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Physical Characterization of Erythromycin Dihydrate, Anhydrate and Amorphous Solid and Their Dissolution Properties[1)]

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Three erythromycin solids were characterized. In differential scanning calorimetry (DSC), the anhydrate exhibited only a melting endotherm at 193 °C. The dihydrate was desolvated at temperatures between 59 and 105 °C and liquefied at 124—130 °C. The dehydration product remained crystalline up to 124 °C (polarizing microscopic observation). When the dihydrate was heated at 135 °C and cooled to room temperature, an amorphous solid having a glass transition temperature of 106 °C was produced. The monohydrate reported by Allen *et al.* was found in this study to be a desolvation product of the chloroform solvate.

The aqueous solubilities for the dihydrate and the anhydrate were determined by a conventional method, while that for the amorphous solid was estimated by measuring the dissolution rate by means of a rotating disk method. The temperature dependence of solubility for each form was such that the solubility increased with decrease in temperature. The heats of solution increased with temperature and the plots against temperature for the three forms could be fitted to a straight line. The slope of the plot appeared to be identical among the three forms. This result suggested that the above peculiar temperature dependence of solubility might result from some intermolecular interaction in the aqueous solution.

Keywords——erythromycin; amorphous solid; solubility; thermal behavior; activation energy; glass transition

Erythromycin forms solvates with water or various organic solvents such as acetone, chloroform, ethanol, *n*-butanol and isopropanol.[2-6)] Many kinds of solvates can easily be desolvated by mild heat treatment or even by leaving them under room conditions.[2-6)] Accordingly, industrial raw material is often a desolvation product. Such a property of erythromycin sometimes causes problems in tabletting or in the disintegration and dissolution of tablets. In addition, since erythromycin is usually formulated as enteric-coated tablets, phase transition can be expected to occur during contact with organic solvents in the coating process.

Commercial erythromycin is usually available as the dihydrate. The dihydrate can be obtained by recrystallization from an aqueous solution or from an aqueous acetone solution at a temperature below about 50 °C.[2-5)] By heating an aqueous suspension of the dihydrate powder at 55—100 °C[3,4)] or by drying the dihydrate powder for one week at 105 °C in a vacuum oven,[6)] an anhydrate can be prepared. The anhydrate is the only crystalline phase that is not solvated.

Two kinds of monohydrate were first reported by Shtolts *et al.*[4)] Pelizza *et al.*,[5)] however, considered these monohydrates to be the anhydrate and an amorphous form with adsorbed water. Subsequently, Allen *et al.*[6)] also reported a monohydrate the origin of which was unknown. Apart from the monohydrate, Pelizza *et al.*[5)] described a form which showed an endotherm at 140 °C on the DSC heating curve. However, our preliminary experiments suggested that the monohydrate reported by Allen *et al.*[6)] was a desolvation product of the

chloroform solvate and the nature of the hypothetical form reported by Pelizza *et al.*[5] remains uncertain.

The present study was started to examine the relation between the thermal and thermodynamic properties of pharmaceutical solids and the temperature dependence of their aqueous solubilities, especially with regard to amorphous solids. Such a fundamental investigation was considered to be necessary for overcoming various problems in tablet manufacture and tablet dissolution. As to erythromycin solids, conflicting observations in the literature required reconciliation. In addition, little is known of the properties of erythromycin amorphous solid or about its aqueous solubility.

In this work, the dihydrate, the anhydrate and the amorphous form were characterized by measuring their X-ray powder diffraction patterns and infrared spectra (IR), and by differential scanning calorimetry (DSC), thermogravimetry (TG) and the hot-stage polarizing microscopy, and their phase transitions are discussed. The dissolution properties and the aqueous solubilities of erythromycin were also studied in detail.

Experimental

Materials——Erythromycin USP grade was supplied by Sumitomo Chemical Co., Ltd., as a dihydrate. The dihydrate was recrystallized from an aqueous solution at 50 °C. The anhydrate was prepared by boiling an aqueous suspension of dihydrate for 2 h. The dihydrate was not converted into anhydrate at 90 °C, in contrast to the description by Pelizza *et al.*[5] The amorphous form was obtained by heating the dihydrate for 2 h at 135 °C in an oven and then cooling to room temperature. The transparent amorphous product was powdered by grinding in an agate mortar. The chloroform solvate was obtained by recrystallization from chloroform solution.

Instrumentation——X-Ray diffraction patterns were recorded with a Rigakudenki X-ray diffractometer, Geigerflex 2011 or Miniflex 2005 (Ni filter, CuK_α, 30 kV, 10 mA; time constant 1 s; scanning rate 2 °C/min). IR spectra were recorded as mulls in liquid paraffin (Nujol) on a Hitachi 260-30 spectrometer. DSC thermograms were recorded on a Shimadzu SC-30 thermal analyzer at a heating rate of 10 °C/min under a dry nitrogen flow of 35 ml/min. Samples, 5—10 mg, were crimped into aluminum cells. Thermogravimetric analysis was performed with a Shimadzu DTG-30 thermal analyzer in air. The hot-stage polarizing microscopy was performed with an Olympus POM polarizing microscope at a heating rate of 1—2 °C/min.

