

Intermolecular Association Model of Antifungal Polyene Macrolide via Concerted =C–H···O Interactions: X-ray Crystal Structure Analysis and Ab Initio Molecular-Orbital Calculations of Chainin

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The absolute configuration of chainin (**1**), a polyene macrolide antibiotic, was determined by X-ray crystal analyses, and the conformational stability of planar 28-membered ring was confirmed by an ab initio quantum chemical calculation. The most prominent feature observed in the crystal structure was the concerted =C–H···O interactions formed between four conjugated olefinic CH groups and five hydroxyl oxygen atoms of neighboring molecules. The character of the interaction was investigated by IR measurements and quantum chemical calculations, leading to the conclusion that these concerted interactions are powerful enough to form defined intermolecular association of polyene macrolide. Characteristically, this intermolecular association mode allows the proposal of a barrel-stave assembly, comprising eight parallel or antiparallel pairs of **1** via =C–H···O interactions, which serves as a possible ion-permeable membrane channel structure of polyene macrolide, which may be responsible for the antifungal activity in cell membranes.

Many polyene macrolides produced by *Streptomyces* species show potent antifungal properties, and provide clinically important or efficient therapies against animal and human infectious diseases caused by pathogenic fungi.[1] Amphotericin B,[2] nystatin A1,[3] and pimaricin[4] are the most popular polyene macrolides used for treating fungal infections. It has been postulated that these macrolides target the cytoplasmic membranes of fungi, where they interact selectively with ergosterol, causing a disorganization of the membrane structure, leading to a leakage of cellular materials and, consequently, the cellular death of fungi. Their ability to form ion-permeable membrane channels when they associate with cellular membranes is the primary cause of their activity.[5] Despite their clinical use for over thirty years, the channel structures that they form in membranes and their selective affinity mechanism for sterol, on the basis of an experimental molecular explanation at the atomic resolution, have not yet been clarified, although many studies have investigated the association of polyene macrolides with membranes of various compositions.[6]

To solve these pending subjects, crystal structure analyses of polyene macrolides appear to be essential, because it is expected that the intermolecular association mode formed in their aggregated crystal structures could provide a clue to channel structure formation in the membrane and sterol interacting. Despite being such important information sources, only two such reports on amphotericin B (**3**)[7] and roxaticin[8] have been reported so far. Therefore, we determined the crystal structure of chainin (**1**) of disparate nature and activity,[9] and compared the intermolecular association mode with related compounds to clarify the specific interaction force intrinsic to the polyene macrolide.

Chainin (**1**), a pentaene macrolide antibiotic isolated from *Chainia* species,[10] has the amphiphilic structure of five linearly arranged polar OH groups and a hydrophobic conjugated pentaene structure. We are interested in the crystal structure of **1** because it has the simplest structure among polyene macrolides; thus, the molecular packing pattern in the crystal could be expected to reflect the intermolecular association mode due to the amphiphilic structural elements of **1**, which would be related to the functionally important channel formation of polyene antibiotics in cell membranes. In this paper, we report on the detailed X-ray results of **1**. Furthermore, we discuss **1** in terms of its molecular conformation stability and its concerted =C–H···O interactions observed in its crystal structure, which was studied by quantum-chemical ab initio calculations. A barrel-stave assembly comprising eight **1** molecules linked with intermolecular =C–H···O interactions is proposed as a possible ion-permeable membrane channel structure in this paper. These data are important for understanding the structure-activity relationship of the polyene macrolide. The chemical structures used in this work are shown in Fig. 1.

Results and Discussion

Molecular Conformation of Chainin in the Crystal Structure. The molecular conformation of **1** in the crystal structure is shown in Fig. 2. Because the flack χ parameter[11] ($= -0.014$), which was calculated from an X-ray single-crystal structure analysis of *p*-bromobenzene sulfonate of chainin

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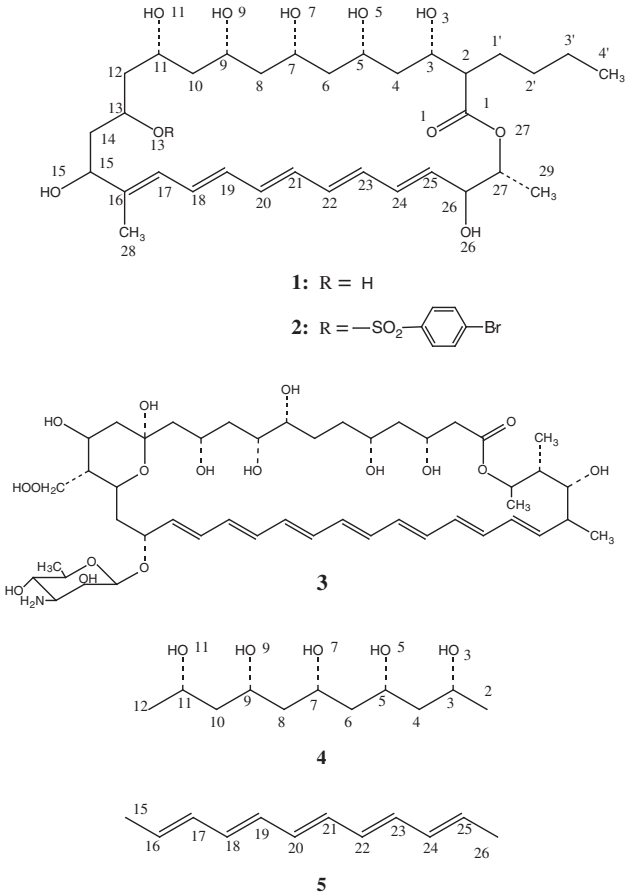


