Asymmetric Transformation. III.[1] Crystal Properties and Structures of a 1,4-Benzodiazepinooxazole Derivative

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Three forms of crystals, the optically active α-form and the optically inactive β- and γ-forms, of 10-bromo-11*b*-(2-fluorophenyl)-2,3,7,11*b*-tetrahydrooxazolo[3,2-*d*][1,4]benzodiazepin-6(5*H*)-one (1) were obtained by different crystallization procedures. The α-form crystal was estimated to be the most stable and the least soluble crystal among them by comparison of the thermodynamic properties of these crystals and the mutual transformations among these crystal forms. X-Ray analyses elucidated that the α-form crystal was an enantiomer, and the β- and γ-form crystals were racemates. Consequently, if a suitable crystallizing condition is selected, it is possible for compound 1 to crystallize as more stable and less soluble crystals of one enantiomer (prior to crystallization of racemates) from methanol solution. These crystal properties of 1 are essential for the second-order asymmetric transformation to occur.

Keywords 1,4-benzodiazepinooxazole; optically active crystal; preferential crystallization; asymmetric transformation; second-order asymmetric transformation; enantiomer; racemate; X-ray crystallography

In the previous communication,[2] we reported the phenomenon that optically active crystals of 10-bromo-11*b*-(2-fluorophenyl)-2,3,7,11*b*-tetrahydrooxazolo[3,2-*d*]-[1,4]benzodiazepin-6(5*H*)-one (**1**) were crystallized from the reaction mixture under achiral conditions, and formation of the optically active crystals was ascribed to preferential crystallization accompanying asymmetric transformation. This crystallization-induced asymmetric transformation is also called the second-order asymmetric transformation,[3] and is usually observed in the case of readily interconvertible diastereomers. Few examples of this phenomenon have been reported in the case of enantiomers.[4] Thus, this rare phenomenon, second-order asymmetric transformation in enantiomers, was studied in more detail. In the previous paper,[1] we reported kinetic studies on the racemization of **1** in methanol, showing that the two enantiomers of **1** were interconverted to each other sufficiently quickly for second-order asymmetric transformation to occur. The process of formation of the optically active crystals of **1** in methanol is illustrated in Chart 1.

For a full understanding of the ability of compound **1** to crystallize preferentially as optically active crystals of one of the two enantiomers, the present study deals with the modes of crystallization of **1** and the physicochemical properties of the three forms of crystals obtained. The thermodynamic stability and solubility in methanol of these crystalline forms were compared and are discussed in relation to the results of crystallization in various solvents, differential scanning calorimetry and X-ray analyses.

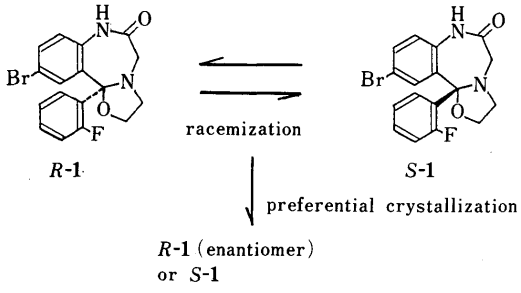

Chart 1. Preferential Crystallization of **1** Accompanying Racemization

Results and Discussion

Formation of the Three Crystalline Forms of 1 When optically active crystals of **1** synthesized according to the procedure described in the previous communication[2] were recrystallized from 20 volumes of methanol by rapid cooling in an ice bath, needle crystals (mp 180—185 °C) were obtained in about 70% yield. These crystals did not show optical rotation in dioxane and had a different infrared (IR) spectrum from that of the optically active crystals. We named the optically active prismatic crystals as α-form (an enantiomer of **1**) and the optically inactive needle crystals as β-form, which is a racemic modification.

The rate of the racemization of **1** was rather slow in aprotic solvents as described in the previous paper.[1] Accordingly, crystallization of **1** was also tested in such aprotic solvents as ethyl acetate and toluene. The optically active α-form of **1** usually gave the original α-form crystals, as expected, but the optically inactive crystals of β-form gave a new crystalline form of prisms, when crystallization was performed in toluene at temperatures between 20 and 50 °C. These new crystals did not show optical rotation in dioxane and had a different IR spectrum from β-form; they are called γ-form, which seems to be a polymorphic form of β-form. When β-form crystals were recrystallized from ethyl acetate or more favorably from a mixture of ethyl acetate and toluene around 45 °C, almost optically inactive crystals were obtained, but they had the same IR spectrum as the α-form crystals; hereafter we call them optically inactive α-form crystals. On the other hand, when the crystallization proceeded quickly at a low temperature or from a concentrated solution, the β-form crystals were always obtained from all the solvents tested; therefore, the formation of β-form crystals might be kinetically controlled.

