Chem. Pharm. Bull. **37**(10) 2786—2789 (1989) Vol. 37, No. 10

Epimerization and Racemization of Some Chiral Drugs in the Presence of Cyclodextrin and Liposomes

Yukio Aso,* Sumie Yoshioka and Yasushi Takeda

National Institute of Hygienic Sciences, 1–18–1 Kamiyoga, Setagaya-ku, Tokyo 118, Japan. *Received March 20, 1989*

The effects of α-, β-, γ- and dimethyl-β-cyclodextrins (α-, β-, γ- and DM-β-CyDs) and liposomes on epimerization or racemization of etoposide, ethiazide and carbenicillin were examined kinetically. α- and β-CyDs accelerated both epimerization and hydrolysis of carbenicillin. They had no effect on epimerization of etoposide, and did not affect racemization and hydrolysis of ethiazide. DM-β-CyD retarded epimerization of etoposide, hydrolysis of picroetoposide (which is an epimer of etoposide), and racemization and hydrolysis of ethiazide, but had no effect on epimerization and hydrolysis of carbenicillin. γ-CyD retarded epimerization of etoposide and hydrolysis of picroetoposide. On the other hand, γ-CyD accelerated epimerization of carbenicillin. It is suggested that the formation of inclusion complexes between CyDs and etoposide, picroetoposide and ethiazide inhibited the attack of bases such as OH^- and buffer components, thereby retarding epimerization, racemization and hydrolysis. On the other hand, α-, β- and γ-CyDs increased the reactivity of carbenicillin through the OH group, accelerating its epimerization and hydrolysis. Liposomes retarded epimerization of etoposide, hydrolysis of picroetoposide and racemization of ethiazide. Liposomes did not affect epimerization and hydrolysis of carbenicillin. These differences in the effect of liposomes on reactivity may be interpreted in terms of the solubility of the drugs.

Keywords epimerization; racemization; hydrolysis; kinetics; etoposide; picroetoposide; ethiazide; carbenicillin; cyclodextrin; liposome

It is well known that for many chiral drugs, one of the optical isomers is different in pharmacological and pharmacokinetic properties from the other.[1,2] The safer and more effective isomer of the two should obviously be administered. It is thus of great importance to elucidate factors affecting epimerization and racemization in order to provide a stable formulation of the desired isomer.

Acid and base catalysis have been found to affect epimerization and racemization of many chiral drugs, including pilocarpine hydrochloride,[3] tetracycline,[4] oxazepam,[5] etoposide,[6] cefsulodine[7] and moxalactam.[8] Human serum albumin (HSA) affects epimerization or racemization of carbenicillin, ethiazide, etoposide.[9]

Cyclodextrins (CyDs) and liposomes have been known to affect the stability of many drugs. Dehydration of prostaglandin E_2 to prostaglandin A_2 and degradation of carmoful are retarded through the formation of the inclusion complex with methylated-β-CyDs[10] β-CyD accelerates hydrolysis of benzylpenicillin and its derivatives.[11] Liposomes affect the stability of 2-diethylaminoethyl *p*-nitrobenzoate and procaine.[12,13] CyDs and liposomes are thus considered to affect epimerization or racemization of chiral drugs.

In this study, the effects of α-, β-, γ- and dimethyl-β-CyDs on epimerization and racemization of etoposide, ethiazide and carbenicillin were examined kinetically and compared with the effects of liposomes. The mechanisms of acceleration or retardation by cyclodextrin and liposomes were also considered.

Materials and Methods

Materials α-, β-, γ-, DM-β-CyDs, egg lecithin and carbenicillin were obtained from Wako Pure Chemical Industries (Osaka, Japan). Ethiazide was a gift from Tokyo Tanabe Pharmaceutical Co. (Tokyo, Japan), and etoposide and picroetoposide were gifts from Nippon Kayaku Co. (Tokyo, Japan). Other chemicals used were of reagent grade. Resolution of the drug isomers was carried out by high-performance liquid chromatography (HPLC).[9]

Preparation of Liposomes Unilamellar liposomes were prepared from egg lecithin by a sonication procedure as described by Finer *et al.*[14] The lecithin was suspended in the buffer solution for kinetic studies and ultrasonically irradiated with a probe-type sonicator (model UR-200P, Tomy Seiko Co., Tokyo, Japan) at 20 kHz and 4 °C under nitrogen. To calculate the volume fraction of lipid phase, the density of lipid was taken as 1.02, and the molecular weight, 800.[12]

