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Note

Conformational evaluation and detailed ^{1}H and ^{13}C NMR assignments of eremophilanolides

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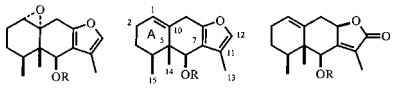
Extensive application of 1D and 2D NMR methodology, combined with molecular modeling, allowed the complete ^{1}H and ^{13}C NMR assignments of eremophilanolides from *Senecio toluccanus*. Comparison of the experimental ^{1}H, ^{1}H coupling constant values with those generated employing a generalized Karplus-type relationship, using dihedral angles extracted from MMX and DFT calculations, revealed that the epoxidized eremophilanolides 1 and 2 show conformational rigidity at room temperature, whereas molecules 3–6, containing an isolated double bond, are conformationally mobile. Copyright © 2004 John Wiley & Sons, Ltd.

KEYWORDS: NMR; ^{1}H NMR; ^{13}C NMR; 2D NMR; *Senecio toluccanus*; Senecionae; Asteraceae; sesquiterpenelactones; eremophilanolides; furanoeremophilanes; conformational analysis

INTRODUCTION

The large genus *Senecio* (Asteraceae) has been studied extensively for their secondary metabolites. Pyrrolizidine alkaloids, eremophilanolides and cacalolides are particularly characteristic from species of this genus.[1] It is well documented that pyrrolizidine alkaloids are toxic to humans and livestock,[2] and recently it has been reported that some furanoeremophilanes and cacalolides show moderate antibacterial[3] strong insect antifeedant[4] and antihyperglycemic[5] activity. Furanoeremophilanes with an ester group at C-6 are frequently found, whereas furanoeremophilanes with a hydroxyl group at C-6 are uncommon in nature.[1]

As part of our chemical study of species of the genus *Senecio*,[6] we collected *S. toluccanus* from the mountain region of Morelia, Michoacan, Mexico. Their roots gave 1,10-epoxy-6-hydroxyeuryopsin (**1**),[7] 6-hydroxyeuryopsin (**3**)[7–9] and toluccanolide A (**5**),[7,10] and the corresponding acetyl derivatives **2**,[7,11] **4**[7] and **6**[10] were obtained by reaction with acetic anhydride. Compounds **2**–**5** are described as natural products[9–11] and were also obtained by biogenetic-type conversions from the eudesmanolide alantolactone,[7] this being the first time that **1** is described as a natural product.



1 R = H **2** R = Ac **3** R = H **4** R = Ac **5** R = H **6** R = Ac

Literature surveys show the existence of a large group of this kind of eremophilanolides but no publication gives complete ^{1}H NMR assignments, since the $CH_2(2)$, $CH_2(3)$ and CH(4) signals appear in a narrow chemical shift range, and in consequence there have not been studies concerning the conformation of these compounds in solutions. ^{13}C NMR data have been reported for only a small number of these compounds. Therefore, and in continuation of our studies of sesquiterpenes,[12,13] we decided to perform the complete assignment of the ^{1}H and ^{13}C NMR spectra as well as a conformational evaluation of **1**–**6**.

RESULTS AND DISCUSSION

From the hexane extracts of *S. toluccanus*, compounds **1**, **3** and **5** were obtained, which is interesting because they belong to the small group of eremophilanolides with a hydroxyl group at C-6.

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1,10-Epoxy-6-hydroxyeuryopsin (**1**)[7] was identified by comparison of the ^{1}H NMR data with those described for a compound obtained by chemical transformation of an alantolactone. The described data give no information about the signals corresponding to the $CH_2(2)$—$CH_2(3)$—CH(4) fragment, or for the $CH_2(9)$ signals, and ^{13}C NMR data are not reported. Complete ^{1}H and ^{13}C NMR assignments (Tables 1 and 3, respectively) were made with the aid of COSY, gHSQC, gHMBC, and NOESY experiments combined with molecular modeling. The ^{1}H NMR spectrum of **1** shows the furan proton at δ 7.08 (brq, 1.5 Hz) coupled to the methyl group at δ 2.09 (brd, 1.5 Hz), while the H(1) signal was observed at δ 3.09 (brd, 4.8 Hz) which, in the COSY experiment, only showed correlation with the signal at δ 1.88 assigned to H(2β). This indicates that the dihedral angle between H(1) and H(2α) is ca 90° and confirms the α-orientation of the epoxide ring. The methylene signals, the signal due to H(6) and the methyl group signals were assigned to the values given in Table 1.

