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CONFORMATION OF O-METHYLATED AMYLOSE AND CYCLODEXTRINS*

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Abstract—The conformation of the glucopyranose units of O-methylated amylose, cyclodextrins and of model O-methylated glucoses and di-glucoses was investigated by PMR and IR spectroscopy. The C_1—H and C_2—H bonds of O-methylated α-glucoses and of α-1, 4-linked di- and poly-glucoses were found to be equatorial and axial, respectively and consistent with the chair "C1" conformation of the glucopyranose units.

The hydrogen bonding of partially methylated compounds in nonpolar (CCl_4, $CHCl_3$) as well as in polar (DMSO) solvents was also studied. The OH at C-3 of 2,6-di-O-methylated α- and β-cyclodextrin was found to be intramolecularly hydrogen-bonded to the oxygen of the methoxyl at C-2 of the adjacent unit. This internal hydrogen bond is solvent- and concentration-independent and accounts for the resistance to methylation of the OH at C-3 of cyclodextrins and probably also amylose.

THE conformation of polysaccharides has an important bearing on their chemicophysical properties. As far as amylose and its oligomers (dextrins) are concerned, evidence has accumulated favouring the chair "C1" conformation of the α-D-glucopyranose units.[1–5] These units are moderately free to rotate about the glycosidic C_1—O and the C_4—O bonds and a number of chain conformations can be obtained by varying the angles of rotation between the monomeric units. While in the case of the "V" crystalline modification of amylose (as taken in its inclusion complexes) evidence of a helical conformation has been provided by X-ray diffraction studies,[6] the conformation of the more extended polymeric chain of the "A" or "B-type" crystalline modifications (as found in native amylose) has not yet been determined. Somewhat conflicting reports have been published concerning the preferred conformation of the amylose chain in solution.[7] However, the present trend is to assume that the backbone conformation of the polymer in water as well as in DMSO is closely related to that in the helical crystalline complexes.[7] Such a helix is likely to be stabilized by internal hydrogen bonds between the hydroxyls at C-2 and C-3 of contiguous glucose units, as suggested by X-ray diffration studies on the cyclohexa-amylose (α-cyclodextrin) complex with potassium acetate[5] and by PMR and IR spectra of amylose and cyclodextrins in DMSO solution.[2]

Since the substitution of OH hydrogens with non-hydroxyl bearing groups may remove the internal hydrogen-bonding stabilization of the polymer chain and since the bulkiness of the substituent may affect the shape of the monomeric units, the

* Part III of *Conformation of amylose and its derived products*. Part I and II: Refs. 1 and 2.

conformation of O-methylated amylose and cyclodextrins was investigated by PMR and IR spectroscopy. In the case of O-methylated cyclodextrins, the assumption was made that the steric restraint by the macrocyclic structure would prevent large deviations from the symmetrical alignment of the monomeric units. A particular attention was paid to partially O-methylated products, which, on account of their solubility both in nonpolar and in polar solvents, provide a unique opportunity of studying the intromolecular hydrogen bonding by the most effective approach, i.e. the study of solvent- and concentration-dependence of the OH absorptions.

The PMR spectra provide information on the conformation of the glucopyranose units by studying the resonance of the anomeric (C_1H) proton, from which the orientation (equatorial or axial) of the C_1—H bond and the dihedral angle between the C_1—H and C_2—H bonds can be derived.[8] In the case of partially methylated products, the H-bonding can be investigated by studying the OH resonances in DMSO solution.[2] The IR spectra provide information on the orientation of the C_1—H and C_1—O bonds[1] and on the H-bonding in solid phase as well as in polar and non-polar solvents.[9]

The permethylated monomer (D-glucose) and dimer (maltose) as well as a number of partially O-methylated glucoses were investigated as models for permethylated amylose and cyclodextrins. Also partially O-methylated amylose and cyclodextrins were studied.

RESULTS

(1) *PMR spectra*

PMR data are reported in Table 1 for O-methylated glucoses and in Table 2 for O-methylated glucosides, including amylose and cyclodextrins derivatives. Fig. 1

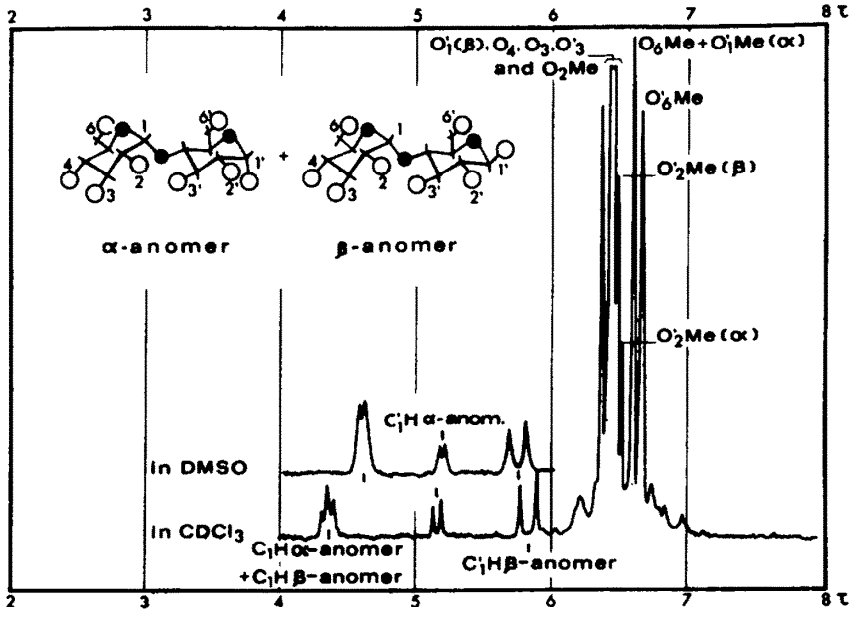

FIG. 1 PMR spectrum of methyl hepta-O-methylmaltosides ● oxygen; ○ methoxyl

shows the PMR spectrum of methyl hepta-O-methylmaltosides (α and β) and Fig. 2 those of 2,6-di-O-methyl-β-cyclodextrin and tri-O-methyl-β-cyclodextrin.

The OH *resonances* were identified as those sensitive to deuteration, temperature variation or addition of trifluoroacetic acid.[2,10] In DMSO solution the assignments to individual hydroxyls were based on previous assignments for unsubstituted sugars[2,11] and on consideration of the shift induced by adjacent methoxyls. The assignments in $CDCl_3$ solution were made only for 2,3,6-tri-O-methyl-α-D-glucose, 2,3,4,6-tetra-O-methyl-α- (and β-) D-glucose and di-O-methylated cyclodextrins.

