Chem. Pharm. Bull. **43**(3) 476—482 (1995) Vol. 43, No. 3

Preliminary Preformulation Studies of a 2-(3,4-Dimethoxyphenyl)ethylamine Derivative for Oral Administration at an Exploratory Stage of New Drug Development

Masami MORITA,*[,a] Toru HOSOKAMI,[b] Tutomu KONNO,[a] Koji KINUNO,[a] Eiichi MAFUNE,[a] and Norio TAKASUGI[a]

Developmental Research Laboratories[a] and Exploratory Research Laboratories III,[b] Daiichi Pharmaceutical Co., Ltd., 16–13, Kitakasai 1-chome, Edogawa-ku, Tokyo 134, Japan. Received August 25, 1994; accepted November 15, 1994

Preliminary preformulation studies of a 2-(3,4-dimethoxyphenyl)ethylamine derivative were investigated. The hydrochloride form showed incompatibility with the excipients used for oral dosage forms. There were several crystal forms of the free base, namely, α-anhydrate, β-anhydrate, monohydrate, and trihydrate. The trihydrate form was unstable. The degree of crystallinity of the β-anhydrate form was difficult to control. The monohydrate form was difficult to manufacture with constant quality.

The serum levels of the compounds in rats were almost related to the dissolution rates in the JP 1st disintegration medium from the discs. The serum level of α-anhydrate was the lowest. However, the dissolution rates from the formulations of α-anhydrate were improved. After oral administration of the improved formulation, the serum level of α-anhydrate in beagle dogs was almost triple that after the oral administration of the capsule of the hydrochloride form.

Key words preformulation; exploratory stage; stability; compatibility; absorption

There have been several reports discussing the importance of the salt selection for an ionic drug candidate.[1–3] It is also important that the physicochemical properties of a series of new drug candidates, as well as salt forms,[1] are preliminarily evaluated in the selection of a drug candidate.

We reported that *N*-[2-(3,4-dimethoxyphenyl)ethyl]-2-phenylaminoacetamide hydrochloride had significant antiulcer activity.[4] During the exploratory research, a 2-(3,4-dimethoxyphenyl)ethylamine derivative was synthesized and preliminary preformulation studies were performed. In this paper, several physicochemical and biopharmaceutical properties of the derivative were investigated.

Experimental

Materials 3-[[[2-(3,4-Dimethoxyphenyl)ethyl]carbamoyl]methyl]-aminobenzamide hydrochloride (**1**) (Fig. 1) was synthesized as described in a previous paper.[4]

Measurements of Melting Point (mp) and Proton Nuclear Magnetic Resonance (^{1}H-NMR) Spectra, and Element Analysis Measurements of mp and ^{1}H-NMR spectra, and element analysis, were performed with the same apparatuses described in a previous paper.[4] Splitting patterns are designated as followed: s, singlet; br s, broad singlet; br, broad; d, doublet; dd, double doublets; t, triplet; q, quartet; m, multiplet.

Preparations of α-Anhydrate, β-Anhydrate, Monohydrate, Trihydrate and Amorphous Sample of Free Base The α-anhydrate of the free base of **1** was prepared as follows. After adding **1** (6.0 g, 0.015 mol) to a mixture of $CHCl_3$ (250 ml) and 5% aqueous Na_2CO_3 (200 ml), the resulting mixture was stirred at room temperature for 30 min. The organic layer was separated from the aqueous layer, washed with water, and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the residue was recrystallized from a mixture of MeOH and Et_2O to give 4.7 g (87%) of α-anhydrate as colorless crystals, mp 133—135 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.62 (2H, t, J=7.3 Hz), 3.29 (2H, br q, J=6.8 Hz), 3.64 (2H, d, J=5.9 Hz), 3.70 (3H, s), 3.71 (3H, s), 6.07 (1H, t, J=5.9 Hz), 6.61—7.16 (7H, m), 7.21 (1H, br s), 7.79 (1H, br s), 7.87 (1H, br t, J=5.9 Hz). *Anal.* Calcd for $C_{19}H_{23}N_3O_4$: C, 63.85; H, 6.48; N, 11.76. Found: C, 63.59; H, 6.40; N, 11.71.

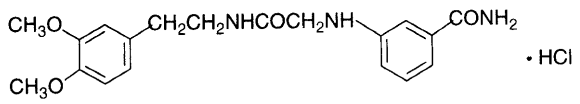

Fig. 1. Chemical Structure of **1**

The trihydrate of a free base of **1** was prepared as follows. Compound **1** (15.0 g, 0.038 mol) was dissolved in water (1.5 l) at 60 °C with stirring. After standing overnight at room temperature, the resulting precipitate was collected by filtration. The crystals were air-dried at room temperature to give 12.7 g (81%) of trihydrate as colorless needles, mp 54—56 °C. *Anal.* Calcd for $C_{19}H_{23}N_3O_4 \cdot 3H_2O$: C, 55.46; H, 7.10; N, 10.21. Found: C, 55.29; H, 7.15; N, 10.32.

β-Anhydrate was prepared by desiccating the trihydrate under reduced pressure over $CaCl_2$ at room temperature overnight. An amorphous sample was prepared by desiccating the trihydrate under reduced pressure over P_2O_5 at 50 °C for 30 min. The monohydrate was prepared by storing the trihydrate at 40 °C and 75% R.H. for 7 d.

