Special issue research article

MRC

Received: 9 January 2015 Revised: 3 March 2015 Accepted: 17 March 2015 Published online in Wiley Online Library: 1 July 2015

(wileyonlinelibrary.com) DOI 10.1002/mrc.4246

Complete ^{1}H NMR assignment of cedranolides†

Nury Perez-Hernandez,[a] Barbara Gordillo-Roman,[b] Daniel Arrieta-Baez,[c] Carlos M. Cerda-Garcia-Rojas[b] and Pedro Joseph-Nathan[b]*

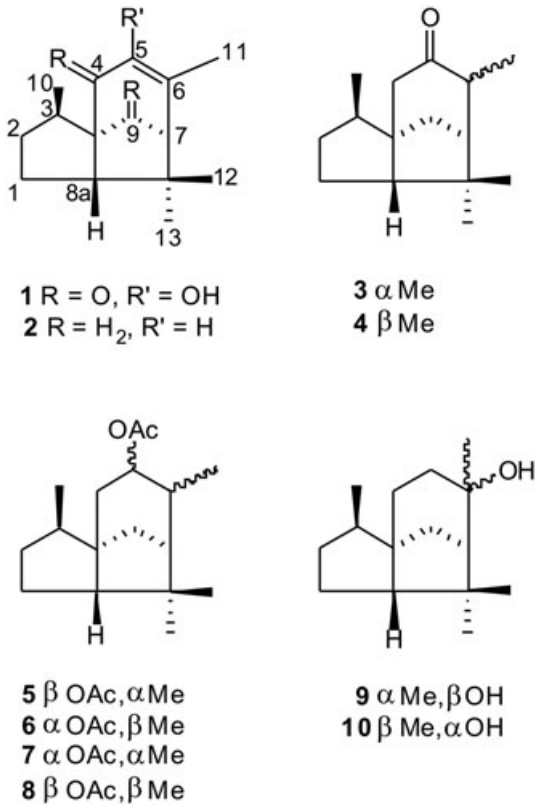
Complete and unambiguous ^{1}H NMR chemical shift assignment of α-cedrene (2) and cedrol (9), as well as for α-pipitzol (1), isocedrol (10), and the six related compounds 3–8 has been established by iterative full spin analysis using the PERCH NMR software (PERCH Solutions Ltd., Kuopio, Finland). The total sets of coupling constants are described and correlated with the conformational equilibria of the five-membered ring of 1–10, which were calculated using the complete basis set method. Copyright © 2015 John Wiley & Sons, Ltd.

Keywords: cedrane derivatives; iterative ^{1}H NMR analysis; PERCH spin–spin simulation; conformational behavior; ^{1}H-^{1}H coupling constants; CBS-4M method

Introduction

The cedranolide family is composed of a series of natural sesquiterpenes, which contain the tricyclo[5.3.1.0^{1,5}]undecane ring system. The primary compounds of this chemical group are economically important α-cedrene (**2**) and cedrol (**9**), which were first isolated in 1841 from red cedar wood *Juniperus virginiana*.[1] α-Cedrene (**2**) is an approved food preservative that shows antimicrobial,[2] trypanocidal,[3] insecticidal,[4] and hepatoprotective properties,[5] while cedrol (**9**) is a fragrance ingredient used in cosmetics, perfumes, and cleaning products.[6] Recent studies have shown that inhalation of cedrol (**9**) modulates the autonomic activity via the central nervous system.[7] The structure elucidation of α-cedrene (**2**) and cedrol (**9**)[8] was achieved more than a century after their isolation. These sesquiterpenes have been the subject of numerous studies focused to built the tricyclo[5.3.1.0^{1,5}]undecane skeleton,[9] being the arene-olefin meta-photocycloaddition strategy one of the most recognized synthetic protocols.[10]

Description of the spectral properties of **2** and **9** has also been a goal of some studies, like the complete assignment of ^{13}C NMR spectra.[11] Partial ^{1}H NMR assignments of α-pipitzol (**1**), α-cedrene (**2**), cedrol (**9**), and isocedrol (**10**) are also available.[12] Even though there is vast synthetic work and identification of cedranolides in several essential oils, the full assignment of their ^{1}H NMR spectra, including the knowledge of the coupling constant values, has not been carried out up to now. Thus, in this work, we describe the complete ^{1}H NMR assignment of cedranolides **1–10** using the iterative full spin analysis available in the PERCH NMR software (PERCH Solutions Ltd., Kuopio, Finland). Additionally, the coupling constants were contrasted with the conformational preferences of cedranolides **1–10** using density functional theory at the complete basis set method (CBS-4M, where M stands for the Minimal population localization), level to calculate optimized geometries and then derive Boltzmann distributions.



