In Situ Observation of the Four-step Dehydration Process of the 1 β -Methylcarbapenem Antibiotic CS-834 Crystal by X-Rays

Katsuhiro KOBAYASHI,^{*,a,c} Hiroshi FUKUHARA,^a Tadashi HATA,^b and Yuji OHASHI^c

^a Process Development Laboratories, Sankyo Co., Ltd.; 1–12–1 Shinomiya, Hiratsuka, Kanagawa 254–8560, Japan:

^b Biomedical Research Laboratories, Sankyo Co., Ltd.; 1–2–58 Hiromachi, Shinagawa-ku, Tokyo 140–8710, Japan: and

^c Department of Chemistry and Materials Science, Tokyo Institute of Technology; 2–12–1 O-okayama, Meguro-ku, Tokyo 152–8551, Japan. Received June 4, 2003; accepted August 22, 2003

The 1 β -methylcarbapenem antibiotic CS-834 takes six crystalline forms depending on ambient conditions. The X-ray powder diffraction revealed that the dihydrate crystal (B2-form) was changed to the monohydrate (B1-form) through the intermediate form (B2'-form). The monohydrate form was then changed to the dehydrate (B0-form) through the intermediate B1'-form. The progress of the dehydration along the needle axis (*c*-axis) was observed under a microscope. When a single crystal of the B2-form was mounted on a diffractometer and the humidity was reduced, the crystal was gradually changed to the various dehydration forms with retention of the single crystal. The crystals of B2- to B0-forms form isostructures to each other except the solvent water molecules. In the crystal structure of the B1-form, the pivaloyloxymethyl moiety is disordered. One is nearly similar to that of the B2-form, while another is similar to that of the B0-form. Each crystal structure consists of a columnar arrangement of CS-834 along the *c*-axis, and the water molecules are located between the columns and form a characteristic hydrogen bond network. When the water molecules leave the crystal, the columns slide slightly following the slight conformational change in the pivaloyloxymethyl groups and are connected by another type of hydrogen bond network. Such a rearrangement of the hydrogen bond network should be a motive force of the phase change to the next step due to the dehydration. Since the hydrogen bond network extends along the *c*-axis, the dehydration proceeds along the *c*-axis as observed microscopically.

Key words hydrate; dehydration; 1 β -methylcarbapenem antibiotic; single-crystal X-ray analysis; hydrogen bond system; crystal structure

Bulk drug substances frequently can be obtained as crystalline hydrates rather than as anhydrides, because they can crystallize more easily,¹⁾ or are transformed into hydrates by contact with water molecules from hydrate excipients in the formulation process.²⁾ The hydrate crystals often transform into different solids such as amorphous or dehydrate form in the process of drying or storage under low relative humidity (RH).^{3–6)} Generally, the resulting dehydrate crystals can be converted to the different hydrate forms depending on the surrounding RH and temperature. It is also well known that different hydrate drugs show different physicochemical properties,^{7,8)} such as bioavailability, phase stability, and dissolution rate. For the development of pharmaceuticals using hydrous bulk drug substances, many efforts have been made to investigate the changes in the physicochemical properties in the process of phase transformation to different hydrate states. The prediction of hydration properties has therefore been a long-standing subject of great interest in pharmaceutical science.

A compound of 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 possessing potent and broad-spectrum antibacterial activities and excellent stability against dehydropeptidase-I (Fig. 1).⁹⁾

It crystallizes as an anhydrate form (A-form) from anhydrous solutions and as a dihydrate (B2-form) from an aqueous solution. The B2-form undergoes the multistep transformation following the loss of water molecules upon heating, as shown in Fig. 2.¹⁰⁾ This figure indicates that the B2-form is transformed to the dehydrate form (B0-form) through the B2'-, B1-, and B1'-forms, where the B1-form was the mono-

hydrate form whereas the B2'- and B1'-forms were intermediate ones between the B2- and B1-forms, and between the B1- and B0-forms, respectively. The possible crystal transformation of CS-834 is summarized in Fig. 3.

