Circular Dichroism Study on Inclusion Complexes of β-Cyclodextrin with Anti-Inflammatory Fanamates

Keyphrases □ Cyclodextrin-fanamate inclusion complexes—circular dichroism studies □ Anti-inflammatory fanamate-cyclodextrin inclusion complexes—circular dichroism studies □ Circular dichroism—analysis of cyclodextrin-fanamate inclusion complexes □ Complexes, inclusion (cyclodextrin-fanamates)—stoichiometric ratio, stability constants, circular dichroism

To the Editor:

Cyclodextrins are known to form inclusion complexes with various drug molecules (1–7). When optically inactive compounds interact with optically active compounds, new circular dichroism (CD) bands are induced in the absorption bands of optically inactive compounds (8). Thakkar *et al.* (9) recently reported that barbiturates generated the induced CD upon binding to β-cyclodextrin (cycloheptaamylose) and estimated the stability constants of the inclusion complexes from the CD bands. Azo dye, iodine, and benzoic acid also have been shown to generate extrinsic Cotton effects upon binding to cyclodextrins (10–13).

Since the induced Cotton effects can give useful information on the nature of inclusion complexes (9–13), we studied, by means of a CD technique, the interactions of β-cyclodextrin with anti-inflammatory fanamates such as flufenamic, mefenamic, and meclofenamic acids in aqueous solution. In this communication, we report that the analysis of these induced CD bands could afford the conformational elucidation of the guest molecules of fanamates within the cavity of β-cyclodextrin.

The CD[1] and UV[2] spectra were taken in 0.1 *M* phosphate buffer (pH 7.0) at 25°. The optical anisotropy factor ($g = \Delta\epsilon/\epsilon$), which is proportional to the magnitude of the induced Cotton effects, was calculated from the following expression:

$$g = \frac{[\theta]}{3300 \times \epsilon} \qquad \text{(Eq. 1)}$$

where $[\theta]$ is the molar ellipticity, and ϵ is the molar absorption coefficient of the drug in the presence of β-cyclodextrin (1.0×10^{-2} *M*) at the maximum wavelength of the CD spectrum.

Figure 1A shows the effect of β-cyclodextrin on the UV absorption spectrum of flufenamic acid. The absorption maximum of the drug shifted to a longer wavelength in the presence of β-cyclodextrin, with concomitant change in molar absorption coefficients. Figure 1B shows the CD spectrum for flufenamic acid in the presence of β-cyclodextrin, with positive peaks at 287 and 328 nm. Since intrinsic Cotton effects of β-cyclodextrin itself exhibit below 220 nm, the observed optical activity above 220 nm can be attributed to the induced Cotton effect of flufenamic acid, generated by the formation of an inclusion complex with β-cyclodextrin. The binding of β-cyclodextrin with other fanamates, mefenamic acid and meclofenamic acid, also showed similar changes in UV spectra and generated the induced CD bands.

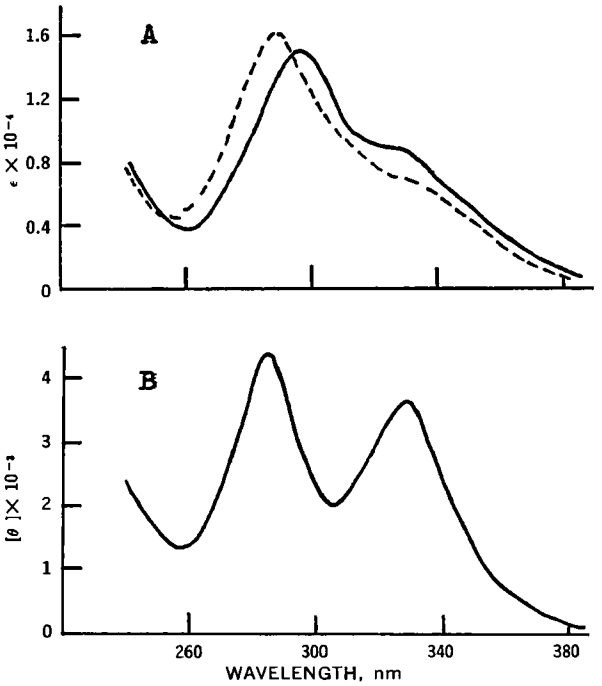
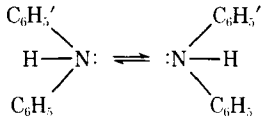


Figure 1—(*A*) *Effect of β-cyclodextrin* (1.0×10^{-2} M) *on UV spectrum of flufenamic acid* (5.0×10^{-5} M). *Key: - - -, flufenamic acid alone; and ——; flufenamic acid plus β-cyclodextrin.* (*B*) *CD spectrum of flufenamic acid* (5.0×10^{-5} M) *in the presence of β-cyclodextrin* (1.0×10^{-2} M).

To determine what factors are important for the generation of the induced Cotton effects, interactions of other *N*-phenylanthranilates and their related compounds with β-cyclodextrin were studied. Table I summarizes the *g* values obtained for these compounds bound to β-cyclodextrin. With the exception of anthranilic acid, all compounds studied generated the induced Cotton effects. This may indicate that the diphenylamine moiety in drugs is essential to generate the optical activity. It is assumed from these findings that the uncomplexed diphenylamine, which resonates between two pyramidal structures (Scheme I), is fixed in one conformation by the formation of the inclusion complex.

