopyranosyl)α-D-glucose]			5·15(3·0)			5·33(4·5); 5·48(6·0); 5·70(4·0[a]) doublet doublet triplet
α-Maltose[4-O(α-D-glucopyranosyl)α-D-glucose][b]		3·70(4·5)	[c]		4·75(*c*)	[c]
β-Maltose[4-O(α-D-glucopyranosyl)β-D-glucose	3·40(6·5)		5·01(3·0)	5·70[d](6·5; 6·5[d]) pair of doublets	4·63(<2); 4·66(6·5[a])	[5·07; 5·15; 5·21; 5·57][e]
α-Cellobiose[4-O(β-D-glucopyranosyl)α-D-glucose][b]		3·72(4·5)	[c]	[c]	[c]	[c]
β-Cellobiose[4-O(β-D-glucopyranosyl)β-D-glucose]	3·42(7·0)			5·60[d](7·0; 7·0[d]) pair of doublets 5·71(6·5)[d]	4·82(3·5)	[5·06; 5·12; 5·39; 5·46; 5·54][e]
α-Gentiobiose[6-O(β-D-glucopyranosyl)α-D-glucose][b]		3·84(4·0)	[c]	[c]		
β-Gentiobiose[6-O(β-D-glucopyranosyl)β-D-glucose][b]	3·50(7·0)			[c]		[c]
α-Melibiose[6-O(α-D-galactopyranosyl)α-D-glucose]		3·88(4·0)	5·00–5·22[a]			[5·30; 5·52; 5·61; 5·67; 5·72][e]
β-Melibiose[6-O(α-D-galactopyranosyl)β-D-glucose][b]	3·54(6·5)		[c]			[c]
Maltotriose		[f]	5·00(*f*)		~4·60(*f*); ~4·68(*f*)	[~5·15; ~5·60][e]
Isomaltotriose		[f]	~5·10(*f*)			[~5·30; ~5·65][e]
Maltotetraose		[f]	~5·00(*f*)		~4·60(*f*)	[~5·20; ~5·50][e]
Maltopentaose		[f]	~5·00(*f*)		~4·60(*f*)	~5·50(*f*)
Maltohexaose		[f]	~5·00(*f*)		~4·60(*f*)	~5·50(*f*)
Amylose			4·90(3·0[g])		~4·55(*f*); 4·61(*f*)	5·51(*f*)
α-Cyclodextrin			5·19(3·0[g])		4·55(6·0); 4·59(<2·0)	5·57(*f*)
β-Cyclodextrin			5·17(3·0[g])		4·33(6·0); 4·38(<2·0)	5·62(*f*)
Laminaran				~5·56[g](7·0[g])	4·75(*f*)	[5·31; ~5·50][e]
Linear dextran			5·40[g](2·5[g])			[~5·20; ~5·55][e]
Branched dextran			~5·10[g](*f*) ~5·35[g](*f*)			[~5·60; ~5·70][e]

[a] Value not unequivocally obtainable from the present spectrum. [b] Product obtained by mutarotation and examined in mixture with the anomer. [c] Values not obtainable from the mixture of the anomers. [d] Value obtained from the deuterated product (see Experimental). [e] Prominent peaks from complex resonances. [f] Values not observable in the present spectrum. [g] Value obtained from the 80–120° 100 Mc/s spectrum.

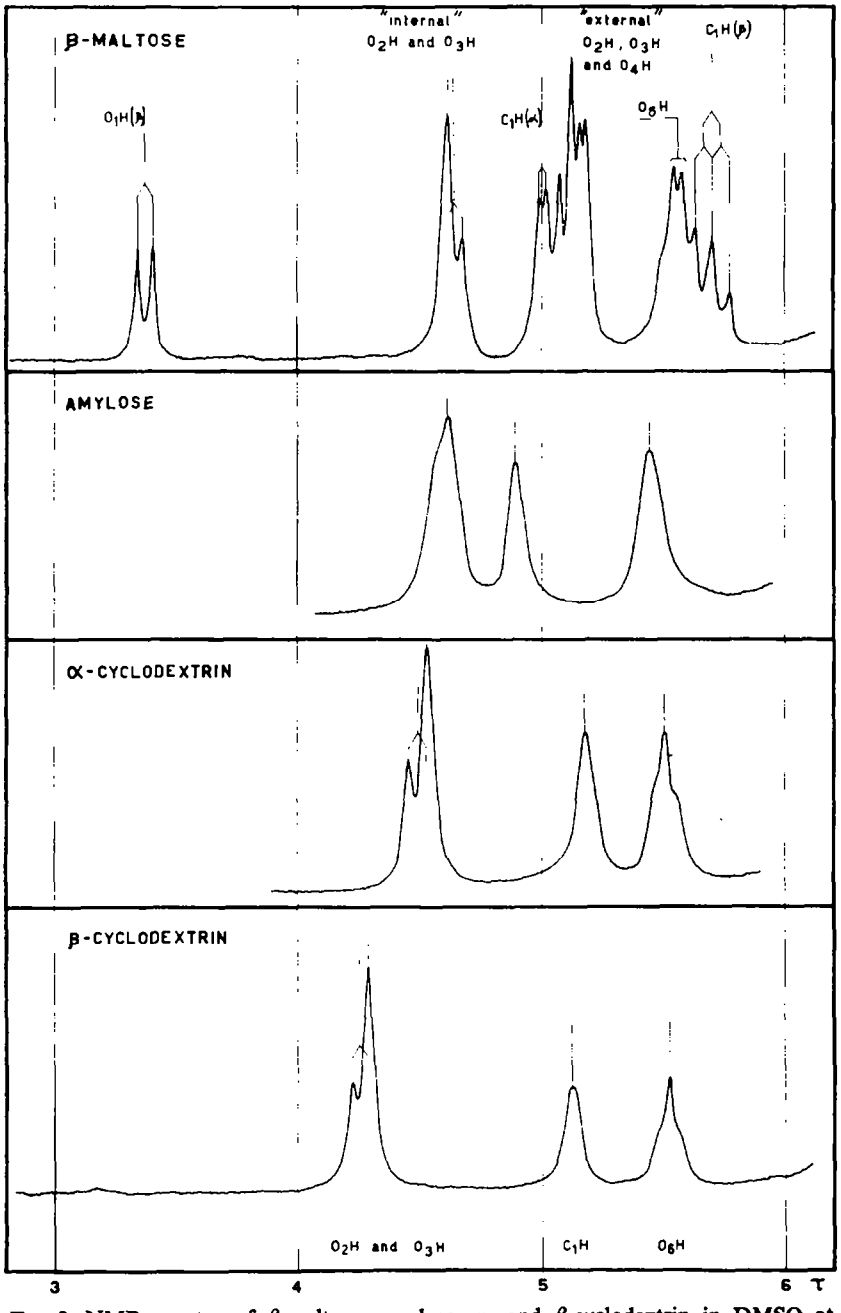


FIG. 2. NMR spectra of β-maltose, amylose, α- and β-cyclodextrin in DMSO at 100 Mc/s at 28°C.

groups in DMSO, a number of representative alcohols were also examined in this solvent and the chemical shifts and coupling constants of their OH resonances are reported in Table 4. As it can be seen from Tables 1, 3 and 4, the hydroxyls without influence from electron-withdrawing groups absorb in DMSO between 5 and 6 τ.* This range is then taken as characteristic for this type of OH groups, that are accessible to DMSO. On the other hand, the hydroxyl of 2,3,3-trimethyl-2-butanol, which is expected to be partially prevented from associating with DMSO because of steric hindrance by the adjacent methyl groups, absorbs at slightly higher field (6·22 τ). The hydroxyls of 1,3-butanediol, 1,4-butanediol and *cis*-1,2-cyclohexanediol, which

TABLE 4. CHEMICAL SHIFTS, τ(ppm) AND COUPLING CONSTANTS (c/s, GIVEN IN PARENTHESES) OF OH RESONANCES OF MODEL HYDROXY COMPOUNDS IN DMSO (10% w/w).