It was reported that the hydrate crystals of disodium adenosine 5'-triphosphate (Na₂ATP) and disodium cytidine 5'-monophosphate (Na₂CMP) also underwent reversible humidity-dependent structural transformation between the higher hydrate and the lower hydrate forms as observed by X-ray crystal structure analysis.^{11,12)} Here, we report the dehydration process of the hydrous crystal of CS-834, with the change in the RH and the structural changes in the transformation observed by single-crystal X-ray analysis.

Experimental

Preparation of Different Crystal Forms. Materials CS-834 of pharmaceutical grade was synthesized in the Process Development Laboratories of Sankyo Co., Ltd.

Preparation of the Dihydrate (B2-Form) The A-form crystals were dissolved in a 3% aqueous ethyl acetate solution at 40 °C. The solution was filtrated, evaporated, cooled to about 0 °C, and kept at 0 °C overnight. The crystals were collected by filtration and washed with 3% aqueous ethyl acetate. The crystals were kept at 25 °C and under about 60% RH for 3 d.

Preparation of Single Crystals of the B2-, B2'-, B1-, and B0-Forms Single crystals of the B2-form were obtained from an aqueous ethyl acetate solution by slow evaporation. Single crystals of the B2'-, B1-, and B0-forms were obtained from the B2-form by keeping under the controlled conditions

Fig. 1. Chemical Structure of CS-834

* To whom correspondence should be addressed. e-mail: katsuh@hira.sankyo.co.jp

Fig. 2. Change in Powder X-Ray Diffraction Patterns upon Heating: (a) at 30 °C (B2-Form); (b) at 35 °C (B2'-Form); (c) at 50 °C (B1-Form); (d) at 60 °C (B1'-Form); (e) at 80 °C (B0-Form); and (f) after Annealing at 120 °C

The measurement was performed at 25 °C (A-form).

Fig. 3. Phase Interconversion with Preparation Conditions of Modifications of CS-834

as follows: the B2'-form, under less than 16% RH at 25 °C; the B1-form, under 11% RH at 20 °C; and the B0-form, under 0% RH at 20 °C. Relative humidity was controlled using silica gel (0% RH) and saturated aqueous solutions of CaBr_2 (16% RH) and $\text{LiCl} \cdot \text{H}_2\text{O}$ (11% RH).¹³⁾

Analytical Methods. Morphological Observation Optical microscopic observation were performed on a model AMX180 polarizing microscope with a rotating stage (Nikon Co., Ltd.). The B2-form single crystal was placed on a glass slide, and the slide was stored under 16% RH at 25 °C. The size of the single crystal was $1.5 \times 0.2 \times 0.1 \text{ mm}^3$.

Powder X-Ray Diffraction under Various RH Conditions Each sample was packed in a sample holder and the diffraction pattern was recorded on a model Rint 2200 diffractometer (Rigaku Denki Co., Ltd.) equipped with a Rigaku humidity controller. The diffraction data in the 2θ range of 3–40° were measured using a scintillation counter with graphite-monochromated $\text{CuK}\alpha$ radiation (40 kV and 40 mA) with a scan step of 0.02° and scan rate of $4^\circ/\text{min}$.

X-Ray Structure Determination of the Crystal Forms The X-ray diffraction measurements of the B2-, B1-, and B0-forms were carried out at -50°C on a Rigaku R-Axis RAPID system equipped with an imaging-plate scanner using graphite-monochromated $\text{MoK}\alpha$ radiation. Unit cell parameters were obtained from least-squares refinement of the setting angles of reflections based on the data collected.

The X-ray crystal data of the B2'-form were collected at -100°C in the ω - 2θ scan mode with a four-circle X-ray diffractometer (Rigaku AFC-7R) using graphite-monochromated $\text{CuK}\alpha$ radiation. Unit cell parameters were refined with 20 reflections. The intensities were corrected for Lorentz and polarization effects.