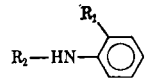
By application of the continuous variation method to the induced Cotton effects, the stoichiometric ratio of the complexes was found to be 1:1 for β-cy-



Scheme I

[1] Jasco model 20A recording spectropolarimeter.
[2] Hitachi EPS-3T spectrophotometer.

Table I—Optical Anisotropy Factors of *N*-Phenylanthranilates and Their Related Compounds Bound to β-Cyclodextrin



Compound		UV Maximum		CD Maximum		
R_1	R_2	Wavelength, nm	ε, × 10^{-4}	Wavelength, nm	$\Delta\epsilon^a$	g^b, × 10^4
COOH	H	240	0.74			
		310	0.30		(Not observed)	
H	C_6H_5	283	1.40	273	1.21	1.03
COOH	C_6H_5	291	1.26	285	2.36	1.90
		332	0.53	332	0.61	1.15
$COOCH_3$	C_6H_5	287	1.40	293	2.06	1.62
		343	0.64	335	0.78	1.28
COOH	2-COOH—C_6H_4	—	—	233	1.00	0.81
		—	—	242	−0.58	−0.73
		289	1.17	292	−1.45	−1.27
		338	0.73	345	−0.23	−0.32
COOH	3-CF_3—C_6H_4 (Flufenamic acid)	292	1.46	287	1.30	0.93
		320	0.86	328	1.09	1.36
COOH	2,3-Xylyl (Mefenamic acid)	283	0.70	280	−0.33	−0.29
				315	0.61	1.13
		336	0.46	335	0.73	1.16
COOH	2,6-Dichloro-*m*-tolyl (Meclofenamic acid)	285	0.48	275	0.43	0.91
		320	0.43	320	0.03	0.07

[a] Molar ellipticity (deg cm^2 $dmole^{-1}$). [b] Optical anisotropy factor (see text).

clodextrin and fanamates. Stability constants ($K_{1:1}$) determined by the CD method for β-cyclodextrin with flufenamic, mefenamic, and meclofenamic acids were 1300, 630, and 450 M^{-1}, respectively. In contrast to β-cyclodextrin, α-cyclodextrin (cyclohexaamylose) showed no appreciable complex formation. This suggests that the cavity size of α-cyclodextrin is not large enough to include the bulky guest molecules of drugs.

In the series of flufenamic, mefenamic, and meclofenamic acids, the magnitude of the *g* value at the CD bands of the longest wavelength (320–340 nm) agreed with the order of stability constants of the complexes. The stereospecific nature of the *ortho*-substituents in two aromatic rings apparently is responsible for the magnitude and/or sign of the induced CD bands. Meclofenamic acid, in particular, was much less influenced by the asymmetric environment than were other compounds, which could be due to the bulky chlorine and methyl substituents. An NMR study (7, 14) may provide further information on the mode of inclusion of these drug molecules within the β-cyclodextrin cavity. Carbon 13 NMR experiments on these problems are now being conducted in our laboratory.

(1) J. Cohen and J. L. Lach, *J. Pharm. Sci.*, **52**, 132(1963).
(2) J. L. Lach and J. Cohen, *ibid.*, **52**, 137(1963).
(3) J. L. Lach and T. F. Chin, *ibid.*, **53**, 69(1964).
(4) W. A. Pauli and J. L. Lach, *ibid.*, **54**, 1745(1965).
(5) J. L. Lach and W. A. Pauli, *ibid.*, **55**, 32(1966).
(6) T. Higuchi and J. L. Lach, *J. Amer. Pharm. Ass., Sci. Ed.*, **43**, 349(1954).
(7) A. L. Thakkar and P. V. Demarco, *J. Pharm. Sci.*, **60**, 652(1971).
(8) B. Bonich, *J. Amer. Chem. Soc.*, **89**, 6143(1967).
(9) A. L. Thakkar, P. B. Kuhen, J. H. Perrin, and W. L. Wilham, *J. Pharm. Sci.*, **61**, 1841(1972).
(10) K. Sensse and F. Cramer, *Chem. Ber.*, **102**, 509(1969).
(11) D. French, J. G. Foss, M. Carville, and J. R. Runyon, *Amer. Chem. Soc. Abstr.*, **153**, C-21(1967).
(12) J. R. Runyon, Ph.D. thesis, Iowa State University, Ames, Iowa, 1968.
(13) K. Takeo and T. Kuge, *Stärke*, **24**, 281(1972).
(14) P. V. Demarco and A. L. Thakkar, *Chem. Commun.*, **1970**, 2.

Ken Ikeda[x]
Kaneto Uekama
Masaki Otagiri

Faculty of Pharmaceutical Sciences
Nagoya City University
Tanabe-dori, Mizuho-ku
Nagoya, Japan

Masahiro Hatano

Chemical Research Institute of Non-aqueous Solutions
Tohoku University
Sendai, Japan

Received January 29, 1974.
Accepted for publication April 10, 1974.

[x] To whom inquiries should be directed.

Preliminary Observations on Cardiac Activities of *Cannabis sativa* L. Root Extracts

Keyphrases □ *Cannabis sativa* L. root extracts—cardiac activity of hexane, chloroform, ethanol, water, and hydrochloric acid extracts □ Marijuana—cardiac activities of *Cannabis sativa* L. root extracts □ Cardiac activity—hexane, chloroform, ethanol, water, and hydrochloric acid extracts of *Cannabis sativa* L. roots

To the Editor:

Rodger (1) reported that whiskey extracts (about 45% alcohol) of *Cannabis* roots have been used by American Indians and others to treat "dropsy." Additionally, the extracts were reported to have a digi-