dioxane: $\epsilon^{\text{shoulder}}_{382\ m\mu}$ 94, $\epsilon^{\text{min}}_{395\ m\mu}$ 84, $\epsilon^{\text{max}}_{405\ m\mu}$ 95, $\epsilon^{\text{min}}_{415\ m\mu}$ 63, $\epsilon^{\text{max}}_{425\ m\mu}$ 80; R.D. (Fig. 4) in dioxane (c 0.078): $[\alpha]_{700}$ +15°, $[\alpha]_{589}$ +70°, $[\alpha]_{423}$ −900°, $[\alpha]_{413-407}$ +250° (plateau), $[\alpha]_{393-380}$ +530° (plateau), $[\alpha]_{358}$ +370°, $[\alpha]_{350}$ +400°, $[\alpha]_{335}$ +350°, $[\alpha]_{315}$ +360°.

Anal. Calcd. for $C_{11}H_{14}N_2O_2$: C, 64.06; H, 6.84; N, 13.58. Found: C, 63.82; H, 6.31; N, 13.49.

N-Nitroso-N-methyl-O-acetyl-D-lactamide (VIII).—Commercially available (California Corporation for Biochemical Research, Los Angeles) calcium D-lactate tetrahydrate was transformed into the anhydrous acid, thence the acid chloride (with phosphorus trichloride) and then the N-methylamide, which exhibited m.p. 68–69° after recrystallization from ether–petroleum ether; $[\alpha]_D$ +6.9° (c 5.5 in chloroform), $\lambda^{\text{Nujol}}_{\text{max}}$ 3.02, 5.78 and 6.06 μ.

Anal. Calcd. for $C_6H_{11}NO_3$: C, 49.64; H, 7.64; N, 9.65. Found: C, 49.86; H, 7.64; N, 9.69.

The nitroso derivative VIII was obtained in nearly quantitative yield as a yellow oil, λ_{max} 5.7–5.8 μ; ultraviolet in dioxane: $\epsilon^{\text{shoulder}}_{390\ m\mu}$ 67, $\epsilon^{\text{max}}_{404\ m\mu}$ 111, $\epsilon^{\text{min}}_{413\ m\mu}$ 69, $\epsilon^{\text{max}}_{420\ m\mu}$ 97; R.D. (Fig. 4) in dioxane (c 0.615): $[\alpha]_{700}$ +14°, $[\alpha]_{589}$ +13°, $[\alpha]_{440}$ −120°, $[\alpha]_{435}$ −149°, $[\alpha]_{432.5}$ −170°, $[\alpha]_{422.5}$ −150°, $[\alpha]_{417.5}$ −276°, $[\alpha]_{405}$ −101°, $[\alpha]_{375}$ +263°, $[\alpha]_{310}$ +42°.

Anal. Calcd. for $C_6H_{10}N_2O_4$: C, 41.38; H, 5.79; N, 16.09. Found: C, 41.91; H, 5.99; N, 15.80.

[CONTRIBUTION FROM THE HORMEL INSTITUTE, UNIVERSITY OF MINNESOTA, AUSTIN, MINN.]

The Association of α- and β-Cyclodextrins with Organic Acids[1]

BY HERMANN SCHLENK AND DONALD M. SAND

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Solubilities of organic acids have been determined in order to study their association with α- and β-cyclodextrins in water. The cyclodextrins increase the solubilities of acids which easily form crystalline inclusion complexes with them (caproic to lauric acid). Acids from which such complexes have not been obtained, or have been obtained only under extreme conditions, still may exhibit increased solubility (benzoic and iodobenzoic acids). Acids having still larger diameter are much less, if at all, affected (durylcarboxylic to 4-durylbutyric acids), and show extremely high component ratios cyclodextrin/acid in solution. The association of the acids which brings about the higher solubility obviously follows an inclusion mechanism. The compatibility of the whole acid molecule with the void inside the cyclodextrins is a deciding factor. Fatty acids follow the principle of preferential placing in crystalline dehydrated complexes of cyclodextrins. They are mostly "crowded," while "loose" packing is encountered with benzoic and p-iodobenzoic acids. I^- outranks other ions in preventing the association of organic acids with α-cyclodextrin. This is in accord with the great affinity of I^- to amylose-type carbohydrates, which has been demonstrated by other investigators. The optical rotations of cyclodextrins are changed when they are associated with other molecules. The changes appear to be related to the structure of the latter.

Introduction

The terms "inclusion compound" or "clathrate" have been coined to describe the positional relationship of two components which form certain types of crystals. Often the components are designated as host and guest, again in reference to the solid state. Some evidence for association of host with guest molecules in the liquid phase has been advanced for urea and straight-chain aliphatic compounds[2,3] which are potential guest molecules in the crystalline phase. Complete dissociation in solution, however, has been claimed for the complexes of deoxycholic acid.[4] More recently, association phenomena in aqueous solutions have been studied utilizing α-, β- and γ-cyclodextrins (α-, β-, γ-CDX), or in more significant nomenclature, cyclohexa-, -hepta- and -octaamylose.[5] While usually several host molecules are needed to provide the encasing structure, one molecule of cyclodextrin offers the geometry necessary for inclusion. With it, inclusion is not necessarily restricted to the crystalline phase, but may also take place in solution. Under this assumption, Cramer, Lautsch and co-workers investigated reactions inducing optical asymmetry, rates of dehydrogenation, enzymatic processes, spectra of dyestuffs and oxidation–reduction potentials in solutions of cyclodextrins.[6] The greater part of these and similar investigations[7–9] was carried out with reference to the mode of enzyme action. Much less effort was made to detail the steric conditions which might influence the association of cyclodextrins with other molecules in solution. Steric considerations are a major objective of this report which is restricted to the association of cyclodextrins with molecules of rather simple structure.

It has been found that the solubility of benzoic acid is increased by the presence of α- and β-cyclodextrins. The presence of ions greatly influences the solubilities and, implicitly, the association.[10] Therefore, it was desirable to avoid methods that involve buffer or other extraneous components in the associating systems. Experiments with aminobenzoic acids and some other acids having amino groups showed it advisable to correlate only results obtained from one type of compound. Organic acids without additional functional groups were chosen here and their solubilities were taken as a criterion for association with α- and β-cyclodextrins in water.

(1) This investigation was supported by grants of the U. S. Atomic Energy Commission, of the Air Force Office of Scientific Research, Air Research and Development Command, and by the Hormel Foundation. Presented, in part, at the 128th Meeting of the Am. Chem. Soc., Minneapolis, Minn., Sept., 1955.

(2) W. Schlenk, *Ann.*, **565**, 204 (1949).

(3) L. C. Fetterly, Thesis, 1950, Univ. of Washington.

(4) H. Sobotka and S. Kahn, *Biochem. J.*, **26**, 898 (1932); H. Sobotka, *Chem. Revs.*, **15**, 358 (1934).

(5) D. French, "Advances in Carbohydrate Chemistry," Vol. 12, Academic Press, Inc., New York, N. Y., 1957, p. 189; D. French and R. E. Rundle, *J. Am. Chem. Soc.*, **64**, 1651 (1942)

(6) *E.g.*, F. Cramer and coworkers, *Ber.*, **92**, 378 (1959); **86**, 1576, 1582 (1953); *Ann.*, **579**, 17 (1953); W. Lautsch and coworkers, *Kolloid-Z.*, **144**, 82 (1955); **153**, 103 (1957); *Z. Naturforsch.*, **11b**, 282 (1956); W. Broser and coworkers, *Z. Naturforsch.*, **8b**, 711, 722 (1953); **10b**, 121 (1955).

(7) A. R. Todd, *Chemistry & Industry (London)*, 802 (1956).

