The detailed properties of Y′ and Y″ proteins are now under investigation, and will be reported later.

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Physical Properties of Pyrimidine and Purine Antimetabolites. I. The Effects of Salts and Temperature on the Solubility of 5-Fluorouracil, 1-(2-Tetrahydrofuryl)-5-fluorouracil, 6-Mercaptopurine, and Thioinosine

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The effects of sodium chloride, sodium sulfate, and sodium iodide on the solubility of 5-fluorouracil, 1-(2-tetrahydrofuryl)-5-fluorouracil, 6-mercaptopurine, and thioinosine were studied. Sodium chloride and sulfate salted-out these antimetabolites while sodium iodide salted-in them. The solubility of these antimetabolites at various temperatures was also measured. There was no indication of the transition in the stable form in the temperature range studied.

We have earlier observed that alkylxanthines (caffeine, theophylline, theobromine, and caffeine derivatives) and uracil exhibited anomalous salting behaviors.[2)] Since pyrimidine and purine antimetabolites are also highly polar compounds, their physical properties in aqueous solutions can be different from those of slightly polar nonelectrolytes in water. Probably because of the highly polar nature of these antimetabolites, they are not solubilized by surfactants such as polysorbate 80 and sodium lauryl sulfate and not included into the interior cavity of the β-cyclodextrin molecule to any significant extent.[3)]

Since activity rather than concentration is important in partition (which applies to extraction from biological fluids into organic solvents prior to assay) and permeation (which applies to the controlled delivery), the salting behaviors of 4 pyrimidine and purine antimetabolites were examined. The effects of temperature were also determined in order to examine if there is any transition in the stable form with the change in temperature as was observed in ampicillin,[4)] cyclacillin,[5)] and sulfanilamide.[6)]

Experimental

Materials——5-Fluorouracil, 1-(2-tetrahydrofuryl)-5-fluorouracil (THFFU), and thioinosine were gifts from Kyowa Hakko Co., Taiho Pharmaceutical Co., and Morishita Seiyaku Co., respectively, 6-Mercaptopurine hydrate was purchased from Sigma Chemical Co. Sodium sulfate (anhydrous), sodium chloride, sodium iodide, and sodium thiosulfate (pentahydrate) were all of reagent grade and purchased from Wako Pure Chemical Industries.

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1) Location: *Kita-12, Nishi-6, Kita-ku, Sapporo 060, Japan.*
2) K. Kakemi, H. Sezaki, T. Mitsunaga, and M. Nakano, *Chem. Pharm. Bull.* (Tokyo), **18**, 724 (1970).
3) Y. Arakawa and M. Nakano, unpublished data.
4) J.W. Poole and C.K. Bahal., *J. Pharm. Sci.*, **57**, 1945 (1968).
5) J.W. Poole and C.K. Bahal, *J. Pharm. Sci.*, **59**, 1265 (1970).
6) K. Sekiguchi, Y. Tsuda, and M. Kanke, *Chem. Pharm. Bull.* (Tokyo), **23**, 1353 (1975).

Measurements of the Solubility in Salt Solutions——Salt solutions (4 ml) were placed in 5 ml capacity vials and an excess amount of drug was added. Each vial was then sealed with a Teflon-lined screw cap. The vials were shaken horizontally in a waterbath (Taiyo Incubator Model M^{1N}) maintained at 30.0° for a period of about 20 hours. Since the pKa values of 5-fluorouracil and THFFU are in the neutral pH range (8.0[7] and 7.7[8] respectively), care has been taken to maintain the pH of salt solutions less than 6 in order to ensure that essentially all of the drug was present in unionized form. For every drug-salt combination, at least 3 salt concentrations were used. When iodide solutions were used, they were protected from oxidation by 1 mM sodium thiosulfate. After equilibration, the solutions were quickly filtered through a sintered-glass disk. An aliquot of the filtrate was then appropriately diluted for spectrophotometric assay (Hitachi Perkin-Elmer Model 139) at the wavelength of the maximum absorbance of each antimetabolite. Experimental salting-out constants were calculated according to the following Setschenow equation.[9]

$$\log \frac{S^\circ}{S} = k_{app} C_s$$

where S and S° are the molar solubility in the salt solution and in pure water, respectively, C_s the molar concentration of the electrolyte, and k_{app} the empirical salting-out parameter.

Measurements of Solubility at Different Temperatures——An excess amount of each antimetabolite was equilibrated for more than 10 hours at various temperatures in a water-jacketed-beaker connected to a circulating waterbath. Adequate mixing was obtained by stirring with a magnetic stirrer. Subsequently the suspension was quickly filtered through a sintered-glass disk and the filtrate was assayed for the dissolved drug. The assay procedures were as described for the measurements of solubility in salt solutions.