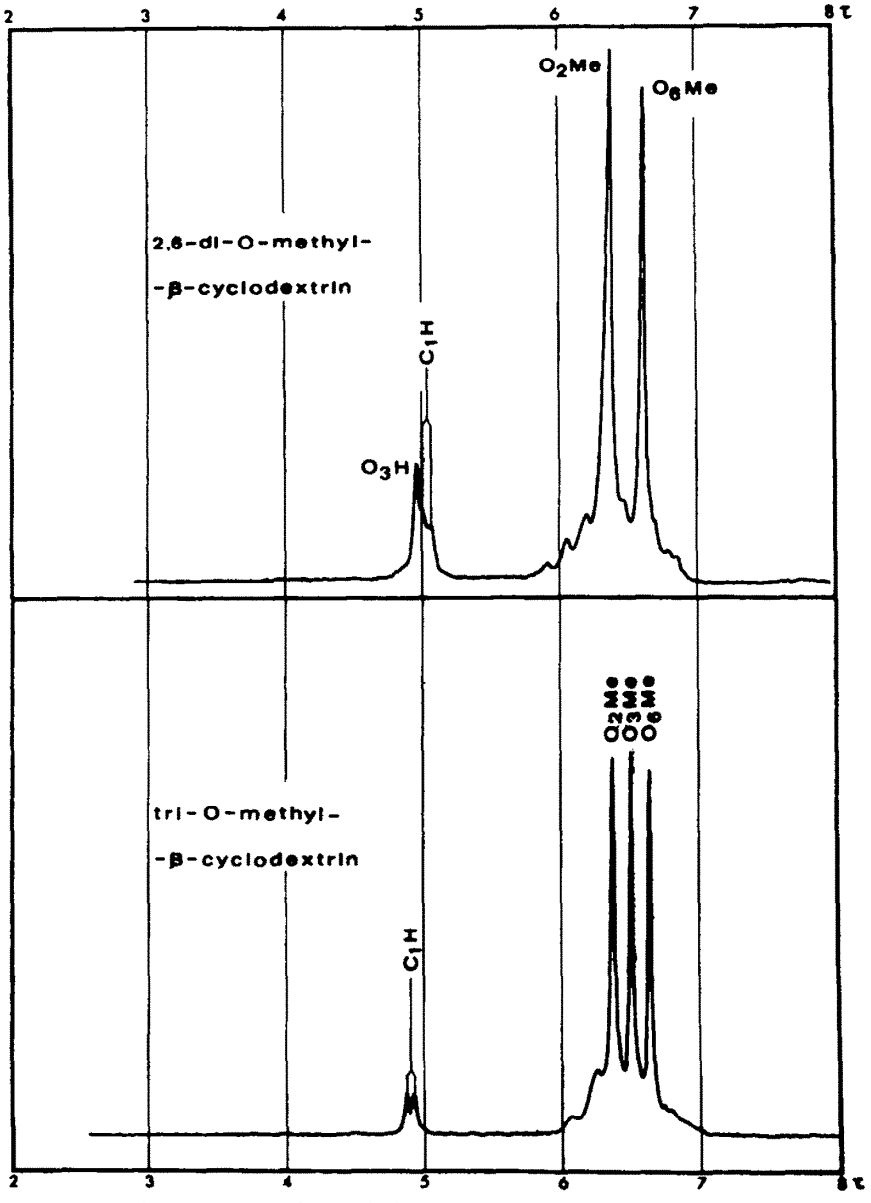

FIG. 2 PMR spectra of di- and tri-O-methylated β-cyclodextrin in $CDCl_3$.

As the OH signals in chloroform are strongly concentration-dependent, the chemical shifts are reproducible only at the concentrations used. In the case of di-O-methyl-cyclodextrins, the OH signals in $CDCl_3$ were found to be concentration independent.

The C_1H *resonances* were readily identified as the only non-hydroxyl signals at field lower than 6 τ. As previously reported,[2,11] they are displayed as doublets when the OH at C-1 is substituted. Otherwise, they appear as a pair of doublets due to the coupling of the C_1H proton both with C_2H and O_1H protons.

The OMe *resonances*, displayed between 6·32 and 6·65 τ in $CDCl_3$ and between 6·50 and 6·78 τ in DMSO-d_6 as sharp singlets superimposing the non anomeric CH signals, were assigned on the basis of correlations with the position and the orientation of the substituents.*

From data in Tables 1 and 2 the following observations can be made:

(a) C_1H *and* OH *proton resonances of* O-*methylated glucoses and* 1,4-*diglucoses* (*ring B*†). From the comparison between unsubstituted and partially O-methylated sugars in DMSO both C_1H and OH signals appear affected by adjacent MeO groups.

The C_1H *signal* in DMSO shifts upfield on methylation at O_1 for both α- and β-anomers (+0·37 ppm for α- and +0·25 ppm for β-glucose). On methylation at O_2 a downfield shift (−0·21 ± 0·04 ppm) is observed for α-anomers. The effects are additive, being the shift for the 1,2-O-methylated α-anomers about +0·18 ppm. The data available for β-anomers do not permit a correlation to be established. Me groups at O_3, O_4 and O_6 apparently do not affect the C_1H resonances; however, the highest downfield shifts in the range above mentioned are observed for the products with the largest number of MeO groups.

The C_1H resonances in $CDCl_3$ are displayed at field lower than in DMSO for the compounds unsubstituted at O_1. ($\Delta_{DMSO-CDCl_3}$ = +0·17 to +0·29). O_1-methylated compounds display a smaller or practically no solvent effect (methyl 2,3-di-O-methylglucoside +0·14; permethylated α-glucose +0·02; permethylated α- and β-maltose +0·04 and −0·07 ppm, respectively).

The splitting of the C_1H signal by coupling with C_2H is of the order of 3 c/s for the α- and 7·5 c/s for the β-anomers both in DMSO and $CDCl_3$.

The OH *signals* in DMSO move to lower field upon methylation of the adjacent hydroxyls. The following effects (expressed as τ-difference, Δ_{OH}) are observed for O-methylated α-glucoses (unsubstituted O_1H):

(1) one OMe substituent in alpha position with respect to the OH produces a −0·13 to −0·16 ppm shift (Δ_{O_4H} and Δ_{O_2H} for 3-O-methyl-α-glucose: −0·16);

(2) two OMe groups (in alpha and alpha′ position) produce about twice the above shift (Δ_{OH} for 2,4,6-tri-O-methyl-α-glucose = −0·34); the O_6Me of 2,3,6-tri-O-methyl-α-glucose (Δ_{O_4H} = −0·34) behaves as adjacent to O_4H);

(3) two OMe groups (in alpha and beta position) give a −0·15 to −0·19 shift (Δ_{O_1H} for 2,3-di-O-methyl-α-glucose = −0·15; for hepta-O-methylcellobiose = −0·16; for 2,3,6-tri-O-methyl-α-glucose = −0·19);

* Assignments of the methoxy-resonances were also reported by Barker *et al.*[12] for a number of O-methylated sugars in DMF solution. In this solvent the OMe resonances are displaced about 0·07 ppm upfield with respect to $CDCl_3$ and about the same amount downfield with respect to DMSO-d_6.

† The diglucose ring bearing OH or OMe at C-1 is designated ring B. The diglucose ring bearing the glycosidic linkage is designated ring A.

(4) three OMe groups (in alpha, beta and gamma position, respectively) give an even larger shift (Δ_{O_1H} for 2,3,4,6-tetra-O-methyl-α-glucose = −0·23).

The above data show that a sequence of OMe groups produces a larger shift on the resonance of an OH adjacent to the sequence than one isolated OMe group does.

From the few data available for β-anomers, it appears that in this case the shift towards lower field of the O_1H signal is about 0·04 ppm larger than that observed for α-anomers. The downfield shift also appear larger for O-methylated methyl glucosides than for O-methylated glucoses (Δ_{O_2H} for methyl α- (and β-) glucoside = −0·21; Δ_{O_4H} for methyl 2,3-di-O-methyl-α-glucoside = −0·28). This last example confirms that also OMe groups in beta and gamma position with respect to the OH contribute to the downfield shift of its signal.

