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Studies on Drug Nonequivalence. VII.[1] Bioavailability of Acetohexamide Polymorphs

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The thermodynamic values of two polymorphic forms of acetohexamide (form I and form II) were calculated from solubility measurements. The transition temperature and the heat of transition were estimated to be 154°, and 230 cal./mol, respectively.

In vivo absorption studies of these polymorphs were carried out in four beagle dogs, and bioavailability parameters were determined from the blood concentration *versus* time curves. The polymorphic form of acetohexamide did not affect the bioavailability.

Keywords——acetohexamide polymorphism; solubility; transition temperature; thermodynamic parameters; oral administration; bioavailability

In the previous paper, the authors reported that acetohexamide exists in two polymorphic forms.[1] This paper deals with the thermodynamic properties of these polymorphs as well as their bioavailability in beagle dogs. It was found that there was no significant difference in the bioavailability of the two forms.

Experimental

Materials——Acetohexamide polymorphs I and II were prepared as described previously,[1] and identified by infrared spectrophotometry (IR), X-ray diffractometry, differential scanning calorimetry (DSC), and polarizing microscopy. The particle sizes of acetohexamide used for solubility and bioavailability studies were in the range of 62—75 μm. The sample powder for bioavailability studies was prepared as a mixture with corn starch to enhance the wettability and dispersibility of the powder.

Solubility Measurement——Excess acetohexamide was placed in 40 ml of 20% EtOH in a 500 ml flask, and agitated at 300 rpm in a water bath maintained at 20°, 25°, 30°, or 35°. Aliquots of the solution were removed at appropriate times, filtered through a membrane filter (pore size, 0.45 μ), and then diluted for spectrophotometric assay at 249 nm.

Bioavailability Study——Four male beagle dogs, weighing 9.0 to 12.4 kg were used in a two-way crossover design with a dose interval of one week. One gram of sample powder (250 mg of acetohexamide) suspended in 50 ml of 0.001 N HCl was administered through a stomach tube to dogs fasted for 18 hr. Heparinized blood samples were taken at 0.5, 1, 2, 3, 4, 6, 8, and 10 hr after dosing. The plasma samples were frozen and stored at −20° until assay.

Plasma Concentration Measurement——Concentrations of acetohexamide and its active metabolite, hydroxyhexamide, in plasma were determined by the high-pressure liquid chromatography (HPLC) method developed by Takagishi *et al.*[3] This procedure is as follows. Two ml of 1/10 M phosphate buffer (pH 5.0), then 5 ml of $AcOEt/C_6H_6$ (1 : 1) solution were added to 0.2 ml of plasma containing 20 μg of tolazamide (internal standard). The mixture was shaken for 10 min and centrifuged, and then 2 ml of the organic phase was taken and evaporated to dryness under a nitrogen flow. The residue was dried under a vacuum in a desiccator for 30 min. The dried substance was dissolved in 100 μl of acetonitrile/H_2O (1 : 1) solution and 10 μl of the solution was injected. The apparatus used was a high-pressure liquid chromatograph (Waters, model ALC/GPC 204) equipped with a variable wavelength photometric detector (Shimadzu, model SP-1). The conditions for analysis were as follows. Column, 15 cm/4 mm i.d.; packing, Lichrosorb RP-8 (5 μm);

1) Part VI: K. Kuroda, T. Yokoyama, T. Umeda, and Y. Takagishi, *Chem. Pharm. Bull.* (Tokyo), **26**, 2565 (1978).
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3) Y. Takagishi, K. Sato, and H. Maekawa, *Yakugaku Zasshi*, in preparation.

mobile phase, acetonitrile/0.2% acetic acid (1: 1); flow rate, 60 ml/hr; wavelength, 235 nm; sensitivity, 0.16 a.u.f.s.

Results and Discussion

Solubility Behavior

The solubility curves of acetohexamide polymorphs I and II at various temperatures are shown in Fig. 1. Form II dissolved faster than form I, and the apparent equilibrium solubility was about 1.2 times that of form I. The transition status from form II to form I was examined in terms of the X-ray diffraction patterns, DSC, and by polarizing microscopy before and after the solubility measurements. The transition did not occur within 3 hr in the medium. The solubility ratios at various temperatures were plotted according to van't Hoff's equation. It is possible to calculate the transition temperatures of the two crystal forms from two straight lines.[4] As shown in Fig. 2, the estimated transition temperature was 154.° The temperature range of 20—35° was selected in this study because of relative ease of measurement. This transition temperature approximately corresponded to that obtained by DSC, *i.e.* 157°.[1] The heats of solution for the two polymorphic forms were calculated from the slope of each straight line. The thermodynamic values calculated for each form are listed in Table I. It is clear that the difference in thermodynamic properties between forms I and II is small.