Results and discussion

Even though the partial ^{1}H NMR assignments available[12] for cedranolides **1**, **2**, **9,** and **10**, constitute a useful tool for their

* *Correspondence to: Pedro Joseph-Nathan, Departamento de Química, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Apartado 14-740, México, D. F., 07000 Mexico. E-mail: pjoseph@nathan.cinvestav.mx*

† *In honor of Professor William F. Reynolds.*

a *Escuela Nacional de Medicina y Homeopatía, Instituto Politécnico Nacional, Guillermo Massieu Helguera 239, México, D. F., 07320 Mexico*

b *Departamento de Química, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Apartado 14-740, México, D. F., 07000 Mexico*

c *Centro de Nanociencias y Micro y Nanotecnologías, Instituto Politécnico Nacional, México, D. F., 07738 Mexico*

identification in synthetic or phytochemical studies, the complete evaluation of the ^{1}H NMR coupling constants is necessary to understand structural and conformational aspects of this sesquiterpene family. Recently, the ^{1}H NMR iterative full spin analysis methodology, integrated into the PERCH NMR software,[13] has successfully been used for the complete ^{1}H NMR assignment of natural products.[14] This analysis is based on the principle of integral transforms[15] in which the product of a frequency-domain spectrum is multiplied by a set of functions leading to a set of integral transforms, which are computed by the principal component regression method to obtain the corrected spectral parameters (δ, J_{HH}).[13] By using this methodology, the complete spectral data

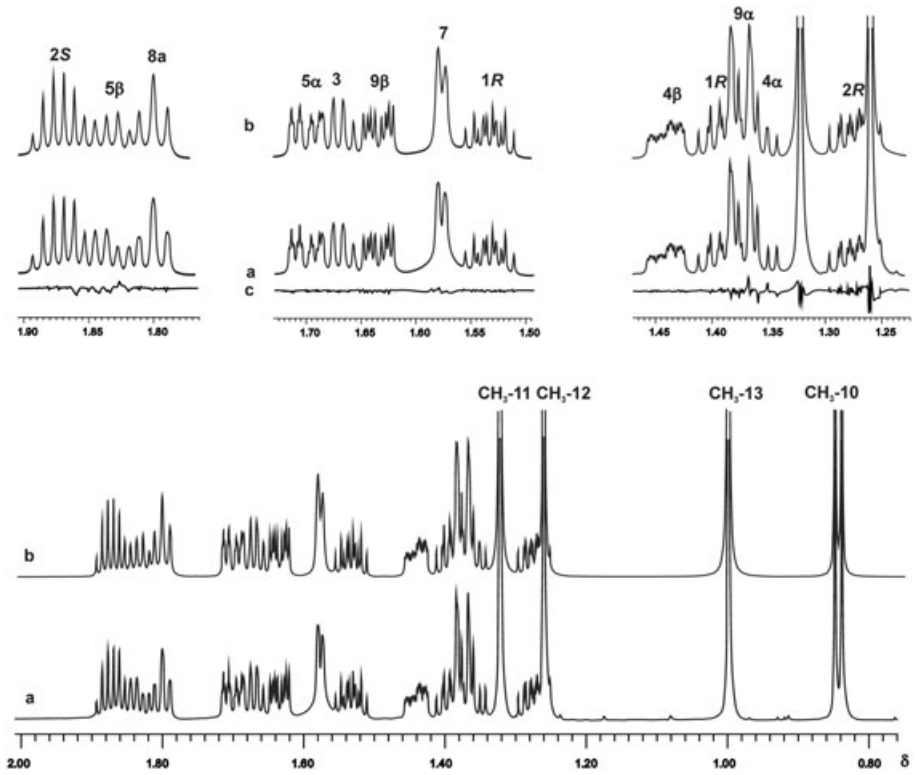


Figure 1. Comparison of experimental (a), PERCH calculated (b), and residual (c) 750-MHz NMR spectra of cedrol (**9**).

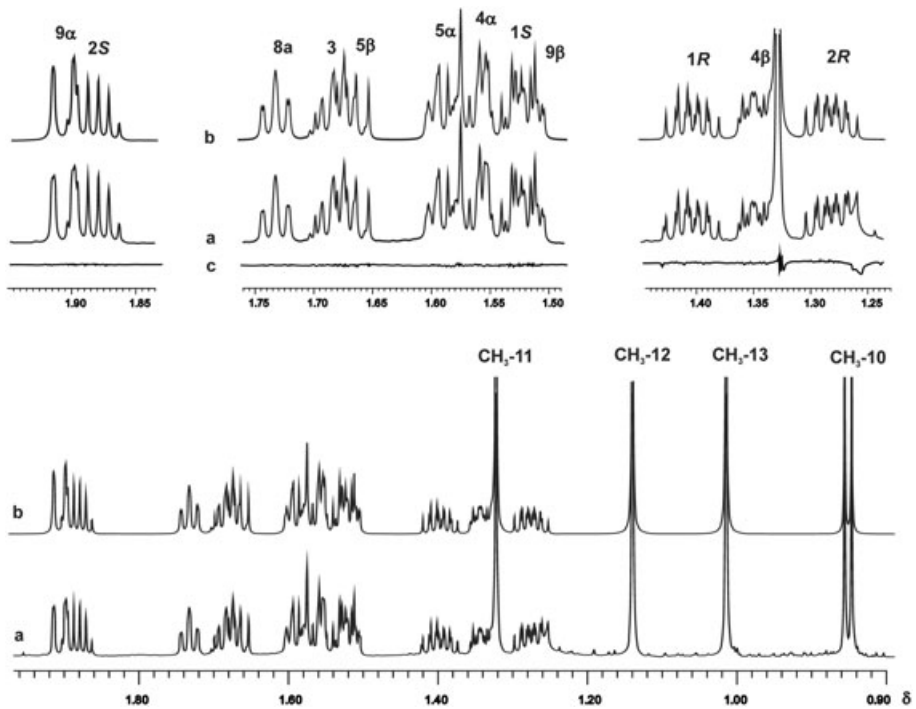


Figure 2. Comparison of experimental (a), PERCH calculated (b), and residual (c) 750-MHz NMR spectra of isocedrol (**10**).

