[Chem. Pharm. Bull. 26(8)2565—2568(1978)]

UDC 615.453.2.011.3.015.1.073 : 548.33

Studies on Drug Nonequivalence. VI.[1] Physico-chemical Studies on Polymorphism of Acetohexamide[2]

Koji Kuroda, Teruyoshi Yokoyama, Tsuneo Umeda,[3a] and Yasushi Takagishi[3b]

Hospital Pharmacy, Kobe University School of Medicine[3a] and Production Division, Shionogi & Co., Ltd.[3b]

(Received December 19, 1977)

Form I, form II, and $CHCl_3$-II type of acetohexamide are obtained by recrystallization from different solvents. X-ray diffraction patterns, infrared spectra, and differential scanning calorimeter curves of the $CHCl_3$-II type are identical with those of form II, but $CHCl_3$-II type contains a chloroform molecule, which cannot be removed by normal drying condition, and $CHCl_3$-II type seems unsuitable for medical use. The solubility of form II is 1.2 times that of form I.

Keywords——acetohexamide; polymorphism; optical characteristic; solubility; DSC–TG curves

Acetohexamide is a sulfonylurea compound, used as an oral antidiabetic agent which is effective in controlling blood glucose in properly selected patients with maturity-onset diabetes. It is hardly soluble in water. The polymorphs of relatively insoluble drugs may often affect their bioavailability.[4] In a series of studies on drug nonequivalence, we found that acetohexamide exists in two polymorphic forms. This paper reports the preparative method and physicochemical properties of acetohexamide polymorphs. Form I was obtained by recrystallization from ethanol, methanol, or acetone, and form II from either 50% ethanol or chloroform. From the reasons mentioned in a postscript, form II recrystallized from chloroform is referred to as $CHCl_3$–II type and that prepared from 50% ethanol simply as form II.

Experimental

Material——Acetohexamide polymorphs were prepared as follows: Form I: Acetohexamide (0.5 g) was dissolved in 20 ml of warm EtOH, MeOH, or $(CH_3)_2CO$, and permitted to crystallize at a room temperature. The long plate crystals were collected by filtration, and dried at 105° *in vacuo*.

Form II: Acetohaxamide (0.6 g) was dissolved in 400 ml of EtOH–H_2O (1:1) solution on a water bath for 1 hr at 85°, and the solution was allowed to stand over night a room temperature, allowing to crystallize at 5°. The long plate crystals were collected by filtration and dried at 105° *in vacuo*.

$CHCl_3$-II Type: Acetohexamide (0.3 g) was dissolved in 25 ml of hot $CHCl_3$, and the solution was allowed to cool to a room temperature. The small plate crystals were collected by filtration, and dried at 120° *in vacuo*.

Identification of Polymorphs——Polymorphic forms of acetohexamide were determined by using X-ray diffractometry (Rigaku Denki Geigerflex, Ni filtered Cu-Kα radiation), infrared (IR) spectra (Hitachi 345), and differential scanning calorimeter-thermogravimetric analysis (DSC-TG) (Rigaku Denki, heating rate 5°/min).

Determination of Optical Characteristic of Polymorphs——Optical characteristics were determined by the immersing method described by Watanabe *et al.*[5]

1) Part V: K. Kuroda, T. Yokoyama, and T. Umeda, *Kobe Journal of Medical Sciences*, 22, 255 (1976).
2) This work was presented at the 27th Annual Meeting of the Kinki Branch, Pharmaceutical Society of Japan, Kobe, November, 1977.
3) Location: *a) Kusunoki-cho, Ikuta-ku, Kobe, 650 Japan; b) Imafuku, Amagasaki, 660 Japan.*
4) *cf.* J.K. Haleblian, *J. Pharm. Sci.*, 64, 1269 (1975).
5) A. Watanabe, Yu. Tanaka, and Yo. Tanaka, *Chem. Pharm. Bull.* (Tokyo), 25, 2239 (1977).

Determination of Solubility——About 300 mg of the sample powder was placed in 150 ml of distilled water in a 500 ml flask and the mixture was agitated at 300 rpm. An aliquot of the solution was taken by a glass syrnge at intervals, filtered through a membrane filter of 0.45 μm pore size, and a carefully measured aliquot was diluted for spectrophotometric assay[6] at 249 nm.

During the measurement of solubility behaviors, any polymorphic transition did not occur at solid phase in the suspension, which was confirmed by DSC and X-ray diffraction.

Results and Discussion

IR Spectra and X-Ray Diffraction Patterns

As shown in Fig. 1 and 2, IR spectra and X-ray diffraction patterns of forms I and II are entirely different, but the characteristic pattern of form II was similar to that of $CHCl_3$–II type.