Annealing Experiments——A dihydrate solid sample was heated at a rate of 10 °C/min in the DSC apparatus. When the temperature reached 100 to 119 °C, this temperature was held constant for 20 or 40 h and then the sample was cooled to room temperature. The annealed was scanned at a rate of 20 °C/min and the DSC curve was recorded. This high scanning rate of 20 °C/min was suitable for observing a small change in the heat capacity at the glass transition temperature.

Solubility Measurement——An excess amount of erythromycin solid was added to water (5—40 ml) in a flask. The suspension was incubated at a regulated temperature of 30—80 °C for a stated time and the whole was immediately filtered to remove the remaining solid with a Millipore GSWP-025-00 filter of 0.22 μm pore size. The above procedure was performed at every sampling time and temperature and repeated three times at every point of measurement. The concentration in the filtrate was measured by a procedure similar to the one described by Allen *et al.*[6] The absorbance ($\lambda = 236$ nm) of the adequately diluted solution was determined with a Hitachi 100-10 spectrophotometer and compared to the absorbance of a reference solution treated similarity. The residue on the filter was observed on the hot-stage polarizing microscope at a heating rate of 1—2 °C/min.

Initial Dissolution Rate by the Rotating Disk Method[7]——A disk of 2 mm thickness was prepared by compressing the powder (472 mg) at a load of 4 t in a cylindrical die of 15.5 mm inner diameter, which was placed on a flat surface. The cylinder holding the compressed disk was placed on the end of a stirring shaft powered by a variable speed motor, immersed in 100 ml of distilled water, phosphate buffer (1/30 M KH_2PO_4–Na_2HPO_4) or Menzel buffer (1/20 M Na_2CO_3–1/10 M $NaHCO_3$) in a cylindrical flat-bottomed flask of 500 ml capacity and then rotated at 600 rpm. The dissolution fluid was maintained at constant temperature and a sample of 1 ml was withdrawn at intervals of 5 min. The volume of dissolution fluid was kept constant by adding a volume equal to that remove for sampling.

Results and Discussion

Characterization of the Dihydrate

The dihydrate sample used in the present experiments was recrystallized once from an aqueous solution at 50 °C. The X-ray diffraction pattern shown in Fig. 1-a coincides well

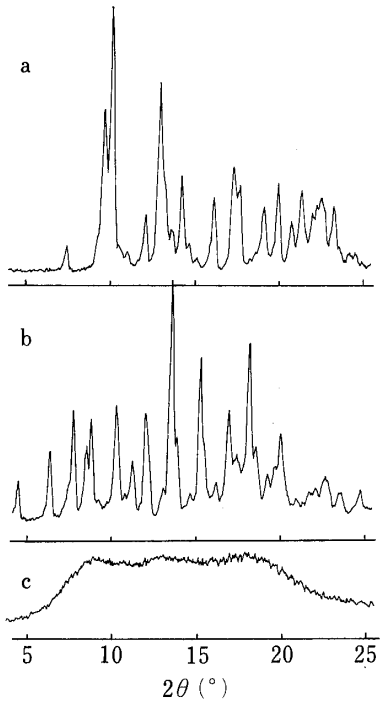

Fig. 1. X-Ray Diffraction Patterns of the Three Forms of Erythromycin

a, dihydrate; b, anhydrate; c, amorphous form.

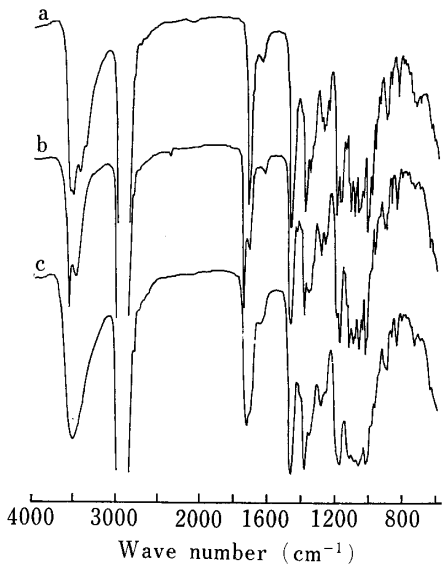

Fig. 2. Infrared Spectra of the Three Forms of Erythromycin (Nujol Mull)

a, dihydrate; b, anhydrate; c, amorphous form.

with the results reported by Croley *et al.*[3] and Rose.[8] The IR spectrum is shown in Fig. 2-a.

Figure 3-a shows the DSC heating curve of erythromycin dihydrate. A sample was scanned up to 105 °C (the solid line), immediately cooled to room temperature and again scanned up to 205 °C (the dotted line). The DSC curve in the temperature range above 105 °C was not affected by preheating up to 105 °C. A wide endotherm below 105 °C clearly corresponds to the evaporation of water from the dihydrate. For a dihydrate sample which had been heated at 100 °C for 11 h in an oven and immediately mixed with liquid paraffin, the X-ray diffraction pattern and the IR spectrum exhibited no distinctive change. On polarizing microscopic observation, no phase transition was observed at any temperature below 124 °C and liquefaction to an isotropic liquid was first observed at 127—130 °C. This liquefaction clearly corresponds to a small endotherm on the DSC curve (Fig. 3-a). These results show that the desolvation product of the dihydrate retains its crystalline structure up to about 124 °C.

