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Some Properties and the Inclusion Behavior of Branched Cyclodextrins[1)]

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Maltosyl (G_2)- and maltotriosyl (G_3)-cyclodextrins (CDs), synthesized from maltose or maltotriose and α-, β- or γ-CD with *Pseudomonas* isoamylase or *Klebsiella aerogenes* pullulanase were purified by high-performance liquid chromatography, and their solubilities in water and in various concentrations of methanol in water, specific rotations, and hemolytic activities were studied. G_2- and G_3-CDs, as well as glucosyl (G_1)-CDs, were much more soluble than each parent CD in water and methanol aqueous solutions. The hemolytic activities of the branched CDs decreased with increasing side chain length; CD > G_1-CD > G_2-CD > G_3-CD. The inclusion behavior of the branched CDs with slightly soluble or insoluble drugs in aqueous solution and in the solid state was examined by the solubility method and the differential scanning calorimetry. The complexation abilities of G_1-, G_2-, G_3-CD and their parent CD in a series appeared to be almost the same and the stabilities of complexes in water were practically unaffected by the length of the side chain. However, the enhancement of solubility of poorly water-soluble drugs by branched CDs was much more marked than that by the parent CD.

Keywords——glucosyl-cyclodextrin; maltosyl-cyclodextrin; maltotriosyl-cyclodextrin; solubility; optical rotation; hemolytic activity; inclusion complex; solubility method; differential scanning calorimetry; phase solubility diagram

Previously, we reported[2)] on the inclusion behavior of three glucosyl-cyclodextrins (CDs), 6-*O*-α-D-glucosyl-α-CD (G_1-α-CD), 6-*O*-α-D-glucosyl-β-CD (G_1-β-CD), and 6^A, 6^D-di-*O*-α-D-glucosyl-β-CD ($2G_1$-β-CD) obtained from the mother liquor of a large-scale preparation of β-CD with *Bacillus ohbensis* cyclomaltodextrin glucanotransferase. After that, maltosyl (G_2)- and maltotriosyl (G_3)-CDs were synthesized from maltose or maltotriose and CDs through the reverse action of *Pseudomonas* isoamylase[3)] or *Klebsiella aerogenes* pullulanase.[4)]

This paper deals with the solubilities in water and in methanol aqueous solution (aq. soln.), specific rotations, and hemolytic activities of these branched CDs, 6-*O*-α-maltosyl-α-CD (G_2-α-CD), 6-*O*-α-maltosyl-β-CD (G_2-β-CD), 6-*O*-α-maltosyl-γ-CD (G_2-γ-CD), 6-*O*-α-maltotriosyl-α-CD (G_3-α-CD), 6-*O*-α-maltotriosyl-β-CD (G_3-β-CD) and 6-*O*-α-maltotriosyl-γ-CD (G_3-γ-CD), and also describes their inclusion behavior, compared with those of glucosyl (G_1)-CDs (including G_1-γ-CD prepared from G_3-γ-CD by enzymic hydrolysis) and the parent CDs.

Experimental

Materials——G_2-CDs and G_3-CDs were synthesized from maltose or maltotriose and CDs through the reverse action of *Pseudomonas* isoamylase or *Klebsiella aerogenes* pullulanase and purified by gel filtration on columns of Sephadex G-15, Toyopearl HW 40S and Bio-Gel P-2,[3)] then further purified by high-performance liquid

chromatography (HPLC) on a LiChroprep RP-18 or an Asahipak GS-302 column.[5] G_1-α-CD and G_1-β-CD were isolated and purified according to the reported method.[6] G_1-γ-CD was prepared from G_3-γ-CD by hydrolysis with *Rhizopus delemar* glucoamylase GIII.[7] α-CD and β-CD were used after recrystallization from water. γ-CD, which was supplied by Sanraku Incorporated, was purified by HPLC. Nitrazepam, mp 224—226 °C (dec.), was kindly supplied by Shionogi & Co. Ltd. Phenobarbital (JPX grade) was purified by recrystallization, mp 174—178 °C. The other drugs used were of reagent grade: betamethasone, mp 231—234 °C (dec.); dehydrocholic acid, mp 237 °C; digitoxigenin, mp 253 °C; digitoxin, mp 256—257 °C; digoxin, mp 235 °C (dec.); estriol, mp 282 °C; griseofulvin, mp 220 °C; 1-monooleoyl-*rac*-glycerol (1-monoolein), mp 35 °C; vitamin D_3, mp 84—85 °C; vitamin K_3, mp 105—107 °C; vitamins E and K_1, both oils. All other materials were of analytical-reagent grade. Deionized and double-distilled water was used throughout the study. Reagent-grade organic solvents used for HPLC were freshly distilled and filtered through a 0.45-μm membrane filter.