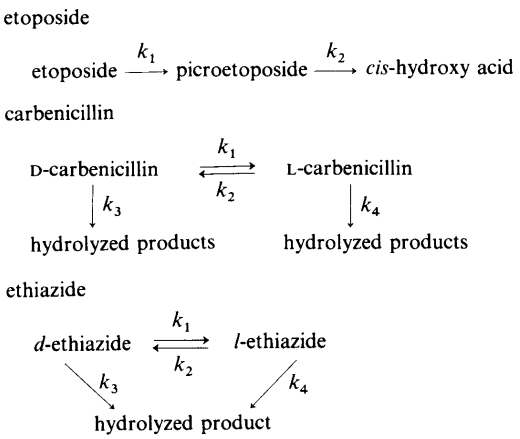

Chart 1

Kinetic Methods The degradation rates of the drugs were measured in buffer solutions at 37 °C and pH 7.4 (0.0667 M phosphate buffer) for ethiazide and carbenicillin, and pH 10.0 (0.2 M borate buffer) for etoposide and picroetoposide. Ionic strength was adjusted to 0.2 with NaCl. The reaction was started by the addition of methanol solutions of ethiazide and etoposide or an aqueous solution of carbenicillin to the solution containing CyD (0—0.05 M) or liposomes (0—2.5%). The final concentration of the drugs in the reaction mixture was 2×10^{-5} M for carbenicillin and ethiazide and 1×10^{-5} M for etoposide. The final concentration of methanol added was 0.5%. Aliquots of the reaction mixture were withdrawn at appropriate intervals and were assayed for the drugs and their degradation products by HPLC.[9]

Estimation of Epimerization or Racemization and Hydrolysis Rate Constants of the Drugs The rate constants for the drugs were estimated from the time-concentration data of both optical isomers of each drug according to the outline shown in Chart 1.[9] A non-linear curve-fitting program was used for the estimation.[15]

Estimation of the Formation Constant of the Drug–CyD Complex and the Rate Constant for the Included Drug When a drug and CyD forms 1 : 1 complex, the observed reaction rate, k_{obs}, can be represented by Eq. 1.[16]

$$k_{obs} = \frac{k_0 + k_c \times K_c \times [\mathrm{CyD}]}{1 + K_c \times [\mathrm{CyD}]} \quad (1)$$

where k_0 and k_c are rate constants for the drug in the solution and that of the drug included in CyD, respectively, and K_c is the formation constant of drug-CyD complex. [CyD] is the concentration of CyD and is approximately equal to the initial concentration of CyD, $[CyD]_0$, when an excess amount of CyD exists in the solution. The values of k_0, k_c and K_c in Eq. 1 were estimated from the dependence of the reaction rate on CyD concentration by non-linear curve fitting.

Estimation of the Rate Constant of the Drug Bound to Liposomes When the reaction of the drug in the presence of liposomes is represented by the partition model, the observed rate constant can be expressed by Eq. 2.[12]

$$k_{obs}=\frac{k_0\times(1-V)+k_c\times K_l\times V}{(1-V)+K_l\times V} \quad (2)$$

where k_0 and k_c are rate constants for the drugs in the solution and in the lipid phase, respectively, K_l is the partition coefficient of the drug and V is the volume fraction of the lipid phase. The values of k_0, k_c and K_l in Eq. 2 were estimated in a similar manner to that used in the analysis of the reaction in the presence of CyD.

Estimation of the Formation Constants of the Drug–CyD Complex by Ultraviolet (UV) Spectroscopy Absorbance of the drugs was measured in the same buffer as used for the kinetic studies in the presence of CyDs at various concentrations. The spectra were recorded immediately after adding a methanol solution of etoposide, picroetoposide or ethiazide, or an aqueous solution of carbenicillin to the buffer. Measurement was carried out twice. Degradation of the drug during measurement was not detected. The change in the absorbance of the drugs at an appropriate wavelength in the presence of CyD was analyzed according to Scott's equation.[17]