(8) W. Lautsch and coworkers, *Kolloid-Z.*, **161**, 2, 10, 28 and 36 (1958).

(9) F. Cramer and W. Dietsche, *Ber.*, **92**, 1739 (1959).

(10) H. Schlenk and D. M. Sand, Abstracts of Papers, 128th Meeting, Am. Chem. Soc., Minneapolis, Minn., Sept. 1955, p. 50-O.

In connection with other work we were interested in increasing the solubility of straight-chain fatty acids in water. Their solubilities and their complexes with cyclodextrins are dealt with in section I. Other sections concern cyclodextrin and benzoic acid (II), substituted benzoic acids (III), the influence of ions on solubility (IV) and observations on the optical rotations of cyclodextrins when in the state of association (V).

Experimental and Results

I. Cyclodextrins and Normal Aliphatic Acids. Materials.—The procedures of Freudenberg[11] and French[12] were essentially followed for preparing α- and β-cyclodextrin. The materials had $[\alpha]^{35}D$ +149.5° to 150° and +162°, respectively, and yielded clear solutions with water.

Ethyl esters of commercial hexanoic to dodecanoic acids were prepared by azeotropic distillation with benzene, using toluene- or naphthalenesulfonic acids as catalysts. The crude esters, after removal of catalyst and solvent, were distilled under reduced pressure in an inert atmosphere, discarding first and last fractions. Batches of 1–1.2 kg. of such esters then were fractionated in a 25 mm. × 1.2 m. heligrid column (Podbielniak, Inc.). Purified N_2 was bubbled through the liquid to assure smooth boiling while a Todd vacuum pressure regulator provided constant pressure. Distilling periods were from 35 to 55 hr. and 100-ml. fractions were taken, except when the column head temperature warranted smaller cuts. Between 70 and 90% of the distilled materials had constant boiling points and refractive indices. Fractions were combined to obtain 100- to 250-g. samples which then were saponified cold with KOH in ethanol under N_2. The free acids were recovered in low boiling petroleum ether (Skellysolve F) and recrystallized twice at temperature between −30 and −50°. The acids were then distilled under N_2 at reduced pressure in 65–75-g. portions through a 12-cm. Vigreux column and 4 fractions were taken. The temperatures did not significantly change during the distillation which lasted 8 to 10 hr. and the center portion of distillate, amounting to approximately 70% of total, was collected in two fractions which were used for these studies.

Ethyl undecenoate was the source for undecanoic acid. After fractionation in the heligrid column, it was hydrogenated and the above procedure followed.

Gas–liquid chromatography of the methyl esters did not reveal contaminants. Freezing points and solubilities in water confirmed the values of the literature[13,14] which now are generally accepted. The suitability of these acids for our purposes was further indicated when amounts in excess of the solubility by 20% and 500% were equilibrated with water. The titration values of the aqueous phases agreed within less than 1% for the shorter chain acids.

Acids higher than lauric were obtained from the Hormel Foundation, or from commercial supplies, or were synthesized. In the latter two cases, they underwent rigorous purification, controlled by m.p. and gas–liquid chromatography.

Equilibration.—Solubility experiments were made with hexanoic to dodecanoic acids at 31.3 ± 0.05°. The acids were added to the aqueous solutions of cyclodextrins in Pyrex reagent bottles, mostly of 500-ml. size. It was necessary to determine the appropriate amounts of reagents in preliminary experiments since the presence of a large excess of solid or of lipid phase makes difficult the withdrawal of pure aqueous samples. Freshly boiled water was used and all procedures were carried out under N_2 as far as practical. The glass stoppered bottles were suspended into a bath from a vibrating rod. Equilibria were approached from higher as well as lower temperatures and were reached, with fatty acids only, within 24 hr. In presence of cyclodextrins up to three days may be required for equilibrating the three phases, lipid, solid complex and aqueous solution. All samples were run in triplicate and equilibrium was considered as established only when results did not change significantly over a period of 48 hr. Sedimentation periods as long as 10 hr. were necessary with C_{11}- and C_{12}-acids to obtain clear aqueous phases before sampling. Often it was of advantage to mount filter cloth over the tip of the sampling pipet.

Analysis of Solutes.—Transfer pipets calibrated for total volume were used for sampling. They were rinsed with ethanol to avoid loss of sample by precipitation on the wall of the pipet at room temperature. Titrations were carried out under N_2 with KOH of concentrations between 0.1 and 0.02 *N*. Tests showed that in hot solutions containing 25 to 50% ethanol, the cyclodextrins do not interfere with the use of phenolphthalein. For the solubilities of C_{10}- to C_{12}-acids in water, the average deviation from the mean was 3, 11 and 18%, respectively, as determined by 8 or more titrations. The reproducibility is better in the presence of cyclodextrins, because of the higher concentration of the acids.

Two methods were applied to determine the concentrations of cyclodextrins. The solubilities of β-cyclodextrin in mixtures of water and ethanol were determined gravimetrically and the results are given in Fig. 1.

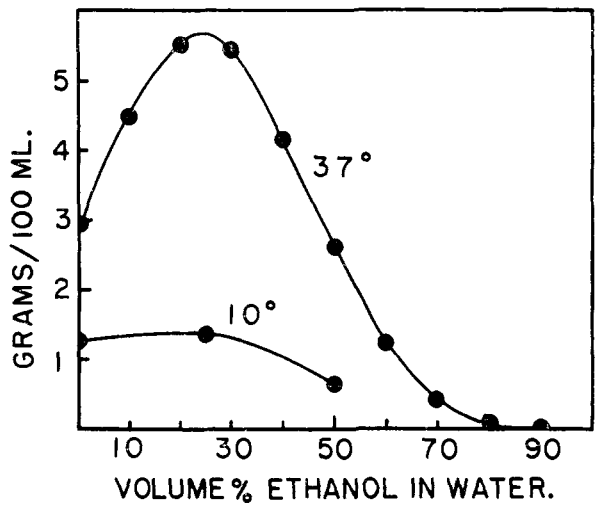

Fig. 1.—The solubility of β-cyclodextrin in mixtures of ethanol and water.

The concentration of cyclodextrins in solutions with C_6- to C_9-acids were also determined gravimetrically. Aliquots were dried over P_2O_5 at 1 mm. pressure, weighed and their acid portion titrated. However, at low concentrations of cyclodextrin, as they occur with longer chain acids, this procedure is impractical.

A method originally designed by Somogyi for the determination of blood sugar[15] was used in such cases. Under the conditions chosen here, the time for 50% hydrolysis of cyclodextrins to glucose is 7 to 8 min. and the reaction is virtually complete after 30 min. Some differences of reaction rates were encountered with the α- and β-compounds[16] and with their complexes. Therefore a standard time of 50 min. was chosen to achieve complete hydrolysis in all cases. The following example details the adjustments to be made for the determination of the resulting glucose.

After equilibration and sedimentation, samples of 10 ml. were drawn from the aqueous mixtures of α-cyclodextrin with decanoic acid and diluted to 50 ml. From this, aliquots of 2 and 3 ml. were taken, transferred to 10-ml. volumetric flasks and 1 ml. of H_2O was added to the 2-ml. aliquot. Hydrolysis was carried out by adding 2 ml. of 10 *N* H_2SO_4 and immersing the flasks in a boiling water-bath for 50 min. After cooling in ice, the hydrolysates were neutralized to the phenolphthalein end-point by adding, first, slightly less than 2 ml. of 10 *N* NaOH, and then,

(11) K. Freudenberg, E. Plankenhorn and H. Knauber, *Ann.*, **558**, 1 (1947).

(12) D. French, M. L. Levine, J. H. Pazur and E. Norberg, *J. Am. Chem. Soc.*, **71**, 353 (1949).

