Physicochemical Analyses of Phase Transition and Dehydration Processes of a New Oral 1*β*-Methylcarbapenem Antibiotic Agent, CS-834

Katsuhiro KOBAYASHI,[a,d] Hiroshi FUKUHARA,[a] Isao KAWAMOTO,[b] Tadashi HATA,[c] Akiko SEKINE,[d] Hidehiro UEKUSA,[d] and Yuji OHASHI*,[d]

[a] *Process Development Laboratories, Sankyo Co. Ltd.; 1–12–1 Shinomiya, Hiratsuka, Kanagawa 254–8560, Japan:* [b] *Medicinal Chemistry Research Laboratories, Sankyo Co. Ltd.;* [c] *Biomedical Research Laboratories, Sankyo Co. Ltd.; 1–2–58 Hiromachi, Shinagawa-ku, Tokyo 140–8710, Japan: and* [d] *Department of Chemistry and Material Sciences, Tokyo Institute of Technology; 2–12–1 O-okayama, Meguro-ku, Tokyo 152–8551, Japan.*

Received July 16, 2002; accepted September 4, 2002

The characterizations of the anhydrate (A-form), monohydrate (B1-form), and dihydrate (B2-form) of CS-834 were investigated by powder X-ray diffraction, differential scanning calorimetry (DSC), thermogravimetry-differential thermal analysis (TG-DTA), infrared spectroscopy, and Karl Fischer moisture titration. The typical DSC curve of the B2-form showed five endothermic peaks at 35.0, 46.4, 56.2, 99.2, and 190.4°C and an exothermic peak at 123.4°C. In TG-DTA analysis, the three peaks at 35.0, 46.4, and 56.2°C had a total weight loss of 7.3%, corresponding to the release of two water molecules. From morphological observation under thermomicroscopy, the endothermic peak at 99.2°C was attributed to the melting of the dehydrous crystals (B0-form) and the exothermic peak at 123.4°C to the recrystallization to the A-form crystals. The endothermic peak at 190.4°C was due to the melting of the A-form crystals. After incubation for 6.0 h at 35, 50, 60, and 80°C, the powder X-ray diffraction patterns of the B2-form indicated that it was converted into the A-form *via* the B1-form and B0-form. Thus CS-834 exists in homologous hydrous crystal forms in multiple-phase transformations with the dehydration of two water molecules.

Key words pseudopolymorph; dehydration; phase transition; 1*β*-methylcarbapenem; powder X-ray diffractometry; thermal analysis

A drug, substance can take various forms, such as an amorphous, polymorph, hydrate, and solvate, according to conditions of the manufacturing processes, for example, drying,[1,2] grinding,[3,4] and compressing.[5,6] Depending on the drug form, the pharmaceutical physical properties, bioavailability,[7—9] stability,[10] dissolution rate,[11,12] and moisture,[13,14] which are factors to consider in the design of dosage form, are affected. Therefore it is important to analyze the characterization of the bulk substance and to select the suitable form for formulation.

Pivaloyloxymethyl (1*R*,5*S*,6*S*)-6-[(*R*)-1-hydroxyethyl]-1-methyl-2-[[(*R*)-5-oxo-pyrrolidin-3-yl]thio]-1-carbapen-2-em-3-carboxylate, CS-834, was developed as an orally effective 1*β*-methylcarbapenem antibiotic agent with strong antibacterial activities against a wide range of bacteria and excellent stability against dehydropeptidase-I (Fig. 1).[15] CS-834 exists in three modifications, as an anhydrate (A-form), a monohydrate (B1-form), and a dihydrate (B2-form).[16] The anhydrate is crystallized from an anhydrous solvent. On the other hand, the dihydrate is obtained by crystallization from an aqueous solution, and is converted into the monohydrate by drying under reduced pressure. The crystal structures of the anhydrate[17] and the dihydrate[18] by single crystal X-ray analysis were reported in previous papers.

