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The Determination of (α-Bromoisovaleryl)urea in Plasma and the Bioavailabilities of Its Polymorphic Forms in the Rat[1)]

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A simple and sensitive determination method for (α-bromoisovaleryl)urea in plasma was developed by using gas–liquid chromatography with an electron capture detector. (α-Bromoisovaleryl)urea was extracted from plasma with ethyl ether and ethanol in a ratio of 5:1 (volume); the recovery was 97.5%. The plasma concentrations were determined by the internal standard GLC-ECD method without further clean-up by column chromatography. The detection limit was about 0.5 μg/ml in 0.1 ml plasma. In order to determine the effect of polymorphism (form I, form II, and form III) of (α-bromoisovaleryl)-urea on the bioavailabilities, plasma levels in rats after intraduodenal administration were investigated. The areas under the blood concentration curves (AUC) and the mean dissolution times (MDT) were calculated to determine the extent of bioavailability and the rate of bioavailability, respectively. The results indicated that the polymorphic states of (α-bromoisovaleryl)urea did not show significantly different bioavailabilities.

Keywords——(α-bromoisovaleryl)urea; determination; polymorph; bioavailability; plasma level; AUC; MDT

Introduction

(α-Bromoisovaleryl)urea has been used as a sedative or a hypnotic, but its biological fate has not been much studied. Kojima[2)] determined the blood concentration in rabbits after oral administration by using gas–liquid chromatography with an electron capture detector. This method is very complicated because of the need for column chromatography for the removal of biological contaminants from samples. Ito *et al.*[3)] also used column chromatography for clean-up of biological samples from rats. Recently, Okamoto *et al.*[4)] reported a determination method using high-performance liquid chromatography and the blood concentration curves in dogs, but a large volume of sample was needed in this method. The determination method developed in this study is superior because it is simple and offers high recovery and high sensitivity (the limit of detection was 0.05 μg in 0.1 ml plasma sample).

It has been reported by the authors that (α-bromoisovaleryl)urea has three polymorphic forms (form I, form II, and form III),[5)] and the dissolution properties of these polymorphic forms were described.[6)] Form II transformed to form I rapidly in water at 37°C. This transition was delayed by gelatin, and the dissolution rate and the solubility of form II were larger than that of form I. The differences of free energies at 37°C were fairly small among these polymorphic forms.

There have been some reports on the effect of the solubilities,[7)] the dissolution rates,[8)] and the free energies[9–11)] of polymorphic forms on the bioavailabilities. In order to investigate whether the differences of the free energies, the dissolution rates, and the solubilities of (α-bromoisovaleryl)urea polymorphs influenced their bioavailabilities, the plasma concentration in rats after intraduodenal administration was determined by the new method. The time courses of the blood concentration are analyzed in detail in this paper.

Experimental

Preparation and Identification of (α-Bromoisovaleryl)urea Polymorphs——(α-Bromoisovaleryl)urea polymorphs (form I, form II, and form III) were prepared and identified as described in the previous paper.[5)]

Animak Experiments——Wistar male rats, 180—220 g body weight, were fasted for one night before the experiment. The animals were cannulated in the duodenal lumen and the femoral artery under ether anesthesia. About 10 mg of powdered (α-bromoisovalery)urea (100—170 mesh) was suspended in 0 25 ml of 5% gelatin solution and administered into the duodenum through the cannula with a syringe. After the administration, the remaining suspension was washed into the duodenum with 3 ml of water. In the case of administration of aqueous solution, (α-bromoisovaleryl)urea was dissolved in water at the concentration of 2.0 mg/ml, and 5.0 ml of the solution was administered intraduodenally. At appropriate times after the administration, about 0.25 ml of blood was collected in a heparinized microtube from the femoral artery through the cannula. Each blood sample was centrifuged at 3000 rpm for 10 min and 0.1 ml of plasma sample was collected.

