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Enantioselective Distribution of Amino-Alcohols in a Liquid–Liquid Two-Phase System Containing Dialkyl L-Tartrate and Boric Acid

Yoshihiro ABE,* Tomoko SHOJI, Michi KOBAYASHI, WANG Qing, Naoko ASAI, and Hideyuki NISHIZAWA

Kyoritsu College of Pharmacy, 1–5–30 Shibakoen, Minato-ku, Tokyo 105, Japan.

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Racemic amino-alcohols such as pindolol, propranolol, alprenolol and bucumolol enantiomers exhibited different distribution behaviors in a two-phase system consisting of a chloroform solution of didodecyl L-tartrate and an aqueous solution of boric acid. It seemed that a borate complex of the 1,2-diol group of the tartrate and the amino-alcohol was formed in the system. In the case of pindolol, one enantiomer was preferentially extracted into the organic phase (×2.20) at equilibrium.

Key words didodecy L-tartrate; boric acid; amino-alcohol; enantioseparation; liquid–liquid extraction

Tartaric acid and its derivatives are widely used for enantioseparation in liquid[1] or gas chromatography.[2] However, there have been no reports of enantioseparation by the simultaneous use of tartaric acid derivatives with boric acid, although it is well-known that boric acid forms complexs with 1,2-diols or β-amino-alcohol groups. We previously reported a successful continuous enantioseparation method for racemates using dual-flow countercurrent extraction.[3] To widen the applicability of this method, the distribution of racemate enantiomers in a liquid–liquid two-phase system in the presence of chiral compounds has been investigated. We found that didodecyl L-tartrate exhibited a distinctive chiral discrimination with respect to racemic β-amino-alcohols in the liquid–liquid two-phase system in the presence of boric acid. A similar phenomenon has been observed in the presence of dialkyl L-tartrate and sodium hexafluorophosphate, where it was estimated that the tartrate formed a complex with an amino-alcohol in the organic phase due to hydrogen bonding.[4] When boric acid is present, however, we believed that a different type of chiral recognition would occur. Namely, that a borate complex (Fig. 1) would formed for the following reasons: (1) boric acid can be extracted into organic solvents in the presence of 1,2- or 1,3-diols,[5] (2) boric acid forms a bisdiol borate (2 : 1 complex) or trisdiol diborate (3 : 2 complex) in chloroform after azeotropic removal of water,[6] (3) the 1 : 2 complex of ethyleneglycol dissolves in benzene or chloroform, although the 3 : 2 complex is insoluble in chloroform,[6] and, from our own results, (4) about 31% of boric acid is extracted into the organic phase when there are high concentrations of an amino-alcohol in the two-phase system, (5) the enantioselective distribution is not observed when no boric acid is present in buffered solutions with the same pH, (6) carbonyl groups of the tartrate are not necessary for enantioselective distribution,[7] and (7) at a concentration of 100 mM tartrate, no enantioselective distribution of (±)-pindolol was observed when boric acid was replaced by sodium hexafluorophosphate.

In this report, we describe the enantioselective distribution of amino-alcohols in a liquid–liquid two-phase system where we believed that a borate complex would be formed between β-amino-alcohols and dialkyl L-tartrates in the organic phase.

Experimental

Pindolol, propranolol hydrochloride, alprenolol hydrochloride, dimethyl L-tartrate, diethyl L-tartrate and dibutyl L-tartrate were purchased from Aldrich Chemical Co. (U.S.A.). L-Tartaric acid, 1-propanol, 1-octanol, 1-dodecyl alcohol, cyclohexanol, benzyl alcohol, thionylchloride, boric acid and chloroform were obtained from Wako Pure Chemicals (Osaka), and used without further purification. Bucumolol was kindly supplied by Sankyo Co., Ltd. (Tokyo). After addition of 1 M sodium hydroxide solution, an aqueous solution of propranolol hydrochloride was extracted with ether to obtain propranolol free base. This was recrystallized from dichloromethane and *n*-hexane.