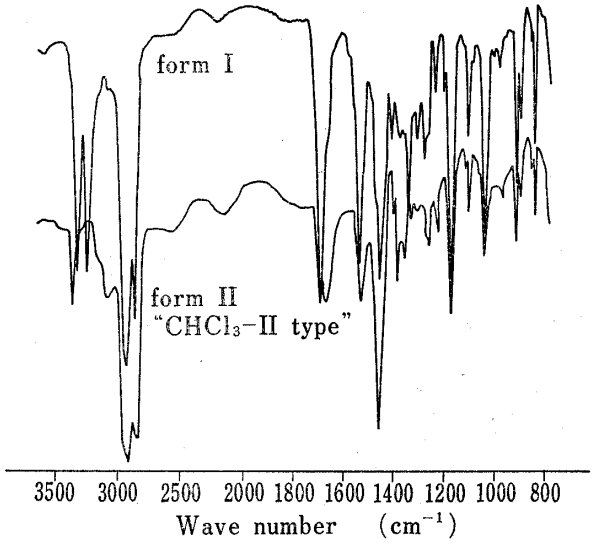

Fig. 1. Infrared Absorption Spectra of Acetohexamide Polymorphs (Nujol)

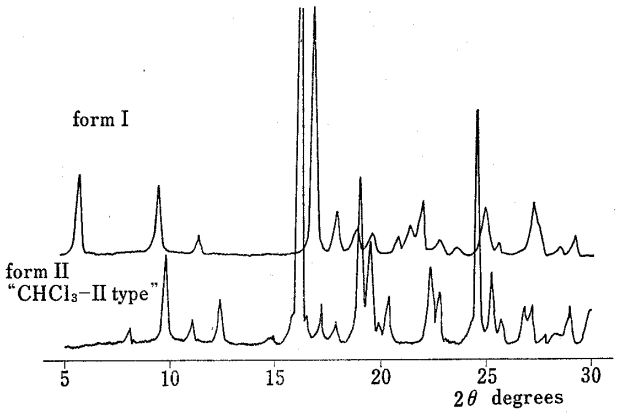

Fig. 2. X-Ray Diffraction Patterns of Acetohexamide Polymorphs

DSC-TG Curves and Elementary Analysis

As shown in Fig. 3, DSC curves of form I shows only one peak corresponding to the melting point, but those of form II and $CHCl_3$–II type show different thermograms with two endothermic peaks. The first endothermic peak comes at the period of the transition from form II to form I at 164—169°, and the second peak is attributed to the melting point. TG curve of form II is obviously different from that of $CHCl_3$–II type, *i.e.*, $CHCl_3$–II type dried at 120° shows a continuous decrease in weight to the extent of about 3% from 121—164°. This extent of decrease depended on the drying condition.

The elementary analytical values of forms I and II agreed closely with theoretical values for acetohexamide, as shown in Table I. On the contrary, it was found that $CHCl_3$–II type contained chlorine.

From the results of DSC–TG curves and elementary analysis, it is considered that chloroform molecule binds strongly with acetohexamide molecule in the $CHCl_3$–II type crystal nonstoichiometrically and cannot be removed by normal drying condition, such as heating *in vacuo*. Therefore, $CHCl_3$–II type is unsuitable for medical use.

Optical Characteristic and Solubility Behavior

The optical characteristic of forms I and II is entirely different, as summarized in Table II.

6) E.F. Salim and W.W. Hilty, *J. Pharm. Sci.*, **56**, 385 (1967).

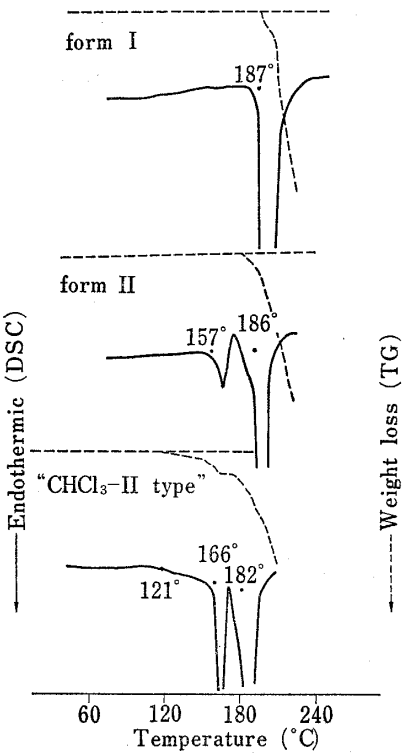

Fig. 3. DSC-TG Curves of Acetohexamide Polymorphs (heating rate 5°/min)

——: DSC curve, ----: TG curve.

Table I. Elementary Analysis of Acetohexamide Polymorphs

Modification	C	H	N	O	S	Cl
Form I	55.60	6.18	8.52			—
Form II	55.49	6.19	8.62	19.80	9.97	—
$CHCl_3$-II type	54.22	6.11	8.32	18.60	9.61	2.81
Calc.	55.54	6.21	8.64	19.73	9.89	—

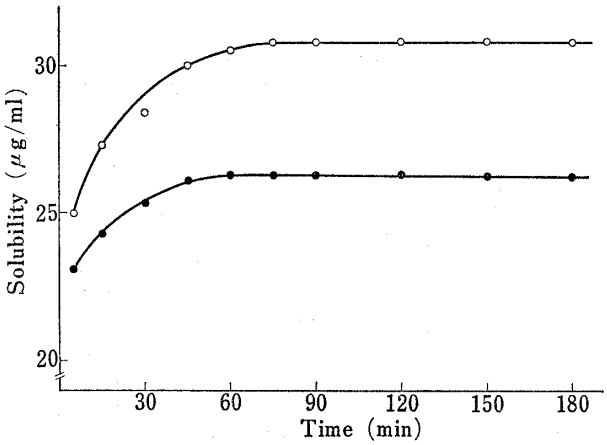

Fig. 4. Solubility Curves of Acetohexamide Polymorphs in Water at 37°

—●—: form I, —○—: form II.

