Dissolution Behavior and Solubility of Anhydrous and Dihydrate Forms of Wy-4508, an Aminoalicyclic Penicillin

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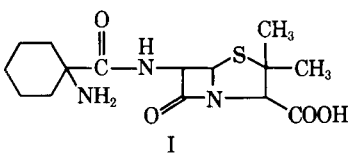
Abstract □ Anhydrous and dihydrate forms of an aminoalicyclic penicillin (Wy-4508) were compared for solubility in distilled water at temperatures ranging from 7 to 60°. Differences were noted in the physical–chemical properties of these two forms. Below the transition temperature, 61°, the anhydrous form was found to be significantly more soluble than the dihydrate. In addition, the solubility of the anhydrous crystal was shown to be inversely related to temperature. The thermodynamic properties noted for the two forms of the antibiotic have been experimentally evaluated.

Keyphrases □ Penicillin, aminoalicyclic (Wy-4508)—anhydrous, dihydrate forms, solubility □ Temperature effect—aminoalicyclic penicillin solubility □ Dissolution—aminoalicyclic penicillin, anhydrous, dihydrate □ Thermodynamic properties—anhydrous, dihydrate aminoalicyclic penicillin

Many organic and inorganic compounds exist in separate crystalline forms having different physical–chemical properties. Higuchi (1) has pointed out that the resulting variation in thermodynamic properties associated with the differences in crystal forms may be of considerable pharmaceutical importance. The present report is concerned with an investigation of the differences in the physical–chemical properties of two forms of an aminoalicyclic penicillin, Wy-4508 [6-(1-aminocyclohexanecarboxamido)penicillanic acid] (I).



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Specifically, the solubilities in distilled water of the anhydrous and dihydrate forms of this penicillin were determined, and the thermodynamic properties of these two compounds were experimentally evaluated.

Much of the past work reported on the physical–chemical properties of crystalline hydrates has been concerned with inorganic compounds, as illustrated by the studies of Taylor and Henderson (2) on various hydrates of calcium nitrate and of Hill (3) on calcium sulfate. Recently, several investigations concerned with studies of organic molecules in the anhydrous and hydrated forms have been reported. Two of the hydrated forms of phenobarbital, as well as the anhydrous form, were examined by Eriksson (4) for apparent solubility in water as a function of time. Shefter and Higuchi (5) reported the relative dissolution rates of solvated and nonsolvated crystalline forms of several types of compounds of pharmaceutical interest, including steroids and xanthines. These workers also determined the thermodynamic properties of several crystal systems. In a recent report by the present authors (6), the dissolution and solubility behavior of anhydrous and trihydrate forms of the semisynthetic penicillin, ampicillin, was reported.

EXPERIMENTAL

Apparatus—The following were used: a constant-temperature water bath equipped with a Unitherm Haake constant-temperature circulator[1] and a rotating-bottle apparatus,[2] Swinney hypodermic adapter,[3] Millipore filters (pore size 0.45 μ),[3] and 120-ml. amber bottles with polyseal caps.[4]

Compounds—In all the experiments, anhydrous Wy-4508 (Wyeth Laboratories batch C-10777, m.p. 181–182°) was used. The dihydrate form of Wy-4508 was prepared from the anhydrous material by preparing a saturated solution of the penicillin in 1.0 *N* hydrochloric acid and precipitating the hydrated form at pH 7. IR spectra, differential thermal analysis, and Karl Fischer moisture determinations were obtained for the material and conclusively characterized the anhydrous and dihydrate forms.

Dissolution Procedure—An excess of drug, 4 g., in the appropriate form was added to 50 ml. of distilled water previously equilibrated to the desired temperature. The bottles were rotated at a constant speed in a water bath maintained at the appropriate temperature. Samples withdrawn at definite intervals were filtered through a Millipore filter and diluted immediately to avoid any precipitation of the penicillin in the filtered samples due to supersaturation.

The penicillin content was determined by means of the following iodometric titration procedure. To 2.0-ml. aliquots containing 1–3 mg. of Wy-4508, 2.0 ml. of 1 *N* sodium hydroxide was added, and samples were allowed to stand at room temperature for 15 min. At the end of the time, 2.0 ml. of 1.2 *N* HCl was added, followed by 10 ml. of 0.01 *N* iodine. After 15 min., the excess of iodine was titrated, using 0.01 *N* sodium thiosulfate. For the blank determinations, 10 ml. of 0.01 *N* iodine was added to a 2.0-ml. sample and titrated immediately.