Table 1. Hydrogen NMR chemical shifts of the methylcyclopentane portion of **1–10** (in parts per million from TMS)

Compound	1*R*	1*S*	2*R*	2*S*	3	Me-10	8a	RMS[a]
1	1.510	1.769	1.656	1.906	2.408	1.382	2.112	0.099
2	1.386	1.578	1.358	1.851	1.743	0.838	1.694	0.037
3	1.430	1.585	1.314	1.881	1.862	0.841	1.620	0.049
4	1.403	1.564	1.310	1.899	1.882	0.860	1.697	0.050
5	1.442	1.570	1.311	1.820	1.698	0.815	2.163	0.066
6	1.379	1.530	1.283	1.838	1.725	0.835	1.821	0.049
7	1.397	1.536	1.272	1.852	1.720	0.846	1.798	0.099
8	1.418	1.535	1.283	1.814	1.686	0.806	2.283	0.073
9	1.389	1.533	1.274	1.873	1.671	0.843	1.800	0.087
10	1.397	1.526	1.276	1.883	1.680	0.851	1.732	0.061

[a]Root mean square for the comparison of experimental and calculated ^{1}H NMR spectra.

for all hydrogen atoms of a target molecule can be determined by iterative minimization of the difference between the simulated and experimental spectra.

Thus, the ^{1}H NMR iterative full spin analysis of cedranolides **1–8** was made using the PERCH software. The 500-MHz FIDs were Fourier transformed into frequency-domain spectra employing the preparation module (PAC). In addition, molecular models for **1–8** were prepared in the Molecular Modeling Software (MMS) module, submitted to Monte Carlo search, and the minimum energy conformers were used to predict a preliminary ^{1}H NMR spectrum for each cedranolide. There is no advantage if the initial molecular model imported into the MMS module originates from molecular dynamics or from a Monte Carlo protocol, because PERCH predicts the initial spectrum using molecular mechanics. Manual refinement of the simulation is always required. Some known chemical shifts and coupling constants were manually fed before starting the iteration process in the parameter editor of the PMS module. The

Table 2. Hydrogen NMR chemical shifts of the methylcyclohexane portion and the *gem*-dimethyl signals of **1–10** (in parts per million from TMS)

Compound	4α	4β	5α	5β	6	7	9α	9β	Me-11	Me-12	Me-13
1	—	—	—		—	2.836	—	—	2.059	1.079	1.029
2	2.167	1.782	5.220		—	1.744	1.380	1.653	1.670	1.019	0.950
3	2.364	2.208	—		2.667	1.675	1.845	1.722	1.131	0.981	0.962
4	2.331	2.331	—		2.545	1.982	1.753	2.035	1.194	1.007	0.958
5[a]	1.898	1.536	4.172		2.111	1.428	1.293	1.522	1.039	1.113	0.952
6[a]	1.214	1.999		5.055	1.811	1.665	1.219	1.756	1.037	1.184	0.956
7[a]	1.454	1.690		5.152	2.339	1.521	1.383	1.430	0.880	1.138	0.944
8[a]	1.825	1.716	5.186		2.062	1.614	1.101	1.870	1.056	1.285	0.971
9	1.361	1.440	1.701	1.836	—	1.577	1.375	1.634	1.322	1.260	0.999
10	1.573	1.340	1.590	1.675	—	1.557	1.906	1.519	1.322	1.140	1.014

[a]Methyl of acetyl group: **5**, 2.049; **6**, 2.049; **7**, 2.037; and **8**, 2.089.

Table 3. Coupling constants of the methylcyclopentane portion of **1–10** given in Hertz[a]