In order to determine the conformation of **1**, the minimum energy structure was calculated by means of MMX molecular modeling.[14] Two global minimum structures were found, **1a** [E_{MMX} = 40.59 kcal mol^{-1} (1 kcal = 4.184 kJ)] and **1b** (E_{MMX} = 35.15 kcal mol^{-1}), which were submitted to density functional theory (DFT) calculations.[15] The optimized structure of the most stable theorical conformation **1b**, shown in Fig. 1, has a total energy value of E_{DFT} = −809.25346 hartree (1 hartree = 627.5095 kcal mol^{-1}).[16] The observed coupling constant values are in good agreement with those calculated for **1b** from the H—C—C—H dihedral angles using a generalized Karplus-type relationship[17] (Table 2). In addition, the NOESY experiment showed correlation between H(4) and H(6), between Me(14) and H(3β) and H(9β) and between Me(15) and H(3α), which is in agreement with the conformation depicted in Fig. 1. These data indicate that the A ring of **1** exists almost exclusively in a conformation intermediate between half-chair and envelope, with Me(15) in a pseudo-equatorial position, as reflected by the polar set of parameters[18] Q = 0.502, θ = 52.50°, Φ = 17.07°, which were calculated from the DFT coordinates using the RICON program.[19]

The acetyl derivative **2**[7,11] was prepared by treating **1** with acetic anhydride in pyridine. The ^{1}H NMR signals for **2** were similar to those for **1**, except for the H(6) chemical shift, which now appears at δ 6.35 and by the presence of the acetyl group signal at δ 2.17 (Table 1). The ^{13}C NMR data

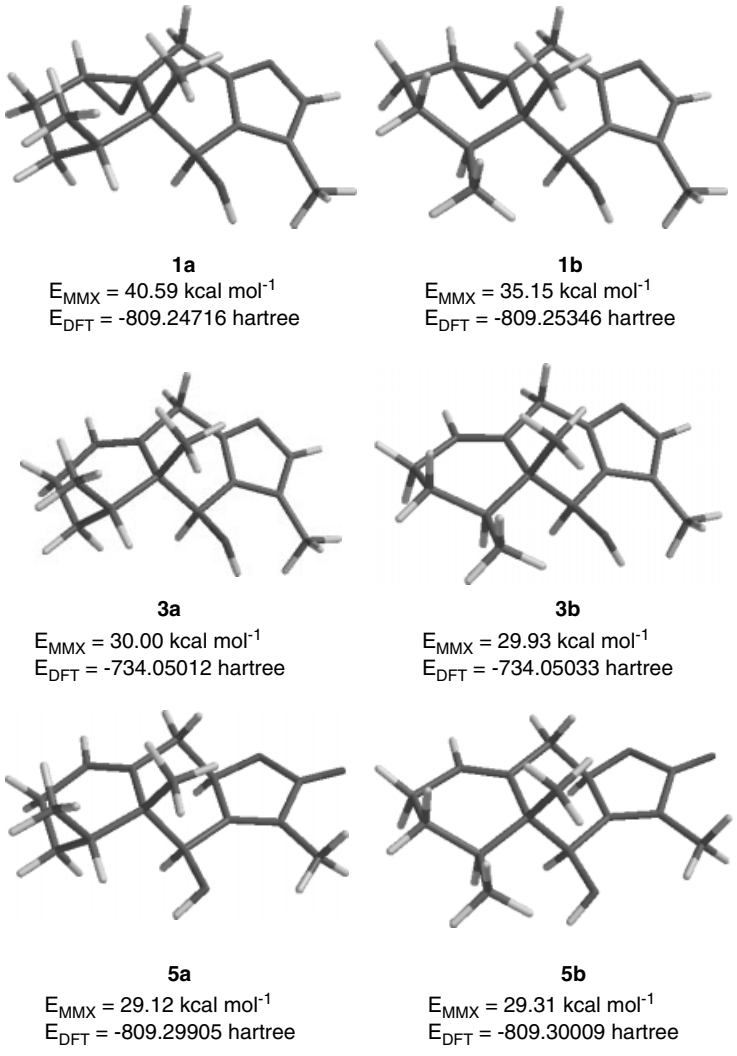

Figure 1. Density functional theory (B3LYP/6–31G*) molecular models of eremophilanolides **1**, **3** and **5**.