Dipropyl L-tartrate was prepared by condensation of the acid with 1-propanol using thionylchloride and then purified by distillation under reduced pressure (method A). The other dialkyl L-tartrate was prepared by esterification of L-tartaric acid with alcohol in benzene using a catalytic amount of *p*-toluene sulfonic acid in a Dean–Stark apparatus (method

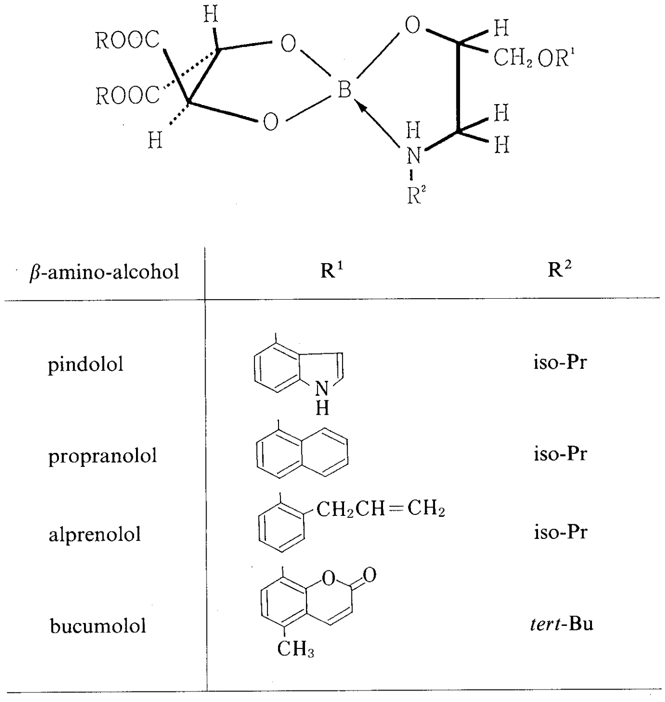

Fig. 1. Postulated Structure of the Borate Complex of Didodecyl L-tartrate (R = dodecyl) and β-Amino Alcohol in the Organic Phase

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* To whom correspondence should be addressed.

Table I. Melting Points, Optical Rotations and Distirbution Ratios of Dialkyl L-Tratrates

R	Preparation method[a]	mp (bp/Torr)°C[b]	$[\alpha]_D^{25}$ [c]	Distribution ratio[d]	
				B^+	B^-
CH_3	P	57—60 (dec.)[e]	21 (c=2.5, H_2O)[e,f]	0.12	0.14
C_2H_5	P	(280)[e]	8.5 (neat)[e,g]	2.11	2.25
n-C_3H_7[h]	A	(176/10 Torr)	20.9	14.7	9.91
n-C_4H_9[i]	A	(194/10 Torr)	18.6	≫	
n-C_8H_{17}[j]	B	41	12.3	≫	
n-$C_{12}H_{25}$[h]	B	64	9.7	≫	
Cyclohexyl[l]	B	67	21.7	≫	
Benzyl[m]	B	58	11.7	≫	

a) P, purchased sample. Method A: condensation using thionyl chloride. Method B: acid catalyzed esterification involving removing water by azeotropic distillation with benzene. *b*) Uncorrected. *c*) c=2.0, acetone, 25 °C. *d*) Distribution ratios are expressed as C_{org}/C_{aq}, where C_{org} and C_{aq} are the concentrations of tartrate in the organic and aqueous phase, respectively. They were determined by an evaporation-weighing procedure. Thus 5 ml of the organic phase at the equilibrium state was evaporated to dryness and weighed to obtain C_{org}. Then C_{aq} was calculated from the concentration of tartrate at the beginning (C_o) and C_{org}. B^+ and B^- indicate the presence of boric acid (B^+) or not (B^-) in the aqueous phase. Distribution of boric acid into the organic phase in the presence of tartrate was shown to be negligible by the titration experiment. In the case of ≫, the distribution ratio of the tartrate was over 50. *e*) "Catalog and Handbook of Fine Chemicals," Aldrich Chemical Co. *f*) 22 °C. *g*) 20 °C. *h*) bp 181 °C/22 Torr, $[\alpha]_D^{23}$ 6.8 (c=5.4917, $CHCl_3$), *Beil.*, **3**, 516 (1921). *i*) bp 175 °C/5 Torr, $[\alpha]_D^{20}$ 11.5 (neat), lit. *a*). *j*) mp 43—44 °C. $[\alpha]_D$ 13 (c=1.3, acetone), ref. 4*a*). *k*) mp 53—55 °C *Beil.*, E III, **3**, 1022 (1962). *l*) mp 53—54, $[\alpha]_D^{22}$ 14.9 (c=1.04 methanol), H. Heldin, K. J. Lindner, C. Pettersson, W. Lindner, R. Rao, *Chromatographia*, **32**, 407 (1991). *m*) mp 67—69 °C, $[\alpha]_D^{25}$ 12.6 (c=0.3976, ethanol), H.L. Smith, E. S. Brown, J. D. Smith, J. Andrako, *J. Pharm. Sci.*, **54**, 1269 (1965).