2,3,3-Trimethyl-2-butanol		6·22 s
Cyclohexanol		5·61 d (4·0)
2-Hydroxymethyl-tetrahydropyran		5·57 t (5·0)
1,3-Butanediol		5·67 t (4·5); 5·65 d (4·5)
1,4-Butanediol		5·51 t (5·0)
cis-1,2-Cyclohexanediol		5·89 d (3·0)
5-Hydroxy-1,4-naphtoquinone		−1·75 s
	in $CDCl_3$:	−1·86 s
syn-Ethyl-2(*o*-chlorophenyl)-3-hydroxy-acrylate		−1·20 s
	in CCl_4:	−2·22 d (13·0)
Tetrahydro-cochliobolin A[16]		4·40* s; 1·17* d (7·0)*
	in $CDCl_3$:	5·20 s; 1·37 s
Tetrahydro-cochliobolin B[16]		5·98 s; 4·94 s; 1·14 d (7·0)

s = singlet; d = doublet; t = triplet. * A. Fiecchi, private communication.

in nonpolar solvents are intra H-bonded,[14] absorb in DMSO in the range above mentioned. This suggests that the intramolecular association is mainly replaced by H-bonding with the solvent. However, as shown in Table 4, some intra H-bonds as in juglone (5-hydroxy-1,4-napthoquinone), in *syn* ethyl-2(*o*-chlorophenyl)-3-hydroxyacrylate[15] and in tetrahydro-cochliobolins[16] appear unaffected by DMSO and the corresponding OH resonances are displayed at low field, their chemical shifts being practically the same as in $CDCl_3$.

Solvent and temperature effects on OH *and* C_1H *proton resonances*

In order to obtain information on the type and strength of the association involving the "internal" hydroxyls of maltosides, we have investigated the behavior of the OH and C_1H resonances of β-cyclodextrin toward a change in the solvent characteristics. This product was chosen because the downfield shift from 5 τ of its O_2H and $O_3'H$ resonances is larger than that of any other maltosides so far investigated. Moreover, the lack of reducing hydroxyl groups avoids complications due to possible mutarotation.

Some solvents of different dielectric constants were added to DMSO solutions of

* Hydroxyl proton resonances of cyclohexanol and a number of related alcohols in DMSO have been recently reported (C. P. Rader, *J. Amer. Chem. Soc.* **88**, 1713 (1966)).

[14] G. C. Pimentel and A. L. McClellan, *The Hydrogen Bond*, p. 97. W. H. Freeman and Co., San Francisco (1960).

[15] G. Cignarella, L. Mariani and E. Testa, *Gazz. Chim. Ital.* **95**, 831 (1965).

[16] L. Canonica, A. Fiecchi, M. Galli Kienle and A. Scala, *Tetrahedron Letters*, 1211 and 1329 (1966).

β-cyclodextrin and the chemical shifts of these solutions were compared with those of some reference alcohols in the same solvent mixtures. The variation of the OH resonances of β-cyclodextrin on addition of acetonitrile, dioxane and water to its solutions in DMSO is compared in Table 5 to that experienced by model alcohols such as cyclohexanol and 2-hydroxymethyl-tetrahydropyran (HMTP). It can be seen that dioxane and acetonitrile shift upfield the OH resonances, the variation being not of the same order for all the hydroxyls investigated. In fact, OH resonances of cyclohexanol and HMTP display a somewhat larger shift than the O_6H signal of β-cyclodextrin, while the O_2H and O_3H signals of the latter product are affected only a little. Water shifts the OH resonances downfield. However, the shift experienced by the O_2H and O_3H signals of β-cyclodextrin is still smaller than that of the O_6H signal of the same product and that of the hydroxyl proton of cyclohexanol and of HMTP.

TABLE 5. SOLVENT DEPENDENCE OF HYDROXYL PROTON RESONANCES (τ VALUES) OF CYCLOHEXANOL, 2-HYDROXYMETHYL-TETRAHYDROPYRAN (HMTP) AND β-CYCLODEXTRIN AT 38°.
(CHEMICAL SHIFT DIFFERENCES WITH RESPECT TO DMSO ARE GIVEN IN PARENTHESES)

	Cyclohexanol	HMTP	β-Cyclodextrin		
			O_2H and O_3H	O_6H	C_1H
DMSO/CH_3CN 1:1 v/v	6·01(+0·40)	5·92(+0·35)	4·40(+0·02)	5·78(+0·16)	5·12(−0·05)
DMSO/Dioxane 1:1 v/v	5·78(+0·17)	5·79(+0·22)	4·38(0·00)	5·72(+0·10)	5·12(−0·05)
DMSO	5·61	5·57	4·38	5·62	5·17
DMSO/H_2O 9:1 v/v	5·25(−0·36)	5·33(−0·24)	4·31(−0·07)	5·44(−0·18)	5·16(−0·01)

A preliminary investigation of the temperature effect on α- and β-cyclodextrin and amylose was also undertaken. Figure 3 shows the 100 Mc/s spectra of β-cyclodextrin run at 28° and 80°, from which it is apparent the upfield shift of the OH resonances, as a result of the diminished strength of solvent–solute association. The C_1H signal remains practically at the same field at the two temperatures and its splitting is well observable at 80°. The coupling constant is 3·0 c/s, the same previously suggested by us from the band shape in the 38° 60 Mc/s spectrum.[12b] Essentially the same splitting was measured for α-cyclodextrin and amylose. Contrary to CH resonances, OH signals tend to broaden and to coalesce as a result of the weakened H-bonding. The shift induced by increasing the temperature is slightly smaller for the O_2H and O_3H peaks than for the O_6H peak. Also dextran and laminarin spectra were run at high temperature, as shown in Fig. 4. In both compounds the splitting of the C_1H signal, as measured from the high temperature spectra (2·5 and 7·0 c/s, respectively), is consistent with the "Cl" conformation of the glucopyranose units.

Hydroxyl IR *absorption*

The IR absorption of OH groups is quite sensitive to H-bonding effects. The most characteristic hydroxyl band, due to the stretching vibration of the O–H bond (νO–H) is usually found in the 3650–3200 cm^{-1} range. Its frequency is lowered and its intensity and width are usually increased by H-bonding. This effect has been widely investigated in non-associative solvents, which allows inter- and intramolecular associations of hydroxylated molecules to be studied over the appropriate range of concentrations.[14]

