

## Equilibrium Studies on 2-(5-Methylthien-3-yl)-2,5-dihydro-3H-pyrazolo[4,3-c]quinolin-3-one (S-135) in *n*-Octanol

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The partition equilibrium of 2-(5-methylthien-3-yl)-2,5-dihydro-3H-pyrazolo[4,3-c]quinolin-3-one (S-135) in *n*-octanol (*n*-OcOH)–water system was studied. S-135, which was developed as a drug for activating depressed brain function, could be easily partitioned into *n*-OcOH from water with a coefficient ( $p$ ) of about 40 as the ratio of the concentration in the organic phase to the aqueous phase. In alkaline solution above pH 11, S-135 with a  $pK_a$  value of 9.44 decreased the partition ratio up to about 5 with an increase in unextractable sodium salt dissociated in the aqueous phase. When extracted in more alkaline solution above pH 13, the distribution increased to  $p = 15$ , in spite of the fact that sodium salt alone was present. The distribution ratio increased as the concentrations of *n*-OcOH and NaOH increased in the mixed solvent system of *n*-OcOH, *n*-hexane and aqueous alkaline solution. The acid–base equilibrium in *n*-OcOH and the partition equilibrium between *n*-OcOH and the strongly alkaline system were examined to determine the species extracted in *n*-OcOH. The extracted species of S-135 was not a monosodium salt but a complex salt associated with both the solvent molecule and NaOH. The salt isolated from the 5N NaOH solution containing NaOH in its structure differed in physico-chemical character from the monosodium salt, thus supporting the validity of the partition equilibrium.

**Keywords** pyrazoloquinoline; pyrazoloquinoline Na salt; partition equilibrium; partition coefficient; *n*-octanol; acid–base equilibrium

The partition coefficient ( $p$ ) of the compounds for *n*-octanol (*n*-OcOH) and a water system has been measured as a parameter to aid understanding of the structure–activity relationship.<sup>1,2)</sup> *n*-OcOH is regarded as an organic solvent that mimics the hydrophobic nature of the biomembrane well.<sup>3–6)</sup> The potential structure as a drug has been predicted from the relationship of the pharmacological activity and the  $\log p$  value. The receptor binding affinity and brain uptake of benzodiazepine drugs have been studied in relation to the lipophilicity, such as  $\log p$  and the retention index of high performance liquid chromatography.<sup>7,8)</sup> 2-(5-Methylthien-3-yl)-2,5-dihydro-3H-pyrazolo[4,3-c]quinolin-3-one (S-135) was developed as a drug to activate depressed brain function. This compound binds to benzodiazepine receptor and exhibits pharmacological activities which are the opposite of the benzodiazepine-like actions.<sup>9,10)</sup> The weak acid S-135 with  $pK_a$  9.44 was found to be pharmacologically active but the methylated S-135, which is not an acid, bound the receptor very weakly, resulting in little activity. In considering the importance of the acidic character of S-135, our attention was focussed on the physico-chemical characters of S-135 in connection with the pharmacological difference between the acid and the non-electrolyte. Xie *et al.* reported that the accumulation of benzodiazepine in adipose tissue was influenced by the basicity ( $pK_a$ ) of the compound.<sup>11)</sup> In the present study, the acid–base equi-

librium in *n*-OcOH and the distribution equilibrium of S-135 from water to *n*-OcOH were investigated. Not only the free form of S-135 but also its sodium salt was distributed in *n*-OcOH. Interestingly, the sodium salt was extracted from a strongly alkaline solution above 1N, in which no nonionizable species exists. Terada and his colleagues reported the distribution equilibria of the ionizable compounds phenol and methyl orange,<sup>6,12)</sup> and clarified the equilibria formed by co-distribution of the nonionizable species and its ion from aqueous solution to *n*-OcOH. Few reports have been published on the distribution equilibrium of the sodium salt of the drug alone and its lipophilicity. The affinity of the sodium salt of S-135 to sodium ion provides information on the pharmacological activity of S-135 anion dissociated *in vivo*.

### Experimental

**Apparatus** A Hiranuma reporting titrator, Model Comtite-101, connected with Radiometer glass and calomel electrodes (G222C and K901) was used for potential measurement. Two spectrophotometers, a Shimadzu model UV 190 and a Hitachi model 330, were used for the absorbance measurement of the liquid sample and the solid sample, respectively. A Hitachi spectrofluorometer, model MPF-4 was used for the fluorescence measurement of the solid sample. Infrared spectra were recorded on a Model IR-700 spectrophotometer from Japan Spectroscopic. The differential scanning calorimetric curve was measured with a Perkin-Elmer DSC-7.

**Reagents and Materials** The chemicals were of reagent grade. Alcohols (MeOH, EtOH and *n*-OcOH) were of special grade and dehydrated with molecular sieves (type MS4ANRG 8 × 12, Unionshouwa, Co., Ltd.).

(a) Preparation of Sodium Methyl 4-Hydroxynicotinate: To a solution of 0.35 g of methyl 4-hydroxynicotinate (1) in  $\text{CHCl}_3$ –MeOH (1 : 5, 80 ml) was added an equimolar amount of 1M NaOH. The solution was evaporated to dryness and recrystallized from MeOH– $\text{CH}_3\text{CN}$ , affording 170 mg of the salt as white powder.