Fig. 1. Chemical structures of chainin (**1**), its *p*-bromobenzene sulfonate derivative (**2**), amphotericin B (**3**), and model compounds **4** and **5** used for the =C–H···O interaction, together with the atomic numberings of **1**, **4**, and **5**.

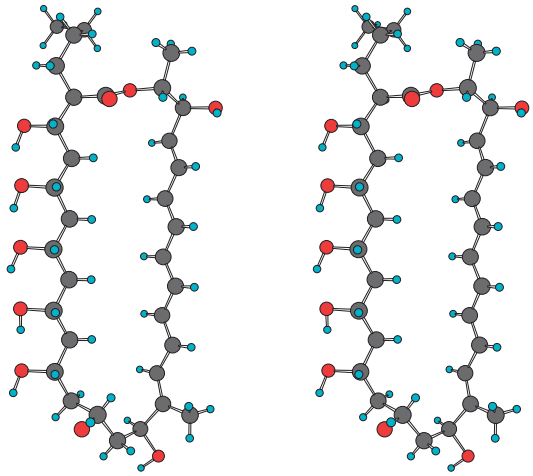


Fig. 2. Stereoscopic molecular conformation of **1**, viewed perpendicular to the plane of OH groups.

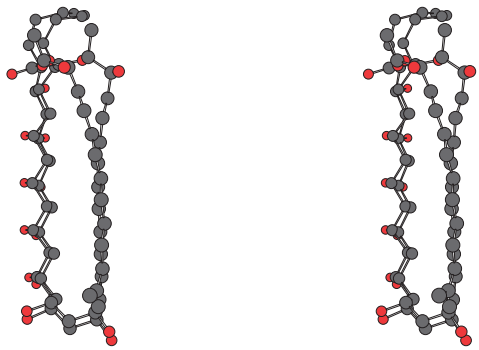


Fig. 3. Stereoscopic conformational comparison between the X-ray (back figure) and the geometry-optimized (front figure) structures of **1** molecule. The averaged shift between the same atoms of both molecules is 0.613 Å.

(**2**), was almost equal to zero, it was clear that the absolute structures of **1** and **2** should be as shown in Fig. 1. The molecule is characterized by a planar orientation of five OH groups on one side and a conjugated unsaturated plane on the other side, forming an extended rectangular 28-membered ring approximately 5 Å in width and 15 Å in length. The pentaene chromophore is composed of five conjugated double bonds from C15 to C26 [1.434–1.496 Å for single bond and 1.324–1.351 Å for double bond] and is in the *trans* configuration of a fully extended plane. The saturated chain from C2 to C12 on the opposite side of the macrolide ring as the chromophore has a single bond of 1.506–1.527 Å and forms the *trans* extended conformation [torsion angle = 173.4–182.1°], and the plane of five OH groups takes a dihedral angle of 26.7° with respect to the pentaene unsaturated plane. Because this dihedral angle was 6.4° in **2**, it is suggested that the rotation of the unsaturated plane is allowed to some extent within the 28-membered ring of chainin. The H positions of the OH groups were all determined by the difference Fourier map. Five OH groups from O3 to O11 [C–O = 1.420–1.450 Å] participated in the intramolecular O–H···O hydrogen-bonded six-membered ring formation. Since the hydrogen-bonded donor–acceptor pairs between these neighboring OH groups were opposite for **1** and **2**, such a concerted six-membered ring formation could depend on the hydrogen-bond formation of the terminal OH, i.e., the terminal O11H is hydrogen-bonded to O1, which is translated by a two-fold screw symmetry of the parent atom in the crystal of **1**, whereas the terminal O3H is hydrogen-bonded to O26, which is translated by one unit cell in the crystal of **2**.

Conformational Stability of Extended 28-Membered Ring of Chainin. To investigate the energy stability and rigidity of the planar 28-membered ring structure of **1** observed by X-ray crystal analysis, geometry optimization was performed using ab initio quantum chemical calculations with the Gaussian 98 program system (in vacuo).[12] The superimposition of the starting X-ray and optimized structures of **1** is shown in Fig. 3. The results clearly indicate that the extended and rectangular conformation of the 28-membered ring is stable and relatively rigid, and is based on the intrinsic character of **1**. The largest discrepancy was observed at a torsion angle of C24–C25–C26–C27 (−126.6° for X-ray structure and −100.43° for optimized structure), and the remaining angles were all within ±12°. This means that any constraint imposed on the ring structure is mainly dissolved at the C25–C26 bond; the bond length (=1.507 Å) of the optimized structure shows that the olefinic unsaturated conjugation is completely terminated at the C25 atom.

