antitumor results obtained in these two bioassays. In order to make a more direct comparison, we intend to carry out *in vivo* and *in vitro* bioassays using the same tumor cells in both cases.

At this time, it is not clear why the glucosyl monoesters were, in general, less effective than the analogous derivatives of disaccharides. However, this may be accounted for, presumably, by the difference in the hydrophile-lipophile-balance (HLB) between them, in view of our previous observation that the di- and higher-substituted disaccharide esters (which possessed larger HLB values) tended to have poorer antitumor effects in both bioassay systems[4]: it is apparent that the HLB values of the glucosyl monoesters are approximately equal to those of the corresponding diesters of disaccharides. Since the disaccharide-monoester preparations used in our previous antitumor tests were mixtures consisting of various positional isomers, the influence of the acyl location on the antitumor activities has not yet been elucidated. However, by analogy with our present findings, it seems probable that acyl location is an important factor for the biological activities of disaccharide-monoesters.

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Dissolution Properties of Salt Forms of Berberine

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The dissolution properties of hydrochloride, sulfate, and iodide salts of berberine were compared. The apparent solubility and dissolution rate of the hydrochloride salt were less than those of the sulfate salt in media resembling GI fluids with respect to pH. The common ion effect on the solubility of berberine hydrochloride was also investigated.

Keywords——berberine; salt form; solubility; dissolution rate; common ion effect

Salt formation is known to influence a number of physicochemical properties of the parent drug, including solubility, dissolution rate, stability, and hygroscopicity. These properties, in turn, affect the bioavailability and formulation characteristics of the drug. The appropriate selection of the most suitable salt form can offen greatly increase the medicinal value of a given drug.

Berberine is a potential antidiarrheal agent, which is administered orally as the hydrochloride salt. It is only soluble in water to the extent of 0.94,[1] 2.16,[2] and 2.3[3] mg/ml at 20°, 30°, and 37°, respectively. Variation of the pH value of the solvent does not change the solubility of berberine hydrochloride because it is the salt of a quaternary ammonium ion. In addition, it is expected that the solubility of berberine hydrochloride will be less than would be disirable in GI fluid because of the abundance of chloride ions.[4] Incomplete dissolution of berberine hydrochloride in the GI tract is likely in view of this poor solubility and common ion effect of chloride ions.[5-8]

On the other hand, other salt forms of berberine such as sulfate,[9] iodide,[10] and tannate[11] are known in the literature. However, little systematic work has been done on the solubility and dissolution rate of salt forms of berberine. The purpose of the present study was to determine the comparative dissolution properties of three salt forms of berberine: the hydrochloride, iodide, and sulfate.

Experimental

Materials——Berberine hydrochloride dihydrate and berberine sulfate trihydrate were obtained from Wako Pure Chemical Industries. They were used without further purification. Berberine iodide was prepared by the method of Kametani *et al.*[10] *Anal.* Calcd for $C_{20}H_{18}INO_4$: C, 51.85; H, 3.92; N, 3.02. Found: C, 51.77; H, 3.81; N, 2.79. Salts were identified by melting point determination Mitamura Riken micro hot-stage apparatus) and elemental analysis. The size of the crystals in the solubility and dissolution studies was not controlled.

Solubility Determination——Apparent solubilities of the salt forms were measured by shaking excess salt at 25° and 37° in water overnight.[8] Aqueous berberine concentrations were determined spectrophotometrically.

Aqueous solutions of NaCl, KCl, and Na_2SO_4 were prepared at concentrations up to 0.01 M, and the apparent solubility of berberine hydrochloride in these solutions was also determined at 37°. Experimental salting-out constants were calculated according to the Setschenow equation.[12]

$$\log S_0/S = kC$$

where S and S_0 are the solubility in the salt solution and that in pure water, respectively; C the molar concentration of the electrolyte, and k the empirical salting-out constant.

