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Ion Pair Extraction of Trimethoprim with Sulfonphthalein Dyes[1]

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Ion pair extraction of a basic drug (trimethoprim) with sulfonphthaleins (BTB, BCG and BPB) was examined on the basis of equation (1).

$$\frac{[\mathrm{IH}^-]_\mathrm{w}}{Q} = \left\{\frac{(p+1)K_{\mathrm{BH}^+}}{K_{\mathrm{ex}}f}\right\}\frac{1}{(a_\mathrm{H})_\mathrm{w}} + \frac{1}{K_{\mathrm{ex}}f^2} \qquad (1)$$

The molar ratio, Q, of the extracted ion pair ($Q=[\mathrm{BH^+IH^-}]_\mathrm{org}/(C_\mathrm{B}-[\mathrm{BH^+IH^-}]_\mathrm{org})$) was represented as a function of the hydrogen ion activity ($(a_\mathrm{H})_\mathrm{w}$) and the concentration of the sulfonphthalein monoanion ($[\mathrm{IH}^-]_\mathrm{w}$). The extraction constant, K_ex, of the equilibrium (2) was estimated from equation (1).

$$\mathrm{BH^+_w} + \mathrm{IH^-_w} \underset{}{\overset{K_\mathrm{ex}}{\rightleftarrows}} \mathrm{BH^+IH^-_{org}} \qquad (2)$$

The log K_ex values of TMP-BTB were found to be 5.26, 7.08 and 8.10 in benzene–H_2O, 1,2-dichloroethane–H_2O and $CHCl_3$–H_2O systems, respectively. The log K_ex values of TMP-BCG and TMP-BPB in the 1,2-dichloroethane–H_2O system were found to be 5.89 and 4.95, respectively. The effect of pH on the ion pair extraction is discussed in terms of equation (1).

The pH values suitable for the assay of TMP with BTB by means of ion pair extraction were found to be in the range of 4 to 6.

Keywords——trimethoprim; ion pair extraction; sulfonphthalein dyes; effect of pH on ion pair extraction; extraction constant

Organic bases and quarternary ammonium compounds form ion pairs with some acidic dyes, such as sulfonphthaleins.[3–8] These compounds can be determined by extracting the ion pair into an organic solvent. The ion pair extraction method has been effectively used as a means of drug analysis because it offers high sensitivity and simplicity. It has been pointed out that the formation of such ion pairs is highly dependent on pH.[9,10] Schill[4] studied the effect of pH on the ion pair formation of amine derivatives with bromothymol blue (BTB). He suggested that the optimum pH for the determination was in the range from 7 to 8. An ion pair extraction method for the assay of trimethoprim (TMP)[11] using BTB was developed by the investigators of F. Hoffmann-La Roche and Co., A. G.[12] They selected pH 7.5 as a suitable condition for the assay. However, it has been found by us that only 44% of the TMP-BTB ion pair is extracted at this pH. Our studies developed out of

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our interest in determining the optimum pH values for the formation of ion pairs between TMP and some sulfonphthaleins.

Theoretical

The acid-base equilibria of the base and the acidic dye in aqueous solution are shown as equilibria (I), (II) and (III), respectively.

$$\mathrm{BH^+} \overset{K_{\mathrm{BH^+}}}{\rightleftarrows} \mathrm{H^+} + \mathrm{B} \qquad \text{(Equil. I)}$$

$$\mathrm{IH_2} \overset{K_{\mathrm{IH_2}}}{\rightleftarrows} \mathrm{H^+} + \mathrm{IH^-} \qquad \text{(Equil. II)}$$

$$\mathrm{IH^-} \overset{K_{\mathrm{IH^-}}}{\rightleftarrows} \mathrm{H^+} + \mathrm{I^{2-}} \qquad \text{(Equil. III)}$$

where B and $\mathrm{BH^+}$ are the base and its conjugate acid, respectively, and $\mathrm{IH_2}$, $\mathrm{IH^-}$ and $\mathrm{I^{2-}}$ are the uncharged species and the two kinds of dissociated anions of the sulfonphthalein dye. When an immiscible organic solvent is added to an aqueous solution of the base containing the dye, the ion pair, $\mathrm{BH^+IH^-}$, is transferred into the organic phase. Extraction of the ion pair is expressed by the equilibrium,

$$\mathrm{BH^+}_{\mathrm{w}} + \mathrm{IH^-}_{\mathrm{w}} \overset{K_{\mathrm{ex}}}{\rightleftarrows} \mathrm{BH^+IH^-}_{\mathrm{org}} \qquad \text{(Equil. IV)}$$

where the subscripts, w and org, refer to the aqueous and the organic phases, respectively.

