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Brasilane-Type Sesquiterpenoids from *Laurencia obtusa*

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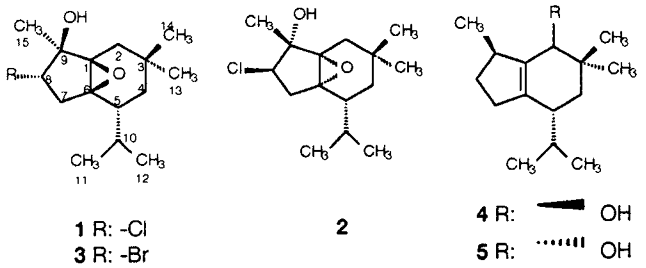
ABSTRACT



1 R: -Cl
3 R: -Br

2

4 R: OH
5 R: OH

Three novel halogenated rearranged sesquiterpenes (1–3) have been isolated along with brasilenol (4) and epibrasilenol (5) from the organic extract of the red alga *Laurencia obtusa*, collected at Symi island in the Aegean Sea, Greece. The new metabolites possess the unusual skeleton of brasilane and contain the unprecedented 1,6-epoxy moiety. The structures of the new natural products, as well as their relative stereochemistry, were established by means of spectral data analyses, including two-dimensional NMR experiments along with molecular calculations.

Red algae of the genus *Laurencia*, present in most coastal waters around the world, receive continuous attention because they seem to be an endless source of structurally unusual secondary metabolites.[1]

In the course of our ongoing investigations toward the isolation of biologically active compounds from marine organisms of the Greek seas,[2] we examined specimens of *Laurencia obtusa* collected off the coasts of Symi Island. Freeze-dried organism was extracted with CH_2Cl_2/MeOH, and the extract was subjected repeatedly to chromatographic separations to finally afford three novel rearranged sesquiterpenes (**1**−**3**) as minor components, along with brasilenol (**4**) and epibrasilenol (**5**).

Compound **1** was purified by means of HPLC separations and isolated as a colorless oil.[3] Both ^{13}C NMR data and HRFAB-MS measurements supported the molecular formula $C_{15}H_{25}O_2Cl$. The EI mass spectrum revealed molecular ion $[M]^+$ isotopic peaks at *m/z* 272 and 274 with intensities of

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(1) (a) Faulkner, D. J. *Nat. Prod. Rep*. **2001**, *18*, 1−49. (b) Guella, G.; Pietra, F. *Helv. Chim. Acta* **2000**, *83*, 2946−2952. (c) König, G. M.; Wright, A. D. *J. Nat. Prod*. **1994**, *57*, 477−485.

(2) (a) Iliopoulou, D.; Vagias, C.; Harvala, C.; Roussis, V. *Phytochemistry* **2002**, *59*, 111−116. (b) Iliopoulou, D.; Roussis, V.; Pannecouque, C.; De Clercq, E.; Vagias, C. *Tetrahedron* **2002**, (in press).

(3) **Data for 1:** colorless oil; $[\alpha]_D$ +44.11 (*c* 0.17, $CHCl_3$); UV λ_{max} (*n*-hexane) (log ϵ) 203.0 (2.61) (nm); IR($CHCl_3$) λ_{max} 3579, 2931, 1460, 1389, 1366, 1203, 1097, 979, 842 cm^{-1}; HRFAB-MS (*m/z*) 255.1502 $[M - H_2O + H]^+$ (calcd for $C_{15}H_{24}O^{35}Cl$ 255.1517); ^1H NMR (C_6D_6, 400 MHz) δ 3.93 (1H, brt, $J = 8.2$ Hz, H-8), 2.39 (1H, dd, $J = 13.6, 7.5$ Hz, H-7β), 1.85 (1H, m, H-5), 1.79 (1H, brd, $J = 15.0$ Hz, H-2β), 1.69 (1H, dd, $J = 13.6, 9.2$ Hz, H-4α), 1.53 (1H, m, H-10), 1.42 (1H, s, 9 −OH), 1.37 (1H, d, $J = 14.7$, H-2α), 1.12 (3H, s, H-15), 1.06 (3H, s, H-14), 1.04 (1H, brdd, $J = 12.9, 5.4$ Hz, H-4β), 0.79 (3H, s, H-13), 0.73 (3H, d, $J = 6.8$ Hz, H-11), 0.67 (1H, dd, $J = 12.9, 12.9$ Hz, H-4α), 0.56 (3H, d, $J = 6.8$ Hz, H-12); ^{13}C NMR (C_6D_6, 50 MHz) δ 79.8, 71.3, 66.7, 65.8, 38.9, 37.7, 35.8, 34.3, 33.3, 29.2, 27.6, 26.6, 22.2, 19.7, 18.5; EIMS 70 eV, *m/z* (rel intensity %) 274, 272 $[M]^+$ (0.6:1.7), 231, 229 $[M - C_3H_7]^+$ (31: 97), 213, 211 (4:12),

