

X-ray Diffraction Analysis of Nonequilibrium States in Crystals: Observation of an Unstable Conformer in Flash-Cooled Crystals

Jun Harada and Keiichiro Ogawa*

Contribution from the Department of Chemistry, Graduate School of Arts and Sciences, The University of Tokyo, Komaba, Meguro-ku, Tokyo 153-8902, Japan

Received August 29, 2003; E-mail: ogawa@ramie.c.u-tokyo.ac.jp

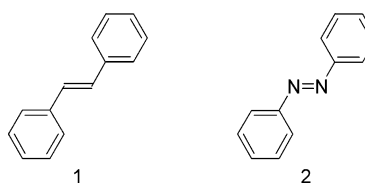
Abstract: Some molecules with a molecular skeleton similar to that of stilbenes and azobenzenes show orientational disorder in the crystals. If the disorder is dynamic, a conformational interconversion takes place through a pedal motion. In this study X-ray diffraction analyses of (*E*)-stilbene (**1**) and azobenzene (**2**) were carried out at various temperatures. We succeeded in observing thermodynamic nonequilibrium states that were generated by fast freezing of the conformational interconversion at low temperatures. The populations of the two conformers in crystals of **1** at 90 K varied with the cooling rate. Flash cooling of a crystal of **2** from room temperature to 90 K made it possible to observe the minor unstable conformer that does not exist in the equilibrium state at the same temperature.

Introduction

Solid-state conformational changes have been increasingly studied in a wide range of fields in chemistry.¹ Among several methods that have been applied to the subject, X-ray diffraction analysis is one of the most powerful. When conformational changes take place in crystals, each molecule adopts one of several possible, alternative conformations. When a dynamic equilibrium is established through conformational interconversions, the populations of the conformers vary with temperature according to the Boltzmann distribution law.² X-ray diffraction studies give access to populations by analysis of diffraction data with a disorder model, i.e., the refinement of the crystal structure as a superposition of conformers with different occupancy factors. Thus, variable-temperature X-ray diffraction measurement and analysis of the disorder give us not only static molecular structures but also substantial information on the dynamic aspects of molecules in the crystals. The disorder analysis has been applied to various types of reversible processes.³ Recent advances in instrumentation, such as area detectors, have made it possible to collect a series of accurate X-ray diffraction data at various temperatures within a reasonable experimental time.

In equilibrating systems, the Boltzmann distribution law tells us that the population of an energetically unfavorable conformer increases as the temperature is raised. Thus, even if the population of a minor conformer is below the limit of detection

at or below room temperature, at higher temperatures it could increase to a detectable level. The thermodynamic equilibrium state at high temperature can be frozen if the system is flash cooled and the unstable minor conformer detected in the resultant nonequilibrium state at a low temperature. In this study we succeeded in X-ray diffraction analyses of thermodynamic nonequilibrium states in flash-cooled crystals of (*E*)-stilbene (**1**) and azobenzene (**2**) and, thus, in observing an unstable conformer that cannot normally exist in the equilibrium state at low temperature.



We have studied the conformational changes of stilbenes and azobenzenes in crystals using disorder analysis.^{4,5} In the crystal structures of these compounds, an orientational disorder (a misorientation of the molecules in the crystal lattice) was often observed (Figure 1).^{6–8} At the disordered site, the molecules

- (1) (a) Gavezzotti, A.; Simonetta, M. *Chem. Rev.* **1982**, *82*, 1–13. (b) Gavezzotti, A.; Simonetta, M. In *Organic Solid State Chemistry. Studies in Organic Chemistry 32*; Desiraju, G. R., Ed.; Elsevier: Amsterdam, 1987; Chapter 11. (c) Harris, K. D. M.; Aliev, A. E. *Chem. Br.* **1995**, 132–136. (d) Bürgi, H. B. *Faraday Discuss.* **2002**, *122*, 41–63.
- (2) If intermolecular interactions in the crystals are large enough to influence the temperature dependence of the populations, other models such as a Bragg–Williams approximation may have to be considered. Bragg, W. L.; Williams, E. J. *Proc. R. Soc. London, Ser. A* **1934**, *145*, 699–730.