The splitting of the O_1H signal is of the order of 4·5 c/s for the α- and of 6·5 c/s for the β-anomers, like for the unsubstituted products.[2] Lack of data does not permit a generalization to be made on the signal splitting of the non-anomeric hydroxyls.

(b) C_1H *and* OH *proton resonances of* O-*methylated* 1,4-*diglucoses (ring A) and* α-1,4-*polyglucoses.* As shown in Table 3, the resonances of the C_1H *proton* belonging to the ring "A" of α-1,4-di- and poly-glucoses shift to lower field on methylation at O_2. Practically no shift is shown by the β-1,4-linked hepta-O-methylcellobioses.

In Table 3 the chemical shift difference between the C_1H signals in DMSO and in $CDCl_3$ ($\Delta_{DMSO-CDCl_3}$) is also reported. Only for O-methylated methyl maltosides and amylose the shift is within the range observed for O-methylated glucoses (+0·17 to +0·29 ppm). Di- and tri-O-methylated α-cyclodextrin and tri-O-methylated β-cyclodextrin display a shift consistently lower (about +0·08 ppm). No solvent effect is shown by di-O-methylated β-cyclodextrin and by O-methylated cellobioses.

The C_1H peak splitting is of the order of 3·0 c/s for the α-1,4- and of 7·0 c/s for the β-1,4-linked products in both DMSO and $CDCl_3$.

For the OH *signals* the following shifts (Δ_{OH}) are observed upon methylation of the adjacent hydroxyls: amylose (degree of substitution = 2·3) −0·21 and +0·33; 2,6-di-O-methyl-α-cyclodextrin = +0·78; 2,6-di-O-methyl-β-cyclodextrin = +0·68 ppm.*

(c) *Methoxy-proton resonances.* The O_1Me resonance of methyl α-glucoside in DMSO (6·78 τ) is displayed 0·17 ppm upfield with respect to the β-anomer (6·61 τ). Practically the same difference was found in D_2O solution (6·64 and 6·48 τ).[4] The present data show that the O_1Me signal of α-anomers shifts about 0·06 ppm downfield on methylation at O_2.

The O_6Me signals are shown at the highest field within the range characteristic of OMe resonances. The diglucoses hepta-O-methylcellobioses and methyl hepta-O-methylmaltosides show two separate signals for the O_6Me and O'_6Me groups.

As long as the anomeric carbon atom bears a OH or OMe substituent, α-anomers give the O_2Me signal at 6·46–6·52 τ in $CDCl_3$ and at 6·64–6·67 τ in DMSO-d_6, and β-anomers at 6·51–6·55 τ in DMSO-d_6; no sufficient data are available in $CDCl_3$ for β-anomers. When the anomeric center is α-1,4-linked with another glucose residue, the O_2Me signal shifts about 0·10 ppm downfield with respect to the above given range.

The O_3Me and O_4Me resonances occur at the lowest field within the range of the OMe signals, and are practically unaffected by the configuration at C-1.

* Differences are given with respect to the O_3H signal of the unsubstituted products (Ref. 2).

(2) *IR spectra*

The IR spectra of solid films O-methylated amylose and cyclodextrins (α and β) are shown in Fig. 3. The IR frequencies are given in Table 4 together with the assignments of the main bands, for which advantage has been taken of the assignments for unsubstituted carbohydrates[1, 13] and of the established correlations between IR spectra and structure of non-carbohydrate compounds.

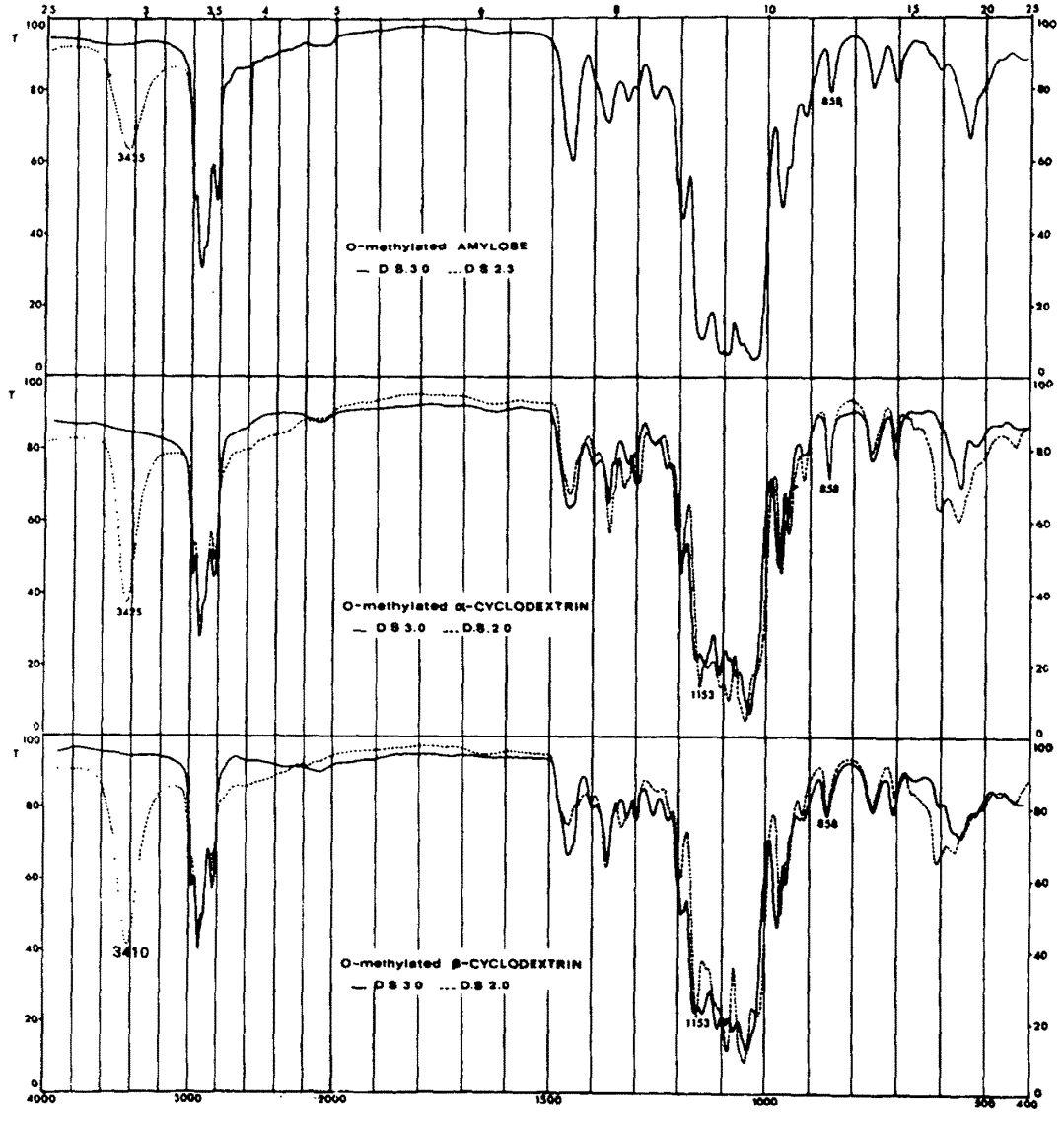

FIG. 3 IR spectra of O-methylated amylose and cyclodextrins (solid films from $CHCl_3$ solution).

