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Polymorphism of Methyl (*E*)-3-Phenyl-2-propen-1-yl 1,4-Dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate (FRC-8411)

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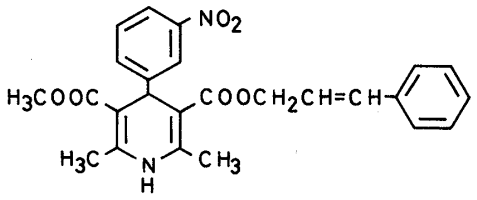
Two crystal forms of the new dihydropyridine derivative, methyl (*E*)-3-phenyl-2-propen-1-yl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate (FRC-8411), were obtained by recrystallization from methanol. These crystal forms were identified by using powder X-ray diffractometry, infrared spectroscopy, differential scanning calorimetry (DSC) and thermogravimetry. By means of DSC, the melting points of forms I and II were found to be 140 and 121 °C, respectively.

Forms I and II, having a similar particle size distribution, were administered orally or intravenously to spontaneously hypertensive rats. In the case of oral administration, the hypotensive action of form I was milder than that of form II and tachycardia was not observed after administration of form I.

Keywords——methyl (*E*)-phenyl-2-propen-1-yl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate (FRC-8411); dihydropyridine derivative; powder X-ray diffraction; IR spectrum; thermal analysis; pharmacological effect

Methyl (*E*)-3-phenyl-2-propen-1-yl 1,4-dihydropyridine-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate (FRC-8411, Chart 1) is a new antihypertensive dihydropyridine derivative with a gradual onset and long duration of action.[1] FRC-8411 exists as water-insoluble yellow crystals. FRC-8411 is a *trans* isomer and a racemic compound.



FRC-8411

Chart 1

A number of studies on polymorphism of various drugs have appeared in the literature.[2] Different polymorphs of drugs are known to possess different bioavailabilities.[3] In this study, the crystalline form of FRC-8411 was investigated and the existence of two polymorphic forms (forms I and II) was confirmed. Methods for the preparation of both polymorphs and details of the antihypertensive action of forms I and II, having a similar particle size distribution, in spontaneously hypertensive rats are presented.

Experimental

Materials——FRC-8411 was synthesized as reported previously.[4] Solvents used were of analytical reagent grade. The polymorphic forms were prepared as follows.

Form I: FRC-8411 (10 g) was dissolved in 80 ml of methanol at 80 °C and the solution was stirred at room temperature. The resulting crystals were collected by filtration and dried in a vacuum. Form I also could be recrystallized from ethanol, acetonitrile, ethyl acetate and isopropyl alcohol.

Form II: FRC-8411 (10 g) was dissolved in 80 ml of methanol at 80 °C and the solution was rapidly cooled to 2 °C with stirring. The resulting crystals were collected by filtration and dried in a vacuum. Form II also could be recrystallized from benzene, toluene and tetrahydrofuran–hexane (1 : 1) mixture.

Characterization of Polymorphic Forms——Polymorphs of FRC-8411 were examined by powder X-ray diffraction analysis, infrared (IR) spectroscopy, differential scanning calorimetry (DSC), thermogravimetry (TG) and elemental analysis. Powder X-ray diffractometry was carried out using a Rigaku Denki Geigerflex model 2027 diffractometer with Ni-filtered Cu-$K\alpha$ radiation. IR spectra were obtained by the KBr disk method with a Hitachi 295 infrared spectrophotometer. Differential scanning calorimetry measurements were carried out using a Perkin-Elmer DSC-1B with a scanning speed of 4 °C/min. The sample weight was about 2 mg in a liquid cell. TG was done using a Shimadzu DT-20B unit at a heating rate of 5 °C/min. A Perkin-Elmer 240B instrument was used for elemental analysis. For examining the presence of impurities and decomposition products, proton nuclear magnetic resonance (^{1}H-NMR) spectra in $CDCl_3$ were obtained with a Varian XL-300 NMR spectrometer. Determination of optical rotation was done with a Union Giken PM-101 polarimeter.

Particle Size Distribution of Forms I and II——Particle size distribution was determined by using a Shimadzu SA-CP3 centrifugal particle size analyzer. A 0.2% aqueous solution of sodium hexametaphosphate was used as the dispersion medium.