Procedure for Dissolution Studies——The apparent dissolution rate of crystalline powder was determined at 37° as described previously.[8] The experiments were performed at pH 1.0 (0.1 N HCl), 2.0 (0.01 N HCl), and 3.0 (0.001 N HCl) in dilute hydrochloric acid or pH 7.4 in a buffer system consisting of KH_2PO_4, Na_2HPO_4, and sufficient NaCl to make the mixture isotonic. Experiments were duplicated or triplicated, and the mean values were obtained. All data are expressed as free base equivalent.

Results and Discussion

Table I summarizes the solubility data obtained for each salt at 25° and 37°, together with the melting points. It is clear that the sulfate salt is approximately 50—100 times more soluble than the hydrochloride salt of berberine. Berberine iodide is less soluble than the hydrochloride salt.

Berberine sulfate has a lower melting point than the hydrochloride and iodide salts, suggesting that the enhanced solubility of this salt is due in part to decreased crystal lattice energy.

To investigate the decrease in solubility of berberine hydrochloride due to the common ion effect, it was decided to measure the solubility of the hydrochloride salt as a function of chloride ion concentration. Setschenow plots for berberine hydrochloride in the presence of various salts are shown in Fig. 1. Sodium chloride showed a salting-out effect in the hydrochloride solution, indicating the existence of a significant common ion effect owing to the

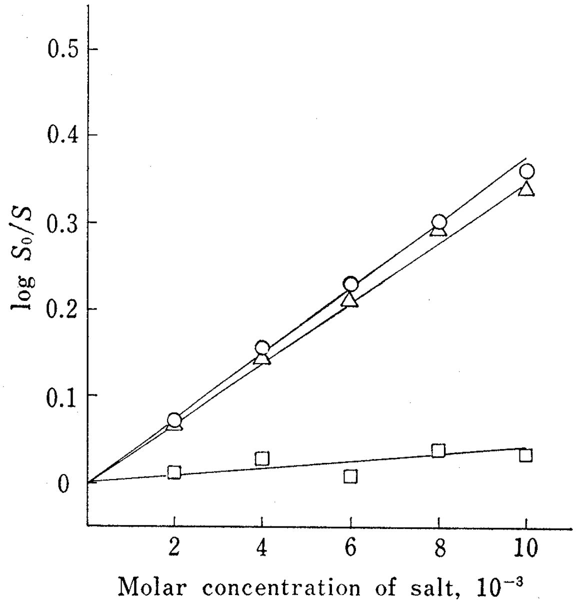

Fig. 1. Setschenow Plots for Berberine Hydrochloride in Salt Solutions at 37°

—○—: NaCl, —△—: KCl, —□—: Na_2SO_4.

Table I. Apparent Solubilities and Melting Points of Salt Forms of Berberine

Salt form	mp (dec.)	Temp.	Apparent solubility[a] (mg/ml)	pH[b]
Hydrochloride	180—182°	25°	1.41	5.19
		37°	2.64	4.93
Sulfate	140—142°	25°	79.58	5.59
		37°	277.00	4.73
Iodide	255—258°	25°	0.07	6.53
		37°	0.10	5.88

a) All data are expressed as free base equivalent.
b) pH of saturated solutions.

Table II. Salting-out Constants of Salt Forms of Berberine at 37°

Salt form	Salt	Salting-out constant, *k*
Hydrochloride	NaCl	36.67
Hydrochloride	KCl	35.00
Hydrochloride	Na_2SO_4	3.75
Sulfate	NaCl	1.67

addition of excess chloride, which significantly reduces the dissociation of the hydrochloride salt, reducing its solubility. A similar salting-out effect was noted with KCl. Since the data for the two salts are similar, specific effects due to the cation are considered to be negligible. The presence of Na_2SO_4, on the other hand, appears to have little influence on the solubility of berberine hydrochloride. In addition, the solubility of the sulfate salt was only slightly affected by the addition of NaCl, as shown in Table II, which summarizes the salting-out constants obtained. These results indicate that the solubility of berberine hydrochloride decreases considerably with increase in chloride-ion concentration.