The IR absorption of O-methylated amylose and cyclodextrins is similar. The better resolution observed for the spectra of cyclodextrin derivatives is mainly a reflection of the lower mol wt and, for solid samples, of the higher crystallinity of the oligomers with respect to the polymer. A common feature of the spectra shown in Fig. 3 is the "C_1-group" band at 858 cm^{-1}. Partially methylated products show an O—H stretching band whose frequency (νOH) diminishes going from amylose (3435 cm^{-1}) to α-cyclodextrin (3425 cm^{-1}) to β-cyclodextrin (3410 cm^{-1}).

The νOH frequencies of partially methylated amylose and cyclodextrins are compared in Table 5 with those of a number of methylated glucoses and glucosides in dilute CCl_4 solution, in concentrated DMSO solution and in the solid phase. The most intense O—H bands of methylated glucoses and glucosides in dilute CCl_4 solution (0·05%) are displayed between 3535 and 3605 cm^{-1}, a region characteristic of intramolecularly hydrogen-bonded hydroxyls.[13,14]

The OH frequencies in dilute CCl_4 solution have been already reported by Michell and Higgins[14] for compounds 1 and 3 of Table 5, and our data are in substantial agreement with theirs. The assignments of the individual frequencies to specific intramolecular H-bonds was made according to the Michell and Higgins approach.[14] A common feature of methylated glucoses in dilute CCl_4 solution is a weak, broad band near 3420 cm^{-1} ascribed to the contribution of a small number of self-associated molecules (dimers). This band is not observed for compounds that have the O_1H hydroxyl substituted. As the solute concentration increases (e.g. 2,3,4,6-tetra-O-methylglucose), the high-frequency bands of the intramolecularly bonded hydroxyls significantly lower in intensity and the dimer becomes the strongest one.

The νOH band of methylated cyclodextrins is found at the same low frequency (α-cyclodextrin: 3430 cm^{-1} and β-cyclodextrin: 3420 cm^{-1}) both in diluted and in concentrated CCl_4 solution. From data reported in Table 6, it is also evident that the intensity of the band does not practically change upon a 200-fold variation of the concentration. Because of solubility limitations, the spectrum of partially methylated amylose was taken only in $CHCl_3$, wherein the main νOH band was found at 3410 cm^{-1}. 2-6-di-O-methylated α- and β-cyclodextrin give in $CHCl_3$ the same values as in CCl_4.

In DMSO solution only one model compound, methyl 2,3-di-O-methyl-α-glucoside, displays a νOH band within the range 3400–3320 cm^{-1}, characteristic for unsubstituted sugars of the hydroxyls H-bonded to the solvent.[2] Compounds with an unsubstituted O_1H adjacent to a O_2Me group absorb at lower frequencies (3275–3230 cm^{-1}). From the data available no generalization is possible for non-anomeric hydroxyls.

Partially methylated amylose gives in DMSO a complex νOH absorption, with one component band at frequency (3450 cm^{-1}) higher than the usual range for unsubstituted sugars and the remaining components substantially within the usual range. The νOH frequency of 2,6-di-O-methylated cyclodextrins does not change going from CCl_4 to DMSO solution.

As expected on account of the complex H-bonding (both intra- and intermolecular) often found in the solid state, in this phase no relationships are apparent between the νOH frequency of the model compounds and the position of the unsubstituted hydroxyls. It is significant that di-O-methylated cyclodextrins display in the solid state νOH frequencies that are nearly the same as in DMSO.

DISCUSSION

(1) *Conformation of the glucopyranose units and flexibility of the polymeric chain.*

It has been established that equatorial and axial C_1H protons resonate at different fields because they are differently shielded by the magnetically anisotropic C_2—C_3 and C_5—O_5 bonds.[15] Due to the observed effect of OMe groups at C-1 and C-2 on the C_1H proton of α-glucose derivatives, the range taken as characteristic of equatorial C_1—H bonds for unsubstituted carbohydrates in DMSO (4·83–5·35 τ)[2] extends upfield to 5·45 τ and downfield to 4·61 τ. The C_1H signals of O-methylated β-anomers were found between 5·58 and 5·95 τ, i.e. substantially within the field characteristic of axial C_1—H bonds of the unsubstituted compounds. The values of O-methylated α-maltose, amylose and cyclodextrins are within the equatorial range.

The equatorial orientation of the C_1—H bond of methylated α-maltose, amylose and cyclodextrins is further supported by the IR absorption of the "C_1-group" near 858 cm^{-1} and, in the case of 2,6-di-O-methylated cyclodextrins, by the band at 1153 cm^{-1}, which falls within the range of axial C_1—O bonds.[1] In permethylated products the last absorption is split in two components (probably because of coupling with a C—OMe vibration) and is not useful for correlation purposes.

The splitting of the C_1H PMR signal, of the order of 3·0 c/s for all the derivatives of α-glucose and of 7·5 c/s for those of β-glucose, suggests a C_1—H/C_2—H dihedral angle of about 60° and about 180°, respectively. These data, together with the finding that the C_1—H bond is equatorial in α-anomers and axial in β-anomers, strongly support the "C1" chair conformation for the pyranose units of all the investigated compounds.

For a more adequate interpretation of the chemical shift of the C_1H proton, factors other than the equatorial or axial orientation are to be taken into account. Among these factors, which may lend to some superposition of the equatorial and axial ranges, Glass has suggested the electronegativity of the substituent at C_1 to be of primary importance.[4] Although no superposition of the ranges above mentioned was observed for our model compounds, the highest values within these ranges are actually given by methyl glucosides. This fact can be accounted for by the electron-releasing effect of the Me group, which shields the C_1H proton. Also the influence of the magnetic anisotropy of the O_1—Me bond may be regarded as contributing to the shielding of the C_1H proton.

The downfield shift of the C_1H signal of O_2-methylated products may be also accounted for by a magnetic anisotropy effect. Actually the O_2—Me bond in its more probable conformations appears always oriented in such a way with respect to the C_1—H bond to produce a long-range deshielding of the C_1H proton.

As previously observed for unsubstituted carbohydrates[2,4] also in the case of O-methylated derivatives the C_1H signal of cyclodextrins is found at field consistently higher than that of O-methylated amylose and maltose. The high values of cyclodextrins were suggested by Glass to be probably related to ring current effects.[4] An alternative explanation can be that the magnetic anisotropy effect associated with the O_1—C'_4 bond produces a larger shielding of the C_1H proton in cyclic structures than in linear ones. Owing to steric restrictions in the macrocycles, the glucopyranose units of cyclodextrins are expected to closely approach an alignment in which the C_1—H bond of every unit eclipses[16] the O_1—C'_4 bond of the contiguous unit. In this arrangement the O_1—C'_4 bond would have to produce a shielding of the C_1H proton. The

chain of linear maltosides is more flexible than that of cyclodextrins and is able to take a conformation that relieves the Van der Waals interaction between the C_1H and C'_4H protons through rotation of the glucopyranose units about the glycosidic bonds. This departure from the quasi-eclipsed (syn-periplanar) conformation of cyclodextrins implies a reduction of the shielding of the C_1H proton. In conclusion, it is suggested that the chemical shift of the C_1H signal, besides being indicative of the orientation of the anomeric C_1—H bond with respect to the glucopyranose unit is also dependent on the angles of internal rotation between the adjacent units, i.e. on the chain conformation.