Table 1. Atomic Charges (esu) of Conjugated Double Bonds and Hydroxyl Groups, Calculated from the Optimized Structure of **1**

Conjugated double bond							
Atom	Charge	Atom	Charge	Atom	Charge	Atom	Charge
C17	−0.15899	C18	−0.13729	C19	−0.13367	C20	−0.13698
H17	0.12680	H18	0.13500	H19	0.13254	H20	0.13338
C21	−0.13310	C22	−0.13664	C23	−0.13145	C24	−0.12592
H21	0.13405	H22	0.13356	H23	0.13569	H24	0.14216
C25	−0.18620						
H25	0.15823						
Hydroxyl group of saturated chain							
Atom	Charge	Atom	Charge	Atom	Charge	Atom	Charge
O3	−0.69017	O5	−0.70712	O7	−0.70923	O9	−0.71034
H3	0.38097	H5	0.38709	H7	0.38836	H9	0.38429
O11	−0.68911						
H11	0.34907						

Crystal Packing of Chainin Molecules. No solvents were included in the **1** and **2** crystals. The crystal structure is characterized by a herringbone arrangement of molecules along the *c*-direction (Fig. S1), where the respective molecules incline to each other at an angle of approximately 130°. Two long molecular axes of conjugated double bonds and saturated chains are arranged in a stackwise fashion with a two-fold screw symmetry along the *b*-direction and unit-cell translation symmetries along the *a*-direction, forming two-dimensional layers expanded in the *a*- and *b*-directions. The layers expanding in the *c*-direction are connected by van der Waals contacts with the neighboring butyl (C1′–C4′) and methyl (C29) side chains. These hydrophobic channels appear not to be strong enough to fix the stacking layers, because the crystals easily crack in the direction perpendicular to the *c*-axis.

Intermolecular Association Mode of Chainin Observed in Crystal Structure. The antifungal activity of polyene macrolides is believed to be due to the ability to form ion-permeable membrane channels.[5] To investigate the possible channel structure in cellular membranes, a crystal-structure analysis of **1** was carried out, hoping that the intermolecular interaction formed in the crystal reveals the essential driving-force of the channel structure formation. Consequently, a molecular packing pattern analysis suggested two characteristic association modes (Fig. S2). These modes would have originated in the intermolecular association of **1**, rather than in the crystal-packing requirement, because the charge distribution of the respective atoms that participated in these intermolecular associations showed electrostatic coupling in the optimized structure (Table 1).

One characteristic association mode is the stacking alignment between the conjugated unsaturated double bonds (C17–C25) and the five hydroxyl groups (O3–O11) arranged almost on a plane with an average spacing of 4.2 Å (Fig. 4). Although the planes of the unsaturated double-bonded and saturated extended backbone chains slightly overlap, the plane consisting of five OH groups is nearly parallel to the conjugated unsaturated plane (dihedral angle = 26.7°) and the oxygen atoms lie almost on the plane; the distances of O(hydroxyl)···C(double bond) atoms are in the range of 4.4–5.1 Å. Since these distances

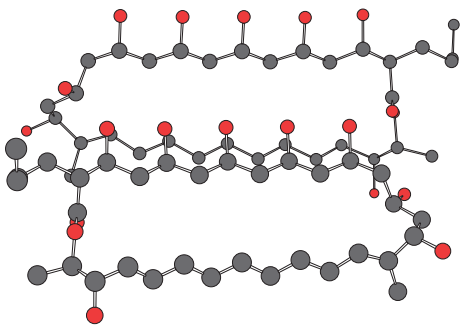


Fig. 4. Molecular association via stacking between conjugated unsaturated double bonds and five hydroxyl groups, translated by two-fold screw symmetry of **1** along *b*-direction.

are significantly longer than the minimum van der Waals contact (=3.1 Å), the single electrostatic OH···π interaction between the hydroxyl group and the double bond could be fairly weak. However, this figure suggests that five concerted interactions produce a detectable intermolecular association.