The structures were solved by direct methods using program SIR97,¹⁴⁾ and full-matrix least-squares refinement on F^2 was completed using the program SHELXL-97.¹⁵⁾ Nonhydrogen atoms were refined anisotropically. In the B2'-form, the oxygen atoms of water molecules were refined isotropically. In the B1-form, minor atoms in the disordered pivaloyloxymethyl group were also refined isotropically. As many hydrogen atoms as possible were located in the difference Fourier map. The positions of the remaining

hydrogen atoms were assigned geometrically. All hydrogen atoms were refined isotropically. The experimental and crystallographic data are summarized in Table 1.

For the B1- and B0-forms, the intensity data were collected after many trials. However, the crystallinity was not satisfactory considering from the diffraction pattern. The diffraction spots were expanded and split. Although the integration area of each spot was expanded to include the whole spot, the R -merge factor became 15.1% and 15.3% for the B1- and B0-forms, respectively. This caused the considerably high R values for the two crystal forms.

Results

Transformation Conditions from the B2-Form to Various Hydrate Forms To prepare single crystals of various hydrates, the transformation of crystalline powder of the B2-form was investigated using humidity-controlled powder X-ray diffractometry. The change in powder X-ray diffraction pattern is shown in Fig. 4. The B2-form was transformed to the B2'-form under 20% RH at 25 °C for 10 h and to the B1-form under 10% RH at 25 °C for 2 h. The powder X-ray diffraction patterns of these samples were identical with those of the B2'- and B1-forms, respectively. However, the B1-form could not be further transformed into the B1'-form under 6% RH at 25 °C for 2 months.

The B2'-form produced instantly reverted to the B2-form under RH higher than 22% at 25 °C. The B1-form was transformed into the B2-form when the RH increased to more than 80% at 25 °C. The anhydrate crystal (A-form), however, was not transformed into any of the hydrates under RH of 40% to 80% for 1 month.

Morphological Change of the B2-Form Crystal Optical microscopy was used to observe the crystal transformation caused by the loss of water molecules from the B2-form. As shown in Fig. 5, the B2-form crystal became opaque, indicating dehydration of the B2-form. The opaque regions expanded from the (001) and the (00 $\bar{1}$) faces, which are both ends of the needle, and then spread along the c -axis in both directions. Such an optical change should be closely related to the crystal structure.

Crystal Structures of the B2- and B2'-Forms The crystal structure of the B2-form at 23 °C was determined previously.¹⁶⁾ To compare the crystal structure of the B2-form

Fig. 4. Change in Powder X-Ray Diffraction Patterns of the B2-Form at Various RH Values at 25 °C: (a) 30% RH, after 2 h; (b) 20% RH, after 10 h; (c) 10% RH, after 2 h; (d) 6% RH, after 2 Months

The B2-form was transformed to the B2'-form under 20% RH at 25 °C for 10 h. After storage under 10% RH at 25 °C for 2 h, the B2-form was converted into the B1-form, which could not be further transformed under 6% RH at 25 °C for 2 months. The hydrate crystal forms were assigned to characteristic diffraction peaks, of which the values were: B2-form: 6.8, 18.5, and 23.2°; B2'-form: 7.2, 18.7, 21.7, 22.5, and 23.7°; B1-form: 7.5, 20.7, 22.7, and 23.4°; B1'-form: 7.8, 18.8, 20.4, and 23.4°; and B0-form: 8.0, 19.5, 21.4, and 23.8°.

Fig. 5. Morphological Change of the B2-Form in Dehydration: (a) 0 h; (b) 3 h; (c) 12 h; and (d) 2 d

A single crystal of the B2-form was stored under approximately 16% RH at 25 °C. The long axis of the needle crystal was parallel to the *c*-axis from the X-ray structure analysis of the B2-form.

with those of other hydrates, the structure of the B2-form was determined at $-50\text{ }^{\circ}\text{C}$ using X-ray structure analysis. The crystal data and the experimental details are summarized in Table 1. The molecular and crystal structures at $-50\text{ }^{\circ}\text{C}$ are shown in Figs. 6a and 7a, respectively. A hydrogen bonding chain of water molecules is formed along the two-fold screw axis parallel to the *c*-axis, and the CS-834 molecules are hydrogen-bonded to the chain. The unit-cell dimensions of B2'-form changed significantly compared with those of the B2-form; the lengths of the *b*- and *c*-axes length became 4.6% shorter and 3.9% longer than the corresponding ones of the B2-form, respectively. However, the *a*-axis changed little