Determination Procedure——Point nine ml of water, 0.5 ml of internal standard [(α-bromo-*n*-caproyl)-urea[6]] aqueous solution, and 6 ml of solvent mixture (ethyl ether: ethanol=5: 1) were added to 0.1 ml of plasma sample in a centrifuge tube. The mixture was shaken for 5 min. After centrifugation at 3000 rpm for 10 min, 5 ml of the upper layer was evaporated to dryness. The residue was dissolved in 0.2 ml of acetone. Two μl of this acetone solution was injected into the gas–liquid chromatograph (GLC). All the solvents used in this experiment were purchased from Kanto Chem. Co., Inc. (Tokyo) as specially prepared reagents for pesticide residue analysis.

GLC Conditions——A gas chromatograph (Shimadzu Seisakusho, Ltd., type GC-5A, Kyoto) equipped with a column of 5% PEG 20M/Chromosorb G AW DMCS (60—80 mesh), 3 mm×1 m, and an electron capture detector (ECD) was used. The column temperature was 200°C and flow rate of carrier gas (N_2) was 80 ml/min.

Results and Discussion

Determination of (α-Bromoisovaleryl)urea in Plasma

The chromatograms of blank plasma (0.1 ml) and of a typical sample are shown in Fig. 1. Even though no clean-up by column chromatography was done, there was little interference, as shown in Fig. 1. The two peaks of (α-bromoisovaleryl)urea and (α-bromo-*n*-caproyl)urea internal standard were clearly separated under these conditions, and the retention times were 2.2 and 3.4 min, respectively. The average recovery of (α-bromoisovaleryl)urea from plasma of rats was 97.5% with the extractive solvent mixture (ethyl ether: ethanol=5: 1). The detection limit was about 0.5 ng.

The calibration curve for the experimental range is shown in Fig. 2. The regression line was obtained by the least-squares method; $Y=0.0783\,X-0.0128$, $r=0.999$. The coefficient of variation was 0.085 when the peak ratio was 0.2 ($n=3$), and 0.013 when the peak ratio was between 0.4 and 1.5 ($n=9$), respectively.

This method for determination of (α-bromoisovaleryl)urea in plasma is simpler and more sensitive than previous methods,[2-4] because up to 0.5 μg/ml of (α-bromoisovaleryl)urea in 0.1 ml of plasma can be measured satisfactorily without any interference, even though no pretreatment by column chromatography is carried out.

Animal Experiments

In order to determine the effect of polymorphism on the bioavailability of (α-bromoisovaleryl)urea, animal experiments were carried out by using the determination method described above. It is known that the drug absorption is affected by food and gastric emptying rate. In order to exclude these factors, the sample was directly administered into the duodenum of rats fasted for one night.

Figure 3 shows the plasma levels of (α-bromoisovaleryl)urea after the intraduodenal administration of an aqueous solution of (α-bromoisovaleryl)urea.

Figures 4—6 show the plasma levels after intraduodenal administration of form I, form II, and form III polymorphs of (α-bromoisovaleryl)urea, respectively. All the plasma concentration values were corrected for body weight and dose. The bioavailability parameters for solution, form I, form II, and form III are summarized in Table I.

Since the maximum plasma concentration time (T_{max}) of solution was significantly ($p=0.05$) smaller than those of all the crystals, it seems reasonable to consider that the dissolution