(b) Preparation of Sodium Salt of 3-Iodo-4-hydroxypyridine: To a solution of 1 g of 3-iodo-4-hydroxypyridine (2) in MeOH (10 ml) was added an equimolar amount of 1M NaOH. The solution was evaporated to dryness and dissolved in EtOH (10 ml). After filtration with a Hyflo Super-Cell (Johns-Manville Sales Corp.), ether was added until turbidity

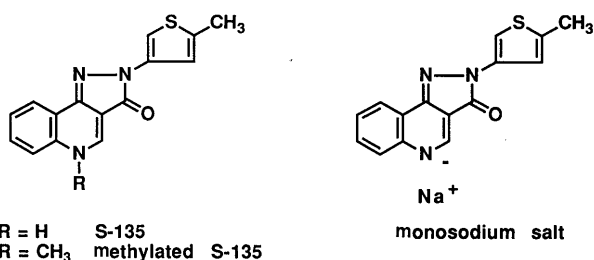


Fig. 1. Structures of S-135 (Anion) and Its *N*-Methylated Compound

was observed. The mixture, allowed to stand overnight, yielded 800 mg of the salt as white needles.

The two salts were identified as monosodium salts by elemental analysis.

(c) Preparation of Sodium Salt of S-135: To a suspension of 2.8 g of S-135 (10 mmol) with 10 ml of 1 M NaOH was added 50 ml of EtOH. After stirring for 1 h, the dissolved solution was evaporated to dryness under reduced pressure. The residue was dissolved with 20 ml of EtOH and then 300 ml of Et<sub>2</sub>O was added in portions, giving white crystals. After being washed several times, the crystals were dried for 5 h *in vacuo* affording 2.6 g.

*Anal.* Calcd for C<sub>15</sub>H<sub>10</sub>N<sub>3</sub>NaOS · 1/3H<sub>2</sub>O: C, 58.24; H, 3.48; N, 13.59; Na, 7.43; S, 10.36. Found: C, 58.11; H, 3.51; N, 13.47; Na, 7.78; S, 10.51.

(d) Preparation of the Complex Salt of S-135: To a solution of S-135 (0.5 g) in 1 M NaOH (10 ml) was added 5 M NaOH (50 ml). After this had been allowed to stand at room temperature, the deposited crystals were obtained by filtration and washed twice with H<sub>2</sub>O to give the complex salt as pale yellowish white needles.

*Anal.* Calcd for C<sub>15</sub>H<sub>10</sub>N<sub>3</sub>NaOS · 1/2NaOH · 2.5H<sub>2</sub>O: C, 48.91; H, 4.24; N, 11.41; Na, 9.36. Found: C, 48.76; H, 4.13; N, 11.49; Na, 9.40.

**Measurement of pK<sub>a</sub> Values** The pK<sub>a</sub> values, determined by the usual method, were 9.44 for S-135, 9.50 for **1** and 9.49 for **2**. Compound **1** was dissolved in 10% methanolic solution because of its low solubility.<sup>13)</sup>

**Measurement of Potential of Alcoholic Solutions of S-135, **1** and **2**** An alcoholic solution (MeOH, EtOH and *n*-OcOH) containing a mixture of S-135 with an equimolar amount of its sodium salt was prepared to make 5.0 × 10<sup>-3</sup> M (50 ml). The solution was diluted with the alcohol to prepare several solutions in the range of 5 × 10<sup>-3</sup>—1 × 10<sup>-5</sup> M. The glass electrode was dipped in the cell. A calomel electrode was immersed in a separate compartment filled with the alcohol saturated with LiCl. A salt bridge containing the alcohol saturated with LiCl was placed between the cell and the compartment. The solution was stirred with a stirrer. When the potential (*E*) value was stable at a constant value (15—20 min), it was recorded.

The *E* measurement of equimolar mixtures of compound **1** and its salt, and compound **2** and its salt in the three alcohols were conducted in the same manner as that of S-135.

**Measurement of Distribution Ratio** (a) Effect of *n*-OcOH Concentration on Distribution: The sodium salt of S-135 (about 7 mg) was accurately weighed into a 50-ml volumetric flask, dissolved it in, and diluted to mark with MeOH (stock solution). One ml of the stock solution was pipetted into a 50-ml centrifuge tube and the solvent was evaporated off. The residue was dissolved in 20.0 ml of 0.1 N NaOH and then the mixed solvent *n*-OcOH—*n*-hexane (20.0 ml) was added. The 0.1 N NaOH solution used was saturated with the mixed solvent by shaking both phases, and the solvent used was saturated with 0.1 N NaOH before use. The mixture was shaken with a shaker for 30 min. After being centrifuged at 2500 rpm for 10 min, the organic phase was discarded with an aspirator. The absorbance at 284 nm was read using the treated 0.1 N NaOH as a blank. The calibration curve of the sodium salt of S-135 was made using the standard solution prepared with the treated 0.1 N NaOH.

(b) Effect of NaOH Concentration on Distribution: The residual sodium salt obtained in the manner described above was dissolved in NaOH solutions (20.0 ml) of varying concentrations from 0.1 to 5 N which were saturated with *n*-OcOH—*n*-hexane (20%, v/v). Next, 20 ml of *n*-OcOH—*n*-hexane (20%, v/v) saturated with the aqueous phase was added and shaken for 30 min. The extraction and absorbance measurement were done as described above.

Shaking and centrifugation were done at room temperature (25 ± 2 °C). All the samples in (a) or (b) were treated at the same time to assure the same conditions.