(only 1.0% shorter) after the transformation to the B2'-form. The characteristic powder diffraction peak at 7.2° corresponds to the (020) reflection. The molecular structure with atom numbering and the crystal structure are shown in Figs. 6b and 7b, respectively. There are three types of water molecule per CS-834 molecule. The final occupancy factors of the disordered ones, O31, O32, and O33, were fixed at 0.22, 0.22, and 0.78, respectively. The numbers of water molecules per CS-834 molecule in the B2'-form were 1.22 from occupancy parameters. The B2'-form had two types of hydrogen bond scheme because the distances of the O31–O33 atoms and the O32–O33 atoms were very short. The O31 and O32

Table 1. Experimental and Crystallographic Data

Parameter	B2-form	B2'-form	B1-form	B0-form
Chemical form	Dihydrate	Intermediate form	Monohydrate	Dehydrate
Formula	$C_{20}H_{32}O_9N_2S_1 \cdot 2H_2O$	$C_{20}H_{28}O_7N_2S_1 \cdot nH_2O$	$C_{20}H_{30}O_8N_2S_1 \cdot 1H_2O$	$C_{20}H_{28}O_7N_2S_1$
Formula weight	476.56	—	458.53	440.51
Radiation used (Å)	MoK α , 0.71069	CuK α , 1.5418	MoK α , 0.71069	MoK α , 0.71069
Temperature (°C)	−50	−100	−50	−50
Size (mm ³)	0.25×0.65×0.05	0.30×0.60×0.10	0.20×0.50×0.05	0.15×0.30×0.03
Crystal form, color	Needle, colorless	Needle, white	Needle, white	Needle, white
Crystal system	Orthorhombic	Orthorhombic	Orthorhombic	Orthorhombic
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell constant				
<i>a</i> (Å)	15.4185 (7)	15.23 (1)	14.815 (2)	14.792 (4)
<i>b</i> (Å)	25.660 (1)	24.45 (1)	23.358 (3)	22.195 (6)
<i>c</i> (Å)	6.0412 (2)	6.18 (1)	6.9825 (6)	6.685 (1)
<i>V</i> (Å ³)	2390.1 (2)	2301 (5)	2416.3 (4)	2194.7 (9)
<i>Z</i>	4	4	4	4
Calcd. density (g/cm ³)	1.324	—	1.260	1.333
μ (mm ^{−1})	0.186	0.172	0.179	0.191
Absorption correction	Multi-scan	None	Multi-scan	Multi-scan
<i>F</i> (000)	1016	1016	976	936
<i>R</i> _{int} (%)	4.14	17.22	16.17	17.25
θ Range for data collection (°)	1.59—27.48	3.42—64.84	1.74—27.47	1.65—27.48
Index range: min/max	−18/19, −33/33, −7/7	0/17, 0/28, −6/0	−19/19, −30/30, −9/8	−19/19, −27/28, −8/7
Refinement method	Full-matrix/ <i>F</i> ²	Full-matrix/ <i>F</i> ²	Full-matrix/ <i>F</i> ²	Full-matrix/ <i>F</i> ²
Restraints	2	3	3	0
No. of reflections	5350	2068	4697	4436
No. of reflections used (<i>I</i> ₀ > 2σ(<i>I</i> ₀))	4993	1153	2070	2265
Parameters	386	290	306	272
<i>R</i> 1 (%)	3.3	10.4	15.4	15.2
<i>wR</i> 2 (%)	7.5	28.7	37.7	36.8
Goodness of fit	1.052	1.057	1.015	1.012
Weight scheme	$w = 1/[\sigma^2(F_o^2) + (0.0388P)^2 + 0.4138P]$ where $P = (F_o^2 + 2F_c^2)/3$	$w = 1/[\sigma^2(F_o^2) + (0.1761P)^2 + 7.4575P]$ where $P = (F_o^2 + 2F_c^2)/3$	$w = 1/[\sigma^2(F_o^2) + (0.1870P)^2 + 7.8185P]$ where $P = (F_o^2 + 2F_c^2)/3$	$w = 1/[\sigma^2(F_o^2) + (0.1808P)^2 + 6.8111P]$ where $P = (F_o^2 + 2F_c^2)/3$
$\Delta\rho_{\max}$, $\Delta\rho_{\min}$ (e Å ^{−3})	0.16, −0.19	0.59, −0.36	0.40, −0.47	0.55, −0.43
Extinction method	SHELXL	None	None	None
Extinction coefficient	0.0054 (8)	—	—	—
Diffractometer	R-AXIS RAPID	AFC-7R	R-AXIS RAPID	R-AXIS RAPID