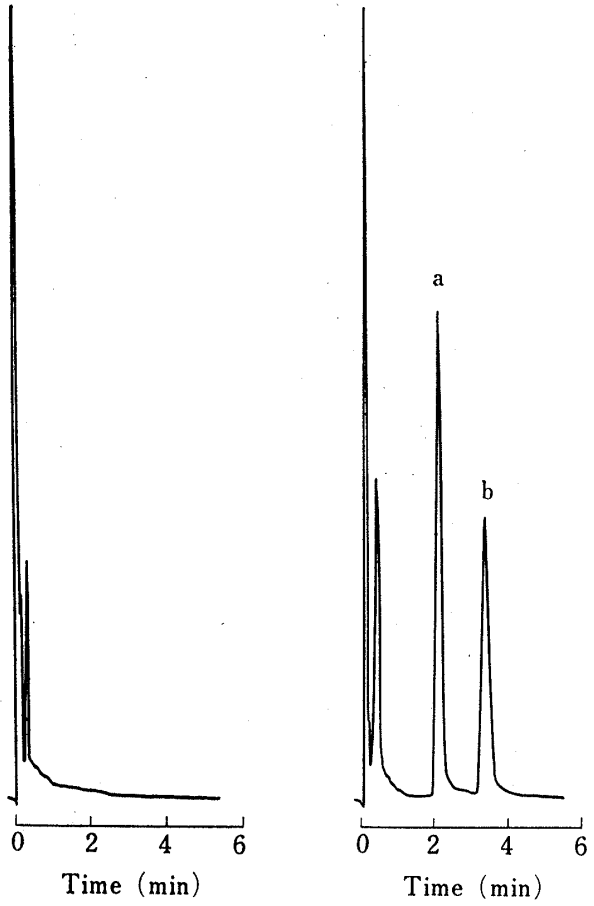

Fig. 1. Gas Chromatograms of Blank Plasma (Left) and Test Sample (Right)

Peaks; a: (α-bromoisovaleryl)urea, 45 ng, b: (α-bromo-*n*-caproyl)urea (IS), 45 ng.
Sens.: 100 MΩ range: 0.16 V.

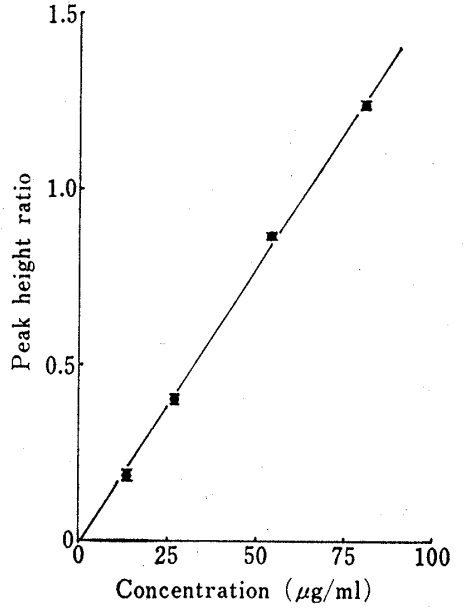

Fig. 2. Calibration Curve of (α-Bromoisovaleryl)urea concentration in 0.1 ml of plasma

$Y=0.0783X-0.0128$, $r=0.9985$ ($n=3\times4$).

process was rate-limiting in the absorption. The peak plasma level (C_{max}) of solution was significantly ($p=0.05$) larger than those of all crystals. The areas under the plasma concentration curves (AUC), which indicate the extent of bioavailability, showed no significant differences ($p=0.05$) among the three polymorphs and aqueous solution. This result also shows that the gelatin does not affect the absorption of (α-bromoisovaleryl)urea at the concentration used in the present experiment.

When the crystals suspended in gelatin solution in order to delay the transformation of crystal form, as described in the previous paper,[6] were directly administrated into the duodenum, the three polymorphic forms of (α-bromoisovaleryl)urea showed no difference in the extent of bioavailability. It is assumed that the small differences in free energy among the polymorphs of (α-bromoisovaleryl)urea[6] do not affect the absorbability, in accord with the inference of Aguiar *et al.*[9] for mefenamic acid.

Mean *in Vivo* Dissolution Time (MDT)

The statistical moment theory[12] was used in this study for comparing the *in vitro* dissolution behavior reported in the previous paper[6] to the *in vivo* dissolution behavior, which may affect the rate bioavailabilities of polymorphs.

The area under the concentration time curve (AUC_∞) and the area under the moment curve ($AUMC_\infty$) from zero time to infinity were calculated by means of linear trapezoidal and extrapolation equations as follows,