The DSC curve shown in Fig. 3-a is fairly different from those of Pelizza *et al.*[5] and Allen *et al.*;[6] that is, in their thermograms, (1) the dehydration showed a relatively sharp peak at 120 °C, (2) the endotherm at 124 °C was not separated and (3) there appeared a broad exotherm at 160—190 °C and a sharp endotherm at 195 °C. It is not clear how the commercially available dihydrate used as a sample by Allen *et al.*[6] had been prepared. Their dihydrate crystals shown in the photographs had a rounded and blocky shape. Their crystal habits were also remarkably different from those of our crystals, which have a sharp-cornered and rodlike/rectangular shape. When the DSC sample was increased in amount and tightly crimped in an aluminum cell, the dehydration peak rose to 120 °C at the maximum. In such a case, the melting peak at 130 °C appeared only as a small shoulder, as reported by Allen *et al.*[6] These facts suggest that the above discrepancies (1) and (2) in the thermograms might have resulted from differences in the preparation method of DSC samples.

When the dihydrate contained a trace of anhydrate, a crystalline phase was developed in

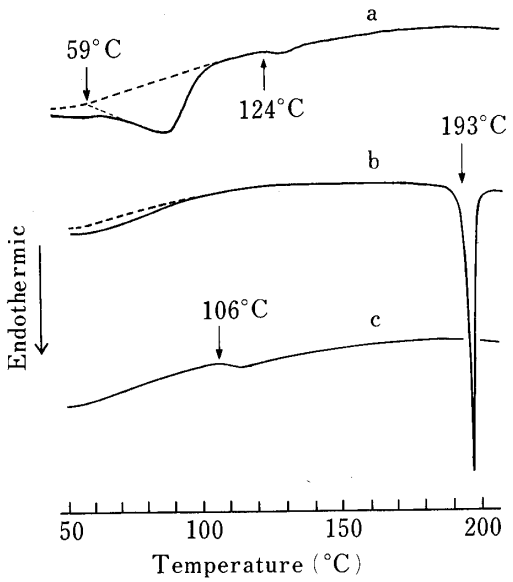

Fig. 3. DSC Curves of the Three Forms of Erythromycin at the Heating Rate of 10 °C/min

a, dihydrate; b, anhydrate; c, amorphous form.

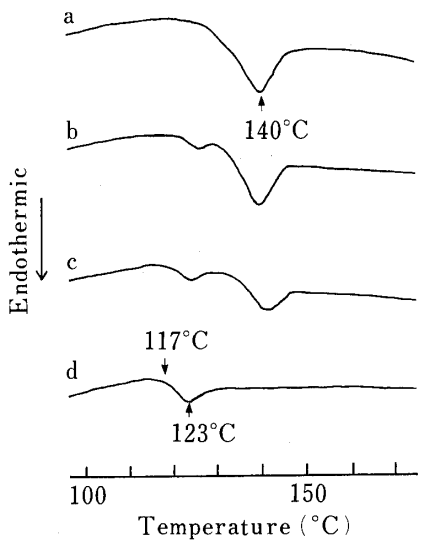

Fig. 4. DSC Curves at the Heating Rate of 20 °C/min for Annealed Dihydrate Samples

Annealing conditions: a, 40 h at 100 °C; b, 20 h at 109 °C; c, 20 h at 114 °C; d, 20 h at 119 °C. Sample weight: 10.0 mg.

the isotropic liquid at 160—190 °C and disappeared at 191—195 °C. With such a dihydrate, the DSC curve showed a broad exotherm at 160—190 °C and a sharp endotherm at 190—193 °C. Thus, the above crystalline phase is clearly the anhydrate (Fig. 3-b). These results indicate that the melt of dihydrate produced at 124—130 °C is a "supercooled liquid." The third discrepancy resulted from this property of the melt and a contaminant in the dihydrate.

A relatively large size of crystals was obtained by recrystallization from aqueous acetone solution. When these crystals were subjected to DSC, the melting peak rose to 140 °C at the maximum, but the dehydration peak rose only slightly. This DSC curve was very similar to the one that Pelizza *et al.*[5] had reported as evidence for their so-called hypothetical form. It is possible that the endotherm peak at 140 °C in the DSC curve reported by Pelizza *et al.*[5] is not due to a new form, but to the melting of the dehydration product of the dihydrate into an isotropic liquid.

Characterization of the Anhydrate

The anhydrate sample was prepared by leaving the dihydrate in boiling water for 2 h. The X-ray diffraction pattern, the IR spectrum and the DSC heating curve are shown in Figs. 1-b, 2-b and 3-b, respectively. These results agree well with the literature data.[3-6]

Amorphous Form and the Glass Transition

The amorphous sample was prepared by heating the dihydrate at 135 °C for 2 h in an oven followed by cooling to room temperature. The X-ray diffraction pattern, the IR spectrum and the DSC heating curve for the amorphous form are shown in Figs. 1-c, 2-c and 3-c, respectively. When the dihydrate was heated not at 135 °C, but at 160 °C, the cooled material become muddy owing to anhydrate recrystallize therein.