The objective of the present investigation was to analyze the phase transition and dehydration processes of the three crystal forms of CS-834 using powder X-ray diffractometry, differential scanning calorimetry (DSC), thermogravimetry-differential thermal analysis (TG-DTA), and infrared (IR) spectroscopy.

Experimental

Preparation of CS-834 Anhydrate (A-Form) Colorless A-form crystals were prepared from an ethanol solution by evaporation and collected by filtration.

Preparation of CS-834 Dihydrate (B2-Form) B2-form crystals were obtained by recrystallization with 3% aqueous ethyl acetate solution at 0 °C. The crystals were dried at 25 °C and 60% relative humidity (RH) for 3 d.

Preparation of CS-834 Monohydrate (B1-Form) B1-form crystals were prepared by drying the B2-form crystals under reduced pressure at room temperature over night and kept at 25 °C and 40% RH for 3 d.

Powder X-Ray Diffractometry Samples were packed into a glass holder and the diffraction patterns were measured on a model Rint 2200 diffractometer (Rigaku Denki Co. Ltd.) fitted with a scintillation counter with a monochromerter by Cu-Kα radiation, a voltage of 40 kV, a current of 40 mA, a 2θ range of 3—40°, a scan step of 0.02°, and at a rate of 4°/min. Powder X-ray diffraction data at various temperatures were also recorded using the diffractometer equipped with a Rigaku temperature attachment and controller. The temperature was raised to 35, 50, 60, and 80 °C at a heating rate of 10 °C/min and each temperature was maintained for 6 h.

DSC DSC curves were recorded on a model DSC3100 instrument (Mac Science Co. Ltd.). Approximately 3 mg of each sample in an open pan was heated from 0 to 230 °C at 5 °C/min with a nitrogen gas flow rate of 100 ml/min unless otherwise stated.

TG-DTA TG-DTA curves were recorded on a model TG-DTA 2000 instrument (Mac Science Co. Ltd.). About 3 mg of samples in an open pan were heated from room temperature to 230 °C at 3 °C/min with a nitrogen gas flow rate of 20 ml/min.

FT-IR Spectrometry IR spectra were obtained with a Perkin-Elmer infrared spectrometer model 2000. A disc pellet was prepared for each sample.

Water Content Titration Water content was determined using the Karl Fischer (KF) method (model MKA-210, Kyoto Denshi Kogyo Co. Ltd.). Samples (100—300 mg) were accurately weighed and quickly transferred to the titration vessel containing anhydrous methanol prior to titration, then they were titrated by KF reagent SS® (Mitsubishi Chemical Co. Ltd. Japan).

HPLC Analysis Content of samples was measured using a Shimadzu

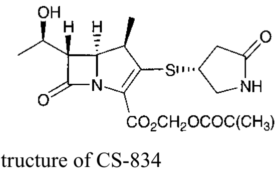

Fig. 1. Chemical Structure of CS-834

---

* To whom correspondence should be addressed. e-mail: yohashi@cms.titech.ac.jp

HPLC system (LC-10A series) using an ODS column and 0.02 M phosphate buffer (pH 7.0)/acetonitrile (60 : 40, v/v) solution as the mobile phase.

Morphological Observation Phase transformations were observed under a thermomicroscope. (Micro Melting Point Apparatus, Yanagimoto Co. Ltd.). Small amount of samples were heated from room temperature to 200 °C at a heating rate of about 3 °C/min.

Results and Discussion

Identification of Modifications The elemental and water content analyses of the three modifications are summarized in Table 1 with the calculated values. These observed values are in good agreement with the theoretical values.

The powder X-ray diffraction profiles of the modifications are shown in Fig. 2. Their profiles differed significantly. The specific X-ray diffraction peaks for the anhydrate (A-form) were observed at $2\theta=4.8$, 9.6 14.2, 16.8, 17.6, and 18.8°, for the dihydrate (B2-form) at $2\theta=6.9$, 20.6, and 21.4°, and for the monohydrate (B1-form) at $2\theta=7.5$, 22.7, and 23.5°.

