Molecular Conformation of Swinholide A, a Potent Cytotoxic Dimeric Macrolide from the Okinawan Marine Sponge *Theonella swinhoei*: X-ray Crystal Structure of Its Diketone Derivative

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The absolute configuration of swinholide A, a potent cytotoxic dimeric macrolide from marine sponge, was determined by the X-ray crystal analysis of its di-*p*-bromobenzylated diketone derivative. The crystal structure further revealed the conformational characteristic of the 44-membered ring structure, which is probably related to the cytotoxic activity. A possible stereostructure of swinholide A is proposed based on the present X-ray result, and its biological implications are also discussed.

Introduction

Research efforts devoted to the structural evaluation of marine natural products received increasing interest because of the variety of biological activities of these compounds.[1] In the search for bioactive substances from the Okinawan marine sponge *Theonella swinhoei*, several new compounds possessing novel structural features have been isolated.[2,3] Swinholide A, a potent cytotoxic macrolide, is one of the metabolites and has a 44-membered dimeric dilactone skeleton.[3] The chemical structures of the swinholide family so far elucidated[3,4] are given in Figure 1.[5] These macrolides all have a common or similar dimeric ring structure. It has been shown[6] that their cytotoxic activities are dependent on the ring structures and on the functional OH groups bound to them. For example, swinholide A exhibits high cytotoxic activity for L1210 (IC_{50} = 0.03 μg/mL) and KB (IC_{50} = 0.04 μg/mL) tumor cells, while the activity of isoswinholide A is significantly lowered (IC_{50} values are 0.12 and 1.4 μg/mL for L1210 and KB cells, respectively). This means that the spatial ring conformation is probably related to the activity, although little is known about the compound's mode of action. Thus, we believe that study on the swinholide molecular conformation provides basic information about its biological activity.

Recently we succeeded in crystallizing swinholide A as its diketone derivative (1) shown in Figure 2. This paper deals with its crystal and molecular structure. The conformational characteristics of swinholide A are also considered. A preliminary X-ray analysis was previously reported.[7]

Results and Discussion

Absolute Configuration. The absolute configuration of 1 was determined by the Bijvoet method[8] using the anomalous dispersion of Cu radiation by the C, O, and Br atoms. All reflections of 45 Friedel's pairs showing the significant Bijvoet differences agreed with the configuration shown in Figure 2. Although the Bijvoet differences are, of course, influenced by the absorption and extinction effects, it was clearly suggested that the absolute stereochemical designations of the 28 chiral carbon centers in 1 are 7*S*,7′*S*, 9*R*,9′*R*, 13*S*,13′*S*, 15*S*,15′*S*, 16*R*,16′*R*, 17*S*,17′*S*, 19*R*,19′*R*, 20*S*,20′*S*, 21*S*,21′*S*, 22*R*,22′*R*, 24*S*,24′*S*, 27*S*,27′*S*, 29*R*,29′*R*, 31*S*,31′*S*; the 7*S*,7′*S* configuration of 1 was also independently determied by the chemical method.[3b,7]

Molecular Dimension and Conformation. The averaged standard deviations for the bond lengths and angles, tables of which are available in the supplementary material, were 0.01 Å and 0.6°, respectively. The values are large because of the high thermal motions of individual atoms and of the relatively small M/N value (=6618/1136), where M and N are numbers of observed reflections and variables used for refinement, respectively. However, the uncertainty for these bonding parameters would not influence the description on the molecular conformation of 1 significantly.