The equilibrium constants of the equilibria (I), (II), (III) and (IV) are defined as:

$$K_{\mathrm{BH^+}} = \frac{[\mathrm{H^+}]_{\mathrm{w}}[\mathrm{B}]_{\mathrm{w}} f_{\mathrm{H^+}}}{[\mathrm{BH^+}]_{\mathrm{w}} f_{\mathrm{BH^+}}} \qquad (1)$$

$$K_{\mathrm{IH_2}} = \frac{[\mathrm{H^+}]_{\mathrm{w}}[\mathrm{IH^-}]_{\mathrm{w}} f_{\mathrm{H^+}} f_{\mathrm{IH^-}}}{[\mathrm{IH_2}]_{\mathrm{w}}} \qquad (2)$$

$$K_{\mathrm{IH^-}} = \frac{[\mathrm{H^+}]_{\mathrm{w}}[\mathrm{I^{2-}}]_{\mathrm{w}} f_{\mathrm{H^+}} f_{\mathrm{I^{2-}}}}{[\mathrm{IH^-}]_{\mathrm{w}} f_{\mathrm{IH^-}}} \qquad (3)$$

$$K_{\mathrm{ex}} = \frac{[\mathrm{BH^+IH^-}]_{\mathrm{org}}}{[\mathrm{BH^+}]_{\mathrm{w}}[\mathrm{IH^-}]_{\mathrm{w}} f_{\mathrm{BH^+}} f_{\mathrm{IH^-}}} \qquad (4)$$

The partition coefficient, p, is,

$$p = \frac{[\mathrm{B}]_{\mathrm{org}}}{[\mathrm{B}]_{\mathrm{w}}} \qquad (5)$$

In these equations, $K_{\mathrm{BH^+}}$, $K_{\mathrm{IH_2}}$ and $K_{\mathrm{IH^-}}$ are the dissociation constants and K_{ex} is the so-called extraction constant, which represents the facility of ion pair formation. The activity coefficient is shown as f for the corresponding ion. Under the condition that the volumes of the aqueous and organic phases are equal, a quantity, Q, is defined as

$$Q = \frac{[\mathrm{BH^+IH^-}]_{\mathrm{org}}}{[\mathrm{BH^+}]_{\mathrm{w}} + [\mathrm{B}]_{\mathrm{w}} + [\mathrm{B}]_{\mathrm{org}}} \qquad (6)$$

A relationship between Q and $[\mathrm{H^+}]_{\mathrm{w}}$ can be derived from Eqs. (1), (4), (5) and (6).

$$\frac{[\mathrm{IH^-}]_{\mathrm{w}}}{Q} = \left\{ \frac{(p+1)K_{\mathrm{BH^+}}}{K_{\mathrm{ex}} f_{\mathrm{IH^-}} f_{\mathrm{H^+}}} \right\} \frac{1}{[\mathrm{H^+}]_{\mathrm{w}}} + \frac{1}{K_{\mathrm{ex}} f_{\mathrm{IH^-}} f_{\mathrm{BH^+}}} \qquad (7)$$

Eq. (7) means that a plot of $[\mathrm{IH^-}]_{\mathrm{w}}/Q$ against $1/(a_{\mathrm{H}})$ (a_{H}: hydrogen ion activity, $[\mathrm{H^+}]f_{\mathrm{H^+}}$) should be linear with a slope of $\{(p+1)K_{\mathrm{BH^+}}\}/(K_{\mathrm{ex}} f_{\mathrm{IH^-}})$ and with an intercept of $1/(K_{\mathrm{ex}} f_{\mathrm{IH^-}} f_{\mathrm{BH^+}})$, if the ionic strength of the aqueous phase is kept constant.