Table 1. ^{1}H NMR data and HMBC correlations of eremophilanolides **1**–**6**[a]

	1		**2**[b]	
H	δ_H (J, Hz)	HMBC	δ_H	HMBC
1	3.09 (brd, 4.8)	2, 3	3.09	2, 3
2α	2.03 (ddd, 13.0, 11.9, 6.4)	1, 3, 4	2.04	1, 3
2β	1.88 (dddd, 13.0, 6.4, 4.8, 1.1)	1, 3, 4	1.91	1, 3
3α	1.35 (dddd, 15.0, 6.4, 2.2, 1.1)	2, 4, 5	1.34	2, 4, 15
3β	1.77 (dddd, 15.0, 11.9, 10.0, 6.4)	2, 4, 5	1.89	2, 4, 15
4	2.01 (dqd, 10.0, 7.3, 2.2)	2, 3	1.57	5, 10, 15
6	4.87 (brd, 2.2)	4, 5, 7, 8, 14	6.35	4, 5, 7, 8, $COCH_3$
9α	2.14 (brd, 16.8)	7, 8, 10	2.20	5, 7, 8, 10
9β	3.18 (brdd, 16.8, 2.2)	7, 8, 10	3.22	5, 7, 8, 10
12	7.08 (brq, 1.5)	7, 8, 11	7.08	7, 8, 11
13	2.09 (brd, 1.5)		1.87	7, 11, 12
14	1.14 (s)	4, 5, 6	1.19	4, 5, 6
15	1.11 (d, 7.3)	3, 4, 5	1.08	3, 4, 5
Ac			2.17 (s)	CH_3CO
	3[c]		**4**[b]	
	δ ^{1}H (J, Hz)	HMBC	δ ^{1}H	HMBC
1	5.63 (ddd, 4.7, 3.1, 2.0)	3, 5, 9	5.66	2, 3, 5, 9
2α	1.88 (ddddd, 17.7, 5.5, 4.7, 4.4, 2.0)	1, 3, 4, 10	1.92	1, 3, 4
2β	2.08 (ddddd, 17.7, 9.7, 5.2, 3.1, 3.0)	1, 3, 4, 10	2.07	1, 3, 4
3α	1.69 (dddd, 13.0, 9.7, 5.5, 3.4)	1, 2, 4, 5, 15	1.88	1, 2, 5
3β	1.45 (dddd,13.0, 5.6, 5.2, 4.4)	1, 2, 4, 5, 15	1.43	1, 2, 5
4	2.08 (qdd, 7.1, 5.6, 3.4)		1.78	2, 5, 6, 10
6	4.66 (brs)	4, 5, 7, 8, 14	6.11	4, 5, 7, 8, $COCH_3$
9α	2.95 (brd, 17.1)	1, 5, 7, 8, 10	2.98	1, 5, 7, 8, 10
9β	3.39 (dddd, 17.1, 3.0, 2.0, 2.0)	1, 5, 7, 8, 10	3.40	1, 5, 7, 8, 10
12	7.03 (brq, 1.1)	7, 8	7.01	7, 8, 11
13	2.06 (brd, 1.1)	7, 12	1.83	7, 11, 12
14	0.98 (s)	6, 10	1.03	4, 5, 6, 10
15	1.02 (d, 7.1)	3, 4, 5	0.94	3, 4, 5
Ac			2.13 (s)	$COCH_3$
	5		**6**[b]	
	δ_H (J, Hz)	HMBC	δ_H (J, Hz)	HMBC
1	5.76 (ddd, 3.8, 3.3, 1.8)	2, 3, 9	5.83	2, 3, 5, 9
2α	2.01 (m)	1, 3, 4, 10	2.03 (dddd, 17.5, 8.4, 5.2, 3.8)	1, 3, 4, 10
2β	2.01 (m)	1, 3, 4, 10	2.05 (dddd, 17.5, 6.6, 4.8, 3.3)	1, 3, 4, 10
3α	1.58 (dddd, 13.7, 6.6, 5.2, 2.8)	1, 2, 4, 5, 15	1.61	1, 2, 4, 5, 15
3β	1.42 (dddd, 13.7, 8.5, 8.4, 4.8)	1, 2, 4, 5, 15	1.40	1, 2, 4, 5, 15
4	1.93 (dqd, 8.5, 6.8, 2.8)		1.76	2, 3,5, 6, 15
6	4.47 (brd, 1.5)	4, 5, 7, 11, 14	5.55	4, 5, 7, 11, 14, $COCH_3$
8	4.43 (brddq, 10.5, 6.8, 1.8)	7, 11	4.56	7, 9, 11
9α	2.73 (brdd, 12.5, 6.8)	1, 5, 7, 8, 10	2.81	1, 5, 7, 8, 10
9β	2.14 (ddd, 12.5, 10.5, 1.8)	1, 5, 7, 8, 10	2.15	1, 5, 7, 8, 10
13	2.05 (dd, 1.8, 1.5)	7, 11, 12	1.89	7, 11, 12
14	0.91 (s)	4, 5, 6, 10	0.97	4, 5, 6, 10
15	1.10 (d, 6.8)	3, 4, 5	1.01	3, 4, 5
Ac			2.21 (s)	$COCH_3$