Table 1. Elemental Analysis and Water Content of the Modifications

Modification		Elemental analysis (%)				Water content (%)
		C	H	N	S	
Anhydrate	Found	54.25	6.15	6.32	7.37	0.2
(A-form)	Calcd	54.53	6.41	6.36	7.28	0
Monohydrate	Found	52.55	6.57	6.06	6.93	4.0
(B1-form)	Calcd	52.39	6.60	6.11	6.99	3.9
Dihydrate	Found	50.53	6.72	5.87	6.92	7.5
(B2-form)	Calcd	50.41	6.77	5.88	6.73	7.6

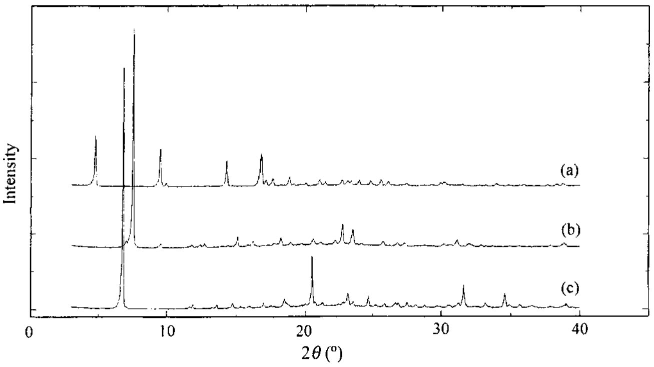

Fig. 2. Powder X-Ray Diffraction Patterns of Three Modifications of CS-834

(a) Anhydrate: A-form, (b) monohydrate: B1-form, (c) dihydrate: B2-form.

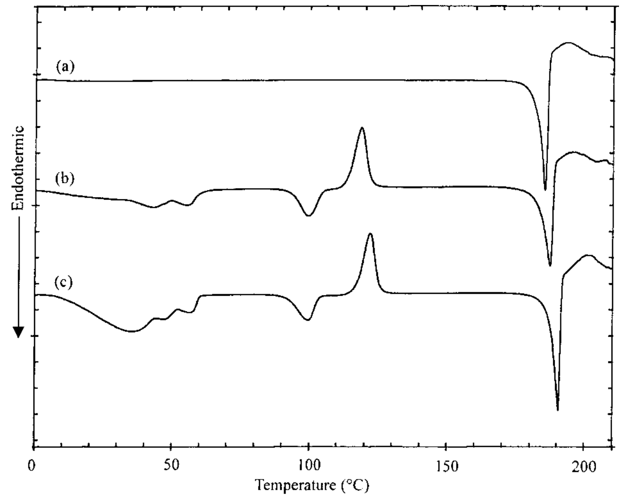

Fig. 3. DSC Curves of Three Modifications of CS-834

(a) Anhydrate: A-form; (b) monohydrate: B1-form; (c) dihydrate: B2-form.

DSC curves of the modifications are shown in Fig. 3. The A-form showed only one endothermic peak at 185.7 °C due to melting. The B2-form showed five endothermic peaks at 35.0, 46.4, 56.2, 99.2, and 190.4 °C and an exothermic peak at 120.8 °C. The DSC curve of the B1-form was similar to that of the B2-form except for the endothermic peak at 35.0 °C. The DSC curves indicated that the hydrates of CS-834 undergo multiple-phase transformations as the temperature is raised.

IR spectra of modifications of CS-834 are shown in Fig. 4. IR patterns of modifications were slightly different in the region of 1600—1800 cm^{-1}. The absorptions at 3000—3500 cm^{-1} of the two hydrates (B2-form and B1-form) were particularly different from that of the anhydrate (A-form) due to the presence of solvent water molecules. The combined results of all physical methods, *i.e.*, powder X-ray analysis and thermal analysis, made it easy to identify the three modifications of CS-834.

