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METHYLATED CYCLOAMYLOSES (CYCLODEXTRINS) AND THEIR INCLUSION PROPERTIES

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ABSTRACT

2,3,6-Tri-*O*-methyl and 2,6-di-*O*-methyl derivatives of cyclohexa- and cyclohepta-amylose (2,3,6-tri-*O*-methyl- and 2,6-di-*O*-methyl-α- and -β-cyclodextrin) have been prepared and shown to be versatile complexing agents. Their complexes in aqueous solution are usually more stable than the corresponding complexes of unsubstituted cycloamyloses. The methylated cycloamyloses also form crystalline complexes, the stability of which depends on the size and shape of the guest molecule. The decomposition temperature of the crystalline complexes with homologous *n*-alkanes, which is relatively high, increases with increasing chain-length of the hydrocarbon. The shift of the i.r. carbonyl band of oleic acid in its solid complex with methylated α-cyclodextrin probably reflects inclusion of the fatty acid in the monomeric form. The methylated cycloamyloses, when used as the stationary phases for g.l.c. or dissolved in a conventional stationary phase, affect the retention times of organic compounds in a manner which suggests that inclusion phenomena are operative.

INTRODUCTION

The inclusion properties of cyclodextrins (cycloamyloses) have been extensively investigated[1–3]. The complexes, which are formed in the solid state and in solution, consist of guest molecules held in the cavity of the macrocycle of the host cyclodextrin and are stabilised by Van der Waals forces, and, to a lesser extent, by dipole–dipole interactions. Hydrogen bonding seems to play a role only in crystalline complexes. Inclusion complexes in aqueous solution are thought to be further stabilised by hydrophobic interactions, *i.e.*, by the tendency of the solvent water to push hydrophobic solutes of suitable size and shape into the essentially hydrophobic cavity, in order to attain the "most probable structure" of the solvent and the minimum energy in the overall system[2,3].

We now report on the modification of cyclodextrins by methylation; methyl

groups were not expected to obstruct the macrocycle cavities. Cyclodextrins have been reported to stimulate fatty acid synthesis by yeasts *via* formation of inclusion complexes with palmitoyl-CoA, and this stimulation was substantially increased by etherification of cyclodextrins[6,7]. Also, since methylated cyclodextrins are fairly soluble in water[4], it is possible to compare their complexing properties in aqueous solution with those of the parent molecules and to study the effect on crystalline-complex formation of reducing or removing the ability to hydrogen-bond.

RESULTS AND DISCUSSION

Tri-*O*-methyl-α- and -β-cyclodextrins, prepared by the Hakomori methylation procedure[8], had the same n.m.r. and i.r. characteristics as the derivatives obtained by exhaustive methylation using the Kuhn and Trischmann method[9,10], when the same solvent was used for crystallisation. However, the use of batches of light petroleum having different g.l.c. profiles gave crystals with different melting-points, all markedly different from those reported[11] when this solvent was used for crystallising tri-*O*-methylcyclodextrins obtained by methylation in liquid ammonia. It was realised subsequently that the components of this solvent co-crystallise with the methylated cyclodextrin to form stable inclusion-complexes, the melting point of which varies with the nature of the guest molecule. Crystallisation from *n*-hexane yielded complexes having constant and reproducible melting-points. Solvent-free complexes could be obtained only after extensive drying (*e.g.*, 40 h at 80°/10 mmHg to remove *n*-hexane from its crystalline complex with tri-*O*-methyl-β-cyclodextrin).

N.m.r. spectroscopy of the di-*O*-methylcyclodextrins suggested that the D-glucose residues were substituted at positions 2 and 6, rather than positions 3 and 6 as indicated by other workers[12], and also showed the presence of a small proportion of the permethylated monomers. Confirmation of 2,6-substitution was obtained as follows. When the di-*O*-methylcyclodextrin was subjected to hydrolysis followed by reduction with borohydride, the product consumed ~2 mol. of periodate, yielding formic acid but no formaldehyde; hence, it must have been mainly 2,6-di-*O*-methylglucitol together with ~10% of 2,3,6-tri-*O*-methylglucitol. Treatment of the hydrolysis products of the di-*O*-methylcyclodextrin with phenylhydrazine gave 6-*O*-methyl-D-glucose phenylosazone, the product expected from 2,6-di-*O*-methyl-D-glucose[13]. G.l.c.–m.s. of the trimethylsilylated derivative of the main hydrolysis-product showed that HO-3 was not methylated.