Results

Effects of Cyclodextrins on Epimerization and Racemization Figure 1 shows the time-course of epimerization of etoposide in the presence and in the absence of γ- and DM-β-CyD. γ- and DM-β-CyDs retarded epimerization of etoposide. γ- and DM-β-CyDs also retarded hydrolysis of picroetoposide, which is an epimer of etoposide. α- and β-CyDs had no effect on epimerization of etoposide or hydrolysis of picroetoposide. The observed reaction rates of etoposide and picroetoposide decreased with increase in the concentration of γ- and DM-β-CyD, and appeared to reach a plateau at high concentrations. DM-β-CyD retarded racemization and hydrolysis of ethiazide. α-, β- and γ-CyDs did not affect racemization and hydrolysis of ethiazide. The formation constant of CyD–drug complex, K_c, and the rate constant for included drug, k_c, were estimated from CyD concentration dependence of the observed reaction rate according to Eq. 1, and are summarized in Table I. Figures 2 and 3 show typical dependences of the observed reaction rate of etoposide, picroetoposide and ethiazide on the CyD concentration according to Eq. 3, on the assumption that [CyD] can be approximated by $[CyD]_0$. The plots are linear in all cases, indicating the formation of a 1 : 1 complex between the drugs and CyDs.

$$\frac{[CyD]_0}{k_0-k_{obs}}=\frac{1}{k_0-k_c}[CyD]_0+\frac{1}{K_c\times(k_0-k_c)} \quad (3)$$

Figure 4 shows the time-course of epimerization and hydrolysis of carbenicillin in the presence and in the absence of γ-CyD. In contrast to the case of etoposide and ethiazide, α-, β- and γ-CyD accelerated epimerization and hydrolysis of carbenicillin, as shown in Fig. 4. DM-β-CyD had no effect on epimerization and hydrolysis of carbenicillin. The dependence of the reaction rate of carbenicillin on CyD concentration indicated the formation of a 1 : 1 complex between carbenicillin and α-, β- or γ-CyD. Figure 5 shows typical plots of the observed epimerization rates of carbenicillin according to Eq. 3 in the presence of γ-CyD.

UV Spectra of the Drugs in the Presence of CyDs UV spectra of etoposide and picroetoposide were changed in the presence of γ- and DM-β-CyD as shown in Fig. 6. α-

TABLE I. Rate Constants Estimated in the Presence of CyD and Formation Constants of Drug–CyD Complexes

Drug	Additive	k_c (min^{-1})	k_0[a] (min^{-1})	k_c/k_0	K_c (l/mol)
Etoposide					
Epimerization	γ-CyD	2.31×10^{-3}	3.69×10^{-2}	0.0626	34.8
	DM-β-CyD	1.90×10^{-3}		0.0516	47.9
Picroetoposide					
Hydrolysis	γ-CyD	2.21×10^{-3}	1.51×10^{-2}	0.146	55.8
	DM-β-CyD	6.78×10^{-4}		0.0449	195
d-Ethiazide					
Racemization	DM-β-CyD	9.52×10^{-4}	6.09×10^{-3}	0.156	71.6
Hydrolysis	DM-β-CyD	7.85×10^{-4}	2.12×10^{-3}	0.370	
l-Ethiazide					
Racemization	DM-β-CyD	1.30×10^{-3}	6.09×10^{-3}	0.213	70.2
Hydrolysis	DM-β-CyD	4.93×10^{-4}	2.37×10^{-3}	0.208	
D-Carbenicillin					
Epimerization	α-CyD	7.08×10^{-4}	4.88×10^{-4}	1.45	68.9
	β-CyD	1.20×10^{-3}		2.46	48.1
	γ-CyD	2.53×10^{-3}		5.18	33.9
Hydrolysis	α-CyD	3.47×10^{-4}	6.83×10^{-5}	5.08	
	β-CyD	2.13×10^{-4}		3.11	
	γ-CyD	1.51×10^{-4}		2.22	
L-Carbenicillin					
Epimerization	α-CyD	1.03×10^{-3}	5.70×10^{-4}	1.81	48.8
	β-CyD	1.36×10^{-3}		2.39	47.6
	γ-CyD	2.32×10^{-3}		4.07	57.3
Hydrolysis	α-CyD	4.83×10^{-4}	1.03×10^{-4}	4.69	
	β-CyD	7.75×10^{-4}		7.52	
	γ-CyD	2.64×10^{-4}		2.56	

a) Estimated from data in the absence of CyD.