Thermal Properties of CS-834 Modifications The hydrates of CS-834 have multiple transformations with the release of water molecules considering based on the DSC curves. Kitaoka *et al.* reported that the heating rate and gas flow rate influenced the formation of pseudopolymorphs in DSC measurement.[19] Therefore the effect of the atmospheric conditions on the DSC curve of the dihydrate was investigated to confirm that the peaks were reproducible. The DSC curves of the dihydrate under a nitrogen gas flow rate of 0—100 ml/min are shown in Fig. 5.

All peaks of the B2-form in Fig. 3 appeared under various measurement conditions. As the gas flow rate increased, the three endothermic peaks between the range of 30—80 °C shifted to lower temperature. Therefore the peak shift indicates that the peaks were related to the release of water molecules. A nitrogen gas flow rate of 20 ml/min or less gave good resolution in the DSC curve. To correlate the DSC peaks with the release of water molecules during the transition, TG-DTA was measured under the same conditions as DSC measurement (heating rate of 3 °C/min with a nitrogen

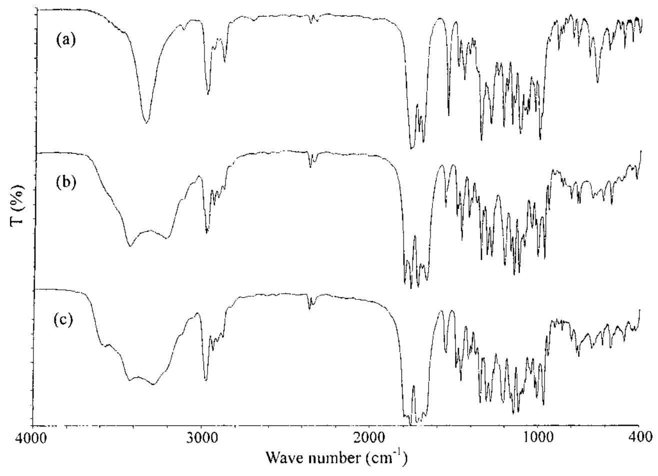

Fig. 4. IR Spectra of Three Modifications of CS-834

(a) Anhydrate: A-form; (b) monohydrate: B1-form; (c) dihydrate: B2-form.

gas flow rate of 20 ml/min), as shown in Fig. 6. The DTA thermograms were compatible with the DSC thermograms.

The A-form showed no weight loss and no peaks up to the melting point of 190 °C. The B1-form showed a total weight loss of 3.8%, and the B2-form had a total weight loss of 7.3% in the range of 30—80 °C, corresponding to the release of solvent water molecules (Calcd at 3.9% and 7.6%, respectively). In the B2-form, the three endothermic peaks in the DTA thermogram had weight loss as follows: peaks at 42.6 °C, 0.67 eq; at 64.2 °C, 0.20 eq; and at 77.6 °C, 0.09 eq. In the B1-form, the two endothermic peaks at 62.3 and 85.2 °C in the DTA thermogram corresponded to a weight loss of 0.26 and 0.20 eq, respectively. In the B1-form, however, the water molecules of 0.5 eq were volatilized up to 45.3 °C due to the low humidity conditions of TG-DTA measurement. Since the dehydration of the B1-form occurred under initial thermal analysis, the transformation into the B1-form from the B2-form dose not completely follow the conversion to a dehydrate from the B1-form. Therefore it was confirmed that the three endothermic peaks between 30—80 °C in the DTA and DSC thermograms occurred due to dehydration, but it is difficult to assign the endothermic peaks to transformation between the stoichiometric hydrous crystal forms. On the other hand, the crystals melted at 98 °C and recrystallized at 113 °C in the morphological observation of the B2-form under thermomicroscopy. The newly grown crystals melted again at 183 °C. Therefore it is suggested that the endothermic peak at 99.2 °C and the exothermic peak at 120.8 °C in the DSC curve shown in Fig. 5 correspond to the melting of the dehydrous crystals and recrystallization into another crystal form, respectively. To confirm the dehydration of two water molecules, the B2-form was heated at 120 °C for 6 h and the purity and water content were measured by HPLC and KF water content titration, respectively. HPLC indicated thermal decomposition of the sample was only 2.2%, but the water content of this sample decreased from 7.6 to 0.1%. It became clear that the DSC peaks at 30—80 °C corresponded to the dehydration of two water molecules and that these water molecules were more easily released at a low temperatures under fast nitrogen gas flow.