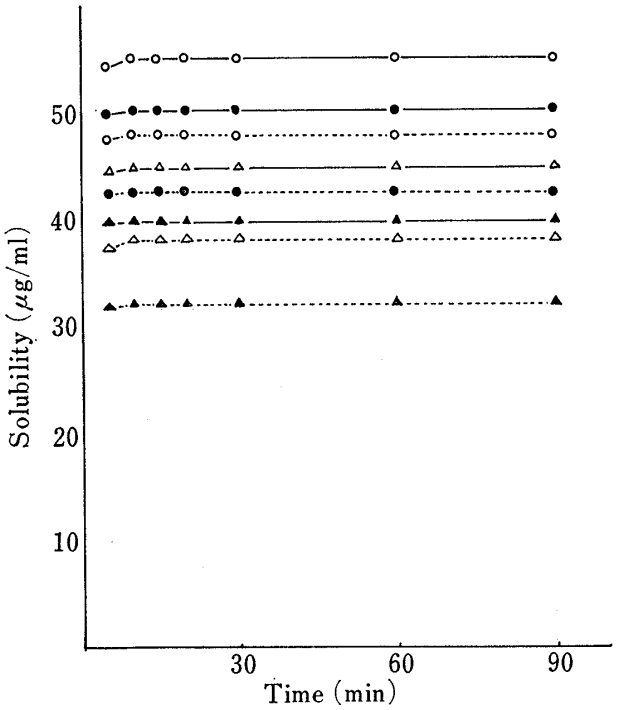

Fig. 1. Solubility Curves for Acetohexamide Polymorphs in 20% Ethanol at Various Temperatures

------: form I, ———: form II,
—○—: at 35°, —●—: at 30°, —△—: at 25°,
—▲—: at 20°.

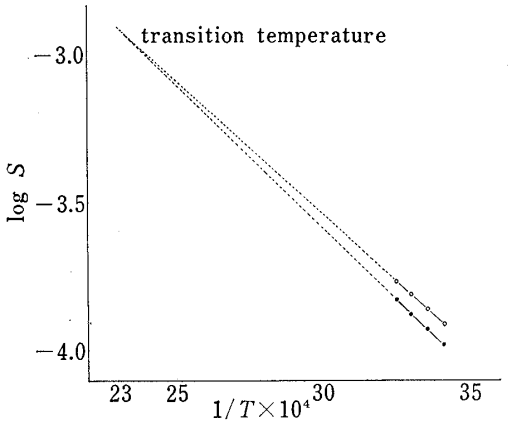

Fig. 2. The van't Hoff's plots for Acetohexamide Polymorphs I and II in 20% ethanol

s: solubility, ●: form I, ○: form II.

Bioavailability Studies

The plasma levels of acetohexamide and hydroxyhexamide after oral administration to beagle dogs are shown in Fig. 3, and the bioavailability parameters for forms I and II are

4) A.J. Aguiar and J.E. Zelmer, *J. Pharm. Sci.*, **58**, 983 (1969).

Table I. Thermodynamic Values calculated for Acetohexamide Polymorphs I and II

	Transition temperature to form I	Heat of solution kcal/mol	Heat of transition cal/mol	ΔG cal/mol
Form I	154°	4.15	230	89.9
Form II		3.92		

summarized in Table II. The mean peak values of plasma acetohexamide were *ca.* 70 μg/ml for form II and *ca.* 62 μg/ml for form I, and the areas under the plasma concentration-time curves (AUC) were *ca.* 219 μg·hr/ml for form II and *ca.* 213 μg·hr/ml for form I.

These results led the authors to conclude that forms I and II probably show no difference in bioavailability.

Aguiar and Zelmer[4] suggested that large difference in the free energy content of polymorphs, as was demonstrated in the case of chloramphenicol palmitate ($\Delta G = -774$ cal./mol), may significantly affect the absorption and resulting blood levels, and that a small difference, as was found with mefenamic acid ($\Delta G = -251$ cal./mol), may not affect the absorbability of the drug. ΔG for the two crystal forms of acetohexamide in the present study was only 89.9 cal./mol. Thus, according to Aguiar and Zelmer, only a slight difference in absorption would be expected. Our results support this.

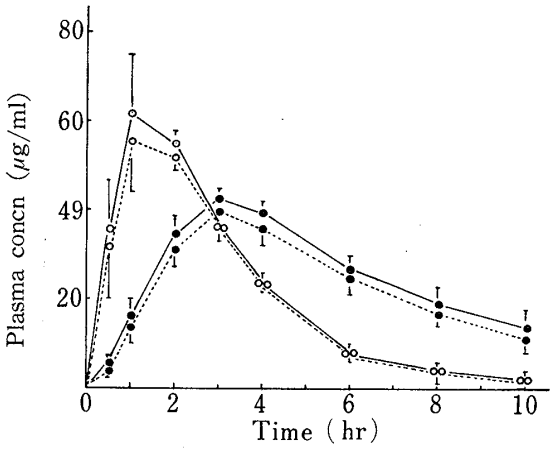

Fig. 3. Plasma Levels of Acetohexamide and Hydroxyhexamide following the Oral Administration of Acetohexamide

·····: form I, ———: form II,
○: acetohexamide, ●: hydroxyhexamide.

Table II. Plasma Levels of Acetohexamide and Hydroxyhexamide following the Oral Administration of Acetohexamide

Drug administered	Substance measured in plasma	Peak time (hr)	Peak level (μg/ml)	AUC[a] (μg·hr/ml)
Form I	Acetohexamide	1.50±0.29	62.38±9.45	212.53±21.62
	Hydroxyhexamide	3.50±0.29	40.30±3.61	235.81±31.15
	Total sulfonylurea			448.29±50.79
Form II	Acetohexamide	1.50±0.29	70.28±9.88	218.90±10.63
	Hydroxyhexamide	3.50±0.29	43.68±1.21	258.26±23.90
	Total sulfonylurea			477.12±15.59

a) AUC was measured up to 10 hr after administration.