The Antihypertensive Actions of Forms I and II——Male spontaneously hypertensive rats (SHR, 14—16 weeks old, Nihon Rat Animal Center) were used. The rats were fasted for 14 h before the start of experiments. Systolic blood pressure was measured in a conscious state by a tail cuff plethysmographic method using an electrosphygmomanometer (Narco, PE-300) at 0, 1, 3, 7 and 24 h after oral drug administration. Heart rate was measured simultaneously with the blood pressure by a heart rate counter (Nihon Koden AT-601G) triggered by the tail pulse pressure. Forms I and II (2 mg), having a similar particle size distribution, were each suspended in 5% gum arabic aqueous solution (5 ml) and administered orally at a dose of 2 mg/kg.

Results and Discussion

Physico-Chemical Properties of FRC-8411 Crystal Forms

The powder X-ray diffraction patterns of the two crystal forms are shown in Fig. 1. The patterns are distinctly different, suggesting the existence of different crystal forms. Figure 2 shows the IR spectra of the two crystal forms. Curve (a) shows the IR spectrum of form I. The absorption bands at 3320, 1650 and 685 cm^{-1} were assigned to the secondary amine (ν_{NH}), the dihydropyridine structure ($\nu_{C=C}$) and the disubstituted phenyl ($\delta_{out\,CH}$), respectively. Curve (b) shows the IR spectrum of form II. In comparision with the IR spectrum of form I, the secondary amine (ν_{NH}) band is shifted to lower frequency, from 3320 to 3290 cm^{-1}, and its absorption intensity is also decreased. Further, the dihydropyridine structure ($\nu_{C=C}$) band is

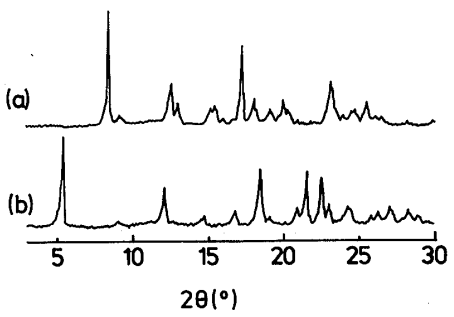


Fig. 1. Powder X-Ray Diffraction Patterns of Polymorphs of FRC-8411

(a) form I, (b) form II.

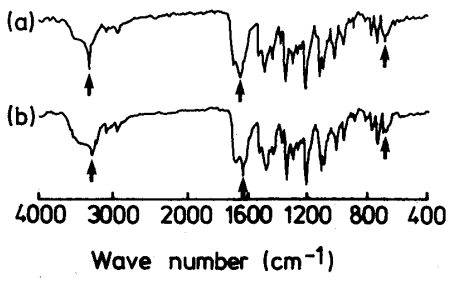


Fig. 2. Infrared Spectra of Polymorphs of FRC-8411

(a) form I, (b) form II.

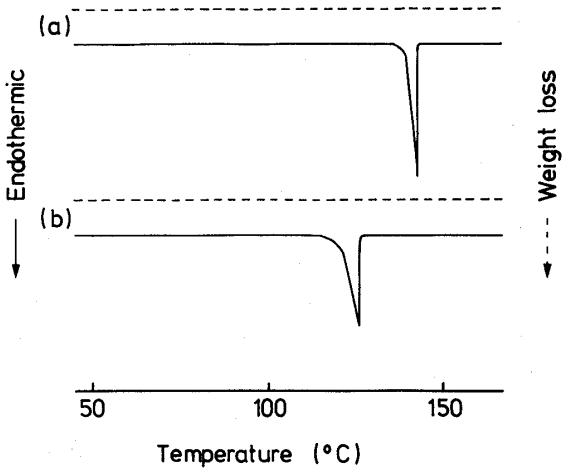


Fig. 3. DSC-TG Curves of Polymorphs of FRC-8411

——, DSC curves; ------, TG curves.
(a) form I, (b) form II.

TABLE I. Elementary Analysis of Polymorphs of FRC-8411

Form	Formula	Analysis (%) Calcd (Found)		
		C	H	N
I	$C_{25}H_{24}N_2O_6$	66.96 (66.70	5.39 5.32	6.25 6.13)
II	$C_{25}H_{24}N_2O_6$	66.96 (66.73	5.39 5.37	6.25 6.15)

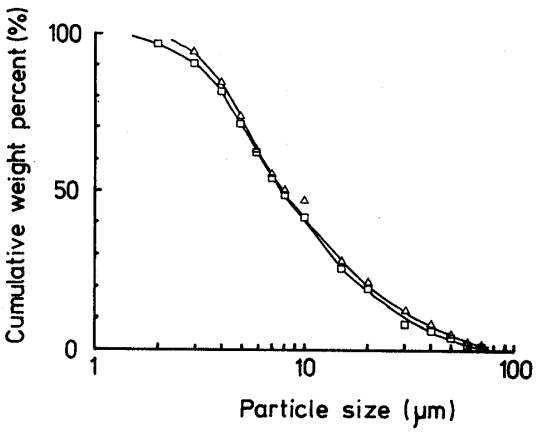


Fig. 4. Particle Size Distribution Curves of Polymorphs of FRC-8411

□, form I; △, form II.