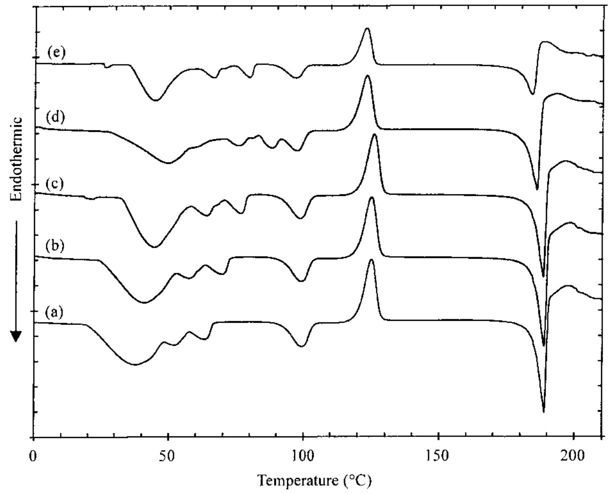

Fig. 5. Effect of N_2 Gas Flow Rate and Heating Rate on DSC Curves of the B2-Form

(a) N_2 gas flow rate: 100 ml/min, heating rate: 5 °C/min; (b) 50 ml/min, 5 °C/min; (c) 20 ml/min, 5 °C/min; (d) no nitrogen flow rate, 5 °C/min; (e) 20 ml/min, 3 °C/min.

Powder X-Ray Diffraction Analysis under Heating Conditions Powder X-ray diffraction under heating conditions was measured to analyze the transformation of the dihydrate form to the dehydrous form. A change in powder X-ray diffraction patterns was observed with increasing temperature, as shown in Fig. 7. The powder diffraction pattern at 35 °C showed a new diffraction pattern (B2′-form), which was confirmed to be the pattern of the B1-form at 50 °C. At 60 and 80 °C, new profiles of the B1′-form and B0-form, respectively, were also seen. The resulting B0-form was transformed into the B1′-form by cooling to 50 °C. Cooling of the B1′-form to 30 °C caused rapid retransformation to the B1-form. Their transformations were ascribed to absorption of water vapor in the laboratory atmosphere. The B2′-form and B1′-form could be interpreted as meta-stable intermediates of stoichiometric crystal forms. Characteristic diffraction peaks of the three new crystal forms were observed at 2θ= 7.2, 21.7, and 22.5° for the B2′-form, at 2θ=7.8, 23.4, and 24.8° for the B1′-form, and at 2θ=8.0, 23.9, and 24.6° for the B0-form. Therefore powder X-ray diffraction analysis indicated that CS-834 takes five homologous crystal forms with various amounts of solvent water.

It became clear that the three endothermic peaks at 35.0, 46.4, and 56.2 °C are due to the release of the two water molecules of the B2-form crystals. The B1′-form has not been