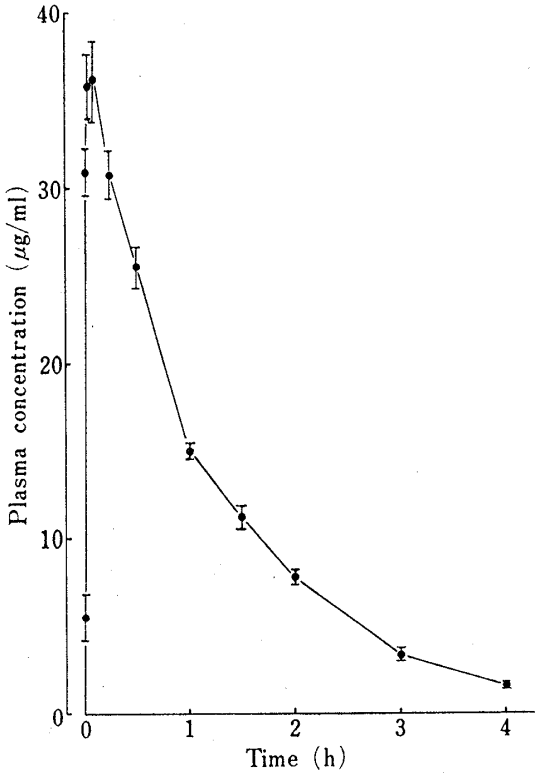

Fig. 3. Plasma Levels after Intraduodenal Administration of Aqueous Solution of (α-Bromoisovaleryl)urea

Points are means ± S.E. of four experiments.

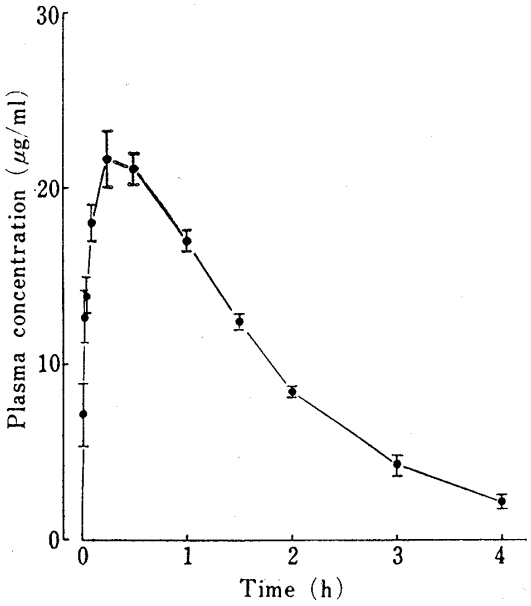

Fig. 4. Plasma Levels after Intraduodenal Administration of Form I of (α-Bromoisovaleryl)urea suspended in Gelatin Solution

Points are means ± S.E. of four experiments.

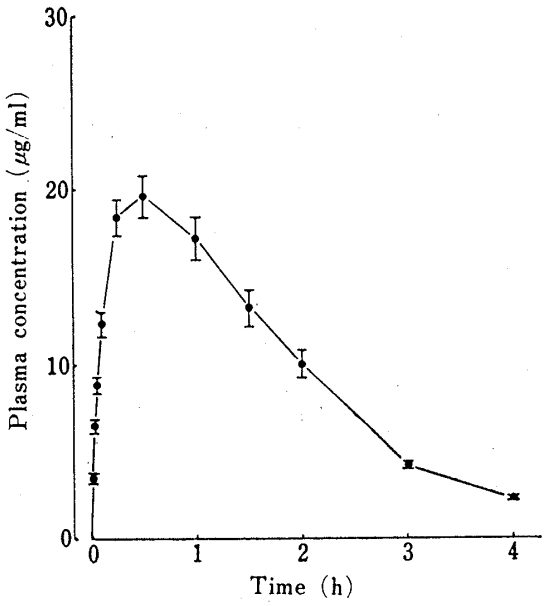

Fig. 5. Plasma Levels after Intraduodenal Administration of Form II of (α-Bromoisovaleryl)urea suspended in Gelatin Solution

Points are means ± S.E. of four experiments.

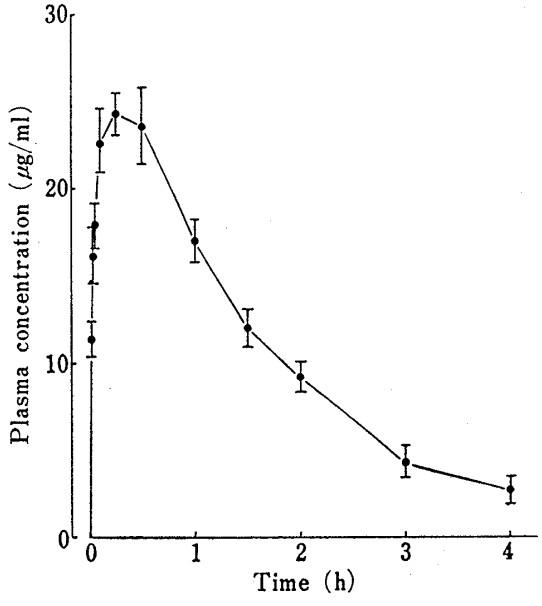

Fig. 6. Plasma Levels after Intraduodenal Administration of Form III of (α-Bromoisovaleryl)urea suspended in Gelatin Solution

Points are means ± S.E. of four experiments.