Results and Discussion

Extraction of TMP-BTB

The ion pair of TMP with BTB was formed at pH below 8.5 in the presence of the monoanion (IH^-) of BTB. The solutions of TMP and BTB were added to buffer solutions ranging in pH from 5.4 to 8.5. The ionic strength (μ) of the solution was adjusted to 0.2 by the addition of NaCl. Benzene, 1,2-dichloroethane and $CHCl_3$ were used for extraction of the ion pair from the aqueous solution. After extraction, the Q value and the concentration of the monoanion in the aqueous phase, $[IH^-]_w$, were determined. Eq. (6) was converted into Eq. (8) using the total concentration of the base, C_B,

$$Q = \frac{[BH^+IH^-]_{org}}{C_B - [BH^+IH^-]_{org}} \qquad (8)$$

where $C_B = [BH^+]_w + [B]_w + [BH^+IH^-]_{org} + [B]_{org}$. The ion pair concentration, $[BH^+IH^-]_{org}$, was measured spectrophotometrically and the Q value was calculated. A calibration curve for $[BH^+IH^-]_{org}$ in a given solvent was made using the 1: 1 complex of TMP and BTB, which was prepared separately. When K_{IH^-} and the molar extinction coefficients for the monoanion (IH^-) and the dianion (I^{2-}) of the dye, ε_{IH^-} and $\varepsilon_{I^{2-}}$, are known, $[IH^-]_w$ is calculable from Eq. (3).

A plot of $[IH^-]_w/Q$ against $1/(a_H)_w$ for the benzene–H_2O system is shown in Fig. 1. A good linear relationship was obtained (Table I). The K_{ex} value was estimated from the intercept ($1/(K_{ex} f_{IH^-} f_{BH^+})$) of the straight line; log $K_{ex} = 5.30 \pm 0.02$. According to Debye and Hückel, the activity coefficient of uni-univalent electrolytes in aqueous solution (at 25°) is related to the ionic strength by[13]

$$-\log f = \frac{0.512\sqrt{\mu}}{1+1.6\sqrt{\mu}} \qquad (9)$$

$$(f = f_{IH^-} = f_{BH^+} = f_{H^+})$$

The log K_{ex} value can also be estimated from the slope of the straight line using

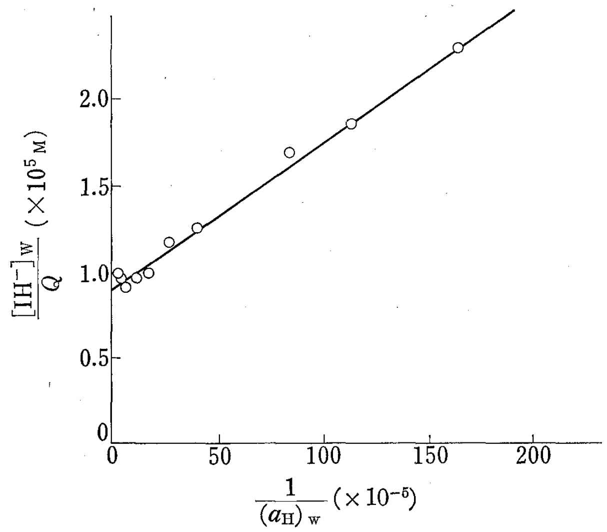

Fig. 1. Plot of $[IH^-]_w/Q$ against $1/(a_H)_w$ for TMP-BTB in the Benzene–H_2O System

TABLE I. Relationship between $[IH^-]_w/Q$ and $1/(a_H)_w$ for TMP-BTB

	Solvent system		
	Benzene/H_2O	1,2-Dichloroethane/H_2O	$CHCl_3/H_2O$
Slope	8.115×10^{-13}	6.537×10^{-14}	3.528×10^{-14}
Intercept	9.226×10^{-6}	3.93×10^{-7}	—[b]
Correlation coefficient (r)	0.995	0.998	0.998
Standard deviation (σ)	0.455×10^{-6}	1.10×10^{-7}	1.695×10^{-7}
n	10	11	11
log K_{ex} (from the slope)	5.26 ± 0.03[a]	7.08 ± 0.02[a]	8.10 ± 0.02[a]
log K_{ex} (from the intercept)	5.30 ± 0.02	6.7 ± 0.2	

a) 95% confidence limit.
b) Intercept=0.