A stereoscopic view of the molecular conformation of 1 is shown in Figure 3. The 44-membered ring structure, which is shown with the ellipsoidal circles and thick bonds, assumes a severely folded conformation with turns at the dihydropyran rings and at the C(21) and C(21′) atoms and is shaped like a twisted saddle. This kind of folding pattern has also been observed in the related cyclic compounds such as patellamide D, a cytotoxic cyclic peptide from a marine tunicate.[9] The lines between the O(9) and O(9′) atoms and between the C(21) and C(21′) atoms have the distances of 11.66 and 10.16 Å, respectively, and are separated by 7.51 Å to each other. The C(22)–C(31) and C(22′)–C(31′) segments protrude over the folded ring structure, and their ring moieties come close to each other. Since there is no intramolecular hydrogen bond in the molecular conformation of 1, this round conformation

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[1] For example: (a) Faulkner, D. J. *Nat. Prod. Rep.* **1990**, *7*, 269, and previous papers. (b) Rinehart, K. L. *Pure Appl. Chem.* **1989**, *61*, 525. (c) Kitagawa, I., *Yakugaku Zasshi* **1988**, *108*, 398 (review).

[2] (a) Kitagawa, I.; Kobayashi, M.; Lee, N. K.; Shibuya, H.; Kawata, Y.; Sakiyama, F. *Chem. Pharm. Bull.* **1986**, *34*, 2664. (b) Kitagawa, I.; Lee, N. K.; Kobayashi, M.; Shibuya, H. *Ibid.* **1987**, *35*, 2129.

[3] (a) Kobayashi, M.; Tanaka, J.; Katori, T.; Matsuura, M.; Kitagawa, I. *Tetrahedron Lett.* **1989**, *30*, 2963. (b) Kobayashi, M.; Tanaka, J.; Katori, T.; Matsuura, M.; Yamashita, M.; Kitagawa, I. *Chem. Pharm. Bull.* **1990**, *38*, 2409.

[4] (a) Kobayashi, M.; Tanaka, J.; Katori, T.; Kitagawa, I. *Chem. Pharm. Bull.* **1990**, *38*, 2960. (b) Tanaka, J.; Higa, T.; Kobayashi, M.; Kitagawa, I. *Ibid.* **1990**, *38*, 2967.

[5] Expressions for the chemical structures of swinholide family are changed here from those given in previous papers,[3,4] since the atomic arrays of hydroxyl groups in the present expressions are more alike to those obtained by the X-ray study.

[6] Kitagawa, I.; Kobayashi, M.; Kawazoe, K.; Katori, T.; Tanaka, J.; Higa, T.; Ishida, T.; Doi, M.; Sasaki, T. Presented at the 32th Symposium on the Chemistry of Natural Products, Chiba, Japan, Oct. 1990; symposium Paper pp 127–134. The details will be reported elsewhere.

[7] Kitagawa, I.; Kobayashi, M.; Katori, T.; Yamashita, M.; Tanaka, J.; Doi, M.; Ishida, T. *J. Am. Chem. Soc.* **1990**, *112*, 3710.

[8] Bijvoet, J. M.; Peerdeman, A. F.; van Bommel, A. J. *Nature* **1951**, *168*, 271.

[9] Schmitz, F. J.; Ksebati, M. B.; Chang, J. S.; Wang, J. L.; Hossain, M. B.; van der Helm, D.; Engel, M. H.; Serban, A.; Silfer, J. A. *J. Org. Chem.* **1989**, *54*, 3463.