(13) A. W. Ralston and C. W. Hoerr, *J. Org. Chem.*, **7**, 546 (1942).

(14) D. M. Eggenberger, F. K. Broome, A. W. Ralston and H. J. Harwood, *ibid.*, **14**, 1108 (1949).

(15) M. Somogyi, *J. Biol. Chem.*, **160**, 61 (1945).

(16) D. French, N. L. Levine and J. H. Pazur, *J. Am. Chem. Soc.*, **71**, 356 (1949).

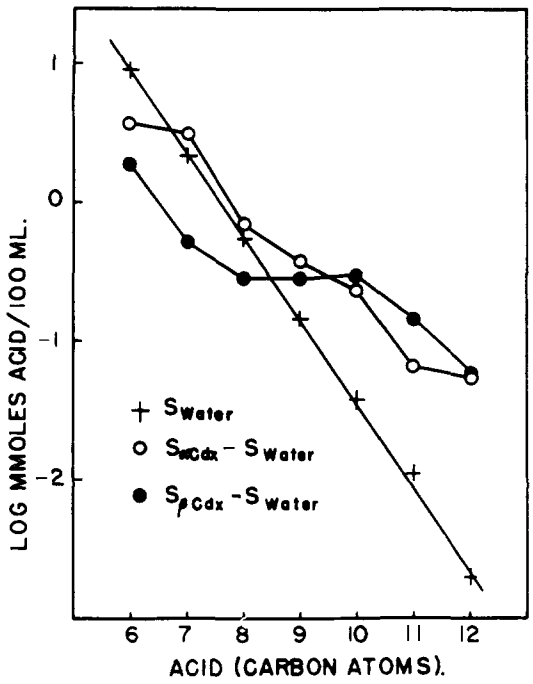

Fig. 2.—Log solubility of fatty acids in water and of their increase in solubility in presence of cyclodextrins ($acid_{total - blank}$).

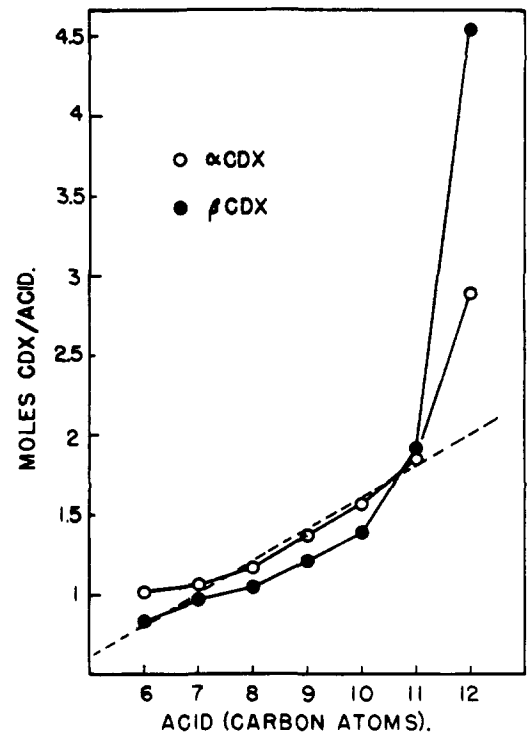

Fig. 3.—Mole ratios of cyclodextrin/fatty $acid_{(total - blank)}$ in water. The broken line has a slope 1 CDX/5 CH_2.

dropwise, 1 *N* NaOH. After making up to 10-ml. volume, 5 ml. were withdrawn and placed into 25 × 200 mm. test-tubes, together with 5 ml. of copper reagent[15] containing 4.95 mg. of KIO_3. The tubes were loosely covered with marbles, heated in boiling water for 20 min. and then immersed in ice-water. Two ml. of 2.5% KI solution and 1.5 ml. of 2 *N* H_2SO_4 were added to yield clear solutions after shaking. Titrations were carried out in the usual manner with 0.05 *N* $Na_2S_2O_3$ and referred to those of standards which had been run concurrently. After equilibration from higher temperature with aqueous decanoic acid for 144 hr., the concentration of α-CDX was found to be 0.355 and 0.360%. Reaching equilibrium from low temperature, after 260 hr., the concentration was found to be 0.355% and 0.356%.

The results were reproducible within 2% and agreed within that limit with gravimetric results when the hydrolysis–reduction procedure was applied to samples containing C_6- to C_9-acids. The concentration of standards, which were glucose and cyclodextrin, with or without organic acids, must not deviate more than 25% from that of the unknown solutions. Knowing the approximate concentration of cyclodextrin in samples to be analyzed facilitates also their proper dilution or the selection of a suitable amount of KIO_3 in Somogyi reagent. The data of these analyses are given in Table I. Figure 2 shows solubilities of fatty acids in water and their additional solubilities with cyclodextrins. Figure 3 shows the ratios of cyclodextrin/acid (total − blank).

TABLE I

SOLUBILITIES OF NORMAL ALIPHATIC ACIDS AND CYCLODEXTRINS IN WATER (31.3°)

Acid	Mmoles acid in 100 ml. of H_2O	Mmoles acid in 100 ml. of H_2O + α-CDX	Mmoles acid in 100 ml. of H_2O + β-CDX	Mmoles CDX in 100 ml. of H_2O + acid α-CDX	Mmoles CDX in 100 ml. of H_2O + acid β-CDX
C_6	8.83	12.40	10.69	3.64	1.57
C_7	2.19	5.31	2.70	3.31	0.49
C_8	0.55	1.24	0.84	0.84	.30
C_9	.15	0.53	.44	.51	.35
C_{10}	.04	.27	.35	.36	.44
C_{11}	.011	.08	.16	.12	.29
C_{12}	.002	.06	.06	.16	.27

Analysis of Crystalline Complexes.—The crystalline complexes were either filtered from the equilibrated solutions or prepared separately in larger amounts. Tridecanoic and higher acids were suspended in aqueous solutions of cyclodextrins, repeatedly heated and shaken at room temperature for several days.

The filtered adducts were contaminated with free acids which were held back by the powdery crystals even when the free floating lipid phase had been removed, for example, by centrifugation. Washing with organic solvents partly extracted the included moiety, and the solvents may be themselves included. For these reasons, we resorted to the distillation method previously used.[17] A deficiency of this procedure is that the complexes are dehydrated, whereas originally at least some of them form as hydrates. Besides, it may be questioned whether the volatilities of contaminating and included fatty acids are distinct enough to permit analyses of the latter only. Some detailed data to this point are given below, showing that purification by distillation is not arbitrary.

The crystals were dried at atmospheric pressure in a desiccator over P_2O_5 and then subjected to the heat-vacuum treatment.[17] Complexes of hexanoic and heptanoic acids were analyzed after bringing them to constant weight at room temperature. With other acids, temperatures were between 50 to 130°, according to chain length. Most of the unbound acids distilled during the first 10–15 min., but the procedure was continued until virtually no additional condensation could be observed during a period of 15 min. In repeated determinations, temperature and time were varied. The powdery complexes were separated from the distilled acids by cutting the distillation tube. The amounts of cyclodextrins and of tightly bound fatty acids were then determined by weight and titration.

Variations of the distilling conditions had little influence on the results, as the following typical examples show. The ratio, CDX/acid, in the β-CDX–C_6 complex, after 1 month over P_2O_5 *in vacuo*, was 0.62; after an additional 8 months, it was 0.63. The ratio for composition of α-CDX-C_7 samples, after 1 month under the same conditions, was 1.08; after 1 hr. heating to 82–85° at 1 mm., it was 1.09, and after 2 hr. at 86–89° at 1 mm. it was 1.09 also.

(17) H. Schlenk, D. M. Sand and J. A. Tillotson, *J. Am. Chem. Soc.*, **77**, 3587 (1955).