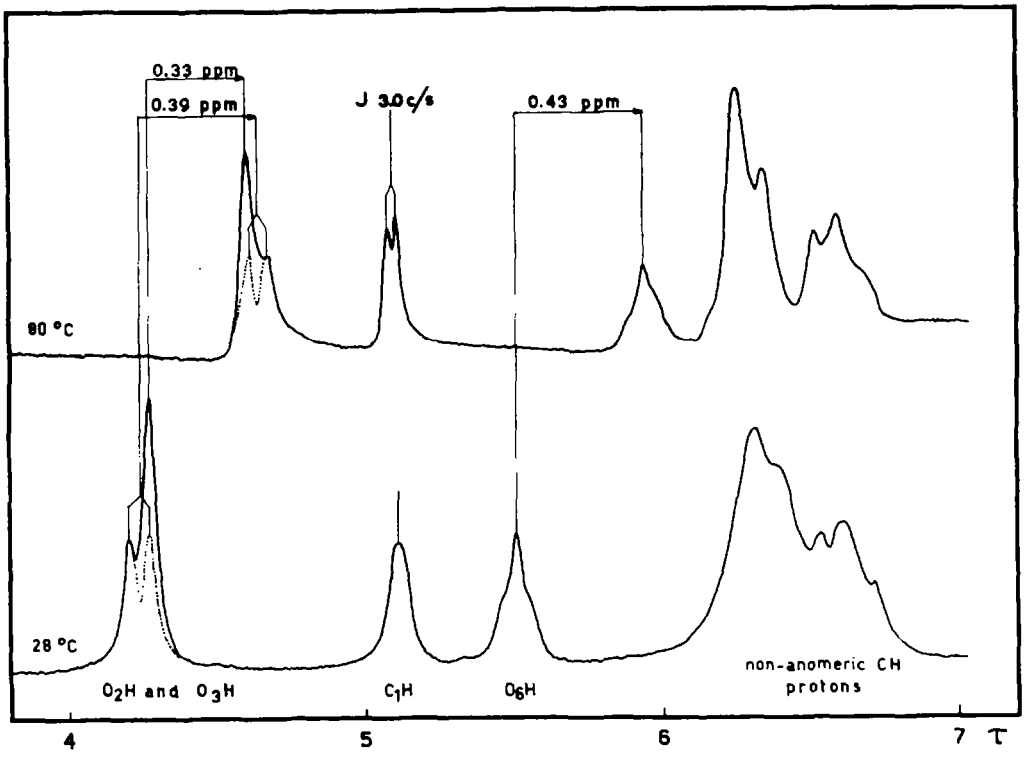


FIG. 3. Temperature dependence of β-cyclodextrin proton resonances in DMSO-d_6 at 100 Mc/s.

The νO—H bands of three alcohols (cyclohexanol, HMTP and *cis*-1,2-cyclohexanediol), taken as simple models of sugars, are shown in Fig. 5. Cyclohexanol shows the spectral behavior of a simple alcohol, displaying in very dilute CCl_4 solution a sharp band at 3625 cm^{-1}, typical of the "free" hydroxyl.[14] In concentrated solutions in the same solvent, cyclohexanol molecules are almost completely self-associated, as revealed by the marked reduction in intensity of the "free" O—H band and by the presence of the intense broad band at lower frequency, typical of intra H-bonded alcohols. Also HMTP in dilute CCl_4 solution gives a sharp band. However, this band occurs in the highest part of the range of intra H-bonds, as reported by Barker *et al.*,[17] who assumed that the O—H group of HMTP is bonded to the ring oxygen, as shown in the figure. In concentrated CCl_4 solution such intra H-bonds, although mostly replaced by the inter H-bonds of dimeric species, are, however, more evident than the "free" hydroxyl of cyclohexanol at the same concentration. *cis*-1,2-Cyclohexanediol in dilute CCl_4 solution displays bands at 3626 and 3585 cm^{-1}, the first attributable to the "free" O—H and the second to the intra H-bonded O—H. As shown in the same figure, all the three alcohols in DMSO solution display a single intense band near 3370 cm^{-1}, revealing a complete association of their OH groups with the

[17] S. A. Barker, J. S. Brimacombe, A. B. Foster, D. H. Whiffen and G. Zweifel, *Tetrahedron* 7, 10 (1959).

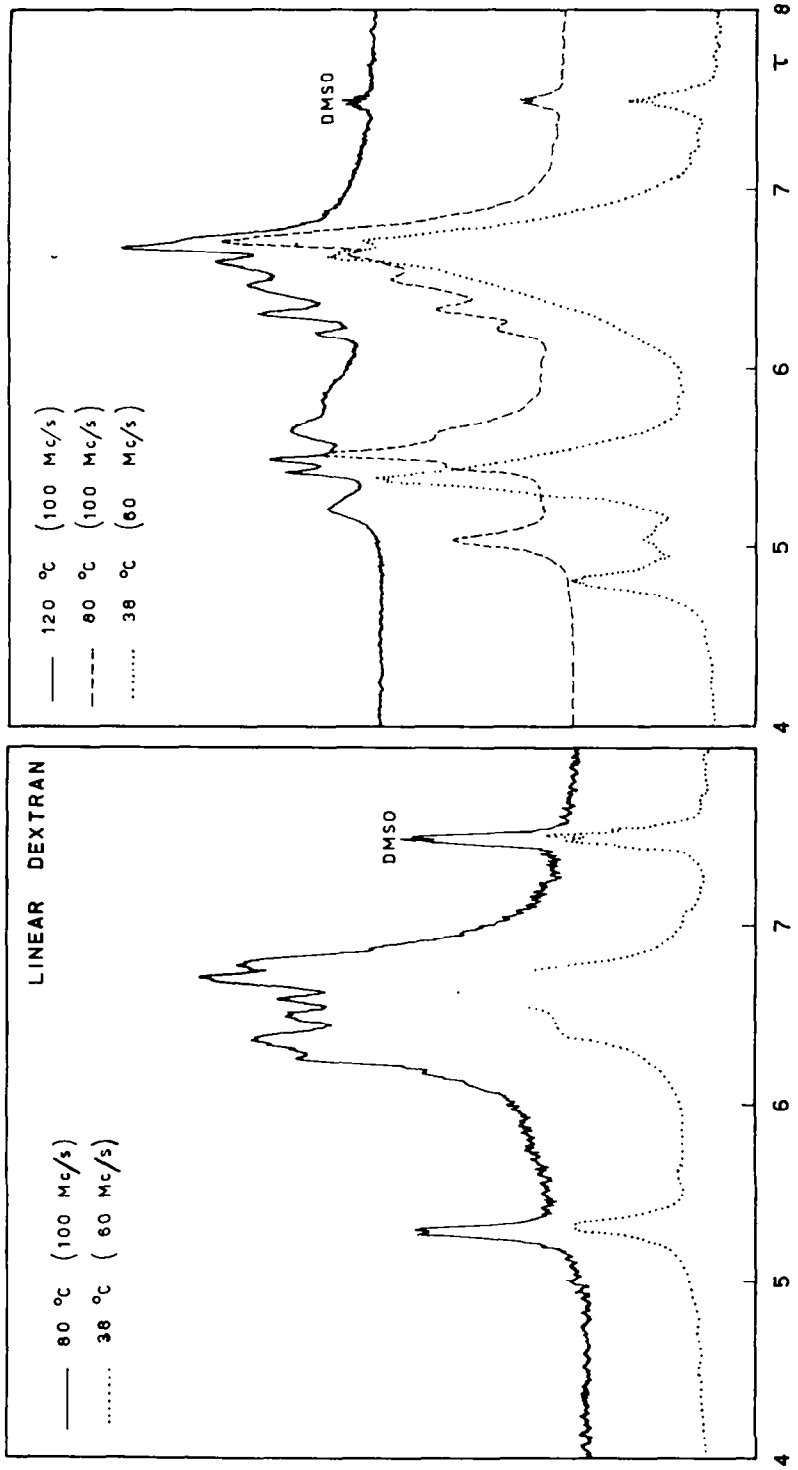


FIG. 4. NMR spectra of (a) linear dextran and (b) laminaran in DMSO-d_6 at different temperatures.

