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A Macrocyclic Dimeric Diterpene with a C_2 Symmetry Axis

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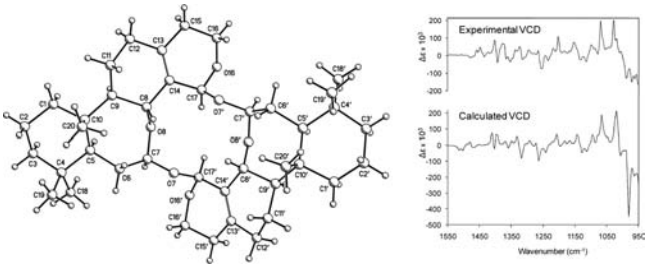
ABSTRACT



An unprecedented macrocyclic dimeric diterpene containing a C_2 symmetry axis was isolated from *Acacia schaffneri*. This compound, named schaffnerine, was characterized as (5*S*,7*S*,8*R*,9*R*,10*S*,17*S*,5′*S*,7′*S*,8′*R*,9′*R*,10′*S*,17′*S*)-7,8:7,17′:16,17:17,7′:7′,8′:16′,17′-hexaepoxy-7,8-*seco*-7′,8′-*seco*-dicassa-13,13′-diene (1) from its spectroscopic data. Comparison of its experimental vibrational circular dichroism spectrum with that calculated using density functional theory, at the B3LYP/DGDZVP level, assigned its preferred conformation and absolute configuration. The latter was confirmed by evaluation of the Flack and Hooft parameters obtained after single-crystal X-ray diffraction analysis.

Acacia schaffneri (Leguminosae) is a tree that grows abundantly in some states of Mexico where it bears the popular names of "huizache" and "huizache chino".[1] This species serves as supply of firewood, and building materials for rural constructions, and it is traditionally used to alleviate stomach pain and toothache.[2] *A. schaffneri* has been the source of three *seco*-oxacassane diterpenes,[3] which are rare in nature; only five of them and two derivatives are known to date.[3−5] During our search for new metabolites of this tree, we isolated from the $CHCl_3$ extracts of its aerial parts an unusual dimeric diterpene that was named schaffnerine (**1**) (Figure 1).

Its structure, containing a novel 12-membered heterocyclic ring with a C_2 symmetry axis, was determined from its physical and spectroscopic data, its relative stereochemistry was secured by single-crystal X-ray diffraction analysis, and its absolute configuration and conformation were accomplished by comparison of the vibrational circular dichroism (VCD) spectra with that obtained using density functional theory (DFT) calculations. In addition, evaluation of Flack and Hooft X-ray parameters was useful to reinforce the absolute configuration of **1**.

Systematic column chromatography fractionation of the $CHCl_3$ extracts of the aerial parts of *Acacia schaffneri*, collected from the municipality of Zempoala, in Hidalgo

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(1) Rico, L. *Bot. Mag.* **2002**, *19*, 152–159.
(2) González-Elizondo, M.; López-Enríquez, I. L.; González-Elizondo, M. S.; Tena-Flores, J. A. *Plantas Medicinales del Estado de Durango y Zonas Aledañas*; Instituto Politécnico Nacional: Mexico City, 1997; p 92.
(3) Manríquez-Torres, J. J.; Torres-Valencia, J. M.; Gómez-Hurtado, M. A.; Motilva, V.; Garcia-Mauriño, S.; Avila, J.; Talero, E.; Cerda-García-Rojas, C. M.; Joseph-Nathan, P. *J. Nat. Prod.* **2011**, *74*, 1946–1951.
(4) Joshi, K. C.; Bansal, R. K.; Sharma, T.; Murray, R. D. H.; Forbes, I. T.; Cameron, A. F.; Maltz, A. *Tetrahedron* **1979**, *35*, 1449–1453.
(5) Lin, A. S.; Lin, C. R.; Du, Y. C.; Lübken, T.; Chiang, M. Y.; Chen, I. H.; Wu, C. C.; Hwang, T. L.; Chen, S. L.; Yen, M. M.; Chang, F. R.; Wu, Y. C. *Planta Med.* **2009**, *75*, 256–261.