The other characteristic association mode is the one-dimensional molecular alignment, where the parallel alignments between the unsaturated double-bonded and saturated extended chains are repeated with an average interval of approximately 5.0 Å (Fig. 5). It is clear that this characteristic association feature originates from the chemical structure of **1**. The one-dimensional molecular alignment is primarily formed by the concerted conjunction with weak interactions between the conjugated =C–H groups and the oxygen atoms of the neighboring OH groups translated by one-unit cell, which is further strengthened by a O26–H···O3 hydrogen bond (2.780 Å). Characteristically, the C–H groups of unsaturated double bonds locate near the center of two neighboring OH groups of the partner molecule, thus participating in a bifurcated C–H···O interaction. The ranges of intermolecular H···O distances and C–H···O angles are of 2.816–3.440 Å (average 3.059 Å) and 145–166° (average 155°), respectively. Although these H···O distances are significantly longer than the minimum van der Waals contact (=2.6 Å), the concerted interactions produce

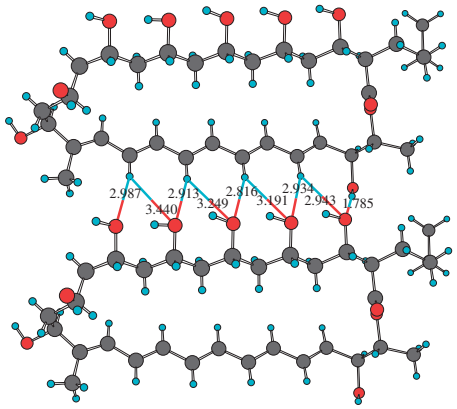


Fig. 5. Intermolecular H···O distances (Å) between olefinic =C–H groups of original **1** and hydroxyl O atoms of **1** translated by one-unit cell along *a*-axis.

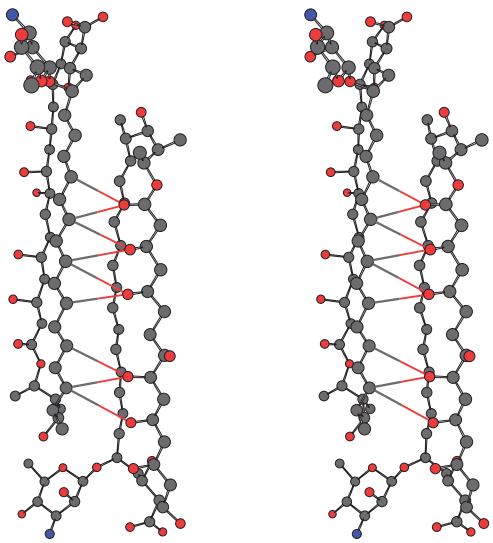


Fig. 6. Stereoscopic view of intermolecular association of **3** via =C–H···O interactions (thin lines) formed in the crystal structure. The concerted =C–H···O interactions are formed between the two neighboring molecules related with the one-unit cell translation after the operation of a two-fold screw symmetry.

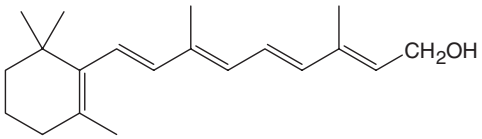
remarkable force that induces a definite association of **1** molecules, because similar intermolecular associations have also been observed in the **2** and **3**[7] crystal structures (Fig. 6). Therefore, it would be reasonable to consider that this concerted =C–H···O interaction is a common structural element for intermolecular assemblies based on the structural feature of polyene macrolide antibiotics.

IR Spectral Study of Intermolecular C–H···O Interactions. To estimate the strength of interactions between the unsaturated double bonds and the OH groups, changes in the stretching wavenumbers of olefinic C–H and hydroxyl O–H groups were determined by the IR measurement. The results are given in Table 2. The O–H and C–H stretching frequencies of the crystal of **1** in KBr were reduced by $\Delta\nu = -120$ and $-22\,\mathrm{cm}^{-1}$, respectively, as compared with the values unchanged by the repeated dilutions of **1** in a $CHCl_3$ solution. Also, the C–H stretching frequency in the crystal of **1** was

Table 2. Stretching Wavenumbers in cm^{-1}, Observed at 20 °C

Sampling	ν_{O-H}	$\nu_{=C-H}$
Dilute solution of **1** in $CHCl_3$[a)]	3472	2960
Crystal (in KBr) of **1**	3352	2938
Crystal (in KBr) of **7**		2986

a) Convergent value obtained upon serial dilution of $CHCl_3$.



7

Chart 1.

reduced by approximately $\Delta\nu = -48\,\mathrm{cm}^{-1}$, as compared with that in the crystal of **7** (Chart 1), measured under the same conditions. These shifts towards the lower frequency side clearly indicate the existence of appreciable =C–H···O interactions in the crystal of **1**.

Ab Initio Calculations of Concerted Multiple =C–H···O Interactions. Noncovalent intermolecular forces play a major role in determining the structures of biological macromolecules and in mediating their biological functions. For intermolecular interactions, the focus has been on hydrophobic interactions, hydrogen bonding, and ion pairing. Among these interactions, hydrogen bonds of the type X–H···Y (X = O, N and Y = O, N, halogen) are generally considered as major forces in the selective or specific molecular recognition, or both. However, it has been increasingly recognized that weak interactions, such as C–H···O,[13] C–H···N,[14] C–H···π,[15] N–H···C,[16] cation···π,[17] and anion···π[18] interactions, also play an important role in the stabilization and functions of biological macromolecules. Thus, further structural authentication of an as yet undiscovered weak interaction is important, because it is necessary to reveal the complex biological function of biomolecules. Here, the X-ray structure of **1** showed that the concerted =C–H···O interactions or the stacking interactions between the conjugated double bonds and the multiple hydroxyl groups could represent a hitherto unrecognized, but significant, contribution to the intermolecular association of chainin (**1**) or its related polyene macrolide antibiotics, such as amphotericin B (**3**). These types of interactions, in which the lone pairs of O atom in the OH group chelate in part to a H atom covalently bound to an unsaturated carbon atom or stacks on unsaturated π bonds, have not been well recognized as weak interactions.

