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Induced circular-dichroism spectra of complexes of cyclomalto-oligosaccharides and azobenzene derivatives*†

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ABSTRACT

The circular-dichroism spectra of the inclusion complexes of 9 types of cyclodextrin (CDs) with 6 kinds of azobenzene derivatives generally show a single peak, but sometimes form a splitting pattern in the first $\pi \rightarrow \pi^*$ region. All single patterns show a positive sign; indicating that the long and slim azobenzene derivatives are incorporated into the cavity from the long-axis side. The splitting patterns change in sign and magnitude according to the substitution on the component molecules and establish the formation of various stacking modes. Plots of the lengths of the azo dyes *vs.* the molar circular-dichroism coefficients suggest that the substituent on the CD torus is an important factor in causing splitting and deciding the sign of the split-type Cotton effects. The foregoing splitting may arise from exciton interaction of two molecules of the chromophoric dye each in the form of a 1:1 complex and in the cyclomaltooctaose (γCD) complex.

INTRODUCTION

When achiral azo dyes form complexes with cyclodextrin (CDs), the complexes exhibit optical activity. As this optical activity is induced by interaction between the CDs and the guest molecules, the shape and the sign of the circular dichroism (c.d.) spectra of those complexes should provide information concerning their structures. From the sign of the c.d. spectra, it is possible to estimate the orientation of the chromophore in the cavity of the CD if the direction of the electric dipole-moment in the chromophore is known [1-3].

C.d. spectra often display splitting patterns. This pattern arises mainly from spatial interaction between two or more chromophores. Natural anthocyanidin 3,5-diglucosides show such splitting in neutral aqueous solution. A minor change of substituents on the anthocyanidin alters the chiral direction of stacking and clarifies the chiroptical stacking of anthocyanidin chromophores[4,5].

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Azo dyes also showed induced splitting of their c.d. spectra in solutions containing CDs, polypeptides, and 1-cyclohexyl-3-(2-morpholoethyl) carbodiimide metho-*p*-toluenesulfonate (CMC), suggesting the occurence of stacking [6–11]. In the previous paper, a water-soluble cyclomaltohexaose (αCD)–epichlorohydrin (Ep) polymer product having a relative molecular mass of < 2000 was isolated and its complex with Methyl Orange showed split-type Cotton effects in the induced $\pi \rightarrow \pi^*$ band of the $N = N$ group. Related complexes containing αCD ethylene glycolbis(epoxypropyl) ether (αCD-DiEp) showed reversal of signs of the splitting pattern [12].

In this paper, azobenzene derivatives were used and c.d. spectra were investigated of all inclusion compounds with cyclomalto-hexa-, hepta-, and -octaoses (α-, β-, and γ-CDs), 2,6-di-*O*-methyl and 2,3,6-tri-*O*-methyl-βCDs (DM-βCD and TM-βCD), and low molecular-mass fractions of water-soluble α-, β-, and γ-CD polymer products.

The aim was to determine what information the c.d. spectra can give on the structure of the CD complexes.

MATERIALS AND METHODS

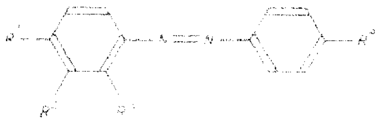
Materials and instruments used were as described previously [12a]. Azo dyes tested are listed in Table I.

In measurements of visible and c.d. spectra, $\sim 10^{-4}$ mol guest molecules and $\sim 10^{-3}$ mol host molecules were dissolved in 0.1M phosphate buffer at pH 6.0. For sparingly soluble compounds, the concentrations of azo dyes included were estimated from calibration curves of the dyes dissolved in 1:1 ethanol–water. The host:guest ratio in the complexes was assumed [9,13a,c] to be the 1:1, except for the γCD complexes where the ratio was 1:2.

TABLE I

Guest molecules tested

Azo dyes



	R^1	R^2	R^3	R^4
1 Tropaeolin 00	$NaSO_3$	H	H	NH-C_6H_5
2 Metanil Yellow	H	$NaSO_3$	H	NH-C_6H_5
3 Methyl Orange	$NaSO_3$	H	H	NMe_2
4 4-Dimethylaminoazobenzene	H	H	H	NMe_2
5 2-{[4-(Dimethylamino)phenyl]azo}benzoic acid	H	H	CO_2H	NMe_2
6 Azobenzene	H	H	H	H

RESULTS AND DISCUSSION

The c.d. spectra. – The azo dye–CD complexes induce single and split c.d. spectral patterns at the $\pi \rightarrow \pi^*$ band of the N = N group (Fig. 1 and Table II). The single patterns have a positive sign. The split patterns show positive and negative split-type Cotton effects. In the former case, the molar circular-dichroism coefficient ($\Delta\varepsilon$) of the longer wavelength extremum is positive and that at the shorter wavelength is negative, and in the latter case, the signs become reversed. The $\Delta\varepsilon$ values were plotted against the backbone lengths of the azo dyes (Fig. 2 a–c).