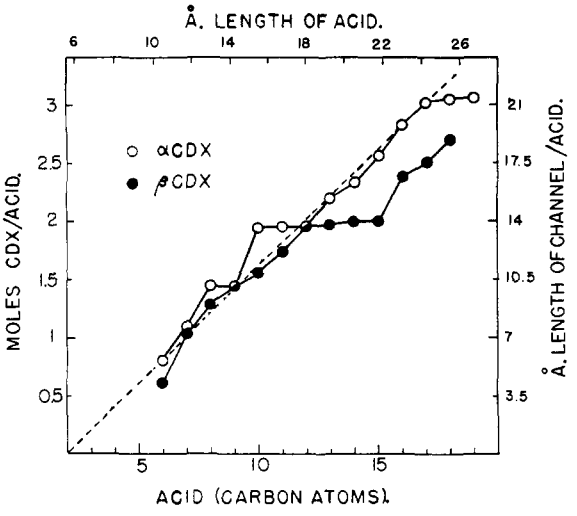

Fig. 4.—The composition of dehydrated complexes of cyclodextrins with fatty acids. The Å. scale of fatty acids is derived from Stuart–Briegleb models by measuring them in stretched form. The height of 1 CDX is approximately 7 Å. according to the models and this value was used in the right hand scale. The broken line has the slope 1 CDX/5 CH_2.

The ratio for α-CDX–C_{18} complex, after 4 hr. at 129° at 1 mm., was 3.03 and after 6.5 hr. at 135° it was 3.07.

The mole ratios of dehydrated complexes with C_6- to C_{19}-acids are shown in Fig. 4.

II. Cyclodextrins and Benzoic Acid.—Procedures used with benzoic acid were essentially the same as described under I. When the complex precipitated, the concentration of cyclodextrin in solution was determined as described there. Otherwise solutions were prepared by adding a known volume of water to a suitable amount of cyclodextrin and its concentration was calculated without considering any changes of volume.[18] Data on benzoic acid and α-cyclodextrin in water are shown in Fig. 5; those on benzoic acid and β-cyclodextrin are compiled in Table II.

TABLE II

SOLUBILITY OF BENZOIC ACID IN AQUEOUS SOLUTIONS OF β-CYCLODEXTRIN

Temp., °C.	Mmoles in 100 ml. of β-CDX	Mmoles in 100 ml. of Bz.A.	Moles $\frac{\beta\text{-CDX}}{\text{Bz.A. (total} - \text{blank), in solution}}$	Moles $\frac{\beta\text{-CDX}}{\text{Bz.A.}}$ in solid complex
2.5–3	0	1.33	..	..
	0.245	1.58	0.98	0.98
10	0	1.70	..	..
	0.414	2.15	0.92	0.80[a]
18	0	2.18	..	
	0.882	3.10	0.96	No complex
29	0	3.07	..	..
	0.902	4.01	0.96	No complex
	1.65	4.87	0.92	0.93
40	0	4.58	..	..
	2.98	7.83	0.92	0.98

[a] This value was verified several times.

After several attempts, the complex of α-cyclodextrin with benzoic acid was prepared as follows. A mixture of 2.51 g. of α-cyclodextrin (2.20 g. anhydrous) and 0.362 g. of benzoic acid in 5 ml. of CO_2-free water was dissolved by heating. Slow cooling to 1° followed by 2 days in the refrigerator yielded a precipitate consisting of benzoic acid and other crystals. After the usual purification by heat and vacuum, the composition of the crystals was 2.0 α-CDX/benzoic acid. The ratio of the solutes in the clear filtrate was found to be 0.97 α-CDX/acid. Another possible method of preparation became apparent when an aliquot of the filtrate was slowly evaporated and the residue dried over P_2O_5 *in vacuo*. After reaching constant weight, the ratio of components was 1.92.

(18) The addition of 770 mg. (0.8 mmole) of α-cyclodextrin to 10 ml. of H_2O increased the volume by about 3.4%.

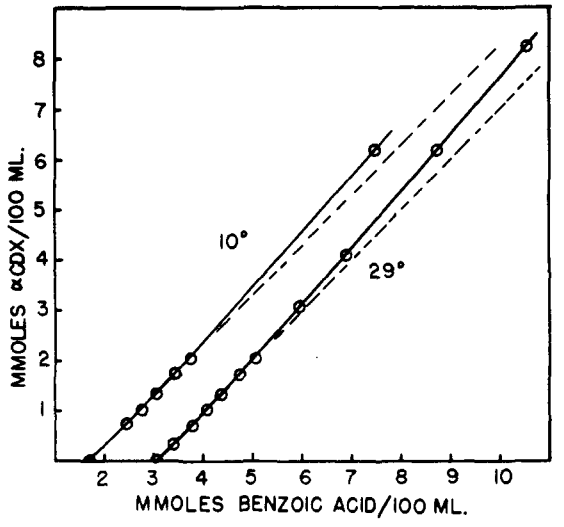

Fig. 5.—The solubility of benzoic acid in presence of α-cyclodextrin. The broken lines show the ratio 1 α-CDX/acid.

III. Cyclodextrins and Substituted Benzoic Acids.—Commercial *o*-, *m*- and *p*-iodobenzoic acids were crystallized repeatedly from water and ethanol solvents. Commercial durene (1,2,4,5-tetramethylbenzene), recrystallized from ethanol, served as starting material for the preparation of durylcarboxylic,[19] -acetic, 3-durylpropionic and 4-durylbutyric acids.[20] The uncorrected m.p.'s (Fisher–Johns m.p. app.) of the latter acids were 179.5–180°, 205–205.2°, 173–173.5° and 142.8–143.2°, respectively. As with the short-chain aliphatic acids, solubility criteria were taken whenever possible to ascertain the purity of the acids.

Equilibrations and analyses were carried out as described under I and II. The results with α-cyclodextrin are listed in Table III, those with β-cyclodextrin in Table IV.

IV. α-Cyclodextrin, Benzoic Acid and Salts.—Equilibration samples were prepared as under II, except that KI required more rigorous precautions against air. Large test-tubes with ground joints were equipped with gas inlets and outlets, similar to gas washing bottles. The closed test-tubes were rinsed with N_2 immediately after filling or withdrawing a sample. They were also covered with black tape to reduce illumination. Solutions containing KI stay colorless under such conditions. The results are listed in Table V.

V. Optical Rotations.—Equilibrated solutions as described under I were used for measuring $[\alpha]^{25}D$ of cyclodextrins in presence of C_6- to C_{10}-acids. The individual concentrations, as derived from Table I, are between 3.5 and 0.35% cyclodextrin. The optical rotation of α-cyclodextrin with benzoic acid was measured at 1% concentration of carbohydrate (10.3 m*M*) with 0.388% benzoic acid (31.8 m*M*) which corresponds to the values of Fig. 5. Other acids were added to solutions of 1% α-cyclodextrin until an oily phase remained undissolved at 31°. These acids were of commercial source, redistilled or recrystallized, but their purity was not established as rigorously as that of the fatty acids.

When the concentration of cyclodextrins in water was varied between 0.5 and 2%, $[\alpha]^{25}D$ remained unchanged. Adjustment of the *p*H to 1.7 by addition of H_2SO_4 did not change the rotations within 2 hours.

The results of measurements at the D-line are listed in Table VI.

(19) M. S. Newman and H. A. Lloyd, *J. Am. Chem. Soc.*, **74**, 2672 (1952).

(20) R. R. Aitken, G. M. Badger and J. W. Cook, *J. Chem. Soc.*, 331 (1950).