- (3) (a) Goldsmith, L. M.; Strouse, C. E. *J. Am. Chem. Soc.* **1977**, *99*, 7580–7589. (b) Sim, G. A. *J. Chem. Soc., Chem. Commun.* **1987**, 1118–1120. (c) Sim, G. A. *Acta Crystallogr., Sect. B* **1990**, *46*, 676–682. (d) Destro, R. *Chem. Phys. Lett.* **1991**, *181*, 232–236. (e) Braga, D.; Farrugia, L. J.; Grepioni, F.; Senior, A. *J. Chem. Soc., Chem. Commun.* **1995**, 1219–1220. (f) Wilson, C. C.; Shanklan, N.; Florence, A. *J. Chem. Soc., Faraday Trans.* **1996**, *92*, 5051–5057. (g) Ogawa, K.; Kasahara, Y.; Ohtani, Y.; Harada, J. *J. Am. Chem. Soc.* **1998**, *120*, 7107–7108. (h) Wilson, C.; Iversen, B. B.; Overgaard, J.; Larsen, F. K.; Wu, G.; Pali, S. P.; Timco, G. A.; Gerbeleu, N. V. *J. Am. Chem. Soc.* **2000**, *122*, 11370–11379. (i) David, W. I. F.; Ibberson, R. M.; Dennis, T. J. S.; Hare, J. P.; Prassides, K. *Europhys. Lett.* **1992**, *18*, 219–225.
- (4) Harada, J.; Ogawa, K.; Tomoda, S. *Acta Crystallogr., Sect. B* **1997**, *53*, 662–672.
- (5) Harada, J.; Ogawa, K. *J. Am. Chem. Soc.* **2001**, *123*, 10884–10888.

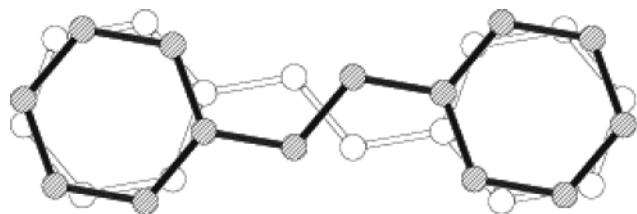


Figure 1. Orientational disorder for stilbenes and azobenzenes.

adopt two conformations related by an approximate 2-fold rotation about the longest axis of the molecules.

Recent studies have revealed that the disorder in stilbenes and azobenzenes is dynamic and is ascribed to a conformational interconversion in the crystals taking place through a pedal motion: a pair of benzene rings moving like the pedals of a bicycle (Figure 2).^{4,5,9–11} The pedal motion was also reported to be a key process of the photochromism of salicylidene-anilines¹² and the photodimerization of *trans*-cinnamides¹³ in crystals. In this study, we carried out X-ray diffraction analyses of crystals of **1** and **2** at various temperatures and observed nonequilibrium states produced by freezing the pedal motion at low temperatures.

Experimental Section

Sample Preparation. Compound **1** was purchased from Tokyo Kasei Co., and colorless crystals were obtained by recrystallization from methanol, mp 125.0–125.1 °C. Compound **2** was purchased from Tokyo Kasei Co., and red crystals were obtained by recrystallization from hexane, mp 68.0–68.4 °C. Melting points were determined on a micro-hot-stage apparatus and are uncorrected.

X-ray Diffraction Analysis. All of the diffraction measurements were carried out using a Bruker SMART 1000 CCD area detector system with graphite-monochromated Mo K α radiation ($\lambda = 0.71073$ Å) ($2\theta_{\max} = 55.0^\circ$). The temperature of the crystals was controlled using a Cryostream (Oxford Cryosystems) open-flow gas cryostat.¹⁴ The temperature in the nozzle was held constant within ± 0.2 K during the measurement. About 2500 frames of data were collected for each data set using a narrow-frame method with scan widths of 0.3° in ω and 10 s exposure times. The frames were integrated with Bruker SAINT V6.02A. Unit cell parameters were determined by least-squares refinement of the three-dimensional centroids of several thousand reflections. The intensities were corrected for Lorentz and polarization effects. A semiempirical absorption correction was applied to the data using the SADABS program. The structure was solved by direct method with SHELXS-97.¹⁵ Structures were refined by full-matrix least squares