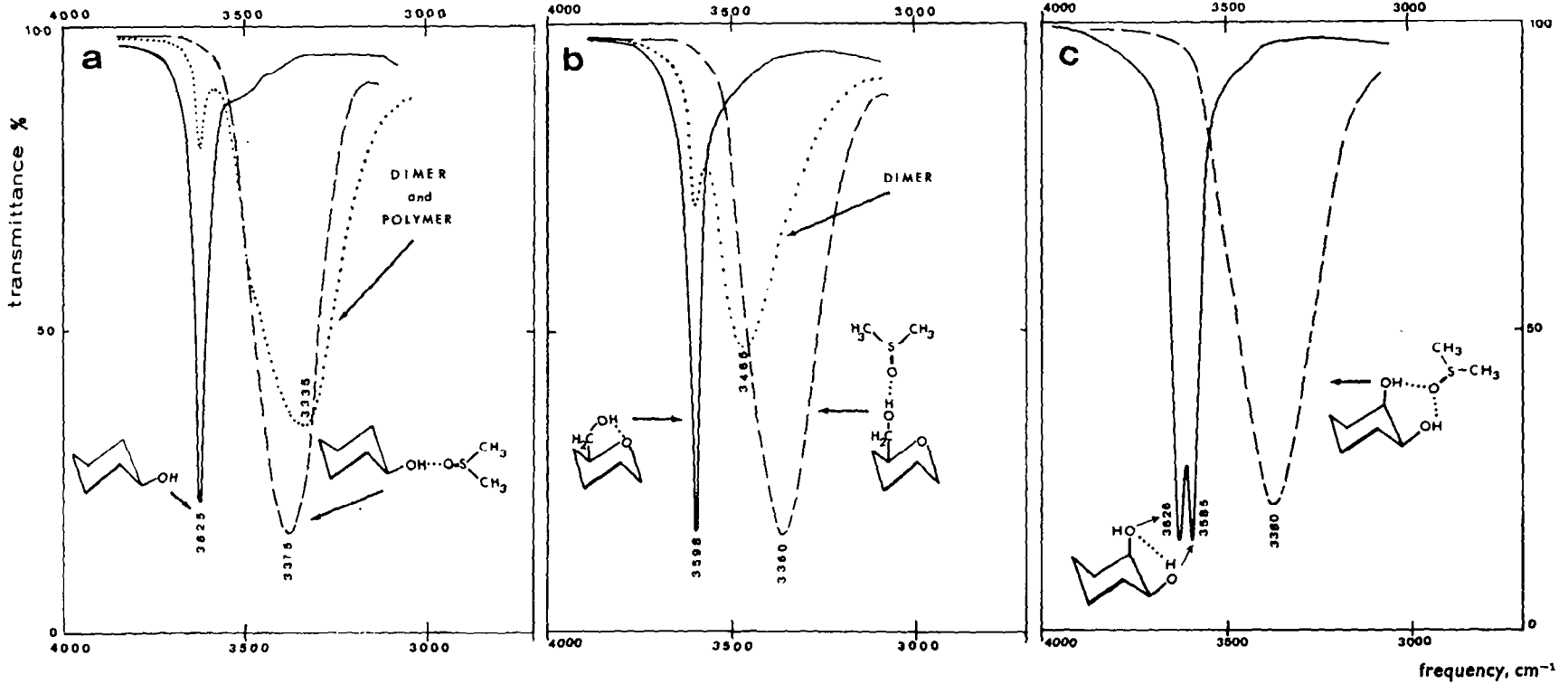


FIG. 5. IR νO—H bands of model alcohols in CCl_4 and DMSO; (a) cyclohexanol; (b) 2-hydroxymethyl-tetrahydropyran; (c) *cis*-1,2-cyclohexanediol. The association for *cis*-1,2-cyclohexanediol with DMSO can alternatively involve two solvent molecules.

——— dil. CCl_4 solution (0·04% w/v, 50 mm silica cell)

····· conc. CCl_4 solution (15% w/v, 0·05 mm silica cell)

– – – – DMSO solution (15% w/v for (a) and (b) and 10% w/v for (c))

solvent. The frequency and width of the band show no detectable variation within the investigated concentration range (from about 1 to 15%, w/v*).

Frequencies, intensities and half band widths of the νO—H bands for a number of alcohols and saccharides in DMSO are given in Table 6. Like cyclohexanol, HMTP and *cis*-1,2-cyclohexanediol, most simple alcohols and saccharides in DMSO solution give an O—H band in the range 3400—3300 cm^{-1}. That this range is to be taken as characteristic of O—H groups associated to the solvent through H-bonding is further substantiated by the fact that the hardly accessible hydroxyl of 2,3,3-trimethyl-2-butanol, that was shown to give its OH proton resonance at a field higher

TABLE 6. FREQUENCY (νO—H), HALF BAND WIDTH ($\Delta\nu 1/2$) AND MOLAR ABSORPTIVITY PER OH GROUP AT THE BAND MAXIMUM (a) OF THE O—H STRETCHING ABSORPTION OF MONO-, DI-, OLIGO- AND POLYGLUCOSES AND SOME MODEL HYDROXYLATED COMPOUNDS.

Compounds	νO—H (cm^{-1})	$\Delta\nu 1/2$ (cm^{-1})	a ($M^{-1} \cdot cm^2$)
α-D-Glucose	3340	220	105
β-D-Glucose	3340	220	110
Methyl-α-D-glucoside	3340	205	111
Methyl-β-D-glucoside	3340	210	114
α,α′-Trehalose	3345	200	108
β-Cellobiose	3330	230	102
Maltose*	3335	232	100
Maltotriose	3340	247	90
Isomaltotriose	3340	220	96
Amylose	3340; 3250 sh	255	98
α-Cyclodextrin	3355; 3270 sh	260	90
β-Cyclodextrin	3355; 3275 sh	277	93
Laminaran	3340	255	93
Linear dextran	3340	225	95
2,3,3-Trimethyl-2-butanol	3415	150	110
Cyclohexanol	3415	150	119
2-Hydroxymethyl-tetrahydropyran	3355	180	107
1,3-Butanediol	3380	165	125
1,4-Butanediol	3375	165	130
cis-1,2-Cyclohexanediol	3380	190	85
trans-1,4-Cyclohexanediol	3380	160	115
5-Hydroxy-1,4-naphtoquinone	3115	very broad	130
α-D-Galactose	3330	230	95
α-D-Xylose	3320	215	103

sh = shoulder

* Since a mixture of α- and β-maltose displays a band identical to that of the β-anomer, the same values are assumed for both anomers.

than 6 τ, absorbs in DMSO at a somewhat higher frequency (3415 cm^{-1}). From these NMR and IR results it can be inferred that the strength of H-bonding is the main factor which determines the downfield shift of the OH resonances of alcohols having no electronegative substituents. The chelated hydroxyl of juglone absorbs at consistently lower frequency, thus revealing that its strong intra H-bond is not

* Because of the band intensity, higher concentrations would require cells thinner than 50 μ. Concentrations lower than 1% would require cells thicker than 0·5 mm and the solvent absorption would be difficult to be compensated.

broken by the solvent. The band of α-glucose is quite symmetrical and displayed in the range of hydroxyls H-bonded to DMSO, while the band of maltose and maltosides is no longer symmetrical. An inflexion on the low-frequency side suggests the presence of an additional absorption, which appears to increase in the order maltose, maltotriose, amylose, α- and β-cyclodextrin. As shown in Fig. 6, there is a linear relationship between the width of the IR νO—H band and the NMR chemical shifts of the "internal" O_2H and $O_3'H$ for α-maltose, amylose, α- and β-cyclodextrin.