These results show that preferential formation of the optically active α-form crystal in methanol depends, to a certain extent, on the relative velocities of crystallization and racemization, and if a much slow crystallization rate (compared with the rate of racemization) is selected at a definite temperature, it is possible to obtain crystals of one enantiomer which have high optical purity. The optically

TABLE I. Crystallization of **1** from 20 Volumes of Methanol

Used crystal $[\alpha]_D^{20}$ (°)		Seed crystal $[\alpha]_D^{20}$ (°)		Temp. (°C)	Precipitated crystal $[\alpha]_D^{20}$ (°)		Recovery (%)
α-Form	−208	—		40	α-Form	−269	70
α-Form	−208	α-Form	−320	40	α-Form	−309	72
α-Form	−208	α-Form	+321	40	α-Form	+206	78
α-Form	−208	α-Form	0	40	α-Form	+35	66
α-Form	+307	—		0	β-Form	0	74
β-Form	0	—		40	α-Form	+242	55
γ-Form	0	—		40	α-Form	−177	58
γ-Form	0	—		40	α-Form	+227	49
γ-Form	0	—		40	α-Form	+81	63

TABLE II. Crystallization of **1** from AcOEt, Toluene and iso-ProOH

Used crystal $[\alpha]_D^{20}$ (°)		Solvent (Times)	Temp. (°C)	Precipitated crystal $[\alpha]_D^{20}$ (°)		Recovery (%)
α-Form	+320	AcOEt (×20)	25	α-Form	+326	54
β-Form	0	AcOEt (×6)/ toluene (×4)	45	α-Form	0	—
α-Form	0	AcOEt (×20)	45	α-Form	+18	—
α-Form	0	AcOEt (×10)	25	β-Form	—	—
α-Form	0	AcOEt (×10)	0	β-Form	0	76
α-Form	0	Toluene (×20)	50	γ-Form	0	49
α-Form	0	Toluene (×20)	0	β-Form	—	89
β-Form	0	Toluene (×10)	60	γ-Form	—	—
α-Form	0	iso-ProOH (×15)	60	α-Form	−20	72
α-Form	0	iso-ProOH (×15)	40	α-Form	−3	77
α-Form	0	iso-ProOH (×20)	25	γ-Form	—	—

active crystals were carefully recrystallized from methanol at 40 °C, in the usual manner to obtain large prisms. The optical rotation of a single crystal thus obtained was measured in dioxane to give the largest specific rotation, $[\alpha]_D^{20}$ +344°. The modes of crystallization in methanol are listed in Table I and the results of serial crystallizations in ethyl acetate, toluene and isopropanol are shown in Table II.

Characterization of α-, β- and γ-Form Crystals The IR spectra and the X-ray diffraction patterns of the three kinds of crystalline forms of **1** are shown in Figs. 1 and 2, respectively. These observed IR spectra and X-ray diffraction patterns show crystallographic differences which are sufficient to characterize these racemic modifications. The results that the IR spectra and the X-ray diffraction patterns of the optically active ($[\alpha]_D^{20}$ +309° (dioxane)) and of the inactive α-form crystals were identical support the view that the optically inactive α-form is a conglomerate, while on the other hand, both the β- and the γ-form crystals were suggested to be racemates.

Thermal behavior of the crystals was investigated by differential scanning calorimetry (DSC) measurements. Temperatures and enthalpy changes of fusion of each crystalline form are listed in Table III.