Compound	$^2J_{1R,1S}$	$^3J_{1R,2R}$	$^3J_{1R,2S}$	$^3J_{1R,8a}$	$^3J_{1S,2R}$	$^3J_{1S,2S}$	$^3J_{1S,8a}$	$^2J_{2R,2S}$	$^3J_{2R,3}$	$^3J_{2S,3}$	$^3J_{3,Me\text{-}10}$
1	−12.52	10.20	5.84	8.94	5.78	3.79	8.21	−12.17	10.25	5.67	7.12
		11.22	*5.76*	*9.62*	*5.64*	*2.43*	*7.28*		*10.73*	*4.86*	
2	−13.00	5.78	6.59	5.80	6.45	8.49	9.47	−12.12	5.30	6.06	7.18
		6.40	*6.23*	*6.22*	*6.30*	*6.66*	*7.80*		*6.24*	*5.79*	
3	−12.87	8.00	6.10	7.52	5.95	6.17	8.81	−12.13	7.32	6.00	7.12
		8.60	*6.01*	*8.06*	*6.00*	*4.71*	*7.63*		*8.18*	*5.63*	
4	−12.80	8.56	6.24	8.11	6.04	5.49	8.70	−12.31	7.82	5.97	7.12
		8.96	*6.01*	*8.49*	*6.00*	*4.35*	*7.34*		*8.01*	*5.77*	
5	−12.71	6.16	6.41	6.09	6.32	8.22	9.37	−12.17	5.58	6.11	7.18
		6.16	*6.13*	*6.29*	*6.29*	*6.73*	*8.24*		*6.67*	*5.48*	
6	−12.71	8.26	6.21	8.63	6.05	5.81	7.76	−12.23	7.66	6.01	7.14
		8.33	*6.05*	*7.96*	*6.07*	*4.93*	*7.62*		*7.87*	*5.58*	
7	−12.65	8.10	6.19	7.89	5.99	5.96	8.69	−12.12	7.46	6.12	7.14
		8.18	*6.05*	*7.83*	*6.07*	*5.08*	*7.65*		*7.74*	*5.81*	
8	−12.95	5.84	6.57	6.28	6.22	7.96	9.31	−12.51	5.59	6.17	7.19
		6.38	*6.31*	*6.29*	*6.68*	*6.38*	*8.28*		*6.20*	*5.93*	
9	−12.59	8.10	6.13	7.79	6.22	5.69	8.65	−12.34	7.70	5.97	7.06
		8.50	*6.00*	*8.04*	*6.00*	*4.80*	*7.57*		*8.03*	*5.74*	
10	−12.66	8.19	6.20	7.75	6.02	5.97	8.67	−12.13	7.54	6.01	7.15
		8.42	*6.09*	*8.02*	*5.01*	*6.11*	*7.70*		*7.94*	*5.86*	

[a]Values in italics are vicinal coupling constants derived from Altona calculations.

total-line-shape-fitting mode was set in the PERCHit shell, and spin simulations were carried out until obtaining a root-mean-square (RMS) error smaller than 0.1%. In the case of cedrol (**9**) and isocedrol (**10**), the additional two hydrogen atom signals in the high-field region of the spectra complicated the spin system to a point where we were unable to perform the complete assignments at 500 MHz, and therefore, we recurred to measurements at 750 MHz. This higher frequency measurements allowed to complete the assignment task as is shown in Fig. 1 for cedrol (**9**) and Fig. 2 for isocedrol (**10**). A visual comparison of Figs 1 and 2 immediately reveals that although **9** and **10** only differ in the stereogenic center having the tertiary alcohol, their ^{1}H NMR spectra are quite different showing the sensitivity of the method to minor structural modifications, which are best evidenced by studies performed at high magnetic fields. In the case of cedranolides **1–8**, the 500-MHz spectra comparisons are shown in the Supporting Information, which also contains the final PERCH output files for all compounds.

The complete sets of ^{1}H NMR chemical shifts of **1–10** are shown in Tables 1 and 2, while the coupling constants are shown in Tables 3 and 4. For compounds **2**, **9,** and **10**, some previously

Table 4. Coupling constants of the methylcyclohexane portion of **1–10** given in Hertz

Compound	$^{2}J_{4\alpha,4\beta}$	$^{3}J_{4\alpha,5}$	$^{3}J_{4\beta,5}$	$^{4}J_{4\beta,9\beta}$	$^{3}J_{5,6}$	$^{3}J_{6,7}$	$^{3}J_{6,11}$	$^{3}J_{7,9\alpha}$	$^{3}J_{7,9\beta}$	$^{2}J_{9\alpha,9\beta}$	$^{4}J_{12,13}$
2	−16.66	2.55	3.96	−1.24	—	—	—	0.01	4.00	−10.83	−0.44
3	−15.96	—	—	−3.09	—	2.08	7.45	0.07	4.61	−12.48	−0.24
4	−14.80	—	—	−2.90	—	3.25	7.01	0.02	4.73	−11.98	−0.54
5	−14.66	6.89	1.58	−2.74	1.17	1.96	7.45	0.81	4.82	−11.84	−0.42
6	−11.98	9.77	6.51	−2.52	11.27	2.53	7.21	1.02	4.74	−11.59	−0.30
7	−11.79	11.27	6.56	−2.08	6.99	3.17	7.08	0.60	4.68	−11.98	−0.52
8	−14.72	5.94	0.89	−2.82	5.47	2.97	7.54	1.08	4.92	−11.52	−0.46
9	−12.85	—	—	−2.97	—	—	—	0.05	5.09	−12.49	−0.86
10	−12.80	—	—	−2.70	—	—	—	0.82	4.59	−11.94	−0.40

For **2**: $^{5}J_{4\alpha,11} = 2.32$; $^{5}J_{4\beta,11} = 1.87$; and $^{4}J_{5,11} = -1.53$.
For **3**: $^{4}_{4\beta,6} = -1.58$ and $^{4}J_{6,9\beta} = -1.76$.
For **5** and **7**: $^{4}J_{6,9\beta} = -1.36$ and −1.13, respectively.
For **9**: $^{3}J_{4\alpha,5\alpha} = 5.80$; $^{3}J_{4\alpha,5\beta} = 6.86$; $^{3}J_{4\beta,5\alpha} = 1.53$; $^{3}J_{4\beta,5\beta} = 6.54$; $^{2}J_{5\alpha,5\beta} = -13.33$; $^{4}J_{5\alpha,7} = -1.57$; and $^{4}J_{7,8a} = -0.31$.
For **10**: $^{3}J_{4\alpha,5\alpha} = 5.82$; $^{3}J_{4\alpha,5\beta} = 12.58$; $^{3}J_{4\beta,5\alpha} = 1.38$; $^{3}J_{4\beta,5\beta} = 6.53$; $^{2}J_{5\alpha,5\beta} = -14.59$; $^{4}J_{5\alpha,7} = -1.87$; and $^{4}J_{7,8a} = -0.52$.