As derived from comparison of PMR spectra in $CDCl_3$ and in DMSO (shown in Fig. 1 for hepta-O-methylmaltoside) the chemical shift of C_1H protons belonging to the rings methylated at O_1 are little affected by the solvent. As far as the "A"-ring (the one bearing the glycosidic linkage) is concerned, no solvent effect is shown by the β-1,4-diglucoses α- and β-cellobiose (linked through an equatorial glycosidic bond) while the α-1,4-diglucoses α- and β-maltoside and the polymer amylose (linked through an axial glycosidic bond) display a consistent solvent effect. Although the shift of the C_1H signal in DMSO with respect to $CDCl_3$ for methylated glucoses containing unsubstituted OH groups can be explained mainly in terms of hydrogen-bonding effects, the solvent-dependence of the C_1H signal of O-methylated α-1,4-linked linear di- and polyglucoses is probably attributable mainly to a solvent-induced change of the chain conformation. In fact, the $\Delta_{DMSO-CDCl_3}$ shift is well reduced in O-methylated α-cyclodextrins (which are assumed to be only moderately free to depart from the eclipsed chain conformation) and falls to zero for O-methylated β-cyclodextrins (which, on account of results on unsubstituted compounds,[2] are taken to be even more tightly constrained in the above conformation).

(2) *Hydrogen bonding of partially O-methylated compounds*

As pointed out by Michell and Higgins,[14] every OH of partially methylated glucoses is close to at least one oxygen atom of the molecule. Consequently, in dilute solution in nonpolar solvents all the OH groups of these compounds are intramolecularly H-bonded.[14] These H-bonds are fairly weak, since in concentrated CCl_4 solution they are broken and replaced by intermolecular associations.

In polar solvents like DMSO the hydroxyls are usually H-bonded to the solvent and their resonances are considered as mainly affected by the strength of the H-bonding.[2] The observed downfield shift of the resonances of the OH protons adjacent to OMe groups seems to be associated, at least in part, to a stronger H-bonding with the solvent. The IR νOH absorption, which for unsubstituted sugars is usually found in the range 3400–3320 cm^{-1},[2] is actually shifted to lower frequencies in most methylated compounds. The largest shift is given by the O_1H of O_2-methylated compounds (νOH = 3275–3230 cm^{-1}). This behaviour is not surprising, since the O_1H is expected to be the most strongly H-bonded hydroxyl[2] and the IR data show that it is able to give intermolecular associations even in very dilute CCl_4 solution (dimer band in the 3420 cm^{-1} region). Because of the few data available and of the broadness of the νOH band of compounds containing more than one OH group, no generalization can be made for the hydrogen-bonding of non-anomeric hydroxyls. A possible explanation of a stronger H-bond in O-methylated than in unsubstituted sugars is that in the last compounds the bulky solvent molecules are prevented by mutual

repulsion to approach the OH groups closely, while a OH surrounded by OMe groups (less bulky than $OH\cdots O{=}S\begin{smallmatrix}Me\\Me\end{smallmatrix}$ systems) is more effective in actracting a DMSO molecule.

Although the splitting of the PMR OH peaks is assumed as dependent on the

stereochemistry of the C—O system,[2,17,18] no information can be obtained until a relationship of the Karplus type[19] is derived for this group. However, as long as O_1H signals are concerned, substantially the same splitting is observed for unsubstituted as well as for O_2-methylated glucoses (about 4·5 c/s for α-anomers and about 6·5 c/s for β-anomers) and it is implied that the O_1H bond is oriented substantially in the same way in the two types of products.

Molecular models of 2,6-di-O-methylated cyclodextrins built with "C1" units show that the OH at C-3 of every unit is likely to be H-bonded to the O_2' of the contiguous unit. A schematic picture of this H-bond is given in Fig. 4. The PMR data of the O_3H proton of dimethylated cyclodextrins are not clearly indicative by themselves of an intramolecular H-bonding. In fact, the O_3H-signals in DMSO fall within the range of the hydroxyls essentially H-bonded to the solvent.[2] Insufficient data are available on OH resonances in $CDCl_3$ to obtain useful correlations. However, since the OH signals in $CDCl_3$ are usually found at field higher than 6 τ, the low-field OH signals of dimethylated cyclodextrins in this solvent (5·09 τ for the α- and 4·95 τ for the β-cyclodextrin derivative) indicated a H-bond stronger than the usual. Both the low frequency and the solvent- and concentration-independence of the IR νOH band of 2,6-di-O-methylated cyclodextrins provide a clear cut evidence of an intramolecular hydrogen bond not broken by polar solvents nor replaced by molecular self-association. The slightly lower frequency and the higher intensity of the νOH band of the β- with respect to the α-cyclodextrin derivative indicate a stronger H-bond for the former compound, and parallel the findings on unsubstituted cyclodextrins.[2]

An internal H-bonding of the same type seems also given by partially O-methylated amylose, as it is suggested by the νOH absorption in DMSO in the 3450–3410 cm^{-1} region and by the two low-field PMR OH signals, tentatively attributed to "residual" O_2H and O_3H groups respectively H-bonded to the oxygen atoms at C-3 and C-2 of adjacent residues. No further information on the complex inter- and intra-molecular H-bonding pattern of partially methylated amylose is at present available. However, it is probable that an internal H-bond similar to that found for the dimethylated cyclodextrins stabilizes a helical conformation of the amylose chain, as suggested for the unsubstituted polymer.

The internal H-bonding probably plays an important role in determining the relative reactivities of the hydroxyls of amylose and cyclodextrins during methylation. The OH at C-3 of amylose is reported to be the most resistant to methylation with dimethyl sulphate,[20] and we found the same for cyclodextrins (Experimental). To account for the low reactivity of the O_3H group of amylose, Croon suggested a polymer conformation involving boat "3B" units and an intramolecular H-bond between every OH at C-3 and adjacent ring oxygen.[21] On the other hand, from PMR and IR data[2] we found that an intramolecular H-bond involving the hydroxyls at C-3 and C-2 is formed in amylose and cyclodextrins built with the energetically

favoured "C1" units. As reported in the experimental part, the resistance to methylation with dimethyl sulphate increases going from amylose, to α-, to β-cyclodextrin. Since the IR νOH frequency indicates an intramolecular H-bond whose strength increases in the same order, it can be inferred that the internal H-bonding is the main factor affecting the resistance to methylation of the OH at C-3.

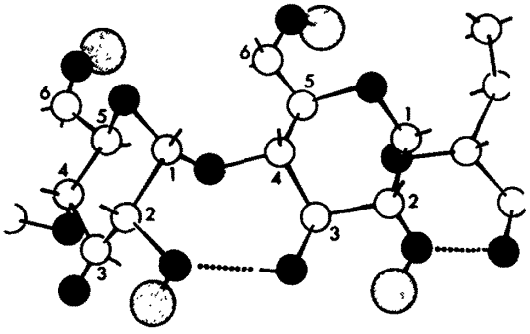

FIG. 4 Partial perspective view of a 2,6-di-O-methylated cyclodextrin. ○ carbon; ● oxygen; ○ methyl.

EXPERIMENTAL

The PMR spectra were taken on a Varian A-60 spectrometer (60 Mc/s) with TMS as internal reference (τ = 10·00 ppm). 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 temp was 38° and the concentration of the carbohydrates was about 10%. IR spectra were run on a Perkin–Elmer mod. 337 grating spectrophotometer equipped with a Perkin Elmer-Hitachi servo-recorder with suitable gears as described in Ref. 1.