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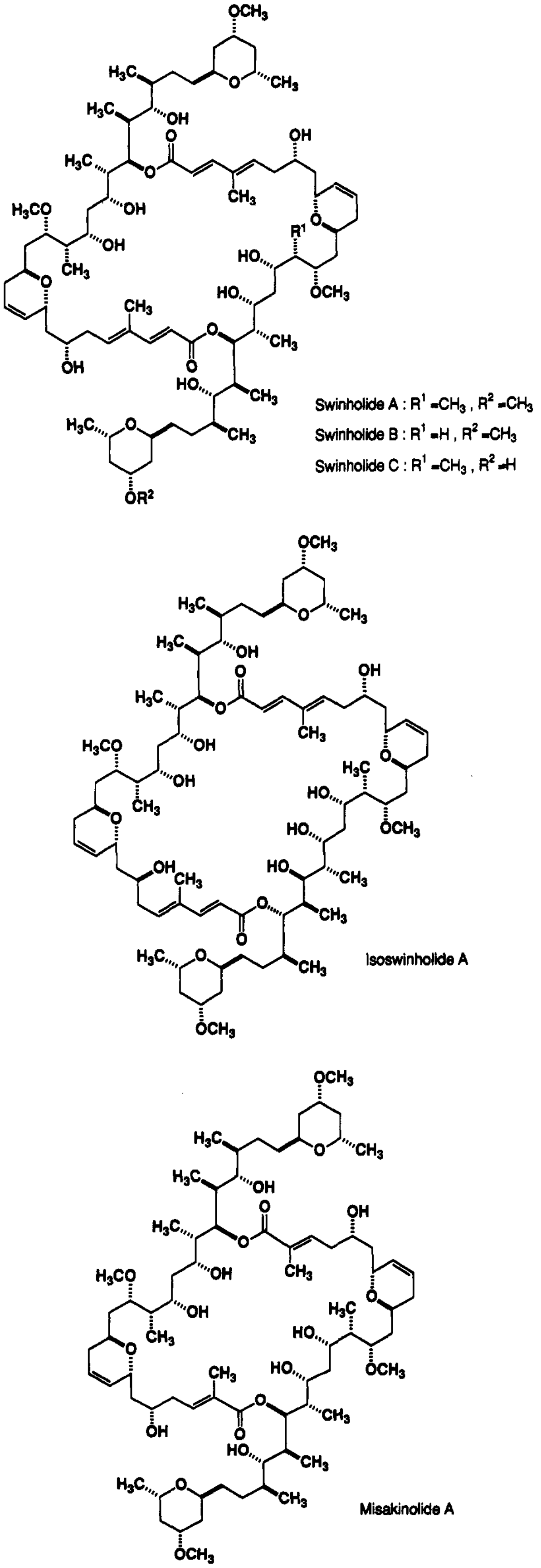

Figure 1. Chemical structures of swinholide family.

observed is mainly held by the van der Waals interaction.

Both of the tetrahydropyran rings in the side chains adopt a chair conformation, and the dihydrogen rings within the ring structure [C(9)–C(13)] are in the half-chair conformation with C(9)-axial and C(13)-equatorial orientations. The ring conformation itself appears to be stable and not to be significantly distorted by the external factors such as crystal packing, because all of ring torsion angles (see Table I) are in the energetically stable trans or gauche orientation. A similar conformational characteristic is observed in a related monomeric macrolide, scytophycin C[10] (Chart I). The atomic arrangement in the structure

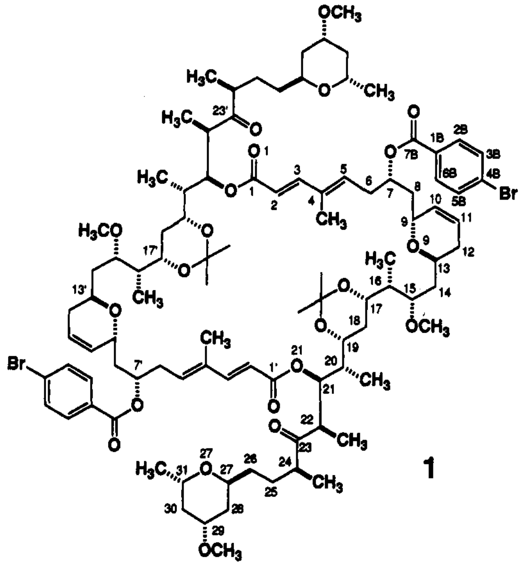
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Figure 2. Chemical structure of 1, along with the atomic numbering used for X-ray study. The numbering for the left half of 1 dimer structure is represented with a prime.