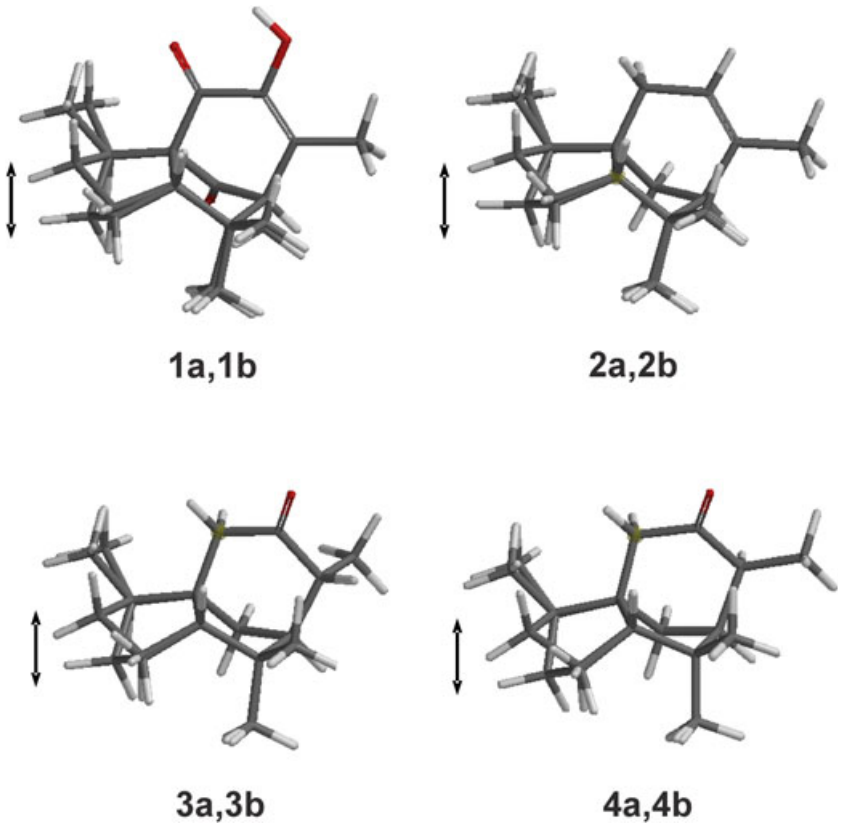


Figure 3. Conformers of **1–4** calculated using the CBS-4M method. Arrows indicate torsions at specific groups.

reported[12a] chemical shift values were very similar to those determined in the present study. For compounds **1**, **3–8**, the complete assignments of ^{1}H NMR chemical shifts are reported here for the first time. As could be expected, the chemical shifts of the five-membered ring, which contains the C10 methyl group, are quite comparable in the complete family of cedranolides. The major differences were found in the six-membered ring, which contains different substituent patterns at the C4, C5, and C6 positions. No additional NMR experiments, like 2D or NOE measurements, were carried out for the total assignment of the cedranolides. Some *gem*-dimethyl signals were initially assigned using published data,[12a] although these data are not required because the correct