The PMR and IR solvents ($CDCl_3$ and DMSO-d_6 from C.I.B.A., Basel, Switzerland; CCl_4 from C. Erba, Milano, Italy) were stored over activated Linde molecular sieves 4A for at least 48 hours before use, and the H_2O content checked by IR spectrophotometry.

O-methylated glucoses and glucosides were either from Koch-Light, Colnbrook, Bucks, England (Compounds 1 and 9 of Table 1 and 1, 2, 3 and 5 of Table 2) or kindly supplied by Dr. N. K. Richtmyer of National Institutes of Health, Bethesda, Md., U.S.A. (compounds 5, 7, 8 and 11 of Table 1 and 4 of Table 2).

Permethylated maltose and amylose were prepared by the procedure reported by Kuhn and Trischmann for the complete methylation of starch[22] with Me_2SO_4 and BaO in a 1:1 mixture of DMF and DMSO. After a 48 hr reaction, maltose (Koch-Light) was completely methylated, while amylose (M.W. 150·000, Koch-Light) yielded a product with degree of substitution (D.S.) = 2·3, by OMe analysis. Permethylated amylose (microcrystalline powder, soluble in $CHCl_3$ and DMSO, insoluble in H_2O and petroleum ether, $[\alpha]_D^{20}$ in DMSO +180) was obtained by remethylating the D.S. 2·3 amylose for an additional 24 hr and crystallizing the crude product from a concentrated $CHCl_3$ soln by adding pet. ether (40–60°). The crude permethylated maltose was an oil consisting of a mixture of α- and β-anomers of the permethylated (D.S = 8, $[\alpha]_D^{20}$ in $CHCl_3$ +120) and incompletely methylated products. The separation from partially methylated products was accomplished by high-vacuum distillation; no attempts were made to separate α- and β-anomers.

Di-O-methylated α-cyclodextrin and di-O-methylated β-cyclodextrin were obtained by the above procedure from unsubstituted cyclodextrins (obtained by *b. macerans* cleavage of starch[23]). The methylated products were crystallized from $CHCl_3$ and addition of pet. ether (40–60°). The β-cyclodextrin derivative was further crystallized from hot water. While occasionally a product with a somewhat higher D.S. (2·1–2·3) was obtained from α-cyclodextrin, no further enhancement of D.S. was obtained from β-cyclodextrin even after a 7 days reaction. By hydrolysis with H_2SO_4 the products yielded only 2,6-di-O-methyl-D-glucose (glass, $[\alpha]_D^{20}$ in $CHCl_3$ +44), identified by PMR in DMSO, making use of the correlations developed in the present work (α-anomer: O_1H 3·75 τ, C_1H 4·88 τ, O_2Me 6·66 τ, O_6Me 6·77 τ, β-anomer: O_1H 3·35 τ, C_1H 5·65 τ, O_2Me 6·55 τ, O_6Me 6·77 τ). The products were then identified as 2,6-di-O-methyl

TABLE 1. CHEMICAL SHIFTS, τ (ppm) AND COUPLING CONSTANTS (c/s, GIVEN IN PARENTHESES) OF SOME O-METHYLATED GLUCOSES IN $CDCl_3$ AND DMSO-d_6 AT 38°

Compounds	O_1H doublet		C_1H pair of doublets[a]		Non-anomeric OH's secondary : doublets primary : triplet			O-Me singlets		
	$CDCl_3$	DMSO-d_6	$CDCl_3$	DMSO-d_6	$CDCl_3$	DMSO-d_6	assign.	$CDCl_3$	DMSO-d_6	assign.
(1) 3-O-methyl-α-D-glucose	*b*	3·78 (4·5)	*b*	5·08[c] (4·5; ~2[d])	*b*	5·12 (~3) 5·44 (~5) 5·70 (5·5)	O_4H O_2H O_6H	*b*	6·51	O_3Me
(2) 3-O-methyl-β-D-glucose[e]	*b*	3·45 (6·5)	*b*	5·68[c] (6·5; 7·0[d])	*b*	*f*		*b*	6·51	O_3Me
(3) 2,3-di-O-methyl-α-D-glucose[g]	*b*	3·70 (4·5)	*b*	*b*	*b*	*b*		*b*	*b*	
(4) 2,3-di-O-methyl-β-D-glucose[g]	*b*	3·30 (6·6)	*b*	*b*	*b*	*b*		*b*	*b*	
(5) 2,3,6-tri-O-methyl-α-D-glucose	6·08 (3·0)	3·66 (4·5)	4·65 (3·0; 3·0)	4·90 (4·5; 3·0[d])	7·03 (2·0)	4·94 (5·0[c])	O_4H	6·35 6·48 6·58	6·55 6·67 6·76	O_3Me O_2Me O_6Me
(6) 2,3,6-tri-O-methyl-β-D-glucose[e]	*h*	*h*	5·35[d](*h*; 7·0[d])	5·64[d] (*h*; 7·0[d])	*h*	*h*		*f*	6·52 6·55 6·76	O_2Me O_3Me O_6Me
(7) 2,4,6-tri-O-methyl-α-D-glucose	*i*	3·73 (4·5)	4·64 (*i*; 3·5)	4·90 (4·5; ~3)	*i*	5·06(*l*)	O_3H	6·43 6·48 6·59	6·60 6·67 6·76	O_4Me O_2Me O_6Me
(8) 2,4,6-tri-O-methyl-β-D-glucose[e]	*i*	3·29 (6·5)	5·39 (*i*; 7·5)	*f*	*i*	*f*		*f*	6·55 6·60 6·76	O_2Me O_4Me O_6Me
(9) 2,3,4,6-tetra-O-methyl-α-D-glucose[g]	5·80 (3·0)	3·62 (4·5)	4·69 (3·0; 3·0)	4·87 (4·5; 3·0)	—	—		6·37 6·45 6·47 6·60	6·54} 6·58} 6·66 6·73	O_3Me O_4Me O_2Me O_6Me

(10) 2,3,4,6-tetra-O-methyl-β-D-glucose[a]	5·15 (5·5)	3·24 (6·5)	5·45 (5·5; 7·0)	5·62 (6·5; 7·0)	—	—	*f*	6·51	O_2Me
								6·54	O_3Me
								6·58	O_4Me
								6·73	O_6Me
(11) Hepta-O-methyl-α-cellobiose	*i*	3·58 (4·5)	4·69 (*i*; 3·5)	4·93 (4·5; 3·0)	—	—	6·38	6·50	O_2Me
			5·70 (7·0)	5·70 (7.0)			6·41	6·55	O_3Me
							6·45	6·58	$O_3'Me$
							6·47	6·58	O_4Me
							6·47	6·66	$O_2'Me$
							6·59	6·68	O_6Me
							6·62	6·74	$O_6'Me$
(12) Hepta-O-methyl-β-cellobiose[e]	*i*	3·21 (6·5)	5·38[d] (*h*; 7·0[d])	5·58 (6·5; 7·0)	—	—	*f*	6·50	O_2Me
			5·70 (7·0)	5·70 (7·0)				6·55	O_3Me
								6·58	$O_3'Me$
								6·58	O_4Me
								6·55	$O_2'Me$
								6·68	O_6Me
								6·74	$O_6'Me$

[a] Doublet when the coupling with O_1H has been removed and for the C_1H of the non-reducing ring of diglucoses.
[b] Spectrum not taken.
[c] Attribution made from the spectrum at higher temperature.
[d] Value observed after addition of 5% CF_3COOH.
[e] Values obtained from an anomeric mixture.
[f] Value not obtainable from the anomeric mixture.
[g] Values from W. Mackie and A. S. Perlin, *Canad. J. Chem.* **44**, 2039 (1966); 0·10 ppm were subtracted from the original values for taking into account the different referencing.
[h] Value not observable because of the presence of CF_3COOH.
[i] Value not observable in the present spectrum.
[l] Broad absorption.