The change at 106 °C on the DSC curve (Fig. 3-c) is clearly attributable to the glass transition, since the heat capacity increase at this temperature. The amorphous form is therefore a supercooled liquid at 106—193 °C and a glass below 106 °C. On the other hand, the powdered amorphous sample liquefied at 127—130 °C as determined on the polarizing microscope. This result means that at temperatures below 127 °C the molecules can not have

sufficient motional degree of freedom to flow as a liquid. This might be related to the finding that the dehydrate crystalline and could not liquefy up to 124 °C at the heating rate of 10 or 1—2 °C/min.

In relation to this problem, DSC of annealed sample was performed. The data are shown in Fig. 4. When the heating rate was set at 20 °C/min, the melting of the dihydrate and the glass transition of the amorphous solid were exhibited at 140 and 117 °C, respectively. The samples annealed at 109—119 °C for 20 h showed the glass transition (Fig. 4). Clearly, this phenomenon resulted from gradual conversion to the amorphous form. Figure 4 shows that the rate of the conversion decreasing temperature and that the conversion can be induced only at temperatures above the glass transition, because at 100 °C no change can be detected. Thus, the liquefaction of the dihydrate microscopically observed at 127—130 °C is different from a first order phase transition such as the melting of the anhydrate.

IR Spectra (C=O Stretching Vibration)

In the IR spectra shown in Fig. 2, the C=O stretching absorption most clearly exhibits the difference among the three forms. Erythromycin has two types of C=O bondings on the macrolide ring, ketone and lactone. Figure 5 shows the C=O stretching absorption on an enlarged scale. The C=O stretching absorption of free lactone at 1741 cm^{-1} appears with the anhydrate but does not with the dihydrate.[2,9] On the other hand, with the dihydrate the absorption peak of lactone shifts to 1721 cm^{-1}, clearly owing to intermolecular hydrogen-bonding. This hydrogen-bonded absorption was unaltered by dehydration. The peak at 1710 cm^{-1} for the anhydrate (only a small shoulder can be observed with the dihydrate) was due to the C=O stretching vibration of ketone.[2,9] The broad peak for the amorphous solid shows that the molecules take a variety of orientations.

Dehydration of the Dihydrate

Figure 6 shows an example of TG and differential thermogravimetry (DTG) curves for a dihydrate sample. The decrease in weight by 4.6% corresponds to about 2 mol of water. According to Horowitz *et al.*,[10] this dehydration process conformed to first-order kinetics in the range of 0.55—3.55% decrease in weight. The calculated values of the activation energy (E_a) and the reference temperature (T_s) at which the dehydration is $(1-1/e)$ completed are plotted against the weight of sample in Figs. 7-a and b, respectively. The values of E_a and T_s

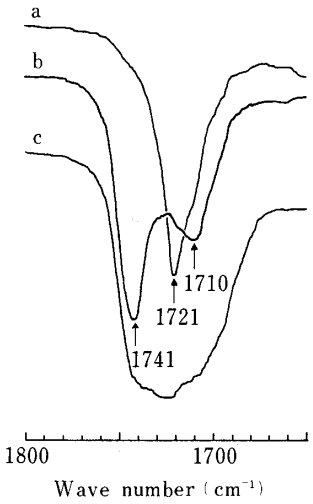

Fig. 5. IR Spectra (C=O Stretching Vibration) of the Three Forms of Erythromycin (Nujol Mull)

a, dihydrate; b, anhydrate; c, amorphous form.

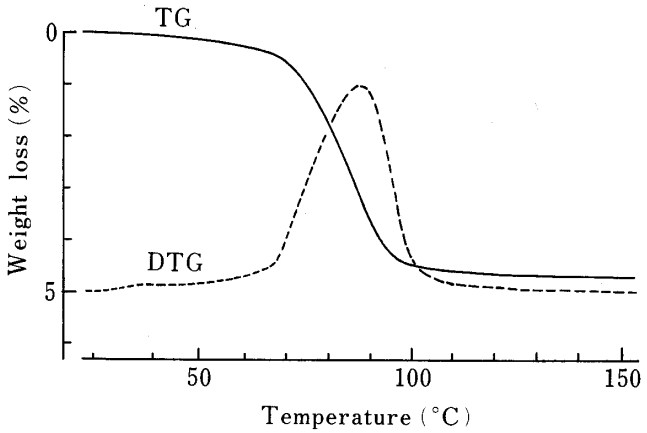

Fig. 6. Thermogravimetric Change of Erythromycin Dihydrate

Heating rate: 10 °C/min. Sample weight: 8.45 mg.