Table II. Distribution Ratios (D_1, D_2) and Separation Factors (α) of Amino-alcohols in the L-DDT Chloroform–100 mM Aqueous Boric Acid Two-Phase System

Amino-alcohol	Distribution ratio of amino-alcohol enantiomers					
	A			B		
	D_1	D_2	α	D_1	D_2	α
Pindolol	0.791	1.744	2.20	0.093	0.145	1.56
Propranolol	0.170	0.432	2.54	1.565	4.508	2.88
Alprenolol	0.158	0.359	2.27	1.787	4.262	2.38
Bucumolol	—[a]	—[a]	—	0.356	0.851	2.39

Two phase system: A, 100 mM boric acid aqueous solution (pH 5.2)–100 mM L-DDT in $CHCl_3$; B, 100 mM boric acid in 50 mM phosphate buffer (pH 5.2)–100 mM L-DDT in $CHCl_3$. The concentration of amino-alcohol was 0.5 mM in the total volume of both phases. *a*) Distribution ratio was too small to measure.

B). The physical properties of the dialkyl L-tartrates obtained are summarized in Table I.

The extraction experiments on the racemic amino alcohols were performed in test-tubes fitted with glass stoppers using the two-phase system, with or without L-tartrates in the organic phase, and with boric acid in the aqueous phase. After shaking in a water bath thermostatted at 25 °C for 16 h, the two phases were separated and the enantiomers in the aqueous phase were analyzed by HPLC. The distribution ratios of the sample enantiomers were determined chromatographically.[3] These are expressed as:

$$D=\frac{[aa]_{org}}{[aa]_{aq}}$$

where $[aa]_{org}$ and $[aa]_{aq}$ are the concentrations of amino-alcohol enantiomers in the organic and aqueous phases, respectively.

For HPLC analyses, a JASCO 980 HPLC system (Japan Spectroscopic Co., Ltd., Tokyo) was used equipped with Rheodyne sampling valve (20 μl) and a Chiralcel OD-R column (4.6 i.d. × 250 mm) for pindolol and propranolol (Daicel, Japan; eluent, 0.1 M potassium hexafluorophosphate : acetonitrile = 60 : 40) or Ultron ES-PhCD (4.6 i.d. × 250 mm) for alprenolol and bucumolol (Shinwa Chemical Industry, Japan; eluent, 20 mM phosphate buffer pH 4.6 : acetonitrile = 80 : 20 or 75 : 25).

After shaking (16 h) an equal volume (50 ml) of aqueous boric acid (100 mM) and of a chloroform solution of didodecyl L-tartrate (L-DDT, 100 mM), with or without propranolol (50 mM free base in the organic phase), the boric acid in 15 ml aqueous phase was titrated with 0.1 M sodium hydroxide with using glycerin and phenolphthalein as an indicator.[8]

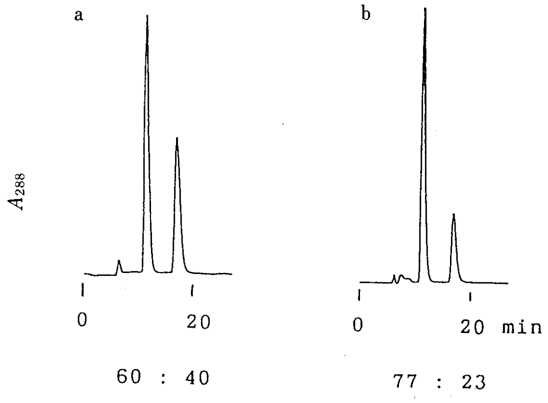

Fig. 2. Chromatograms of the Aqueous Phases After Batch Extraction (a, One Time; b, Five Times) of (±)-Pindolol with a Chloroform Solution of L-DDT (100 mM)

The concentration of pindolol was 0.5 mM, and the aqueous phase was 100 mM with respect to boric acid. The ratio of the areas of the chromatographic peaks are indicated below.

Results and Discussion

Enantioselective distribution was observed in the liquid–liquid two-phase system composed of a chloroform solution of didodecyl L-tartrate (100 mM) and an aqueous boric acid (100 mM) solution (Fig. 2 and Table II).

To investigate the enantioselectivity, L-DDT was used as a separator, and (±)-pindolol was used for the racemates unless otherwise stated.