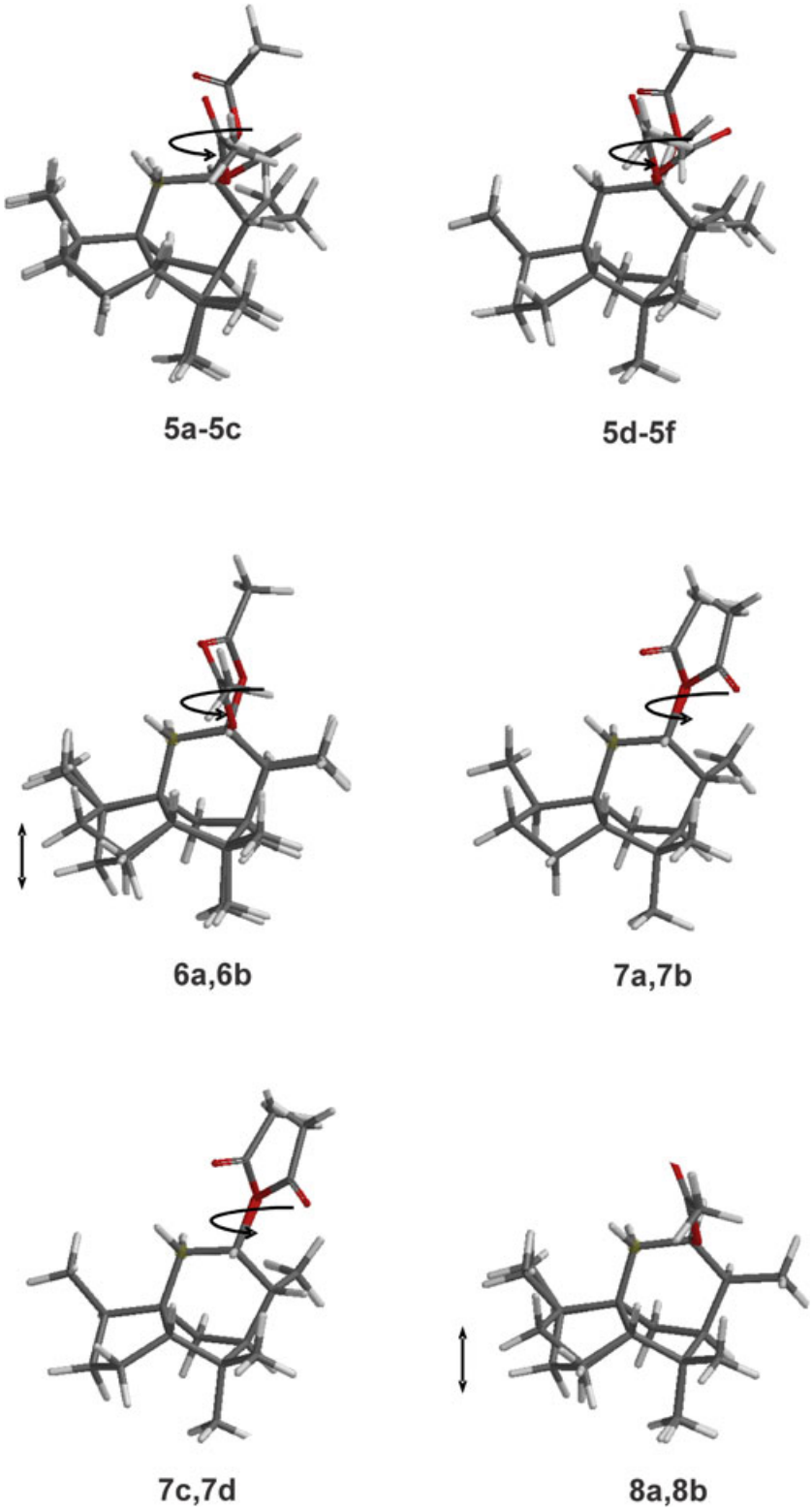


Figure 4. Conformers of **5–8** calculated using the CBS-4M method. Arrows indicate torsions at specific groups.

assignments follow using the lowest RMS value for both alternatives. For instance, in the case of **9**, the incorrect assignment of the individual methyl group signals provides RMS = 0.090%, while the correct assignment provides RMS = 0.087%. In all cases, Me-12 appeared at higher frequencies than Me-13. It is relevant to mention that the 500-MHz experimental spectra were acquired with a resolution better than 0.4 Hz and those at 750 MHz with a magnetic homogeneity better than 0.7 Hz. Thus, the chemical shift values are reported with three significant digits after the decimal point, although the PERCH software provides them with six. In the case of the coupling constant values, the PERCH software provides four significant figures after the decimal point, and therefore, the values are reported with two figures after the decimal point.

Although previous spectral assignment helps to have a better approximation for the initial calculated spectra, inversion of diastereotopic hydrogen atoms, which is frequently needed, can be ascertained by evaluation of the RMS values, as was carried out for sesquiterpene benzoquinones[14b] and pyrrolizidine alkaloids.[14d] Concerning the coupling constant values, by evaluating the data in Table 3, it can be observed that the $J_{1R,2S}$, $J_{1S,2R}$, and $J_{2S,3}$ vicinal coupling constants of the C1-C2-C3-C3a-C8a five-membered ring are comparable within the entire series, averaging 6.2, 6.1, and 6.0 Hz, respectively, while $J_{1R,2R}$, $J_{1R,8a}$, $J_{1S,2S}$, $J_{1S,8a}$, and $J_{2R,3}$ show notable differences from compound to compound. According to these values, compounds **1–10** can be divided into two main groups, one composed of **2**, **5,** and **8**, with average values of 5.9, 6.1, 6.1, 9.4, and 5.5 Hz for $J_{1R,2R}$, $J_{1R,8a}$, $J_{1S,2S}$, $J_{1S,8a}$, and $J_{2R,3}$, respectively, and the other group constituted by **3**, **4**, **6**, 7, **9,** and **10**, with *J* values of 8.2, 7.8, 5.9, 8.6, and 7.6 Hz for the same coupling constants, respectively. In turn, α-pipitzol (**1**) stands alone with *J* values of 10.2, 8.9, 3.8, 8.2, and 10.2 Hz for those coupling constants, because it is the only compound having a carbonyl group at C4. These findings can be explained on the basis of different conformational behaviors. In previous works, the conformational preferences for cedranolides **5–10** were described using geometry optimization and populations from density functional theory calculations at the B3PW91/DGDZVP level of theory.[16] These studies provide consistent results to estimate vibrational properties, giving successful comparisons between experimental and calculated IR and Vibrational Circular Dichroism (VCD) spectra. However, when the dihedral angles and conformational populations were employed in the present work to obtain the averaged calculated vicinal coupling constants using the Altona software,[17] the correlation with the coupling constants listed in Table 3 was only reasonable.

