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Physico-Chemical Properties and Bioavailability of Benoxaprofen Polymorphs[1)]

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The different crystal forms of benoxaprofen were obtained by recrystallization from several solvents. These crystal forms were identified by using X-ray diffractometry, infrared spectroscopy, differential scanning calorimetry and thermogravimetry. It was found that benoxaprofen exists in two polymorphic forms and that form II is more stable than form I.

The thermodynamic values of the two polymorphic forms of benoxaprofen were calculated from solubility measurements. The transition temperature and the free energy difference (ΔG) were estimated to be 98.0 °C and −232 cal/mol, respectively.

The solubility of form I was about 1.5 times greater than that of form II. However, there was no significant difference in the bioavailability of the two polymorphic forms in rabbits.

Keywords——benoxaprofen; polymorphism; thermodynamic parameter; solubility; bioavailability

Benoxaprofen (2-(4-chlorophenyl)-α-methyl-5-benzoxazole-acetic acid) is one of the newest nonsteroidal anti-inflammatory drugs with relatively prolonged action. However, the occurrence of polymorphic forms of benoxaprofen has not been reported so far.

In the present study, the crystal form of benoxaprofen was investigated and two polymorphic forms were confirmed. The physico-chemical properties of these crystal forms and their relative bioavailabilities in rabbits are also presented.

Experimental

Materials——The polymorphic forms were prepared as follows: 1) Form I: Benoxaprofen (5 g) was dissolved in 100 ml of ethanol at 85 °C and the solution was rapidly cooled to 2 °C. The resulting crystals were collected by filtration and dried in a vacuum.

2) Form II: Benoxaprofen (5 g) was dissolved in 30 ml of acetone at 50 °C and allowed to recrystallize at room temperature. The resulting crystals were collected by filtration, and dried in a vacuum.

Identification of Crystal Forms——Two crystal forms were identified by using X-ray diffractometry (Rigaku Denki, Geigerflex model 2011 B, Ni filter, Cu-K_α radiation, 40 kV, 10 mA, 120000 cpm, infrared spectrophotometry (IR, Hitachi, model 260-30, in Nujol), differential scanning calorimetry (DSC, Perkin-Elmer, model DSC-2C), and thermogravimetry (TG, Perkin-Elmer, model TG-2).

Solubility Measurements——Each crystal form (100 mg) was placed in 100 ml of 1/15 M phosphate buffer (pH 7.0) maintained at various temperatures (20, 25, 30, 35 and 40 °C), and shaken at 80 strokes per min. Samples were taken at appropriate intervals and filtered through a Millipore filter (pore size: 0.45 μm). After dilution with distilled water, the concentrations of benoxaprofen were measured at 305 nm with a spectrophotometer (Hitachi, model 200-20).

Animal Experiments——White male rabbits (weighing from 2.8 to 3.5 kg) were used in this study. The stomach-emptying rate of rabbits was controlled according to the method reported by Maeda *et al.*[2)] Benoxaprofen polymorphs (15 mg per kg body weight suspended in 30 ml of distilled water) were orally administered to the stomach-emptying-rate controlled rabbits. Blood samples were taken from an ear vein at appropriate intervals. The

plasma samples were frozen and stored at −5 °C until assay.

Measurements of Benoxaprofen in Plasma——The high-performance liquid chromatography (HPLC) method developed by Fleitman *et al.*[3] was applied with a slight modification as follows: one ml of 1 N HCl and 6 ml of benzene–cyclohexane (85 : 15) solvent were added to 0.5 ml of plasma. The mixture was shaken for 10 min and centrifuged at 5000 rpm for 10 min, and then 5 ml of the organic phase was taken and evaporated to dryness under a gentle nitrogen flow. Benzene (0.5 ml) was added to the residue and mixed with a vortex mixer, and the benzene was evaporated off again. The residue was dissolved in 150 μl of methanol containing 650 μg/ml of diazepam (internal standard), and 20 μl of the solution was injected into the HPLC apparatus (Shimadzu LC-3A with a Shimadzu SPD-2A detector). The conditions for analysis were as follows: column, 4 mm i.d. × 25 cm; packing, Partisil 10 ODS; mobile phase, acetonitrile–1/10 M acetic acid (6 : 4); flow rate, 0.7 ml/min; wavelength, 305 nm; sensitivity, 0.005 a.u.f.s.