Complexes were prepared by crystallising the methylated cyclodextrins from a solvent that could also function as a guest, or from a solution of the guest in a non-complexing solvent such as chloroform. ^{1}H-N.m.r. spectroscopy of solutions in trideuteriomethyl sulphoxide usually served to confirm co-crystallisation of the guest and host, and to determine the approximate stoichiometry of the complex. Small aromatic molecules (such as benzene and mono-substituted benzenes) gave 1:1 crystalline complexes, also confirmed by u.v. spectroscopy, suggesting a clathrate-type structure. Long-chain molecules (alkanes and fatty acids with $n > 4$) gave non-

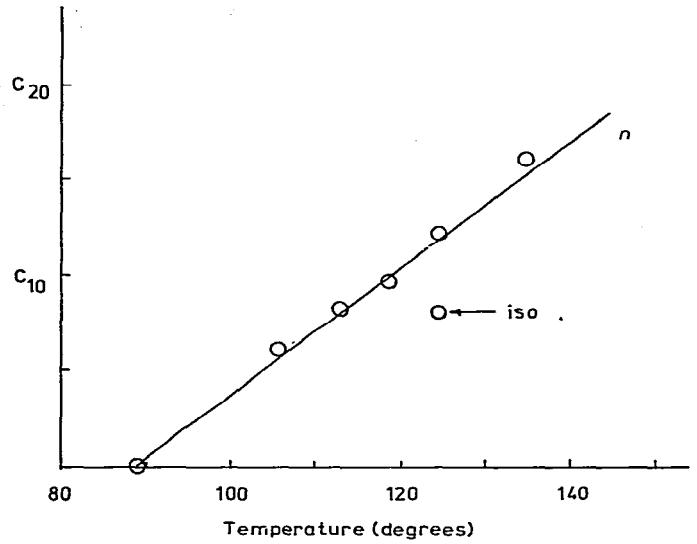

Fig. 1. Decomposition temperature for tri-*O*-methyl-β-cyclodextrin and its crystalline complexes with some *n*-alkanes (C_6–C_{16}) and iso-octane, as a function of chain length of the complexed hydrocarbon.

stoichiometric complexes, containing ~10% of guest. Such behaviour is essentially the same as that of unsubstituted cyclodextrins, the inclusion complexes of which with *n*-alcohols and fatty acids having $n > 4$ form channel structures in which the dextrin molecules are aligned coaxially[14,15].

The thermal stability of the crystalline complexes, as determined by differential scanning calorimetry (d.s.c.), varied over a wide range of temperatures and energies of decomposition, depending on the nature of the guest molecules. The complexes re-formed from the melt on cooling, provided that the guest was not volatilised at the melting-temperature of the complex. When only part of the guest molecules volatilised from the system, the decomposition temperature of the re-formed complex was usually lower than that of the original, probably reflecting the existence of partially filled channels. More than one endotherm was sometimes observed for partially re-formed complexes. Fig. 1 shows that the decomposition temperature of a homologous series of complexes of tri-*O*-methyl-β-cyclodextrin with *n*-paraffins increases with increasing chain-length of the latter. The complexes of the β-derivative with branched hydrocarbons generally had higher thermal stability than those with the linear isomers, as shown in Fig. 1 for *n*- and iso-octane. Such behaviour is not unexpected, on account of the better fit of branched isomers in the rather large cavity of β-cyclodextrin, which has an internal diameter of ~7.5 Å as deduced from space-filling models.

The crystalline complexes involving hydrocarbons must be stabilised essentially by Van der Waals forces. When the guest contains a suitable group, such as carboxyl, hydrogen bonding is possible. For the crystalline complexes of fatty acids, existence of a hydrogen bond different from that (1705 cm^{-1}) involved in the usual dimeric

structure should be reflected in a shift of the i.r. carboxyl-band. No such shift was observed for the complex of oleic acid with di-*O*-methyl-β-cyclodextrin. However, there was shift to 1738 cm^{-1} for the complex of di-*O*-methyl-α-cyclodextrin. This frequency is typical for monomeric carboxyl-groups in an ether-like environment[16], probably reflecting the constraints associated with the smaller cavity of the α derivative. It is unlikely that there would be a hydrogen bond involving a $-CO_2H$ oxygen and the HO-3 of 2,6-di-*O*-methylcyclodextrin, since HO-3 is bonded to MeO-2 of a neighbouring unit[9].