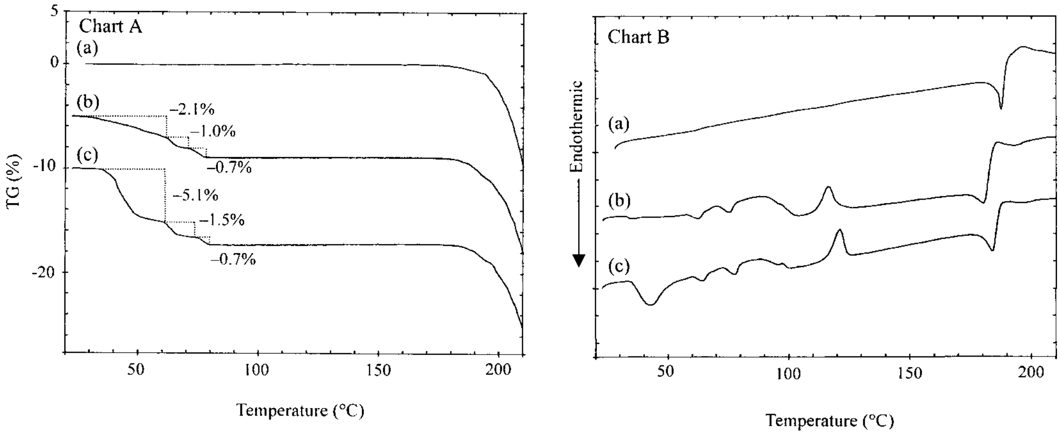

Fig. 6. TG-DTA Curves of Three Modifications

Chart A, TG thermograms and Chart B, DTA thermograms: (a) anhydrate (A-form); (b) monohydrate (B1-form); (c) dihydrate (B2-form).

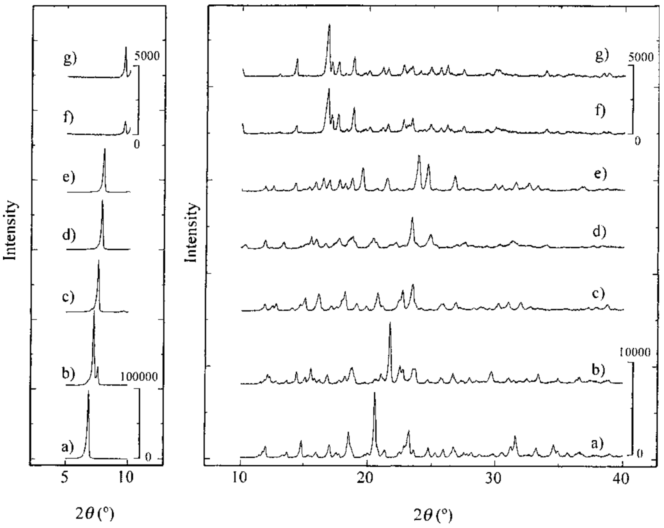

Fig. 7. Powder X-Ray Diffraction Patterns of the B2-Form Heated at Various Temperatures

(a) B2-form, (b) 35 °C, (c) 50 °C, (d) 60 °C, (e) 80 °C, (f) after annealing at 120 °C, the measurement was performed at 25 °C, (g) A-form (control sample).

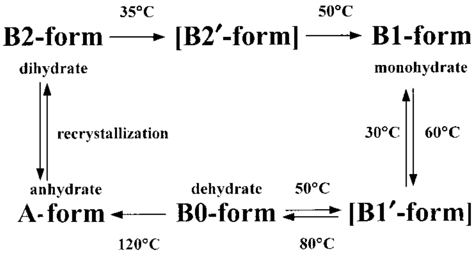

Fig. 8. Phase Interconversion with Preparation Conditions of Modifications of CS-834

isolated because it is unstable under the usual laboratory conditions. It is difficult to assign the three endothermic peaks to the transformation of each crystal form. The endothermic peak at 99.2 °C is caused by the melting of the B0-form crystals. The exothermic peak at 120.8 °C is attributed to the recrystallization of the A-form crystals. The endothermic peak at 190.4 °C is due to the melting of the A-form crystals.

As the powder X-ray diffraction pattern at 120 °C showed an unclear profile due to the irregularity of the sample surface caused by the thermal transformation, the data were collected again after quenching to room temperature and resampling. The diffraction pattern obtained was consistent with that of the A-form. The possible transformation of the dihydrate is summarized in Fig. 8.