Table I. Conformational Torsion Angles (deg) of 1 and Deviation from C_2 Symmetric Structure

bond	torsion angle[a]		difference
	Ring Structure		
C(1)–C(2)	168.9 (7)	171 (1)	2.1
C(2)–C(3)	−176.0 (9)	−176.1 (1)	0.1
C(3)–C(4)	171.2 (9)	169 (1)	2.2
C(4)–C(5)	−178.6 (9)	−171 (1)	7.6
C(5)–C(6)	105.7 (8)	109 (1)	3.3
C(6)–C(7)	175.7 (7)	169.3 (9)	6.4
C(7)–C(8)	160.9 (8)	161.3 (9)	0.4
C(8)–C(9)	178.2 (9)	174.9 (9)	3.3
C(9)–C(10)	−117 (1)	−109 (1)	8.0
C(10)–C(11)	−1 (1)	−5.9 (8)	4.9
C(11)–C(12)	21 (1)	16.7 (8)	4.6
C(12)–C(13)	−164.5 (9)	−166.7 (8)	2.2
C(13)–C(14)	−170.7 (9)	−171.1 (9)	0.4
C(14)–C(15)	89.6 (8)	103.1 (7)	13.5
C(15)–C(16)	−173.4 (8)	−174.1 (7)	0.7
C(16)–C(17)	172.8 (8)	173.4 (7)	0.6
C(17)–C(18)	−176.4 (8)	−173.4 (7)	3.0
C(18)–C(19)	177.8 (8)	169.0 (7)	8.8
C(19)–C(20)	176.5 (8)	175.1 (6)	1.4
C(20)–C(21)	−173.9 (7)	−171.6 (6)	2.3
C(21)–O(21)	121.1 (7)	119.0 (5)	2.1
	Side Chain		
C(21)–C(22)[b]	−165.8 (8)	177.3 (6)	11.5
C(22)–C(23)	85.7 (9)	98.7 (7)	13.0
C(23)–C(24)	−160.1 (9)	−160.0 (9)	0.1
C(24)–C(25)	74.6 (9)	76 (1)	1.4
C(25)–C(26)	−174 (1)	−168 (1)	6.0
C(26)–C(27)[c]	86 (1)	172 (1)	86.0

[a] Torsion angles given in the second column correspond to those of the primed atoms in the dimer structure of 1. [b] C(20)–C(21)–C(22)–C(23). [c] C(25)–C(26)–C(27)–C(28).

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[10] Ishibashi, M.; Moore, R. E.; Patterson, G. M. L.; Xu, C.; Clardy, J. *J. Org. Chem.* **1986**, *51*, 5300.

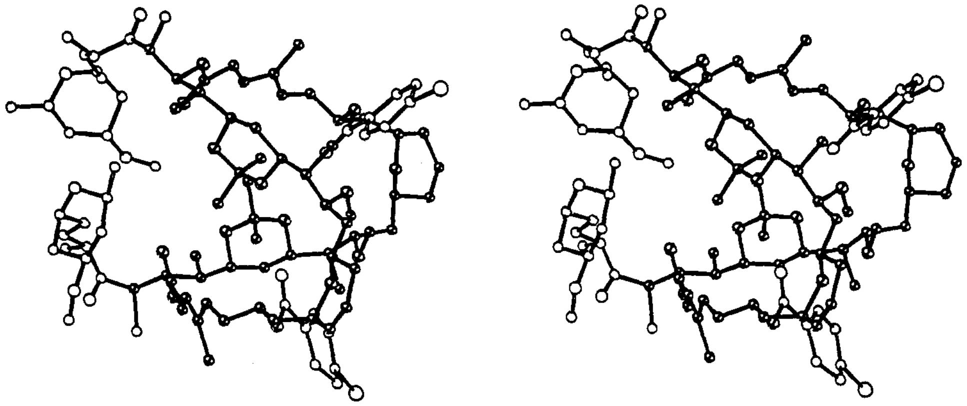
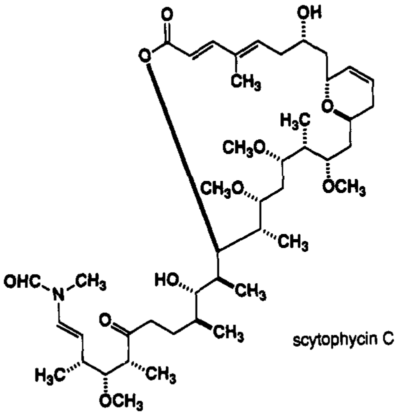