TABLE III

SOLUBILITY OF SUBSTITUTED BENZOIC ACIDS IN AQUEOUS SOLUTIONS OF α-CYCLODEXTRIN (31.3°)[a,b]

Acid	Mmoles in 100 ml. of α-CDX	Mmoles in 100 ml. of Acid	Solubility: total / blank	Moles α-CDX / acid (total − blank)
Benzoic (29°)[b]	0	3.07	(1.94)	(1.07)
	3.09	5.96		
o-Iodobenzoic	0	0.44	(1.44)	(15.4)
	2.94	.63		
m-Iodobenzoic	0	.053	(20)	(3.1)
	3.09	1.05		
p-Iodobenzoic[b]	0	0.011	15	4.4
	0.754	.170		
Durylcarboxylic	0	.25	(1.07)	(122)
	2.06	.27		
Durylacetic	0	.018	(1.1)	(1000)
	2.06	.020		
3-Durylpropionic	0	.004	(1.5)	(1000)
	2.06	.006		
4-Durylbutyric	0	.004	(3)	(290)
	2.06	.011		

[a] Data obtained from solutions not in equilibrium with crystalline complex are listed in parentheses. [b] The crystalline complex α-CDX–benzoic acid is described in the text. Of the others, *p*-iodobenzoic acid was the only one to yield a crystalline complex. It has the component ratio 2 α-CDX/acid.

TABLE IV

SOLUBILITY OF SUBSTITUTED BENZOIC ACIDS IN AQUEOUS SOLUTIONS OF β-CYCLODEXTRIN (31.3°)[a]

Acid	Mmoles in 100 ml. of β-CDX	Mmoles in 100 ml. of Acid	Solubility: total / blank	Moles β-CDX / acid (total − blank) in solution	Moles β-CDX / acid, in solid complex
Benzoic (29°)	0	3.07	1.59	0.92	0.93
	1.65	4.87			
o-Iodobenzoic	0	0.44	1.18	2.49	0.83
	0.18	.51			
m-Iodobenzoic	0	.053	20	1.73	1.03
	1.79	1.085			
p-Iodobenzoic	0	0.01	21	5.43	1.03
	1.14	.22			
Durylcarboxylic	0	.25	(1.84)	(8.43)	Not formed
	1.76	.46			
Durylacetic	0	.018	(3)	(48)	Not formed
	1.77	.054			
3-Durylpropionic	0	.004	(6)	(77)	Not formed
	1.77	.028			
4-Durylbutyric	0	.004	(13)	(38)	Not formed
	1.77	.050			

[a] Data obtained from solutions not in equilibrium with crystalline complex are listed in parentheses.

Optical rotations at shorter wave lengths were measured in a Rudolph spectropolarimeter using the symmetrical angle technique, with manual recording of galvanometer readings. A Hanau S-81 mercury lamp was the light source. The rotations of samples containing cyclodextrins and heptanoic acid were measured at 15 wave lengths between 578 and 254 mμ, those containing benzoic acid at 10 wave lengths between 578 and 294 mμ. The curves are plain positive[21] within these ranges and are so far sufficiently characterized by the values given in Table VII.

(21) C. Djerassi, "Optical Rotatory Dispersion," McGraw-Hill Book Co., Inc., New York, N. Y., 1960, p. 12.

TABLE V

EFFECT OF SALTS ON THE SOLUBILITY OF BENZOIC ACID IN AQUEOUS SOLUTIONS OF α-CYCLODEXTRIN (29°)[a]

Solutes α-CDX	Solutes 1 *M* salt	Total Bz.A. in soln.	Total loss of Bz.A. from soln.	Loss of Bz.A. assoc. with α-CDX
0	0	30.8		
26.5	0	55.3		
0	KCl	23.1	7.7	
26.5	KCl	46.2	9.1	1.4
0	KBr	24.7	6.1	
26.5	KBr	47.1	8.2	2.1
0	KI	27.8	3.0	
26.5	KI	43.6	11.7	8.7

[a] All amounts are mmoles/liter, except 1 *M* salt.

TABLE VI

THE OPTICAL ROTATION OF CYCLODEXTRINS IN PRESENCE OF ORGANIC ACIDS OR OF INORGANIC SALTS[a]

Acid	$[\alpha]^{25}D$ α-CDX	$[\alpha]^{25}D$ β-CDX	Acid or inorganic salt	α-CDX
None	+149.6°	+162.0°	Benzoic	+165
Hexanoic	+141.1	+157.0	2,3-Hexenoic	+160
Heptanoic	+138.3	+157.3	3,4-Hexenoic	+143°
Octanoic	+136.2	+157	2,3-Isopentenoic	+158
Nonanoic	+133	+159	Sorbic	+183
Decanoic	+134.5	+158	Ethyl sorbate	+181
			1 *M* KCl	+150[b]
			1 *M* KBr	+146[b]
			1 *M* KI	+126[b]

[a] Solutions of α-methylglucoside, $[\alpha]^{25}D + 158.5°$, in aqueous 1 *M* KCl, KBr, KI, 0.287% benzoic acid or 0.25% heptanoic acid showed rotations $[\alpha]^{25}D$ between +157° and +159°. [b] These values are corrected for change of volumes upon addition of salts.

TABLE VII

THE OPTICAL ROTATION OF CYCLODEXTRINS AT SHORT WAVE LENGTHS IN PRESENCE OF BENZOIC AND OF HEPTANOIC ACIDS

CDX	Acid	$[\alpha]^{25}_{254}$	$[\alpha]^{25}_{294}$
1% α	None	+1160°	+741°
1% α	0.388% bz. acid	....	+950
1% α	0.40% C_7-acid	+1070	+690
1% β	None	+1165	+798
1% β	0.378% bz. acid	....	+900
1% β	0.303% C_7-acid	+1100	+713

Discussion

Cycloamyloses may contain the glucose units in boat form[22] B-1 to B-3,[23] or in chair form.[5,24] Stuart–Briegleb models[25] of cyclodextrins constructed from glucose in boat form are more flexible than those containing the glucose units in chair form. In both cases the insides of the macrocycles are made up by three zones that are quite different from each other, as pointed out in the legend of Fig. 6a and 6b.

It appears likely that the role of the zones in association varies with compounds of different chemical character. Such considerations are in line with the experiences mentioned in the introduction that caused us to limit this study to carboxylic acids.

(22) K. Freudenberg and F. Cramer, *Ber.*, **83**, 296 (1950).
(23) R. E. Reeves, *J. Am. Chem. Soc.*, **71**, 215 (1949).
(24) D. French and R. L. McIntire, *ibid.*, **72**, 5148 (1950).
(25) G. Briegleb, "Methoden der organischen Chemie," E. Müller ed., Thieme Verlag, Stuttgart, Germany, 4th ed., vol. III/a, 1955, p. 545.

The solubility of β-cyclodextrin in mixtures of ethanol and water shows a maximum at about 25 vol. % of ethanol which is indicative of association of the carbohydrate with this solvent (Fig. 1). It is also known that ethanol and many other solvents are easily included in crystalline cyclodextrins. Therefore solubility experiments were made in water only, although this limited the range of compounds accessible to measurement by titration.

I. The solubilities of the homologs caproic to lauric acid are increased when cyclodextrins are present in their aqueous solution (Table I). The factors relating their solubility in presence of cyclodextrin to the corresponding blank solubility increase from 1.2 to 30 but they are not large enough to cancel completely the normal decrease of solubility with extending chain length. Glucose, α-methyl glucoside or maltose did not influence the solubilities of such acids.

The physical properties of homologous, nonpolar compounds normally follow a straight logarithmic function[26] and this rule is fulfilled by the fatty acids[27] which change their solubility in water by constant logarithmic increments. The rule is not applicable however when cyclodextrins are present in the aqueous medium. The irregularity of their influence becomes particularly apparent when only the additional solubilities, $S_{total} - S_{blank}$, are considered (Fig. 2). The solubilities of higher acids cannot be extrapolated, but one can anticipate that they are increased by cyclodextrins also.