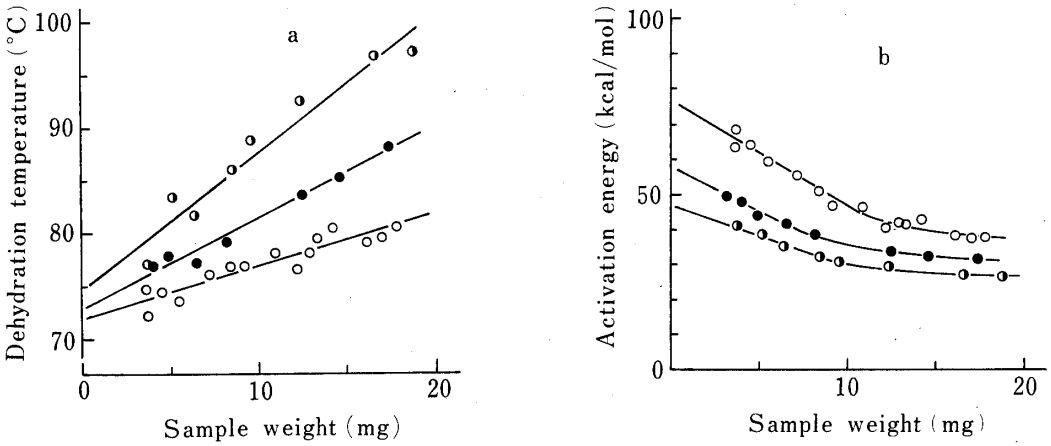

Fig. 7. Activation Energy (a) and Temperature at Maximum Slope (b) for Desolvation of Erythromycin Dihydrate

Heating rate (°C/min): ○, 2; ●, 5; ◑, 10.

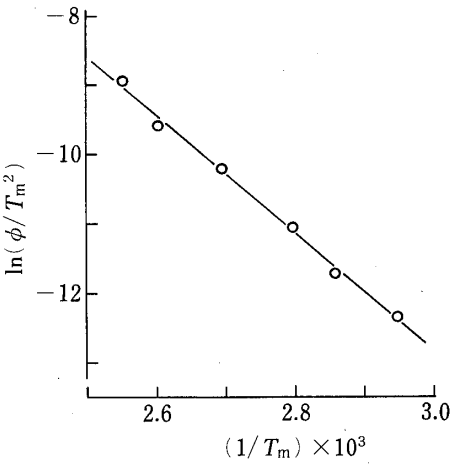

Fig. 8. Kissinger Plots for Desolvation of Erythromycin Dihydrate by DSC

T_m, temperature of peak (°K); ϕ, heating rate (°K/min).

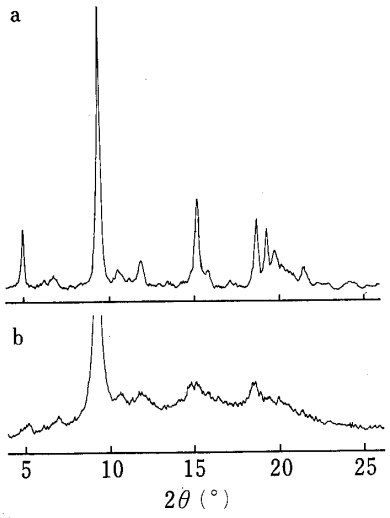

Fig. 9. X-Ray Diffraction Patterns of the Chloroform Solvate and the Desolvated Solid of Erythromycin

a, chloroform solvate; b, desolvated solid, prepared by permitting the chloroform solvate to stand under room conditions for 2 d.

lineary extrapolated to zero weight and zero heating rate were 81.2 kcal/mol and 71.2 °C.

On the other hand, in the range above 3.55% weight decrease the Horowitz plot showed a very low activation energy. The peak of the dehydration endotherm on the DSC curve appeared in this range of weight decrease. From the Kissinger plot shown in Fig. 8,[11] the activation energy was obtained as 16.8 kcal/mol. The reason for the existence of these very different activation energies is not clear.

Monohydrate Reported by Allen

In the present study, no monohydrate was found by recrystallization from aqueous solutions at any temperature between 2 and 100 °C or after any heat treatment tested. The samples used by Allen *et al.*[6] were not well defined as regards origin, preparation or treatment. Their X-ray diffraction pattern showed a considerably low degree of crystallinity, including a halo pattern characteristic of the amorphous form shown in Fig. 1-c.

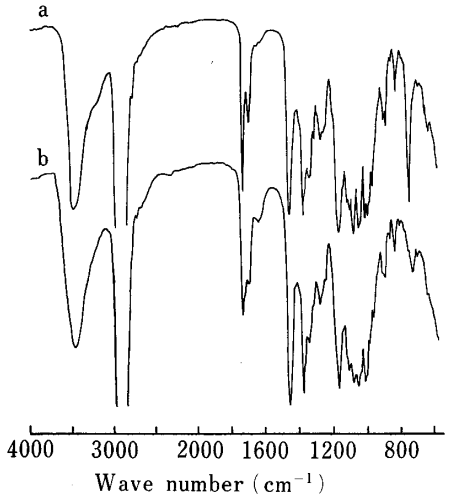

Fig. 10. IR Spectra of Chloroform Solvate and the Desolvated Solid of Erythromycin

a, chloroform solvate; b, desolvated solid.