Fig. 6. Molecular Structures of CS-834 in Hydrates: (a) B2-Form; (b) B2'-Form; (c) B1-Form; and (d) B0-Form

Displacement ellipsoids are given at the 50% probability level. In the B2'-form, the O31–O33 of water molecules were disordered and thus refined isotropically. The occupancy factor of each was: O31, 0.22; O32, 0.22; and O33, 0.78. The B1-form was disordered with respect to the pivaloyloxymethyl moiety except for the C24 atom. The atoms in the major part corresponded to the O21–C27 atoms. The O21B–C27B atoms corresponded to the minor part. The occupancy factor of the major part was 0.69.

Fig. 7. Crystal Structures of the Hydrates Viewed along the c -Axis: (a) B2-Form; (b) B2'-form; (c) B1-Form; and (d) B0-Form

The dashed lines represent hydrogen bond interactions. In the B1-form, the crystal structure model of the major part is shown.

water molecules formed the same hydrogen bond scheme as the B2-form. It is obscure why such a hydrate form is stable and the reproducibility is maintained.

Crystal Structures of B1- and B0-Forms The unit-cell dimensions of the B1-form changed significantly from those of the B2-form; the lengths of the a - and b -axes were 3.9% and 9.0% shorter than those of the B2-form, respectively, while the c -axis was 13.5% longer. This means that the 2θ value of the characteristic diffraction peak due to the (020) reflection becomes 7.6° . The molecular and the crystal structures are shown in Figs. 6c and 7c, respectively. The pivaloyloxymethyl group except for the C24 atom is disordered with the occupancy factors of 0.69 and 0.31 for the major and minor components, respectively. The hydrogen bonding forms a chain through the pyrrolidine amide moiety along

the two-fold screw axis parallel to the c -axis.

The cell dimensions of the B0-form are similar to those of the B1-form; the lengths of the a - and b -axes are 3.3% and 13.0% shorter than those of the B2-form, respectively, while the c -axis is longer by 11.1%. The 2θ value of the characteristic diffraction peak due to the (020) reflection becomes 8.0° . The molecular structure and the crystal structure are shown in Figs. 6d and 7d, respectively. There is no peak corresponding to the solvent water molecule around the amide carbonyl group and the hydroxyl group in the difference Fourier map. There are two hydrogen bonds between the molecules.

Molecular Structure of CS-834 in Each Crystal Form

The crystal structure of the A-form at 23°C was reported previously.¹⁷⁾ The molecular and crystal structures are shown

in Fig. 8. The molecular structures in various crystal forms were compared with each other, including the A-form. The conformation of the C3–C17 bond of the A-form is different from those of the hydrates and B0-forms. The torsion angle of N4–C3–C17–O19 of the A-form is $2.9(2)^\circ$, while those of the B2-, B2'-, B1-, and B0-forms are $-159.7(1)^\circ$, $-161.6(6)^\circ$, $-174(1)^\circ$, and $-173.5(9)^\circ$, respectively. Such difference in the torsion angle is the reason why the A-form