Results and Discussion

X-Ray Diffraction Patterns and IR Spectra

The X-ray diffraction patterns of the two crystal forms are shown in Fig. 1. The patterns were distinctly different, suggesting the existence of different crystal forms. The IR spectra of

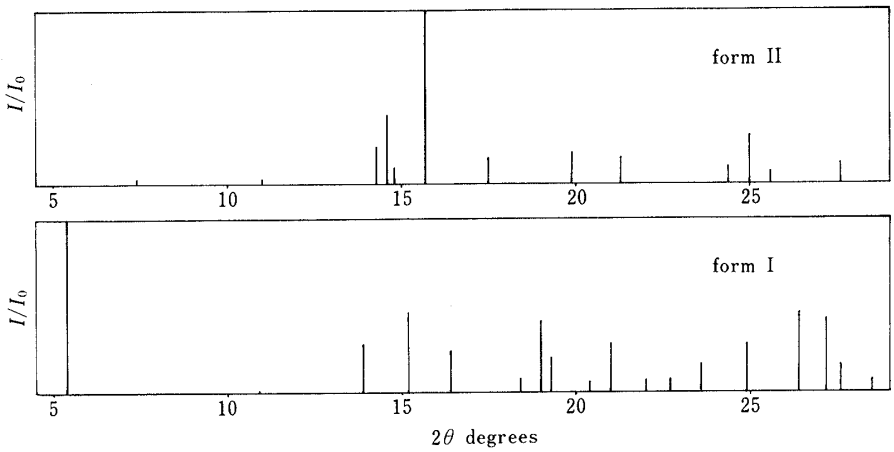

Fig. 1. X-Ray Diffraction Patterns of Benoxaprofen Polymorphic Forms

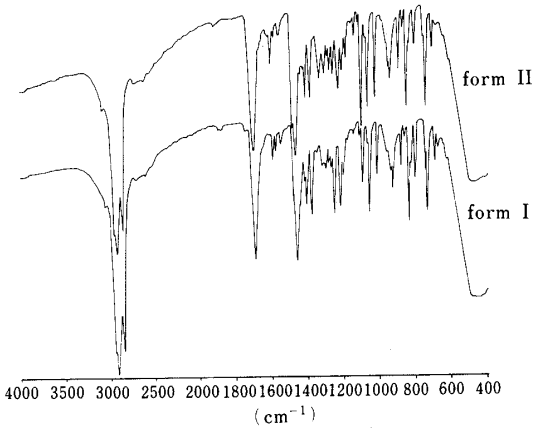

Fig. 2. IR Spectra of Benoxaprofen Polymorphic Forms (in Nujol)

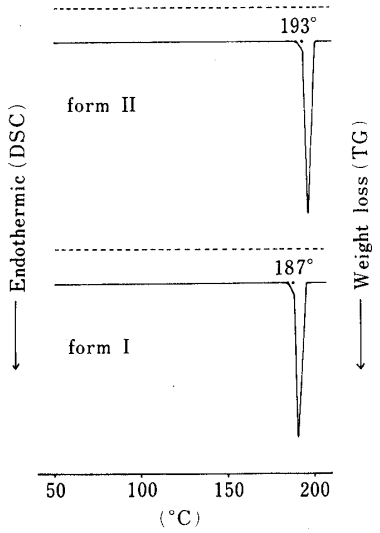

Fig. 3. DSC-TG Curves of Benoxaprofen Polymorphic Forms

——, DSC curves; ----, TG curves.
Form I: sample weight 2.80 mg, heating rate 20 °C/min.
Form II: sample weight 2.33 mg, heating rate 20 °C/min.

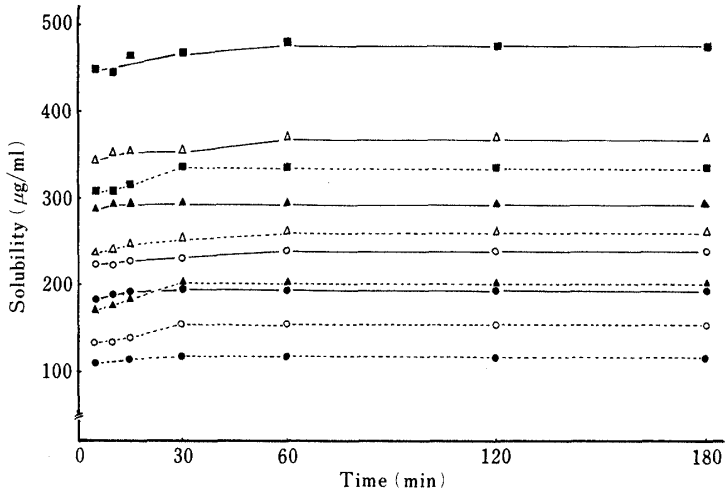

Fig. 4. Solubility Curves for Benoxaprofen Polymorphic Form in 1/15 M Phosphate Buffer (pH 7.0) at Various Temperatures

——, form I; – – – –, form II; ●, 20 °C; ○, 25 °C; ▲, 30 °C; △, 35 °C; ■, 40 °C.