RESULTS AND DISCUSSION

The solubility of the anhydrous form of Wy-4508 at 7, 20, 25, 30, 40, 50, and 60° is shown in Fig. 1. Similar data at 10, 20, 25, 30, 40, 50, and 60° for the dihydrate form of this agent are illustrated

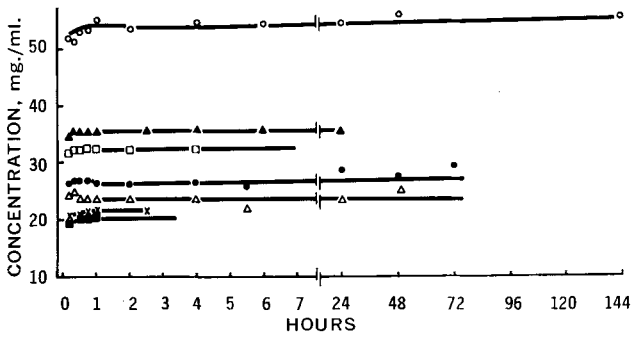

Figure 1—*Solubility of the anhydrous form of Wy-4508 in distilled water at temperatures ranging from 7 to 60°. Key:* ○, *7°;* ▲, *20°;* □, *25°;* ●, *30°;* △, *40°;* ×, *50°; and* ■, *60°.*

[1] Brinkmann Instruments, Westbury, N. Y.
[2] E. D. Menold Sheet Co., Lester, Pa.
[3] Millipore Corp., Bedford, Mass.
[4] Erno Products, Philadelphia, Pa.

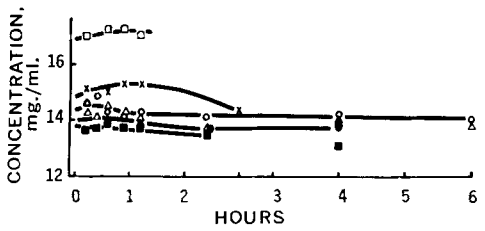

Figure 2—*Solubility of the dihydrate form of Wy-4508 in distilled water at temperatures ranging from 10 to 60°. Key: ○, 10°; ▲, 20°; ■, 25°; ●, 30°; △, 40°; ×, 50°; and □, 60°.*

in Fig. 2. These figures show the concentration of the antibiotic attained in solution as a function of time in the presence of the excess of the solid phase in the appropriate form and under essentially constant agitation. An inverse relationship between temperature and solubility for the anhydrous form of this drug was observed. The dihydrate form of the drug shows no significant change in solubility between 10 and 40°. The negative heat of solution noted for this compound is consistent with the data reported for the anhydrous form of the amphoteric penicillin, ampicillin (6).

An inverse relationship between solubility and temperature is not usually observed, especially with organic compounds. However, from the data presented here and previously (6), it appears that such a relationship (negative heat of solution) is characteristic of the anhydrous form of amphoteric penicillins.

The data shown for the solubility in water for the two forms of Wy-4508 investigated suggest that the equilibrium solubilities observed are good approximations of the true solubility of these crystals. Therefore, the measurements made at the several temperatures permit calculation of the thermodynamic values involved in the transition of the anhydrous form to the dihydrate. The thermodynamic relationship involving polymorphism and solubility is extensively discussed in reports by Shefter and Higuchi (5) and Higuchi *et al.* (7).

A classical van't Hoff-type plot of the apparent equilibrium solubilities observed at the various temperatures of this investigation showed a reasonably good linear relationship for both forms of the antibiotic for temperatures up to 40° (Fig. 3). At the higher temperatures, a deviation from linearity was observed for both forms of Wy-4508. This is probably due to degradation of the penicillin at these higher temperatures.