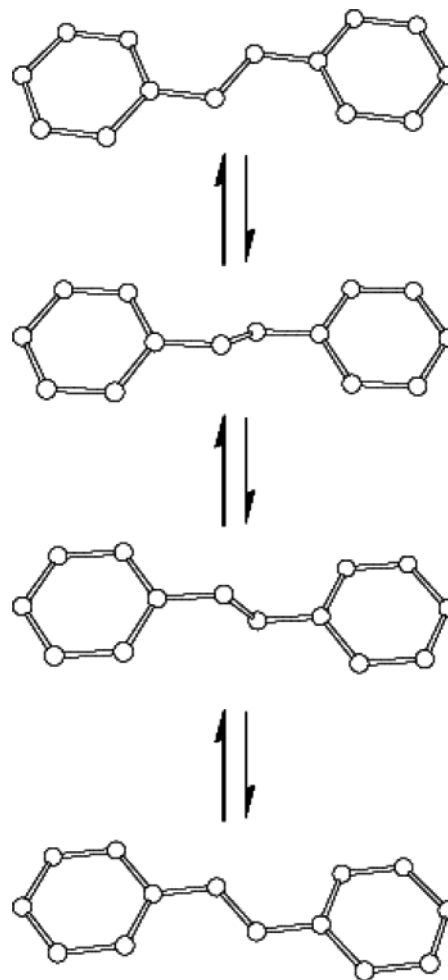


Figure 2. Pedal motion of stilbenes and azobenzenes.

on F^2 using SHELXL-97.¹⁶ For nondisordered molecules, all H atoms were located from difference Fourier electron density maps and refined isotropically, and all C and N atoms were refined anisotropically. Details of the structure refinement of the disordered crystals have been deposited as Supporting Information. The crystal and experimental data are summarized in Table 1.

X-ray Analysis of Crystals Cooled by Different Methods to 90 K. The diffraction measurement of a crystal of **1** at 90 K was carried out three times using the same crystal. Each time the crystal was cooled using a different method. (i) The crystal was cooled slowly at the cooling rate of 1 K/min, and then X-ray diffraction measurement was carried out at 90 K (the analyzed data are referred to as 90 K1 hereafter). (ii) After the diffraction measurement the crystal was warmed to room temperature at the rate of 1 K/min. The crystal and the goniometer head where the crystal was mounted were detached from the goniometer. The temperature of the nitrogen gas flow of the Cryostream Cooler, with which the diffractometer was equipped, was adjusted to 90 K. The nitrogen gas flow was deflected by a glass slide placed slantingly over the nozzle of the Cryostream Cooler. The goniometer head with the crystal was remounted on the goniometer, and the glass slide was taken off to release the gas flow. By this method, the crystal was flash-cooled to 90 K. Then, X-ray diffraction analysis was carried out (90 K2). (iii) The crystal was warmed to room temperature at the rate of 1 K/min for annealing, followed by slow cooling to 90 K at the rate of 1 K/min. Afterward X-ray diffraction analysis was carried out at 90 K (90 K3). Data acquisition, integration, and reduction were performed

- (6) (a) Finder, C. J.; Newton, M. G.; Allinger, N. L. *Acta Crystallogr., Sect. B* **1974**, *30*, 411–415. (b) Bernstein, J. *Acta Crystallogr., Sect. B* **1975**, *31*, 1268–1271. (c) Hoekstra, A.; Meertens, P.; Vos, A. *Acta Crystallogr., Sect. B* **1975**, *31*, 2813–2817. (d) Bouwstra, J. A.; Schouten, A.; Kroon, J. *Acta Crystallogr., Sect. C* **1984**, *40*, 428–431.
- (7) (a) Brown, C. J. *Acta Crystallogr.* **1966**, *21*, 146–152. (b) Bouwstra, J. A.; Schouten, A.; Kroon, J. *Acta Crystallogr., Sect. C* **1983**, *39*, 1121–1123.
- (8) Brown, C. J. *Acta Crystallogr.* **1966**, *21*, 153–158.
- (9) (a) Ueda, Y.; Nakamura, N.; Chihara, H. *J. Phys. Soc. Jpn.* **1988**, *57*, 4063–4064. (b) McGeorge, G.; Harris, R. K.; Batsanov, A. S.; Churakov, A. V.; Chippendale, A. M.; Bullock, J. F.; Gan, Z. *J. Phys. Chem. A* **1998**, *102*, 3505–3513.
- (10) (a) Saito, K.; Yamamura, Y.; Kikuchi, K.; Ikemoto, I. *J. Phys. Chem. Solids* **1995**, *56*, 849–857. (b) Saito, K.; Okada, M.; Akutsu, H.; Sorai, M. *Chem. Phys. Lett.* **2000**, *318*, 75–78.
- (11) Galli, S.; Mercandelli, P.; Sironi, A. *J. Am. Chem. Soc.* **1999**, *121*, 3767–3772.
- (12) Harada, J.; Uekusa, H.; Ohashi, Y. *J. Am. Chem. Soc.* **1999**, *121*, 5809–5810.
- (13) (a) Ito, Y.; Hosomi, H.; Ohba, S. *Tetrahedron* **2000**, *56*, 6833–6844. (b) Ohba, S.; Hosomi, H.; Ito, Y. *J. Am. Chem. Soc.* **2001**, *123*, 6349–6352.
- (14) Cosier, J.; Glazer, A. M. *J. Appl. Crystallogr.* **1986**, *19*, 105–107.
- (15) Sheldrick, G. M. *Acta Crystallogr.* **1990**, *A46*, 467–473.