General Methods——Melting points were measured with a micro melting point apparatus (Yanagimoto, Kyoto, Japan) and are uncorrected. Optical rotations were determined with a DIP-360 digital polarimeter (JASCO, Tokyo, Japan). The pH measurements were carried out on an M-8 pH meter (Horiba, Kyoto, Japan). Lyophilization was carried out with an FD-1 freeze-dryer (Tokyo Rika, Tokyo, Japan). A UVIDEC-610C double-beam spectrophotometer (JASCO) was used for the determination of absorbances. HPLC analyses were performed using a Familic-300S HPLC pump, a model VL-614 injector, and a UVIDEC-100V variable-wavelength ultraviolet (UV) detector (all from JASCO). The columns used were a Finepak SIL-C_{18} (250 × 4.6 mm i.d.) (JASCO), a YMC-Pack A-802 C_4 (150 × 4.6 mm i.d.), a YMC-Pack A-212 C_8 (150 × 6 mm i.d.) and a YMC-Pack Al-312 ODS (150 × 6 mm i.d.) (all from Yamamura Chemical, Kyoto, Japan). Preparative HPLC was carried out using a Twincle pump and a VL-611 injector (both from JASCO) with an R-401 refractive index (RI) monitor (Waters Assoc., Milford, MA, U.S.A.). For purification of branched CDs, a column packed with LiChroprep RP-18 (5—20 μm, 300 × 20 mm i.d.) (Yamazen, Osaka, Japan) and an Asahipak GS-320 (500 × 7.6 mm i.d.) (Asahi Kasei, Tokyo, Japan) were used. The purity of each preparation was checked by HPLC which was performed on a Hibar LiChrosorb NH_2 (250 × 4 mm i.d.) (Merck, Darmstadt, F.R.G.) with acetonitrile–water (60 : 40) as an eluent by the use of an 880-PU Intelligent HPLC pump (JASCO), a model 7125 injector (Rheodyne, Cotati, CA, U.S.A.) and an SE-31 RI monitor (Showa Denko, Tokyo, Japan).

Solubility Studies——Solubilities of Branched CDs: The solvent (water or various concentrations of methanol aq. soln.) was carefully added in portions of 0.01—0.1 ml to a glass vessel containing 500 mg of lyophilized branched CDs, and the volume of the solvent required for complete dissolution of the CDs within 30 min at 25 ± 1 °C by vigorous shaking for 30 s periods at 5-min intervals was measured. For reference, the solubilities of lyophilized α-, β- and γ-CD were determined in the same way.

Estimation of Complex-Forming Ability of Branched CDs by the Solubility Method[8]: This was done according to the previously described procedure.[2] HPLC conditions for determination of the drugs newly used in this work are shown in Table I.

Preparation of Solid Complexes and Thermal Analysis——The solid complexes were prepared by mixing equimolecular amounts of a CD and a drug in water. The mixture was shaken at 30 °C for 24 h, and filtered through a 0.2-μm membrane filter to remove excess drug, then the filtrate was lyophilized. The drug content in the freeze-dried sample was determined by HPLC. For example, when 135 mg (0.38 mmol) of 1-monoolein and 425 mg (0.38 mmol) of G_1-α-CD were mixed in 5 ml of water, the 1-monoolein/G_1-α-CD molar ratio in the solid complex obtained was 1/4. The solid complexes were used for thermal analysis, which was done using a Thermo Flex DSC-8230B (Rigaku, Tokyo, Japan). The scanning temperature range was −40—300 °C and the scanning speed was 5 °C/min.

Determination of Hemolytic Activity——This was carried out in the same manner as described before.[2]

TABLE I. Conditions of Drug Determination by HPLC

Drug	Column	Eluent $CH_3OH : H_2O$	Flow rate (ml/min)	Detection at (nm)	Retention time (min)
Betamethasone	YMC-Pack A-212 C_8	63 : 37	1.0	240	9.0
Dehydrocholic acid	YMC-Pack A-212 C_8	65 : 35[a]	1.0	285	8.0
Digoxin	YMC-Pack AL-312 ODS	62 : 38	1.0	220	9.5
1-Monooleoyl-*rac*-glycerol (1-Monoolein)	YMC-Pack A-802 C_4	80 : 20	1.0	210	11.5

a) 0.05% CH_3COOH was used instead of H_2O. See the previous paper[2] for determination of the other drugs.