To confirm the strength of these interactions quantitatively, the structures of **4** and **5** (Fig. 1), the geometries of which were optimized by ab initio quantum chemical calculations using the Gaussian 98 program system (in vacuo), were arranged so as to achieve the same orientation as the corresponding structural moieties of the two neighboring **1** molecules observed in the crystal structure (Fig. 5), as shown in Fig. 7; hereafter, this molecular arrangement of **4** and **5** is named **6**. Obviously, the atomic charges of the optimized structures of **4** and **5** show that the molecular orientation of **6** is induced by electrostatic interactions between the olefinic C–H proton

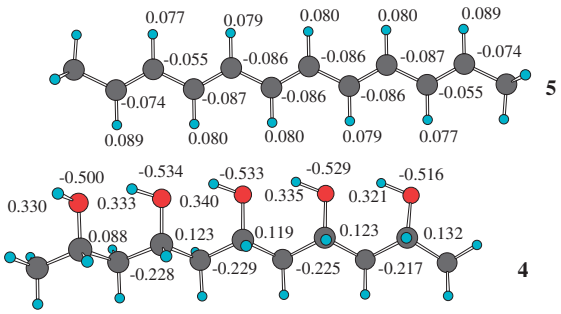


Fig. 7. Optimized structures and atomic charges (esu) of **4** and **5**. Both structures are arranged in the same orientation as the corresponding structural moieties between two neighboring **1** molecules shown in Fig. 5. The atomic charges of **4** are given for the OH groups and saturated backbone carbon atoms.

and the hydroxyl oxygen atoms, where the charges of the olefinic hydrogen atoms (0.077–0.089) are coupled to the hydroxyl oxygen atoms (−0.500–−0.534). To investigate whether the orientation of **6** is the most stable structure, the geometry of **6** was optimized using ab initio calculations; and the optimized structure of **6** is shown in Fig. 8.

The optimized structure of **6** was considerably different from the starting structure concerning the spatial orientation between **4** and **5**. In particular, the O–H bonds were almost at right angles to the olefinic C–H bonds (dihedral angle = 111.2°), which is in contrast with the nearly parallel orientation between both bonds at the starting structure (dihedral angle = 167.7°). During the optimization steps, the OH groups of **4** and the olefinic C–H groups of **5** approached each other gradually, until they were linearly aligned and had H···O distances of 2.229–2.484 Å (average, 2.356 Å) and C–H···O angles of 144.9–166.0° (average, 157.1°). Since this distance is considerably shorter than the sum of the van der Waals radii of 2.6 Å, it is apparent that the weak =C–H···O interactions at the starting stage changed to =C–H···O hydrogen bonds at the optimized stage. The total energy (−1251.675827 A.U.) of the optimized structure was more stable by −25.076 kcal mol^{-1} than the sum of the total energies of the optimized structures of **4** (−794.679728 A.U.) and **5** (−456.956136 A.U.), indicating structural stabilization due to multiple =C–H···O hydrogen bonds, where an energy of −5.015 kcal mol^{-1} per hydrogen bond is stabilized.

On the other hand, the stacking alignment with an average spacing of 4.2 Å between the conjugated unsaturated double bonds and the linearly arranged OH groups arranged almost on a plane was also considered as a characteristic association mode that originates from the chemical structure of **1** (Fig. 4). Because optimized calculations were not performed for this stacked association, no definite conclusion could be drawn. However, a comparison of the respective atomic charges in the optimized structures of **4** and **5** suggests that the stacking alignment is a **1**-specific association mode via the electrostatic interactions between the H atoms of olefinic C–H bonds and the O atoms of hydroxyl OH groups.

Possible Channel Structure of Eight Chainin Molecules. From the above-mentioned results, the concerted =C–H···O interactions were suggested to be an important force in the intermolecular association of polyene macrolides, although the existence of these interactions has been hitherto unrecognized. On the other hand, it is generally believed that the major biochemical function of polyene macrolide is based on the formation of an ion-permeable channel across a lipid bilayer.[5] The present results have led us to imagine that such channel formation may be achieved by a parallel or antiparallel arrangement of polyene macrolide via the concerted =C–H···O interactions between the multiple unsaturated double bonds and the linearly arranged OH groups within the molecular structure. Although details of the molecular architecture of the ion-permeable channel have not yet been fully established, a barrel-stave assembly consisting of eight pairs of molecules has been proposed as a possible channel structure of polyene macrolide.[5,19] Crystal analyses of **1**, **2**, and **3** showed three different intermolecular association patterns via the =C–H···O interactions (Fig. 9a), and the formation of the eight-pair barrel-stave assembly of **1** is possible by a combination of these patterns (as illustrated in Fig. 9b); such an assembly may be more easily formed under a nonpolar lipid membrane environment.