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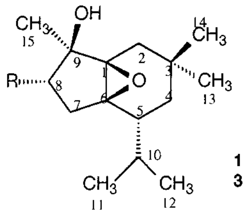
Table 1. ^{1}H (400 MHz) and ^{13}C (50 MHz) NMR Data of Compounds **1**−**3** in $CDCl_3$

	1		**2**		**3**	
position	δ ^{1}H	δ ^{13}C	δ ^{1}H	δ ^{13}C	δ ^{1}H	δ ^{13}C
1		71.4		66.9		70.8
2β	1.85 (brd, 14.5)	33.0	1.80 (dd, 15.0, 2.0)	34.8	1.87 (brd, 15.0)	33.3
2α	1.58 (d, 14.5)		1.49 (d, 15.0)		1.59 (d, 15.0)	
3		29.1		28.7		29.3
4β	1.21 (ddd, 12.7, 5.2, 1.6)	35.4	0.97 (ddd, 13.3, 5.1, 2.0)	32.5	1.21 (ddd, 12.9, 5.1, 1.4)	35.2
4α	0.88 (dd, 12.7, 12.7)		1.15 (dd, 13.3, 12.6)		0.88 (dd, 12.9, 12.9)	
5	1.95 (m)	38.5	1.79 (m)	38.6	1.95 (m)	38.7
6		67.5		66.6		68.2
7β	2.67 (dd, 13.9, 7.3)	37.3	1.66 (dd, 14.0, 9.5)	38.5	2.69 (dd, 13.9, 7.5)	38.5
7α	1.84 (dd, 13.9, 9.2)		2.72 (dd, 14.0, 7.3)		1.99 (dd, 13.9, 9.3)	
8	3.88 (dd, 9.2, 7.3)	65.2	3.91 (dd, 9.5, 7.3)	65.0	3.92 (dd, 9.3, 7.5)	57.7
9		80.1		80.3		79.8
10	1.92 (m)	27.6	1.97 (m)	29.1	1.93 (m)	27.8
11	0.98 (d, 6.6)	22.3	0.93 (d, 7.1)	20.9	0.98 (d, 6.6)	22.2
12	0.84 (d, 6.6)	18.7	0.90 (d, 7.1)	18.2	0.84 (d, 6.6)	18.8
13	0.95 (s)	34.3	0.92 (s)	31.6	0.95 (s)	34.4
14	0.96 (s)	26.4	0.80 (s)	26.3	0.96 (s)	26.4
15	1.22 (s)	19.5	1.22 (s)	19.6	1.27 (s)	21.8
-OH	1.56 (s)				1.80 (s)	

2.9/1, indicating the presence of one chlorine atom. The IR spectrum exhibited strong absorptions for a tertiary hydroxyl group (3579 cm^{-1}) and an isopropyl group (1389 and 1366 cm^{-1}). The ^{13}C NMR spectrum along with the DEPT and HSQC experiments showed the presence of three oxygenated tertiary carbons and a quaternary carbon, three methines, three methylenes, and five methyls. Three carbons were bonded to oxygen, resonating at δ 80.1 (s), 71.4 (s), and 67.5 (s) or 65.2 (d), and one was chlorinated, resonating at δ 65.2 (d) or 67.5 (s). Furthermore, the ^{1}H NMR spectrum revealed the signals of one methine group bonded to oxygen or halogen at δ 3.88 (dd), two tertiary methyls of an isopropyl moiety at δ 0.84 (3H, d, 6.6 Hz) and δ 0.98 (3H, d, 6.6 Hz), and three quaternary methyls at δ 0.95, 0.96, and 1.22. With an unsaturation degree of 3, the structure was suggested to contain three rings. The presence of five methyls excluded the common four methyl skeletons of most sesquiterpenes and suggested a bicyclic brasilane structure.[4]

Since on the basis of the molecular formula there are only two oxygens in the molecule, the structure must contain one hydroxy and one ether functionality. The presence of only one hydroxyl group was confirmed by silylation of a small quantity of **1** with MSTFA (*N*-methyl-*N*-trimethylsilyltrifluoro acetamide).