Preparation of Solid Dispersion with Macrogol 6000 Amorphous sample (5 g) and macrogol 6000 (5 g, JP grade) were mixed in a beaker and the mixture was heated to melt at 60 °C. After cooling to room temperature, the solidified sample was ground to granules in a mortar.

Preparation of Suspension Samples A suspension was prepared in a 0.5% aqueous sodium carboxymethylcellulose solution by the same method for the oral administration of rat experiments as described in a previous paper.[4]

Preparation of Formulation Samples of α-Anhydrate with Improved Dissolution The recipes for the formulations are shown in Table I. The preparation methods were as follows. Formulation No. 2: α-Anhydrate

TABLE I. Recipes of the Formulation of α-Anhydrate

	Formulation No.		
	2	31	32
α-Anhydrate (mg)	200	200	200
Hydroxypropylcellulose (mg)	4	4	4
Microcrystalline cellulose (mg)	8	141	192
Corn starch (mg)	8	—	—
Polyoxyl 40 stearate (mg)	—	2	—
Low substituted hydroxypropylcellulose (mg)	—	148	50
Dibasic calcium phosphate (mg)	—	—	50
Magnesium stearate (mg)	—	5	4
Total (mg)	220	500	500

* To whom correspondence should be addressed.

was ground by jet-milling, then the ground sample was coated by spraying with a 5% aqueous hydroxypropylcellulose solution. All excipients were mixed with α-anhydrate coated by hydroxypropylcellulose in a mortar and were filled in a capsule. Formulation No. 31: Hydroxypropylcellulose and polyoxyl 40 stearate (JP grade) were used as coating agents for jet-milled α-anhydrate and microcrystalline cellulose (JP grade), respectively. Microcrystalline cellulose was wetted using a polyoxyl 40 stearate aqueous solution and was mixed in a mortar. A wet mixture was air-dried overnight. All materials were mixed in a mortar and were filled into a capsule. Formulation No. 32: All excipients were mixed with α-anhydrate coated by hydroxypropylcellulose in a mortar, and the mixture was compressed using circular, flat-faced 9 mm punches to make a tablet.

Water Content Water content was determined by the Karl Fisher method using a model MK-A2 titrator (Kyoto Electronics Co.).

Thermal Analysis Differential scanning calorimetry (DSC) thermograms were measured with a DSC instrument (Perkin-Elmer Model DSC-1B). Indium was used for calibrating DSC. A sample (3 mg) in a closed pan was scanned at a heating rate of 2 °C/min. Thermogravimetry (TG) was performed with a TG instrument (Shimazu Model DT-30). The sample (10 mg) was scanned at the same heating rate as that of DSC.

Infrared Spectroscopy (IR) IR spectra were recorded using a spectrometer (Hitachi Model 285) as a KBr disk.

Powder X-Ray Diffractometry (XRD) XRD patterns were measured with a diffractometer (A Geiger Flex 2012, Rigaku Denki Co., Ltd.). The operating conditions were the same as reported in a previous paper.[5]

Calculations of the Degree of Crystallinity and the Disorder Parameter The degree of crystallinity, X_{cr}, and the disorder parameter, k, were determined with a Burroughs 7800 computer by the automated computing procedure reported in a previous paper.[5]

Measurement of pK_a pK_a of the compound in methanol was determined by the spectrophotometric method[6] using the absorbance at 218 nm. A Hitachi 124 Spectrophotometer was used.

Measurements of Moisture Sorption and Crystal Transformation Studies Samples were stored at 25 and 40 °C in a saturated aqueous solution of various inorganic salts for 30 d. Weight change was measured during the storage. Polymorphic transformation, desolvation, and solvation during the storage were confirmed by XRD. For crystal transformation studies, samples were also stored at 50 and 100 °C.

Measurements of Solubilities Solubilities were measured by the same method as described in a previous paper.[4]

Measurements of Dissolution Rates 1) Rotating Basket Method: The dissolution rates of the formulation and powder (200 mg) were measured by the JP XI rotating basket method. The dissolution medium was JP XI, 1st fluid (pH1.2). Its volume and temperature were 900 ml and 37±0.5 °C, and the rotation speed was 50 rpm. Aliquots (5 ml) of the solution were withdrawn at appropriate intervals with a syringe. Samples were filtered through a 0.45 μm Millipore membrane. The dissolution of the compound into the medium was measured by the absorbance at 221 nm using a Hitachi 557 Double Wavelength Double Beam Spectrophotometer.

2) Paddle Method: The sample (100 mg) was compressed using circular, flat-faced 13 mm punches until the compressive force became 5 t/cm^2, and this state was maintained for 30 s. Then, the disk was ejected. The bottom and edge of the disk were sealed by polymer adhesives (Bond Alon α, Toa Gousei Kagaku Co.) and adhered to the stainless-steel metal. The sample was placed at the bottom of a 1000 ml round-bottomed flask. A dissolution test was performed by the JP XI paddle method. The dissolution medium was JP XI, 1st fluid (pH1.2). Its volume and temperature were 900 ml and 37±0.5 °C, and the rotation speed was 50 rpm. The sampling and assay methods were the same as described in the section 1) rotating basket method.