Fig. 8. Molecular Structures of CS-834 in the Anhydrate (A-Form)¹⁷⁾

was not transformed into the B2-form even when stored under high RH for more than 1 month. The B1-form has two disordered conformations. The conformer with the major occupancy factor of 0.69 has a similar structure to that of the B2- or B2'-form, while the minor conformer is similar to that of the B0-form. The major conformer has a torsion angle of C22–O21–C20–O19 of $79(2)^\circ$, which is in accordance with the corresponding ones of the B2- and B2'-forms [$78.4(2)^\circ$ and $79.8(8)^\circ$, respectively]. The same torsion angle of the minor conformer is $-77(3)^\circ$, which is in agreement with that of the B0-form of $-78(1)^\circ$. Thus the change in molecular conformation was also caused by dehydration after the disordered water molecule O31 and O32 completely leave the crystal. The cooperative motion of dehydration and conformational change probably makes it possible to maintain the single crystal form from the B2-form to the B0-form.

Discussion

Dehydration Process of the Dihydrate to the Dehydrate

There is a characteristic hydrogen bond network in all crystal structures between the columnar arrangement of CS-834 and the water molecules. For example, the B2-form has an infinite hydrogen bond network composed of the two water molecules O31 and O32 along the two-fold screw axis parallel to the *c*-axis. Figure 9 is a schematic drawing of the molecules along the two-fold screw axis. The N11–H and carbonyl oxygen, O16, of the amide group and the O30 of the hydroxy group in the CS-834 molecule form hydrogen bonds with the

Fig. 9. Crystal Packing of the B2-Form along Two-Screw Axis

The functional groups with hydrogen bond interactions alone are represented to give a clear view in this figure.

water network.

When the B2-form is transformed to the B2'-form, the minor component of O31 and O32 occupies a similar position to that observed in the B2-form and forms the same hydrogen bond network as that in the B2-form. On the other hand, the major component of O33 forms a hydrogen bond with N11. Moreover, it directly forms a hydrogen bond with O16 of one neighboring molecule and with O8 and O30 of the other neighboring molecule.

When the B2'-form is transformed to the B1-form, the disordered water molecules, O31 and O32, are completely lost. The columnar structure of CS-834 molecules move to form a hydrogen bond for an interaction between the N11-H group and the O16 atom of the neighboring molecule. A hydrogen bond network is also formed along the two-fold screw axis along the *c*-axis. The O16 atom maintains the hydrogen bond with the water molecule O34 which has hydrogen bonds with O30 and O8.

When the B1-form is transformed to the B0-form, the water molecule O34 is completely lost. The O30 atom directly forms a hydrogen bond with O16. The hydrogen bond network along the two-fold screw axis is thus conserved. It was impossible to collect the intensity data of the B1'-form, and it is difficult to assume the crystal structure between the B1- and B0-forms. The B1'-form may be a disordered structure composed of the B1- and B0-forms, assuming that the B1'-form is an intermediate structure as observed in the B2'-form. The reason why such intermediate structures as the B2'- and B1'-forms are stable remains unresolved.

The CS-834 hydrates form a columnar structure along the *c*-axis. Since the B2-form crystal gradually became opaque along the *c*-axis from both edges of the crystal during the transformation to the B2'-form, the solvent water molecules may exit the crystal along the *c*-axis. The water molecules were located in the hydrophilic area composed of the amide and hydroxyl groups. Even if the water molecules were gradually released, the CS-834 and water molecules can form hydrogen bonds to develop a columnar structure along the *c*-axis, changing the partner of the hydrogen bonds. Since a similar hydrogen bond network along the two-fold screw axis is maintained in the dehydration process from the B2- to B0-form, the crystalline lattice is considered to be conserved. It is clear that the conformational change in CS-834 plays an important role in maintaining the single crystal form.