The αCD series give the largest $\Delta\varepsilon$ values in all CD–azo dye complexes and almost all c.d. spectra show splitting (Fig. 2a). The complexes with αCD-Ep gave negative split-type Cotton effects and the $\Delta\varepsilon$ values of both extrema are the same. The crossing points of the c.d. couplet and the absorption maxima in the visible spectra are practically coincident. The complexes with αCD-DiEp show positive split-type Cotton effects and the $\Delta\varepsilon$ values of the extrema are not the same (Fig. 1).

Fig. 2b shows that the complexes with the βCD derivatives give a single shape, except for those with TM-βCD. Substitution by an *O*-methyl group at O-2 and O-6 in βCD lengthens [14a,15] the cavity from ~8 to ~11 Å and enhances the inclusion ability. This fact suggests that the driving force for inclusion of the guest molecule into the cavity is not hydrogen bonding, but van der Waals (and/or hydrophobic) interactions. Further substitution by *O*-methyl groups at the inner side of βCD (the 3-OH group) narrows the inner diameter of the cavity and causes negative split-type Cotton effects. In the **1**–TM-βCD complex, the $\Delta\varepsilon$ values of both extrema in the splitting pattern are the same, but in the **2**–TM-βCD complex, the $\Delta\varepsilon$ of negative sign is larger than that of the positive one. Shorter azo dyes are not included by TM-βCD.

Fig. 2c shows that the γCD series form complexes only with long-sized azo dyes (**1**–**3**). Table I shows that the **3**–γCD complex gives a λ_{max} of 435 nm, 24 nm shorter than that of **3** itself in the visible spectrum, and does not cause splitting in the c.d. spectrum. The **1**– and **2**–γCD complexes give λ_{max} values of 426 and 417 nm, 17 nm shorter than those of **1** and **2** themselves, and give a small splitting in the c.d. spectra. In the latter complexes, the $\Delta\varepsilon$ values at longer wavelength are large whereas those at shorter wavelength are small. The c.d. spectra of the αCD-DiEp complexes are similar to those of the **1**– and **2**–γCD complexes, but the λ_{max} values of the visible spectra in the former move 1–8 nm to longer wavelength than those of **1** and **2** themselves. In the γCD complexes, the host–guest complexation ratio is 2:1 and 2:2 (ref. 13a), and in the αCD-DiEp complexes it is 1:1. The λ_{max} values in the visible spectra may thus be used to differentiate between host–guest stoichiometries.

Azo dye **6**, which has the fundamental skeleton of the azo dyes studied in this work, does not necessarily form complexes with all CDs. Neither can dye **4** be included by β-, γ-, and TM-βCDs. Substitution at both benzene rings permits inclusion into α-, β-, and γ-CDs.