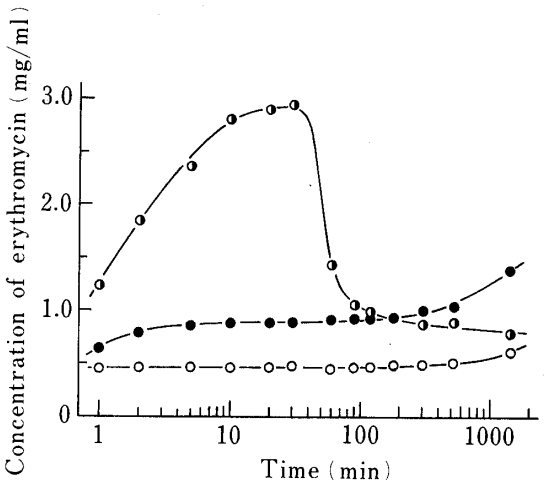

Fig. 11. Concentration–Time Curves for the Three Forms of Erythromycin in Distilled Water at 37 °C

○, dihydrate; ●, anhydrate; ◑, amorphous form. A solid sample was suspended in water and the concentration in the filtrate was measured at various times.

Erythromycin is also available commercially as a desolvation product of the chloroform solvate. Figures 9 and 10 show the X-ray diffraction patterns and the IR spectra, respectively, of a chloroform solvate and its desolvation product prepared by leaving the solvate under room conditions 2 d. Samples prepared by leaving the solvate for 24 d or by keeping it *in vacuo* for 24 h, showed X-ray diffraction patterns and IR spectra that retained features similar to those in Figs. 9-b and 10-b, respectively, thought the characteristics those of the amorphous form (Figs. 1-c and 2-c). In Fig. 9-b, the broadened peak $2\theta = 9.3°$ remains distinctive. However, on heating at 100 °C this disappeared very easily and the IR spectrum also coincided well with that of the amorphous solid (Fig. 2-c).

The chloroform solvate exhibited two distinct C=O stretching absorption peaks at 1740 and 1705 cm^{-1}, as did the anhydrate (Figs. 10-b and 5-b); the C=O stretching vibration of lactone of the chloroform solvate was free from hydrogen-bonding. When the solvate was permitted to stand under room conditions, the absorbance at 1740 cm^{-1} gradually decreased and that at 1720 cm^{-1} (hydrogen-bonded) was correspondingly increased. As a result, the IR spectrum in the full range came to agree well with that of the monohydrate reported by Allen *et al.*[6] (Fig. 10-b). The X-ray diffraction pattern for the desolvation product shown in Fig. 9-b also agreed well with that of their so-called monohydrate.

The polarizing microscopic observation and the DSC analysis revealed that the chloroform solvate was desolvated below 90 °C, simultaneously being converted to an amorphous form in the original shape, and the amorphous solid liquefied at 126—130 °C. This thermal behavior is similar to the description give for the monohydrate by Allen *et al.*[6]

These results suggest that the monohydrate reported by Allen *et al.*[6] was actually a desolvation product of the chloroform solvate with some degree of crystallinity. The monohydrate reported by Shtolts *et al.*,[4] prepared from a chloroform solvate, was probably the same kind of desolvation product.

Dissolution Behavior and Solubility

Figure 11 shows the time courses of dissolution of the three forms at 37 °C in water. The dihydrate, which is most stable, exhibits the lowest solubility of the three. The anhydrate shows about twofold higher solubility than the dihydrate. The transition of the anhydrate to

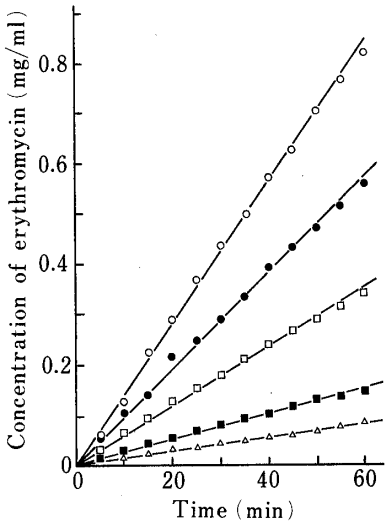

Fig. 12. Concentration–Time Curves for the Dihydrate of Erythromycin in Distilled Water and Phosphate Buffers at 30 °C by the Rotating Disk Method

pH values of buffers were as follows: ○, 7.0; ●, 7.3; □, 7.7; ■, 8.3; △, in distillled water.

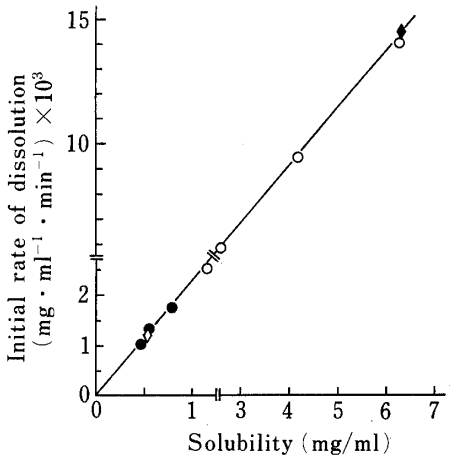

Fig. 13. Relation between the Solubility and the Initial Rate of Dissolution from the Rotating Disk at 30 °C

○, dihydrate in the phosphate buffer; ●, dihydrate in Menzel buffer; ◇, dihydrate in distilled water; ◆, anhydrate; the solubility was estimated by the method described in Table I.