Results and Discussion

Solubility

Table II shows the solubilities of branched CDs in water at 25, 40 and 55 °C together with data on α-, β- and γ-CDs. All branched CDs are much more soluble than the respective parent CDs. In particular, the solubilities of G_1-, G_2- and G_3-β-CD are very high compared to the solubility of β-CD: at 25 °C about 50, 65 and 60 times greater, respectively. In the case of branched α-CDs, the solubilities of G_1-, G_2- and G_3-α-CD are higher than that of α-CD by factors of about 4.5, 1.8 and 6.0, respectively, at 25 °C. Although the reason for the low solubility of G_2-α-CD compared with the other branched CDs has not been clarified yet, G_2-α-CD is particularly apt to crystallize, and moreover, the concentrated solution tends to gel. γ-CD has been the most soluble CD known until now, but branched γ-CDs are even more soluble than γ-CD.

As a general rule, the solubility of a solid increases with increasing temperature.

Table II. Solubilities of CDs in Water

CD	Glucose unit	Solubility (mmol/ml × 10^2)		
		25 °C	40 °C	55 °C
α-CD	6	18[a]	21	47
G_1-α-CD	7	80[a]	103	119
G_2-α-CD	8	24	35	54
G_3-α-CD	9	107	122	137
β-CD	7	1.6[a]	3.1	4.4
G_1-β-CD	8	77[a]	77	133
G_2-β-CD	9	104	104	122
G_3-β-CD	10	94	94	110
γ-CD	8	20	43	64
G_1-γ-CD	9	98	101	118
G_2-γ-CD	10	94	94	114
G_3-γ-CD	11	85	85	104

a) These data were obtained in experiments using larger amounts of sample (500 mg) than those (300 mg) used in the previous work and are slightly different from the data previously reported.[2]

Table III. Solubilities of CDs in Methanol Aqueous Solutions at 25 °C

CD	Solubilities (mmol/ml × 10^2)					
	5%	10%	20%	30%	40%	50%
α-CD	7.3	4.1	2.3	1.3	1.0	0.7
G_1-α-CD	90	108	108	108	108	108
G_2-α-CD	77	77	64	64	64	55
G_3-α-CD	111	84	84	84	84	84
β-CD	1.3	1.0	0.6	0.5	0.4	0.3
G_1-β-CD	110	92	86	86	82	77
G_2-β-CD	98	82	78	76	73	69
G_3-β-CD	88	73	70	69	66	62
γ-CD	17	15	13	4.8	3.1	1.7
G_1-γ-CD	98	80	76	76	73	69
G_2-γ-CD	88	73	69	69	66	62
G_3-γ-CD	80	67	64	62	60	56

However, increases in the solubilities of branched β-CDs and γ-CDs with the rise of temperature from 25 to 40 °C were negligible, and commonly an increase of temperature had less effect on the solubilities of branched CDs than on those of non-branched CDs.

The solubilities of CDs in various concentrations of methanol aq. soln. at 25 °C are listed in Table III. It was of great interest that the solubilities of the parent CDs in methanol aq. soln. markedly decreased with increasing methanol content in the solvents, while there were no marked differences between the solubilities of branched CDs in water and those in methanol aq. soln., though the latter was rather higher than the former in the cases of G_1-α-, G_2-α- and G_1-β-CD.

Optical Rotation

Specific rotations of branched CDs and the parent CDs are summarized in Table IV. Those of the first three members in the α-CD series appear to be additive. On the other hand, those of the three branched β-CDs are almost the same and are 10° higher than that of β-CD, while the values of all members in the γ-CD series are similar to each other. The partial regularity in the relationship between the specific rotation and the molecular weight of each member of the α-CD series may reflect the relatively rigid ring structure, while the increase of ring size brings about an increase of flexibility in the ring structure and the bond angles of β- and γ-CD rings change irregularly with lengthening of the side chain, so that the linkage rotation varies, resulting in loss of additivity in specific rotations.