DISCUSSION

(1) *Conformation and hydrogen bonding of glucose in* DMSO

Both NMR and IR spectra show that all the hydroxyl groups of α- and β-glucose in DMSO are strongly H-bonded to the solvent. All the intra H-bonds expected in the highly hydroxylated structure of glucose[18] are then broken by DMSO. As the electron-withdrawing effect of the ring oxygen makes the anomeric hydroxyl proton

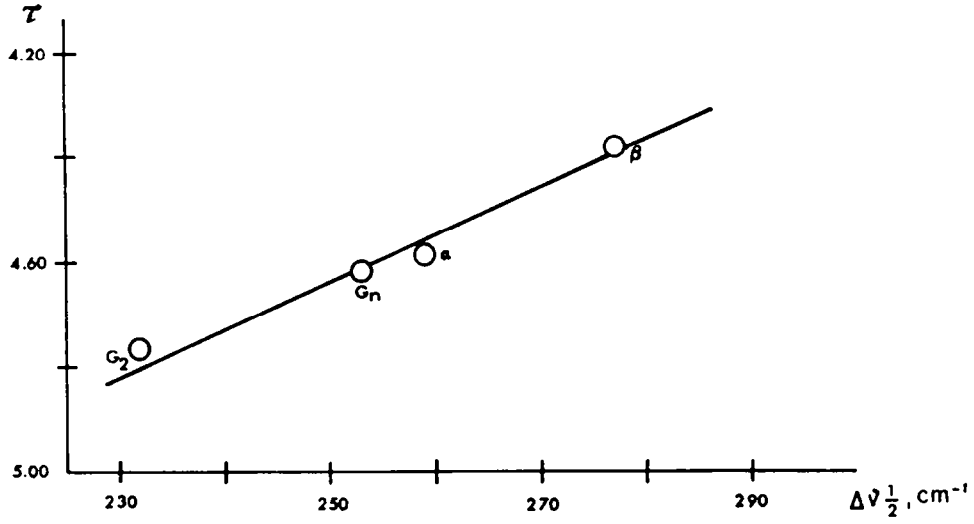
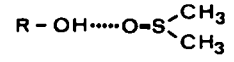


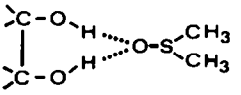
FIG. 6. Relationship between the chemical shift of the O_2H–$O_2'H$ resonances and half band width of the IR νO—H band.
G_2 = α-maltose; G_n = amylose; α = α-cyclodextrin; β = β-cyclodextrin.

more "acidic" than the other hydroxyl protons, the former is expected to be more strongly H-bonded. The large downfield shift displayed by the O_1H signal due to the inductive effect could thus be increased by H-bonding. On the other hand, no significant difference was found in frequency, width and intensity-per-OH-group of the IR νO—H band of glucose and the corresponding methylglucosides. However, the lack of IR evidence of a stronger association of the anomeric hydroxyl can be considered as mainly due to an insufficient sensitivity of the IR absorption to reveal a slight difference in H-bonding of an individual hydroxyl. The fact that the non-anomeric hydroxyls of α-glucose give individual NMR peaks with characteristic splitting suggests a slightly different geometry and strength of their H-bonds. On the other hand, β-glucose shows apparently equivalent O_2H, O_3H and O_4H protons in this respect.

[18] A. J. Michell and H. G. Higgins, *Tetrahedron* **21**, 1109 (1965).

A H-bond involving one OH group per one DMSO molecule, as shown in (a), is the most obvious association for a monohydroxylated compound. When two vicinal hydroxyls are available, one DMSO molecule might associate with both of them, as shown in (b), thus forming a 7-membered ring. An association of the second type was recently proposed by Rao and Foster for cis (axial, equatorial) diolic system like the two hydroxyls at C_1 and C_2 of α-glucose and α-xylose.[19] These authors suggested that such "complex" formation caused a distortion of the pyranose "C1" ring with a decrease of the C_1H/C_2H dihedral angle. The same authors later noticed that their hypothesis was based on an erroneous assignment of the O_1H doublet to the C_1H proton.* Our H_1H_2 coupling constant values (Table 2) definitely suggest that the conformation of glucoses and xyloses in DMSO is substantially the same as in D_2O, i.e. the "C1", which apparently leaves enough room around the hydroxyls for associating with the bulky DMSO molecules. Moreover, the fact that the resonance

a 

b 

of the anomeric hydroxyl is found at about the same field for α-glucose and 2-deoxy-α-glucose (3·85 and 3·96 τ, respectively) and for β-glucose and 2-deoxy-β-glucose (3·50 and 3·58 τ, respectively), the coupling constants being substantially the same (4·0–4·5 and 6·5 c/s, respectively), suggests that the anomeric hydroxyls of these compounds are H-bonded in a similar way. Due to the expected sensitivity of the OH peak chemical shift and splitting towards the strength and the geometry of the H-bonding, the O_1H resonances of glucoses and their corresponding 2-deoxy derivatives would have been significantly different if the compounds were H-bonded in a different way. Consequently, since 2-deoxyglucoses cannot give with DMSO an association of the (b) type, the anomeric hydroxyl of glucoses should associate with one DMSO molecule as in (a).

A rather constant difference (0·3–0·4 ppm) was found between O_1H resonances of α- and β-anomers, the latter absorbing at lower field. Having assumed the "C1" conformation for the pyranose unit, "equatorial" O_1H protons thus absorb at lower field than the "axial", having a behavior similar to C_1H protons.[20] This difference in chemical shift may correspond to a difference in the strength of H-bonding with DMSO or merely to a magnetic anisotropy effect, as it was suggested[21] between axial and equatorial CH protons. Because of the above-mentioned insensitivity in revealing slight differences in H-bonding strength between the individual hydroxyls of glucoses, the IR νO—H band cannot provide a conclusive answer to the question.

As far as non-anomeric OH protons are concerned, it appears that their chemical

* V. S. R. Rao, private communication.

[19] V. S. R. Rao and J. F. Foster, *J. Phys. Chem.* **69**, 656 (1965).

[20] R. U. Lemieux, R. K. Kullnig, H. J. Bernstein and W. G. Schneider, *J. Amer. Chem. Soc.* **80**, 6098 (1958).

[21] L. M. Jackman, *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*, pp. 115–119. Pergamon Press, London (1959).

shifts are no longer dependent only on their axial or equatorial orientation. The different accessibility of the individual hydroxyls to the solvent molecules is most probably the main factor affecting the OH resonances. A rather complex H-bonding with DMSO is expected for polyhydroxylated molecules. A possible H-bonding pattern for α-glucose is shown in Fig. 7. The picture should be considered, however, purely speculative and drawn only in the attempt to accommodate the DMSO molecules in such a way as to give the best solvation of glucose, while maintaining the bulky solvent molecules sufficiently apart from each other and from the glucopyranose ring. No attention is paid to the exact geometry of the DMSO molecule, which has been considered non-planar.[1] Also the location of the hydroxyl protons is not considered in the figure.

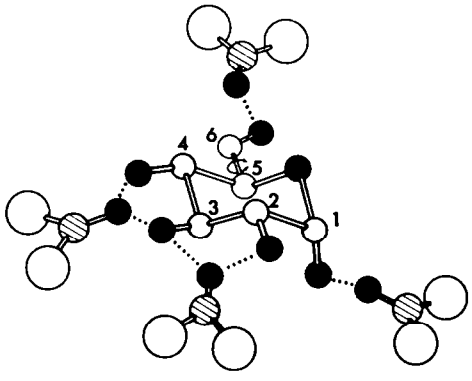


FIG. 7. Possible H-bonding of α-D-glucose in DMSO.
○ carbon; ● oxygen; ● sulphur; ○ methyl. Hydrogens are not shown.