Cyclodextrins and the methylated derivatives form inclusion complexes in aqueous solution[5-7]. When the guest has chromophores and is polarisable, complexation in solution can be detected by spectral shifts in the u.v.–visible range, as shown for unsubstituted cycloamyloses[3,17]. Significant shifts were observed when methylated cyclodextrins were added to solutions containing *p*-nitrophenolate ion and 2,6-dichlorophenol–indophenol. *p*-Nitrophenol fits better (*i.e.*, complexes stronger) in the six-membered α- than in the seven-membered β-cyclodextrin[14,17]. However, the spectral shift observed on addition of an excess of tri-*O*-methyl-β-cyclodextrin was even larger ($\Delta\lambda$ 18 nm) than that ($\Delta\lambda$ 15 nm) on addition of unsubstituted α-cyclodextrin[17]. Also, the bathochromic shift of 2,6-dichlorophenol–indophenol (at pH 8) was consistently larger when the methylated derivatives of β-cyclodextrin were added ($\Delta\lambda$ 16 nm) than in the presence of the same amount of unsubstituted β-cyclodextrin ($\Delta\lambda$ 8 nm).

The di- and tri-*O*-methylated cyclodextrins produced substantially the same shifts, but none was produced by linear oligosaccharides (starch hydrolysate d.p. ~20), indicating that complexation requires a host macrocycle. That complexation is a true inclusion phenomenon can probably be ascertained by n.m.r. spectroscopy, by observing selective shifts of H-3 and H-5, which project inside the macrocycle, in the presence of guest molecules[18]. However, no attempts have yet been made to rationalise n.m.r. shifts for the methylated cyclodextrin systems, since, due to interference by the methoxyl-proton signals, the signals of H-3 and H-5 are not easily observed. Moreover, the signals of the other protons (H-1,2,4,6) are also occasionally shifted on addition of some compounds capable of forming complexes, probably reflecting a reorientation of the methoxyl groups around the C–O–Me bonds, suggested by the fact that one or two methoxyl signals also shift. This effect was also observed in other studies[5].

Quantitative data on the strength of the complexes in solution have been obtained only for Methyl Orange, using a spectrophotometric method[14]. In a strongly acidic solution (in which minor pH changes do not cause any spectral changes), the absorbance at 508 nm dramatically decreased on addition of α-cyclodextrin, an effect interpreted in terms of a different site of protonation when the Methyl Orange molecule is included in the dextrin[19]. Similar hypochromic shifts were also observed on addition of di- and tri-*O*-methyl-α-cyclodextrin. However, these methylated derivatives also caused a large hypsochromic shift (~50 nm), indicating a much more pronounced polarisation of the included molecule.

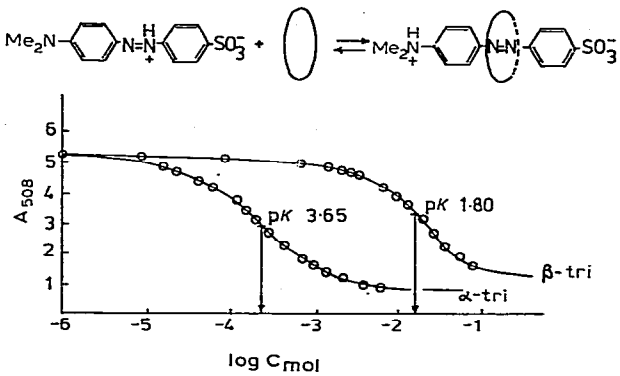

Fig. 2. Absorbance of the visible band of Methyl Orange (in 8mM HCl) as a function of the concentration of tri-*O*-methyl-α- and -β-cyclodextrin.

In Fig. 2, the decrease of the 508-nm absorbance is plotted as a function of the concentration of tri-*O*-methyl-α- and -β-cyclodextrin. The dissociation constants of the corresponding complexes, as derived from the inflexion points of the curves[19], are also shown. Due to uncertainty about the stoichiometry of the complexes (both 1:1 and 2:1 complexes appear to be formed by α-cyclodextrin with Methyl Orange in neutral solutions[17]), the dissociation constants should be regarded as apparent. However, assuming the same stoichiometry for the complexes of unsubstituted and methylated dextrins, the stability of the complex with tri-*O*-methyl-α-cyclodextrin (log K_{diss} 3.65) appears to be substantially greater than that of α-cyclodextrin (pK_{diss} 2.89, in agreement with literature data[19,20]). The stability of the corresponding complex with tri-*O*-methyl-β-cyclodextrin (pK_{diss} 1.80) is much lower than that with the α derivative, as expected from the looser fit of the guest in the larger β-macrocycle.