Hemolytic Activity

Figure 1 shows the hemolytic effect of branched CDs on human erythrocytes in isotonic solution, compared with that of each parent CD. It is known that the hemolytic activity of CDs is in the order of β- > α- > γ-CD.[9] The hemolytic activity of G_1-β-CD was about the same as that of β-CD, but those of the other branched CDs were lower than that of each parent CD, and became weaker with lengthening of the side chain.

TABLE IV. Specific Rotations of CDs

CD	$[\alpha]_D^{25}$ (°)	CD	$[\alpha]_D^{25}$ (°)	CD	$[\alpha]_D^{25}$ (°)
α-CD	+148.8	β-CD	+158.7	γ-CD	+174.8
G_1-α-CD	+154.1	G_1-β-CD	+168.2	G_1-γ-CD	+178.5
G_2-α-CD	+161.0	G_2-β-CD	+168.7	G_2-γ-CD	+175.4
G_3-α-CD	+159.1	G_3-β-CD	+168.5	G_3-γ-CD	+177.5

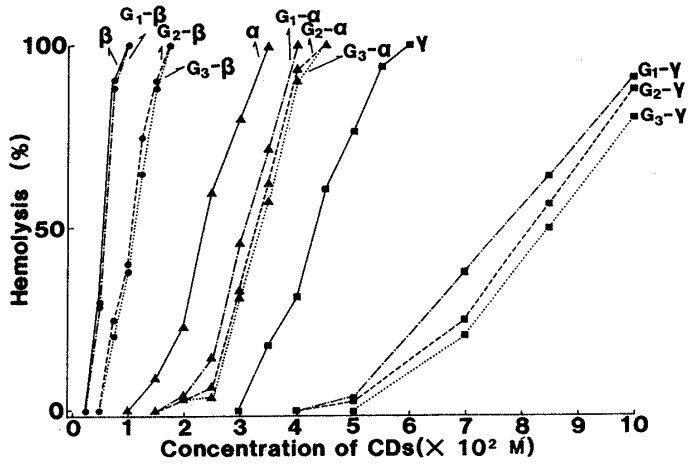

Fig. 1. Hemolytic Effects of CDs on Human Erythrocytes in 0.1 M Isotonic Phosphate Buffer (pH 7.4) at 37 °C

Inclusion Behavior

The complex-forming ability of branched CDs with fourteen poorly water-soluble (slightly soluble and insoluble) drugs in water was studied mainly by the solubility method and was compared with that of each parent CD. CDs are known to form inclusion complexes with a variety of guest molecules in solution and in the solid state. The minimum requirement for inclusion complex formation is a size compatibility between host and guest molecules.[10] Usually, the CD complexes thus formed are stabilized by various intermolecular forces such as hydrophobic interaction, van der Waals forces, hydrogen bonding, and others.[11]

Tables V—VII show the values of apparent stability constant (K), estimated from Eq. 1 based on the assumption that a 1 : 1 complex was initially formed and calculated from the initial rising portion of the solubility diagrams.[8]

$$K = \frac{\text{slope}}{\text{intercept} \times (1 - \text{slope})} \quad (1)$$

The complexation abilities of G_1-, G_2-, G_3-CD and their parent CD in a series appear to be almost the same, since they have the same cavity dimensions, and the stabilities of

TABLE V. Apparent Stability Constants (M^{-1}) of Slightly Soluble Drug–α-CD Complexes Determined by the Solubility Method in Water at 30 °C

Guest molecule	Host molecule			
	α-CD	G_1-α-CD	G_2-α-CD	G_3-α-CD
Betamethasone	260	260	260	260
Dehydrocholic acid	170	180	190	200
Digitoxigenin	1700	2200	2000	1800
Digitoxin	350	340	330	330
Digoxin	250	250	250	250
Estriol	—	—	—	—
Griseofulvin	—	—	—	—
Nitrazepam	30	30	30	30
Phenobarbital	30	30	30	30
Vitamin K_3	40	40	40	40

TABLE VI. Apparent Stability Constants (M^{-1}) of Slightly Soluble Drug–β-CD Complexes Determined by the Solubility Method in Water at 30 °C

Guest molecule	Host molecule			
	β-CD	G_1-β-CD	G_2-β-CD	G_3-β-CD
Betamethasone	5400	5300	4900	4600
Dehydrocholic acid	6500	6500	6500	6500
Digitoxigenin	130000	130000	130000	130000
Digitoxin	37000	38000	38000	38000
Digoxin	27000	27000	26000	26000
Estriol	37000	38000	36000	35000
Griseofulvin	30	20	20	20
Nitrazepam	130	140	130	130
Phenobarbital	1400	1400	1400	1400
Vitamin K_3	190	190	190	190