Table II. Refractive Indices and Related Properties of Acetohexamide Polymorphs

Modification	Crystal system	Refractive indices n_1	Refractive indices n_2	Remarks
Form I	Triclinic	1.555 ($n_{\alpha'}$)	1.643 ($n_{\gamma'}$)	Long plate Extinction inclined (41—45°)
Form II	Monoclinic	1.571 (n_α)	1.601 (n_β)	Long plate Extinction parallel Elongation positive

Fig. 4 shows the solubility behavior of forms I and II in distilled water at 37°. The apparent equilibrium solubility of form II is about 1.2 times that of form I.

Transition of form II to form I did not occur within 3 hr in the solution.

Results of these experiments gave the following conclusion:

(1) Characteristic patterns of IR spectra, X-ray diffraction, and DSC curves of $CHCl_3$–II type agreed with those of form II, but $CHCl_3$–II type would be unsuitable for medical use, since this crystal contained the chloroform molecule nonstoichiometrically and could not be removed by normal drying condition.

(2) The solubility of form II was 1.2 times that of form I.

Recently, Girgis-Takla and Chroneos[7] reported the two polymorphic forms of acetohexamide. They obtained form A by recrystallization from glacial acetic acid and form B from

7) P. Girgis-Takla and I. Chroneos, *J. Pharm. Pharmac.*, **29**, 640 (1977).

chloroform. Our study has been done independent of their report, and form A and form B described by them are similar to form I and $CHCl_3$–II type, respectively, in our study. Effect of the nature of the medium on the dissolution rate and *in vivo* bioavailability are currently being investigated in our laboratories.

[Chem. Pharm. Bull. 26(8)2568—2571(1978)]

UDC 615.272.2.015.42.076.9 : 591.11.05

A Remarkable Elevation of Serum Calcium Concentration Induced by Ligation of Bile Duct

Masayoshi Yamaguchi and Takeo Yamamoto

Shizuoka College of Pharmaceutical Science[1)]

(Received February 1, 1978)

The change of calcium concentrations in the serum, liver, and bile of rats was investigated after a single oral administration of calcium chloride. The administration of calcium produced a corresponding increase of calcium concentration in the serum and liver, and this increase was dose dependent. A remarkable elevation of calcium content in the bile was caused by the administration of calcium. By the ligation of bile duct, the calcium concentration in the serum was markedly enhanced after the administration of calcium (10 and 50 mg/100 g) when compared with that of the sham-operated rats. These results suggest that the excretion of calcium into the bile prevents the elevation of calcium concentration in the serum after the absorption of calcium from the intestine.

Keywords——serum calcium; liver calcium; bile calcium excretion; hypercalcemic effect by bile duct ligation; calcium metabolism in hepatic bile system

There are several studies in relation to the regulation of serum calcium after the calcium absorption from the intestine.[2,3)] However, the mechanism to decrease the calcium concentration in the serum increased by the ingestion of calcium containing diet has not been fully resolved. On the other hand, the physiological significance of the bile excretion of calcium absorbed from the intestine is little known reportedly, although a large amount of calcium is contained in the bile. The present study was therefore undertaken to investigate the alteration of calcium concentration in the serum, liver, and bile of rats orally administered calcium chloride. We found that calcium is excreted into the bile and that the serum calcium concentration is markedly enhanced by the ligation of bile duct.

Materials and Methods

Animals——Male Wistar rats, weighing approximately 120 g, were used. The animals were kept at a room temperature of 25±1° and fed commercial laboratory chow containing 7.4% carbohydrate, 1.1% calcium and 1.1% phosphate (Oriental Test Diet Co., Tokyo) and tap water *ad libitum* until the day of testing.

Drug——Calcium chloride was dissolved in demineralized water to the concentration of 10 or 50 mg of Ca/ml. This solution was given by a single oral administration (1 ml/100 g) to rats. Control rats was administered orally demineralized water.

Surgical Procedures——The abdomen was opened by a incision after the intraperitoneal administration of 25% urethane (0.6 ml/100 g). The common bile duct was then cannulated with PE-10 tubing and the bile duct ligated, and the incision was closed with wound clips. The animals were put on the thermostatic

1) Location: *2–1, Oshika 2-chome, Shizuoka, 422 Japan.*
2) G. Coen, G. F. Mazzuoli, I. Antonozzi, and A. Scada, *Metab. Clin. Exp.*, **23**, 709 (1974).
3) R. Swaminathan, J. Ker, and A.D. Care, *J. Endocrinol.*, **61**, 83 (1974).