[a] 300 MHz, $CDCl_3$, TMS as internal standard.

[b] Multiplicities and coupling constant values are as in the corresponding non-acetylated molecule, unless otherwise stated.

[c] Chemical shifts from the spectrum in pyridine-d_5: δ 5.56 (H1), 1.83 (2α), 2.03 (2β), 1.70 (3α), 1.38 (3β), 2.37 (H4), 4.87 (H6), 3.04 (H9α), 3.47 (H9β), 7.32 (H12), 2.27 (Me13), 1.18 (Me14), 1.09 (Me15).

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Table 2. Observed[a] and calculated[b] coupling constant values (Hz) and dihedral angles (°) for **1**, **3** and **5**

	1			**3a**		**3b**		**3**		**5a**		**5b**		**5**	
H	Φ	J_{calc}	J_{obs}	Φ	J_{calc}	Φ	J_{calc}	J_{calc}[c]	J_{obs}	Φ	J_{calc}	Φ	J_{calc}	J_{calc}[d]	J_{obs}
1,2α	88.4	0.8	0.0	39.7	5.0	78.3	2.8	4.4	4.7	38.4	5.1	76.8	2.8	3.7	3.8
1,2β	−26.3	6.5	4.8	−75.6	2.9	−37.4	5.1	3.5	3.1	−77.1	2.8	−38.8	5.0	4.1	3.3
2α,3α	−41.5	6.6	6.4	46.6	5.6	−49.0	5.1	5.5	5.5	47.5	5.4	−48.2	5.2	5.3	5.2
2α,3β	−157.2	11.5	11.9	−69.7	1.5	−166.0	12.6	4.5	4.4	−68.9	1.6	−165.3	12.5	7.9	8.4
2β,3α	76.0	0.8	1.1	162.6	12.2	67.4	1.8	9.4	9.7	163.7	12.3	67.9	1.7	6.2	6.6
2β,3β	−39.6	7.0	6.4	46.3	5.6	−49.6	5.0	5.5	5.2	47.2	5.4	−48.9	5.1	5.3	4.8
3α,4	70.3	1.9	2.2	−55.6	3.6	67.4	2.2	3.2	3.4	−57.8	3.3	66.3	2.4	2.8	2.8
3β,4	−174.4	12.2	10.0	60.9	2.8	−176.2	12.3	5.4	5.6	59.0	3.1	−177.6	12.3	8.4	8.5
8,9α										−49.2	5.8	−50.3	5.6	5.7	6.8
8,9β										−166.6	10.6	−166.2	10.5	10.5	10.5

[a] From 300 MHz spectra.

[b] From the dihedral angle obtained from DFT molecular model.

[c] Weighted vicinal coupling constants of conformers **a** and **b** in a 73 : 27 ratio.

[d] Weighted vicinal coupling constants of conformers **a** and **b** in a 42 : 58 ratio.

assignments of **2** (Table 3) were achieved by comparison with the data for **1** and with the aid of gHSQC and gHMBC experiments.