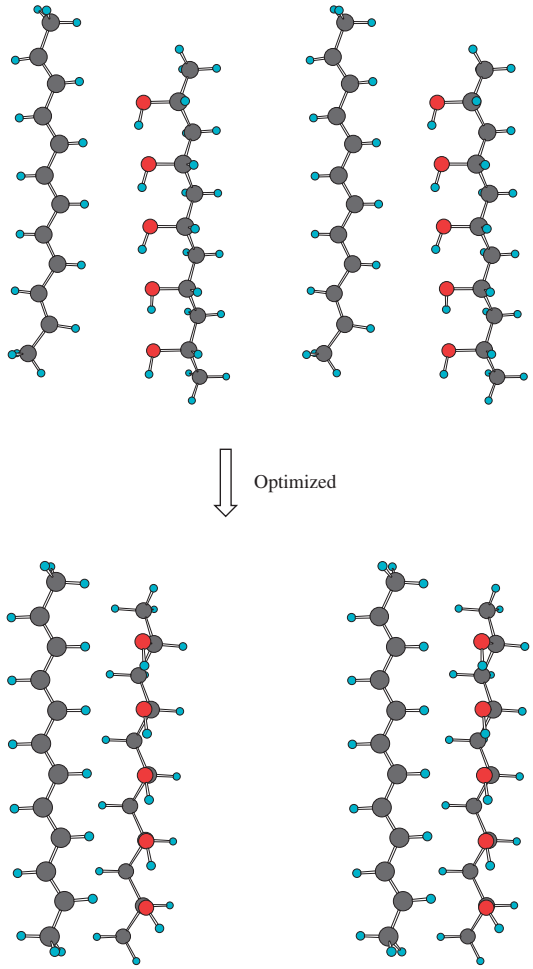


Fig. 8. Stereoscopic views of the starting (upper) and optimized (lower) structures of **6**. The optimized structures of **4** and **5** were used as the starting structure for the geometry optimization of **6**.

The ab initio calculations of concerted multiple =C–H···O interactions indicated the energetic preference of the perpen-

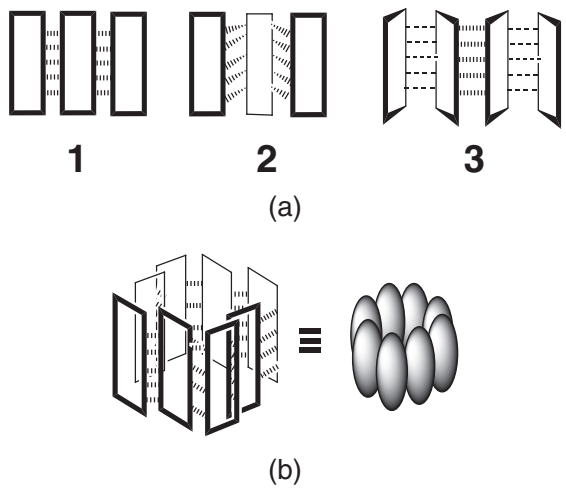


Fig. 9. (a) Three types of association pattern of polyene macrolide molecules via concerted =C–H···O interactions, in which the rectangular or lozenge boxes show the molecules, and the broken lines represent the =C–H···O interactions. (b) A channel model consisting of eight molecules via =C–H···O interactions.

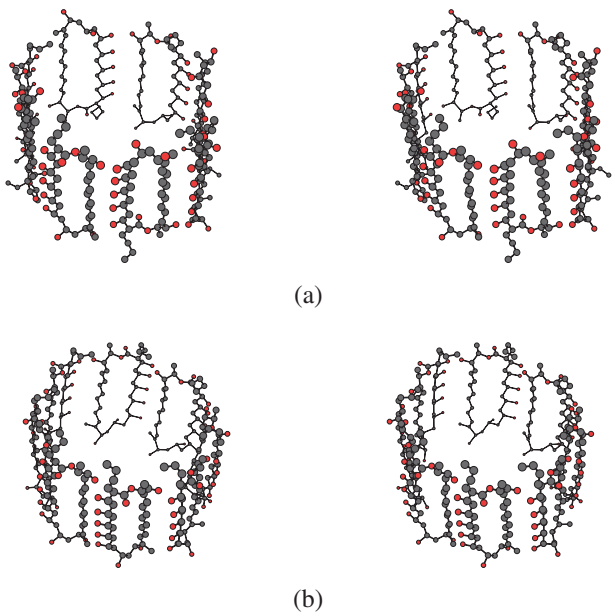


Fig. 10. Stereoscopic view of energy-minimized barrel-stave assembly of eight-pair parallel (a) or antiparallel (b) **1** molecules via multiple intermolecular C–H···O interactions.