Figure 3. Stereoscopic drawing of 1. The 44-membered ring structure is represented by the ellipsoidal circles and thick lines, and the side chain by the open circles and thin lines.

Chart I



is very similar to the monomeric unit of swinholide A, and the configurations of the asymmetric carbons are the same in the two molecules. The use of space-filling models also indicates no significant effect of the ring structure by the attachment of the *p*-bromobenzoates.

Deviation from C_2 Symmetry. Although the chemical structure of 1 consists of the C_2-related dimeric units, the crystal conformation was not related by the symmetry. In the ring structure moiety, however, the deviation from the C_2 symmetry is not significant (see Table I). The largest deviation is found at the torsion angle around the C(14)–C(15) bond, which is probably due to the short contacts between the related methoxy groups. In contrast, the side chains show relatively large conformational deviations, and the torsion angle about the C(26)–C(27) bond corresponds to the largest deviation of 86°. This could be a reason why 1 has no crystallographic C_2 symmetry.

In general, it can be concluded that the C_2 symmetric molecular conformation is essentially a favorable form for 1. The C_2 symmetric conformation of 1 has been confirmed in the solution by the ^{1}H and ^{13}C NMR analyses.[3]

Crystal Packing. Two crystallographically independent methanol molecules exist in the crystal structure and are linked with O(17) and O(17′) atoms by respective hydrogen bonds of 2.87 (1) and 2.879 (8) Å. No intermolecular hydrogen bonds were observed among neighboring molecules, and they were mainly stabilized by usual van der Waals contacts.

Possible Conformation of Swinholide A. Since the amorphous solid of swinholide A has not yet been successful in yielding single crystals, its X-ray analysis is not available. However, the crystal structure of 1 clearly shows the absolute configuration of swinholide A [except for the C(23) and C(23′) asymmetric carbons], together with the conformational characteristics. On the other hand, the *S* configurations at C(23) and C(23′) atoms have been independently determined by the ^{1}H NMR method.[3b,7] Therefore, it is presumable the possible conformation of swinholide A, based on the present result. Assuming that (1) the ring conformation of 1 is not significantly affected by the existence of di-*p*-bromobenzoate moieties and (2) the spatial ring conformation is in a energetically favorable region, a probable conformation of swinholide A molecule, as judged from the torsion angles about the respective bonds, is obtained as shown in Figure 4, where the oxygen atoms are shown with open circles.

It is interesting to note that oxygen atoms in this model conformation are all directed into the interior of the ring structure. The presence of free OH groups and the 44-membered ring structure could be closely related to the cytotoxic activity of swinholide family.[6] Such a conformational characteristic might influence, to some extent, its biological function. However, this proposal is speculative, and the detailed mechanism of action must be subjected to future investigations.

Table II. Summary of Crystal Data and Data Collection

formula	$C_{98}H_{142}O_{22}Br_2{\cdot}2CH_3OH$
Mr	1896.07
space group	$P2_1$
a, Å	14.500 (2)
b, Å	21.249 (3)
c, Å	18.987 (3)
β, °	103.00 (1)
V, Å^3	5700 (1)
Z	2
D(measd), g·cm^{-3}	1.101 (2)
D(calcd), g·cm^{-3}	1.105
absorpt coeff, cm^{-1}	13.86
F(000)	2024
crystal size, mm^3	0.4 × 0.5 × 0.3
T of data collection, °C	15
data collection method	ω–2θ scan
scan speed in 2θ, deg.·min^{-1}	3
scan range in ω, deg	$1.30 + 0.15 \tan \theta$
data range measd, deg	$2 \leq 2\theta \leq 110$
data collected	$h,k,\pm l$
no. of unique data measd	7550
no. of data with $F_o > \sigma(F_o)$ = (=M)	6618
no. of variables (=N)	1136
R	0.075
R_w	0.089