TABLE 2. CHEMICAL SHIFTS. τ (ppm) AND COUPLING CONSTANTS (c/s, GIVEN IN PARENTHESES) OF SOME O-METHYLATED GLUCOSIDES IN $CDCl_3$ AND DMSO-d_6 AT 38°

Compounds	C_1H doublets		Non-anomeric OH's secondary : doublets primary : triplet			O-Me singlets		
	$CDCl_3$	DMSO-d_6	$CDCl_3$	DMSO-d_6	assign.	$CDCl_3$	DMSO-d_6	assign.
(1) Methyl α-D-glucoside	*a*	5·45 (3·0)	*a*	5·22 (5·0)	O_4H	*a*	6·78	O_1Me
				5·32 (4·0)	O_3H			
				5·39 (6·0)	O_2H			
				5·62 (5·5)	O_6H			
(2) Methyl β-D-glucoside	*a*	5·95 (7·0)	*a*	5·04 (4·0)	O_2H	*a*	6·61	O_1Me
				5·16 (*b*)	O_3H			
				5·16 (*b*)	O_4H			
				5·56 (6·0)	O_6H			
(3) Methyl 2,3-di-O-methyl-α-D-glucoside	5·10 (3·0)	5·24 (2·5)	6·20 (*b*)	5·00 (4·5)	O_4H	6·32	6·55	O_3Me
			6·20 (*b*)	5·58 (5·5)	O_6H	6·46	6·67	O_2Me
						6·54	6·71	O_1Me
(4) Benzyl 2,4,6-tri-O-methyl-β-D-glucoside	5·63 (7·5)	5·36 (8·0)	*c*	4·73 (5·0)	O_3H	6·38	6·54	O_2Me
						6·43	6·57	O_4Me
						6·58	6·71	O_6Me
(5) Methyl 2,3,4,6-tetra-O-methyl-α-D-glucoside	5·20 (3·0)	5·22 (3·0)	—	—		6·41	6·55	O_3Me
						6·49	6·60	O_4Me
						6·52	6·67	O_2Me
						6·61	6·73	O_1Me
						6·61	6·73	O_6Me
(6) Methyl hepta-O-methyl-α-maltoside[d]	4·35 (3·0)[e]	4·62 (3·0)[e]	—	—		6·37	6·52	O_2Me
	5·16 (3·5)[f]	5·20 (3·0)[f]				6·43	6·52	O_3Me
						6·45	6·55	O'_3Me
						6·45	6·59	O_4Me
						6·52	6·64	O'_2Me
						6·59	6·71	O_1Me
						6·59	6·71	O_6Me
						6·65	6·77	O'_6Me

(7) Methyl hepta-O-methyl-β-maltoside[d]	4·38 (3·0)[e] 5·84 (7·5)[g]	4·62 (3·0)[e] 5·77 (7·5)[g]	—	—		6·37 6·43 6·45 6·45 6·48 6·49 6·59 6·65	6·52 6·52 6·55 6·58 6·59 6·59 6·71 6·77	O'_1Me O_2Me O_3Me O'_3Me O_4Me O'_2Me O_6Me O'_6Me
(8) O-Methyl-amylose, D.S.=2.3	4·42 (*b*)	4·61 (~3)	~4·85 (*b*) ~5·24 (*b*)	4·40 (*b*) 4·94 (*b*)		6·44 6·44 6·60	6·55 6·59 6·74	O_2Me O_3Me O_6Me
(9) Tri-O-methyl-amylose	4·42 (~3)	4·61 (~3[h])	—	—		6·42 6·47 6·60	6·55 6·59 6·74	O_2Me O_3Me O_6Me
(10) 2,6-di-O-methyl-α-cyclodextrin	4·97 (3·0[h])	5·04 (~3)	5·09 (7·0)	5·37 (<2·0)	O_3H	6·36 6·60	6·52 6·78	O_2Me O_6Me
(11) Tri-O-methyl-α-cyclodextrin	4·95 (3·0)	5·03 (3·0[h])	—	—		6·35 6·50 6·60	6·52 6·62 6·78	O_2Me O_3Me O_6Me
(12) 2,6-di-O-methyl-β-cyclodextrin	5·03 (3·0)	5·03[h](*b*)	4·95 (<2·0)	5·06 (*b*)	O_3H	6·36 6·60	6·52 6·76	O_2Me O_6Me
(13) Tri-O-methyl-β-cyclodextrin	4·89 (3·0)	4·97 (3·0[h])	—	—		6·36 6·50 6·63	6·52 6·63 6·76	O_2Me O_3Me O_6Me

[a] Spectrum not taken.
[b] Broad absorption.
[c] Value not observable in the present spectrum.
[d] Values obtained from an anomeric mixture.
[e] Equatorial C_1H of ring "A" (the one bearing the glycosidic linkage).
[f] Equatorial C'_1H of ring "B" (the one bearing the O_1Me group).
[g] Axial C'_1H of ring "B".
[h] Value observed after addition of 5% CF_3COOH and/or by temperature variation.

derivatives (2,6-di-O-methyl-α-cyclodextrin: m.p. 265°, $[\alpha]_D^{20}$ in $CHCl_3$ +130; d,6-di-O-methyl-β-cyclodextrin: m.p. 312°, $[\alpha]_D^{20}$ in $CHCl_3$ +122).*

Permethylated cyclodextrins (D.S. = 3·0) were obtained from the unsubstituted compounds with MeI in DMF. To 1 g of dextrin in 5 ml DMSO 15 ml MeI and 10 g BaO were added and the suspension was maintained overnight at 40°. After a further addition of MeI and BaO, the suspension was kept 4 days at 40°, the excess MeI was evaporated and the residue extracted with 5 × 40 ml $CHCl_3$. The $CHCl_3$ extracts were concentrated at 50° under vacuum and the methylated dextrin adsorbed on animal charcoal and eluted with benzene. The permethylated products were then crystallized from $CHCl_3$/pet ether. Tri-O-methyl-β-cyclodextrin was further crystallized from H_2O at 80° and vacuum dried at 80°. Analytical data comply with those reported for cyclodextrins permethylated by a procedure in liquid ammonia[24] (tri-O-methyl-α-cyclodextrin: m.p. 205°, $[\alpha]_D^{20}$ in $CHCl_3$ +163; tri-O-methyl-β-cyclodextrin: m.p. 156°, $[\alpha]_D^{20}$ in $CHCl_3$ +163).

TABLE 3. CHEMICAL SHIFT DIFFERENCE (Δ_{C_1H}, ppm) OF THE ANOMERIC PROTON OF O-METHYLATED 1,4-DIGLUCOSES (RING A) AND α-1,4-POLYGLUCOSES WITH RESPECT TO THE UNSUBSTITUTED COMPOUNDS

	Δ_{C_1H} in DMSO*	$\Delta_{DMSO-CDCl_3}$
hepta-O-methyl-α-cellobiose	−0·01	0·00
hepta-O-methyl-β-cellobiose	−0·01	0·00
methyl hepta-O-methyl-α-maltoside	−0·41	+0·27
methyl hepta-O-methyl-β-maltoside	−0·41	+0·24
tri-O-methylamylose	−0·29	+0·19
tri-O-methyl-α-cyclodextrin	−0·16	+0·08
2,6-di-O-methyl-α-cyclodextrin	−0·15	+0·07
tri-O-methyl-β-cyclodextrin	−0·20	+0·08
2,6-di-O-methyl-β-cyclodextrin	−0·14	0·00

* The values of the unsubstituted products are from Ref. 2.