Results and Discussion

Effects of Salts

The effects of sulfate, chloride, and iodide on the solubility of 5-fluorouracil, THFFU, 6-mercaptopurine, and thioinosine are shown graphically, based on the Setschenow equation, in Fig. 1—4. Some of the plots are nonlinear. But the order of the effects of different salts was the same for all of the antimetabolites. In every drug solution, sulfate showed greater salting-out effects than chloride, while iodide exhibited salting-in effects.

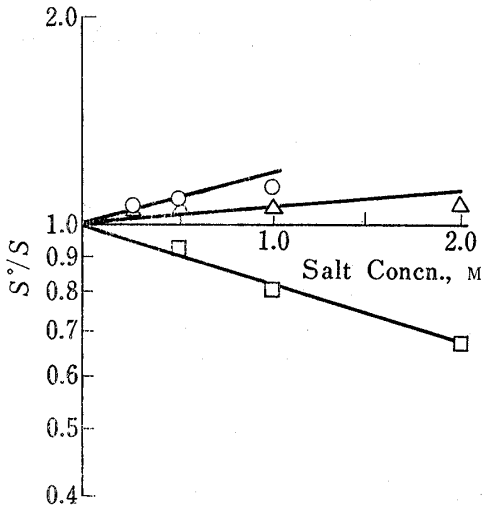

Fig. 1. Setschenow Plots for 5-Fluorouracil at 30°

Key: ○, Na_2SO_4; △, NaCl; and □, NaI

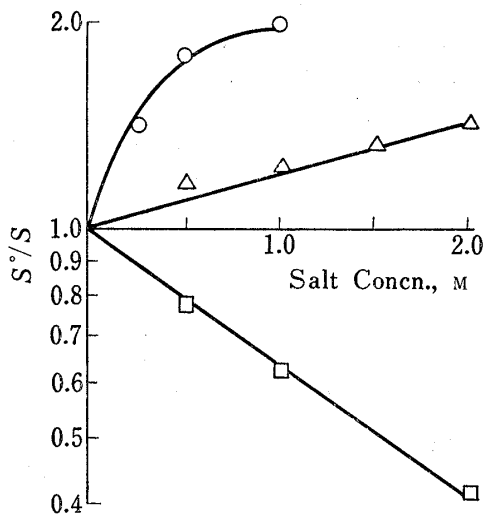

Fig. 2. Setschenow Plots for THFFU at 30°

Key: ○, Na_2SO_4; △, NaCl; and □, NaI

7) B.C. Rudy and B.Z. Senkowski, in "Analytical Profiles of Drug Substances," Vol. 2, K. Florey, Ed. Academic Press, 1973, p. 221.
8) K. Yamagami, A. Akazawa, and M. Yonemoto, Physical-chemical Properties of N_1-(2-Tetrahydrofuryl)-5-fluorouracil, Taiho Pharmaceutical Co.
9) F.A. Long and W.F. McDevit, *Chem. Rev.*, **51**, 119 (1952).

When 5-fluorouracil and THFFU are compared, the solubility of the latter is much more influenced by the salts than the former, suggesting significant changes in the activity coefficient of THFFU in electrolyte solutions probably due to the presence of a hydrophobic tetrahydrofuryl group. The effects of salts on permeation of THFFU through a silicone rubber membrane will be published elsewhere.[10]

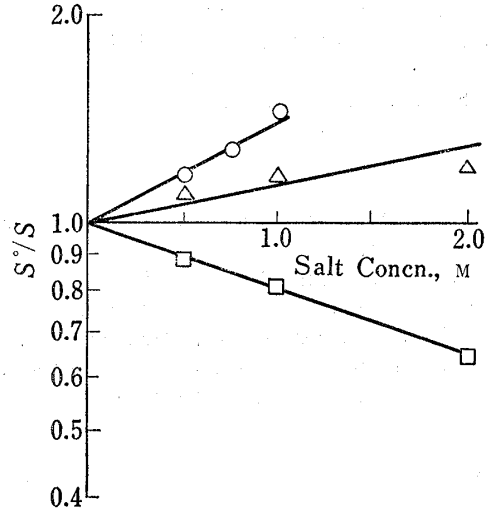

Fig. 3. Setschenow Plots for 6-Mercaptopurine at 30°
Key: ○, Na_2SO_4; △, NaCl; and □, NaI