the dihydrate was not observed by polarizing microscopic examination or in the X-ray diffraction patterns of the residue obtained by filtration of the suspension. The incubation times longer than 200 min, the apparent concentration curves of the dihydrate and the anhydrate increase gradually with time, owing to the decomposition of erythromycin. The amorphous solid exhibits a very high level of apparent solubility, but after incubation for over 30 min the concentration shows a rapid decrease (Fig. 11). It was confirmed from the X-ray diffraction patterns of the solid portion of suspension that the rapid decrease had resulted from conversion to the dihydrate. Thus, the solubility of the amorphous solid could not be determined by this conventional method, but those of the dihydrate and the anhydrate were adequately determinable in terms of the concentration at 1 h.

The solubility of the amorphous solid[12] was next estimated by application of the rotating disk method reported by Nogami *et al.*[7] However, their method of data analysis did not give a good result with the amorphous erythromycin because of rapid phase transition.[7] The Noyes–Whitney equation suggests that the dissolution rate in the sink condition will be proportional to the solubility at constant temperature, surface area and agitation. Figure 12 shows the time course of dissolution of the dihydrate in water or in phosphate buffer solution as determined by the rotating disk method. In every case, the concentration at 1 h is only about 14% of the solubility and the dissolution rate is almost constant. These constant dissolution rates are plotted in Fig. 13 against the corresponding solubilities; a good proportionality is apparent. The data with Menzel buffer are also plotted in Fig. 13.

Figure 14 shows the dissolution profiles for the amorphous solid at various temperatures. At 80 °C the initial rate could not be obtained, since the phase transition had already started within the first 5 min. At every temperature, the crystalline phase was observed on the disk surface after dissolution for 1 h. The profiles shown in Fig. 14 suggest the occurrence of the phase transition at 10—20 min; at that time the dissolution rate is decreased, very rapidly at temperatures above 50 °C.

Table I shows the initial dissolution rate and the solubility of the amorphous solid with the corresponding values for dihydrate and the solubility of anhydrate. The solubilities of the

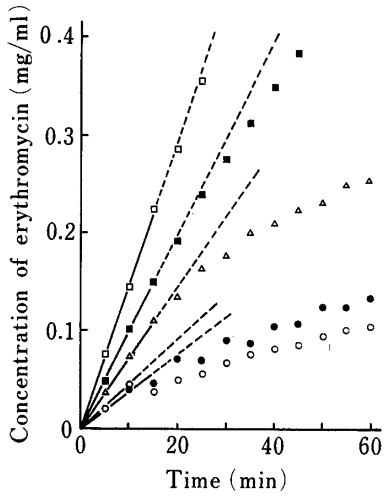

Fig. 14. Concentration–Time Curves for the Amorphous Solid of Erythromycin in Distilled Water at Various Temperatures by the Rotating Disk Method

□, 30 °C; ■, 40 °C; △, 50 °C; ○, 60 °C; ●, 70 °C.

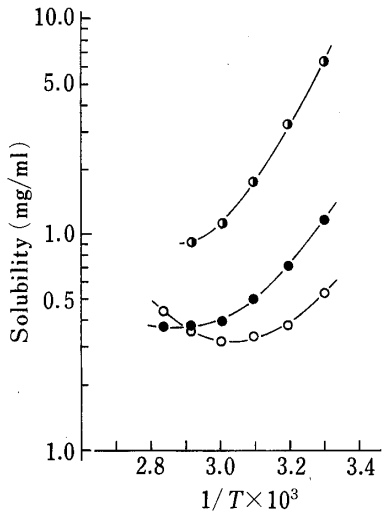

Fig. 15. van't Hoff Plots of Solubility Values for the Three Forms of Erythromycin in Distilled Water

○, dihydrate; ●, anhydrate; ◑, amorphous form.

TABLE I. Solubility (s_i: mg/ml) and Initial Rate of Dissolution (k_i: mg·ml^{-1}·min^{-1}) of Erythromycin

Temperature (°C)	Anhydrate	Dihydrate			Amorphous	
	s_1	s_2	$k_2 \times 10^3$	$s_2/k_2 \times 10^{-3}$	s_3[a)]	$k_3 \times 10^3$
30	1.152	0.528	1.21	0.436	6.32	14.5
40	0.712	0.379	1.15	0.330	3.26	9.87
50	0.499	0.337	1.12	0.301	1.75	6.37
60	0.392	0.319	1.16	0.275	1.12	4.33
70	0.377	0.354	1.36	0.260	0.917	3.40
80	0.371	0.438	1.68	0.261		

a) $s_3 = s_2 \times k_3/k_2$.

amorphous solid were estimated on the basis of the proportionality between the initial dissolution rate and the solubility as shown in Fig. 13. The ratios of the corresponding initial rate (s_2/k_2 in Table I) are not constant, mainly because of the temperature dependence of the diffusion constant.