(6) Schaffnerine (**1**): White prisms from Et_2O; mp 247−249 °C; $[\alpha]_{589}$ −99, $[\alpha]_{578}$ −103, $[\alpha]_{546}$ −117, $[\alpha]_{436}$ −188, $[\alpha]_{365}$ −269, (*c* 0.9, $CHCl_3$); IR ν_{max} ($CHCl_3$) 1467, 1392, 1265, 1134, 1086, 1049, 1026, 970 cm^{-1}; EIMS *m*/*z* (rel. int.) 636 $[M]^+$ (1), 319 (84), 283 (38), 162 (38), 159 (32), 151 (33), 149 (50), 135 (100), 133 (66), 123 (83), 91 (61); HRESI/APCIMS *m*/*z* 659.4281 $[M + Na]^+$ (calcd for $C_{15}H_{18}O_4 + Na^+$ 659.4282).

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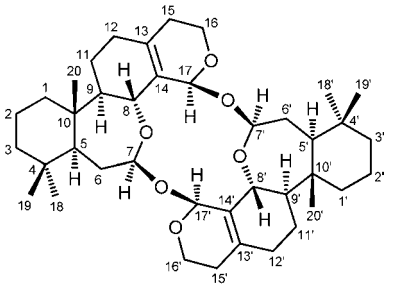

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Figure 1. Structure of schaffnerine (**1**) isolated from *Acacia schaffneri*.

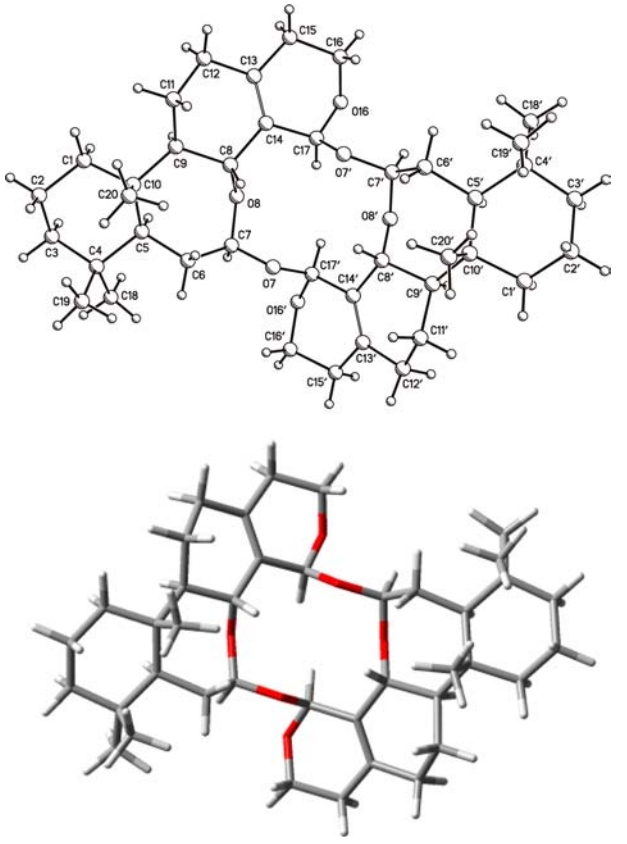

Figure 2. Comparison between the X-ray (top) and the DFT B3LYP/DGDZVP minimum energy (bottom) structures of **1**.

State, México, during March 2009, led to the isolation of schaffnerine (**1**),[6] in addition to known 7,8-*seco*-7,8-oxacassa-13,15-dien-7,17-diol, (−)-7,8-*seco*-7,8-oxacassa-13,15-dien-7-ol,[3] and other known compounds (see Supporting Information), which were identified by NMR spectroscopic analyses including 1D and 2D in comparison with published data. The ^{1}H NMR spectrum of **1** (Table 1) showed two signals owing to acetal hydrogens at δ_H 5.70 (br s, H-17) and 5.26 (dd, J = 10.4, 5.2 Hz, H-7), a methine geminal to oxygen at δ_H 4.52 (br d, J = 8.0 Hz, H-8), and