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13) A. Albert and E.P. Serjeant, "The Determination of Ionization Constants," 2nd ed., Chapman and Hall Ltd., London, 1971, p. 28.

K_{BH^+} and p. The values of K_{BH^+} and p were spectrophotometrically measured separately. The estimated log K_{ex} value, 5.26±0.03, was in fair agreement with that obtained from the intercept of the straight line.

The plots for the other solvent systems, 1,2-dichloroethane–H_2O and $CHCl_3$–H_2O, also yielded straight lines in the same manner. The log K_{ex} values estimated are shown in Table I. The base, TMP, was extracted as its ion pair in the following order of effectiveness: $CHCl_3 > $1,2-dichloroethane$>$benzene.

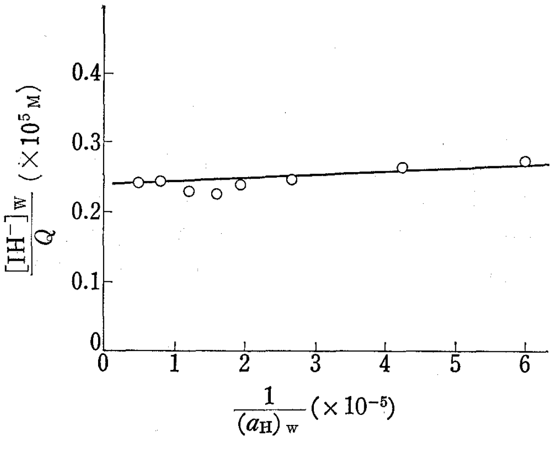

Fig. 2. Plot of $[IH^-]_w/Q$ against $1/(a_H)_w$ for TMP-BCG in the 1,2-Dichloroethane–H_2O System

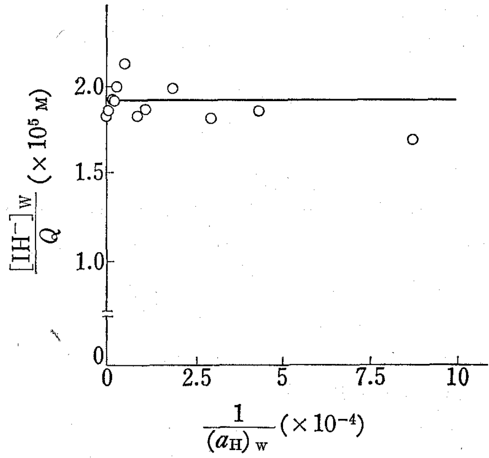

Fig. 3. Plot of $[IH^-]_w/Q$ against $1/(a_H)_w$ for TMP-BPB in the 1,2-Dichloroethane-H_2O System

Extraction of TMP-BCG and TMP-BPB

The formations of the ion pairs of TMP with bromocresol green and bromophenol blue, TMP-BCG and TMP-BPB, in the 1,2-dichloroethane–H_2O system occurred below pH 6 and below pH 5, respectively. The quantity $[IH^-]_w/Q$ was plotted as a function of $1/(a_H)_w$, as shown in Figs. 2 and 3, respectively. Both straight lines were almost parallel to the abscissa, $1/(a_H)_w$. This phenomenon may be explained as follows. Eq. (7) can be converted into the form,

$$\frac{1}{Q} = \frac{1}{K_{ex}f^2[IH^-]_w}\left\{\frac{(p+1)K_{BH^+}}{[H^+]_w}+1\right\} \tag{10}$$

The extractions of TMP-BCG and TMP-BPB were performed at pH 4.7—6.2 and 2.7—4.9, respectively. The partition coefficient, p, and the K_{BH^+} value of TMP were 6.72 and 7.59×10^{-8}, respectively. Because $[H^+]_w \gg (p+1)K_{BH^+}$ under such extraction conditions, Eq. (10) may be simplified as follows.

$$Q = K_{ex}f^2[IH^-]_w \tag{11}$$

Plots of Q against $[IH^-]_w$ are shown in Figs. 4 and 5. Straight lines were obtained, which passed through the origin. The log K_{ex} values were estimated from the slopes of the straight lines, as listed in Table II.