Compatibility Studies with Excipients for Oral Dosage Form The compound (100 mg) and each excipient (100 mg) were uniformly mixed in a mortar. The mixture was stored at 40 °C and 75% R.H. for 30 d. Excipients used were JP grades of lactose, sucrose, corn starch, mannitol, microcrystalline cellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose, magnesium stearate, talc, titanium dioxide, and macrogol 6000. Changes in appearance were observed.

Scanning Electron Microscopy (SEM) SEM photographs were taken with a scanning electron microscope (model JSM-T20, JOEL Co.) to observe the difference between the surface of trihydrate and that of β-anhydrate.

Absorption Studies Male Donryu rats weighing 270 to 340 g and male beagle dogs weighing 13.5 to 15.5 kg were used. All animals were fasted for 24 h prior to drug administration. The absorption studies of rats were the same as described in a previous paper.[4] For beagle dogs, either a capsule, a tablet or 200 mg of α-anhydrate powder was orally administered, and blood samples were collected from a foreleg vein at 0.5, 1, 2, 3, 4, 6, 8 h. The serum sample was analyzed using a high performance liquid chromatography method.[4]

Results and Discussion

Physicochemical Properties of Hydrochloride (1), α-Anhydrate, β-Anhydrate, Trihydrate, Monohydrate and Amorphous Sample The XRD pattern of **1** is shown in Fig. 2. The degree of crystallinity and the disorder of the crystal lattice were 78% and 9.9 Å^2. The latter was very large. Compound **1** is unstable in the air and was transformed to hydrochloride hemihydrate. The value of pK_a was very small (0.86 in MeOH). A short-term compatibility screen of **1** with excipients commonly used in solid dosage forms was conducted. It is shown in Table II that **1** was observed to be incompatible with all excipients, although α-anhydrate was compatible. For **1**, the physicochemical properties, especially the crystal lattice disorder and small pK_a value, may be the reason that hydrochloric acid is easily released by the adsorption of water, and that it reacts with all excipients.

Figure 3 shows the XRD patterns of α-anhydrate, trihydrate, and monohydrate of a free base of **1**. These degrees of crystallinity of were 85, 79 and 85%, respectively. The XRD patterns of β-anhydrate are shown

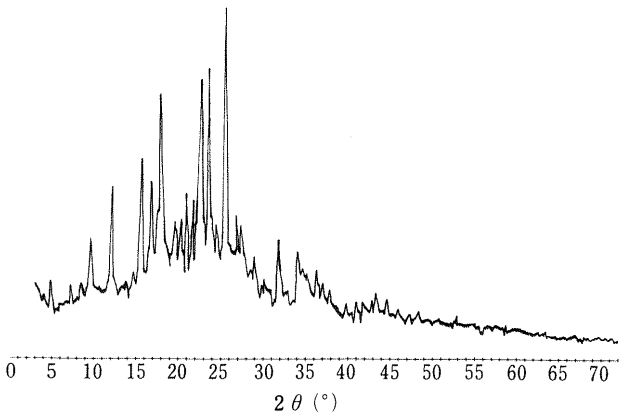

Fig. 2. X-Ray Diffraction Pattern of **1**

TABLE II. Compatibility of **1** and α-Anhydrate with Excipients Used for Oral Dosage Forms at 40 °C and 75% R.H. for 1 Month

Excipients	**1**	α-Anhydrate
No Excipients	+	−
Lactose	++	−
Sucrose	++	−
Corn starch	++	−
Mannitol	++	−
Microcrystalline cellulose	++	−
Hydroxypropylcellulose	++	−
Hydroxypropylmethylcellulose	++	−
Magnesium stearate	++	−
Talc	++	−
Titanium dioxide	++	+
Macrogol 6000	++	−

++, clear color change (yellow brown); +, color change (white yellow); −, no change.

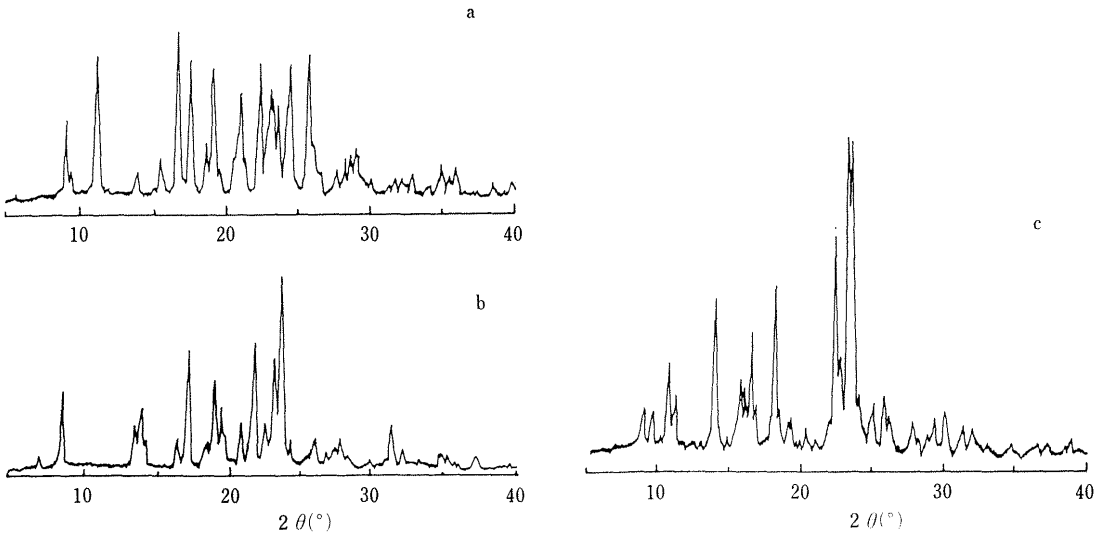

Fig. 3. X-Ray Diffraction Patterns of Trihydrate, Monohydrate, and α-Anhydrate of a Free Base of **1**
a, trihydrate; b, α-anhydrate; c, monohydrate.