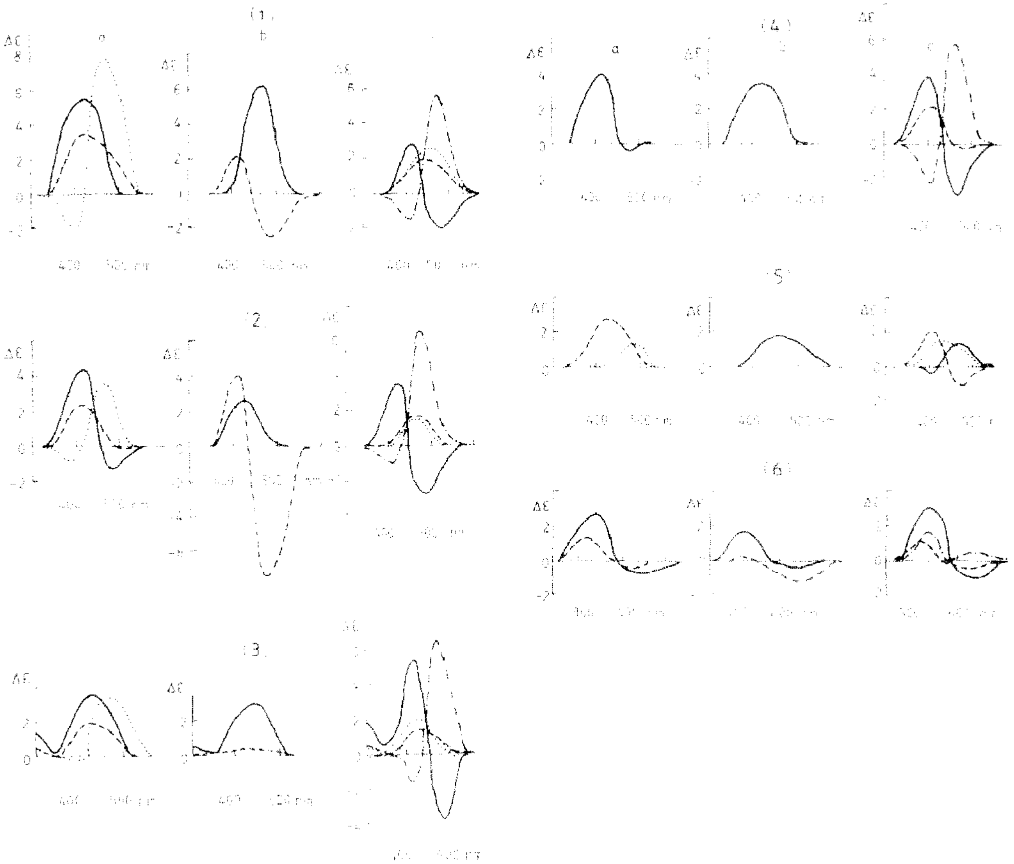


Fig. 1. C.d. spectra of azo dye complexes with cyclodextrins. (a) αCD (—), βCD (- - - -), γCD (····); (b) 2,6-di-*O*-methyl-βCD (—), 2,3,6-tri-*O*-methyl-βCD (- - - -); (c) Products formed with linking agents: αCD-epichlorohydrin (—), βCD-epichlorohydrin (- - - -), γCD-epichlorohydrin (····), and αCD-ethyleneglycol bis(epoxypropyl) ether (-·-·-).

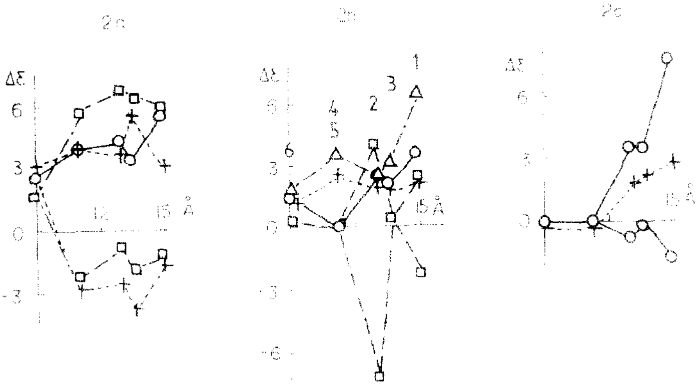


Fig. 2. Correlation between the induced molar circular dichroism coefficient (Δε) of azo dye complexes and the length of the guest molecules (Å): (a) αCD series: αCD (○ — ○), αCD-Ep (+ - - + - - + - -), αCD-DiEp (□ - · □ - · □ - ·); (b) βCD series: βCD (○ — ○), βCD-Ep (+ · · + · ·), DM-βCD (△ - △ -), TM-βCD (□ - - □ - -); (c) γCD series: γCD (○ — ○), γCD-Ep (+ · · + · ·).

TABLE II

Absorption maxima and induced c.d. values of complexes between azo dyes and cyclodextrins[a]

nm_{max}		Dye only	Complex								
			αCD-DiEp	*αCD-Ep*	*αCD*	*TM-βCD*	*βCD-Ep*	*βCD*	*DM-βCD*	*γCD-Ep*	*γCD*
1	λ	443	450	444	442	436	434	434	436	430	426
	CD		470, 410	480, 413	420	485, 410	440	418	467	457	467. 400
	$\Delta\varepsilon$		+5.8, −1.3	−1.8, +3.0	+5.6	−2.3, +2.3	+2.1	+3.5	+6.3	+2.7	+7.8, −1.8
2	λ	434	442	437	433	428	432	426	429	424	417
	CD		465, 405	475, 415	475, 415	467, 405	455	410	420	455	458, 390
	$\Delta\varepsilon$		+6.6, −0.9	−2.7, +3.6	−1.3, +4.3	−7.4, +3.9	+1.7	+2.3	+2.5	+1.6	+3.5, −0.9
3	λ	459	460	463	460	457	450	458	455	440	435
	CD		472, 410	482, 415	420	440	417	420	440	420	462
	$\Delta\varepsilon$		+6.3, −2.1	−3.8, +5.4	+3.4	+0.4	+1.5	+2.0	+3.0	+2.1	+3.4
4	λ	417	423	423	419	419	420	419	420	417	
	CD		460, 404	467, 400	410		405		415		
	$\Delta\varepsilon$		+5.7, −2.3	−2.9, +3.9	+3.9		+2.2		+3.5		
5	λ	432	430	425	432	427	416	416	415	427	436
	CD			468, 400			465, 405	420	450	480, 415	480
	$\Delta\varepsilon$			+1.3, −0.4			−1.1, +2,1	+2.8	+1.8	+0.8, +1.4	+1.3
6	λ	316	324	326	326	322	321	321	321	320	333
	CD		335	332	335	310	316	310	309		
	$\Delta\varepsilon$		+1.6	+3.1	+2.6	+0.2	+1.1	+1.3	+1.7		