Distributions of S-135 in the three systems, CHCl<sub>3</sub>/H<sub>2</sub>O, *n*-OcOH/H<sub>2</sub>O and *n*-OcOH—hexane/H<sub>2</sub>O employed in (b) were performed using a constant ionic strength, μ = 0.2,<sup>14)</sup> 0.2<sup>14)</sup> and 0.1 (NaOH), respectively.

**Measurement of UV Spectrum in Solid State** The monosodium salt of S-135 and the isolated complex salt of S-135, 0.16 mg each, were mixed with 300 mg of NaCl. The mixture (150 mg) was pressed into a tablet with a die for IR measurement using a Hitachi spectrophotometer (model 330) attached to an integral sphere with a 60 mm diameter in which transmitted light through the tablet was collected.

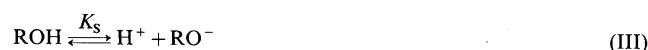
**Measurement of Fluorescence Spectrum in Solid State** The isolated complex salt was powdered and placed on a quartz glass (1.0 × 4.4 × 0.1 cm) and the powder was spread evenly over the entire area of the glass plate. The surface of the powder was covered with a quartz glass of the same shape. The two plates were placed on black paper of the same size and fixed at the edge with a piece of tape. This set of plates was placed diagonally in the square cell holder of a Hitachi spectrophotometer (model MPF-4)

where the rays of excitation light reflected off the surface of the quartz glass.

## Results and Discussion

**Acid–Base Equilibria of S-135 in *n*-OcOH** Organic salt in aqueous solution dissociates completely to form ions, but the dissociation of the salt in organic solvent is repressed as its dielectric constant ( $\epsilon$ ) becomes smaller. Previously we reported the acid–base equilibria of a base and its salt in alcohols<sup>15,16)</sup> to confirm the co-existence of the dissociated ion and the salt in *n*-butyl alcohol ( $\epsilon = 17.1$ ). The salt exists as an ion pair predominantly in a solvent of less than  $\epsilon = 10$ .<sup>17,18)</sup>

Kolthoff and Bruckenstein<sup>19,20)</sup> investigated the acid–base equilibria of a base and its salt in a low dielectric constant solvent, acetic acid ( $\epsilon = 6.13$ ) to estimate [H<sup>+</sup>] potentiometrically. According to their treatment, when we consider the equilibria of an acid and its sodium salt in *n*-OcOH whose dielectric constant is 10.3, the following equilibria occur,



where HX is a weak acid and NaX is its sodium salt.  $K_{\text{HX}}$  is the ionization constant and the two constants  $K'_{\text{HX}}$  and  $K_{\text{NaX}}$  are the dissociation constants of H<sup>+</sup>X<sup>-</sup> and Na<sup>+</sup>X<sup>-</sup>, respectively.  $K_s$  is the autoprotolysis constant of the solvent. The overall dissociation constant  $K_{\text{OHX}}$  is defined as follows:

$$K_{\text{OHX}} = \frac{[\text{H}^+][\text{X}^-]}{[\text{HX}] + [\text{H}^+ \text{X}^-]} = \frac{K'_{\text{HX}}}{1 + \frac{1}{K_{\text{HX}}}} \quad (\text{1})$$

If the acid is too weak to express ( $[\text{HX}] + [\text{H}^+ \text{X}^-] \gg [\text{X}^-]$ ) and very little dissociation occurs from the salt in a low dielectric constant solvent, when a mixture of an acid and its sodium salt is dissolved in the solvent, [H<sup>+</sup>] is calculated from the equation derived from equilibria I, II and III,

$$[\text{H}^+] = \sqrt{\frac{K_{\text{OHX}} C_a (K_{\text{OHX}} C_a + K_s)}{K_{\text{OHX}} C_a + K_{\text{NaX}} C_s}} \quad (\text{2})$$

where  $C_a$  ( $= [\text{HX}] + [\text{H}^+ \text{X}^-]$ ) and  $C_s$  ( $= [\text{Na}^+ \text{X}^-]$ ) are the analytical concentrations of the acid and its salt, respectively. Equation 2 is essentially the same as Kolthoff's equation which was derived under the coexistence of the base and its salt, also showing the [H<sup>+</sup>] formed by the titration of an acid with the strong base. According to Kolthoff's treatment,<sup>21)</sup> Eq. 2 is simplified as Eq. 3, when the acid which is strong enough to give the end point and its salt coexist. Under these conditions,  $K_{\text{OHX}} C_a$  is much greater than  $K_s$ .

$$[\text{H}^+] = \frac{K_{\text{OHX}} C_a}{\sqrt{K_{\text{OHX}} C_a + K_{\text{NaX}} C_s}} \quad (\text{3})$$

The assumption applies for the equilibria of S-135; S-135 was titrated with sodium octanolate in *n*-OcOH to give a large potential change at the end point.