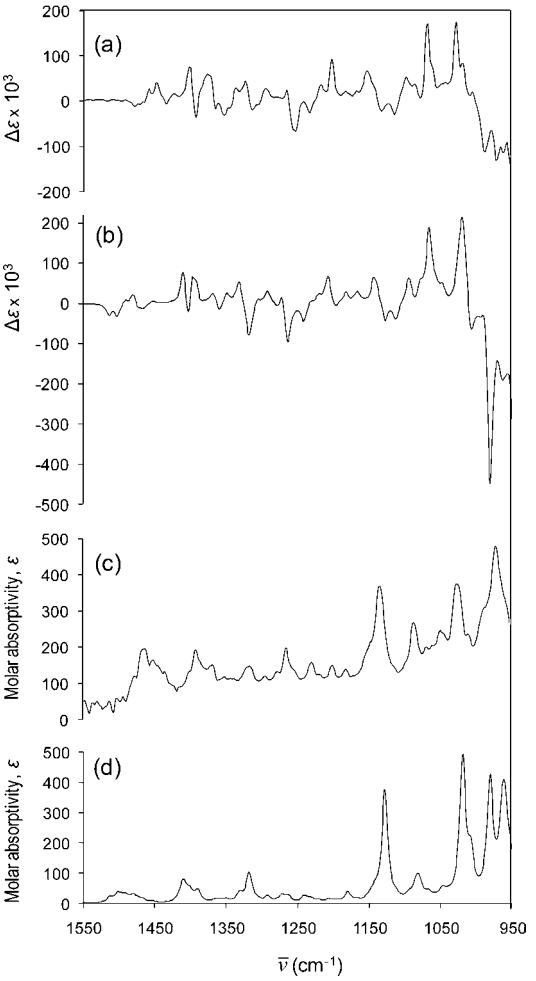

Figure 3. Vibrational spectra of **1**. (a) Experimental VCD in $CDCl_3$, (b) calculated VCD at the B3LYP/DGDZVP level, using anH = 0.981, (c) experimental IR in $CDCl_3$, and (d) calculated IR at the B3LYP/DGDZVP level, using anH = 0.981.

an ABXY system due to the protons of the C-16 methylene geminal to oxygen at δ_H 3.82 (td, J = 11.5, 3.9 Hz, H-16β) and 3.66 (ddd, J = 11.5, 6.6, 1.1 Hz, H-16α) coupled with the hydrogens of the C-15 methylene at δ_H 2.37 and 1.70. This last assignment was confirmed by the ^{1}H−^{1}H COSY experiment. Moreover, signals for three tertiary methyl groups were observed at δ_H 0.88 (Me-20), 0.85 (Me-19), and 0.87 (Me-18). In the ^{13}C and APT NMR spectra, 20 signals were observed, from which two were assigned to quaternary vinyl carbons at δ_C 135.8 (C-13) and 129.6 (C-14), two to acetal carbons at δ_C 94.9 (C-7) and 88.4 (C-17), and one to a methine carbon bearing an oxygen atom at 67.2 (C-8).

At this point, the data suggested the substance under study may be a diterpene-type metabolite. However, a peculiar correlation between the signal at δ_H 5.26, attributed to the acetal proton H-7, and the signal at δ_C 88.4 assigned to the acetal carbon C-17 (actually C-17′),

(7) Cerda-García-Rojas, C. M.; García-Gutiérrez, H. A.; Hernández-Hernández, J. D.; Román-Marín, L. U.; Joseph-Nathan, P. *J. Nat. Prod.* **2007**, *70*, 1167–1172.

Table 1. ^{13}C (100 MHz), ^{1}H (400 MHz), COSY, and HMBC NMR Data for **1** in $CDCl_3$[a]