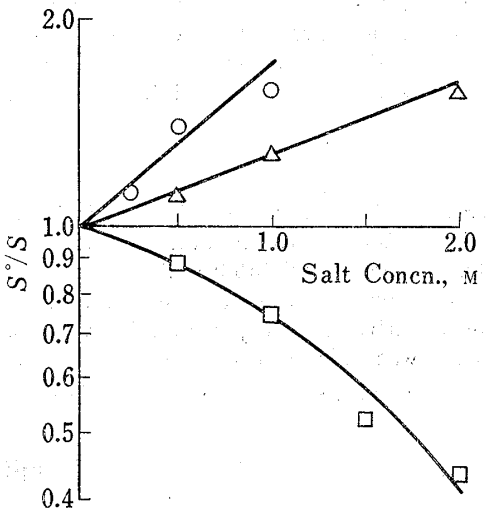

Fig. 4. Setschenow Plots for Thioinosine at 30°
Key: ○, Na_2SO_4; △, NaCl; and □, NaI

When 6-mercaptopurine and thioinosine are compared, the effects of salts were somewhat more pronounced in the latter. Robinson and Grant[11] found more pronounced salt effects in adenosine than in adenine and they attributed the greater degree of salting-in effects in the nucleoside to the larger self-interaction effects expected with the more soluble nucleoside. Since thioinosine is more than 10 times as soluble as 6-mercaptopurine, the similar interpretation may be applicable. The empirical salting-out parameters obtained from Fig. 1—4 are summarized in Table I.

Table I. Empirical Salting out Parameters at 30.0°

Salt	5-Fluorouracil	THFFU	6-Mercaptopurine	Thioinosine
Na_2SO_4	0.058	0.56[a]	0.14	0.23
NaCl	0.014	0.077	0.048	0.097
NaI	−0.087	−0.20	−0.097	−0.13[a]

a) estimated from the initial slope

Effects of Temperature

The solubilities of 4 antimetabolites at various temperatures are shown in Fig. 5 and 6. As shown in Fig. 5, THFFU is slightly more soluble than 5-fluorouracil at 37° in spite of the fact that the former is a hydrophobic tetrahydrofuryl derivative of the latter. This may indicate stronger hydrogen bonds in the solid state of 5-fluorouracil as apparent from the higher melting point of 5-fluorouracil (mp 280—284°)[7] than that of THFFU (mp 167—168°)[8]. The heats of solution obtained from Fig. 5 are 4.5 kcal/mole and 5.3 kcal/mole for 5-fluorouracil and THFFU, respectively.

10) M. Nakano, Y. Arakawa, K. Juni, and T. Arita, *Chem. Pharm. Bull.* (Tokyo), in press.
11) D.R. Robinson and M.E. Grant, *J. Biol. Chem.*, **241**, 4030 (1966).

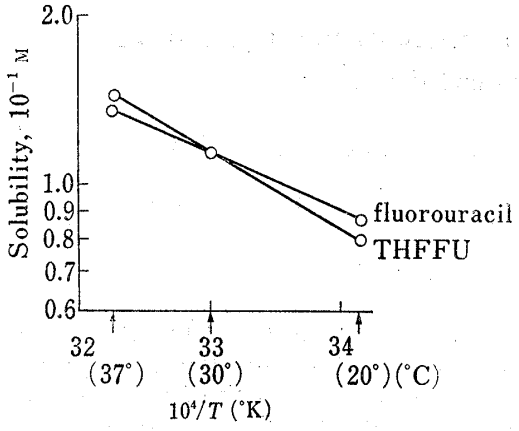

Fig. 5. Solubilities of 5-Fluorouracil and THFFU in Water as a Function of Temperature

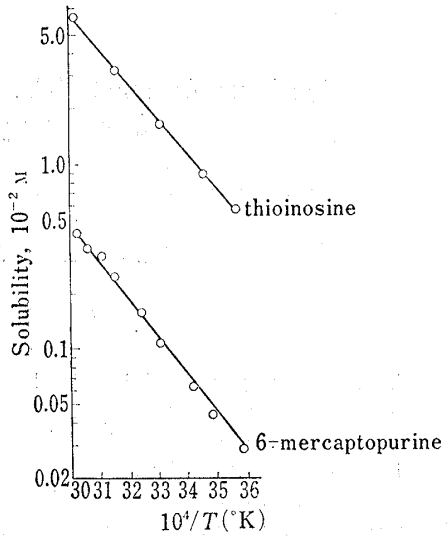

Fig. 6. Solubilities of 6-Mercaptopurine and Thioinosine in Water as a Function of Temperature

As shown in Fig. 6, the solubility of thioinosine is some 10 times greater than that of 6-mercaptopurine. Since the slopes of the van't Hoff type plot are almost parallel, it is expected that their heats of solution are similar. The heats of solution obtained from Fig. 6 are 9.3 kcal/mole and 8.8 kcal/mole for 6-mercaptopurine and thioinosine, respectively. The straight lines in the van't Hoff type plots indicate that the hydrate is the only stable form throughout the temperature range of 6—54° for 6-mercaptopurine and the anhydrous form is the only stable form for thioinosine throughout the same temperature range. If there were transition in the stable form, there would have been a break point in the plot.

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