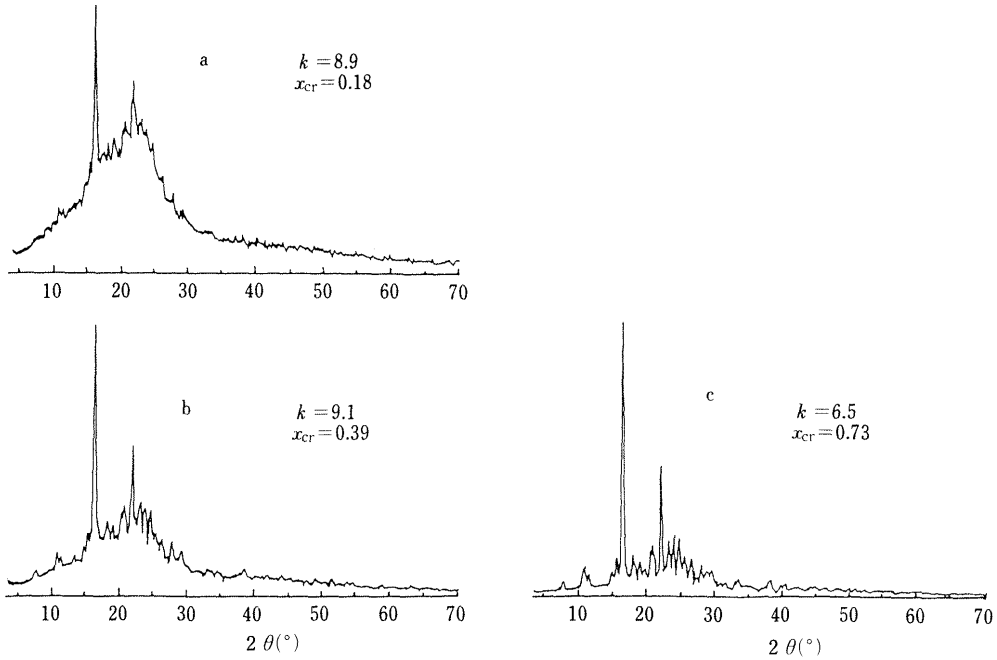

Fig. 4. Effects of Dehydration Conditions of Trihydrate on the Crystal Lattice Disorder and the Degree of Crystallinity of β-Anhydrate
Dehydration conditions: a, at 40°C under reduced pressure over P_2O_5; b, at 30°C under reduced pressure over P_2O_5; c, at room temperature under reduced pressure over $CaCl_2$.

in Fig. 4c. The states of the crystal lattices showed a lot-to-lot variation. The samples shown in Figs. 4a and 4b were prepared under reduced pressure over P_2O_5 at 40 and 30 °C, respectively. At dehydration of the trihydrate, thermal motion of crystal lattice or a variation in reduced pressure may influence the resulting crystalline states of the β-anhydrate obtained. Figure 5 shows SEM photographs of trihydrate, β-anhydrate and **1**. The photograph of β-anhydrate obtained by desiccating trihydrate in the solid-state shows many holes parallel to the long axis. It is well known that there are many hydrate crystals with water tunnels.[7] These observations show that the dehydration process occurs along the axis, and that the lattice of β-anhydrate is the cast-off lattice of the trihydrate. Compound **1** was an aggregate of small particles, as shown in Fig. 5.

When trihydrate was stored at 40 °C and 75% R.H., the trihydrate was transformed to a monohydrate. Figure 6 shows the DSC and TG thermograms. The dehydration of trihydrate occurred from 40 to 70 °C. The TG thermogram indicates several steps in the dehydration process. The TG thermogram of the monohydrate also indicates a several-step dehydration process, from 70 to 110 °C. The DSC thermogram of a monohydrate shows an endothermic peak at 133 °C. After heating to 100 °C, the trihydrate was transformed into an amorphous form. The gradual weight loss and the endothermic peak of **1** on the TG and DSC were observed above 120 °C. These changes are due to degradation.