Experimental Section

Materials. Swinholide A was isolated from the Okinawan marine sponge *Theonella swinhoei*, and 1 was chemically synthesized from swinholide A.[3b]

Crystal Analysis of 1. Well-formed prismatic crystals of 1 were obtained from slow evaporation from the methanol–ethyl acetate mixture. Since transparent crystals become opaque in

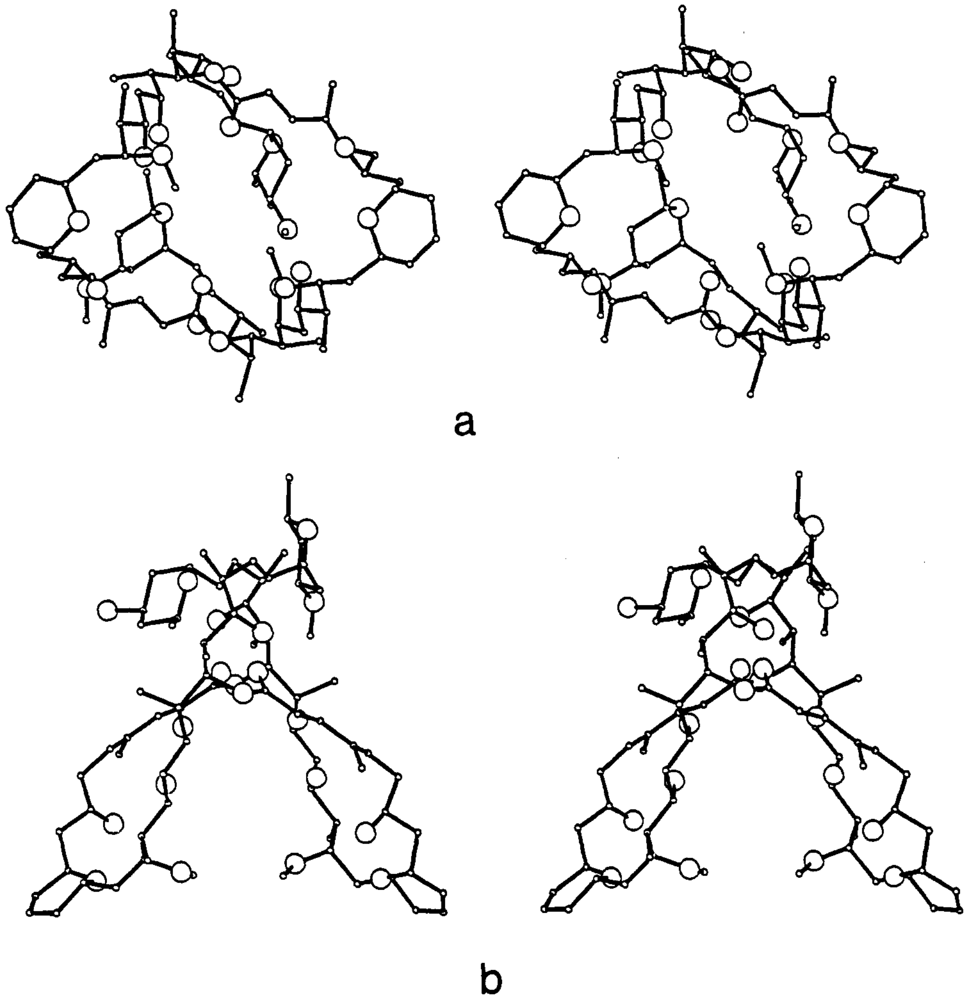


Figure 4. A possible molecular conformation of swinholide A, viewed along the pseudo C_2 symmetry axis of the molecule (a) and perpendicular to it (b). The oxygen atoms are represented by the open circles.