Optimum pH for the Determination of TMP

Since $[H^+]_w$ is proportional to Q in Eq. (7), lower pH is advantageous for TMP determination. Because Eq. (11) is adaptable at low pH, Eq. (8) can be converted to

$$[BH^+IH^-]_{org} = \frac{K_{ex}f^2C_B[IH^-]_w}{K_{ex}f^2[IH^-]_w+1} \tag{12}$$

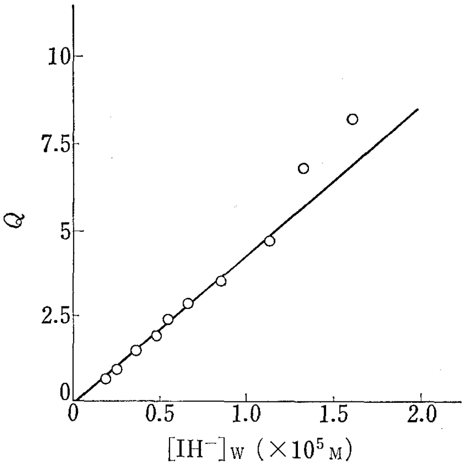

Fig. 4. Plot of Q against $[IH^-]_w$ for TMP-BCG in the 1,2-Dichloroethane–H_2O System

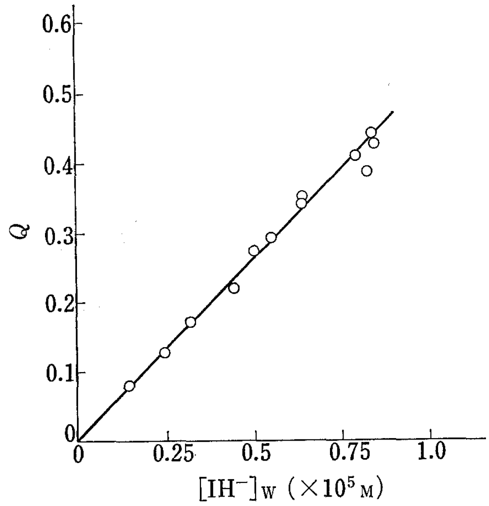

Fig. 5. Plot of Q against $[IH^-]_w$ for TMP-BPB in the 1,2-Dichloroethane–H_2O System

Table II. Relationship between Q and $[IH^-]_w$ for TMP-BTB, TMP-BCG and TMP-BPB

	Ion pair in solvent system		
	TMP-BTB Benzene/H_2O	TMP-BCG (1,2-Dichloroethane/H_2O)	TMP-BPB (1,2-Dichloroethane/H_2O
Slope	7.820×10^4	4.234×10^5	4.877×10^4
Correlation coefficient (r)	0.990	0.997	0.993
Standard deviation (σ)	0.052	0.104	0.015
n	7	8	12
log K_{ex}	5.16 ± 0.08[a]	5.89 ± 0.03[a]	4.95 ± 0.04[a]

a) 95% confidence limit.

If $K_{ex}f^2[IH^-]_w \gg 1$

$$[BH^+IH^-]_{org} = C_B \tag{13}$$

Eq. (13) suggests that the base in the aqueous phase may be completely transferable to the organic phase as an ion pair when the ion, IH^-, is present in a much larger amount than the value $1/(K_{ex}f^2)$. When $[H^+]_w \gg K_{IH^-}$, $[IH^-]_w$ is equal to the total concentration of the dye (C_I). Therefore, when $C_I \gg 1/(K_{ex}f^2)$, all of the TMP will be extracted as an ion pair.