TABLE VII. Apparent Stability Constants (M^{-1}) of Slightly Soluble Drug–γ-CD Complexes Determined by the Solubility Method in Water at 30 °C

Guest molecule	Host molecule			
	γ-CD	G_1-γ-CD	G_2-γ-CD	G_3-γ-CD
Betamethasone	11000	11000	10000	9000
Dehydrocholic acid	800	800	800	800
Digitoxigenin	640000	640000	640000	640000
Digitoxin	78000	78000	76000	75000
Digoxin	29000	26000	24000	22000
Estriol	17000	17000	17000	16000
Griseofulvin	150	150	150	150
Nitrazepam	30	30	30	30
Phenobarbital	110	110	110	110
Vitamin K_3	110	110	110	110

TABLE VIII. Enhancement of the Solubilities of Slightly Soluble or Insoluble Drugs in Water by Complexation with α-CDs at 30 °C

Drug	Solubility in H_2O (mM (μg/ml))	Solubility in 30 mM CD soln. (mM (μg/ml))			
		α-CD	G_1-α-CD	G_2-α-CD	G_3-α-CD
Betamethasone	0.15 (60)	1.32 (520)	1.32 (520)	1.32 (520)	1.32 (520)
Dehydrocholic acid	0.16 (65)	0.87 (350)	0.94 (380)	0.99 (400)	1.04 (420)
Digitoxigenin	0.03 (10)	2.1 (780)	2.5 (920)	2.2 (810)	2.1 (760)
Digitoxin	0.02 (17)	0.24 (180)	0.20 (150)	0.17 (130)	0.17 (130)
Digoxin	0.04 (28)	0.26 (200)	0.26 (200)	0.26 (200)	0.26 (200)
Estriol	0.10 (29)	0.13 (37)	0.13 (37)	0.13 (37)	0.13 (37)
Griseofulvin	0.04 (15)	0.04 (15)	0.04 (15)	0.04 (15)	0.04 (15)
1-Monoolein[a]	—[d]	—[c,d]	6.2 (2200)	5.6 (2000)	5.6 (2000)
Nitrazepam	0.15 (43)	0.46 (130)	0.46 (130)	0.43 (120)	0.43 (120)
Phenobarbital[b]	6.0 (1400)	9.0 (2100)	9.0 (2100)	9.0 (2100)	9.0 (2100)
Vitamin D_3	—[d]	—[d]	—[d]	—[d]	—[d]
Vitamin E	—[d]	—[d]	—[d]	—[d]	—[d]
Vitamin K_1	—[d]	—[d]	—[d]	—[d]	—[d]
Vitamin K_3	0.87 (150)	2.2 (380)	2.2 (380)	2.2 (380)	2.2 (380)

a) The type of solubility curve was Bs in α-CD solution and A in branched α-CD solutions. *b*) The type of solubility curve was A in α-CD and branched α-CD solutions. *c*) Precipitates appeared. *d*) Below the detection limit.

complexes are practically unaffected by the length of the side chain.

However, the enhancement of solubility of poorly water-soluble drugs by G_2- and G_3-β-CD, as well as G_1-β-CD, was much more marked than that by β-CD, as shown in Table IX,

TABLE IX. Enhancement of the Solubilities of Slightly Soluble or Insoluble Drugs in Water by Complexation with β-CDs at 30 °C