In the DSC, each crystalline form showed only one endothermic peak corresponding to melting at 188—191 °C for α-, at 184—187 °C for β- and at 180—185 °C for γ-form. The binary phase diagram between one of the enantiomers and a conglomerate did not show a typical curve as illustrated in the conglomerate, and mixed samples of β- or γ-form crystals with an enantiomer of α-form also did not show a typical melting point phase diagram in the racemate.[3] The reason might be that the melting points of the

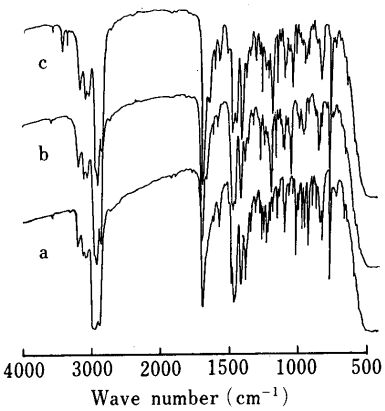

Fig. 1. IR Spectra of the Three Forms of Compound **1** in Nujol
a, form α; b, form β; c, form γ.

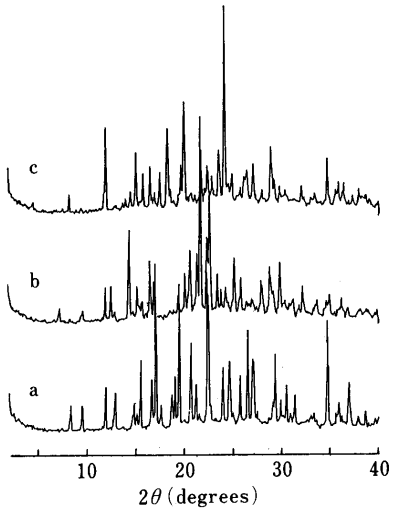

Fig. 2. X-Ray Powder Diffraction Patterns of the Three Forms of Compound **1**
a, form α; b, form β; c, form γ.

TABLE III. Thermodynamic Values for the Three Forms by DSC Measurements

Form	Temperature of fusion (°C)	Enthalpy of fusion (kcal/mol)
α	188.0—191.0	7.80±0.10
β	184.0—187.0	7.13±0.19
γ	180.5—185.0	7.22±0.19

three crystalline forms are very close to each other. Though a mutual structural transformation between the three forms was not observed when each sample was heated in DSC, the α-form might be more thermodynamically stable than the β- and γ-forms if the thermodynamic stability of the crystal is proportional to the melting point.

Solubilities of α-, β- and γ-Form Crystals When β-form crystals were stirred in methanol suspension for several hours at room temperature to measure the solubility, the transformation to the optically active α-form crystals was always observed. Similar transformations from γ- to α-form were also observed in methanol at elevated temperatures (40 and 60 °C). The α-form crystals thus obtained occasionally had rather high degrees of optical rotation. Representative results are collected in Table IV. These

TABLE IV. Transformation between Crystalline Forms in Solvent

Used crystal	Slurry solvent (Times)	Time (h)	Temp. (°C)	Transformed crystal	$[\alpha]_D^{20}$ (°)	Recovery (%)
γ-Form	MeOH (×8)	6	60	α-Form	+315	87
γ-Form	MeOH (×8)	8	40	α-Form	+321	85
β-Form	MeOH (×8)	6	25	α-Form	−176	88
β-Form	MeOH (×20)	7	25	α-Form	+254	70
β-Form	MeOH (×20)	6	25	α-Form	+10	68

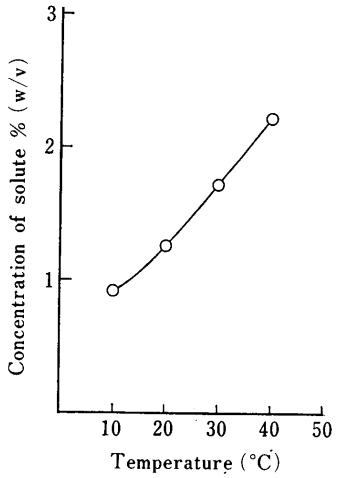

Fig. 3. Saturated Solubility Curve of α-Form Crystals

transformations between the three crystalline forms made the measurement of solubilities difficult; therefore, the solubility of crystals was estimated according to the simplified form (1) of the Schröder–Van Laar equation,[3] where $\Delta_f H$ (kcal/mol) is the enthalpy change of fusion, T_f is the melting point (K) and $[X_A]_{SAT}$ is the saturated solubility expressed as mol fraction at T (K).

$$\ln[X_A]_{SAT} = \frac{-\Delta_f H}{R}\left(\frac{1}{T} - \frac{1}{T_f}\right) \quad (1)$$

This equation suggests that a crystal which has a larger enthalpy of fusion is less soluble than the other when the two have similar melting points. Therefore, α-form crystal seems to be less soluble than β- and γ-form crystals in methanol. The crystal transformations from β- and γ-forms to α-form in methanol suspension are regarded as phenomena due to the differences in solubility and in thermodynamic stability between the α-form and the others. In the case of the α-form crystals, the saturated solubility could be measured and the saturated solubility curve is shown in Fig. 3.