Conclusions

The phase transition and dehydration processes of an orally effective 1β-methylcarbapenem antibiotic agent, CS-834, were studied using physical methods. The DSC measurement indicated that the CS-834 dihydrate has a complicated dehydration process. By measurement of the powder X-ray diffraction pattern under heating, CS-834 can take five hydrous crystalline states that have various amounts of solvent water. Furthermore, the dihydrous B2-form was transformed to the monohydrous B1-form and then the B1-form changed to the dehydrous B0-form through the meta-stable intermediate forms, the B2′-form and the B1′-form, respectively. Finally, the B0-form was thermally transformed into the A-form. In conclusion, investigation of this dehydration process of pharmaceuticals will be helpful for the evaluation of the crystal form in manufacturing and formulation processes.

Acknowledgments The authors are grateful to Dr. Maruyama, Dr. Takebayashi, and Dr. Yoshida of Sankyo Co. Ltd. for their enthusiasm and constructive discussion concerning this work.

References

1) Sugi K., Itaya N., Katsura T., Igi M., Yamazaki S., Ishibashi T., Yamaoka T., Kawada Y., Tagami Y., Otsuki M., Ohshima T., *Chem. Pharm. Bull.*, **48**, 529—536 (2000).
2) Botha S. A., Caira M. R., Guillovy J. K., Lotter A. P., *J. Pharm. Sci.*, **78**, 28—34 (1989).
3) Yajima K., Okihara A., Hoshino M., *Chem. Pharm. Bull.*, **45**, 1677—1682 (1997).
4) Otsuka M., Matsumoto T., Kaneniwa N., *Chem. Pharm. Bull.*, **34**, 1784—1793 (1986).
5) Chan H. K., Doelker E., *Drug Dev. Ind. Pharm.*, **11**, 315—332 (1985).
6) Matsunaga J., Nambu N., Nagai T., *Chem. Pharm. Bull.*, **24**, 1169—1172 (1976).
7) Kimura K., Hirayama F., Uekawa K., *J. Pharm. Sci.*, **88**, 385—391 (1999).
8) Kato Y., Okamoto Y., Nagasawa S., Ueki T., *Chem. Pharm. Bull.*, **29**, 3410—3413 (1981).
9) Aguiar A. J., Krc J. Jr., Kinkel A. W., Samyn J. C., *J. Pharm. Sci.*, **56**, 847—853 (1967).
10) Matsuda Y., Tatsumi E., Chiba E., Miwa Y., *J. Pharm. Sci.*, **73**, 1453—1460 (1984).
11) Bettinetti G., Mura P., Sorrenti M., Faucci M. T., Negri A., *J. Pharm. Sci.*, **88**, 1133—1139 (1999).
12) Uchida T., Yonemochi E., Oguchi T., Terada K., Yamamoto K., Nakai Y., *Chem. Pharm. Bull.*, **41**, 1632—1635 (1993).
13) Imaizumi H., Nanbu N., Nagai T., *Chem. Pharm. Bull.*, **28**, 2565—2569 (1980).
14) Nakamachi H., Yamaoka T., Wada Y., Miyata K., *Chem. Pharm. Bull.*, **30**, 3685—3694 (1982).
15) Miyauchi M., Endo R., Hisaoka M., Yasuda H., Kawamoto I., *J. Antibiot.*, **50**, 429—439 (1997).
16) Kobayashi K., Fukuhara H., Ito S., Furukawa Y., Hata T., "The Annual Meeting of the Crystallographic Society of Japan (in Yokohama)," 1998, p. No. 28.
17) Kobayashi K., Fukuhara H., Kawamoto I., Furukawa Y., Hata T., *Anal. Sci.*, **16**, 555—556 (2000).
18) Kobayashi K., Fukuhara H., Kawamoto I., Ito S., Hata T., *Anal. Sci.*, **17**, 357—358 (2001).
19) Kitaoka H., Wada C., Moroi R., Hakusui H., *Chem. Pharm. Bull.*, **43**, 649—653 (1995).