no.	δ_C	δ_H, mult (J in Hz)	COSY (H → H)	HMBC (H → C)
1α	40.7	0.94, ddd (13.0, 13.0, 3.6)	1β, 2α, 2β	
1β		1.94, ddd (13.0, 4.4, 1.7)	1α, 2α, 2β	
2α	18.7	1.40, m	1α, 1β, 2β, 3α	1, 3
2β		1.47, tt (13.4, 2.9)	2α, 1α, 3α	10
3α	41.6	1.14, td (12.3, 3.4)	2β, 3β, 19	
3β		1.43, m	3α	5
4	34.3	–		
5	47.6	1.28, d (8.7)	6α, 6β	4, 6, 7, 10, 18, 19, 20
6α	31.4	1.65, dd (15.4, 5.2)	5, 6β, 7	4, 5, 7, 10
6β		1.87, ddd (15.4, 10.4, 8.7)	5, 6α, 7	4, 5, 7
7	94.9	5.26, dd (10.4, 5.2)	6α, 6β, 8	8, 17′
8	67.2	4.52, br d (8.0)	9, 12α, 12β, 15α, 15β	13, 14
9	56.4	1.21, m	8, 11α, 11β	8, 10, 11, 12, 20
10	39.0	–		
11α	21.2	1.76, ddd (12.6, 4.0, 2.3)	11β	8, 12, 13
11β		1.30, td (12.6, 4.4),	11α	9
12α	31.8	2.09, br t (14.4)	11α, 11β, 12β	
12β		1.83, ddd (14.4, 3.9, 2.4)	11β, 12α	13, 14
13	135.8	–		
14	129.6	–		
15α	30.2	2.37, br ddd (16.4, 12.1, 7.3)	8, 15β, 16α, 16β, 17	13, 14, 16
15β		1.70, br dt (16.4, 1.9)	8, 15α, 16α, 16β, 17	13, 14
16α	56.2	3.66, ddd (11.5, 6.6, 1.1)	15α, 15β, 16β	15, 17, 13
16β		3.82, td (11.5, 3.9)	15α, 15β, 16α	17
17	88.4	5.70, br s	8, 12α, 12β, 15α, 15β, 16β	7′, 13, 14, 16
18	33.0	0.87, s		3, 4, 5, 19
19	22.3	0.85, s	3α	3, 4, 5, 18
20	15.5	0.88, s		1, 5, 9, 10

[a] Chemical shifts are given for half of the molecule because of its 2-fold rotational symmetry.

Table 2. Relative Energies and Conformational Populations of **1**

conf.	ΔE_{MMFF}[a]	P_{MMFF}[b]	ΔE_{DFT}[c]	P_{DFT}[d]	ΔG_{OPT}[e]	P_{OPT}[f]
1a	0.00	65.0	0.00	98.8	0.00	93.6
1b	0.65	21.7	2.63	1.2	1.59	6.4
1c	0.94	13.3	4.92	–	–	–
1d	5.27	–	4.92	–	–	–

[a] Molecular mechanics energies relative to **1a** with E_{MMFF} = 83.65 kcal/mol. [b] Population in %, calculated from the MMFF energies according to $\Delta E_{MMFF} \approx -RT \ln K$. [c] Single point B3LYP/6-31G(d) energies relative to **1a** with $E_{6\text{-}31G(d)}$ = −1 262 354.37 kcal/mol. [d] Population in % calculated from B3LYP/6-31G(d) energies according to $\Delta E_{6\text{-}31G(d)} \approx -RT \ln K$. [e] Gibbs free energies relative to **1a** with G_{DGDZVP} = −1 261 933.74 kcal/mol. [f] Population in % calculated from Gibbs free energies according to $\Delta G = -RT \ln K$.

observed in the gHMBC spectrum, allowed us to assume the compound had a dimeric structure. Table 1 shows the complete ^{1}H and ^{13}C NMR assignments, which were achieved with the aid of two-dimensional techniques including gCOSY, gHSQC, and gHMBC.

Its HRESI/APCIMS analysis showed $[M + Na]^+$ at m/z 659.4281 (calculated 659.4282 for $C_{40}H_{60}O_6 + Na^+$), confirming the presence of a dimeric compound. The optical activity data of this new natural molecule showed a levorotatory value of $[\alpha]_D$ −99 (c 0.9, $CHCl_3$) in agreement with those of monomeric *seco*-oxacassanes.[3]

The absolute configuration of **1** was established using vibrational circular dichroism (VCD) spectroscopy (see Supporting Information). A molecular model of **1** was subjected to a conformational search following previously described procedures.[3,7] A selection within 10 kcal/mol afforded 14 conformers. Single point DFT calculations with the B3LYP/6-31G(d) level, and narrowing the energy window to a 5 kcal/mol range, left only four conformers with variations in the C(1)−C(2)−C(3)−C(4)−C(5)−C(10) and C(1′)−C(2′)−C(3′)−C(4′)−C(5′)−C(10′) six-membered rings. Geometry optimization of the four

(8) (a) Godbout, N.; Salahub, D. R.; Andzelm, J.; Wimmer, E. *Can. J. Chem.* **1992**, *70*, 560–571. (b) Andzelm, J.; Wimmer, E. *J. Chem. Phys.* **1992**, *96*, 1280–1303.