We considered [H<sup>+</sup>] in the solution containing an

equimolar amount of acid and its salt ( $C_a = C_s$ ). The  $[H^+]$  is equivalent to the concentration of the solution at the half-neutralization point, when acid is titrated with sodium salt of *n*-OcOH (RONa). If  $C_a = C_s = C$ , Eq. 2 is written

$$[H^+] = K_{OHX} \sqrt{\frac{C}{K_{OHX} + K_{NaX}}} \quad (4)$$

When various concentrations of an acid and its salt are present and the change of  $[H^+]$  is followed by the potential ( $E$ ) in the next equation,

$$E = E_0 + \frac{RT}{F} \ln K_{OHX} + \frac{RT}{2F} \ln C - \frac{RT}{2F} \ln(K_{OHX} + K_{NaX}) \quad (5)$$

Eq. 5 means that when  $E$  (mV) is plotted against  $\log C$ , a linear relationship will be obtained and also that the slope of the straight line is 29.5 mV at 25°C. This slope value indicates whether or not the salt of a weak acid in the solvent can dissociate to form ions. In a higher dielectric constant solvent, such as  $H_2O$  and MeOH,  $Na^+X^-$  is

completely dissociated ( $[Na^+] = [X^-] = C_s$ ), and  $[H^+]$  is regulated by the ratio of  $C_a/C_s$ , because

$$K_{HX} = \frac{[H^+][X^-]}{[HX]} = [H^+] \frac{C_s}{C_a} \quad (6)$$

If  $C_a = C_s = C$ ,  $[H^+]$  remains constant irrespective of increasing concentrations of  $C_a$  and  $C_s$ .

The acid-base equilibria of three weak acids and their sodium salts, S-135, compounds 1 and 2 were investigated. The potential was measured for a solution of an equimolar mixture of these acids and their sodium salt. The plot of  $E$  (mV) against  $\log C$  was prepared for three alcoholic solvents, MeOH, EtOH and *n*-OcOH, as shown in Fig. 2. In *n*-OcOH, the plot of the two values of S-135 gave a straight line with a slope of 30.8 mV. This slope value, obtained in the concentration range of  $C_a$  ( $C_s$ ) from  $10^{-5}$  M to  $10^{-3}$  M, was identical with the theoretical value (29.5 mV) presumed from Eq. 5, suggesting little dissociation from S-135 and its sodium salt.

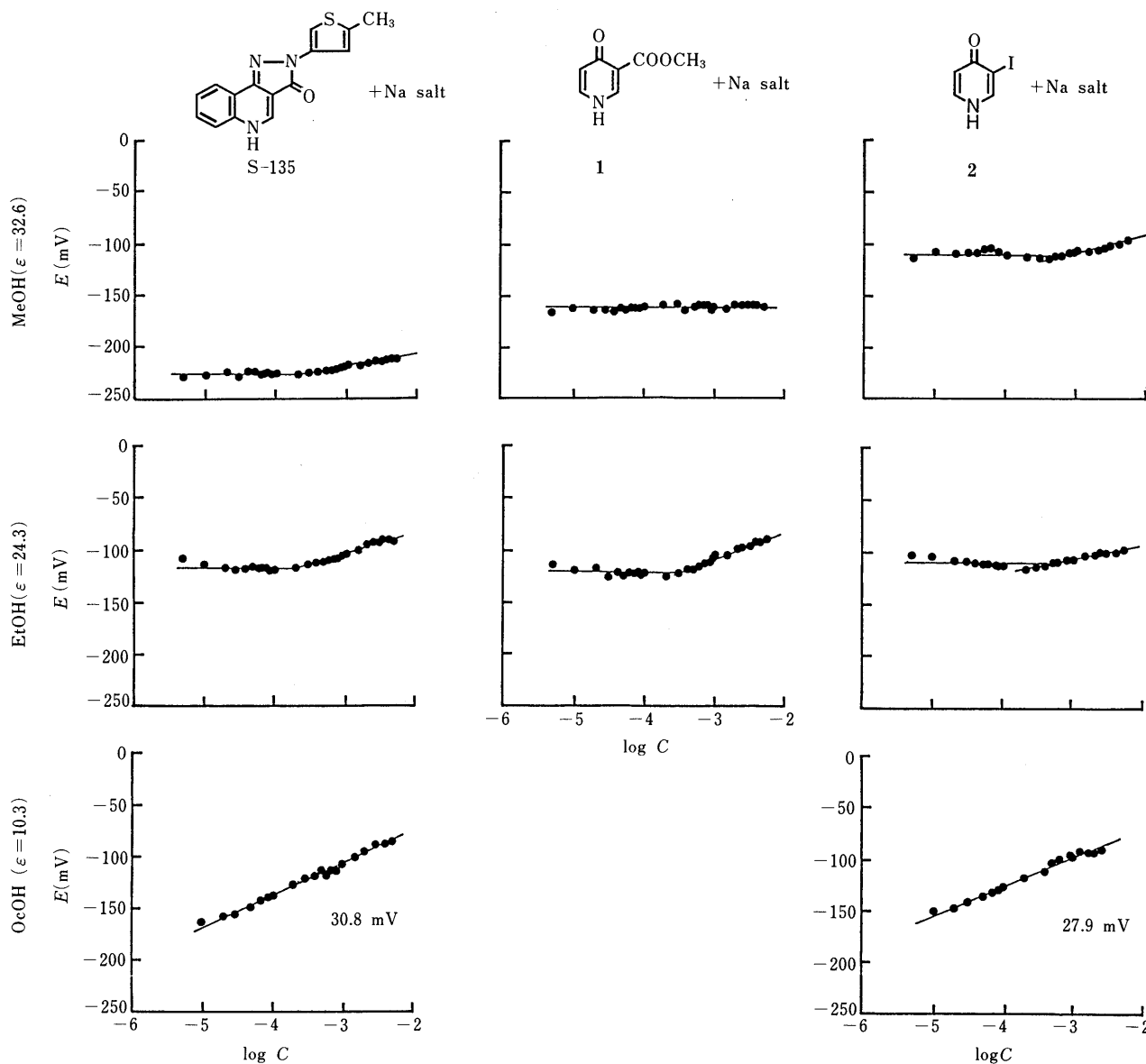


Fig. 2. Plot of  $E$  (mV) against Concentrations of the Solutes in an Equimolar Mixture of Weak Acid and Its Sodium Salt  
 S-135 (*n*-OcOH):  $E = 30.8 \log C_a - 13.1$ ,  $r = 0.995$ ,  $s = 2.50$  ( $n = 20$ ). 2 (*n*-OcOH):  $E = 27.9 \log C_a - 15.4$ ,  $r = 0.993$ ,  $s = 2.54$  ( $n = 17$ ).