Importance of Boric Acid for Enantioselective Distribu-

Table III. Distribution of (±)-Pindolol Enantiomers in the Two-Phase System Composed of 100 mM L-DDT in Chloroform Solution and Some Aqueous Solutions

Aqueous phase	pH	Distribution ratio of the enantiomer of pindolol in the presence of L-DDT		
		D_1	D_2	α
100 mM boric acid	5.2	0.791±0.013	1.744±0.026	2.20
100 mM acetate buffer	5.2	0.051±0.007	0.038±0.008	0.75[a]
100 mM phosphate buffer	5.2	0.048±0.003	0.055±0.006	1.15[a]
100 mM KPF_6	3.2	0.061±0.018	0.053±0.017	0.87[a,b]
100 mM $NaPF_6$	3.5	0.044±0.009	0.041±0.009	0.93[a,b]
500 mM $NaPF_6$	3.9	0.074±0.020	0.071±0.017	0.99[a,b]

Organic phase, 100 mM DDT in $CHCl_3$; pindolol, 0.5 mM in the total volume of the both phases; α, separation factor $= D_2/D_1$, at 25 °C. *a*) Experimental error is large because the values of the distibution ratio are very small. *b*) Distribution experiments were performed at 4 °C.

tion in the System The distribution ratios of pindolol enantiomers were large and enantioselective distribution was obtained when the aqueous phase contained boric acid, but they decreased markedly and the enantioselectivity disappeared when the aqueous phase was replaced by an acetate or phosphate buffer (Table III). In the case of the buffers in Table III, the distribution ratios themselves were very small and there were no enantioselective distribution even in the buffer with a different pH which included no borate ion.

Prelog *et al.* reported the enantioselective distribution of norephedrine in a liquid–liquid two-phase system composed of a 1 M solution of di-5-nonyl L-tartrate in 1,2-dichloroethane and 0.5 M aqueous hexafluorophosphate solution at 4 °C.[4a] Compared with their results, where the selectivities varied from 1.0 ((±)-phenylethyl amine) to 1.51 (norephedrine), enantioselective distribution was observed in our study at much lower concentrations of both of tartrate and boric acid (0.025 M[9] or 0.04 M, *cf.* Table IV). At an L-DDT concentration of 100 mM, no enantioselective distribution of (±)-pindolol was observed even at 4 °C in the presence of 0.5 M sodium hexafluorophosphate. However, an α of 2.5 was obtained when the aqueous phase was replaced by a boric acid solution at 4 °C.

By titrating boric acid in the aqueous phase after extraction with 100 mM L-DDT chloroform solution, it was found that most of the boric acid was retained in the aqueous phase (nearly 99%). However, about 31% of boric acid moved into the organic phase when 50 mM propranolol (free base) was present in the organic phase before extraction. This shows that borate complex formation of L-DDT itself in the organic phase was negligible, but, following the addition of propranolol to the two-phase system, coextraction of boric acid with L-DDT and propranolol occurs.

We recently observed that chiral 1,2-diols such as (2*R*,3*R*)-hydrobenzoin play a similar role in the two-phase system,[7] indicating that the carbonyl groups of tartrate are not necessary for chiral recognition in the system. For such recognition, we postulated that the borate complex shown in Fig. 1 must be the most important factor, although various types of boric acid esters with 1,2-diols are known.[6]

Effect of the Boric Acid Concentration The effect of the boric acid concentration on chiral discrimination was studied (Fig. 3). When the concentration of L-DDT and (±)-pindolol were set at 100 and 0.5 mM, respectively, the changes in the separation factor, α, became small when the molar ratio of boric acid to L-DDT was greater than 0.5.

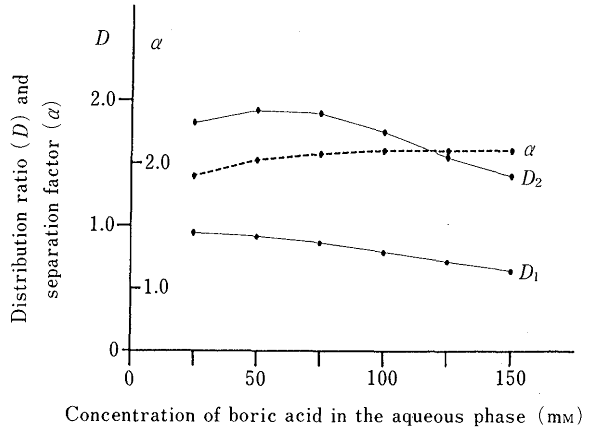

Fig. 3. Effect of the Concentration of Boric Acid in the Aqueous Phase on the Distribution Ratios (D_1 and D_2) and Separation Factors (α) of Pindolol Enantiomers

Organic phase, 100 mM L-DDT in chloroform; sample, 0.5 mM (±)-pindolol in the total volume of the two phases, at 25 °C.