The transition temperature for the anhydrous–dihydrate crystal system of this compound corresponds to the temperature at which the solubility of the two forms is equal. The transition temperature for this system, obtained by extrapolating the straight-line portions

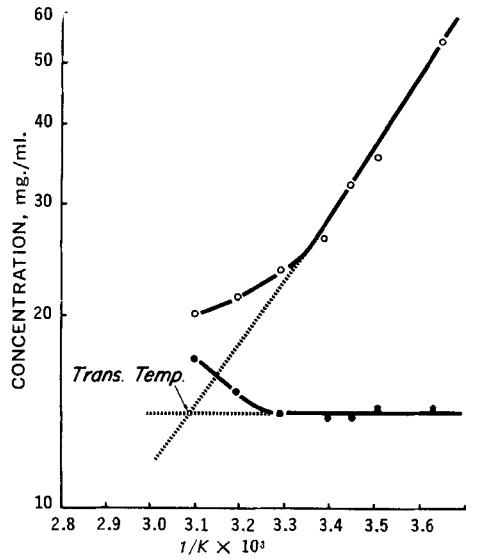

Figure 3—*The van't Hoff-type plot for the anhydrous and dihydrate forms of Wy-4508 in distilled water. Key: ○, anhydrous; and ●, dihydrate.*

of the van't Hoff-type plot, was determined to be 61° (Fig. 3). This plot also points up the inverse relationship between temperature and solubility for the anhydrous form of the drug, whereas the effect of temperatures ranging from 10 to 40° on the solubility of the dihydrate form is insignificant.

The values of the heat of solution for each of the crystal forms were calculated from the slopes of the van't Hoff-type plot and were determined to be −4700 and 0 cal./mole for the anhydrous and dihydrate forms, respectively, The enthalpy of hydration ($\Delta H_{A,H}$) was determined to be −4700 cal./mole. This value is considerably less than that noted for the anhydrous–trihydrate system of ampicillin (6), where the value was determined to be −6400 cal./mole. This is probably due to the fact that only 2 moles of water are involved in the hydration of Wy-4508 as compared to 3 moles of solvent for each mole of ampicillin.

The free energy difference, ΔF_T, between the anhydrous and hydrated forms at constant temperature and pressure is determined by Eq. 1:

$$\Delta F_T = RT \ln \frac{C_s\,(\text{anhydrous})}{C_s\,(\text{dihydrate})} \qquad \text{(Eq. 1)}$$

where C_s is the solubility of the form under consideration at a particular temperature, T, and R is the gas constant. This value, ΔF_T, is a measure of the free energy change involved in a conversion of the anhydrous crystal to the dihydrate crystal. The ΔF_T's at 25 and 37° (corresponding to room and body temperatures) have been determined to be −550 and −390 cal./mole, respectively.

The entropy change, ΔS_T, for the reaction involved in hydrate formation can be calculated by Eq. 2:

$$\Delta S_T = \frac{\Delta H_{A,H} - \Delta F_T}{T} \qquad \text{(Eq. 2)}$$

The values computed for the hydration of the anhydrous to the dihydrate Wy-4508 crystals at 25 and 37° were −13.9 e.u. At the transition temperature of the anhydrous–dihydrate crystal system, ΔF is equal to zero and the entropy change can be calculated by

$$\Delta S\,(\text{trans}) = \frac{\Delta H_{A,H}}{T\,(\text{trans})} \qquad \text{(Eq. 3)}$$

For Wy-4508, ΔS (trans) was determined to be −14.1 e.u. The hydrated species of this system contains 2 molecules of water; the possible intramolecular hydrogen-bond formation between these associated water molecules may account for the relatively large entropy change noted.

The entropy change involved in the fusion of water at 25° is approximately 6 e.u. This decrease in entropy associated with the formation of ice is approximately the same entropy change obtained for the hydration of glutethimide and theophylline (5). Therefore, the energy involved in the transformation of the dehydrated forms of compounds of such types to the hydrate may be related mainly to the decrease in the entropy of water molecules in the hydrate structure. The results obtained for the entropy change in the ampicillin anhydrous–trihydrate system (6), where 3 associated water molecules result in an entropy change of −20 e.u. at 25°, and for the entropy change observed in the present study, where 2 associated water molecules show a change in this value of −13.9 e.u., are consistent with this hypothesis.

The thermodynamic values calculated for the anhydrous–dihydrate Wy-4508 system are summarized in Table I.

The equilibrium solubilities observed in these experiments apparently correspond to the solubilities of anhydrous and dihydrate crystalline phases for the Wy-4508 molecule. At the temperatures

Table I—Thermodynamic Values Calculated for the Anhydrous–Dihydrate Wy-4508 System

Temp.	ΔH, cal./mole Anhydrous	Dihydrate	ΔF_T, cal./mole[a]	ΔS_T, e.u.[a]
	(−4700)	(0)		
25°			−550	−13.9
37°			−390	−13.9
61°			0	−14.1

[a] Calculated for the conversion from the anhydrous to the dihydrate form.