The association states of hydrogen bonding between

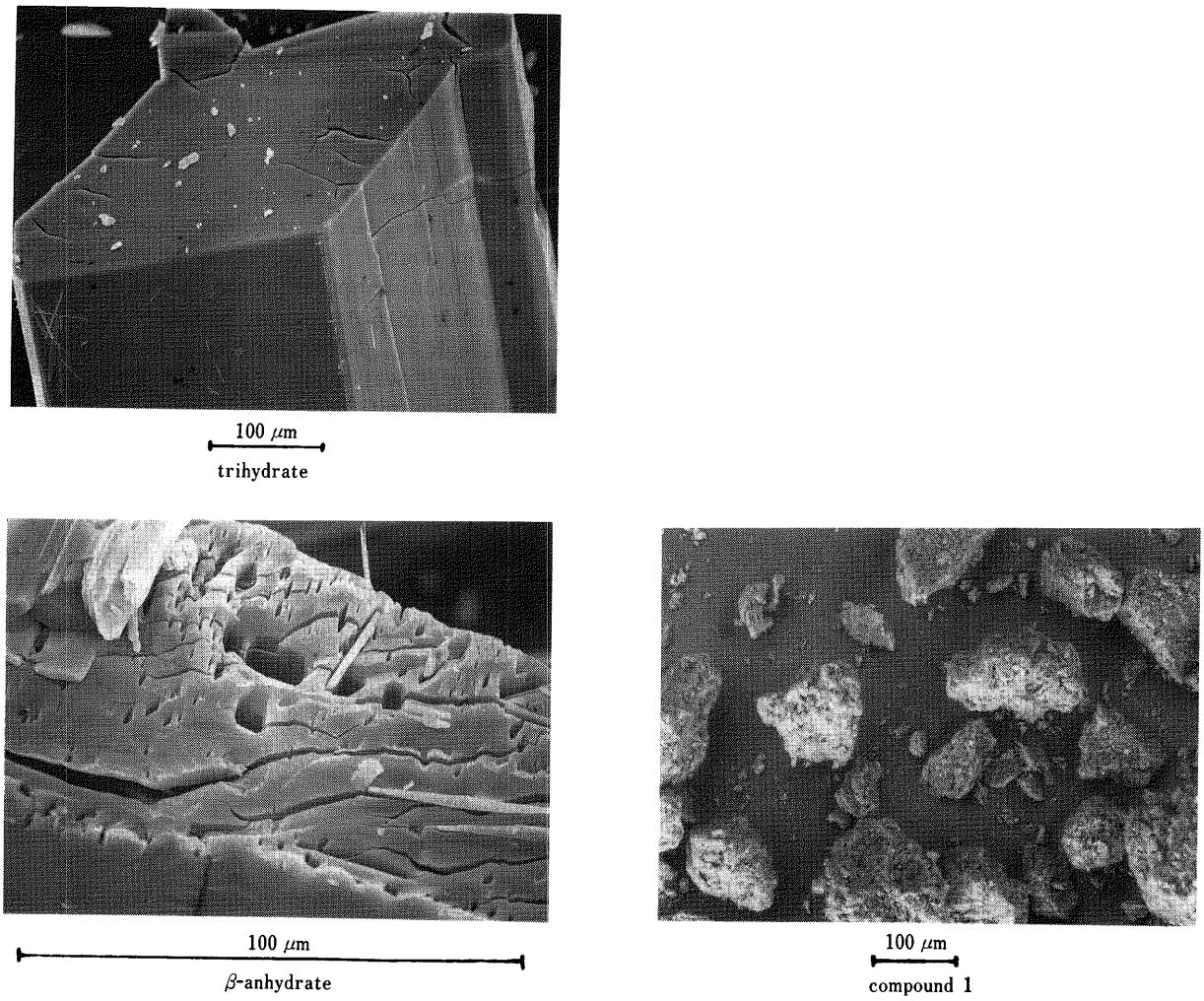

Fig. 5. Difference of Crystal Surface between Trihydrate, β-Anhydrate and **1**

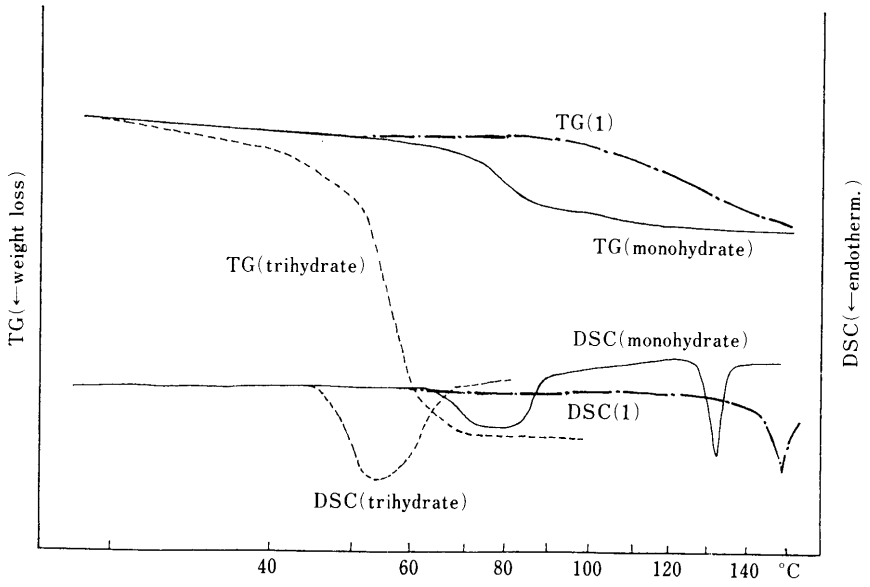

Fig. 6. TG/DSC Thermograms of Trihydrate, Monohydrate and **1**

intermolecular amides in their crystal lattices were examined by IR spectra. As shown in Fig. 7, the absorption of 1690 cm^{-1} on the IR spectra of trihydrate and that of β-anhydrate shows that no association between intermolecular amides was observed. The shift of this peak to 1655—1650 cm^{-1} of the α-anhydrate shows an association between amides, and indicates that α-anhydrate decreases the free energy of crystal lattice by association. This stabilization of the crystal lattice may result in decreasing the solubility. The corresponding peak width of the

monohydrate is rather wide. This indicates that the amides were associated, but that the crystal lattice was distorted by dehydration in the solid-state. This result corresponds with the higher solubility of the monohydrate than that of the α-anhydrate. These regions of the IR spectrum of **1** were similar to that of the α-anhydrate.