The carbon connectivities (Table 1) were traced by a series of two-dimensional NMR homonuclear (COSY, INADEQUATE) and heteronuclear experiments (HMBC, CIGAR) (in both $CDCl_3$ and C_6D_6). The use of CIGAR[5] showed important long-range correlations not visible in HMBC, verifying the proposed structure. For compound **1**, two-dimensional INADEQUATE spectra processed with FRED[6] were also valuable for the confirmation of the brasilane skeleton and unambiguous assignments. The two-dimensional INADEQUATE results showed correlations between C-12 (18.7 ppm) and C-11 (22.3 ppm) with C-10 (27.6 ppm) with a $^{1}J_{C-C}$ of 35.1 Hz in both cases. Furthermore, C-10 (27.6 ppm) is connected to C-5 (38.5 ppm) with $^{1}J_{C-C}$ of 34.6 Hz. C-5 (38.5 ppm) is connected to C-4 (35.4 ppm) with $^{1}J_{C-C}$



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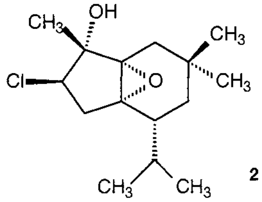
3 R: -Br



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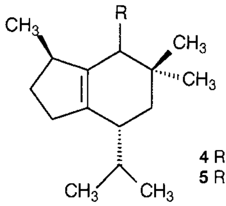

4 R: —OH
5 R: ⋯OH

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[4] (a) Stallard, M. O.; Fenical, W.; Kittredge, J. S. *Tetrahedron* **1978**, *34*, 2077−2081. (b) Caccamese, S.; Amico, V.; Neri, P. *J. Nat. Prod.* **1990**, *53*, 1287−1296. (c) Amico, V.; Caccamese, S.; Neri, P.; Russo, G.; Foti, M. *Phytochemistry* **1991**, *30*, 1921−1927. (d) Wright, A. D.; Konig, G. M.; Sticher, O. *J. Nat. Prod.* **1991**, *54*, 1025−1033. (e) Wright, A. D.; Konig, G. M.; Sticher, O. *Tetrahedron Lett.* **1994**, *35*, 3105−3106.

[5] Krishnamurthy, V. V.; Russell, D. J.; Hadden, C. E.; Martin, G. E. *J. Magn. Reson.* **2000**, *146*, 232−239.

[6] *FRED, Full Reduction of Entire Datasets*, version 2.1; ScienceSoft, LLC; Salt Lake City, Utah.

of 33.9 Hz. C-4 (35.4 ppm) is connected to C-3 (29.1 ppm) with $^1J_{C-C}$ of 34.5 Hz. C-3 (29.1 ppm) is connected to C-14 (26.4 ppm) with $^1J_{C-C}$ of 36.3 Hz. On the other side of the molecule, C-8 (65.2 ppm) is connected to C-7 (37.3 ppm) with $^1J_{C-C}$ of 36.4 Hz. C-7 (37.3 ppm) is connected to C-6 (67.5 ppm) with $^1J_{C-C}$ of 40.6 Hz. C-15 (19.5 ppm) is connected to C-9 (80.1 ppm) with $^1J_{C-C}$ of 34.5 Hz. C-2 (33.0 ppm) is connected to C-1 (71.4 ppm) with $^1J_{C-C}$ of 46.7 Hz. Overall, 10 of the total 16 $^{13}C-^{13}C$ correlations were resolved.

The long-range heteronuclear correlations of C-1 (71.4 ppm) with H-2, H-5, H-7, and H-15; C-6 (67.5 ppm) with H-2, H-4, H-5, H-7, and H-10; C-8 (65.2 ppm) with H-7 and H-15; and C-9 (80.1 ppm) with H-2, H-7, H-8, and H-15, observed in HMBC/CIGAR, confirmed the position of oxygen and halogen at adjacent carbons on the five-member ring. In addition, the correlations of H-11/H-12 with C-10 and C-5; H-13/H-14 with C-3; and H-15 with C-9 confirmed the positions of the methyl groups.