The amount of free acid at equilibrium in the aqueous cyclodextrin–acid medium should be approximately equal to the solubility of the acid in pure water. The ratios, total cyclodextrin/associated acid, have been calculated under this assumption (Fig. 3). In contrast to the composition of the crystalline complexes (Fig. 4) these ratios increase rather steadily and do not indicate the mechanism of association. Some conclusions concerning the latter might be possible by comparing the equilibrium constants of the homologous series. The amounts of dissociated or associated cyclodextrins, however, are not directly accessible from the data. Values derived for associated cyclodextrin from space requirements of models would be arbitrary in view of the great flexibility of the molecules in solution. It is seen also from Figs. 3 and 4, where the identical line 1 CDX = 5 CH_2 may serve as reference, that quite often less cyclodextrin is needed to keep an acid in solution than to bind it in crystalline phase.

The data of Fig. 3 suggests equilibria

$$CDX + \text{acid} \rightleftarrows (CDX\ \text{acid}), \text{ and}$$

for short acids, $(CDX \cdot \text{acid}) + \text{acid} \rightleftarrows (CDX \cdot 2\ \text{acid})$
for long acids, $(CDX \cdot \text{acid}) + CDX \rightleftarrows (2CDX \cdot \text{acid})$ and so on.

The associated species, in turn, are in equilibrium with the crystalline complex. The stability of the crystals, however, depends on the compatibility of the acid molecules with the space offered by the cyclodextrins. Therefore, complexes with a homologous series of guest components will not have properties which change by regular increments.

It has been reported that the law of constant proportions is valid for the formation of cyclodextrin complexes.[28] This is prerequisite for the conclusions drawn from the composition of the complexes, and was confirmed here for conditions where the guest component is in excess.

In crystalline phase, inhomogeneity and therewith the periodicity of forces in a channel make certain sites preferential for the included molecules or for specific groups of them. This is reflected in the composition of a homologous series of complexes. For example, the crystal lattice of thiourea holds 1,6-dicyclohexylhexane in stretched form. However, the channel is wide enough to accommodate 1,7-dicyclohexylheptane and the corresponding derivative of octane "curled" so that the new complexes have the same mole composition and the new guests occupy only the same length of the channel.[29] In choleic acids, part of the channel may remain empty between guest molecules while the next homolog fills the space, keeping again the mole ratio constant.[30] Both crowded and loose packings were found with cyclodextrins. The former applies to their complexes with fatty acids.

When dehydrated, the available length of the channel is always equal to or less than that of the corresponding fatty acid in fully stretched form (Fig. 4). An increase of molar ratios (CDX/acid) at a rate of 1 CDX/5 CH_2 appears significant. However, deviations from such a line are found for α-cyclodextrin with C_8, C_{10} and C_{11} acids, which are assigned to a greater length of the channel than this ratio indicates. Most likely they are more stretched than the other acids. In the case of β-cyclodextrin, deviations from the same line occur with C_{13}- to C_{15}-acids which are packed in the same length of channel as C_{12}-acid. A similar plateau of ratios is indicated for C_{17} and longer acids at a ratio of 3 α-CDX/acid. Constant values are near to 1.5, 2 and 3 cyclodextrin and these values can be considered as the theoretical ones for the particular acids. Association of the carboxyl groups to the wall and/or dimerization of acids can account for the intercept of 5 to 6 Å. at 0 cyclodextrin in Fig. 4.

Models of C_{12}- and C_{15}-acids easily can be adapted to the required length in a glass cylinder of an inner width proportional to that of β-cyclodextrin (see legend to Fig. 6). The same can be demonstrated with the exceptions encountered with α-cyclodextrin.

These results apply strictly to dehydrated complexes only, but it is reasonable to assume such periodicity for their hydrates also.

II. In the series of homologous aliphatic acids the length of the guest molecule was the only variable. The width was varied in the studies with benzoic and related acids. The interaction

(26) I. Langmuir, in "Colloid Symposium," monograph III, Chemical Catalog Co. (Reinhold Publ. Corp.), New York, N. Y., 1935, p. 48; E. L. Skau and R. E. Boucher, *J. Phys. Chem.*, **58**, 460 (1959).

(27) Langmuir's rule compares only equal phases. Lauric acid, however, is solid at the temperature of our experiments.

(28) (a) F. Cramer, *Ber.*, **84**, 851 (1951); (b) F. Cramer and F. M. Henglein, *ibid.*, **90**, 2561 (1957); see, however, (c) F. Cramer, *Angew. Chem.*, **64**, 437 (1952).

(29) W. Schlenk, *Ann.*, **573**, 142 (1951).

(30) W. Schlenk, "Fortschritte der chemischen Forschung," Vol. 2, Springer Verlag, Berlin, 1951, p. 92.

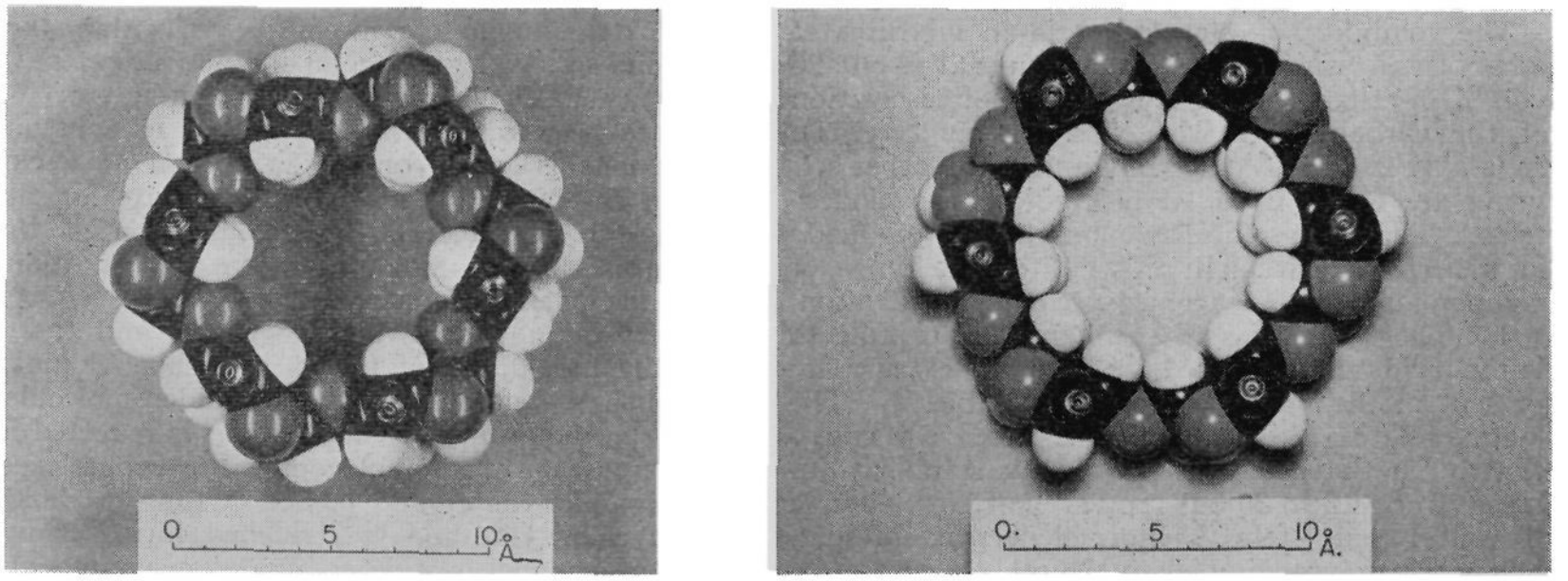

Fig. 6a and b.—Cross sections through models of α-cyclodextrin built from glucose in chair form (a)[5] and in boat form B-1 (b).[22] Six CH_2OH groups which represent the highest layer have been removed from both models to make better visible the inner side of the center layer. In (a) it is lined by CH groups (carbons 3 and 5 of glucose, nearly superimposed in projection) and by O of the maltose linkages. In (b) the inner side is lined by CH groups (carbon 1 and 4 of glucose). The lowest layer is made up essentially by COH (carbons 2 and 3) in (a), and CH (carbon 2) and COH (carbon 3) in (b).