The extraction of TMP-BTB was performed at the lower pH in benzene–H_2O. When the ion pair was extracted at pH 5.35 using TMP (2.1×10^{-5} M) with various concentrations of BTB (0.4×10^{-5}—3.9×10^{-5} M), the relation given in Eq. (11) was adapted. The result of the plot of Q *vs.* $[IH^-]_w$ is shown in Table II. Trimethoprim was recovered in 100% yield at pH 5.0, when C_I was larger than 1.3×10^{-4} M ($K_{ex} = 1.82 \times 10^5$). The extraction of TMP-BTB was also performed in 1,2-dichloroethane–H_2O. Trimethoprim was recovered in 100% yield at pH 4.5—6.1 by using 4.6×10^{-5} M of BTB ($K_{ex} = 1.20 \times 10^7$).

It was concluded from these results that the preferred pH for the assay of TMP by ion pair extraction is in the range of 4 to 6.

Experimental

Apparatus——A Hitachi digital spectrophotometer, model 624, was used for absorbance measurement. A Radiometer pH meter, type TTT1C, was used. All measurements were made at 25°.

Materials——The organic solvents were of reagent grade. Chloroform contained 0.5% EtOH as a stabilizer. Commercial sulfonphthaleins were purified by recrystallization. Trimethoprim was purified by recrystallization from MeOH.

Bromothymol Blue——BTB (Kanto Chemical Co., Inc.) was recrystallized from AcOEt. The crystals, colorless prisms, mp 203—205°, were dried over P_2O_5 *in vacuo* for 2 hr at room temperature. *Anal.* Calcd for $C_{27}H_{28}Br_2O_5S$: C, 51.93; H, 4.52; Br, 25.60; S, 5.14. Found: C, 51.91; H, 4.58; Br, 25.31; S, 4.89.

Bromocresol Green——BCG (Eastman Kodak Co., Ltd.) was recrystallized twice from ether to give pale yellow needles, mp 226—228° (lit.,[14] mp 218—219°). The crystals were dried over P_2O_5 *in vacuo* for 4 hr at 100°. *Anal.* Calcd for $C_{21}H_{14}Br_4O_5S$: C, 36.13; H, 2.02; Br, 45.79; S, 4.59. Found: C, 36.32; H, 1.99; Br, 45.56; S, 4.44.

Bromophenol Blue——BPB (Tokyo Kasei Kogyo Co., Ltd.) was recrystallized from AcOEt–ether to give pale yellow needles. The crystals were recrystallized twice from ether. Pale yellow prisms, mp 265—269° (dec.) (lit.,[15] mp 278° (dec.)). The crystals were dried over P_2O_5 *in vacuo* for 5 hr at 100°. *Anal.* Calcd, for $C_{19}H_{10}Br_4O_5S$: C, 34.06; H, 1.50; Br, 47.71; S, 4.79. Found: C, 34.07; H, 1.51; Br, 47.50; S, 4.75.

Preparation of TMP-BTB——A solution of TMP (0.1 g) in 0.01 N HCl (40 ml) was mixed with an equimolar amount of BTB (0.215 g) in AcOEt. The mixture was shaken and separated. The aqueous phase was extracted with AcOEt (40 ml). The AcOEt solution was combined, washed with H_2O (3 ml) and dried over Na_2SO_4. The solution was evaporated to dryness under reduced pressure. The oily residue was recrystallized from EtOH to give 0.19 g of TMP-BTB as red prisms, mp 214—217°. The crystals were dried over P_2O_5 *in vacuo* for 5 hr at 100°. *Anal.* Calcd, for $C_{41}H_{46}Br_2N_4O_8S$: C, 53.83; H, 5.07; Br, 17.47; N, 6.13; S, 3.51. Found: C, 53.48; H, 5.07; Br, 17.60; N, 6.07; S, 3.67.