The above data were not sufficient for an unambiguous assignment of chlorine on either C-8 or C-6. Thus, there were six possible structures with the ether bridge between the carbons C-1/C-6, C-1/C-8, C-1/C-9, C-6/C-8, C-6/C-9, and C-8/C-9. Careful analyses of NOESY correlations along with the coupling constants of the ^{1}H NMR spectrum and molecular calculations support the assignment of the ether bridge between C-1 and C-6 and the proposed relative stereochemistry of compound **1**. The observed coupling constants of H-8 (9.2, 7.3 Hz) indicated an axial orientation of this proton, and as a consequence, the ether bridge could not be placed on C-8 carbon. Furthermore, NOESY experiments showed a strong interaction between the deshielded methyl H-15 and the axial proton H-7, revealing an axial configuration for H-15. Consequently, the ether bridge could not be placed either at C-9/C-1 or at C-9/C-6 positions.

Moreover, the strong NOE effect between H-2 (δ 1.58) and H-4 (δ 0.88) showed the cis orientation of these protons and suggested a chair conformation for the six-member ring. Further support came from the diagnostic W-coupling (1.6 Hz) between H-2 (δ 1.85) and H-4 (δ 1.21), indicating the equatorial position of these protons. Strong correlations of H-2 (δ 1.85) with both H-13 and H-14 and of H-2 (δ 1.58) only with H-13 suggested equatorial and axial orientations for H-13 and H-14, respectively. A significant correlation between H-2 (δ 1.58) and H-15 revealed their cis (ax−ax) spacial proximity.

The NOE enhancements between H-4 (δ 0.88) and H-10, H-11, and H-12, were observed in the NOESY experiments recorded in C_6D_6 (in this solvent, H-10 was clearly resolved) and confirmed the equatorial conformation of the isopropyl group. This is further supported by a correlation between H-5 and H-7 (δ 2.67) (observed in both $CDCl_3$ and C_6D_6 solvents) that indicated their cis (ax−eq) orientation.

According to the above observations, the structure of metabolite **1** was proposed as (1*R**,5*R**,6*R**,8*S**,9*R**)-8-chloro-1,6-epoxy-5-isopropyl-3,3,9-trimethylbicyclo[4.3.0]-nonan-9-ol.

Compound **2** was isolated as a colorless oil[7] following HPLC purification. The composition of metabolite **2** was deduced to be $C_{15}H_{25}O_2Cl$ from the HRFAB mass spectrum and the NMR data. Comparison of the spectral data of **2** with those of **1** revealed a great similarity in the structures of the two compounds. The EI LRMS and IR spectra were almost identical with those recorded for **1**. The most significant differences between metabolite **2** and **1** were observed in the ^{1}H NMR chemical shifts of methine H-5 (δ 1.79 in **2**; δ 1.95 in **1**) and methyls H-12 (δ 0.90 in **2**; δ 0.84 in **1**) and H-14 (δ 0.80 in **2**; δ 0.96 in **1**) and in the ^{13}C NMR chemical shifts of quaternary carbon C-1 (δ 66.9 in **2**; δ 71.4 in **1**), methine carbon C-10 (δ 29.1 in **2**; δ 27.6 in **1**), and methyl carbon C-13 (δ 31.6 in **2**; δ 34.3 in **1**). These data led to the assumption that compound **2** was a stereoisomer of **1**.The stereochemistry of **2** was resolved by a combination of two-dimensional NOESY data, analysis of coupling constants, and molecular calculations. The coupling constants of H-8 (9.5, 7.3 Hz) indicated an axial orientation of this proton as in compound **1**, and a strong NOE effect between the deshielded methyl H-15 and the axial proton H-7 (δ 1.66) revealed an axial configuration for H-15. The cross-peaks observed in the NOESY spectrum for H-15 to H-14 implied that these protons were cofacial (β), while the 1,6-epoxide occupies the *a*-face of the molecule.