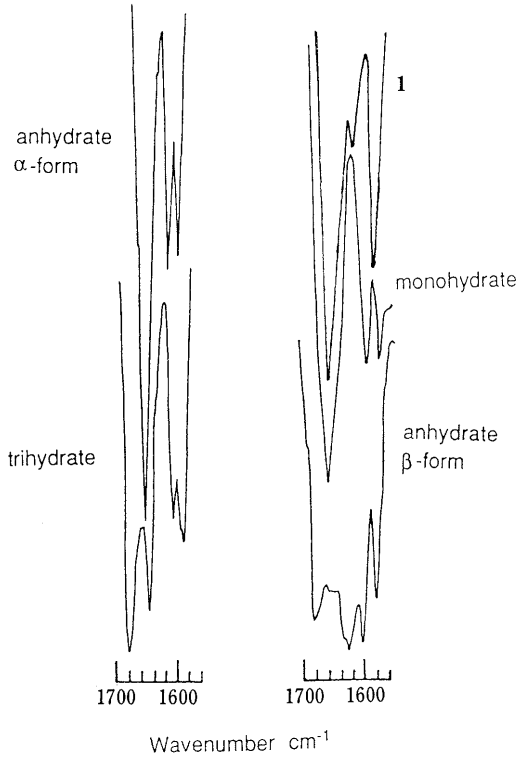

Fig. 7. IR Spectra of Trihydrate, Monohydrate, α-Anhydrate, β-Anhydrate and **1**

TABLE III. Solubilities of **1**, α-Anhydrate, β-Anhydrate, and Trihydrate in Water at Room Temperature

Sample	**1**	α-Anhydrate	β-Anhydrate	Trihydrate
Solubilities (mg/ml)	1.07	0.49	0.73	1.00

The solubilities of **1**, anhydrates and hydrates in water at room temperature are shown in Table III. The stabilization of the crystal lattice observed in the IR spectra corresponds with the rather lower solubility of the α-anhydrate than that of the trihydrate or β-anhydrate.

Moisture sorption curves of α- and β-anhydrates, trihydrate, **1** and the amorphous form at 25 °C for 30 d are shown in Fig. 8. The α-anhydrate and trihydrate show no weight change. However, when the trihydrate was stored at room temperature for 2 years, it transformed to an α-anhydrate. β-Anhydrate had a critical humidity, and over 62% R.H., β-anhydrate transformed to trihydrate. Compound **1** was hygroscopic. On the other hand, the amorphous sample gradually adsorbed moisture according

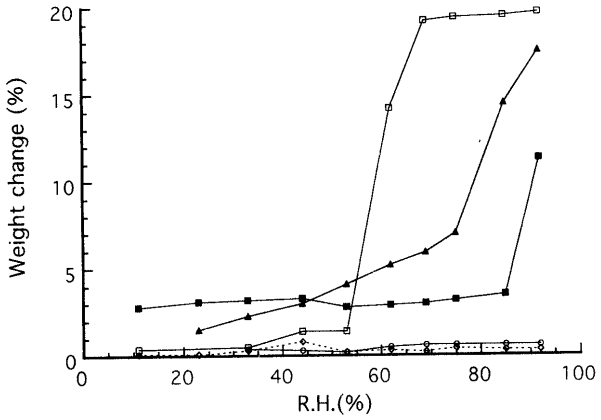

Fig. 8. Comparison of Hygroscopicities of Trihydrate, α-Anhydrate, β-Anhydrate, **1** and the Amorphous Form at 25 °C for 30 d

○—○, trihydrate; ◇···◇, α-anhydrate; □—□, β-anhydrate; ■—■, **1**; ▲—▲, the amorphous form.

TABLE IV. Physical Stability of Amorphous States in Solid Dispersions with Macrogol 6000

Storage condition	Crystalline state
100 °C 6 h	Crystallized to α-anhydrate
50 °C 10 d	Partially crystallized to α-anhydrate
40 °C 75% R.H. 10 d	Partially crystallized to α-anhydrate
25 °C 75% R.H. 10 d	Partially crystallized to α-anhydrate