The macrocycle of (a) is rigid while that of (b) has considerable conformational freedom. In the idealized position of highest symmetry, length and diameter of the channels are approximately the same in both models. In other use of models (see text) the channel of α-cyclodextrin (cyclohexaamylose) was represented by a glass cylinder of 9 cm. i.d., as measured from the model, equivalent to 6 Å. The channel of β-cyclodextrin (cycloheptaamylose) was represented by a cylinder of 12 cm. i.d., equivalent to 8 Å.

of aromatic compounds with cyclodextrins is of some practical interest. Preparative separation of α- and β-cyclodextrins is based on precipitation of the latter as a complex with bromobenzene, while the former remains in the aqueous solution.[11,12,31] The difference has been ascribed to the inability of the aromatic ring to adjust itself to the space inside the smaller α-cyclodextrin.

Solubility measurements of benzoic acid show that it associates with α- as well as β-cyclodextrin. At 10° and 29°, the ratio of 1 α-CDX/1 acid hardly changes over a wide range of concentrations (Fig. 5) nor could an influence of temperature be demonstrated for the association of benzoic acid with β-cyclodextrin[32] between 3° and 40° (Table II). As with the majority of fatty acids, on a molar basis, somewhat more benzoic acid is kept in solution by the β- than by the α-compound. The ratio β-CDX/acid is close to 0.95 in solution and the crystalline complex has approximately the same composition, except in one case.

Although all indications are that benzoic acid associates very strongly with both cyclodextrins, crystallization can be achieved easily only with β-cyclodextrin. Preparation of the α-complex requires extreme conditions, and it has then the composition 2 α-CDX/acid while the ratio in the supernatant still is 1:1. The most plausible explanation is that benzoic acid cannot adjust itself to the shape of crystalline α-cyclodextrin, as has been suggested for some other aromatic compounds. Instead, it adjusts α-cyclodextrin to its own shape, so that an approach to the associating sites becomes possible. It will be seen under V that the influence of benzoic acid on the optical rotation of α-cyclodextrin is distinct from that of the normal aliphatic acids. The conformation which brings about the unusual optical behavior may also cause the difficulty in crystallization. When forced, crystallization takes place at a different component ratio so that benzoic acid becomes compatible with the steric conditions of the solid phase.

III. The data on solubilities of aliphatic and benzoic acids are suggestive of inclusion in solution, but the inference should be verified by probing the properties of molecules for which steric interference can be anticipated. One must expect that the steric requirements in solution are more liberal than in crystals, as already has been indicated in the above discussion on benzoic acid and α-cyclodextrin. Data on iodobenzoic acids, and durylcarboxylic and homologous acids corroborate this (Tables III and IV). The solubilities of the former are increased by both dextrins; apparently their diameters do not exceed the limit which still permits association to a considerable extent. However, *p*-iodobenzoic acid is the only isomer which forms easily a complex with α-cyclodextrin, while all iodobenzoic acids crystallize without difficulty together with β-cyclodextrin. Acids containing a tetramethylphenyl group exceed the dimensions permissible for proper inclusion even in solution. Since durylcarboxylic acid probably is hindered in any type of association with the carbohydrates, the homologous series, including 4-durylbutyric acid, was investigated. The relative

(31) F. Cramer and F. M. Henglein, *Ber.*, **91**, 308 (1958).

(32) A more elaborate concentration–solubility curve was determined for benzoic acid and β-CDX at 29°. With increasing concentration of β-CDX, the solubility of benzoic acid rose proportionately and reached its maximum when β-CDX-bz.a. began to precipitate. The concentration–solubility curve did not show any significant curvature in that area. Upon addition of more β-CDX the amount of crystalline complex increased at the expense of the solid benzoic acid phase, while the concentrations in water and the composition of the solid CDX phase remained constant. Upon addition of β-CDX beyond the point of disappearing benzoic acid the concentration of the acid decreased in solution and in the CDX crystals. It was not determined whether the latter represented one or two phases (*cf.* ref. 28).

increase of solubilities indicates some association with the longer chain acids, but the component ratios are between 100 and 1000 for the α-compound (Table III) and between 8 and 100 for the β-compound (Table IV). With fatty acids of similar length they are around 1 (see Fig. 3). Crystalline complexes of durylcarboxylic acid and its homologs could not be obtained. Several attempts to prepare their β-complexes at concentrations of β-cyclodextrin higher than those listed in Table IV yielded crystals of the pure dextrin.

Molecular models allow a rough evaluation of the possibility for inclusion. A model of benzoic acid fits easily into the cylinder representing the α-channel according to Fig. 6. The benzene ring is necessarily in a position nearly vertical to the plane of the carbohydrate ring. The carboxyl group rotates freely but cannot approach the wall as easily as the carboxyl group of an aliphatic acid. The restricted position of both the hydrocarbon and the carboxyl moiety may bring about a conformational "correction" of the carbohydrate. Models of *o*- and *m*-iodobenzoic acid can be inserted into α-cyclodextrin only under deformation of the macrocycle; they are accepted easily by the β-channel (glass cylinder). Acids substituted with tetramethylphenyl cannot be inserted into the flexible model of α-cyclodextrin and can be fitted only under strong deformation into β-cyclodextrin. These findings are in accord with the results of the actual experiments. However, the limited value of such consideration is evident with *p*-iodobenzoic acid. In models, this compound is under the same positional restrictions as benzoic acid, but the component ratios in solution indicate that *p*-iodobenzoic acid (α-CDX/acid = 4.4) is not as strongly associated as benzoic acid (α-CDX/acid = 1). In the crystalline phase, on the contrary, *p*-iodobenzoic acid is much more apt to combine with α-cyclodextrin than is benzoic acid. Obviously, other factors besides steric limitations are of great importance for crystallization.

It has been found consistently that cyclodextrins either increase the solubility of molecules or are inert. The process of increasing the solubility of a substance having normally a low solubility by means of cosolutes is called either hydrotropy or solubilization.[33] Hydrotropy involves salts of organic acids that, at high concentration, increase the solubility of other compounds by associating with them.[34] Neither high concentration nor salt character are essentials for the solubilizing effect of cyclodextrins. Solubilization involves a multitude of molecules organized to form a micelle which offers the solubilizing environment to the other compound. Molecules of cyclodextrins provide the suitable environment without aggregation. Therefore, they can solubilize without reaching a critical micelle concentration. The phenomenon may be termed as molecular or inclusion solubilization. Such specification should also avoid confusion when other type association or colloidal phenomena involving cyclodextrins, but not necessarily inclusion, will be studied.

IV. When salts are added to solutions of cyclodextrins and compounds that are subject to molecular solubilization, two processes will superimpose. These are the salting-out effect upon the portion of solute which is not associated, and the effect upon the portion which is solubilized by the "molecular micelles." In studies on the former, benzoic acid was one of the preferred substrates. It is salted out from aqueous solutions in amounts which follow the equation log $(S_{water}/S_{salt}) = kC$, where S is the solubility of the acid in the respective medium, C is the molar concentration of salt and k is a constant, which is characteristic for the particular salt. The results fulfill the equation closely when experimental conditions are such that corrections for the dissociated portion of the acid are minimized.[35] Even without such corrections the differences of k are large enough to make apparent the order $k_{KCl} > k_{KBr} > k_{KI}$ for those particular salts.