The Molecular Structures of the Three Crystalline Forms In order to clarify the structural details and symmetries of the three crystals, X-ray analyses were carried out. The crystallographic data for the three forms are given in the experimental section. The molecular structures of the three forms projected on each bromophenyl plane are illustrated in Fig. 4, along with the atomic labels used. As the figure shows, the α-form has a different molecular structure from those of the others, which have similar structures to each other. The major conformational difference between α-form and the others is due to the difference in orientation of the *o*-fluorophenyl ring attached to C(14), that is, the F(15) atom intermolecularly interacts with N(4) in the α-form, and with O(1) in the β- and γ-forms. The interatomic

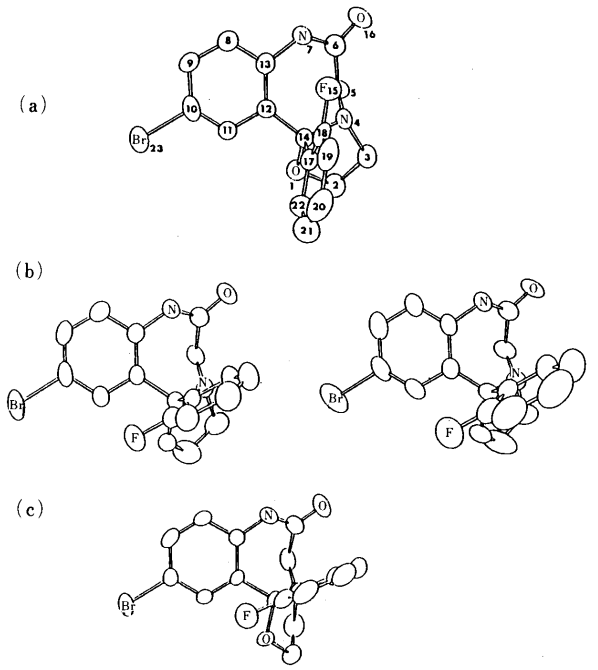

Fig. 4. The Molecular Structures Projected on the Bromophenyl Plane for (a) α, (b) β (Left: β-A and Right: β-B) and (c) γ

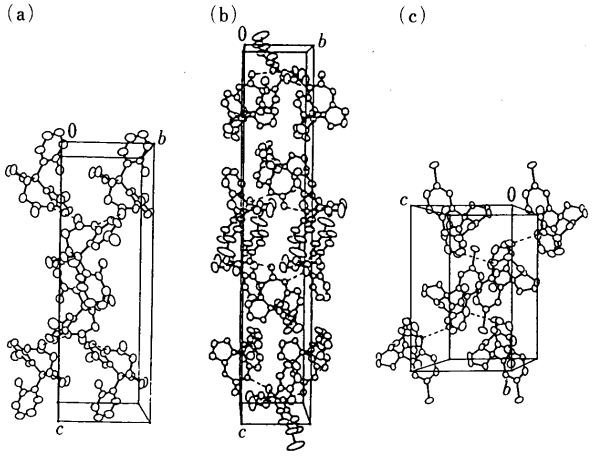

Fig. 5. The Molecular Packings for (a) α, (b) β, and (c) γ
The hydrogen bonds are indicated by dotted lines.

distances between fluorine and these hetero atoms are similar, (F(15)---N(4) = 2.959(10) Å in α, F(15)---O(1) = 2.771(7) Å in β-A, F(15′)---O(1′) = 2.776(8) Å in β-B, F(15)---O(1) = 2.832(7) Å in γ), and close to the sum of van der Waals radii of fluorine and the hetero atoms (F---N = 2.85 Å, F---O = 2.75 Å). The fused 5- and 7-membered rings of these molecules have normal envelope and boat-form conformations, though some characteristic deviations of atoms from the plane are recognized for each molecule.