6-Hydroxyeuryopsin (**3**) was identified by comparison of the ^{1}H and ^{13}C NMR spectra with those described.[7–9] Again, no information about the NMR couplings involving the five hydrogen atoms due to the $CH_2(2)$—$CH_2(3)$—CH(4) fragment is described. Two of these signals, the latter of which turned out to be H(2β) and H(4), when measured in $CDCl_3$, appear overlapped with the Me(13) signal, a situation that makes the assignment difficult. In an effort to distinguish these signals, a ^{1}H NMR spectrum in benzene-d_6 was obtained; however, H(4) and one proton of $CH_2(2)$ appear overlapped at δ 1.90. Therefore, the ^{1}H NMR assignment (Table 1) was made with the aid of data obtained from a spectrum measured in pyridine-d_5, spectral spin–spin simulation,[20] COSY, gHSQC, gHMBC, NOESY and double resonance experiments.

The chemical shifts for the CH(1)—$CH_2(2)$—$CH_2(3)$—CH(4) fragment obtained from the spectrum measured in pyridine-d_5, in which the anisotropic effect[21] shifts the two $CH_2(2)$ protons and the CH(4) proton to δ 2.03, 1.83 and 2.37, respectively, and the estimated approximate two- [^2J(H, H)] and three-bond [^3J(H, H)] spin–spin coupling constant values were used as starting input data in a spectral simulation program.[20] After careful iteration, the traces of the experimental and simulated ^{1}H NMR spectra for the $CH_2(2)$—$CH_2(3)$—CH(4) fragment are shown in Fig. 2. The root mean square (r.m.s.) deviation between experimental and calculated transitions was 0.18 Hz. The coupling constant values extracted from the simulated spectrum in pyridine-d_5, assuming a solvent-independent molecular conformation, were used for the simulation of the ^{1}H NMR spectrum obtained in $CDCl_3$. The final values are given in Table 2 and traces of the experimental and simulated spectra, shown in Fig. 3, reveal that indeed the conformation of the A-ring of **3** remains similar in both solvents.

In the NOESY experiment on **3**, obtained in $CDCl_3$, correlation between the signals at δ 0.98 [Me(14)] and one

Table 3. ^{13}C NMR data of eremophilanolides **1**–**6**[a]

C	**1**	**2**	**3**	**4**	**5**	**6**
1	63.1	62.8	123.8	124.5	128.9	129.9
2	20.0	19.9	22.1	21.7	23.8	23.8
3	23.8	23.5	26.9	26.6	27.6	27.5
4	31.7	32.0	33.1	32.4	35.7	35.6
5	41.2	40.7	43.0	42.4	45.7	44.7
6	69.4	69.7	73.3	72.8	78.2	77.6
7	119.3	116.8	119.7	117.2	160.9	157.5
8	147.5	148.3	150.4	151.0	78.4	78.3
9	30.6	30.5	31.2	31.3	40.2	40.1
10	63.3	63.2	136.6	135.2	133.9	132.8
11	120.4	119.5	120.4	119.5	122.5	122.1
12	138.9	139.0	138.3	138.2	174.7	173.9
13	9.1	8.5	9.2	8.7	9.3	8.7
14	15.1	16.0	15.1	16.4	14.0	14.9
15	15.4	15.3	15.8	15.4	17.5	17.1
Ac		21.0		21.2		21.1
		171.2		171.0		169.8

[a] 75.4 MHz, $CDCl_3$, TMS as internal standard.

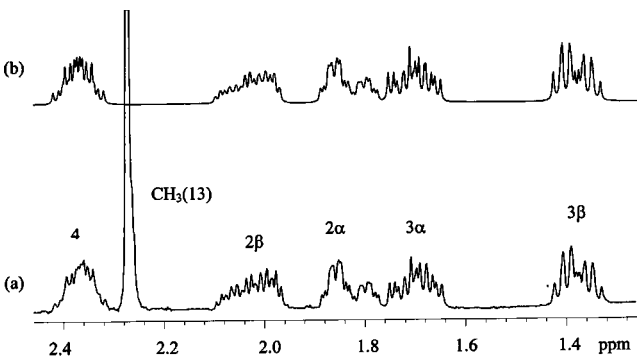

Figure 2. $CH_2(2)$—$CH_2(3)$—CH(4) signals of the 300 MHz ^{1}H NMR spectrum of **3** in pyridine-d_5: (a) experimental; (b) calculated.