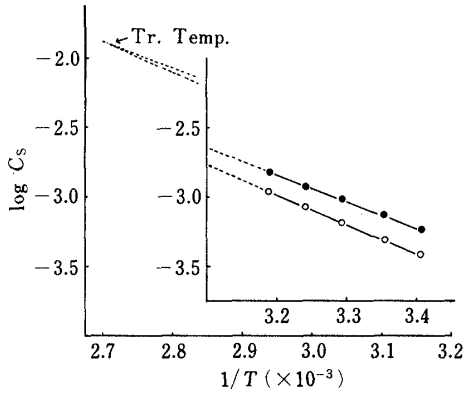

Fig. 5. The van't Hoff's Plots for Benoxaprofen Polymorphic Forms in 1/15 M Phosphate Buffer (pH 7.0)

C_S, solubility; —●—, form I; —○—, form II.

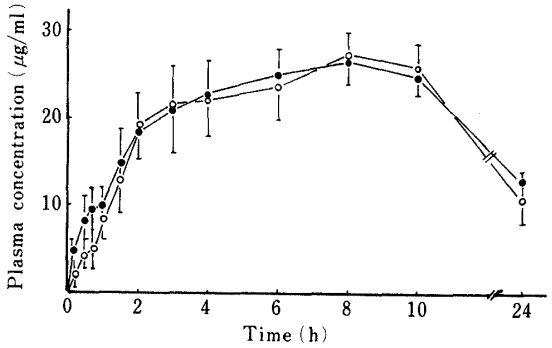

Fig. 6. Plasma Levels of Benoxaprofen Following the Oral Administration of Benoxaprofen Polymorphic Forms to Rabbits

Each point represents the average ± S.E. of six experiments.

—●—, form I; ---○---, form II.

TABLE I. Thermodynamic Values Calculated for Benoxaprofen Polymorphs

	Transition temperature (°C)	Heat of solution (kcal/mol)	ΔG at 25 °C (cal/mol)[a]	ΔS_{298} (e.u.)	ΔS_{trans} (e.u.)[a]
Form II	—	9.37	—	—	—
Form I	98	8.47	−232	−2.41	−2.56

a) Calculated for the conversion to form II.

the two crystal forms are shown in Fig. 2. In the IR spectra, no significant difference of the two crystal forms was observed.

DSC–TG Curves

Figure 3 shows the DSC–TG curves for the two crystal forms. Forms I and II showed only one endothermic peak corresponding to melting points at 187 and 193 °C, respectively.

In the TG curve, no weight decrease could be seen. Therefore, these crystal forms were neither solvates nor hydrates.

Solubility Determination and Thermodynamic Parameters[4]

The solubility curves of the polymorphic forms at various temperatures (20, 25, 30, 35 and 40 °C) are given in Fig. 4. Benoxaprofen is only slightly soluble in acidic solutions. Therefore, phosphate buffer (pH 7.0) was used as a dissolution medium because it provided a suitable solubility range of benoxaprofen. The solubility of form I was about 1.5 times greater than that of form II. These solubility values were plotted according to van't Hoff's equation and straight lines were obtained by the least-squares method, as shown in Fig. 5. The transition temperature was estimated from the intersection point of the two straight lines, and the heats of solution of the two polymorphic forms were calculated from the slopes of the straight lines. The free energy difference (ΔG) between forms I and II was calculated according to the following equation and was estimated to be -232 cal/mol at 25 °C.

$$\Delta G_T = RT \frac{C_s \text{ form II}}{C_s \text{ form I}}$$

Here, C_s is the solubility at a particular temperature. The enthalpy change (ΔH) for the transition of form I to form II was obtained by subtracting the value of the heat of solution of form II from that of form I. The entropy (ΔS) for the transition of form I to form II was calculated by means of the following equation.

$$\Delta S_T = \frac{\Delta H_{I \to II} - \Delta G_T}{T}$$

The thermodynamic values calculated for each form are summarized in Table I.

Plasma Concentrations after Oral Administration of Benoxaprofen Polymorphic Forms

Figure 6 shows the plasma concentration–time curves after oral administration of benoxaprofen polymorphic forms in rabbits. The area under the plasma concentration–time curve [AUC] was calculated according to the trapezoidal rule from time 0 to time 24 h. The [AUC] values of forms I and II were 49.15 ± 118.0 μg·h/ml (mean ± S.E.) and 469.8 ± 51.8 μg·h/ml (mean ± S.E.), respectively. Therefore, no significant difference in bioavailability between forms I and II could be seen. It appears that, since the free energy difference (ΔG) between forms I and II is as small as the ΔG values of mefenamic acid,[4] acetohexamide[5] and tolbutamide,[6] there is no significant effect on the bioavailability, just as was inferred by Aguiar *et al.*[4]

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