The Crystal Structures of the Three Crystalline Forms The molecular packings in the three crystalline forms are shown in Fig. 5. As each molecule in a crystal lattice is arranged according to the space group characterizing the crystal, the space group also defines the symmetry of the crystal. The space group $P2_12_12_1$ for α-form is non-centrosymmetric; this means that a single crystal of α-form consists of only one of the enantiomers which has *R*-configuration or *S*-configuration at the asymmetric center

at C(14). On the other hand, both β- and γ-forms have the same space group $P2_1/c$ which has a center of symmetry, and consequently, each single crystal of them consists of equal numbers of *R*- and *S*-configurational molecules. Moreover, the β-form crystal was found to contain two crystallographically independent molecules (named β-A and β-B) in an asymmetrical unit.

Each crystal forms intermolecular hydrogen bonding between N(7)H and O(16), and the distances are as follows: 2.892(12) Å [N(7)---O(16) $(1-x,\ 1/2+y,\ 1/2-z)$] for α-form, 2.816(9) Å [N(7)---O(16′) $(-1+x,\ -1+y,\ z)$] and 2.844(8) Å [N(7′)---O(16) $(-1+x,\ y,\ z)$] for β-form and 3.230(10) Å [N(7)---O(16) $(x,\ 1/2-y,\ 1/2+z)$, H(7)---O(16) = 2.35(9) Å, N(7)-H(7)---O(16) = 142(2)°] for γ-form. The hydrogen bondings in α- and β-forms have normal distances but the distance is a little longer than usual in the γ-form. In the β-form, an unusual short contact is observed between the oxygen atom of the oxazolidine ring and the bromine atom [O(1)---Br(23) $(1-x,\ 1/2+y,\ 1/2-z)$ = 3.201(5) Å], because the sum of van der Waals radii between these atoms is 3.35 Å.

These results suggest that α-form crystals may be slightly more stable than β- and γ-form crystals.

Conclusion

The results of the present studies on **1** by crystallization, DSC and X-ray analysis indicate that **1** is inclined to form the enantiomer or the conglomerate of α-form, which is more stable and less soluble (at least at the temperature of crystallization) than the racemate of β-form in methanol. These observations suggest that the crystallization process is governed by a second-order asymmetric transformation, that is, a slow crystallization might be able to crystallize only one of the enantiomers due to spontaneous nucleation and subsequent development of the nucleus to an optically active crystal prior to formation of a racemate, as the crystallization always proceeds in a solution equilibrated by racemization.

Experimental

Compound **1** was prepared by the previously reported method.[1,2]

Preparation of the Three Crystalline Forms 1) Optically Active α-Form Crystals: **1** (any form is suitable) is dissolved in 20 volumes of methanol with respect to **1** (v/w) in a flask at 60 °C, then the solution is filtered through cotton to remove particles and the filtrate is placed in a vessel which has about 1.3 times the volume of the filtrate. The vessel is sealed tightly with a stopper and the solution is warmed to 60 °C again and kept at the same temperature for 10 min. Then the vessel is put in a thermostat adjusted at 40 °C for 2 to 3 d. Then the methanol solution is gradually cooled to room temperature and the separated crystals are collected by filtration, washed with cold methanol and dried in a vacuum.

2) Optically Inactive Crystals: (a) α-Form: The β-form crystals (or γ-form) are dissolved in 10 volumes of mixed solvent of ethyl acetate and toluene (3 : 2) at 80 °C and the solution is kept at 45—50 °C. The separated crystals are collected by filtration, washed with the same mixed solvent and dried in a vacuum.

(b) β-Form: **1** (any form is suitable) is dissolved in 20 volumes of methanol under reflux for 30 min and the solution is rapidly cooled to 0 °C by dipping the vessel in an ice bath. Long needles are precipitated immediately, collected by filtration, washed with cold methanol and dried in a vacuum.

(c) γ-Form: The β-form crystals (or optically inactive α-form) are dissolved in 40 volumes of toluene at 60 °C and the solution is kept at 25 °C to form prisms. The crystals are filtered, washed and dried in a vacuum. The same quantity of isopropanol is also suitable for obtaining γ-form crystals in the same way.

Characterization of the Crystalline Forms Each form of **1** was identified by IR spectroscopy and X-ray powder diffraction. The instrument used for IR spectroscopy was a JASCO IR-810 infrared spectrometer. X-Ray powder diffraction analysis was carried out with a Rigaku Denki Geigerflex RAD-r A diffractometer by Ni-filtered CuK_α radiation. Optical rotation was measured on a Perkin-Elmer 243 polarimeter.