- (16) Sheldrick, G. M. *SHELXL-97, program for crystal structure refinement*; Universität Göttingen: Göttingen, Germany, 1997.

Table 1. Crystal Data and Structure Refinements

		Compound 1					
empirical formula		C ₁₄ H ₁₂					
fw		180.24					
temp (K)	250	200	90 (90 K1)	90 (90 K2)	90 (90 K3)		
cryst system	monoclinic						
space group	P2 ₁ /a						
a (Å)	12.3513(5)	12.3207(5)	12.2735(6)	12.2933(7)	12.2763(4)		
b (Å)	5.7088(2)	5.6910(2)	5.6572(3)	5.6612(3)	5.6575(2)		
c (Å)	15.6453(6)	15.5735(6)	15.4399(7)	15.4447(9)	15.4419(6)		
α (deg)	90.0	90.0	90.0	90.0	90.0		
β (deg)	111.935(1)	111.954(1)	112.040(1)	112.103(1)	112.037(1)		
γ (deg)	90.0	90.0	90.0	90.0	90.0		
V (Å ³)	1023.31(7)	1012.78(7)	993.71(8)	995.88(10)	994.13(6)		
Z	4						
reflens colld	12 918	12 842	12 433	12 534	12 523		
indpndt reflens	2355	2331	2284	2287	2285		
R _{int}	0.012	0.018	0.010	0.011	0.014		
data/restraints/params	2355/5/150	2331/5/150	2284/5/150	2287/5/150	2285/5/150		
goodness-of-fit on F ²	1.090	1.110	1.089	1.106	1.108		
R [F ² > 2σ(F ²)]	0.042	0.042	0.040	0.039	0.040		
wR(F ²) (all data)	0.126	0.124	0.106	0.108	0.111		
ρ _{min} (e Å ⁻³)	-0.266	-0.298	-0.325	-0.339	-0.320		
ρ _{max} (e Å ⁻³)	0.254	0.273	0.387	0.374	0.371		
		Compound 2					
empirical formula		C ₁₂ H ₁₀ N ₂					
fw		182.22					
temp (K)	335	300	250	200	150	90 (90 K1)	90 (90 K2)
cryst system	monoclinic						
space group	P2 ₁ /a						
a (Å)	12.217(3)	12.1902(13)	12.1387(6)	12.0949(6)	12.0635(5)	12.0291(7)	12.0365(9)
b (Å)	5.7999(13)	5.7856(6)	5.7622(3)	5.7366(3)	5.7161(3)	5.6930(3)	5.6958(4)
c (Å)	15.261(4)	15.2364(16)	15.1592(8)	15.0923(8)	15.0384(7)	14.9738(9)	14.9790(11)
α (deg)	90.0	90.0	90.0	90.0	90.0	90.0	90.0
β (deg)	112.447(4)	112.426(2)	112.458(1)	112.456(1)	112.454(1)	112.464(1)	112.462(1)
γ (deg)	90.0	90.0	90.0	90.0	90.0	90.0	90.0
V (Å ³)	999.4(4)	993.32(18)	979.90(9)	967.76(9)	958.37(8)	947.62(9)	949.01(12)
Z	4						
reflens colld	12 715	12 407	12 167	11 994	11 870	11 723	11 723
indpndt reflens	2305	2292	2251	2224	2206	2186	2187
R _{int}	0.014	0.017	0.012	0.011	0.011	0.011	0.011
data/restraints/params	2305/5/146	2292/5/146	2251/5/146	2224/5/146	2206/5/146	2186/0/168	2187/0/168
goodness-of-fit on F ²	1.108	1.119	1.101	1.095	1.077	1.073	1.073
R [F ² > 2σ(F ²)]	0.047	0.043	0.040	0.039	0.039	0.033	0.034
wR(F ²) (all data)	0.146	0.134	0.115	0.111	0.106	0.089	0.091
ρ _{min} (e Å ⁻³)	-0.236	-0.186	-0.191	-0.205	-0.266	-0.165	-0.178
ρ _{max} (e Å ⁻³)	0.199	0.210	0.250	0.288	0.296	0.309	0.316

under the same conditions for the three data sets. For a crystal of **2**, two sets of crystal structural data, corresponding to 90 K1 and 90 K2 for **1**, were obtained. The initial 50 frames were collected again at the end of data collection. The analysis of the duplicated reflections shows no significant crystal decay or time evolution during data collection at 90 K.