The conformational populations of **1–10** were therefore further optimized by calculations using the CBS-4M,[18] in which the asymptotic convergence behavior of natural orbitals is used in order to extrapolate the energy limit for an infinitely large basis set. This model is an improved version of the original CBS-4M method and is faster and readily applicable to larger molecules. It computes very accurate energies, which seem to be fundamental in the precise coupling constant averaging of molecules with pseudorotation. At this level of theory, the conformational preference of cedranolides **1–10** is mainly distributed in two groups, as shown in Figs 3, 4 and 5. One group is generated by flipping the C1-C2-C3-C3a-C8a five-membered ring into a twisted-envelope conformation with the methyl group at C3 in a *pseudo*-equatorial orientation, which is composed of models **1a–4a**, **5a–c**, **6a**, **7a–b**, **8a**, **9a–b**, and **10a–c**. The second set of conformers, in which the methyl group at C3 adopts a *pseudo*-axial orientation, is composed of models **1b–4b**, **5d–f**, **6b**, **7c–d**, **8b**, **9c–d**, and **10d–f**. Thus, for compounds

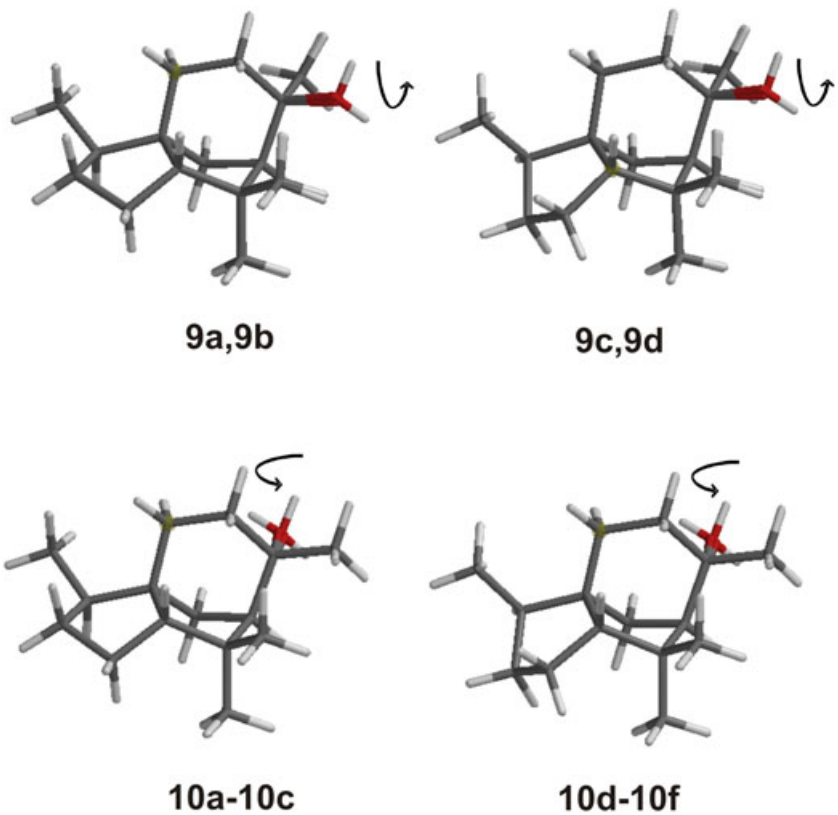


Figure 5. Conformers of **9–10** calculated using the CBS-4M method. Arrows indicate torsions at specific groups.

Table 5. Optimized relative conformer energies ΔG (in kilocalorie per mole) and Boltzmann populations (%) for cedranolides **1–10**

Conformer	ΔG	%	Conformer	ΔG	%
1a	0.00[a]	87.4	**7a**	0.00[g]	49.5
1b	1.15	12.6	**7b**	0.81	12.7
2a	0.05	47.7	**7c**	0.29	30.2
2b	0.00[b]	52.3	**7d**	1.11	7.6
3a	0.00[c]	65.8	**8a**	0.06	47.5
3b	0.39	34.2	**8b**	0.00[h]	52.5
4a	0.00[d]	68.8	**9a**	0.05	31.0
4b	0.47	31.2	**9b**	0.00[i]	33.8
5a	0.04	36.2	**9c**	0.41	17.0
5b	2.65	0.40	**9d**	0.37	18.1
5c	0.00[e]	39.0	**10a**	0.00[j]	28.7
5d	0.69	12.1	**10b**	0.08	25.0
5e	2.16	1.00	**10c**	0.65	9.5
5f	0.74	11.2	**10d**	0.32	16.7
6a	0.00[f]	63.5	**10e**	0.41	14.3
6b	0.33	36.5	**10f**	0.96	5.7

[a] $G^{o} = -507107.15$ kcal/mol.
[b] $G^{o} = -367118.86$ kcal/mol.
[c] $G^{o} = -414288.57$ kcal/mol.
[d] $G^{o} = -414286.787$ kcal/mol.
[e] $G^{o} = -510703.67$ kcal/mol.
[f] $G^{o} = -510704.86$ kcal/mol.
[g] $G^{o} = -510701.53$ kcal/mol.
[h] $G^{o} = -510705.98$ kcal/mol.
[i] $G^{o} = -415034.08$ kcal/mol.
[j] $G^{o} = -415033.65$ kcal/mol.