Measurements of DSC Measurements were done using a Rigaku TCP 10A differential scanning calorimeter. Sample, α-, β-, and γ-form crystals; sample weight, 5.1 mg; sampling time, 3 s; sensitivity, 4 mcal/s; heating rate, 5 °C/min; heating range, 50—300 °C (under N_2 gas). The enthalpies of fusion were calculated by an usual program of thermal analysis. Data are the means of three experiments.

Measurements of the Saturated Solubility About 250 mg of **1** was added to 10 ml of methanol in a test tube and the test tube was dipped in a thermostated bath adjusted to 10 °C. The suspension was stirred for 20 h, then about 1.5 ml of the supernatant was pipetted off and filtered to prepare the first test solution. A second test solution was prepared 5 h later by the same procedure as before to confirm that the solution was saturated. About 500 mg of **1** was added to 10 ml of methanol for the 20 °C measurement, while about 1 g of **1** was used for 30 °C and 1.5 g of **1** for 40 °C. Two test solutions for each temperature were prepared in the same way as described for those at 10 °C. Exactly 1 ml of the test solutions was pipetted and diluted with methanol to give experimental solutions. The concentration of each solution was determined by measuring the ultraviolet (UV) absorbance at the wavelength of 247 nm in comparison with a standard solution. Saturated solubility was calculated from the averaged values.

X-Ray Crystallography The α, β and γ-form crystals used for the X-ray crystallography had dimensions of $0.5\times0.35\times0.15$ mm (α), $0.5\times0.15\times0.1$ mm (β), and $0.5\times0.4\times0.2$ mm (γ). Crystal data are as follows: α-form, $C_{17}H_{14}N_2O_2FBr$, $M_r=377.2$, orthorhombic, space group $P2_12_12_1$, $a=10.333(3)$, $b=7.209(2)$, $c=21.287(1)$ Å; $U=1585.7$ Å^3, $Z=4$, $D_c=1.58$ g·cm^{-3}, λ(MoK_α) = 0.71069 Å, $\mu=26$ cm^{-1}, $F(000)=760$, $T=297$ K. β-Form, $C_{17}H_{14}N_2O_2FBr$, $M_r=377.2$, monoclinic, space group $P2_1/c$, $a=11.616(3)$, $b=7.243(2)$, $c=38.652(9)$ Å; $\beta=91.10(6)$ Å, $U=3251.4$ Å^3, $Z=8$, $D_c=1.54$ g·cm^{-3}, λ(CuK_α) = 1.5418 Å, $\mu=36$ cm^{-1}, $F(000)=1520$, $T=297$ K. γ-Form, $C_{17}H_{14}N_2O_2FBr$, $M_r=377.2$, monoclinic, space group $P2_1/c$, $a=12.617(3)$, $b=14.100(4)$, $c=8.903(2)$ Å; $\beta=104.84(8)$ Å, $U=1531.0$ Å^3, $Z=4$, $D_c=1.64$ g·cm^{-1}, λ(MoK_α) = 0.71069 Å, $\mu=27$ cm^{-1}, $F(000)=760$, $T=297$ K. Intensity data were recorded on a Rigaku four circle automatic diffractometer with graphite monochromated MoK_α radiation ($2\theta<55°$) for α and γ, and CuK_α ($2\theta<130°$) for β. Of 2099, 5600 and 3655 independent reflections measured, only 1444, 4455 and 2361 were considered as observed on the basis of the criterion $F_o>2\sigma(F_o)$ for α, β and γ, respectively. All intensities were corrected for Lorentz and polarization factors but not for absorption. The structures were solved by a combination of the heavy-atom technique and direct methods using MULTAN,[5] and refined by block-diagonal least-squares methods. The positions of the hydrogen atoms were estimated using standard geometry. The final refinements with anisotropic temperature factors for the nonhydrogen atoms lowered the *R* values to 0.056 (weighted $R=0.064$, with the scheme $w=1/\sigma(F_o)$), 0.085 (weighted $R=0.079$, with the scheme $w=1/\sigma(F_o)$), and 0.087 (weighted $R=0.086$, with the scheme $w=1/\sigma(F_o)$) for α, β and γ, respectively.[6]

References and Notes

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6) The following data are available from one of the authors (S.S) upon request: tables of atomic parameters, bond lengths and angles, and observed and calculated structure factors.