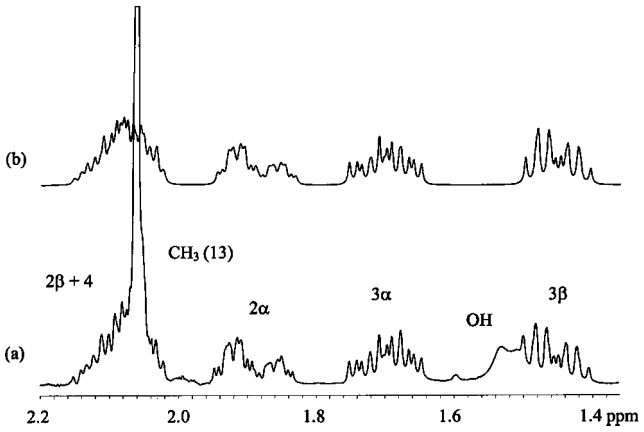

Figure 3. $CH_2(2)-CH_2(3)-CH(4)$ signals of the 300 MHz ^1H NMR spectrum of **3** in $CDCl_3$: (a) experimental; (b) calculated.

proton of $CH_2(9)$ (δ 3.39) assigned to H(9β) was observed. This is further corroborated by the correlation observed between the signals at δ 5.63 and 2.95 assigned to H(1) and H(9α), respectively.

MMX and DFT calculations show two low-energy conformations for **3** (**3a** and **3b** in Fig. 1). Conformation **3a**, with the Me(15) pseudo-axial, was found at E_{MMX} = 30.00 kcal mol^{-1} and the total energy value obtained by DFT was $E_{DFT} = -734.05012$ hartree, and conformation **3b**, with the Me(15) pseudo-equatorial, was found at E_{MMX} = 29.93 kcal mol^{-1} and $E_{DFT} = -734.05033$ hartree. The experimental ^3J(H, H) values for the CH(1)—CH_2(2)—CH_2(3)—CH(4) fragment cannot be explained by a single conformation in solution, and therefore the weighted time-average vicinal coupling constants between these protons were obtained using $^3J_{obs} = n\mathrm{A}(^3J_A) + n\mathrm{B}(^3J_B)$, where nA and nB are the mole fractions of **3a** and **3b**, respectively. Using $J_{2\alpha,3\beta}$, $J_{2\beta,3\alpha}$ and $J_{3\beta,4}$, a 73 : 27 ratio in favor of conformation **3a** was estimated. The calculated and observed coupling constants are given in Table 2. Since the calculated energy difference between the **3a** and **3b** conformations is small, it is reasonable to expect an influence of solvation effects.[22] The calculated values for the puckering coordinates of **3a** and **3b** are Q = 0.483, θ = 51.20°, Φ = 25.53° and Q = 0.491, θ = 53.98°, Φ = 17.41°, respectively.

The acetyl derivative **4**,[7] prepared as above, shows similar ^1H NMR signals to **3**, except for the CH(6) signal, which appears at δ 6.11, and by the presence of the acetyl group signal at δ 2.13. Complete ^1H and ^{13}C NMR assignments of **4** (Tables 1 and 3, respectively) were made after comparing their spectral data with those of **3** and from COSY, gHSQC, gHMBC and NOESY experiments.

Compound **5** was identified by comparison of its physical and spectral data with those reported.[10] The published ^1H NMR spectrum was partially assigned, while the ^{13}C NMR assignments were correct, except for the C-14 and C-15 signals, which were interchanged. The ^1H NMR spectrum showed the signals corresponding to H(6) and H(8) overlapped, and the two signals of CH_2(2) seemed to have very similar chemical shifts and were partially overlapped with the signals of H(4), H(9β), and Me(13), all this making their assignments difficult. However, acetylation of **5** to give **6** caused shifts of the H(4), H(6) and Me(13) signals. This allowed an easy assignment and analysis of the coupling constant values of these signals, which was supported by gHSQC and gHMBC experiments and spectral simulation.