(9) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *Gaussian 03*; Revision C.02; Gaussian, Inc.: Wallingford, CT, 2004.

Table 3. Dihedral or Torsion Angles for the 1,3,7,9-Tetraoxacyclododecane Moiety of **1** and Comparison with Values for Cyclododecanes and 1,3,7,9-Tetraoxacyclododecanes

compound	D_1	D_2	D_3	D_4	D_5	D_6
1 (DFT)[a,b]	−80	+145	−50	−70	+178	−75
1 (XR)[b,c]	−76	+147	−46	−76	+177	−78
BCDPD[d]	−68	+158	−68	−69	+162	−68
TOCD[e]	*−gauche*	*anti*	*−gauche*	*−gauche*	*anti*	*−gauche*

[a] Measured in the minimum energy DFT B3LYP/DGDZVP molecular model of **1**. [b] D_1 corresponds to [(O7−C7−O8−C8) + (O7′−C7′−O8′−C8′)]/2, D_2 to [(C7−O8−C8−C14) + (C7′−O8′−C8′−C14′)]/2, D_3 to [(O8−C8−C14−C15) + (O8′−C8′−C14′−C15′)]/2, D_4 to [(C8−C14−C15−O7′) + (C8′−C14′−C15′−O7)]/2, D_5 to [(C14−C15−O7′−C7′) + (C14′−C15′−O7−C7)]/2, and D_6 to [(C15−O7′−C7′−O8′) + (C15′−O7−C7−O8)]/2. [c] Averaged torsion angles measured in the X-ray diffraction structure of **1**. [d] Averaged torsion angles measured in a series of 33 *N*,*N*′-biscyclododecyl pyromellitic diimides.[15] [e] Estimated for 1,3,7,9-tetraoxacyclododecane.[16]

structures using the DFT B3LYP/DGDZVP level of theory,[8] as implemented in the Gaussian 03 program,[9] and calculation of the harmonic vibrational frequencies yielded one main conformer for the dimeric diterpene **1**.

Table 2 summarizes the thermochemical data for the conformational analysis of **1**, which shows the energy results of the refinement procedure that led to the main conformer **1a**, accounting for 93.6% of the population (Figure 2). The VCD frequencies of the global minimum **1a** were calculated and plotted using Lorentzian bandshapes and bandwidths of 6 cm^{-1}. Figure 3 provides a comparison between the theoretical and experimental VCD spectra of **1**, which shows a remarkable agreement. Quantitative evaluation of this concordance was done using the Compare*VOA* algorithm.[10]

Application of such treatment allowed us to obtain the optimal anharmonicity factor (anH = 0.981) and the IR spectral similarity index S_{IR} = 90.9%. The VCD spectral similarity was S_E = 94.0% for the correct enantiomer, while that for the incorrect enantiomer was S_{-E} = 3.7%, affording a 100% confidence level for the absolute configuration determination.

A single crystal of **1** obtained from a diethyl ether solution was mounted on an X-ray diffractometer equipped with graphite monochromated Cu Kα radiation and a large charge coupled device detector, which allowed collecting the complete sphere of data. Schafferine (**1**) crystallized in the triclinic system, space group *P*1 (see Supporting Information). The molecular structure (Figure 2) was solved by direct methods and refined to a discrepancy index of 4.0%, which is a good value considering compound **1** has 46 non-hydrogen atoms (CCDC deposition number 952249). The complete sphere data set[11] was also used to calculate the Flack parameter,[12] which for the enantiomer shown in Figure 2 was x = −0.07(14), and the Hooft parameter,[13] which was y = −0.091(4). For the inverted structure, the Flack and Hooft parameters were x = 1.03(14) and y = 0.91(4), respectively, from where it follows the correct enantiomer is the one depicted in Figure 2, in agreement with the VCD results.