Results and Discussion

(*E*)-Stilbene (**1**) and azobenzene (**2**) are among the best-known compounds showing orientational disorder in crystals. X-ray diffraction analyses of **1** and **2** have been reported many times by several groups over the past half century.^{6,7,17} The structural features so far reported are as follows: Crystals of **1** and **2** are isomorphous, monoclinic P2₁/a. There are two crystallographically independent molecules in the asymmetric unit (Figure 3). Both of the molecules lie at inversion centers and are almost planar. Molecules at one of the two crystal sites show no disorder (referred to as site 1),¹⁸ whereas those at the

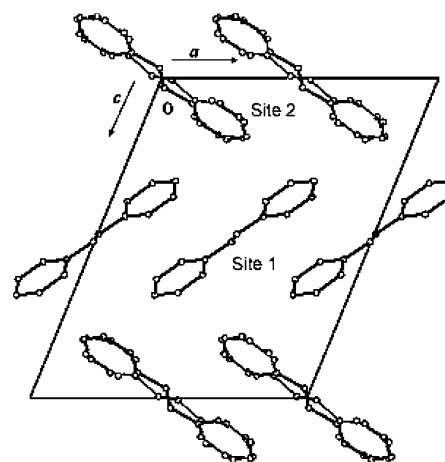


Figure 3. Projection of the crystal structure of (*E*)-stilbene (**1**) along the *b* axis, showing the disorder at site 2.

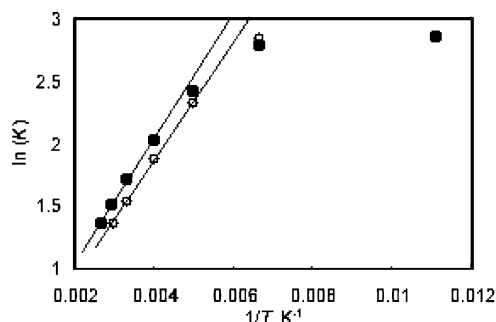
other site do (referred to as site 2). The orientational disorder is dynamic, as deduced from the fact that the occupancy factor of the minor orientation¹⁹ decreases as the temperature is lowered.

- (17) (a) Robertson, J. M.; Woodward, I. *Proc. R. Soc. London, Ser. A* **1937**, *162*, 568–583. (b) Lange, J. J. de; Robertson, J. M.; Woodward, I. *Proc. R. Soc. London, Ser. A* **1939**, *171*, 398–410.
 (18) Site 1 in crystals of **1** shows very weak disorder at or above room temperature.⁵

Table 2. Populations of the Two Orientations at the Disordered Site (Site 2) in the Crystals of (*E*)-Stilbene (**1**) and Azobenzene (**2**)

temp (K)	1	2
373	79.6(4):20.4(4)	^a
340	81.8(4):18.2(4)	^a
335	^a	79.5(4):20.5(4)
300	84.6(3):15.4(3)	82.2(3):17.8(3)
250	88.2(3):11.8(3)	86.6(3):13.4(3)
200	91.8(3):8.2(3)	91.0(3):9.0(3)
150	94.2(2):5.8(2)	94.5(2):5.5(2)
90 (90 K1)	94.5(2):5.5(2)	1:0 ^b
90 (90 K2)	91.0(2):9.0(2)	^c
90 (90 K3)	94.5(2):5.5(2)	^a

^a The data were not collected. ^b The minor orientation was not detected. ^c The minor orientation was detected but not refined.

**Figure 4.** van't Hoff plots ($\ln K$ vs $1/T$) for the two orientations at site 2 in the crystals of (*E*)-stilbene (**1**) (denoted by solid squares) and azobenzene (**2**) (denoted by open circles). K is the ratio of the population of the major orientation to that of the minor orientation.

We reinvestigated the crystal structures of **1** and **2** over temperatures ranging from 373 to 90 K for **1**²⁰ and from 335 to 90 K for **2**, respectively. The temperature of the crystals was lowered at the rate of 1 K/min. The populations of the two orientations at site 2 were determined at each temperature (Table 2). In both crystals, the populations of the two orientations change with changes in temperature. As the temperature was lowered, the population of the major stable orientation increased and that of the minor unstable orientation decreased. In the crystal of **2**, the disorder almost disappeared at 90 K. In contrast, the population of the unstable orientation of **1** did not show significant changes below 150 K, and the disorder persisted to 90 K. In Figure 4, the natural logarithm of the equilibrium constants between the two orientations in the crystals of **1** and **2** are plotted against the reciprocal of temperature. According to the van't Hoff equation, straight lines are expected for the plots when the difference of enthalpy between the two conformers does not change with variation of temperature. The plots for **1** and **2** give almost straight lines at the high-temperature range (373–250 K for **1**, 335–200 K for **2**). At the low-temperature range, however, the plot for **1** increasingly deviates from a straight line. The plot for **2** shows slight nonlinearity at the low-temperature range. The deviation from linearity becomes evident for **1** at a temperature higher than for **2**.