Drug	Solubility in H_2O (mM (μg/ml))	Solubility in 30 mM CD soln. (mM (μg/ml))			
		β-CD	G_1-β-CD	G_2-β-CD	G_3-β-CD
Betamethasone[a]	0.15 (60)	3.3[c] (1300)	13.3 (5200)	13.0 (5100)	12.7 (5000)
Dehydrocholic acid[b]	0.16 (65)	14.9 (6000)	15.4 (6200)	15.4 (6200)	15.4 (6200)
Digitoxigenin[a]	0.03 (10)	0.06[c] (22)	23.0 (8600)	23.0 (8600)	22.7 (8500)
Digitoxin[a]	0.02 (17)	0.92[c] (700)	15.7 (12000)	15.6 (11900)	15.4 (11800)
Digoxin[b]	0.04 (28)	14.1 (11000)	14.1 (11000)	14.1 (11000)	14.1 (11000)
Estriol[a]	0.10 (29)	0.73[c] (210)	16.7 (4800)	16.3 (4700)	16.3 (4700)
Griseofulvin	0.04 (15)	0.05 (17)	0.06 (22)	0.06 (22)	0.06 (22)
1-Monoolein[a]	—[d]	0.02[c] (6)	3.9 (1400)	3.4 (1200)	3.4 (1200)
Nitrazepam	0.15 (43)	0.57 (160)	0.82 (230)	0.78 (220)	0.78 (220)
Phenobarbital[b]	6.0 (1400)	37.9 (8800)	42.2 (9800)	42.2 (9800)	42.2 (9800)
Vitamin D_3[a]	—[d]	0.003[c] (1)	3.6 (1400)	3.4 (1300)	3.4 (1300)
Vitamin E	—[d]	—[c,d]	0.009 (4)	0.009 (4)	0.009 (4)
Vitamin K_1	—[d]	—[c,d]	0.0004 (0.2)	0.0004 (0.2)	0.0004 (0.2)
Vitamin K_3	0.87 (150)	4.2 (730)	5.5 (950)	5.5 (950)	5.5 (950)

a) The type of solubility curve was Bs in β-CD solution and A in branched β-CD solutions. *b*) The type of solubility curve was A in β-CD and branched β-CD solutions. *c*) Precipitates appeared. *d*) Below the detection limit.

especially in the cases of drugs which showed typical BS type solubility curves[8] in β-CD solution. The solubility curves of those drugs in branched β-CDs solutions could all be classified as being of type A,[8] and the solubilities of drugs increased linearly as a function of CD concentration. Similar phenomena were also observed in the systems with the α-CD series (Table VIII) and in the systems with the γ-CD series (Table X). The concentration of CD solution used to obtain the data in Tables VIII—X was 3.0×10^{-2} M, so that β-CD was not completely dissolved, though the formation of soluble complex increases the solubility of β-CD (see Fig. 3(b)). In the case of 1-monoolein, branched-α-CDs showed the largest enhancing effect on the solubility. Branched γ-CDs were the best host compounds for betamethasone, digitoxigenin, digitoxin, digoxin, griseofulvin and vitamin K_1. For all the other compounds tested branched β-CDs were most effective.

Figure 2 shows the phase solubility diagrams obtained for 1-monoolein with the four α-CDs in water at 30 °C. The 1-monoolein-α-CD complex began to precipitate at an α-CD concentration of 2.5×10^{-3} M and therefore the maximum concentration of 1-monoolein in α-CD solution was only 4.0×10^{-6} M. On the other hand, G_1, G_2-, and G_3-α-CD systems show AP type solubility curves and 1-monoolein concentrations in 0.1 M branched α-CDs solutions

TABLE X. Enhancement of the Solubilities of Slightly Soluble or Insoluble Drugs in Water by Complexation with γ-CDs at 30 °C

Drug	Solubility in H_2O (mM (μg/ml))	Solubility in 30 mM CD soln. (mM (μg/ml))			
		γ-CD	G_1-γ-CD	G_2-γ-CD	G_3-γ-CD
Betamethasone[a]	0.15 (60)	5.6[c] (2200)	19.1 (7500)	18.6 (7300)	18.4 (7200)
Dehydrocholic acid[a]	0.16 (65)	2.4[c] (950)	3.7 (1500)	3.7 (1500)	3.7 (1500)
Digitoxigenin[a]	0.03 (10)	17.1[c] (6400)	29.4 (11000)	29.4 (11000)	29.4 (11000)
Digitoxin[a]	0.02 (17)	15.7[c] (12000)	19.6 (15000)	19.6 (15000)	19.6 (15000)
Digoxin[b]	0.04 (28)	15.4 (12000)	15.4 (12000)	15.4 (12000)	15.4 (12000)
Estriol	0.10 (29)	10.8 (3100)	11.4 (3300)	11.1 (3200)	10.8 (3100)
Griseofulvin	0.04 (15)	0.14 (50)	0.16 (55)	0.16 (55)	0.16 (55)
1-Monoolein[a]	—[d]	—[c,d]	0.14 (50)	0.14 (50)	0.14 (50)
Nitrazepam	0.15 (43)	0.43 (120)	0.43 (120)	0.43 (120)	0.43 (120)
Phenobarbital[a]	6.0 (1400)	7.3[c] (1700)	14.6 (3400)	14.6 (3400)	14.6 (3400)
Vitamin D_3[a]	—[d]	0.008[c] (3)	0.04 (15)	0.04 (15)	0.04 (15)
Vitamin E	—[d]	—[c,d]	0.0008 (0.3)	0.0008 (0.3)	0.0008 (0.3)
Vitamin K_1	—[d]	—[c,d]	0.004 (2)	0.004 (2)	0.004 (2)
Vitamin K_3[a]	0.87 (150)	1.0[c] (170)	3.6 (620)	3.6 (620)	3.6 (620)