The foregoing data indicate that *O*-methylation of cyclodextrins increases the strength of their inclusion complexes in aqueous solution, possibly because the methyl groups provide a hydrophobic lining, thereby favouring hydrophobic interactions that stabilise the complexes. In addition, further stabilisation could also be provided by capping of one side of the macrocycle by the methylated primary-hydroxyl groups which, for other types of substitution, significantly improves binding of the guests[21,22]*. The influence of HO-3 is likely to be no more than second-order in the di-*O*-methylcyclodextrins, because of its involvement in intramolecular hydrogen-bonding[9].

Since g.l.c. can be used for detecting molecular interactions, experiments were performed by using methylated cyclodextrins as the stationary phases or by dissolving them in conventional liquid-phases. In order to avoid hydrogen-bonding effects, experiments were limited to the tri-*O*-methyl derivatives, and non-polar compounds (saturated hydrocarbons) were used in the mobile phase. The relatively low melting-

*Hydrophobic interactions might be minor contributors to the stabilisation of these complexes. A recent investigation on the thermodynamics of inclusion complexes of cyclodextrins (as well as their 2,6-di-*O*-methyl derivatives) points to a substantial contribution from Van der Waals–London dispersion forces[23].

point of tri-*O*-methyl-β-cyclodextrin (~89°, see Fig. 1) allowed it to be used as a liquid phase at ~100°. Substantial retention of saturated hydrocarbons was also observed below 89°, but the peaks were severely broadened, probably due, in part, to the uneven crystallisation of the dextrin on the inert Chromosorb support. This effect persisted when the temperature was raised a few degrees above 89°, even after long conditioning of the columns. Above 100°, separation of homologous *n*-alkanes could be obtained. However, complete resolution was achieved only at temperatures well above the boiling point of the hydrocarbons. Increasing resolution with increasing temperature is the opposite trend to that usually observed with ordinary stationary-phases and may reflect inclusion phenomena. This effect was negligible above 130°, leading to normal g.l.c. profiles.

As expected, separations of hydrocarbon mixtures using methylated cyclodextrins dissolved in a conventional stationary-phase (silicone oil) were obtained, even at low temperatures (~40° for hexane isomers), with peak widths similar to those obtained using the silicone oil alone. Addition of the cyclodextrin caused a substantial increase in the retention volume of all the hydrocarbons from 1.0 (at 50°) for *n*-octane to 1.5 on both methylated α- and β-cyclodextrins. For iso-octane, the values became 1.4 for the α- and 1.6 for the β-macrocycle, possibly due to the tighter fit of this hydrocarbon in the β-macrocycle, as also indicated by the thermal stability of the crystalline complexes (Fig. 1).

Cyclodextrin esters of fatty acids have been used[22] as stationary phases in g.l.c., but there was no indication that the retention on these derivatives was affected by inclusion phenomena.

EXPERIMENTAL

General. — I.r. spectra were obtained with a Perkin–Elmer Model 337 spectrophotometer, ^{1}H-n.m.r. spectra with either JEOL JNM-MH-100 or Perkin–Elmer R-32 (90 MHz) spectrometers, u.v.-visible spectra with a Zeiss PMQ-II spectrophotometer, and mass spectra with a Perkin–Elmer Mod. 270 instrument. The g.l.c. experiments were performed with a Perkin–Elmer F-20 gas chromatograph (flame-ionisation detector). Differential scanning calorimetry was performed with a Perkin–Elmer DSC-1 instrument at 16°/min. P.c. of methylated sugars was performed with 1-butanol–ethanol–water (4:1:5, upper layer).

2,3,6-Tri-*O*-methyl-α- and -β-cyclodextrin were prepared by the Hakomori procedure[8]. Physico-chemical data were identical to those of the products obtained by exhaustive, classical methylation[9]. Di-*O*-methyl-α- and -β-cyclodextrin were prepared by the method of Kuhn and Trischmann[10].