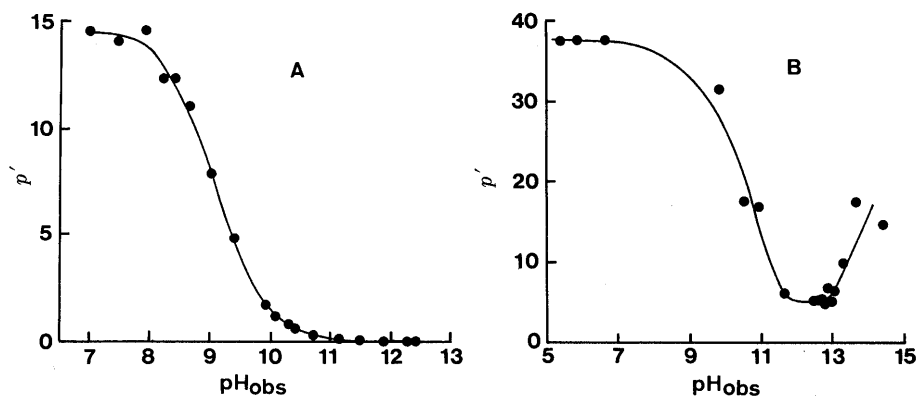


Fig. 3. Distribution Ratio of S-135 from Aqueous Solution to  $CHCl_3$  (A) and  $n-OcOH$  (B)

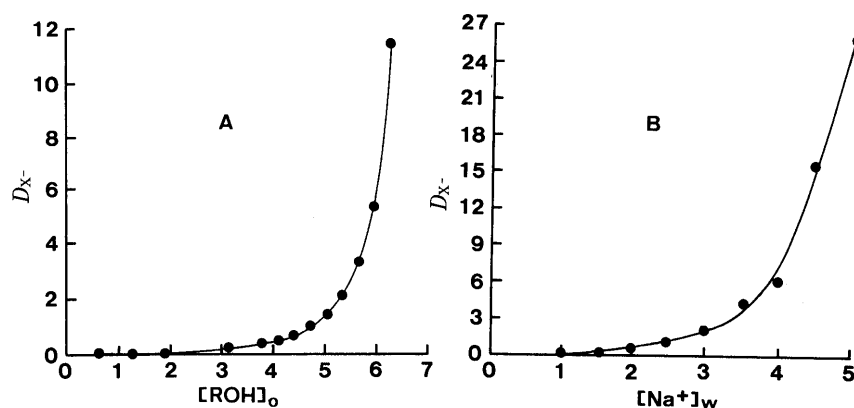


Fig. 4. Distribution Ratio of S-135 from Aqueous NaOH Solution to  $n$ -Hexane- $n$ -OcOH

(A) With various concentrations of  $n$ -OcOH (0.6–6 M). (B) With various concentrations of NaOH (1–5 M).

In MeOH ( $\epsilon = 32.6$ ), which has a higher dielectric constant, little or no change in potential was observed in the same concentration range, indicating complete dissociation of the sodium salt of S-135. In EtOH ( $\epsilon = 24.3$ ), this plot gave almost a linear line but at a higher concentration, a lower slope, suggesting a tendency for the dissociation of the salt to be depressed.

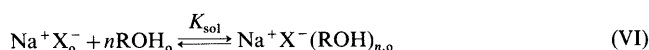
The other two compounds **1** and **2** showed similar results. The slope obtained for **2** in  $n$ -OcOH was 27.9 mV which was close to the theoretical value. No measurement was made of **1** in  $n$ -OcOH owing to the poor solubility. The results show that MeOH is a water-like solvent which allows the electrolyte to dissociate, while  $n$ -OcOH has the character of a low dielectric constant solvent that represses the dissociation of electrolytes such as acetic acid, and that EtOH has an intermediate character in the equilibrium between MeOH and  $n$ -OcOH. Our potentiometric method is generally applicable to measurement of proton in alcoholic solvent.

**Partition of S-135 from Water to Organic Phase** The partition ratio of S-135 from water to  $CHCl_3$  is shown in Fig. 3A. The ratio ( $p'$ ) apparently calculated as  $[S-135]_{in\ organic\ phase}/[S-135]_{in\ aqueous\ phase}$  decreased as it neared pH 8 toward the alkaline region due to the dissociation ( $pK_a = 9.44$ ). The partition of S-135 between  $n$ -OcOH and water, as shown in Fig. 3B, differed from the behavior of  $CHCl_3/H_2O$ : the ratio decreased similarly with the dissociation toward pH 11, but it remained constant ( $p'$  is *ca.* 5) and increased at over pH 13. The  $p'$  value reached about 17 at 1 N NaOH in the aqueous phase.