dicular C–H···O (= ~111°) orientation between the neighboring chainin molecules. Therefore, for model building of the barrel-stave assembly, the symmetric channel structure with the circularly arranged eight optimized geometries of **1** was constructed, where the C–H···O angle was close to 120°. Two types of molecular alignment, parallel and antiparallel, were considered (Fig. 10), and then subjected to energy minimization to eliminate the poor atomic contacts by the AMBER program.[20] Consequently, the parallel assembly was shown to be more stable by 8.3 kcal mol^{-1} than the antiparallel one, although both assemblies were relatively fragile in vacuo. It is important to investigate whether or not such a channel structure exists stably in a cell membrane, because its structure would become labile due to hydrophobic interactions between the polyene moiety of **1** and the hydrocarbon chain of lipid and by hydrophilic interactions between the OH groups of **1** and polar atoms of waters and/or lipid. Therefore, molecular-dynamics simulations of these assemblies in an aqueous solution and the ergosterol-incorporated assemblies in a phospholipid membrane are now in progress to clarify the actual channel model of polyene macrolides.

In conclusion, the present work clarified that concerted weak =C–H···O interactions between unsaturated double bonds and hydroxyl OH groups are structurally important and should be considered as a new structural element for the intermolecular interactions of polyene macrolide antibiotics.

Experimental

Preparation of Chainin. A loopful of a mature slant culture of *Streptomyces* sp. TP-A0625 on Bennet's agar was inoculated into a 500-mL K-1 flask containing 100 mL of the seed medium (pH 7.0) consisting of 1% soluble starch, 0.5% glucose, 0.3% NZ-case, 0.2% yeast extract, 0.5% tryptone, 0.1% K_2HPO_4, 0.05% $MgSO_4 \cdot 7H_2O$, and 0.3% $CaCO_3$. The flask was incubated at 30 °C for 4 days on a rotary shaker (200 rpm). Three-mL aliquots of the seed culture were transferred into one hundred 500-mL K-1 flasks, each containing 100 mL of the production medium (pH 7.0), consisting of 0.5% glucose, 2% glycerol, 2% soluble starch, 1.5% Pharmamedia (Trader's Protein), 0.3% yeast extract, 2.18% KH_2PO_4, 1.48% Na_2HPO_4, and 1% HP-20 resin (Mitsubishi Chemical). Fermentation was carried out at 30 °C for 7 days on a rotary shaker (200 rpm). The fermentation broth (10 L) was extracted with acetone (10 L) and the supernatant was separated from mycelia by centrifugation. The supernatant was evaporated and the resultant aqueous solution was applied to an HP-20 resin column after the pH was adjusted to 7.0. The column was eluted with 80% acetone, the eluate was evaporated and the remaining product was extracted with 1-butanol. The organic layer was then concentrated in vacuo to give a crude extract (3.65 g). The crude extract was washed with 30% aqueous methanol, and **1** (2.09 g) was obtained as a yellow crystalline solid. Recrystallization from CH_2Cl_2–MeOH provided fine yellow needles with melting points of 235–240 °C (decomposition).

Preparation of *p*-Bromobenzenesulfonate Derivative 2. **1** (100 mg, 0.16 mM) was dissolved in a solution of *p*-bromobenzenesulfonyl chloride (200 mg, 0.78 mM) in dry pyridine (20 mL) at 4 °C. After 18 h, the reaction mixture was poured onto ice-water and extracted with ethyl acetate. The organic layer was washed successively with a $CuSO_4$ solution, water, and brine, and dried over anhydrous Na_2SO_4. After filtration, the organic layer was concentrated to give a crude material. It was then sequentially chromatographed on silica gel and an ODS column to afford a derivative (13 mg, 9.4% yield). The ESI-TOF-MS of the derivative gave an $[M + Na]^+$ at m/z 851.1 (88%), 852.1 (38%), 853.1 (100%), and 854.1 (29%). This isotope peak distribution suggested the presence of a bromine atom in the molecule, and thus the molecular formula was determined to be $C_{39}H_{57}O_{12}SBr$. The *p*-bromobenzenesulfonate of **1** was slowly crystallized from methanol–ethyl acetate at 4 °C to afford a light-yellow prism of **2**.