[a] CD: cyclodextrin; DM-βCD: 2,6-di-*O*-methyl-βCD; TM-βCD: 2,3,6-tri-*O*-methyl-βCD; Ep: epichlorohydrin; DiEp: ethyleneglycol bis(epoxypropyl) ether.

Judged from the $\Delta\varepsilon$ values, substitution at the position *para* (**1**) to the N = N group increases the extent of inclusion, but substitution at the *meta* position (**2**) decreases it. Azo dye having *ortho* substituents (**5**) do not form host–guest complexes with the αCD series. The $\Delta\varepsilon$ magnitudes of the single peak in the **1**-complexes are the order: DM-βCD > αCD > βCD > γCD-Ep > βCD-Ep: lengthening of the cavity has a larger effect on $\Delta\varepsilon$ than the size of the inner diameter of the cavity. Only in the **6**-complexes can the peaks in the n → π* region[16] be observed separately, and almost all of them display a negative Cotton effect.

The direction of inclusion. — Comparisons between the experimental and calculated rotational strengths of βCD complexes with the substituted benzenes indicate that these βCD complexes favor axial inclusion in which the long axis of the substituted benzene is parallel to the axis of the βCD cavity, and that the electric transitions polarized along the long axis of the substituted benzenes show positive c.d. spectra, whereas the signs of the short-axis polarized transitions are negative.

In the present azo dye–CD complexes, almost all spectra show positive Cotton effects. It is natural that the long and slim azobenzene derivatives should be included into the cavity from the long-axis side. In fact, ^{1}H-n.m.r. and X-ray work concerning the **3**–αCD complex, establishes that **3** is included into the CD cavity from the hydrophobic *N*,*N*-dimethylaniline side and the N = N group is situated at the neck of the αCD[17c, 18]. The present c.d. spectrum of **3**–αCD complex in the foregoing conformation shows a positive Cotton effect in the first π → π* region.

The exciton-coupled c.d. spectra. — CD complexes often show c.d. splitting patterns (Fig. 1). This pattern may arise mainly from a dipole–dipole interaction between the electric transition-moments of the intramolecular charge-transfer band of the two azo dye molecules[19]. According to this molecular excition theory, the absorption maximum of the visible spectrum and the crossover point of the c.d. couplet should coincide and the $\Delta\varepsilon$ values of both extrema in the splitting pattern should be the same. The magnitudes of the Cotton effect correspond to the magnitude of the transition moment of the chromophore, become inversely proportional to the square of the distance, and depend upon the angle between the interacting chromophores. This theory was originally developed as a tool for determining the absolute configuration or conformation of natural products, and was later extended to the detection of stacking of the molecules.

The exciton splitting and molecular-orbital theories proved that the angle between the transition moments leads to the changes in the c.d. and visible spectra[19]. Calculations of the expected c.d. spectrum were performed by using a model based on the interaction of two transition moments. When the angle between the transition moments is 120°, the calculated c.d. spectrum shows positive split Cotton-effects (positive chirality) and the visible spectrum moves to longer wavelength. On the other hand when the angle is 60°, the calculated c.d. spectrum shows negative split Cotton effects (negative chirality) and the visible spectrum moves to shorter wavelength.

In the present work, when the substituent on the CD is long (DiEp), positive split Cotton-effects appear, and when the substituents on the CD are short (Ep and MeO), negative split Cotton-effects appear. The substituents on the CD torus may play a important role in deciding the angle between the longitudinal axis of the two interacting azo-dye molecules and the tight fitting between the host–guest interaction. On the other hand, when the complexes have only a loose fit, the pattern shows a single Cotton effect.