the air, they were sealed in glass capillaries containing some mother liquid. Oscillation and Weissenberg photographs indicated the crystal to be monoclinic with space group $P2_1$. Unit cell dimensions and diffraction intensities were measured with graphite-monochromated Cu Kα radiation (λ = 1.5418 Å) on a Rigaku AFC-5 computer-controlled diffractometer. Crystal data and parameters for data collection are summarized in Table II. The unit cell parameters were determined by a least-squares fit of 2θ angles for 30 reflections ranging from a 2θ of 30° to 60°. The crystal density was measured by the flotation method using a C_6H_6–CCl_4 mixture. The peak counts were corrected with background counts for 5 s at both ends of the scan range. Four standard reflections were monitored at every 100 reflection intervals throughout the data collection and showed no significant deterioration. The observed intensities were corrected for Lorentz and polarization effects. Absorptional corrections for each reflection were also made using the intensity variation of (0*k*0) reflections with the ϕ scan at χ = 90°.

The structure was solved by the combination of the heavy atom and direct methods using the MULTAN87 program.[11] The positional parameters obtained were then refined by a full-matrix least-squares analysis with isotropic temperature factors and then by a block-diagonal least-squares analysis with anisotropic ones, where the y coordinate of the C(1) atom was fixed during the refinement to define the origin along the y axis. The positions of the geometrically reasonable hydrogen atoms were calculated and included only for the structure factor calculations. The function minimized was $\Sigma w(|F_o| - |F_c|)^2$, where $|F_o|$ is the observed structure factor and $|F_c|$ is the calculated one without taking the anomalous dispersion effects into account. When the value of R $(=\Sigma||F_o| - |F_c||/\Sigma|F_o|)$ was reduced to a constant (=0.09), 45 reflections showing large Friedel differences were selected by the calculation, and the absolute configuration of 1 was determined by the Bijvoet method as described in Results and Discussion. In the final refinement including the anomalous dispersion effects, the weight was calculated as $w = 1.0/[\sigma(F_o)^2 + 0.14022|F_o| - 0.00106|F_o|^2]$, where $\sigma(F_o)^2$ is the standard deviation of the intensity based on counting statistics. The discrepancy indices, R and $R_w(=[\Sigma w(|F_o| - |F_c|)^2/wF_o^2]^{1/2})$ were reduced to 0.075 and 0.089, respectively, for 6618 independent reflections having $F_o^2 \geq \sigma(F_o)^2$, and S $(= [\Sigma w(|F_o| - |F_c|)^2/(M - N)]^{1/2})$ was 0.94. For all crystallographic computations, the UNICS program system[12] was used, the atomic scattering factors and the terms of the anomalous dispersion corrections were taken from ref 13.

All numerical calculations were carried out at the Computer Center, Osaka University of Pharmaceutical Sciences.

Supplementary Material Available: Tables of final atomic coordinates and anisotropic thermal parameters of non-hydrogen atoms and bond distances and angles between non-hydrogen atoms (10 pages). Ordering information is given on any current masthead page.

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(11) Debaerdemaeker, T.; Germain, G.; Main, P.; Tate, C.; Woolfson, M. M. *MULTAN87, Computer Programs for the Automatic Solution of Crystal Structures from X-Ray Diffraction Data*; Universities of York, England, and Louvain, Belgium, 1987.

(12) *The Universal Crystallographic Computing System-Osaka*; The Computation Center, Osaka University: Osaka, Japan, 1979.

(13) *International Tables for X-ray Crystallography*; Kynoch: Birmingham, England, 1974; Vol. IV.