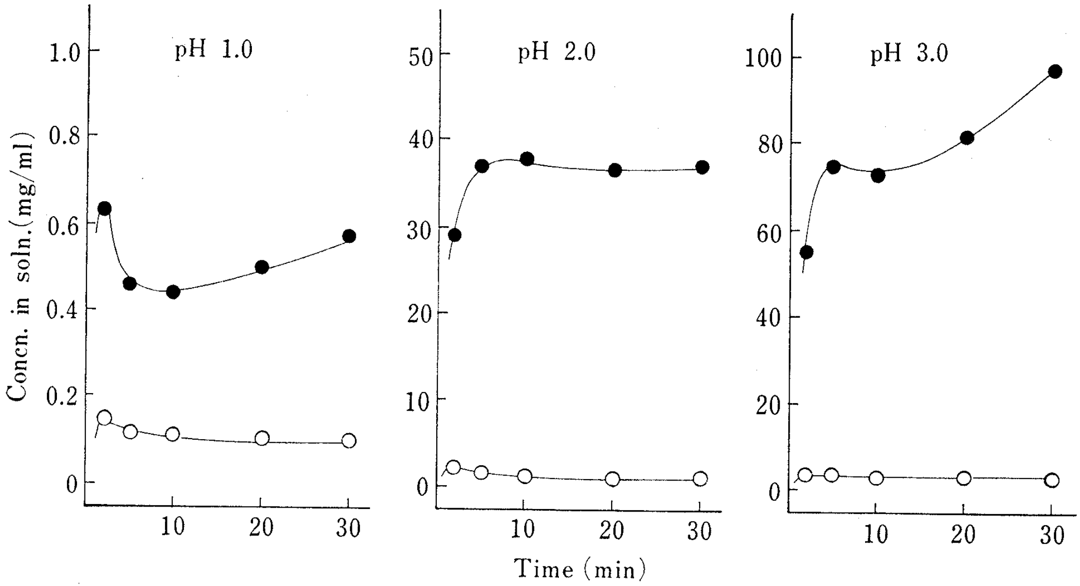

Fig. 2. Dissolution Curves of Hydrochloride (○) and Sulfate (●) Salts of Berberine in Dilute Hydrochloric Acid Solutions at 37°

To substantiate the argument against the use of the hydrochloride salt of berberine due to its poor solubility and the common ion effect, the dissolution behavior of the hydrochloride salt and that of sulfate salt of berberine were compared in media resembling GI fluids with respect to pH. Figure 2 shows the dissolution curves of both salt forms in hydrochloric acid solutions at pH 1.0, 2.0, and 3.0 at 37°. Distinct differences in dissolution behavior were observed between the two salt forms at each pH value, the sulfate salt yielding much greater drug concentrations in solution than the hydrochloride salt.

The dissolution behavior of the two salt forms in pH 7.4 isotonic phosphate buffer was also examined. As shown in Fig. 3, the differences were similar to those in hydrochloric acid solutions; the sulfate salt dissolved in the buffer rapidly and the drug concentration in solution reached 17.8 mg/ml in 30 min.

The results of this study indicate that the apparent solubility and dissolution rate of hydrochloride salt of berberine are considerably less than those of the sulfate salt in chloride-containing media, resulting in incomplete dissolution of the former in the GI tract. In such cases, the use of an alternative salt form, for example the sulfate salt, may improve the dissolution and availability.

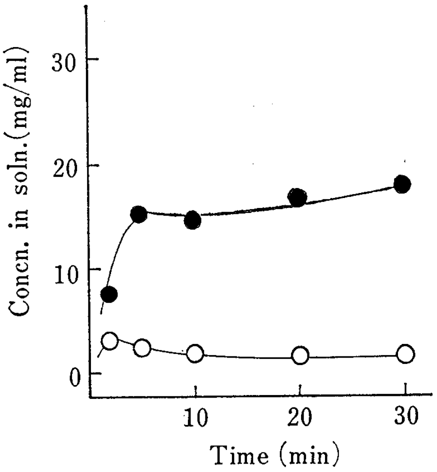

Fig. 3. Dissolution Curves of Hydrochloride (○) and Sulfate (●) Salts of Berberine in pH 7.4 Isotonic Phosphate Buffer at 37°

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