Comparison of the DFT calculated minimum energy conformation and that obtained from the X-ray analysis revealed close similarity (Figure 2). In both cases, the 1,3,7,9-tetraoxacyclododecane macrocycle displayed a slightly distorted [3333] conformation according to proposed nomenclature for cyclododecanes.[14] The dihedral angles measured in the molecular model and the torsion angles from the X-ray structure are in good agreement with literature values for cyclododecanes[15] and 1,3,7,9-tetraoxacyclododecane[16] existing in the [3333] conformation (Table 3).

The anti-inflammatory activity of **1** was studied by measuring[17] cyclooxygenase and inducible nitric oxide synthase levels as well as in vitro production of tumor necrosis factor alpha, showing no significant activity. Cytotoxicity against HT-29 human colon, A-549 lung and UACC-62 skin cancer cell lines was also examined,[18] showing IC_{50} of 80, 62, and 76 μg/mL, respectively.

Compound **1** is a large molecule whose absolute configuration has been determined efficiently by VCD spectroscopy. Also, it is an interesting case of a natural molecule having a C_2 symmetry axis to be studied using Flack and Hooft X-ray parameters. Both methods consistently indicated that the structure and absolute configuration of schaffnerine (**1**) is (−)-(5*S*,7*S*,8*R*,9*R*,10*S*,17*S*,5′*S*,7′*S*,8′*R*,9′*R*,10′*S*,17′*S*)-7,8:7,17′:16,17:17,7′:7′,8′:16′,17′-hexaepoxy-7,8-*seco*-7′,8′-*seco*-dicassa-13,13′-diene.

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Note Added after ASAP Publication. The name of compound **1** stereochemistry was incorrect in the version published asap August 12, 2013; the correct version reposted August 29, 2013.

Supporting Information Available. Experimental details for plant material, extraction, isolation, molecular modeling, VCD calculation, and X-ray analysis of **1**. 1D and 2D NMR spectra, and X-ray and DFT atom coordinates of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

(10) Debie, E.; De Gussem, E.; Dukor, R. K.; Herrebout, W.; Nafie, L. A.; Bultinck, P. *Chem. Phys. Chem.* **2011**, *12*, 1542–1549.
(11) Dolomanov, O. V.; Bourhis, L. J.; Gildea, R. J.; Howard, J. A. K.; Puschmann, H. *J. Appl. Crystallogr.* **2009**, *42*, 339–341.
(12) Flack, H. D.; Bernardinelli, G. *Chirality* **2008**, *20*, 681–690.
(13) Hooft, R. W. W.; Straver, L. H.; Spek, A. L. *J. Appl. Crystallogr.* **2008**, *41*, 96–103.
(14) Dale, J. *Top. Stereochem.* **1976**, *9*, 199–270.
(15) Khorasani, S.; Fernandes, M. A.; Perry, C. B. *Cryst. Growth Des.* **2012**, *12*, 5908–5916.
(16) Borgen, G.; Dale, J. *J. Chem. Soc., Chem. Commun.* **1974**, 484–485.
(17) (a) Camacho-Barquero, L.; Villegas, I.; Sánchez-Calvo, J. M.; Talero, E.; Sánchez-Fidalgo, S.; Motilva, V.; Alarcón de la Lastra, C. *Int. Immunopharmacol.* **2007**, *7*, 333–342. (b) de los Reyes, C.; Zbakh, H.; Motilva, V.; Zubía, E. *J. Nat. Prod.* **2013**, *76*, 621–629.
(18) Velázquez-Jiménez, R.; Torres-Valencia, J. M.; Cerda-García-Rojas, C. M.; Hernández-Hernández, J. D.; Román-Marín, L. U.; Manríquez-Torres, J. J.; Gómez-Hurtado, M. A.; Valdez-Calderón, A.; Motilva, V.; García-Mauriño, S.; Talero, E.; Ávila, J.; Joseph-Nathan, P. *Phytochemistry* **2011**, *72*, 2237–2243.