Table I. Bioavailability Parameters of (α-Bromoisovaleryl)urea Polymorphs after Intraduodenal Administration to Rats

	T_{max} (min)	C_{max} (μg/ml)	AUC[a] (h·μg/ml)
Solution	4.5± 1.5	37.2±4.2	47.0±3.6
Form I	18.8± 6.5	22.6±2.4	45.3±5.5
Form II	20.3±11.8	26.7±3.7	46.0±7.0
Form III	25.0± 7.1	19.9±2.6	44.6±6.3

Each value is the mean S.D. ± of four experiments.
a) Calculated from equation (1) in the text.

$$AUC_\infty = \int_0^\infty C_p dt = \sum\left(\frac{C_n + C_{n-1}}{2}\right)\Delta t + \frac{C_z}{\lambda_z} \quad (1)$$

$$AUMC_\infty = \int_0^\infty tC_p dt = \sum\left(\frac{t_n C_n + t_{n-1}C_{n-1}}{2}\right)\Delta t + \frac{t_z C_z}{\lambda_z} + \frac{C_z}{(\lambda_z)^2} \quad (2)$$

where $t=t_n-t_{n-1}$, $n-1$ and n represent adjacent data points. C_z represents the value of the concentration at the last data point at time t_z. The λ_z is the elimination rate constant estimated by fitting four or five data points in the log-linear portion (1.0, 1.5, 2.0, 3.0, and 4.0 h) to a mono-exponential equation. The mean residence time (MRT) is calculated as follows,

$$MRT = \frac{AUMC_\infty}{AUC_\infty} \quad (3)$$

If one administers suspended crystals into the duodenum, MRT_{crys} will involve the dissolution, absorption and disposition processes. If one administers a solution into the duodenum, MRT_{sol} will involve the absorption and disposition processes. Assuming that the disposition and absorption processes are essentially the same in all cases, one can obtain the mean *in vivo* dissolution time (MDT) as follows,[12]

$$MDT = MRT_{crys} - MRT_{sol} \quad (4)$$

where MRT_{crys} and MRT_{sol} are average values of MRT obtained for administration of suspended crystals and aqueous solution, respectively. In this experiment, since the dissolution process is rate-limiting among the absorption processes as described above, the MDT value is significant. The values calculated using these equations are shown in Table II.

The MDT value of form II was smaller than that of form I. This may reflect the finding that the apparent *in vitro* dissolution rate of form II was about 30% higher than that of form I at 37°C, as shown in the previous paper.[6] On the other hand, MDT of form III was larger than that of form I, as opposed to the previous *in vitro* result that the solubility of form III was higher than that of form I. Because of the extrapolation of C_z and λ_z for estimating AUC_∞

Table II. Parameters of Rate of Bioavailability of (α-Bromoisovaleryl)urea Polymorphs after Intraduodenal Administration to Rats

	AUMC[a] (h²·μg/ml)	MRT[b] (h)	MDT[c] (min)
Solution	59.1± 6.5	1.25±0.08	
Form I	74.1±15.1	1.62±0.14	22
Form II	76.9±28.0	1.55±0.23	18
Form III	74.1± 8.8	1.68±0.15	26

Each value is the mean±S.D. of four experiments.
a) Calculated from equation (2) in the text.
b) Calculated from equation (3) in the text.
c) Calculated from equation (4) in the text.

and $AUMC_\infty$, the MRT value is greatly influenced by C_z and λ_z.[12] However, the errors of estimation should theoretically be canceled out by averaging the values obtained from each experiment. In the present study, it was assumed that the error in C_z was very large because the ultimate concentration was very low, as described above. Therefore, it is difficult to detect differences in the rate bioavailabilities among polymorphs by MDT analysis. Further experiments on this problem are being carried out in our labolatories by pharmacokinetic analysis.

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