a) The type of solubility curve was Bs in γ-CD solution and A in branched γ-CD solutions. *b*) The type of solubility curve was A in γ-CD and branched γ-CD solutions. *c*) Precipitates appeared. *d*) Below the detection limit.

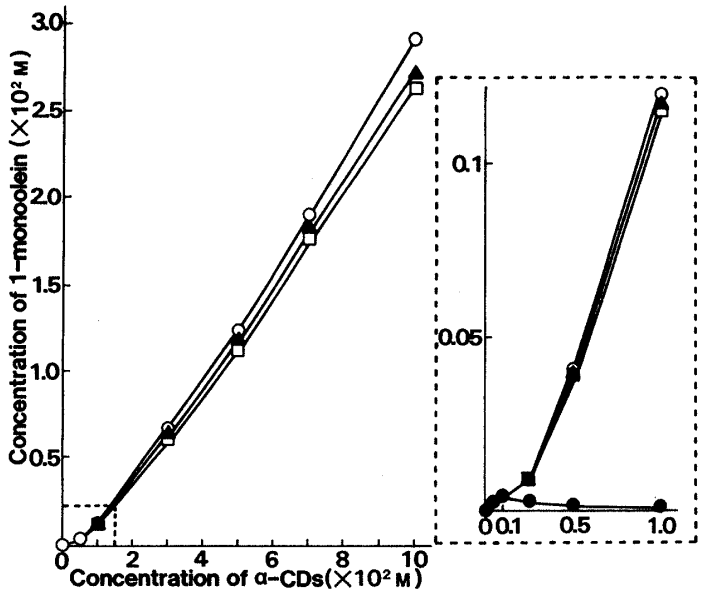

Fig. 2. Phase Solubility Diagrams of 1-Monoolein–α-CD Systems in Water at 30 °C and an Expansion of the Low Concentration Region

●, α-CD; ○, G_1-α-CD; ▲, G_2-α-CD; □, G_3-α-CD.

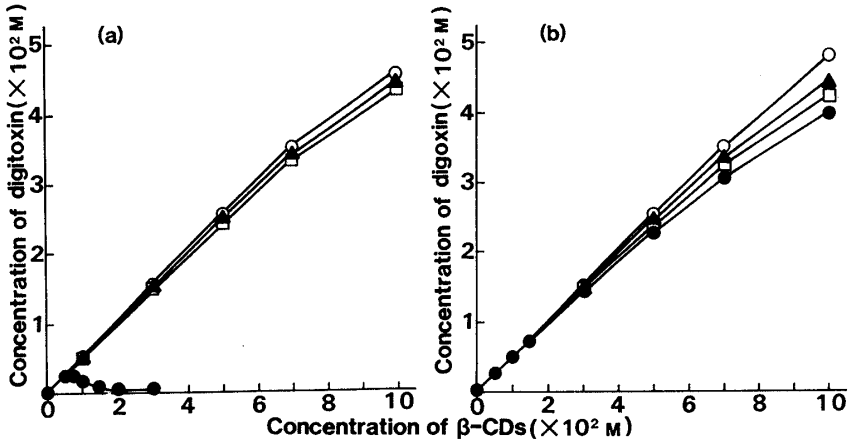

Fig. 3. Phase Solubility Diagrams of Digitoxin–β-CD Systems (a) and Digoxin–β-CD Systems (b) in Water at 30 °C

●, β-CD; ○, G_1-β-CD; ▲, G_2-β-CD; □, G_3-β-CD.