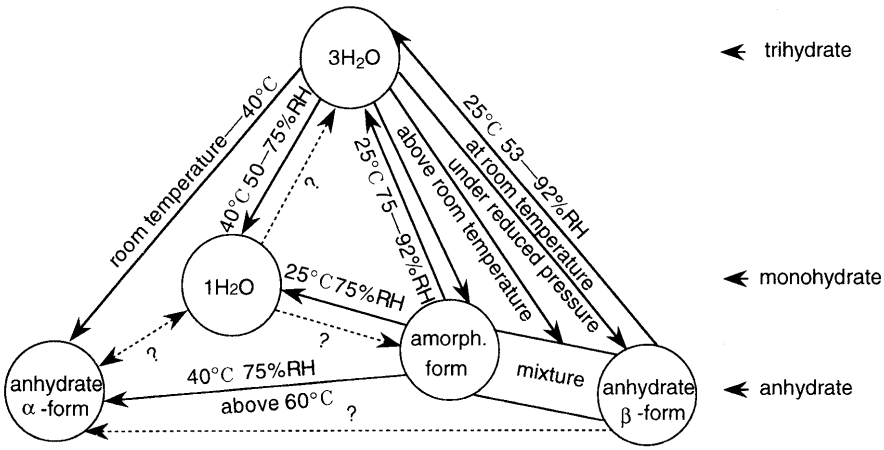

Fig. 9. Phase Transition Diagram

to the increase in relative humidity. The physical stability of the amorphous sample in solid dispersion with macrogol 6000 was examined. It is shown in Table IV. Under accelerated conditions, the amorphous state was not stable. The obtained phase transition diagram is summarized in Fig. 9.

Dissolution Studies and Biopharmaceutical Evaluation It was described[4] that the antiulcer effects of these derivatives bears a close relationship to their bioavailability, and that improved activities depend on their increased absorption and water solubility. The crystal forms of dispersed crystals, concentrations and the pH of the suspensions of various crystal forms were examined. The results are shown in Table V. The solubility of **1** was almost double that of trihydrate or β-anhydrate, and that of α-anhydrate was a half of the solubility of trihydrate or β-anhydrate. The crystal form of the suspended solids was trihydrate in suspension of **1**, trihydrate or β-anhydrate. However, in the case of α-anhydrate, the crystal form was not transformed at all. The dissolution rates of various crystal forms were examined by the paddle method. The results are shown in Fig. 10. The order of dissolution rates was as follows: **1** > β-anhydrate > monohydrate > trihydrate > α-anhydrate. The dissolution rates related well to the apparent solubilities of these crystal forms shown as concentrations in Table V. In Table III, however, the apparent solubilities of **1** and trihydrate were almost the same. These diferences seem to depend on the

Table V. Physico-Chemical Analysis of Suspension Samples for Oral Administration in Animal Experiments

Samples	**1**	α-Anhydrate	β-Anhydrate	Trihydrate
Solid dispersed	Trihydrate	α-Anhydrate	Trihydrate	Trihydrate
Drug concentration (mg/ml)	2.2	0.5	1.1	1.0
pH	0.88	6.25	6.30	6.31

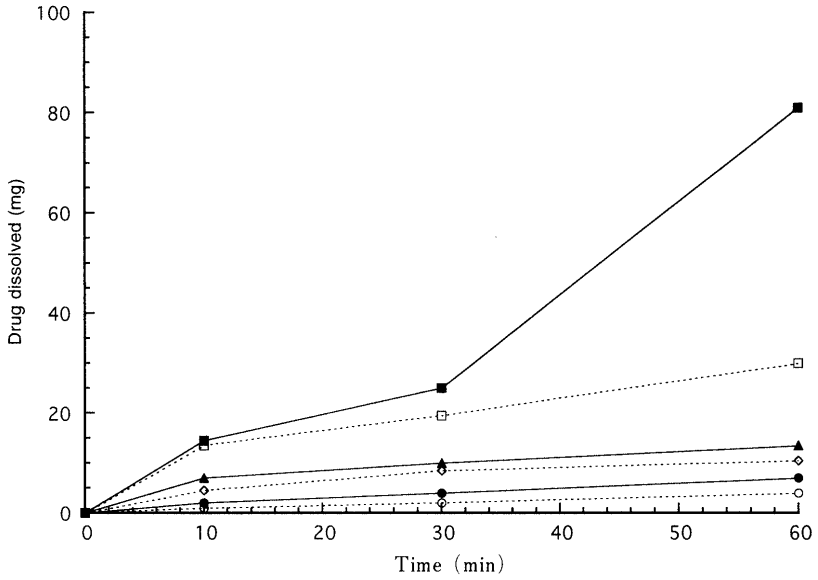

Fig. 10. Dissolution of **1**, Trihydrate, Monohydrate, α-Anhydrate and β-Anhydrate in JP 1st Disintegration Medium at 37 °C from the Disc ($n=3$)
■—■, **1**; □···□, β-anhydrate(ground); ▲—▲, monohydrate; ◇···◇, β-anhydrate(intact); ●—●, trihydrate; ○···○, α-anhydrate.

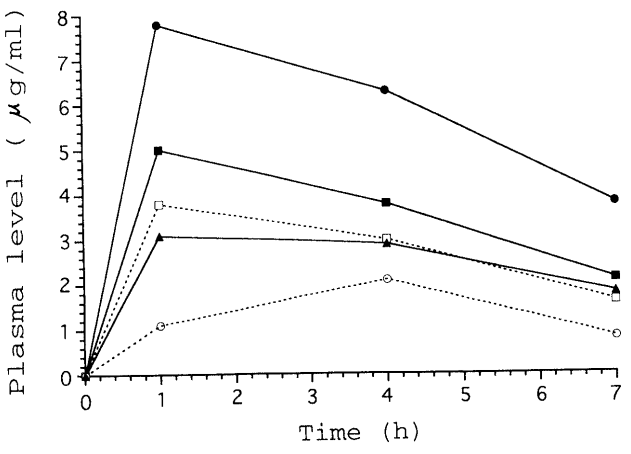

Fig. 11. Mean Serum Concentration of Free Base of **1** after Oral Administration of Various Crystal Forms to Donryu Rats ($n=6$)
■—■, **1**; □···□, β-anhydrate; ▲—▲, monohydrate; ●—●, trihydrate; ○···○, α-anhydrate.

