COMMUNICATION

ChemComm
www.rsc.org/chemcomm

Concerted interaction between conjugated double bond CHs and multiple OHs in polyene macrolide antibiotic chainin: weak =C–H···O interactions responsible for intrinsic molecular assembly†

Yasuko In,[a] Hirofumi Ohishi,[a] Toshimasa Ishida*[a] and Yasuhiro Igarashi*[b]

[a] *Department of Physical Chemistry, Osaka University of Pharmaceutical Sciences, 4-20-1 Nasahara, Takatsuki, Osaka 569-11, Japan. E-mail: ishida@gly.oups.ac.jp; Fax: (+81) 726 90 1068; Tel: (+81) 726 90 1068*

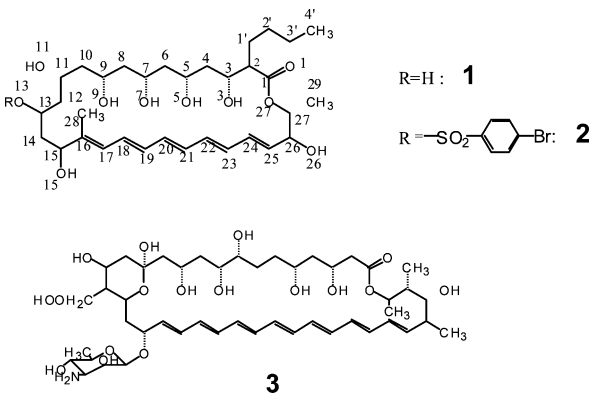
[b] *Biotechnology Research Center, Toyama Prefectural University, 5180 Kurokawa, Kosugi, Toyama 939-0398, Japan. E-mail: yas@pu-toyama.ac.jp; Fax: (+81) 766 56 2498; Tel: (+81) 766 56 7500*

Received (in Cambridge, UK) 18th March 2003, Accepted 3rd June 2003
First published as an Advance Article on the web 12th June 2003

The concerted interactions observed between five conjugated double bond CHs and four hydroxy Os in the crystal of chainin, a polyene macrolide antibiotic, clarified the existence of unprecedented, weak =C–H···O interactions, which is important for forming its intrinsic molecular assembly.

Polyene antibiotics are clinically important as potent antifungal agents. They have amphiphilic structures with multiple polar OH groups and conjugated double bonds within the molecule. Chainin (**1**; Scheme 1), which is a pentaene macrolide antibiotic isolated from *Chainia* species,[1] has four linearly arranged OH groups oriented toward a pentaene structure. We are interested in determining how these amphiphilic structural elements exert their different physicochemical characteristics in terms of molecular conformation and molecular association, because polyene macrolides have been believed to exert their function against a virus or cell through the formation of an ion channel *via* self-formation in the lipid membrane.[2]

Here, we report that the concerted =C–H···O interactions between conjugated double bonds and multiple hydroxy groups represent a hitherto unrecognized, significant contribution to the molecular association of **1** and its related antibiotics. This type of intermolecular interaction, in which the lone pair of O in the OH group chelates in part to two neighboring H atoms covalently bonded to the unsaturated carbon atoms, has not been recognized as a weak, but authentic interaction. However, it is important for the molecular association, because it has been commonly observed in the crystal structures of **1**, its *p*-bromobenzene sulfonate (**2**) and amphotericin B (**3**).[3]



Scheme 1 Chemical structures of **1**, **2** and **3**, together with atomic numbering of **1**.

The molecular conformation of **1**[4] (Fig. 1), the absolute configuration of which was determined by a crystal structure analysis of **2**[5] because there are not enough heavy atoms in **1** for an absolute structure determination, is characterized by the coplanar orientation of four OH groups on one side and the conjugated unsaturated plane on the other side. This forms a planar and rectangular form of approximately 5 Å in width and 15 Å in length. Twelve carbon atoms of the unsaturated plane (C15–C26) forms a *trans*-zigzag plane with dihedral angles of 26.7° and 6.4° with respect to the facing plane of five OH groups for **1** and **2**, respectively, thus allowing a certain extent of freedom in the rotation of the unsaturated plane. The four OH groups participated in forming the concerted intramolecular hydrogen-bonded six-membered ring.

The crystal structure of **1** could be characterized by molecular stacking along the *c*-direction. This one-dimensional molecular packing is primarily formed in conjunction with weak interactions between the conjugated olefinic =C–H moieties on one face of the molecule and the oxygen atoms of the hydroxy groups on the opposite side of an adjacent molecule, translated by one unit cell, in addition to a O(26)–H···O(3) hydrogen bond. Characteristically, the C–H groups are at the mid-position of the OH groups of adjacent molecules, thus forming a bifurcated C–H···O interaction. The intermolecular H···O distances and C–H···O angles are in the ranges of 2.816 to 3.249 Å (average 3.059 Å) and 145 to 166° (average 155°), respectively. Since these H···O distances are significantly longer than the minimum van der Waals contact (= 2.6 Å), in general, it may be considered that there is no specific interaction between the olefinic C–H and OH groups. However, such molecular stacks *via* such concerted =C–H···O interactions have also been observed in the crystal structures of **2** and **3**, and thus these conjugated interactions should be considered as a