To examine the partition behavior of S-135, two experiments were performed. Figure 4A shows the partition ratio of S-135 at 0.1 N NaOH with a mixed solvent of  $n$ -OcOH and  $n$ -hexane at varying solvent ratios. Little or no S-135 was extracted from the solvent with a lower content of  $n$ -OcOH. The extracted ratio increased as the content of  $n$ -OcOH in  $n$ -hexane increased. From a solvent of 20% (v/v)  $n$ -OcOH, S-135 was transferred increasingly to organic phase with varying NaOH concentrations, as shown in Fig. 4B. S-135 in the organic phase increased steeply with increasing concentration of NaOH in the aqueous phase.

**Effect of Concentration of  $n$ -OcOH on Distribution** In alkaline solution strong enough for S-135 to dissociate, S-135, whose  $pK_a$  value is 9.44, exists as an ion in an aqueous NaOH solution of more than 0.1 N. As described above, dissociation of the sodium salt can be repressed to allow it to exist as an ion pair in a low dielectric constant solvent. The distribution of the sodium salt might have occurred by solvation of the sodium salt with  $n$ -OcOH.  $n$ -OcOH is an amphiprotic solvent which will solvate both sodium ion and the counter anion (amphiprotic solvation).<sup>22)</sup> On the other hand,  $n$ -hexane whose dielectric constant is 1.9 will have little or no influence on the interaction with the sodium salt as an inert solvent.<sup>22)</sup> The effect of  $n$ -OcOH was investigated through its concentration change. The organic phase contains water at about 4% or less. The solubility of water in  $n$ -OcOH is about 4% and that in  $n$ -hexane would be less than in  $n$ -OcOH.<sup>23)</sup> Although water content increases with increasing  $n$ -OcOH content, the experiment indicates that

the transference of the sodium salt to *n*-OcOH layer would not be simply due to the dissolution of the salt in H<sub>2</sub>O present in *n*-OcOH, because its concentration in the organic phase was not proportional to the *n*-OcOH concentration. The distribution changed steeply in the narrow concentration range (4.5—6 M) of *n*-OcOH. Since the dielectric constant of the organic phase does not change greatly in the presence of water of less than 4%,<sup>24</sup> the sodium salt will exist as an ion pair associated with *n*-OcOH molecule more stably than with H<sub>2</sub>O. Solvation of the salt with water will lead to the dissociation of the ion pair. The following equilibria were assumed to occur:



where X<sup>-</sup> is S-135 anion and ROH is *n*-OcOH. Subscripts w and o refer to the aqueous and the organic phase, respectively. The equilibrium constants of the equilibria IV—VI are for association, partition and solvation, which are defined as:

$$K_{\text{ass}} = \frac{[\text{Na}^+ \text{X}^-]_w}{[\text{Na}^+]_w [\text{X}^-]_w} \quad (7)$$

$$p = \frac{[\text{Na}^+ \text{X}^-]_o}{[\text{Na}^+ \text{X}^-]_w} \quad (8)$$

$$K_{\text{sol}} = \frac{[\text{Na}^+ \text{X}^- (\text{ROH})_{n,o}]_o}{[\text{Na}^+ \text{X}^-]_o [\text{ROH}]_o^n} \quad (9)$$

The distribution ratio of S-135 species under the equilibria,  $D_{\text{X}^-}$  is

$$D_{\text{X}^-} = C_o/C_w = \frac{[\text{Na}^+ \text{X}^-]_o + [\text{Na}^+ \text{X}^- (\text{ROH})_{n,o}]_o}{[\text{X}^-]_w + [\text{Na}^+ \text{X}^-]_w} \quad (10)$$

where  $C_o$  and  $C_w$  are the concentrations of the total amount of the anion species under the equilibria in the organic and the aqueous phases, respectively, as determined spectrophotometrically.

Combining of Eqs. 7—10 leads to Eq. 11:

$$\frac{D_{\text{X}^-}}{[\text{Na}^+]_w} (1 + K_{\text{ass}}[\text{Na}^+]_w) = pK_{\text{ass}} + pK_{\text{ass}}K_{\text{sol}}[\text{ROH}]_o^n \quad (11)$$

In the aqueous phase, the concentration of the ion pair  $[\text{Na}^+ \text{X}^-]_w$  is much lower than that of the free ion  $[\text{X}^-]_w$ . The assumption,  $1 \gg K_{\text{ass}}[\text{Na}^+]_w$ , is reasonably valid. Equation 11 is simplified as Eq. 12, giving a plot of  $D_{\text{X}^-}/[\text{Na}^+]_w$  against  $[\text{ROH}]_o^n$  which is a straight line.

$$\frac{D_{\text{X}^-}}{[\text{Na}^+]_w} = pK_{\text{ass}} + pK_{\text{ass}}K_{\text{sol}}[\text{ROH}]_o^n \quad (12)$$

The distribution data of S-135 obtained at the various concentrations of *n*-OcOH (Fig. 4A) were analyzed based on Eq. 12. Since the concentration of S-135 used is at the 10<sup>-5</sup> M level and much less than  $[\text{NaOH}]$  in the aqueous phase or  $[\text{ROH}]_o$ ,  $[\text{Na}^+]_w$  and  $[\text{ROH}]_o$  are replaced by the concentrations of NaOH (0.1 M) and *n*-OcOH in the *n*-hexane phase. The plot of  $D_{\text{X}^-}/[\text{Na}^+]_w$  against  $[\text{ROH}]_o^n$  gave a straight line when  $n=5$  with the best linear