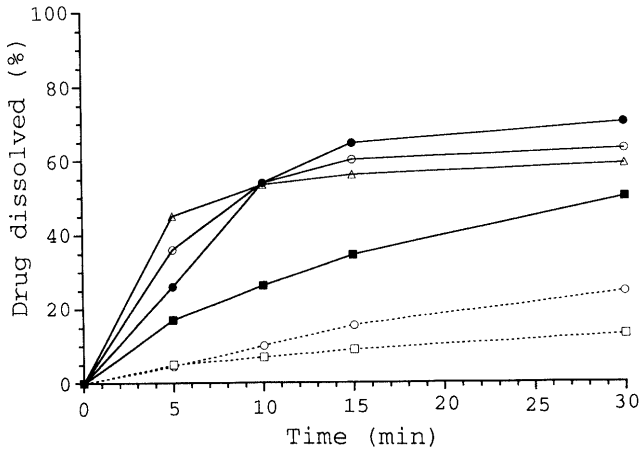

Fig. 12. Improvements of Dissolution of α-Anhydrate in JP 1st Disintegration Medium at 37 °C from Oral Dosage Forms by JP Rotating Basket Method ($n=3$)
■—■, **1**; □···□, β-anhydrate; ○···○, α-anhydrate; ○—○, formulation No. 2; ●—●, formulation No. 31; △—△, formulation No. 32.

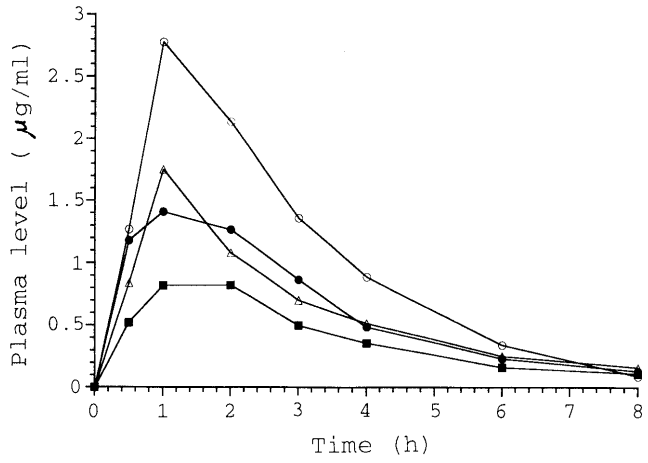

Fig. 13. Mean Serum Concentration of Free base of **1** after Oral Administration of Several Formulations to Beagle Dogs ($n=5$)

■—■, **1**; ○—○, formulation No. 2; ●—●, Formulation No. 31; △—△, formulation No. 32.

dissolution properties of the hydrochloride salt and the free base, as well as the particle sizes, as shown in Fig. 5. It was considered that hydrochloride salt, **1**, released HCl rapidly in the medium and turn to trihydrate. These considerations agreed with the fact that the solid dispersed in a suspension of **1** was trihydrate, as shown in Table V.

After the oral administration of the suspensions of these crystal forms to Donryu rats, serum concentrations were measured and are shown in Fig. 11. The order of serum levels of the compounds was as follows: trihydrate > 1 > β-anhydrate > monohydrate > α-anhydrate. Except for the trihydrate, these results were related to the dissolution rates. Since the size of the trihydrate crystal was very large, a slow dissolution rate was observed. However, in the oral administration of trihydrate homogenized in the medium, very small particles appeared, and the serum level of the trihydrate was higher.

We prepared formulation samples of α-anhydrate with improved dissolution. The dissolution rates of these samples were examined by the rotating basket method. The results are shown in Fig. 12. The dissolution rate of each formulation was faster than those of the powders of the other crystal forms.

After the oral administration of these formulations to beagle dogs, serum concentrations were measured and are shown in Fig. 13. The bioavailabilities of these formulations were improved compared to the α-anhydrate.

References

1) K. R. Morris, M. G. Fakes, A. B. Thakur, A. W. Newman, A. K. Singh, J. J. Venit, C. J. Spagnuolo, A. T. M. Serajuddin, *Int. J. Pharm.*, **105**, 209 (1994).
2) P. L. Gould, *Int. J. Pharm.*, **33**, 201 (1986).
3) S. M. Berge, L. D. Bighley, D. C. Monkhouse, *J. Pharm. Sci.*, **66**, 1 (1977).
4) T. Hosokami, M. Kuretani, K. Higashi, M. Asano, K. Ohya, N. Takasugi, E. Mafune, T. Miki, *Chem. Pharm. Bull.*, **40**, 2712 (1992).
5) M. Morita, S. Hirota, *Chem. Pharm. Bull.*, **30**, 3288 (1982).
6) A. Albert, E. P. Serjeant, "Ionization Constants of Acids and Bases. A Laboratory Manual," 1st ed., tr. by S. Matsuura, Maruzen, Tokyo, 1963, pp. 63—85.
7) S. R. Byrn, "Solid-State Chemistry of Drugs," 1st ed., Academic Press, New York, 1982, pp. 149—188.