Organic Solvent The enantioselective distribution of (±)-pindolol was studied on changing the organic solvent. It was found that dichloromethane, chloroform, chlorobenzene and nitrobenzene were good solvents (Table IV). In order to compare the effect of the organic solvent, the concentration of L-DDT and also that of boric acid was set at 40 mM because of the solubility of L-DDT in some organic solvents.

Effect of Temperature The lower the temperature, the larger the enantioselectivity became (Table V). The distribution ratios were also large at low temperatures. It is known that the total amount of boric acid extracted into the organic phase decreases with increasing temperature when boric acid is extracted with 50% 2-ethylhexanol in a trimethylbenzene mixture.[6c]

Effect of the Ester Alkyl Moiety In the case of dimethyl L-tartrate which distributed mostly into the aqueous phase (D = 0.12) in the two-phase system, enantioselectivity almost disappeared (*cf.* Table I). When

TABLE IV. Effect of the Organic Solvent

Solvent	Distribution ratio of pindolol enantiomers		
	D_1	D_2	α
CH_2Cl_2	0.596	0.964	1.62
$CHCl_3$	0.471	0.727	1.54
CCl_4	0.070	0.088	1.11
ClH_2CCH_2Cl	0.414	0.672	1.62
Cl_3CCH_3	0.165	0.173	1.05
1-Butanol	2.324	2.410	1.04
Diisopropyl ether	0.107	0.114	1.07
2-Butanone	1.312	1.306	0.98
4-Methyl-2-pentanone	0.059	0.056	0.95
Toluene	0.087	0.088	1.01
Chlorobenzene	0.117	0.340	2.91
Nitrobenzene	0.549	0.787	1.43

Aqueous phase, 40 mM boric acid; organic phase, 40 mM L-DDT in the organic solvent; (±)-pindolol, 0.5 mM in the total volume of the both phases; α, separation factor $= D_2/D_1$, at 25 °C.

TABLE V. Effect of Temperature

Temperature (°C)	Distribution ratio of pindolol enantiomers		
	D_1	D_2	α
10	1.170	2.895	2.47
25	0.791	1.744	2.20
40	0.701	1.215	1.73

Organic phase, 100 mM L-DDT in $CHCl_3$; aqueous phase, 100 mM boric acid; (±)-pindolol, 0.5 mM in the total volume of the both phases; α, separation factor $= D_2/D_1$.

TABLE VI. Effect of the Ester Alkyl Moiety

Tartrate alkyl moiety R	Distribution ratio of pindolol enantiomers		
	D_1	D_2	α
CH_3	0.057	0.049	0.86
C_2H_5	0.196	0.422	2.15
n-C_3H_7	0.739	1.713	2.32
n-C_4H_9	0.838	1.874	2.24
n-C_8H_{17}	0.766	1.755	2.29
n-$C_{12}H_{25}$	0.790	1.730	2.20
Cyclohexyl	0.577	1.295	2.24
Benzyl	1.055	2.419	2.29

Organic phase, 100 mM L-DDT in $CHCl_3$; aqueous phase, 100 mM boric acid; (±)-pindolol, 0.5 mM in the total volume of the both phases; α, separation factor $= D_2/D_1$.

the chain length of the alkyl moiety of the ester group became longer than two carbon atoms, where the concentration of tartrates in the organic phase was considered to be large enough compared with that of pindolol, the separation factor (α) was about 2.2, and there was little change in α. This indicates the complex formation occurred some distance from the ester group, which agrees with the recent observation that the 1,2-diol group is important in the enantioselective distribution of amino-alcohols in liquid–liquid two-phase systems composed of chloroform and aqueous boric acid.[7]

Conclusion

In conclusion, we have demonstrated that the enantioselective distribution of enantiomers of β-amino-alcohols occurrs in the liquid–liquid two-phase system. We believe that the borate complex of the diol group of L-tartrate and the amino-alcohol is formed in the organic phase which then results in the enantioselective distribution of the racemates. Application of this finding to the continuous enantioseparation of racemic amino-alcohols using dual-flow countercurrent extraction is now under investigation.

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