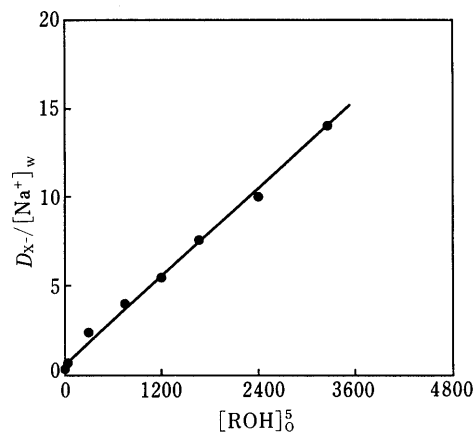


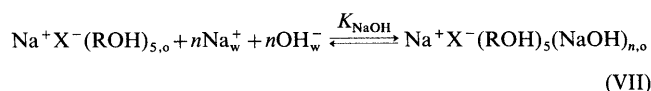
Fig. 5. Plot of  $D_{\text{X}^-}/[\text{Na}^+]_w$  ( $y$ ) against  $[\text{ROH}]_o^5$  ( $x$ ) in the Association Equilibrium between Aqueous NaOH Solution (0.1 N) and *n*-Hexane-*n*-OcOH

$$y = 4.084 \times 10^{-3}x + 0.75, r = 0.998, s = 0.29 (n = 8).$$

relationship, as shown in Fig. 5. This plot at  $n < 5$  gave a parabolic curve, which approached a linear line when the power number was increased to 5. In the case of  $n > 5$ , the points at higher concentrations of *n*-OcOH deviated from the line.

**Effect of Concentration of NaOH on Distribution** As in the salting out effect in solvent extraction observed under strong ionic strength conditions, there may be an increase in distribution. However, the observed phenomenon with increasing NaOH concentration seems unlikely to show a salting out effect because of an abrupt increase in a very narrow concentration range 3.5—5 M. A sigmoidal curve with a small increase in a lower concentration of NaOH, followed by abrupt increase suggests a stoichiometrical change of NaOH shown as an equilibrium between the salt and NaOH.

If distribution occurs in NaOH solution more concentrated than 0.1 N, the extracted salt may differ from  $\text{Na}^+ \text{X}^- (\text{ROH})_n$  as observed above, because the equilibria IV—VI does not change with the NaOH concentration in the aqueous phase. The dependence of  $D_{\text{X}^-}$  upon NaOH concentration experimentally observed in Fig. 4B suggests a new equilibrium in addition to the equilibria IV—VI, as shown here:



The constant  $K_{\text{NaOH}}$  is defined as

$$K_{\text{NaOH}} = \frac{[\text{Na}^+ \text{X}^- (\text{ROH})_s (\text{NaOH})_{n,o}]_o}{[\text{Na}^+ \text{X}^- (\text{ROH})_{s,o}]_o [\text{Na}^+]_w^n [\text{OH}^-]_w^n} \quad (13)$$

The distribution ratio of S-135 species under the equilibria,  $D_{\text{X}^-}$  is

$$D_{\text{X}^-} = \frac{[\text{Na}^+ \text{X}^- (\text{ROH})_{s,o}]_o + [\text{Na}^+ \text{X}^- (\text{ROH})_s (\text{NaOH})_{n,o}]_o}{[\text{X}^-]_w + [\text{Na}^+ \text{X}^-]_w} \quad (14)$$

Combining Eqs. 7—9, 13 and 14 leads to Eq. 15:

$$D_{\text{X}^-} = \frac{\left( \frac{pK_{\text{ass}}K_{\text{sol}}[\text{ROH}]_o^n [\text{Na}^+]_w}{+ pK_{\text{ass}}K_{\text{sol}}K_{\text{NaOH}}[\text{Na}^+]_w [\text{ROH}]_o^n [\text{Na}^+]_w^n [\text{OH}^-]_w^n} \right)}{1 + K_{\text{ass}}[\text{Na}^+]_w} \quad (15)$$

Assuming  $1 \gg K_{\text{ass}} [\text{Na}^+]_w$  described above,

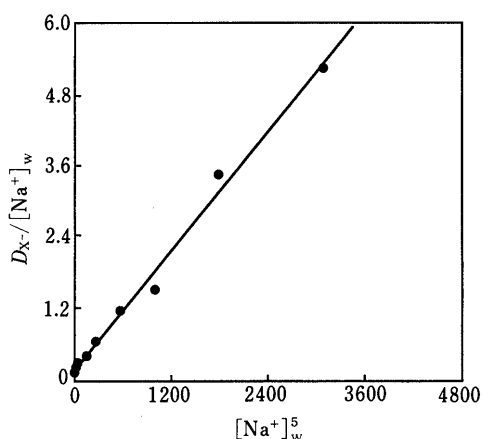


Fig. 6. Plot of  $D_{X^-}/[Na^+]_w$  (y) against  $[Na^+]_w^5$  (x) in the Association Equilibrium between Aqueous NaOH Solution and 20% *n*-OcOH Containing *n*-Hexane Solution

$$y = 1.660 \times 10^{-3}x + 0.22, r = 0.995, s = 0.19 (n = 9)$$

$$\frac{D_{X^-}}{[Na^+]_w} = pK_{ass}K_{sol}[ROH]^5 + pK_{ass}K_{sol}K_{NaOH}[ROH]^5[Na^+]_w^n[OH^-]_w^n \quad (16)$$

Equation 16 shows that the plot of  $D_{X^-}/[Na^+]_w$  against  $[Na^+]_w^n[OH^-]_w^n$  yields a straight line passing through the origin ( $[ROH]_0$  is constant). The distribution data of S-135 obtained from the more concentrated NaOH solution (Fig. 4B) were analyzed based on Eq. 16. Since  $[Na^+]_w$  and  $[OH^-]_w$  are equal and the quantity of the NaOH in the extracted associated salt ( $10^{-5}$  M) is negligible compared with that in the aqueous concentration, the plot of  $D_{X^-}/[Na^+]_w$  against  $[NaOH \text{ concentration}]^{2n}$  was attempted using  $2n = 2-7$ . The best linear relationship was obtained at  $2n = 5$ , as shown in Fig. 6. This plot at  $2n < 5$  showed a parabolic curve, which approached a linear line when the power number was increased to 5. In the case of  $2n > 5$ , the point at lower concentrations of NaOH showed a curve.