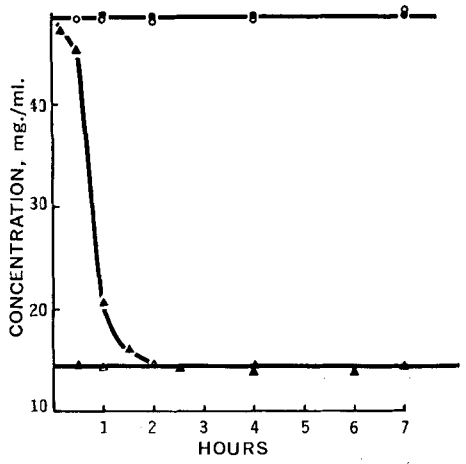

Figure 4—*Influence of seeding anhydrous Wy-4508 with 1 and 10% dihydrate crystals on the solubility in distilled water at 10°. Key:* ○, *anhydrous;* ●, *seeded with 1% dihydrate;* ▲, *seeded with 10% dihydrate; and* △, *dihydrate.*

utilized, there was no evidence of conversion of the more soluble anhydrous form to the less soluble dihydrate species as would be expected strictly from thermodynamic consideration. Undoubtedly, this may be due to steric factors involved in the association of the water molecules in the crystal system and to the relatively high water solubility of this particular amphoteric penicillin. Even at lower temperatures (10°), seeding of the anhydrous form with 1% dihydrate crystals did not result in a rapid conversion of the anhydrous form to the less soluble species. However, when the seed was incorporated at the 10% level, a relatively rapid and complete conversion of the anhydrous to the dihydrate form was observed as shown by the decrease in solubility. These data are summarized in Fig. 4.

SUMMARY

The solubility of the anhydrous and dihydrate forms of Wy-4508 in distilled water has been determined over a temperature range of 7–60°. From the solubility data, the transition temperature for this crystal system was estimated to be 61°. The anhydrous form was found to be significantly more water soluble than the dihydrate at all temperatures below the transition temperature. In addition, the solubility of the anhydrous crystal was shown to be inversely related to temperature (negative heat of solution). The thermodynamic values for the anhydrous–dihydrate Wy-4508 crystal system have been calculated.

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Effects of Disulfiram on Growth, Longevity, and Reproduction of the Albino Rat

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Abstract □ Chronic oral ingestion of disulfiram at concentrations of 1:1000 and 1:2000 in powdered food retards the growth and reproductive capacity of the albino rat; a concentration of 1:1000 also decreases longevity. Enhancement of diet with ascorbic acid has no antidotal action on the growth and reproductive effects. Chronic disulfiram feeding appears to have no significant effects on whole animal oxygen consumption, blood counts, tissue xanthine oxidase activity, and liver molybdenum content. Calcium deposition in the cerebellum was absent.

Keyphrases □ Disulfiram—chronic feeding studies, rats □ Blood cells, number—disulfiram effect □ Reproduction, longevity, body weight—disulfiram effects □ Oxygen consumption—disulfiram effect □ Ascorbic acid effect—disulfiram activity

While single oral doses and chronic feeding of disulfiram in the absence of ethanol are considered relatively nontoxic (1–3), chronic feeding of rats on diets containing disulfiram (1:400–1:10,000) has been reported to retard growth and increase mortality (4). Muscular incoordination was seen in rats chronically fed the 1:400 and 1:1000 diets by the age of 2 years, and microscopic examination of the cerebellum and basal ganglia revealed calcified masses in rats fed 1:400 disulfiram. In addition to its generally accepted inhibitory effect on liver aldehyde oxidase, disulfiram has been reported to have some antithyroid capacity (5). Disulfiram can inhibit the oxygen uptake of rat liver homogenates by apparently acting as a competitive hydrogen acceptor (6). Ascorbic acid overcomes this inhibition and is reported to be effective intravenously in man (7). The metabolic breakdown of disulfiram into diethyldithiocarbamate, an effective chelator, has also been noted (8).

After preliminary work in this laboratory, it was considered important to document the effects of disulfiram in the young rat during the most rapid phases of growth and to see whether longevity and reproductive capacity would be affected.