On principle, one would expect to gain information on the stereochemistry of the hydroxyl protons by considering the splitting of the corresponding NMR signals, which should be related to the dihedral angle between the O—H and the adjacent C—H bond. However, no relationship of this kind has been yet established for OH protons. Since in CH—OH systems splitting values as large as 13 c/s (Table 4) and as low as 2 c/s (Table 3) have been observed, we can infer, by analogy with the behavior of CH—CH systems,[6,11] that the widest observed splitting refers to a hydroxyl *quasi* cis (syn-periplanar[22]) or *quasi* trans (anti-periplanar) to the adjacent ring hydrogen and that the smallest splitting is related to a hydroxyl *quasi* gauche (syn- or anti-clinal). The only attempt to correlate the splitting of an OH signal in DMSO to the location of the proton was made by Ouellette,[23] who proposed a "cisoid configuration" of the O—H bond with respect to the α C—H bond of *cis*-4-t-butylcyclohexanol and a "transoid configuration" for the *trans* isomer. However, Ouellette's claim that the proposed configurations are supported by the coupling constants of the OH signals (3·2 and 4·3 c/s for the *cis* and *trans* isomer, respectively) does not seem to be based on any established relationship between the splitting of the OH signals and the CH/OH dihedral angle. For the reasons given above, the reported values likely correspond to angles intermediate between 0° or 180° and 90°. Moreover, an association of the type suggested for 4-t-butylcyclohexanol, implying an additional bonding between

[22] W. Klyne and V. Prelog, *Experientia* **16**, 521 (1960).
[23] R. J. Ouellette, *J. Amer. Chem. Soc.* **86**, 4378 (1964).

the DMSO sulphur and the hydroxyl oxygen, would bring the solvent molecules just above or below the pyranose ring and would barely accommodate more than two DMSO molecules per one glucose molecule.

(2) *Conformation and hydrogen bonding of di-, oligo- and polyglucoses in* DMSO

The availability of the hydroxyl groups of di-, oligo- and polyglucoses for associating with DMSO is not only dependent on the conformation of the glucopyranose units. The "chain conformation" resulting from the rotation of the glucose units about the glycosidic bonds certainly plays an important role in determining the solvent–solute interactions. Among the polyglucoses investigated in the present work, cyclodextrins are to be regarded as interesting models due to the limited rotation expected for the units of the macrocyclic molecules.

The conformation "C1" appears to be retained by the pyranose units of di- and polyglucoses, as derived from their C_1H resonances. These results are thus similar to those reported by Glass[8] for polyglucoses in D_2O. Most OH resonances of polyglucoses remain in the 5·0–6·0 τ range, suggesting that all their hydroxyls are available for bonding with DMSO. As far as the resonances below 5 τ are concerned, the relationship between the width of the IR νO–H band and the chemical shift of the O_2H and $O_3'H$ resonances, given in Fig. 6, definitely suggests that these resonances can be correlated to a stronger H-bonding. Since the accessibility to DMSO is expected to be reduced rather than increased by building-up polymeric structures, it seems reasonable to infer that the stronger H-bonding of maltosides is intramolecular. This is also suggested by the fact that no downfield shift of OH resonance nor broadening of the IR νO—H band is observed for di- and polyglucoses (such as trehalose and dextrans), whose molecular models do not display a close proximity of hydroxyls of adjacent units. The above intra H-bond will then involve O_2H and $O_3'H$ groups on adjacent units and the proximity of these groups and hence the strength of the internal H-bond will vary according to the conformation of the pyranose units and to the conformation of the polymeric chain.

As mentioned in the introductory part, the chair conformation of the glucopyranose units of cyclodextrins and amylose has long been questioned. The axial glycosidic bond of "C1" glucopyranose units was found to prevent the construction of scale-models of amylose chains.[2,3] Successively, apparently anomalous values of the optical rotations in cuprammonium[2,3] and in aqueous alkali[3,5] led to propose a boat or a skew rather than a chair form of the glucopyranose units of cyclodextrins and amylose. More recent studies on solid cyclodextrins and amylose mostly favored, however, the "C1" conformation of the glucose units, already proposed by Senti and Wittnauer[24] for alkali-amylose. IR spectroscopic solid-phase studies of the amylose–iodine complex[25] and of amylose and cyclodextrins[26] suggested substantially "C1" units. Moreover, α-cyclodextrin in its complex with potassium acetate was recently found by X-ray diffraction studies[27] to possess the glucose units in the "C1" conformation.

On principle, the conformation of a solid is not necessarily retained in solution in

[24] F. R. Senti and L. P. Wittnauer, *J. Amer. Chem. Soc.* **70**, 1438 (1948).
[25] C. T. Greenwood and H. Rossotti, *J. Polym. Sci.* **27**, 481 (1958).
[26] B. Casu and M. Reggiani, *J. Polym. Sci. C*, **7**, 171 (1964).
[27] A. Hybl, R. E. Rundle and D. E. Williams, *J. Amer. Chem. Soc.* **87**, 2779 (1965).

an associative solvent, since small differences in the conformational energy could be overcome by a more favored association with the solvent. The possibility of a conformational change of the pyranose units from the solid to solution should be considered in particular whenever conformations with a low energy difference are taken into account. Previous hypotheses on the conformation of α-1,4-polyglucoses were actually based on the assumption that the stability of the two boat "B1" and "3B" forms of the α-D-glucopyranose ring was comparable to that of the chair "C1" form.[2,3,5] In the light of more recent knowledge on conformational analysis[28] it appears that the energy difference between a chair and a boat (or skew) form is far from being small. Moreover, it is to be pointed out that the reported energy equivlence of the "C1" and the "3B" conformation of glucopyranoses was deduced by considering only a few of the factors affecting the conformational energies. In particular, the destabilizing effect of non-bonding interactions of the "eclipsed" bonds in the "3B" form was neglected by some authors.[5]

The chemical shift and splitting of C_1H signals of cyclodextrins and amylose in DMSO strongly substantiate the "C1" chair conformation. Moreover, as shown in Fig. 8, "C1" chair units are consistent with strong intramolecular H-bonding between O_2H and $O_3'H$ hydroxyls and are expected to be further stabilized by this internal association.* "3B" units, which could also give a $O_2H\cdots O_3'H$ bonding, besides being ruled out by the above energy considerations are also inconsistent with the observed splitting of the C_1H peak and with considerations on the internal H-bonding. In fact, the boat hypothesis states that going from cyclic to linear dextrins the conformation changes from "B1" to "3B".[3] This would imply an increase of the C_1H/C_2H dihedral angle and a closer approach of the O_2H and $O_3'H$ hydroxyls going from cyclic to linear dextrins. On the contrary, we found that α- and β-cyclodextrin and amylose have practically the same C_1H/C_2H dihedral angle and that the internal H-bond is stronger in cyclodextrins than in amylose. Molecular models built with "C1" units show that the internal H-bonding is more likely to be stronger in cyclodextrins than in maltose, in linear dextrins and in amylose. The "internal" O_2H and $O_3'H$ groups of maltose actually seem sufficiently accessible to DMSO, if it is assumed that a slight rotation of the glucose units occurs about the glycosidic bond to relieve the non-bonding interactions of the C_1H and $C_4'H$ bonds. Rotational freedom about the glycosidic bond as well as accessibility of O_2H and $O_3'H$ to DMSO are believed to be progressively reduced going from the dimer to the polymer. Maximum closeness of the above hydroxyls is expected for cyclodextrins due to the cyclic structure which, as stated above, reduces the rotational freedom about the glycosidic link. The strongest intra H-bonding of β-cyclodextrin compared to α-cyclodextrin can arise either from different values of the glycosidic angles or from a slightly different rotation of the glucose units about the glycosidic bond.