There are two possible explanations for the nonlinearity of the plots. The first explanation is based on the assumption that thermodynamic nonequilibrium states are generated by the

freezing of the conformational changes at low temperatures. The freezing of the conformational change during cooling increases the population of the minor orientation compared to the equilibrium state. Thus, the van't Hoff plots become nonlinear and the disorder persists at low temperatures.

The other explanation is based on the assumption that the crystal lattices and, concomitantly, the difference of enthalpy (ΔH) between the two orientations in the crystals change with variation of temperature. There are several possible origins of the change in the ΔH : The magnitude of intermolecular interactions in the crystal can change with intermolecular distances. The ΔH can also change with the populations of the orientations, because the change in the composition of the molecules around a given molecule can change the magnitude of the intermolecular interactions to the molecule. If the extent of such temperature dependence of the intermolecular interactions is different in the two conformers, the ΔH would change with variation of temperature, and thus the plots would become nonlinear.

The latter explanation is not plausible because of the following reasons. If the temperature dependence of ΔH due to the change of the crystal lattices is large enough to be responsible for the nonlinearity of the van't Hoff plots, the nonlinearity should take place at all the temperature range. The nonlinearity in the van't Hoff plots, however, takes place only at the low-temperature range. Therefore, the temperature dependence of ΔH of the two conformers in the crystals, if any, is not large enough to significantly change their populations. The following results strongly support that the conformational changes freeze at low temperatures.

The crystal structure of **1** at 90 K depends on the cooling rate of the crystal. The cell lengths and volume of the flash-cooled crystal (data 90 K2; vide Experimental Section) are larger than those of the slowly cooled crystal (90 K1 and 90 K3). The population of the minor orientation at the disordered site (site 2) also changes with the cooling rate. Figure 5 shows the difference Fourier electron density maps for 90 K1 and 90 K2. Each section contains four carbon atoms of the three central C–Ph and C=C bonds (C1, C7, C7', and C1'). The height of the two residual peaks (peaks A and A'), which correspond to the ethylene carbon atoms of the minor orientation, is 0.36 e \AA^{-3} for 90 K1 and 0.83 e \AA^{-3} for 90 K2. Thus, flash cooling increases the population of the minor orientation: 5.5(2)% for 90 K1; 9.0(2)% for 90 K2. The results for 90 K3 are essentially the same as for 90 K1 (peak height, 0.37 e \AA^{-3} ; occupancy factor, 5.5(2)%) (Table 2).

The cooling rate dependence of the crystal structure can be explained only by assuming that a thermodynamic nonequilibrium state is generated by freezing the conformational change.²¹ Both of the slow cooling and the flash cooling make the conformational change frozen at low temperature. During the flash cooling, the two orientations in the crystal start to deviate from equilibration at a higher temperature than during the slow cooling, because faster conformational interconversion is necessary for equilibration. Therefore, the deviation from equilibrium

(19) For **1** and **2**, in which the benzene rings have no substituents and have 2-fold symmetry, the two conformers that interconvert in the crystal may also be considered as a single conformer in two different orientations.

(20) We have already reported the crystal structures of **1** at 373, 340, 300, and 150 K in the previous paper,⁵ which discusses only the features of site 1.

(21) The freezing of the conformational changes in crystals can be considered as a kind of glass transition. The glass transition is caused by a relaxational effect, through which some process in a disordered stable crystal occurs too slowly at low temperature to permit thermodynamic equilibrium to be established during the laboratory experimental time-scale. (a) Suga, H.; Seki, S. *J. Non-Cryst. Solids* **1974**, *16*, 171–194. (b) Suga, H.; Seki, S. *Faraday Discuss. R. Soc.* **1980**, *69*, 221–240.