* The di-O-methylated β-cyclodextrin was first described by J. Staerk and H. Schlenk. These Authors suggested that the unsubstituted hydroxyls are those at C-2 (149*th A.C.S. Meeting*. Detroit, Spring (1965); *Abstract* 11C, No. 22).

TABLE 4. IR ABSORPTION FREQUENCIES (cm^{-1}) OF O-METHYLATED AMYLOSE AND CYCLODEXTRINS (SOLID FILMS FROM $CHCl_3$ SOLUTIONS)

Tri-O-methyl-amylose	2,6-Di-O-methyl-α-CD	Tri-O-methyl-α-CD	2,6-Di-O-methyl-β-CD	Tri-O-methyl-β-CD	Band assignment
—	3425 m	—	3410 m	—	O—H stretching
2980 w–m	2982 m	2982 m	2982 m	2982 m	antisym.Me stretching
2928 m	2928 m	2928 s	2928 m	2928 s	CH_2 and C— stretching
2900 sh	2900 sh	2900 sh	2900 sh	2900 sh	
2825 w–m	2835 w–m	2820 m	2835 w–m	2835 m	sym. Me stretching
	2818 w		2818 w	2818 sh	
1465 sh	1465 sh		1465 sh		antisym. Me bending
1452 m	1453 m	1453 m	1453 w–m	1455 m	sym. Me bending
		1448 sh		1448 sh	
1365 m	1395 w	1395 w	1405 w	1398 w	C—H benging
	1365 m	1365 m	1365 m	1365 m	
—	1327 m–w	—	1330 m–w	—	O—H in-plane-bending
1320 m–w	1318 sh	1318 w	1315 sh	1318 w	C—H bending or wagging
1300 w	1294 m–w	1302 m–w	1296 m–w	1298 m–w	
1260 w	1255 w, br	1265 sh	1250 w	1260 w	
		1255 w			unassigned
	1220 w	1230 w	1218 w	1230 w	
1204 sh		1205 sh		1205 sh	
1190 m	1194 m	1194 m	1195 m	1194 m	Me rocking
				1187 sh	
1147 s, br	1153 s	1160 s	1155 s	1160 s	antisym. C—O—C(glycosidic)
		1142 s		1142 s	stretching
—	1130 m	1130 sh	1130 sh	—	unassigned
1105 s, br	1105 sh	1108 s	1100 sh	1108 s	antisym. C—O—C(O—CH_3) stretching
1085 s, br	1085 s	1087 m	1087 s	1087 m	
		1074 s		1074 s	coupled C—C and C—O
1060 s	1050 s	1038 s	1048 s	1038 s	stretching
1030 s, br	1020 sh	—	1016 m	1025 sh	
968 m	968 m	970 m	968 m	970 m	Me rocking
950 sh	951 m–w	951 m–w	950 sh	951 m–w	ring vibration
917 w	917 w	917 w	917 w	918 w	unassigned
		912 w		908 w	
858 m–w	858 m–w	858 m–w	858 m–w	858 m–w	C_1-group vibration
762 m–w	762 m–w	762 m	762 m–w	762 m–w	ring breathing vibration
707 m–w	704 m–w	704 m–w	704 m–w	704 m–w	ring vibration
	665 w	665 sh	665 sh	665 sh	
—	—	650 m–br	—	650 m–br	O—H out-of-plane-bending
608 w	605 m–w		608 m–w	603 m–w	
	562 m		567 m–w	573 m	
540 m	545 sh	555 m	540 sh	556 m	ring vibrations
510 sh	505 sh	520 w, br	505 sh	520 m–w	
	435 w	—	435 w	466 w	

s = strong; m = medium; w = weak; sh = shoulder; br = broad.

TABLE 5. OH-STRETCHING FREQUENCIES (cm^{-1}) OF PARTIALLY O-METHYLATED GLUCOSES, GLUCOSIDES, AMYLOSE AND CYCLODESTRINS

Compound	position of the unsubstituted OH_s	in CCl_4 (c = 0·05%)	in CCl_4 (c = 5%)	assignment	in DMSO (c = 10%)	solid (film from DMSO solution)
(1) 2,3,6-Tri-O-methyl-α-D-glucose	1 and 4	3605 m		$O_4H...O_3$	3275 s	3390 sh
		3575 sh		$O_1H...O_2$		3360
		3535		$O_4H...O_6$		
		3420 w, br		dimer		
(2) 2,4,6-Tri-O-methyl-α-D-glucose	1 and 3	3602		$O_3H...O_4$ and $O_3H...O_2$	3275 s	3370
		3570 sh		$O_1H...O_2$		3300 sh
		3420 w, br		dimer		
(3) 2,3,4,6-Tetra-O-methyl-α-D-glucose[a]	1	3610	3610 w	$O_1H...O_2$ (β-anomer)	3230 s	3380
		3574	3575 w	$O_1H...O_2$ (α-anomer)		
		3420 w, br	3430 s	dimer		
(4) Hepta-O-methyl-α-D-cellobiose	1	3614		$O_1H...O_2$ (β-anomer)	3230 s	3430 sh
		3578		$O_1H...O_2$ (α-anomer)		3380
		3420 w, br		dimer		
(5) Methyl 2,3-di-O-methyl-α-D-glucoside	4 and 6	3603	3603 m[b]	$O_4H...O_3$ and $O_6...O_5$	3330 s	3435
		3560 sh	3435 m, br[b]	$O_4H...O_6$		3350 sh
(6) Benzyl 2,4,6-tri-O-methyl-β-D-glucoside	3	3597		$O_3H...O_4$ and $O_3H...O_2$	3448 s	3270
						3190 sh
(7) O-Methyl-amylose (D.S. = 2·3)	2 and 3	—	3410[c]		3450 w	3500 sh
					3390 m	3435
					3320 m	
(8) 2,6-Di-O-methyl-α-cyclodextrin	3	3430	3430[b and c]		3430 s	3425
(9) 2,6-Di-O-methyl-β-cyclodextrin	3	3420	3420[b and c]		3420 s	3410

[a] contains some β-anomer; [b] saturated solution in CCl_4; [c] 1% solution in $CHCl_3$.
w = weak; m = medium; s = strong; sh = shoulder; br = broad.

TABLE 6. CONCENTRATION-DEPENDENCE OF THE INTENSITY OF THE νOH BAND OF 2,6-DI-O-METHYLATED-CYCLODEXTRINS IN CCl_4 SOLUTION AT 25°

c (w/v) %	$\frac{A}{cl}$ * α-Cyclodextrin derivative	β-Cyclodextrin derivative
0·005	4·36	7·25
0·01	4·28	7·28
0·02	4·33	7·20
0·05	4·28	7·19
0·10	4·34	7·22
0·20	4·36	7·27
0·50	4·33	7·27
1·00	4·40	7·28

* A = apparent absorbance; c = concentration, g/100 ml; l = cell thickness, cm.

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