Figure 15 shows van't Hoff plots of the solubility data. The transition between dihydrate and anhydrate was not observed at any temperature. The solubility of the decreases with increase in temperature. With the dihydrate, its solubility took the minimum value at 60 °C.[13] The crossing point of these two solubility curves was about 72 °C. Which is cleary the transition point between these two forms. It is interesting that this transition temperature agree well with the reference temperature of dehydration, 71 °C, extrapolated to zero weight and zero heating rate by the use of the TG data shown in Fig. 7-a.

The estimated solubility of the amorphous solid takes a remarkably high value at each temperature. In addition, the tendency for solubility to increase with decrease in temperature is more marked than with the other forms.

Figure 16 shows the temperature dependence of the partial molar heat of solution, calculated from the slopes of the curves in Fig. 15. The difference of the heats of solution

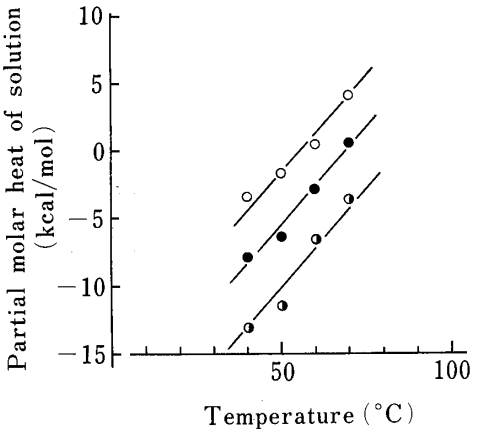

Fig. 16. Temperature Dependence of the Partial Molar Heat of Solution for the Three Forms of Erythromycin in Distilled Water

○, dihydrate; ●, anhydrate; ◑, amorphous form. Data at 30 and 80 °C are omitted since they showed a large scatter.

among the forms are attributable to differences of the molar enthalpies of erythromycin in the solid phases.[14] It is known in many cases that the solid in a glassy state has a heat capacity equal to that of the stable crystalline phase.[15] Hence, the enthalpy difference among the three solid should not be so variable in the range of temperature where the solubilities were determined. From this point of view, the data shown in Fig. 16 were fitted to three parallel lines.[16] The enthalpies of the dihydrate and the anhydrate relative to that of the amorphous solid were −8.6 and −4.0 kcal/mol, respectively. These results suggest that the tendency of the solubility to increase at low temperatures (Fig. 15) may result from some intermolecular interaction in the solution rather than from changes in the properties of the solid phases.

The transition point between two crystalline phases has often been obtained successfully by a linear extrapolation of van't Hoff plots. This method is based on the fact that the heat capacities of both solids should not be very different from one another up to the transition point, because both solids are in the crystalline state. In the case of erythromycin, we could extrapolate the van't Hoff plots on the base of a linear relationship between the heat of solution and temperature (Fig. 16). The extrapolated crossing points of the solubility curves were 97 °C for the dihydrate and the amorphous form and 125 °C for the anhydrate and the amorphous form. The latter is above the glass transition point and therefore the amorphous solid has already become a supercooled liquid. Since the difference of enthalpy between a supercooled liquid and a stable crystalline phase should generally increase up to the melting point,[15] the above simple extrapolation may have no physical significance. For the purpose of clarifying the relations among the solubility curves of erythromycin solids, the thermodynamic properties of the solids must be examined in detail.

A peculiar temperature dependence of aqueous solubility such as is shown in Fig. 15 has already been reported for colchicine,[17] aromatic hydrocarbons such as benzene, toluene, xylene, *etc.*,[18] alcohols such as hexanol and heptanol,[14] hydrocarbons such as methane, ethane, *etc.*[14] and so on.[14] The increase in the heat of solution with temperature (Fig. 16) can be attributed to intermolecular interactions in solution. Shinoda[14] attributed the temperature dependence of solubility of the above hydrophobic compounds in water to hydrophobic hydration. We could not find any other interpretation in published reports for the above peculiar temperature dependence of aqueous solubility.

In the case of erythromycin, its intermolecular interaction in water and the results of a thermodynamic analysis of aqueous solubility will be discussed in the following papers.

Conclusions

1. On DSC, the anhydrate exhibited only a melting endotherm at 193 °C. The dihydrate was dehydrated at temperatures between 59 and 105 °C and liquefied at 124—130 °C. The

dehydration product remained crystalline up to 124 °C (DSC). When the dihydrate was heated at 135 °C and cooled to room temperature, an amorphous solid having the glass transition temperature of 106 °C was produced. Above this glass transition temperature, the dehydration product of the dihydrate was finally converted to an isotropic liquid at a rate that increased with increasing temperature.

2. The so-called monohydrate reported by Allen *et al.* was found in this study to be an desolvation product of the chloroform solvate. In DSC, the endotherm at 140 °C, which was suggested to be evidence of a new form the Pelizza *et al.*, was apparently only a result of retardation in the liquefaction of dihydrate.

3. The heats of solution for the three forms increased with temperature at 40—70 °C. For each form, plots against temperature could be fitted to a straight line with the same slope for the three forms. The enthalpies of the dihydrate and the anhydrate relative to that of the amorphous solid were −8.6 and −4.0 kcal/mol, respectively. These results suggest that the peculiar temperature dependence of erythromycin solubility might result from some intermolecular interaction in the aqueous solution.

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References and Notes

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