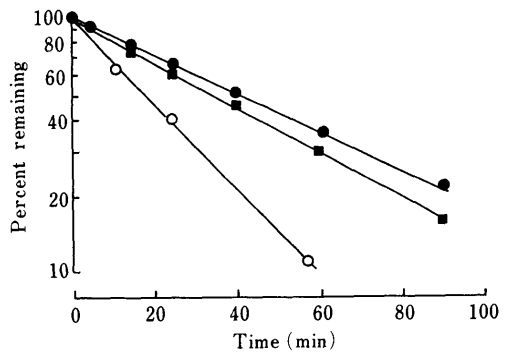

Fig. 1. Time-Courses of Epimerization of Etoposide in the Absence (○) and in the Presence of 0.03 M γ-(■) and DM-β-CyDs (●)

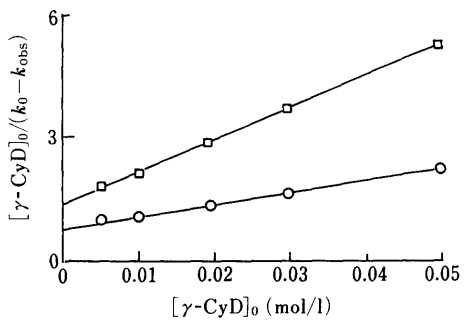

Fig. 2. Effects of γ-CyD on Epimerization of Etoposide (○) and Hydrolysis of Picroetoposide (□) at pH 10.0 and 37 °C

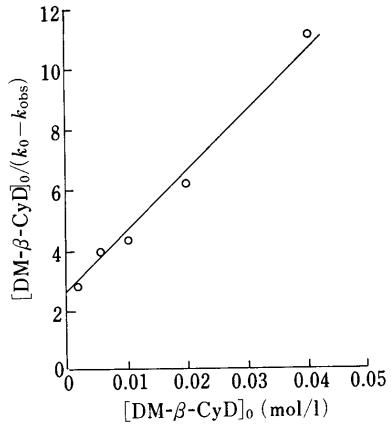

Fig. 3. Effects of DM-β-CyD on Racemization of Ethiazide at pH 7.4 and 37 °C

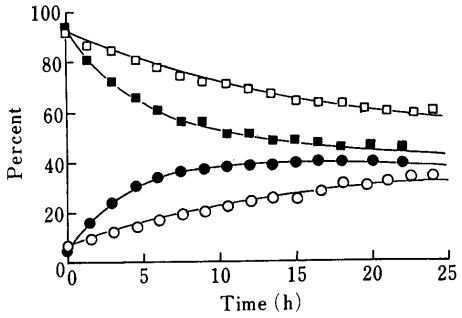

Fig. 4. Time-Courses of Epimerization and Hydrolysis of D-Carbenicillin in the Presence (■, ●) and in the Absence (□, ○) of 0.04 M γ-CyD

□, ■, D-Carbenicillin; ○, ●, L-carbenicillin.

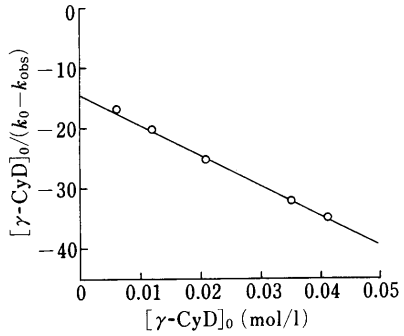

Fig. 5. Effects of γ-CyD on Epimerization of Carbenicillin at pH 7.4 and 37 °C

and β-CyD had no effect on the spectra of etoposide or picroetoposide at the concentration studied. The Scott's plots for the interaction between etoposide or picroetoposide and γ-CyD or DM-β-CyD were linear. The formation constants estimated from the plots agreed with those estimated by the kinetic method. CyDs did not change the UV spectra of ethiazide or carbenicillin under the condition studied.

Effects of Liposomes on Epimerization and Racemization Liposomes retarded epimerization of etoposide and hydrolysis of picroetoposide. Liposomes also retarded racemization of ethiazide, and accelerated hydrolysis of ethiazide at low concentrations of lipid but retarded it at high