During the course of synthesizing the CD-Ep polymers (R-OCH$_2$-CHOH-CH$_2$-O-R), the posibility of producing glyceryl ethers (R-OCH$_2$-CHOH-CH$_2$OH) and polyglyceryl ethers [R-O(CH$_2$-CHOH-CH$_2$O)$_n$CH$_2$-CHOH-CH$_2$OH] as byproducts was discussed[20]. The fact that the αCD-Ep complexes give negative split Cotton-effects and the DiEp complexes show reversal of the signs discounts the formation of such polyglyceryl ethers.

As the **3**–γCD complex includes two **3** molecules in one or two γCDs[13a], the two molecules of **3** must lie parallel in the cavity. When the dihedral angle between the azo dyes is 180°, theoretically no interaction should be evident in the spectrum[19]. This complex shows only a single peak at 415 nm, but exciton splitting causes perturbation at this wavelength, and the second trough is presumably too weak to be observed. In the **1**– and **2**–γCD complexes, the c.d. spectra show positive chirality. The γCD complexes do not align the two azo dye molecules completely parallel to the annular axis of the γCD, and may be distorted.

Azo dye **6** does not show splitting in the $\pi \rightarrow \pi^*$ region with any of the CDs. The portion of azobenzene that is not encapsulated may be too short to interact with other molecules. Interaction between the portions of guest molecules that are not encapsulated may cause splitting of the c.d. spectra.

The molar ratio. — The **2**–TM-βCD complex shows negative exciton splitting. The λ_{max} of the visible spectrum moves 6 nm to shorter wavelength by complex formation. The absorption maximum of the visible spectrum and the cross-over point of the c.d. couplet practically coincide. Continuous-variation plots of the c.d. and visible spectra show the formation of a 1:1 complex (Fig. 3); splitting may be caused by the interaction of two molecules of **2**, each in the form of a 1:1 complex.

The $\Delta\varepsilon$ value in this system is abnormally large in the first Cotton-effect and depends on the TM-βCD:**2** ratio. At higher concentration of the gust (1.6×10^{-4} mol) compared with that of the host molecule (0.4×10^{-4} mol), the splitting pattern shows the same magnitudes in the peak and trough. Upon increasing the amount of TM-βCD, the relative magnitude of the negative peak increases and reaches twice or more its original intensity when the ratio of TM-βCD:**2** is ten. The αCD-DiEp complex series show the same tendency, and the abnormally large $\Delta\varepsilon$ value decreases on dilution. In the **3**–αCD-EP complex in which the $\Delta\varepsilon$ values of both signs are almost the same, the splitting pattern does not depend on the CD:dye ratio. Earlier it was reported that the vicinal tribenzoates of furanoid sugars do not show three Cotton effects but display instead two split Cotton-effects; if the 1,2,3-triol component is disposed such that the chiralities between the 1,2-, 2,3-, and 1,3-dihydroxygroups are all negative or positive, the amplitudes of the first Cotton effect are greatly augumented[21]. In the present case also, more than two complexes may interact.

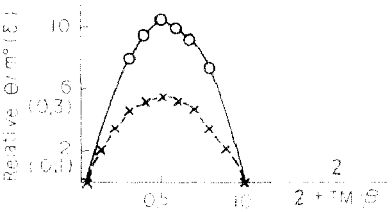


Fig. 3. Continuous variation plots of Metanil Yellow and 2,3,6-tri-*O*-methyl-*β* cyclodextrin complex. -O- ellipticity of the couplet; - × - the absorption coefficient at 429 nm.

Judged from the model of the complexed molecules calculated by the exciton splitting and molecular-orbital theories[19], the negative split Cotton-effects observed in the TM-βCD and αCD-Ep complex series suggest that the angle between the associated two complexes is < 90° and that the positive Cotton effects observed in the αCD-DiEp complex series suggest that this angle is > 90°.

CONCLUSIONS

1. Achiral azo dyes complexed with CDs cause single and split c.d. spectra.

2. All complexes show a positive single sign in the first $\pi \to \pi^*$ region; azo dyes are included into the CD from their long-axis side.

3. Substitution in the host and guest molecules causes splitting of the c.d. maximum. When the CD has long substituents, positive split Cotton-effects appear, when CD has short substituents, negative split Cotton-effects appear. This splitting might be explained by exciton interaction of two molecules of the chromophoric dye in the form of a 1:1 complex and in the γCD. Increasing of the $\Delta\varepsilon$ in the first Cotton effect suggests the association of more than two complexes.

4. In this series, the driving force for inclusion into the cavity is van der Waals (and/or hydrophobic) interactions; hydrogen bonding is not important.

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