It must be pointed out that an intra H-bonding between adjacent glucose units is to be regarded as a stability factor of cyclic structures or large helices (as in "V" amylose) built up with "C1" units. A model of crystalline "V" amylose with an intra bond between the hydroxyls at C_2 and C_3' was suggested by Huggins as early an

* The distances between non-bonded atoms were evaluated by Dreiding molecular models (Büchi, Flawil, Switzerland).

[28] E. L. Eliel, N. L. Allinger, S. J. Angyal and G. A. Morrison, *Conformational Analysis*, Chap. VI. Interscience, New York, N.Y. (1965).

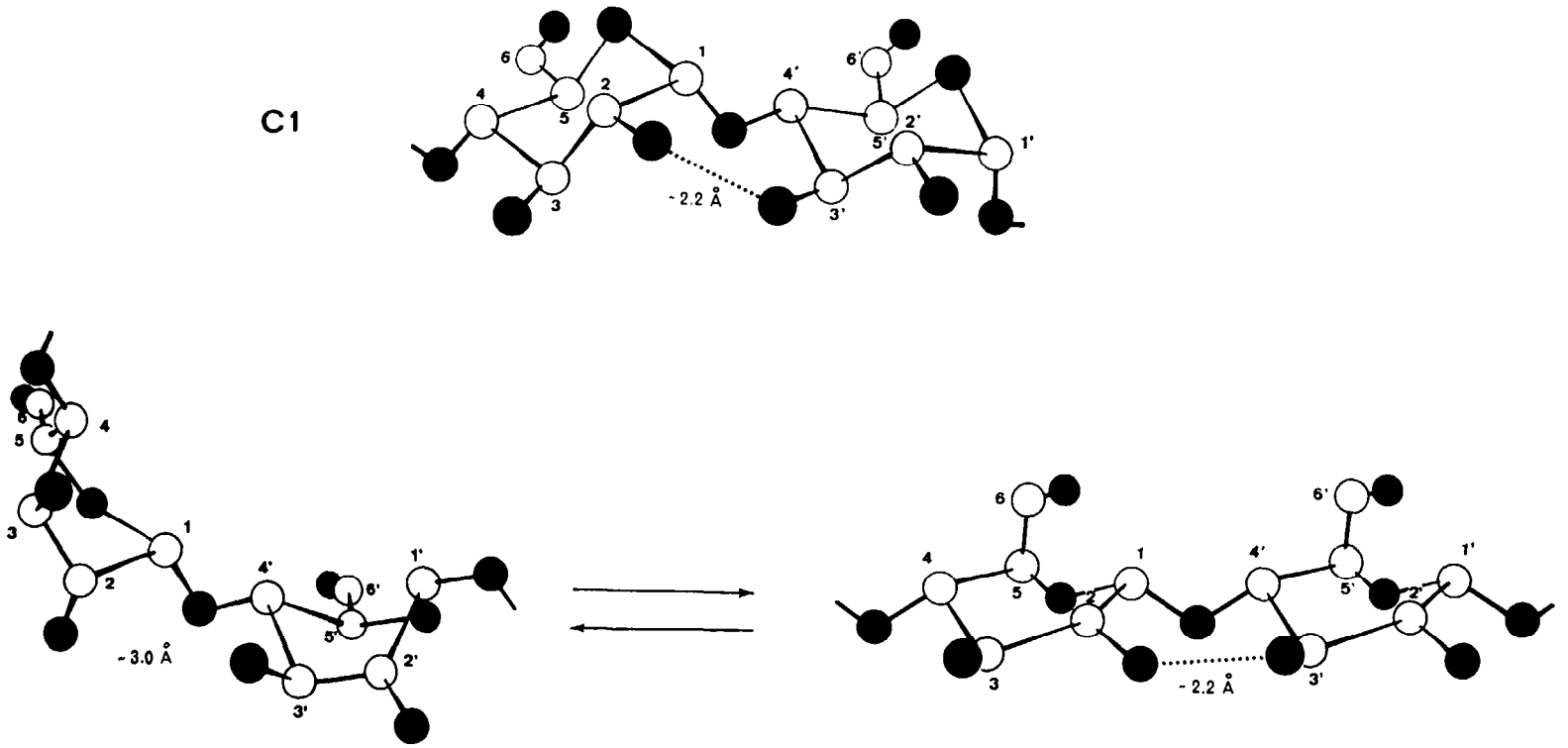


FIG. 8. Dimeric units of α-1,4-linked polyglucoses built with "C1", "B1" and "3B" glucopyranose rings. The most "symmetrical" structures having the C_1—H, C_4—H, C_1'—H and C_4'—H bonds on the same plane are considered.
○ carbon; ● oxygen. Hydrogens are not shown.

ten years ago[29] and recently by Hybl *et al.* on account of X-rays diffraction studies on the α-cyclodextrin potassium acetate complex.[27] The occurrence of a $O_2H\cdots O_3'H$ internal H-bond in DMSO suggests that a helix or at least a coil made of short helical segments is substantially retained by amylose in this solvent. However, no information can be at present acquired regarding the geometry and regularity of the helix.

NMR and IR data suggest for linear dextran a chain conformation with no close approach of the glucose units and for cellobiose and laminarin internal H-bonds involving one hydroxyl group and an acceptor non-hydroxyl oxygen. Possible conformations of the latter two products, where the internal H-bonds form six-membered rings joining successive glucose units, are shown in Fig. 9. A molecular

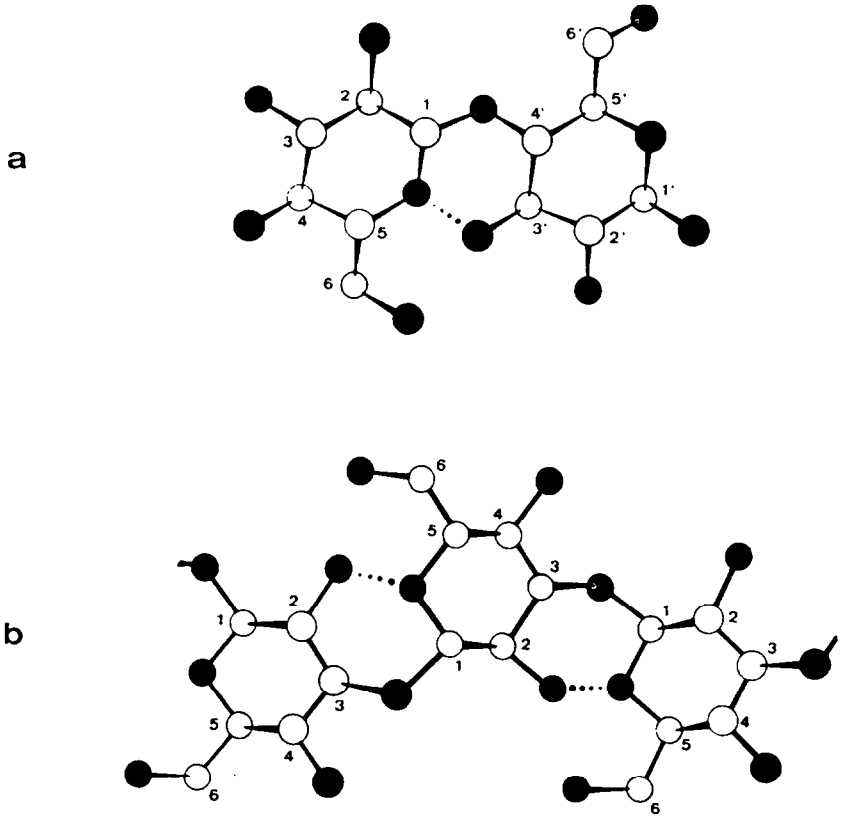


FIG. 9. Chain conformations of (a) cellobiose and (b) laminaran involving "internal" hydrogen-bonds.
○ carbon; ● oxygen. Hydrogens are not shown.