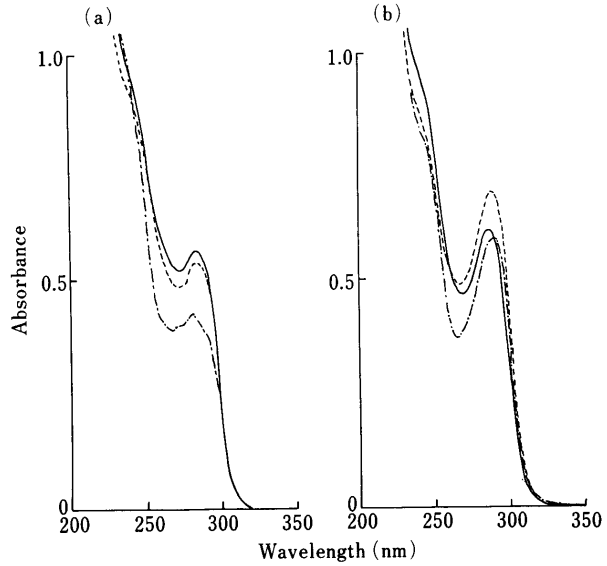

Fig. 6. UV Spectra of Etoposide (a) and Picroetoposide (b) in the Absence (——) and in the Presence of γ- (------) and DM-β-CyD (---)

Etoposide, 8×10^{-5} M; Picroetoposide, 8×10^{-5} M; γ-CyD, 0.03 M; DM-β-CyD, 0.03 M

TABLE II. Rate Constants Estimated in the Presence of Liposomes and Partition Coefficients

Drug	k_c (min^{-1})	k_0 (min^{-1})	k_c/k_0	K_l
Etoposide Epimerization	4.88×10^{-3}	3.69×10^{-2}	0.133	43.7
Picroetoposide Hydrolysis	1.71×10^{-7}	1.51×10^{-5}	1.10×10^{-5}	69.0
d-Ethiazide Racemization	3.20×10^{-9}	6.09×10^{-3}	5.27×10^{-7}	204
l-Ethiazide Racemization	5.63×10^{-9}	6.09×10^{-3}	9.18×10^{-7}	224

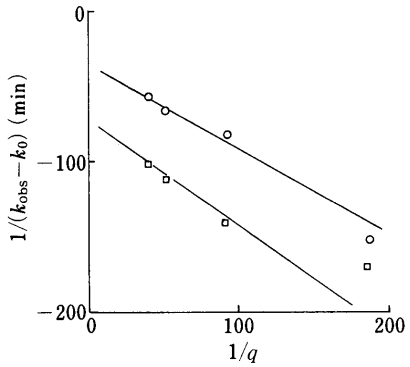

Fig. 7. Effects of Liposomes on Epimerization of Etoposide (○) and Hydrolysis of Picroetoposide (□) at pH 10.0 and 37 °C

concentrations. Epimerization and hydrolysis of carbenicillin were not affected by liposomes. The observed reaction rates for etoposide, picroetoposide and ethiazide were analyzed according to the partition model. The partition coefficient, K_l, and the rate constant for the drug bound to liposomes, k_c, were estimated from the observed reaction rates according to Eq. 2, and are summarized in Table II. Figures 7 and 8 show the dependence of the reaction rates of etoposide, picroetoposide and ethiazide on the volume

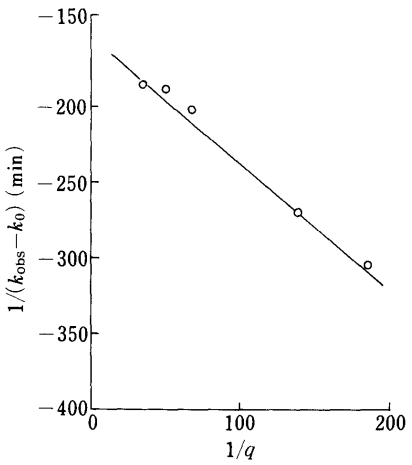

Fig. 8. Effects of Liposomes on Racemization of Ethiazide at pH 7.4 and 37 °C

fraction of the lipid phase according to Eq. 4. The plots were linear with the exception of the plot for picroetoposide at large $1/q$ values.

$$\frac{1}{(k_{obs}-k_0)}=\frac{1}{(k_c-k_0)}+\frac{1}{K_1\times(k_c-k_0)}\cdot\frac{1}{q} \quad (4)$$

where $q=V/(1-V)$.

Discussion

γ-CyD retarded epimerization of etoposide but accelerated that of carbenicillin. This difference may be explained as follows. In the case of etoposide it is considered that proton abstraction at the chiral center by OH^- initiates the epimerization, resulting in the formation of an achiral enol intermediate. It has been reported that CyD inhibits the hydrolysis of some ester compounds by inhibiting the attack of OH^-.[18] Etoposide may form inclusion complexes with γ- and DM-β-CyDs which are less susceptible to the attack of OH^-. On the other hand, in the case of carbenicillin epimerization, it is postulated that the hydroxy group of cyclodextrin may play an important role in the acceleration of the epimerization.This is supported by the fact that the epimerization was not accelerated by DM-β-CyD.