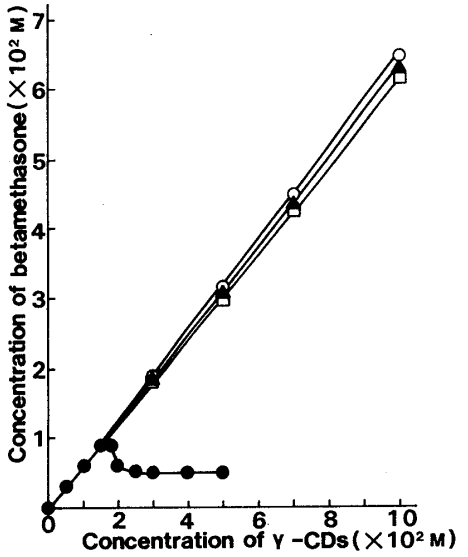

Fig. 4. Phase Solubility Diagrams of Betamethasone–γ-CD Systems in Water at 30 °C

●, γ-CD; ○, G_1-γ-CD; ▲, G_2-γ-CD; □, G_3-γ-CD.

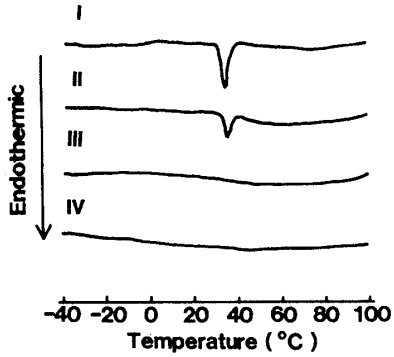

Fig. 5. DSC Thermograms of 1-Monoolein–G_1-α-CD Systems

(I), 1-monoolein alone; (II), physical mixture of 1-monoolein and G_1-α-CD in 1 : 4 molar ratio; (III), 1 : 4 complex of 1-monoolein with G_1-α-CD; (IV), G_1-α-CD alone.

approached 3×10^{-2} M. 1-Monoolein is one of the monoglycerides which are useful as surface-active agents in the pharmaceutical field, the food and cosmetics industries, and others.

Figure 3 shows the phase solubility diagrams obtained for digitoxin (a) and digoxin (b) with the four β-CDs in water at 30 °C. Digitoxin–β-CD and digoxin–β-CD complexes show BS and AN type solubility curves, respectively, whereas G_1-, G_2- and G_3-β-CD systems show AN type solubility curves for digitoxin, as well as for digoxin. The bioavailability of the digitalis glycosides from commercial tablets varies significantly[12] because of the low water solubility[13] and the chemical instability in acidic media.[14] Uekama *et al.*[15] reported an improvement of the oral bioavailability of digoxin by complexation with γ-CD. We expect that branched β- and γ-CDs would show a similar effect on the bioavailability of digoxin, and of digitoxin.

Betamethasone is an adrenocortical hormone that has been widely used in various dosage

forms (tablet, powder, injection, suppository, ointment, eye drops, and others). Otagiri *et al.*[16] reported the effects of β- and γ-CD on release of betamethasone from ointment bases, and suggested that an improvement of topical bioavailability of betamethasone can be obtained by means of inclusion complexation. The phase solubility diagrams of betamethasone with the four γ-CDs in water are shown in Fig. 4.

In Figs. 2—4 slight differences between the solubilities of the guest compound in G_1-, G_2- and G_3-CD solutions of higher concentrations are observed. This phenomenon suggests that steric hindrance between the longer branch and the guest molecule or distortion of the CD ring with the larger branch may tend to decrease the stability of the complex.

Figure 5 shows the differential scanning calorimeter (DSC) curves of 1-monoolein–G_1-α-CD complex, the physical mixture, 1-monoolein alone and G_1-α-CD alone. 1-Monoolein alone and the physical mixture showed an endothermic peak at around 35 °C. However, the endothermic peak disappeared with formation of the complex. This disappearance of the endothermic peak was also observed in the cases of 1-monoolein–G_2-α-CD complex and 1-monoolein–G_3-α-CD complex. The melting point of 1-monoolein is 35 °C, and therefore, handling of this compound at room temperature is not easy. However, 1-monoolein–branched α-CD solid complexes were stable until at least 100 °C. In the cases of dehydrocholic acid–branched β-CD complexes and complexes of betamethasone, digitoxin, digoxin and estriol with branched β- and γ-CDs, similar disappearance of the endothermic peak was observed. These results suggest that these drugs interact with branched β- and γ-CDs in the solid state to form inclusion complexes.

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