arrangement that gives the most "linear" chain as well as a sufficiently close approach of the intra H-bonded hydroxyl to the acceptor oxygen atom, has been considered. The cellobiose conformation shown in Fig. 9a implies a $O_3'H—O_5$ intra H-bond and is similar to the "bent" model of Hermans,[30] who considered his cellobiose

[29] M. L. Huggins, *J. Chem. Educ.* **34**, 480 (1957).
[30] P. H. Hermans, *Physics and Chemistry of Cellulose Fibers*, p. 13. Elsevier, New York, N.Y., (1949).

model as the repeat unit of cellulose. Laminaran could take another chain conformation also implying an internal H-bond between an hydroxyl group and the ring oxygen of the successive glucose unit, but different from that shown in the figure. This second arrangement is similar to that reported for the crystalline 1,3-linked xylan[31] wherein the intra H-bonded hydroxyl is at C_4 instead of at C_2, as it is in the laminarin model in Fig. 9b.

EXPERIMENTAL

Mono- and disaccharides were from commercial sources (B.D.H., Poole, England; Koch-Light, Colnbrook, Bucks, England and Fluka A. G., Buchs, Switzerland). Since their optical rotation and their solid-state IR spectra (nujol mull) conformed to those reported in literature,[32] the products were used without further purification. Alcohols were from the above-mentioned commercial sources and their purity was checked using classical methods and IR spectroscopy.

Linear oligodextrins from maltotriose to maltohexaose were either kindly provided by Dr. W. J. Hoover of Corn Industries Research Foundation, Washington, D.C. or obtained by absorption chromatography on charcoal–celite of an amylose hydrolysate, according to Whelan *et al.*[33] Some overlapping of the effluent peaks of maltopentaose and maltohexaose was observed by optical rotation measurements of the eluate fractions. The measurements were made with a Perkin–Elmer photoelectric polarimeter mod. 141 directly on the eluate, taking advantage of the high sensitivity attainable with the 361 mμ mercury spectral line. Cyclodextrins were prepared by *b.macerans* amylose cleavage of corn and potato starch, according to Cramer.[34] The purity of cyclic and linear dextrins was checked by optical rotation[35] and by round paper chromatography according to Cramer.[36] Reference standards of maltotriose and maltotetraose and of cyclodextrins were kindly provided by Prof. C. E. Weill of Rudgers University and by Prof. D. French of Iowa State University, respectively.

Amylose (M.W. 150,000) was from Koch-Light. Since its optical rotation and IR spectrum were the same as those of a higher molecular weight sample prepared from corn starch using the Schoch's procedure,[37] the same molecular conformation was assumed and the more soluble Koch-Light sample was used in the present investigation.

Isomaltotriose ($[\alpha]_D^{20}$ in $H_2O = +140$) and laminaran ($[\alpha]_D^{20}$ in DMSO $= -19{\cdot}5$) were from Koch-Light. Linear dextran (NRRL B-512F 95% 1- and/or 1,6-linkages by periodate analysis) and branched dextran (NRRL B-742; 57% 1- and/or 1,6; 17% 1,4; 26% 1,3 linkages) were kindly supplied by Dr. A. Jeanes of the Northern Utilization Research and Development Division of the U.S. Department of Agriculture, through the courtesy of Dr. H. F. Zobel.

syn-Ethyl-2(*o*-chlorophenyl)-3-hydroxyacrylate was supplied by Prof. E. Testa of Lepetit Research Laboratories. DMSO was from B.D.H., DMSO-d_6 from C.I.B.A., Basel, Switzerland. Whenever the H_2O content of DMSO, evaluated by IR spectroscopy,[38] was less than 0·1%, the solvent was used without further purification. If necessary, the solvent was dried by vacuum distillation over NaOH pellets and only the middle distilled fraction was used. For IR measurements, which are more sensitive to water impurities than NMR measurements, carbohydrates were dissolved in DMSO in a dry-box and the H_2O content was checked at 1665 cm^{-1}.[38]

D_2O (99·7%) was from Fluka. The deuteration of sugars before their dissolution in DMSO (or DMSO-d_6) was performed by dissolving them in D_2O and pumping off the liquid phase. The process was repeated three times in order to accomplish a complete OH → OD exchange. In the case of reducing sugars the deuteration procedure brought about mutarotation and the final DMSO solution contained both α- and β-anomers. No mutarotation was observed within a reasonable time

[31] R. D. Preston, *Endeavour, It. ed.* **23**, 153 (1964).
[32] "*D.M.S.*" *spectral cards*. Butterworths Scientific Publ., London, England and Verlag Chemie, West Germany.
[33] W. J. Whelan, J. M. Bailey and P. J. P. Roberts, *J. Chem. Soc.* 1293 (1953).
[34] F. Cramer and F. M. Henglein, *Chem. Ber.* **91**, 308 (1958).
[35] D. French, *Adv. Carbohydrate Chem.* **12**, 189 (1957).
[36] F. Cramer and D. Steinle, *Liebigs Ann.* **595**, 81 (1955).
[37] T. J. Schoch, *J. Amer. Chem. Soc.* **64**, 2957 (1942).
[38] B. Casu, G. Gaglioppa and M. Reggiani, *Die Stärke* **17**, 386 (1965).

for the reducing sugars dissolved in dry DMSO. Addition of H_2O (or D_2O) up to 2% did not induce mutarotation and a sufficient deuteration of the dissolved sugar could be accomplished by adding a few drops of D_2O to the usual NMR 0·4 ml DMSO solution.

60 Mc/s spectra were taken on a Varian A-60 spectrometer with tetramethylsilane (TMS) as internal reference (τ = 10·00 ppm). 100 Mc/s spectra were taken on a Varian HA-100 spectrometer, with hexamethyldisiloxane (HMDS) as internal reference (τ = 9·94 ppm, referred to TMS), through the courtesy of Dr. A. Melera (Varian AG, Zürich) and Dr. A. Segre (Istituto di Chimica Industriale, Politecnico, Milano). Coupling constants are given in c/s and were approximated to the nearest half cycle. They are to be considered apparent coupling constants, as they were measured directly from the peak splitting. Unless otherwise stated, the probe temperature was 38° and the concentration of the carbohydrates in DMSO was 10% w/w. IR spectra were run on a Perkin–Elmer mod. 337 grating spectrophotometer, using 0·05 mm cells with BaF_2 windows. Concentrations used for recording the νO—H band in DMSO varied, according to the hydroxyl content of the saccharide, from 3 to 6% (w/v). Frequency values are accurate within ± 3 cm^{-1}.

Acknowledgements—We wish to express our appreciation to all those people mentioned who kindly supplied compounds and to Mr. M. Psallidi for technical assistance. Two of us (B. C. and M. R.) gratefully acknowledge the U.S. Department of Agriculture for a Research Grant (UR-E-15(10)32-FG-It-115).