X-ray Crystal Analyses of 1 and 2. Yellow single crystals of **1** and **2** were prepared from aqueous methanol by slow evaporation at 293 K. X-ray data were collected with a Bruker SMART

APEX CCD camera using graphite-monochromated Mo Kα radiation ($\lambda = 0.71069$ Å) at 263 K. The crystal data are as follows: **1**: $C_{33}H_{54}O_{10}$, MW. 610.76, orthorhombic, $P2_12_12_1$, $a = 8.952(1)$ Å, $b = 9.966(1)$ Å, $c = 37.371(4)$ Å, $V = 3334.2(7)$ Å^3, $Z = 4$, crystal size $= 0.4 \times 0.2 \times 0.1$ mm^3, $D_{calcd} = 1.217$ g cm^{-3}, $F(000) = 1328$, $\mu = 0.089$ mm^{-1}, measured reflections = 20360, R(int) = 0.0636, merged reflections = 7782, reflections $> 2\sigma(I) = 4009$; **2**: $C_{39}H_{57}O_{12}SBr \cdot CH_3OH$, MW. 861.86, orthorhombic, $P2_12_12_1$, $a = 9.589(1)$ Å, $b = 17.720(3)$ Å, $c = 26.227(4)$ Å, $V = 4456.5(11)$ Å^3, $Z = 4$, crystal size $= 0.40 \times 0.08 \times 0.05$ mm^3, $D_{calcd} = 1.285$ g cm^{-3}, $F(000) = 1824$, $\mu = 1.028$ mm^{-1}, measured reflections = 25189, R(int) = 0.0775, merged reflections = 10240, reflections $> 2\sigma(I) = 3896$. Crystal structures were determined by a direct method using the SHELXS-97 program.[21] The positional parameters of non-H atoms were refined using the full-matrix least-squares method with anisotropic temperature parameters using the SHELXL-97 program.[22] The atomic scattering factors and the terms of anomalous dispersion corrections were taken from International Tables for X-ray Crystallography.[23] The H positions of the OH groups were all determined from a difference Fourier map, while those of the other H atoms were calculated based on their stereochemical requirements. They were treated as riding with fixed isotropic displacement parameters (Uiso = 1.2 Ueq for the associated C atoms, or Uiso = 1.5 Ueq for methyl C or O atoms), and were not included as variables for the refinement. The function of $\Sigma w(F_o^2 - F_c^2)^2$ was minimized using the weighting scheme $w = 1/[\sigma^2(F_o^2) + (0.1000P)^2]$, where $P = (F_o^2 + 2F_c^2)/3$. At the final stage of the X-ray analysis, the data for the structure refinement were as follows: **1**: parameter for refinement = 388, $R_1 = 0.0696$ (for $I > 2\sigma(I)$), $wR_2 = 0.1642$ (for all data), GOF = 1.002, shift/esd < 0.002, and flack χ parameter[11] = 0.3(16); **2**: parameter for refinement = 496, $R_1 = 0.0614$ (for $I > 2\sigma(I)$), $wR_2 = 0.1603$ (for all data), GOF = 0.887, shift/esd < 0.003, and flack χ parameter = $-0.014(12)$. These data were deposited with the following designations: **1**: CCDC-204142, **2**: CCDC-204143. These can be obtained free of charge at http://www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge, CB2 1EZ, UK; fax: +44 1223 336033; or deposit@ccdc.cam.ac.uk).

IR Spectra. The infrared (IR) spectra of the samples in the crystalline state (KBr disk) or in chloroform solution were measured using a Perkin-Elmer 1720X spectrometer (FT-IR) equipped with a temperature-controlled cell (20 °C). Scanning parameters: region 4000–400 cm^{-1}, resolution 4 cm^{-1}, 32 scans.

Ab Initio Molecular-Orbital Calculations in Vacuo. To investigate the conformational stability of **1**, the molecular conformation obtained by an X-ray crystal analysis was subjected to geometry optimization. To clarify the nature and strength of concerted =C–H···O interactions between conjugated unsaturated double bonds and hydroxyl groups, two model structures (**4** and **5** in Fig. 1) were designed and their geometries were optimized. Using the optimized structures of **4** and **5**, the same associated model as that observed in the crystal structure of **1** was constructed and subjected to geometry optimization; however, no symmetry constraint was imposed during the optimization. Physicochemical data, such as energies and atomic charges, were evaluated from the respective optimized geometries. All ab initio quantum chemical calculations were performed in vacuo with a double ζ 6-31G basis set at the Hartree–Fock level using the program system Gaussian 98,[12] running on a Compar XP1000 workstation of Cyber Media Center, Osaka University.

On the other hand, the barrel-stave assembly was constructed on a Graphic computer, where the eight parallel or antiparallel molecules of **1** were circularly and symmetrically linked with intermolecular =C–H···O interactions, and was subjected to energy minimization using the steepest descents and successive conjugate gradient algorithms of the AMBER molecular simulation program.[20] Because the ab initio quantum chemical calculation showed that the extended 28-membered ring structure of **1** is rigid and stable, the C–H···O interaction mode (distances and twisting and bending angles) connecting the molecular assembly was optimized as variable parameters.

Supporting Information

Figures S1 (stereo view of herringbone crystal packing of **1** molecules, viewed along the *a*-axis), S2 (two types of molecular association mode, constructed by the crystal packing of **1**), and S3 (stereo view of ladder-like =C–H···O linked molecular arrangement of **1**) in PDF format. These materials are available free of charge on the web at: http://www.csj.jp/journals/bcsj/.

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