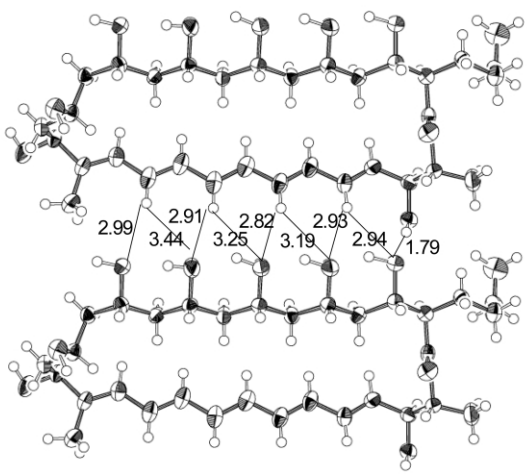


Fig. 1 An ORTEP drawing of two adjacent **1** molecules with 50% probability ellipsoids and intermolecular H···O distances (Å).

† Electronic supplementary information (ESI) available: *ab initio* calculation data for isolated (**4** and **5**) and associated (**6**) model compounds; IR charts of **1** in KBr and chloroform. See http://www.rsc.org/suppdata/cc/b3/b303047d/

DOI: 10.1039/b303047d

 This journal is © The Royal Society of Chemistry 2003

structural element for intermolecular assembly based on the structural features of polyene macrolide antibiotics. The IR spectra also support this =C–H···O interaction. The stretching wavenumbers of the C–H bond are given in Table 1.[6] The O–H and C–H stretching frequencies in crystalline **1** (in KBr) are reduced by $\Delta\nu = -120$ and -22cm^{-1}, respectively, as compared with those of the diluted chloroform solution of **1**. The respective low-field shifts indicate the appreciable O–H···O and =C–H···O interactions, although the strength of the latter would be considerably weakened as compared with conventional hydrogen bonds, because its $\Delta\nu$ is less than one-quarter that of a conventional intermolecular O–H···O hydrogen bond.[7]

In order to confirm this conjugated =C–H···O interaction, the atomic charges of two model compounds (**4** and **5**), in the cases of these being separated and associated in the same manner as chainin in Fig. 1, were calculated at the Hartree–Fock level with the double-ζ 6-31G basis set, performed with the program system Gaussian 98,[8] where the calculations consisted of single points (Fig. 2). When both molecules are associated, it was shown that separation of electronegative and positive charges is caused at the olefinic C–H bond and at hydroxy O–H/O–C bonds. This suggests the existence of intermolecular =C–H···O interactions. The total energy of the associated form was more stable by -14.76 kJ mol^{-1} than the summation of the isolated models, indicating the structural stabilization due to multiple =C–H···O interactions.

The molecular association patterns formed in the crystal structures of **1**, **2**, and **3** are schematically illustrated in Fig. 3.

Table 1 Stretching wavenumbers in cm^{-1}, observed at 20 °C

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

[a] Values unchanged by repeated dilution with $CHCl_3$.

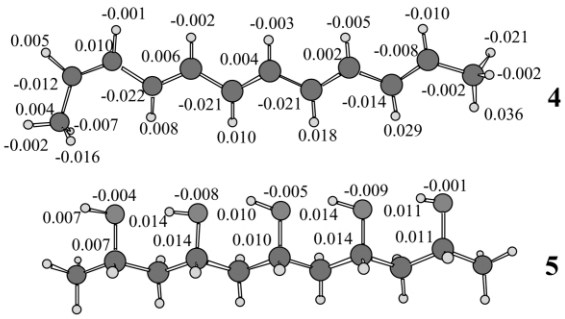


Fig. 2 Difference between the atomic charges (atomic units) of C–H/O–H groups of **4** and **5** in the isolated and associated states.

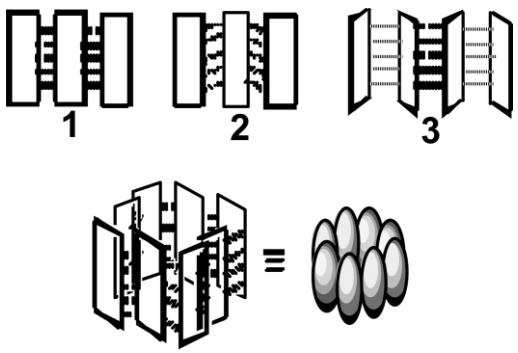


Fig. 3 (Upper) Variation of molecular association formed by =C–H···O interactions. The rectangular or lozenge boxes represent the polyene macrolide molecules of **1**, **2** or **3**. The broken lines represent =C–H···O interactions. (Lower) A tentative column structure.

According to the requirement of the crystal packing, some variations are observed in the molecular assembly. However, a common feature is that these macrolides are arranged in a parallel or antiparallel side-by-side structure by multiple =C–H···O interactions. Generally, it has been considered that their biochemical action is based on the formation of an ion-permeable channel across the lipid bilayer.[9] It is interesting to note that the combination of molecular association patterns of **1**, **2**, and **3** allows column formation without imposing any structural constraint. A tentative model is shown in Fig. 3. Such column formation may be very easy in a nonpolar lipid environment. In conclusion, the conjugated =C–H···O interaction, which has not been remarked upon so far, is functionally important and should be considered as a new structural element for molecular assembly.