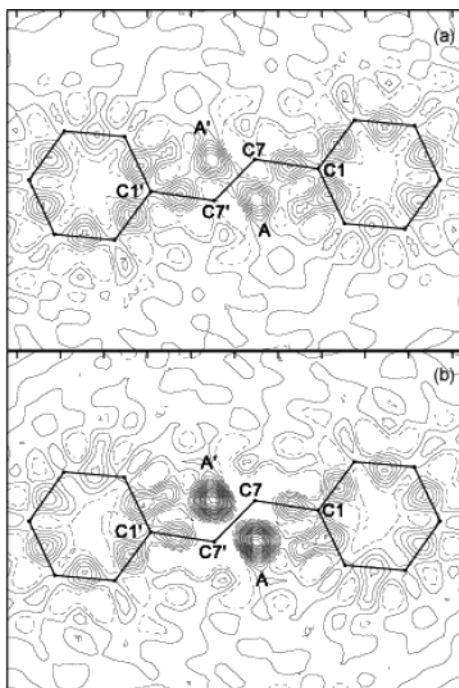


Figure 5. Difference Fourier maps of (*E*)-stilbene (**1**) at site 2: (a) 90 K1 (slowly cooled crystal); (b) 90 K2 (flash-cooled crystal). The section of each map contains C1, C7, C7', and C1'. The contour lines are at 0.05 e Å⁻³ intervals. Negative contours are indicated by broken lines.

state and thus the population of the minor orientation in the crystal flash-cooled to a given temperature is larger than in the crystal slowly cooled to the same temperature.

The crystal structures of azobenzene (**2**) at 90 K also changed with the cooling rate. In the slowly cooled crystal (90 K1), there were no detectable residual peaks corresponding to the minor orientation in the difference Fourier map (Figure 6a).²² In contrast, two residual peaks (peaks A and A')²³ appeared around the N=N bond in the flash-cooled crystal (90 K2) and can be assigned to the nitrogen atoms of the minor orientation (Figure 6b). The results indicate that the minor unstable orientation, which cannot exist in the equilibrium state at 90 K, can exist in the nonequilibrium state in the flash-cooled crystal.

It is noticeable that the freezing of the conformational interconversion brought about only small changes in the crystal structure of **2**. There is no significant difference in the cell dimensions between 90 K1 and 90 K2 (Table 1), and the van't Hoff plot shows only moderate nonlinearity (Figure 4). The results can be explained by assuming that in the crystal of **2** the conformational interconversion takes place faster than in the crystal of **1** and that the thermodynamic equilibrium remains established at a lower temperature. Compared to the crystal of **1**, the population of the minor orientation at the temperature where the conformational interconversion freezes becomes smaller in the crystal of **2**. Thus, the difference between the nonequilibrium state and the equilibrium state at 90 K in **2** is smaller than that in **1**. The fact that the glass transition takes place at a lower temperature for **2** (110 K) than for **1** (170 K)¹⁰ supports this interpretation.

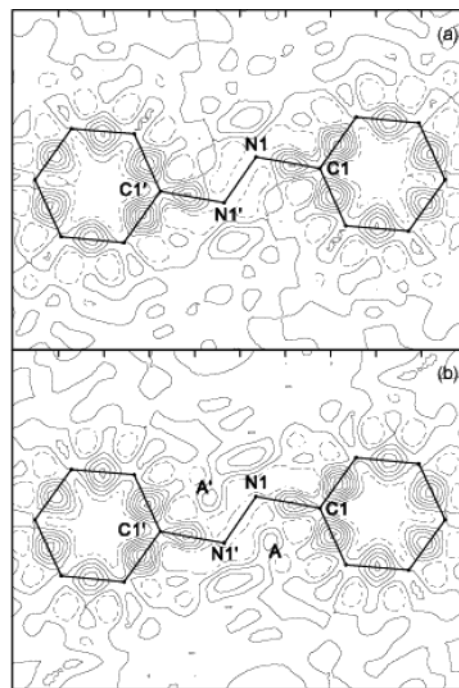


Figure 6. Difference Fourier maps of azobenzene (**2**) at site 2: (a) 90 K1 (slowly cooled crystal); (b) 90 K2 (flash-cooled crystal). The section of each map contains C1, N1, N1', and C1'. The contour lines are at 0.05 e Å⁻³ intervals. Negative contours are indicated by broken lines.

Concluding Remarks

In this study we succeeded in X-ray diffraction analysis of a nonequilibrium state generated by freezing the conformational interconversion in the crystals of stilbene and azobenzene. The populations of the two conformers in the crystal at low temperature vary with the cooling rate, and the minor, less stable orientation, which cannot exist in the equilibrium state at low temperature, can be detected in the nonequilibrium state of the flash-cooled crystal.