The concentration of the solute salt under the extraction equilibrium is  $10^{-5}$  M level, and there is no association between the salt molecules (dimer, trimer). The equilibria that produce solvated dimers  $(NaX)_2(ROH)_n$  and  $(NaX)_2(ROH)_n(NaOH)_n$  were assumed and the distribution data were analyzed by formulating the equations. No relationship was found to explain.

These results indicate that the sodium salt of S-135 after being solvated with coexisting *n*-OcOH in *n*-hexane is extracted from the aqueous phase into the organic phase. The solvated salt with *n*-OcOH becomes more extractable at a concentration of NaOH higher than 1 M, suggesting the formation of a new associated form which contains NaOH as shown in equilibrium VII. The number of associated alcohols agreed with that of the total number of the alkali ions ( $Na^+$  and  $OH^-$ ), suggesting the affinity of NaOH for the sodium salt and the solvation for the charged ions associated with the salt.

**Isolation of the Salt of S-135** S-135 as a weak acidic compound was freely soluble in diluted alkaline solution but its solubility decreased in 5 N NaOH solution. The powder of S-135, when dropped in this solution, changed to an oily lump without dissolving. When left standing, the

oily lump crystallized. This crystalline form was different in physico-chemical character from the monosodium salt separately prepared from the diluted alkaline solution of S-135. Elemental analysis showed  $C_{15}H_{10}N_3OSNa \cdot 1/2NaOH \cdot 2.5H_2O$ . Since UV-spectrophotometry proved that S-135 was not degraded in a strongly alkaline solution and that there was little difference in the UV spectrum in both diluted alkaline solution and 5 N NaOH solution, this crystal was considered to have the structure of the sodium salt.

In order to confirm the difference between this isolated salt and the monosodium salt, solid-state IR, UV and fluorescence spectra were measured. The IR spectra (Nujol) differed, with the monosodium salt absorbing at  $1583 \text{ cm}^{-1}$  as one peak but the isolated crystals absorbing at 1647 and  $1579 \text{ cm}^{-1}$  as sharp peaks. Minor differences (wavenumber and absorption strength) were found in the fingerprint region. The UV absorption in the solid state showed a slight difference between the two salts. The wavelength at the maximum absorption for the monosodium salt was 275 nm with a shoulder at 310 nm while that for the isolated salt was 282 nm. Marked differences were found in fluorescence spectroscopy. When UV light was used to irradiate the isolated salt in the solid state, a strong fluorescence appeared, but this did not happen on the monosodium salt. The wavelength of the maximum fluorescence was 464 nm for the excitation at 397 nm. The differential scanning calorimetric (DSC) curve also showed a difference between the two salts. The large endothermic peaks corresponding to the water in crystals appeared at different temperatures below  $100^\circ\text{C}$ . The monosodium salt had a small and broad endothermic peak near  $250^\circ\text{C}$  and was colored brown at  $280^\circ\text{C}$ , suggesting degradation, while the isolated salt had no endothermic peak near  $250^\circ\text{C}$  and was colored light yellow at  $280^\circ\text{C}$ .

The spectral differences and the different stability in the heated state show that the isolated crystals have a different structure in the solid state from that of the monosodium salt. Formation of the complex salt in aqueous solution also suggests a strong affinity to NaOH of S-135. The mole number of NaOH in the isolated complex salt (0.5 mol/salt) is one-fifth smaller than those of the solvated salt (2.5 mol/salt) under the equilibrium VII. Much easier complexation with NaOH occurred in the presence of *n*-OcOH, forming a more stable solvated salt. Interestingly, the complex salt and the oily lump of S-135 that formed before the crystals appeared, although not freely soluble in 5 N NaOH, dissolved on addition of *n*-OcOH, indicating the possible hydrophobic character of the complex ion. The affinity of the sodium salt of S-135 for NaOH and extractability in *n*-OcOH were proven by an isolation experiment, supporting the partition equilibria formed in a strongly alkaline medium. The complex ion in the solid state, although seeming to have an anion structure, may differ in electronic charge distribution from that of the monosodium salt. Its structure is now under examination.

## Conclusion

The distribution equilibria suggested that the extracted species are an associated form of the sodium salt of S-135 with both solvent *n*-OcOH and NaOH in strong alkaline solution. The formation of the complex salt with NaOH in

a strong alkaline solution as crystals suggested the affinity of NaOH to the sodium salt. Also, the soluble character of the complex salt in solvent preferably *n*-OcOH to water supported the distribution equilibria discussed. The fact that binding character to the receptor of S-135 was distinct from that of non-ionic *N*-methylated S-135, might have some relation with the sodium ion affinity in *in vivo* lipophilic media.

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