Hydrolysis of picroetoposide was retarded by γ- and DM-β-CyD. Racemization and hydrolysis of ethiazide were retarded by DM-β-CyD. The above argument on retardation of etoposide epimerization should also apply to the case of ethiazide and picroetoposide. DM-β-CyD retarded the reaction of etoposide, picroetoposide and ethiazide but β-CyD had no effect. The formation constants of the complexes between β-CyD and these drugs seem to be small under the conditions studied.

The absorbance of picroetoposide at 290 nm was increased by γ-CyD, and was decreased by DM-β-CyD, whereas the absorbance of etoposide was decreased by both γ- and DM-β-CyD (Fig. 6). The effects of CyDs on the UV spectra suggest that picroetoposide interacts with γ-CyD and DM-β-CyD at different sites of the molecule. This speculation is supported by the fact that the formation constants, K_c, of picroetoposide were different with γ-CyD and DM-β-CyD, while those of etoposide were not different between the two CyDs (Table I).

Liposomes retarded the epimerization of etoposide and the hydrolysis of picroetoposide, whereas epimerization and hydrolysis of carbenicillin was not affected. This may be interpreted in the term of solubility. Etoposide and picroetoposide are practically insoluble in water. In the presence of liposomes, they may exist in the lipid phase rather than the water phase, so that the attack of OH^- and water molecules is inhibited.[12] By contrast, the aqueous solubility of carbenicillin is high and this compound is most likely associated with the aqueous, rather than lipid phase, of the liposomes. The effects of liposomes on the racemization and the hydrolysis of ethiazide are complicated, and further experimental investigation may be needed to interpret them.

References

1) D. E. Drayer, *Clin. Pharmacol. Ther.*, **40**, 125 (1986).
2) M. Simonyi, *Med. Res. Rev.*, **4**, 359 (1984).
3) S. Yoshioka, Y. Aso, T. Shibazaki and M. Uchiyama, *Chem. Pharm. Bull.*, **34**, 4280 (1986).
4) R. B. Taylor, D. G. Durham and A. S. H. Shivji, *Int. J. Pharm.*, **26**, 259 (1985).
5) Y. Aso, S. Yoshioka, T. Shibazaki and M. Uchiyama, *Chem. Pharm. Bull.*, **36**, 1834 (1988).
6) Y. Aso, Y. Hayashi, S. Yoshioka, Y. Takeda Y. Y. Kita, Y. Nishimura and Y. Arata, *Chem. Pharm. Bull.*, **37**, 422 (1989).
7) T. Fujita and A. Koshiro, *Chem. Pharm. Bull.*, **32**, 3651 (1984).
8) N. Hashimoto, T. Tasaki and H. Tanaka, *J. Pharm. Sci.*, **73**, 369 (1984).
9) Y. Aso, S. Yoshioka and Y. Takeda, *Chem. Pharm. Bull.*, submitted.
10) K. Uekama and M. Otagiri, "CRC Critical Reviews in Therapeutic Drug Carriers," Vol. 1, CRC Press Inc., Boca Raton, 1987, pp. 1—40.
11) D. E. Tutt and M. A. Schwartz, *J. Am. Chem. Soc.*, **93**, 767 (1971).
12) T. Yotsuyanagi, T. Hamada, H. Tomida and K. Ikeda, *Acta Pharm. Suec.*, **16**, 325 (1979).
13) T. Yotsuyanagi, T. Hamada, H. Tomida and K. Ikeda, *Acta Pharm. Suec.*, **16**, 271 (1979).
14) E. G. Finer, A. G. Flook and H. Hauser, *Biochim. Biophys. Acta*, **260**, 49 (1967).
15) K. Yamaoka, Y. Tanigawara, T. Nakagawa and T. Uno, *J. Pharmacobio-Dyn.*, **4**, 879 (1981).
16) S. Tanaka, K. Uekama and K. Ikeda, *Chem. Pharm. Bull.*, **24**, 2825 (1976).
17) R. L. Scott, *Rec. Trav. Chim.*, **75**, 787 (1956).
18) T. Chin, P. Chung and J. L. Lach, *J. Pharm. Sci.*, **57**, 44 (1968).