Moreover, the chair conformation for the six-member ring was confirmed by the strong NOE effect between H-2 (δ 1.49) and H-4 (δ 1.15) and from the characteristic W-coupling (2.0 Hz) between H-2 (δ 1.80) and H-4 (δ 0.97). A significant correlation was also observed between H-2 (δ 1.80) and H-15 revealing their cis (eq−ax) spacial proximity. Strong correlations of H-2 (δ 1.49) and H-4 (1.15) with H-13 indicated the equatorial orientation of H-13. The NOE interactions between H-5 and H-14 revealed the equatorial conformation of the isopropyl group. This assumption was also supported by a correlation between H-5 to H-7 (δ 1.66) indicating their cis (ax−ax) spacial proximity.

On the basis of the above findings, the structure of metabolite **2** was deduced as (1*S**,5*R**,6*S**,8*R**,9*S**)-8-chloro-1,6-epoxy-5-isopropyl-3,3,9-trimethylbicyclo[4.3.0]-nonan-9-ol.

Compound **3** was isolated, after HPLC purification, in minute quantities as a colorless oil.[8] The molecular formula $C_{15}H_{25}O_2Br$ for **3** was deduced from HRFAB-MS data in combination with the NMR spectra.

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(7) **Data for 2:** colorless oil; $[\alpha]_D$ −7.33 (*c* 0.15, $CHCl_3$); UV λ_{max} (*n*-hex) (log ϵ) 202.4 (2.72) (nm); IR($CHCl_3$) λ_{max} 3579, 2930, 1464, 1388, 1368, 1204, 1075, cm^{-1}; HRFAB-MS (*m/z*) 255.1534 $[M - H_2O + H]^+$ (calcd for $C_{15}H_{24}O^{35}Cl$ 255.1517); ^{1}H NMR (C_6D_6, 400 MHz) δ 3.86 (1H, dd, $J = 9.3, 7.5$ Hz, H-8), 2.37 (1H, dd, $J = 13.6, 7.5$ Hz, H-4α), 1.73 (1H, m, H-10), 1.57 (1H, brd, $J = 15.0$ Hz, H-2β), 1.42 (1H, m, H-5), 1.41 (1H, d, $J = 15.0$ Hz, H-2α), 1.27 (1H, dd, $J = 13.6, 9.3$ Hz, H-7β), 1.14 (1H, dd, $J = 13.0, 13.0$, H-4α), 1.10 (3H, s, H-15), 0.93 (3H, d, $J = 6.8$ Hz, H-12), 0.84 (3H, d, $J = 6.8$, Hz, H-11), 0.77 (1H, m, H-4β), 0.74 (3H, s, H-13), 0.50 (3H, s, H-14); ^{13}C NMR (C_6D_6, 50 MHz) δ 80.0, 66.5, 66.1, 65.5, 38.8, 38.5, 35.1, 32.8, 31.6, 29.3, 28.6, 26.2, 21.0, 19.9, 18.4; EIMS 70 eV, *m/z* (rel intensity %) 274, 272 $[M]^+$ (0.6:1.7), 231, 229 $[M - C_3H_7]^+$ (19:58), 213, 211 (1.6:6.3).

(8) **Data for 3:** colorless oil; $[\alpha]_D$ +12 (*c* 0.15, $CHCl_3$); UV λ_{max} (*n*-hexane) (log ϵ) 207.0 (2.72) (nm); IR($CHCl_3$) λ_{max} 3564, 2954, 1462, 1389, 1366, 1212, 1095, 979, 840 cm^{-1}; HRFAB-MS (*m/z*) 299.1024 $[M - H_2O + H]^+$ (calcd for $C_{15}H_{24}O^{79}Br$ 299.1011); EIMS 70 eV, *m/z* (rel intensity %) 275, 273 $[M - C_3H_7]^+$ (84:85), 257, 255 (5:5).

All spectral characteristics were quite comparable to those of metabolite **1**. Comparison of the spectra indicated metabolite **3** to be the C-8 bromo analogue of metabolite **1**. The relative stereochemistry of **3** based on interpretation of the NOE enhancements and molecular calculations was found to be the same as that in metabolite **1**.

Consequently, the proposed formula for compound **3** is (1*R**,5*R**,6*R**,8*S**,9*R**)-8-bromo-1,6-epoxy-5-isopropyl-3,3,9-trimethylbicyclo[4.3.0]nonan-9-ol.

To date, only 11 representatives[4] of the brasilane family, including the metabolites reported herein, have been isolated, all from *Laurencia* species.