Notes and references

1 K. S. Gopalkrishnan, N. Narasimhachari, V. B. Joshi and M. J. Thirumalachar, *Nature*, 1968, **218**, 597.

2 J. Bolard, *Biochim. Biophys. Acta*, 1986, **864**, 257.

3 X-Ray analyses of polyene macrolide antibiotics have been so far reportred only for **3** (P. Ganis, G. Avitabile, W. Mechlinski and C. P. Schaffner, *J. Am. Chem. Soc.*, 1971, **93**, 4560) and roxaticin (H. Maehr, R. Yang, L.-N. Hong, C.-M. Liu, M. H. Hatada and L. J. Todaro, *J. Org. Chem.*, 1989, **54**, 3816). Although roxaticin contains pentaene double bonds and eight OH groups, the crystal structure was analyzed for the hepta-*O*-acetyl roxaticin. Thus, its molecular packing was not considered in this study.

4 *Crystal data* of **1**: $C_{33}H_{54}O_{10}$, $M = 610.76$, $0.40 \times 0.20 \times 0.10$ mm^3, orthorhombic, $P2_12_12_1$, $a = 8.952(1)$, $b = 9.966(1)$, $c = 37.371(4)$ Å, $V = 3334.2(7)$ Å^3, $Z = 4$, $F(000) = 1328$, $T = 293$ K, $\theta_{max} = 28.29°$, $\rho = 1.217$ g cm^{-3}, μ(Mo Kα) = 0.089 mm^{-1}, measured independent reflections = 7782, reflections of $I > 2\sigma(I) = 4009$, parameters used for refinement = 388, $R_1 = 0.0696$ (for $I > 2\sigma(I)$), $wR_2 = 0.1642$ (for all data), GOF = 1.002. Flack χ parameter = 0.3(16). The positions of H atoms were all located on the difference Fourier map and were treated as riding with fixed isotropic displacement parameters (*U*iso = 1.2*U*eq for the associated C or N atoms, or *U*iso = 1.5*U*eq for methyl C or O atoms); their atomic positions were not included as variables for the refinements. CCDC 204142.

5 *Crystal data* of **2**: $C_{40}H_{61}O_{13}SBr$, $M = 861.86$, $0.40 \times 0.08 \times 0.05$ mm^3, orthorhombic, $P2_12_12_1$, $a = 9.589(1)$, $b = 17.720(3)$, $c = 26.227(4)$ Å, $V = 4456.5(11)$ Å^3, $Z = 4$, $F(000) = 1824$, $T = 293$ K, $\theta_{max} = 28.33°$, $\rho = 1.285$ g.cm^{-3}, μ(Mo Kα) = 1.028 mm^{-1}, independent reflections = 10240, reflections of $I > 2\sigma(I) = 3896$, parameters used for refinement = 496, $R_1 = 0.0614$ (for $I > 2\sigma(I)$), $wR_2 = 0.1852$ (for all data), GOF = 0.887, Flack χ parameter = $-0.014(12)$, indicating that the absolute structures of **1** and **2** are correct (H. D. Flack, *Acta Crystallogr., Sect. A*, 1983, **39**, 876). The positions of H atoms were all located on the difference Fourier map. The refinement of H atoms was performed with the same as that of **1**. CCDC 204143. See http://www.rsc.org/suppdata/cc/b3/b303047d/ for crystallographic data in CIF or other electronic format.

6 FT-IR instrumentation: Perkin-Elmer 1720X spectrometer equipped with a temperature-controlled cell. Scanning parameters: region 4000–400 cm^{-1}, resolution 4 cm^{-1}, 32 scans.

7 E. T. G. Lutz, Y. S. J. Veldhuizen, J. A. Kanters, J. H. Van der Maas, J. Baran and H. Ratajczak, *J. Mol. Struct.*, 1992, **270**, 381.

8 Gaussian 98, Revision A.9, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, V. G. Zakrzewski, J. A. Montgomery, Jr., R. E. Stratmann, J. C. Burant, S. Dapprich, J. M. Millam, A. D. Daniels, K. N. Kudin, M. C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G. A. Petersson, P. Y. Ayala, Q. Cui, K. Morokuma, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. Cioslowski, J. V. Ortiz, A. G. Baboul, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, J. L. Andres, C. Gonzalez, M. Head-Gordon, E. S. Replogle and J. A. Pople, Gaussian, Inc., Pittsburgh PA, 1998.

9 G. Fujii, J.-E. Chang, T. Coley and B. Steere, *Biochemistry*, 1997, **36**, 4959; M. Bagiinski, H. Resat and J. A. McCammon, *Mol. Pharmacol.*, 1997, **52**, 560; N. Matsumori, N. Yamaji, S. Matsuoka, T. Oishi and M. Murata, *J. Am. Chem. Soc.*, 2002, **124**, 4180.