In order to make a total assignment of the ^1H NMR signals, and to determine the minimal energy conformation of **5**, molecular modeling was used as above. As in **3**, MMX and DFT calculations show two-minimal energy conformations for **5** (Fig. 1). The calculated structure **5a** has E_{MMX} = 29.12 kcal mol^{-1} and $E_{DFT} = -809.29905$ hartree and **5b** has E_{MMX} = 29.31 kcal mol^{-1} and $E_{DFT} = -809.30\,009$ hartree. From the weighted time-average ^3J(H, H) a 42 : 58 ratio in favor of the most stable conformation **5b** was calculated. The conformation of the A-ring in **5a** and **5b** is defined in each case by the puckering parameters as between half-chair and envelope (**5a**, Q = 0.490,, θ = 51.25°, Φ = 21.58°; **5b**, Q = 0.483, θ = 54.15°, Φ = 18.05°).

As a general fact, the MMX and DFT calculations reveal that acetylation of **1, 3** and **5** to provide **2, 4** and **6** causes no significative change in the molecular conformation of each pair of compounds. Also, from the analysis of the coupling constant values and the MMX and DFT calculations, it is concluded that the A-rings of **1** and **2** have a single conformation between half-chair and envelope with CH_3(15) in a pseudo-equatorial position, whereas in **3**–**6**, in which the epoxy group at C-1–C-10 is replaced by a double bond, an important conformational dynamic bending in the CH_2(2)—CH_2(3)—CH(4) fragment is observed at room temperature.

A detailed inspection of the ^{13}C NMR data shows that acetylation of **1**, **3** and **5** did not cause significant changes in the chemical shift of the C-6 signal. A polarization of the π-electrons, induced by the acetyl group, was evident from the C-7 chemical shift, which is shifted about 3.5 ppm to lower frequency in all acetylated derivatives (**2**, **4**, **6**), and C-10, which is shifted by about 1 ppm in **4** and **6**. On the other hand, a shielding of about 1 ppm to higher frequency for C-14 in all acetylated compound (**2**, **4**, **6**) is also observed.

EXPERIMENTAL

General

Merck silica gel (230–400 mesh) was used for column chromatography (CC). Molecular models were generated using the MMX force field,[14] as implemented in the PCMODEL program. The structures generated from the PCMODEL program were geometrically optimized by DFT (B3LYP/6–31G*)[15] using the PC Spartan 02 program from Wavefunction (Irvine, CA, USA). The calculated coupling constants were obtained from the H—C—C—H dihedral angles measured in the minimum energy DFT molecular models by means of the Altona equations.[17]

NMR spectra

NMR measurements were carried out using 5 mm probes at 22 °C from $CDCl_3$ solutions, unless stated otherwise, with TMS as the internal standard. Typical 1D ^1H and ^{13}C spectra were acquired under standard conditions on Varian Mercury

spectrometers operated at 300 and 75 MHz, respectively. ^{1}H spectra were obtained using a 4807.7 Hz spectral window with 16 384 data points, using Gaussian apodization for data processing, and ^{13}C spectra were obtained using a spectral window of 18 761.7 Hz with 131 072 data points for the processing. NOESY spectra were generated with a mixing time of 0.8 s, relaxation delay 1.0 s, data matrix 4K × 4K (400 increments to 4 K, zero filling in F_1, 4 K in F_2), 16 transients in each increments, spectral width 3000 Hz. The 2D hydrogen-detected heteronuclear shift correlation spectra were obtained using the gHMQC and gHMBC pulse sequences with 512 time increments, 64 transients were collected for each time increment and a relaxation delay of 1.0 s was always used.

Plant material

Roots of *S. toluccanus* were collected at km 260 of Mexican federal highway No. 15 in April 2002. A voucher specimen (No. 15 004) is deposited in the herbarium of the Universidad Autónoma de Chapingo, Chapingo, Mexico.

Extraction and isolation

Dried and powdered roots of *S. toluccanus* (916 g) were extracted with hexane under reflux (×3). After defatting by precipitation with MeOH, the extract (7.4 g, 0.8%) was chromatographed over silica gel, eluting with hexane and hexane–EtOAc mixtures. The fractions eluted with hexane–EtOAc (9 : 1) afforded **3** (940 mg) as a pale yellow oil, and those fractions eluted with hexane–EtOAc (4 : 1) were further purified by CC to give **1** (11 mg). The fractions eluted with hexane–EtOAc (7 : 3) were rechromatographed by CC to afford **5** (12 mg).

Acetylations of **1**, **3** and **5** were performed in the usual manner with acetic anhydride in pyridine.

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