Hydrolysis of di-O-methyl-β-cyclodextrin. — Crystalline di-*O*-methyl-β-cyclodextrin (0.5 g) was dissolved in 72% sulphuric acid (5 ml) with cooling. The solution was left for 1 h at room temperature, and then diluted with water (40 ml) and kept at 100° for 4 h. The solution was neutralised with barium carbonate, filtered, treated with Amberlite IR-120(H^+) resin, and concentrated to a syrup (524 mg), $[\alpha]_D$

+62° (*c* 2, water). P.c. of the syrup revealed a minor component (10–15%, chromatographically identical to 2,3,6-tri-*O*-methyl-D-glucose) and a major component having slightly lower mobility (R_{Glc} 2.27). The trimethylsilyl derivative of the latter component had the same g.l.c. retention as that described[25] for trimethylsilyl 2,6-di-*O*-methyl-3,4-di-*O*-trimethylsilyl-α,β-D-glucopyranoside, and its mass spectrum was different from that of the alternative isomer, trimethylsilyl 3,6-di-*O*-methyl-2,4-di-*O*-trimethylsilyl α,β-D-glucopyranoside[26].

A portion (80 mg) of the syrup, dissolved in water (5 ml), was treated overnight with sodium borohydride (50 mg). Inorganic ions were removed with Amberlite IR-120(H^+) resin and methanol, and the solution was concentrated to a syrup (70 mg) in which no reducing sugars were detectable by p.c.

The borohydride-reduced hydrolysate (42 mg) was dissolved in a solution of sodium metaperiodate (214 mg) in water (10 ml). The consumption of periodate was determined[27] spectrophotometrically at 222.5 nm and was complete after 1 h (2 mol/mol of sugar, estimated on the basis of 2,6-di-*O*-methylglucitol). Formic acid was estimated by back titration with 0.01M sodium hydroxide (phenolphthalein), and the oxidised solution was tested for formaldehyde by using the chromotropic acid reagent[28].

6-O-Methyl-D-glucose phenylosazone. — (*a*) 6-*O*-Methyl-D-glucose[26] {75 mg, m.p. 133–135°, $[\alpha]_D$ +85→+53° (*c* 0.27, water)} was treated[29] with phenylhydrazine (180 mg) to give 6-*O*-methyl-D-glucose phenylosazone (31 mg), m.p. 176–177° (from aqueous methanol), $[\alpha]_D$ −54° (after 19 h; *c* 0.12, ethanol).

(*b*) The di-*O*-methyl-α-cyclodextrin hydrolysate (197 mg) was treated for 1.25 h with phenylhydrazine (720 mg) and 25% acetic acid (6 ml). The product was collected, and recrystallised several times with methanol–water (2:1) to yield 6-*O*-methyl-D-glucose phenylosazone (31 mg), m.p. and mixture m.p. 170–171°. The products from (*a*) and (*b*) gave identical X-ray powder diagrams and i.r. spectra.

Crystalline complexes. — The complexes of methylated cyclodextrins with liquid hydrocarbons were prepared by crystallising the former from the latter. Complexes with solid hydrocarbons and fatty acids were prepared by crystallisation from saturated solutions in chloroform containing 10% of methylated cyclodextrin.

The crystals were washed with cold ether, and air-dried at room temperature. The approximate stoichiometry of the complexes was usually determined by ^1H-n.m.r. spectroscopy of solutions in trideuteriomethyl sulphoxide, from the relative intensities of signals of the guest and the anomeric proton of the cyclodextrin derivative. The content of aromatic guests was occasionally checked by u.v. spectrophotometry on solutions in ethanol. Traces of residual, volatile guests (*e.g.*, *n*-hexane) in crystalline complexes after controlled heating of the complexes were detected by g.l.c., using the platinum spiral of the pyrolyser unit as the sampler. Low voltages were applied to the spiral, in order to reach the decomposition temperature of the complex (and release the guest) without substantial pyrolysis of the methylated cyclodextrin. Pyrolysis products of small molecular weight could potentially interfere. The complexes of fatty acids were not quantitatively analysed.

Complexation in aqueous solution. — These complexes were investigated by u.v.–visible spectrophotometry[14,17,19]. The dissociation constants of the Methyl Orange complexes were determined as described[19,20] for unsubstituted cyclodextrins.

G.l.c. experiments. — Methylated cyclodextrins as stationary phases were used in stainless-steel columns (2 m × 0.125 in.) of 5% of dextrin (deposited by concentration of a solution in acetone) on silanised Chromosorb-W, or of 15% of silicone oil DC-710 (Perkin–Elmer) containing 10% of dextrin deposited on silanised Chromosorb-W. Columns were preconditioned for at least 4 h under nitrogen at 100° before first use and for 1 h between successive experiments. G.l.c. of hydrocarbon mixtures on methylated-cyclodextrin phases were made under isothermal conditions, with a nitrogen flow of 25–30 ml/min.

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