This paper shows that the flash-cooling technique, which is in widespread use for macromolecular crystallography,²⁴ is useful for structure determination of thermodynamically unstable conformers by X-ray diffraction analysis.²⁵ In equilibrated crystals of molecules that can potentially adopt conformations other than the stable one, the minor unstable conformer can coexist with only a vanishingly small population, if any, at sufficiently low temperature and may be difficult to detect. The population of the unstable conformer increases enough to be detected at high temperatures.²⁶ Thus, heating and then flash

(22) There was no peak corresponding to the nitrogen atoms of the minor orientation among the highest 100 residual peaks obtained by the difference Fourier synthesis.

(23) The height of the peaks is 0.11 e Å⁻³. The peaks A and A' are the largest residual peaks except those corresponding to the bonding electrons of C–C and C–N bonds and of lone pairs of nitrogen atoms.

(24) For X-ray diffraction experiments of macromolecular crystals, the flash-cooling technique is utilized to freeze water inside and surrounding the crystals into a vitreous form without hexagonal ice formation. (a) Hope, H. *Acta Crystallogr., Sect. B* **1988**, *44*, 22–26. (b) Teng, T.-Y. *J. Appl. Crystallogr.* **1990**, *23*, 387–391. (c) Garman, E. F.; Schneider, T. R. *J. Appl. Crystallogr.* **1997**, *30*, 211–237. Although conformational freezing probably takes place in many protein structures during flash-cooling of the macromolecular crystals, it would be difficult to know which parts of the structures are frozen.

(25) Flash-cooling techniques in an Ar matrix have proved to be useful for detection of unstable conformers. Squillacote, M.; Sheridan, R. S.; Chapman, O. L.; Anet, F. A. L. *J. Am. Chem. Soc.* **1975**, *97*, 3244–3246.

(26) Although the population of the unstable conformer at low temperature in the flash-cooled crystals is lower than that at high temperature, the determination of the unstable conformer from diffraction data at high temperature has its own problem due to the following reasons. The intensity of Bragg reflections from crystals at high temperature rapidly falls off at higher scattering angles, because of large thermal motion of atoms and molecules. Thus, only reflections with low angles can be measured with enough accuracy. Moreover, the thermal motion of the molecules blurs the structure and thus the resolution of the disordered conformers is limited.

cooling the crystals causes the high population of the unstable conformer to be preserved in the nonequilibrium state at a low temperature, at least partially. High-quality X-ray diffraction analysis of such crystals at low temperature allows one to determine the structure of the unstable conformer.²⁷ X-ray diffraction analysis has been so far exploited for structure determination of photoinduced unstable species in crystals.²⁸ The X-ray diffraction analysis of flash-cooled crystals is a promising technique for structure determination of thermally induced unstable species.

(27) Application of the flash-cooling technique to the detection of unstable conformers is limited by the relative energies of the conformers and the time scale of the conformational change: The population of the unstable conformer must be high enough to detect at least at high temperature. The rate of the conformational change must be high enough to establish an equilibration at high temperature and low enough to freeze at low temperature. Recent and future developments of instrumentation, e.g. higher quality of diffraction measurement, gas stream at temperature much lower than liquid-nitrogen temperature, and faster collection of diffraction data, would relax the above requirements.

Acknowledgment. This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan and by Mitsubishi Chemical Corp. Fund.

Supporting Information Available: Details of the structure refinement of the disordered crystals, ORTEP diagrams for **1** and **2** (PDF), and X-ray crystallographic data (CIF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

JA038203L

(28) (a) Coppens, P.; Novozhilova, I.; Kovalevsky, A. *Chem. Rev.* **2002**, *102*, 861–883. (b) Kim, C. D.; Pillet, S.; Wu, G.; Fullagar, W. K.; Coppens, P. *Acta Crystallogr., Sect. A* **2002**, *58*, 133–137. (c) Coppens, P. *J. Chem. Soc., Chem. Commun.* **2003**, 1317–1320. (d) Kawano, M.; Sano, T.; Abe, J.; Ohashi, Y. *J. Am. Chem. Soc.* **1999**, *121*, 8106–8107. (e) Kawano, M.; Hirai, K.; Tomioka, H.; Ohashi, Y. *J. Am. Chem. Soc.* **2001**, *123*, 6904–6908. (f) Schlichting, I. *Acc. Chem. Res.* **2000**, *33*, 532–538. (g) Marchivie, M.; Guionneau, P.; Howard, J. A. K.; Chastanet, G.; Létard, J.-F.; Goeta, A. E.; Chasseau, D. *J. Am. Chem. Soc.* **2002**, *124*, 194–195. (h) Money, V. A.; Evans, I. R.; Halcrow, M. A.; Goeta, A. E.; Howard, J. A. K. *J. Chem. Soc., Chem. Commun.* **2003**, 158–159.