The complex, TMP-BTB, was also obtained from EtOH solution containing TMP and BTB in the same molar ratio.[15]

Preparation of TMP-BPB——The complex, TMP-BPB, was extracted with AcOEt in the manner described above (TMP: 0.1 g and BPB: 0.23 g). The solution was allowed to stand for a few hours at room temperature. The crystals appeared as orange needles. Yield, 0.15 g, mp 179—182°. The crystals were dried over P_2O_5 *in vacuo* for 5 hr at 100°. *Anal.* Calcd, for $C_{33}H_{28}Br_4N_4O_8S$: C, 41.27; H, 2.94; Br, 33.29; N, 5.83; S, 3.34. Found: C, 41.13; H, 3.08; Br, 33.08; N, 5.73; S, 3.43.

The complex, TMP-BPB, was also obtained from MeOH solution containing TMP and BPB in the same molar ratio.[15]

Preparation of TMP-BCG——A solution of TMP (0.083 g) in MeOH (20 ml) was mixed with BCG (0.20 g) in MeOH (20 ml). The solution was evaporated to dryness under reduced pressure. The residue was dissolved in a mixture of MeOH and AcOEt. The solution was allowed to stand for a day to give a brown deposit. Recrystallization from MeOH–AcOEt gave 0.022 g of TMP-BCG as orange needles, mp 186—189°. The crystals were dried over P_2O_5 *in vacuo* for 5 hr at 100°. *Anal.* Calcd for $C_{35}H_{32}Br_4N_4O_8S$: C, 42.53; H, 3.26; Br, 32.34; N, 5.67; S, 3.24. Found: C, 42.18; H, 3.34; Br, 32.28; N, 5.66; S, 3.44.

Determination of Ion Pairs——Accurately weighed TMP (6—12 mg) was dissolved in 0.1 N AcOH (3 ml) and diluted with H_2O to 50 ml, then 0.5 ml aliquots of TMP solution were placed in shaking tubes. Buffer solution[16] of various pH's (μ=0.2, 9.0 ml), 2 M NaCl (0.1 ml), sulfonphthalein solution (0.1 ml) and H_2O (0.3 ml) were then added (TMP 2×10^{-5}—4×10^{-5} M; sulfonphthaleins 2×10^{-5}—8×10^{-5} M). Sulfonphthalein solutions were prepared by dissolving accurately weighed sulfonphthaleins (15—50 mg) in 0.1 N NaOH (1—2 ml) and diluting these solutions with H_2O to 10 ml. After mixing the solutions, the pH values were measured. Next, 10 ml of organic solvent (benzene, 1,2-dichloroethane or $CHCl_3$) was placed in the shaking tubes. The tubes were gently shaken by hand and allowed to stand for 30 min, then mechanical shaking was performed for 10 min.[17] The solution was centrifuged. The absorbance of the organic phase was measured at the absorption maximum of the complex (TMP-sulfonphthalein). The concentration of the ion pair, $[BH^+IH^-]_{org}$, was estimated from the calibration curve. The aqueous phase was used for the measurement of $[IH^-]_w$ of the sulfonphthalein.

Calibration Curve——About 3 mg of accurately weighed complex was dissolved in MeOH (0.5 ml) in a 25 ml volumetric flask. The solvent was evaporated to dryness under reduced pressure. The residue was dissolved in the organic solvent and diluted to 25 ml, then 0.5—2.5 ml portions of the solution were pipetted into five 10 ml volumetric flasks and the solvent was added to make 10 ml. The absorbance was measured at the absorption maximum of the complex [(410 nm (in benzene), 407 nm (in 1,2-dichloroethane) and 415 nm (in $CHCl_3$) for TMP-BTB, 415 nm (in 1,2-dichloroethane) for TMP-BCG, and 412 nm (in 1,2-dichloroethane) for TMP-BPB].

Measurement of Sulfonphthalein Ion Concentration (**$[IH^-]_w$**)——The absorbance in the aqueous phase was measured at the absorption maximum of the I^{2-} form of the sulfonphthalein (615 nm for BTB and BCG, and 592 nm for BPB). The concentration, $[IH^-]_w$, was estimated from the absorbance (A) using the equation,

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[15] K. Kigasawa, H. Shimizu, M. Fujino, and T. Kametani, *Yakugaku Zasshi*, **90**, 182 (1970).