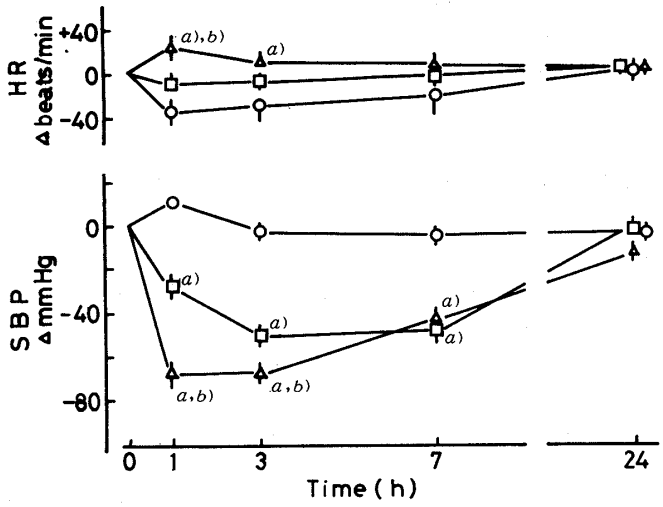


Fig. 5. Effects of Crystal Form on the Systolic Blood Pressure (SBP) and the Heart Rate (HR) after the Oral Administration of Form I (□) and Form II (△) to SHR

Each value indicates the mean ± S.E. of fifteen experiments. *a*) $p<0.05$ as compared with the control group (○), *b*) $p<0.05$ as compared with the form II group.

shifted to lower frequency, from 1650 to 1640 cm^{-1}. As regards the absorptions of the disubstituted phenyl ring, a new band appeared at 705 cm^{-1}, while the absorption intensity at 685 cm^{-1} was decreased. Figure 3 shows the DSC-TG curves of the two crystal forms. Forms I and II showed only one endothermic peak corresponding to the melting at 140 and 121 °C, respectively. In the TG curves, no change in weight was observed below 170 °C. Therefore, these crystal forms are neither solvates nor hydrates.

The elemental analysis data of the two crystal forms coincided well with the theoretical values, as shown in Table I. The same NMR spectra were obtained for the two crystal forms, and the two forms had no optical rotation. It follows from the above that the crystals do not contain impurities or decomposition products, and that the two forms are distinct polymorphs.

Particle size distributions of the two crystal forms are shown in Fig. 4, which indicates the

cumulative percent of oversize particles. There was no significant difference between the two distribution curves, and the median diameters of forms I and II were 8.11 and 8.71 μm, respectively.

Antihypertensive Actions of Forms I and II

Figure 5 shows the changes in blood pressure and heart rate after oral administrations of forms I and II, having a similar particle size distribution, at a dose of 2 mg/kg. Forms I and II both caused a significant hypotension which lasted more than 7 h in SHR ($p<0.05$). However, there was a significant difference in the time-courses of hypotension after the oral administration of the two polymorphic forms in SHR ($p<0.05$). The rate of hypotension appearance after the administration of form I was slower than that after the administration of form II, that is, the maximum hypotension was observed at 3 h after the administration of form I, while it was observed at 1 h after the administration of form II. Moreover, a significant tachycardia was observed after the administration of form II ($p<0.05$), but not after the administration of form I. Thus, there was an apparent difference in the pharmacological effects after the oral administration of the two polymorphic forms to SHR. The two polymorphic forms, disolved in 50% HCO-60 (Nikko Chemicals) ethanol solution and diluted with physiological saline, were also administered intravenously to SHR as control experiments. There was no significant difference in the pharmacological effects.

Different polymorphs of drugs can show different values of importance of dissolution rate, bioavailability, physical stability and chemical stability. In this study, the two polymorphic forms of FRC-8411 were found to show different hypotensive actions after oral (but not intravenous) administration to SHR; the action of form I was milder than that of form II. The results could be explained in terms of different dissolution behavior of the polymorphic forms in the gastrointestinal tract of the rats.

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