Molecular Modeling. The conformational space for compounds **1**−**3** was systematically explored using the Merck[9] (MMFF) force field as implemented in SPARTAN.[10] The energies of the conformers were calculated at the ab initio level using the 6-31G* basis set.

The global minimum energy conformer of molecule **1** [designated **1**_(1)] bears the six−member ring in a chair conformation and the ^{i}Pr group and the Cl atom at equatorial positions (Figure 1). Furthermore, the methyl groups at positions 9 (C-15) and 3*β* (C-14) of the ring system are in an axial orientation, whereas the methyl group at position 3*a* (C-13) is equatorial. The second lowest energy conformer **1**_(2), is calculated to be 4.34 kcal/mol higher in energy than **1**_(1) and differs from the latter only in the conformation of the five-member ring (Figure 2). Hence, in **1**_(2), the Cl atom is axial, while the methyl group at position 9 (C-15) is equatorial.

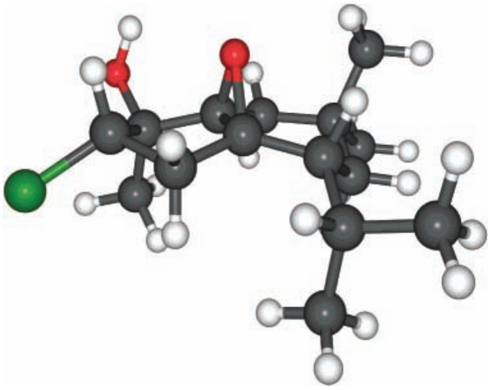

Figure 1. Conformer **1**_(1). Atoms are colored gray (carbon), white (hydrogen), red (oxygen), and green (chlorine).

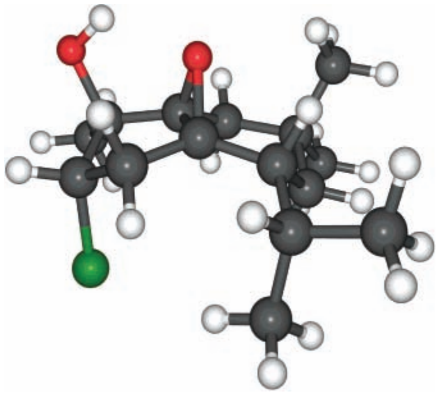

Figure 2. Conformer **1**_(2). Atoms are colored gray (carbon), white (hydrogen), red (oxygen), and green (chlorine).

For conformer **1**_(1), the calculated dihedral angles formed between the hydrogen atoms at positions 5 and 8 (H-5 and H-8, respectively) and their adjacent hydrogen atoms (H-4α/H-4*β* and H-7α/H-7*β*, respectively) are consistent with the corresponding coupling constants measured in the NMR experiments (see Supporting Information). For conformer **1**_(2), the value for the calculated dihedral angle formed between H-4α and H-8 (155.53°) cannot be accounted for by the measured value for the corresponding coupling constant $J_{H-7\alpha/H-8}$ (9.2 Hz). According to the Karplus equations, in the latter case, the value for *J* should have been close to zero. The results of the modeling studies for compound **3** are analogous to those presented above for compound **1**.

The global minimum energy conformer of molecule **2** [designated **2**_(1)] bears the six−member ring in a chair conformation and the ^{i}Pr group and the Cl atom at equatorial positions. Moreover, the methyl groups at positions 9 (C-15) and 3*β* (C-14) of the ring system are in an axial orientation, whereas the methyl group at position 3*a* (C-13) is equatorial. The second lowest energy conformer **2**_(2), is calculated to be 3.34 kcal/mol higher in energy than **2**_(1) and differs from the latter only in the conformation of the five−member ring. Hence, in **2**_(2), the Cl atom is axial, while the methyl group at position 9 (C-15) is equatorial. As for compound **1**, only conformer **2**_(1) accounts for all the NMR data (see Supporting Information).

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Supporting Information Available: Tables of full NMR data for compounds 1 and 2 and molecular modeling. This material is available free of charge via the Internet at http://pubs.acs.org.

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[9] Halgren, T. A. *J. Comput. Chem.* **1996**, *17*, 490−519 and following papers in this issue of *J. Comput. Chem*.

[10] *SPARTAN*, version 5.1.3; Wavefunction, Inc.: 18401 Von Karmen Avenue, Suite 370, Irvine, CA 92612.