Conclusions

The dehydration process of the CS-834 dihydrate crystal was examined using the X-ray powder diffraction method. Changing the RH or temperature, the dihydrate crystal B2-form was transformed to the dehydrate B0-form through the B2'-, B1-, and B1'-forms, and the crystalline lattice was almost completely conserved. The B0-form returned to the B2-form under more than 80% RH. Each structure was determined by single-crystal X-ray analysis at -100°C or -50°C except for the B1'-form. It was impossible to obtain the B1'-

form suitable for X-ray analysis. The four structures of the B2-, B2'-, B1-, and B0-forms are isomorphous, forming a columnar hydrogen bond network along the two-fold screw axis parallel to the *c*-axis, although the number of the solvent water molecules are different. Therefore these four structures are called pseudo-polymorphs. The anhydrate A-form has a different conformation from that of the B0-form. This explains why the A-form cannot be transformed to the B2-form even if maintained under very high RH.

The solvent water molecules occupy the hydrophilic area composed of the amide and hydroxyl groups. Even if the water molecules were gradually released, the CS-834 and water molecules can form hydrogen bonds to develop a columnar structure along the screw axis, changing the partner of the hydrogen bonds. The slight conformational change in the CS-834 molecule plays an important role in the recombination of the hydrogen bonds. The various hydration structures and the conformational change in the component molecule make it possible to retain the single crystal form and undergo a reversible process between the dehydration and hydration processes.

Acknowledgments The authors are grateful to Vice Professor Dr. Hidehiro Uekusa and Dr. Akiko Sekine of the Tokyo Institute of Technology for their constructive discussions and to Dr. Shuichi Ito and Mr. Youji Furukawa of Sankyo Co., Ltd. for technical advice.

References

- 1) Vipagunta S. R., Brittain H. G., Grant D. J. W., *Adv. Drug Del. Rev.*, **48**, 3–26 (2001).
- 2) Kitamura S., Chang L., Guillory J. K., *Int. J. Pharmaceut.*, **101**, 127–144 (1994).
- 3) Wang S. L., Lin S. Y., Chen T. F., *Chem. Pharm. Bull.*, **48**, 1890–1893 (2000).
- 4) Sheng J., Venkatesh G. M., Duddu S. P., Grant D. J. W., *J. Pharm. Sci.*, **88**, 1021–1020 (1999).
- 5) Leung S. S., Padden B. E., Munson E. J., Grant D. J. W., *J. Pharm. Sci.*, **87**, 508–513 (1998).
- 6) Kitaoka H., Wada C., Moroi R., Hakuui H., *Chem. Pharm. Bull.*, **43**, 649–653 (1995).
- 7) Shefter E., Higuchi T., *J. Pharm. Sci.*, **52**, 781–791 (1963).
- 8) Khankari R. K., Grant D. J. W., *Thermchim. Acta*, **248**, 61–79 (1995).
- 9) Miyauchi M., Endo R., Hisaoka M., Yasuda H., Kawamoto I., *J. Antibiot.*, **50**, 429–439 (1997).
- 10) Kobayashi K., Fukuhara H., Kawamoto I., Hata T., Sekine A., Uekusa H., Ohashi Y., *Chem. Pharm. Bull.*, **50**, 1570–1573 (2002).
- 11) Sugawara Y., Kamiya N., Iwasaki H., Ito T., Satoh Y., *J. Am. Chem. Soc.*, **113**, 5440–5445 (1991).
- 12) Sugawara Y., Nakamura A., Iimura Y., Kobayashi K., Urabe H., *J. Phys. Chem. B*, **106**, 10363–10368 (2002).
- 13) Stokes R. H., *Ind. Eng. Chem.*, **41**, 2013 (1949).
- 14) Altomare A., Burla M. C., Camalli M., Cascarano G. L., Giacovazzo C., Guagliardi A., Moliterni A. G. G., Polidori G., Spagna R., *J. Appl. Crystallogr.*, **32**, 115–119 (1999).
- 15) Sheldrick G. M., SHELXL-97, University of Goettingen, Germany, 1997.
- 16) Kobayashi K., Fukuhara H., Kawamoto I., Ito S., Hata T., *Anal. Sci.*, **17**, 357–358 (2001).
- 17) Kobayashi K., Fukuhara H., Kawamoto I., Furukawa Y., Hata T., *Anal. Sci.*, **16**, 555–556 (2000).