In regard to the second process, *i.e.*, the interaction of salts with solubilizers, most publications deal with ionic active agents.[36] Cyclodextrins may be better comparable to non-ionic micelles, but general rules for the effect of anions on such have not yet emerged.[37]

Benzoic acid and α-cyclodextrin were used in our experiments together with KCl, KBr and KI. The addition of sodium benzoate and other measures correcting for dissociation[33] were dismissed. They permit more accurate results for the salting-out of the first type but at the same time might influence the molecular solubilization. The amounts of benzoic acid that were salted out from aqueous solutions by the potassium halides decreased in the proper order (Table V). When they are subtracted from the amount of benzoic acid eliminated by the salts from cyclodextrin solutions, one arrives at the approximate amount of acid that has been expelled from the host. The results show that benzoic acid, when included, is partly protected against the ions. However, the equilibria are markedly affected even in this case where the associating forces are particularly strong. The reversal of eliminating power, KI > KBr > KCl, proves that the ions act upon α-cyclodextrin rather than directly upon the benzoic acid included by it.

I^- is outstanding in splitting or preventing the association, and it outranks by far not only the halides but also other salts. The complex of α-cyclodextrin with oleic acid was spread with its supernatant on a microscope slide, and drops of 5 *M* KI, KBr, KCl, NaI, NaCl, $NaNO_3$, NaH_2PO_4 or 3*M* Na_2SO_4 were added, all components being in about equal amounts. With iodides, oil droplets were released instantaneously from the wet

(33) G. S. Hartley, *Ann. Reports Progr. Chem.* (Chem. Soc. London), **45**, 33 (1949); H. B. Klevens, *Chem. Revs.*, **47**, 1 (1950); M. E. L. McBain and E. Hutchinson, "Solubilization and Related Phenomena," Academic Press, Inc., New York, N. Y., 1955; J. C. Harris, *J. Am. Oil Chemists' Soc.*, **35**, 428 (1958).

(34) C. Neuberg and F. Weinmann, *Biochem. Z.*, **229**, 467 (1930).

(35) G. M. Goeller and A. Osol, *J. Am. Chem. Soc.*, **59**, 2132 (1937).

(36) H. B. Klevens, *ibid.*, **72**, 3780 (1950); P. H. Richards and J. W. McBain, *ibid.*, **70**, 1338 (1948).

(37) T. M. Doscher, G. E. Myers and D. C. Atkins, *J. Colloid Sci.*, **6**, 223 (1951).

paste of complex, while with other salts, droplets were observed only after several minutes or hours.

A similarly unique role of I^- is seen in the reaction of I_2 with starch where it cannot be substituted by other ions when the blue color is to be formed. French and co-workers recently demonstrated that a complex of I^- with the carbohydrate is essential for the development of the visible color.[38] The same authors showed that with α-cyclodextrin a complex α-CDX·I_3^- is formed in solution[39] and quote the preparation of a crystalline complex α-CDX·KI.[40] Prominent interaction of I^- with α-cyclodextrin is also revealed here by the optical rotation where its effect outranks that of Br^- or Cl^- (Table VI). I^- may serve not only to form with I_2 the necessary complex I_3^-, but also to bring about a conformation of starch or α-cyclodextrin which is suitable for association with this species.[39]

V. Different optical rotations with different solvents have been reported by Freudenberg for cyclodextrins,[11] and Cramer mentions that their concentrations cannot be determined exactly by polarimetry when other organic compounds are present in the aqueous solutions.[28b] Our analyses of cyclodextrins and fatty acids in water enabled us to measure the optical rotations under defined conditions (Table VI). The specific rotation of α-cyclodextrin is markedly lowered with increasing chain length of acids and the same holds, though to a lesser degree, for the β-compound. However, when the guest molecule has conjugated unsaturation, the optical rotation of α-cyclodextrin is increased. The specificity is suggested by the values found in the presence of 2,3-hexenoic and 3,4-hexenoic acids. Sorbic acid, or its ester, having the highest conjugation, affect the rotation the most.

Several speculations can be derived from this finding. The shift of optical rotation of an active substrate caused by association with an inactive cosolute may allow the derivation of rules concerning the structure of the latter. The use of optically active solvents is not practical for such a purpose, but cyclodextrin can serve as an associating "solvent" in many cases without introducing an excessive amount of optically active molecules which are not involved in the difference to be measured. The values of Table VI are not the optical rotations of associated cyclodextrins, although in some instances the amount of free host is very small. The rotation changes have opposite signs and this rules out that they result merely from differences in equilibria.

The ratios of optical rotations, α-CDX·heptanoic acid/α-CDX, are 0.93 between 578 and 248 mμ. A slight increase is found for the rotation ratio α-CDX·benzoic acid/α-CDX, from 1.12 to 1.28 over the range 578 to 297 mμ. The region 270 to 275 mμ, where λ_{max} is found for free and associated benzoic acid, could not be covered in the measurements at concentrations that would allow reference to the solubility experiments. The interesting correlation of ultraviolet absorption band of guest and rotatory dispersion of host will be easier with compounds having absorption better suitable than heptanoic or benzoic acids.[41]

Quantitation of the rotation shift may afford a method for assaying the portion of free cyclodextrin in association equilibria. Because absorption in the accessible ultraviolet range is not conditional, guest molecules of simple saturated structures can be screened. A further advantage is the fact that the presence of components extraneous to the equilibria is not required in such measurements.

(38) J. A. Thoma and D. French, *J. Am. Chem. Soc.*, **82**, 4144 (1960).
(39) J. H. Thoma and D. French, *ibid.*, **80**, 6142 (1958).
(40) H. A. Dube, Ph.D. Thesis, 1947, Iowa State College.
(41) C. Djerassi and K. Undheim, *J. Am. Chem. Soc.*, **82**, 5755 (1960).

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Podocarpic Acid Derivatives. Synthesis of Nimbiol

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The conversion of podocarpic acid into O-methylpodocarpane and the latter's transformation into several hydrophenanthrones and a bicyclic ketoacid are described. Attempts to convert the acid and its derivatives to natural diterpenic substances are discussed. The synthesis of nimbiol, *via* 13-methylpodocarpic acid and O,13-dimethylpodocarpane, is portrayed.

As our studies on the total synthesis of the resin acids approached completion, we became interested in their conversion into other diterpenes. *d*-Podocarpic acid (Ia), whose synthesis we recently reported,[3] appeared to be a suitable starting material for the synthesis of ring C substituted aromatic, hydroaromatic and *seco* compounds. The present communication concerns itself with an attempted construction of the phyllocladene (II) nucleus and the synthesis of nimbiol (III).

Phyllocladene.—Two routes were used for the conversion of *d*-podocarpic acid (Ia)[4] into O-methylpodocarpane (Ib). Tosylation of O-methylpodo-

(1) Public Health Service Predoctoral Research Fellow, 1959-1960.
(2) National Science Foundation Coöperative Graduate Fellow, 1959–1960.
(3) E. Wenkert and A. Tahara, *J. Am. Chem. Soc.*, **82**, 3229 (1960).
(4) The natural acid could be isolated readily from rimu oleoresin, kindly supplied by S. B. Penick and Co. In order to obtain other terpenic plant constituents, the isolation procedure of I. R. Sherwood and W. F. Short [*J. Chem. Soc.*, 1006 (1938)] was followed by chromatography of the residues of the ethanol extract from which podocarpic acid had been crystallized. However, no other natural substances were discovered.