[16] H.T.S. Britton and R.A. Robinson, *J. Chem. Soc.*, **1931**, 1456.

[17] The concentration of the extracted BH^+IH^- was constant over shaking times from 5 min to 30 min. However, the concentrations varied substantially if the procedure of standing for 30 min before the mechanical shaking was omitted.

$$[\mathrm{IH}^-]_\mathrm{w}=\frac{A}{\varepsilon_{\mathrm{I}^{2-}}\cdot\dfrac{K_{\mathrm{IH}^-}f_{\mathrm{IH}^-}}{(a_\mathrm{H})_\mathrm{w}f_{\mathrm{I}^{2-}}}+\varepsilon_{\mathrm{IH}^-}} \tag{14}$$

The molar extinction coefficients, $\varepsilon_{\mathrm{IH}^-}$ and $\varepsilon_{\mathrm{I}^{2-}}$, were determined separately by dissolving the sulfonphthaleins in buffer solution ($\mu=0.2$) (pH 2.0 and 10.0 for BTB, pH 2.0 and 8.0 for BCG, and pH 1.0 and 7.5 for BPB).

Measurement of Partition Coefficient——The p values were measured by the method of Ågren *et al.*,[18] according to Eq. (15).

$$\frac{1}{P'\cdot(a_\mathrm{H})_\mathrm{w}}=\frac{1}{p\cdot(a_\mathrm{H})_\mathrm{w}}+\frac{1}{p\cdot f_{\mathrm{BH}^+}\cdot K_{\mathrm{BH}^+}} \tag{15}$$

where P' was defined as

$$P'=\frac{[\mathrm{B}]_\mathrm{org}}{[\mathrm{B}]_\mathrm{w}+[\mathrm{BH}^+]_\mathrm{w}} \tag{16}$$

In order to estimate P', the aqueous phase ($\mu=0.2$) containing TMP and the buffer solution was shaken with the solvent (benzene, 1,2-dichloroethane and $CHCl_3$) for 10 min. The partition was equilibrated by shaking the solution mechanically (using an Iwaki KM shaker, type V-S) for more than 5 min. The log p values for benzene–H_2O and 1,2-dichloroethane–H_2O systems were found to be -0.353 and 0.827, respectively.

When $p \gg P'$, Eq. (15) becomes

$$\frac{1}{P'\cdot(a_\mathrm{H})_\mathrm{w}}=\frac{1}{p\cdot f_{\mathrm{BH}^+}\cdot K_{\mathrm{BH}^+}} \tag{17}$$

In the case of the $CHCl_3$–H_2O system, $1/(P'(a_\mathrm{H})_\mathrm{w})$ was constant regardless of the pH value. The log p value (1.62) was estimated from the mean value of $1/(P'(a_\mathrm{H})_\mathrm{w})$ observed at 9 different pH's.

Measurement of Dissociation Constant——The $\mathrm{p}K_{\mathrm{IH}^-}$ and $\mathrm{p}K_{\mathrm{BH}^+}$ values were measured by the usual spectroscopic method.[19] The ionic strength (μ) of the solution was kept constant ($\mu=0.2$) using 2 M NaCl. Buffer solution ($\mu=0.2$) of various pH's (9.0 ml) and 2 M NaCl (0.1 ml) were added to 0.5 ml portions of the test solution (sulfonphthalein or TMP). The solution was diluted with H_2O to 10 ml. Britton–Robinson's buffer solution[16] was prepared using 2 N NaOH, 0.4 M H_3PO_4, –AcOH and –H_3BO_3. The $\mathrm{p}K_\mathrm{a}$ values for BTB, BCG, BPB and TMP were found to be 7.46±0.01, 4.98±0.01, 4.25±0.01 and 7.12±0.01, respectively.

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[18] A. Ågren, R. Elofsson, and S.O. Nilsson, *Acta Pharm. Suec.*, **8**, 475 (1971).

[19] Ref. 13, pp. 44—64.