3, **4**, **6**, **7**, **9,** and **10**, the conformational preference favored the twisted-envelope model with the *pseudo*-equatorial methyl group at C3, accounting for approximately 65% of the total population (Table 5). In the case of **1**, this percentage was even higher (87.4%), thus explaining the observed differences in the coupling constant values for this substance. Conversely, in compounds **2**, **5,** and **8,** the twisted-envelope conformations with the *pseudo*-axial methyl group at C-3 are favored, accounting for approximately 52% of the population, as summarized in Table 5. This combination agrees with the observed vicinal coupling constants obtained using the PERCH program, giving a good correlation with the calculated coupling constant values predicted by the Altona methodology, when the Boltzmann population at the CBS-4M level of theory is used, as exemplified in Fig. 4 for compounds **5** and **8**. The remaining coupling constants for the C1-C2-C3-C3a-C8a five-membered ring, corresponding to the geminal coupling $J_{1R,1S}$ and $J_{2R,2S}$, are in agreement with previous values reported for compounds **2**, **9,** and **10**, while the 'W-type' couplings, $^{4}J_{8a,9\alpha}$, and $^{4}J_{8a,13}$, as well as $^{3}J_{3,10}$, are reported here for the first time.

The six-membered ring of α-pipitzol (**1**) and α-cedrene (**2**) adopts a half-chair conformation, while in compounds **3–10** with no C5/C6 double bond, this ring adopts a chair conformation. The vicinal coupling constants in the six-membered ring are in an acceptable range considering the typical values for cyclohexane derivatives in a chair conformation.[19] Thus, the axial–axial couplings ($^{3}J_{a,a}$): $J_{4\alpha,5}$ in **6** and **7**; $J_{5,6}$ in **6**; and $J_{4\alpha,5\beta}$ in **9** and **10** provide an average value of 11.9 Hz. The equatorial–equatorial couplings ($^{3}J_{e,e}$): $J_{4\beta,5}$ in **5** and **8**; $J_{4\beta,5\alpha}$ in **9** and **10**; $J_{5,6}$ in **5**; and $J_{6,7}$ in **5** and **7** show the usual average value of 1.4 Hz, while the axial–equatorial couplings ($^{3}J_{a,e}$): $J_{4\alpha,5}$ in **5** and **8**; $J_{4\beta,5}$ in **6** and **7**; $J_{5,6}$ in **7** and **8**; $J_{6,7}$ in **6** and **8**; $J_{4\alpha,5\alpha}$ in **9** and **10**; and $J_{4\beta,5\beta}$ in **9** and **10** show the average value of 6.3 Hz. The vicinal couplings $J_{7,9\alpha}$ and $J_{7,9\beta}$, corresponding to $^{3}J_{a,e}$ and $^{3}J_{e,e}$, respectively, display values of 0.5 and 4.5 Hz, which are slightly outside the expected range, probably due to the ring strain imposed to C-7 as a bridgehead carbon atom. Comparison of the observed coupling constant values of the six-membered ring of **1–10** with the values calculated by the Altona method also provides a good correlation. Finally, the geminal coupling constants $J_{4\alpha,4\beta}$, $J_{5\alpha,5\beta}$, and $J_{9\alpha,9\beta}$, as well as the long-range W-couplings $J_{4\beta,9\beta}$ and $J_{12,13}$ are evidenced for the first time. Considering that W-type coupling constants can take negative or positive sign, again, the criteria were the lower RMS values calculating positive and negative long-range coupling constants. Just to illustrate the point, for **8,** a positive $^{4}J_{4\beta,9\beta}$ value of 2.82 Hz provides RMS = 0.075%, while $^{4}J_{4\beta,9\beta} = -2.82$ Hz provides RMS = 0.073%, and in the case of **9**, $^{4}J_{12,13} = +0.86$ Hz provides RMS = 0.088%, while $^{4}J_{12,13} = -0.86$ Hz provides RMS = 0.087%.

Conclusion

The complete ^{1}H NMR data of ten cedranolides, which include well-known α-cedrene (**2**) and cedrol (**9**), are described by applying an iterative full spin analysis using the PERCH NMR software. The coupling constant values were correlated with conformational preferences of the cedranolides obtained by calculations using the CBS-4M method. This correlation revealed that the conformational preferences of the cedranolides are distributed in two types of conformers: one group in which the C1-C2-C3-C3a-C8a five-membered ring preferentially adopts a twisted-envelope shape with the methyl group at C3 in *pseudo*-equatorial orientation, which is preferred in compounds **3**, **4**, **6**, **7**, **9,** and **10**, and a second group obtained by flipping the five-membered ring with the methyl group at C3 in a *pseudo*-axial orientation, which is preferentially favored in cedranolides **2**, **5,** and **8**. These findings are in good agreement with the coupling constant values obtained by taking into account the Boltzmann population estimated from calculations using the CBS-4M method.

Experimental

Compounds

Sesquiterpenes **1–4** were prepared as previously described,[11] while cedranolides **5–10** were available from recent vibrational circular dichroism studies.[16]

NMR experiments

Chemical shifts (δ) are given in parts per million (ppm), and coupling constants (J) are given in hertz (Hz). Samples of approximately 10 mg of each sesquiterpene were placed in 5-mm tubes, dissolved in 0.9 ml of $CDCl_3$ and degassed by slowly bubbling an Ar stream under ultrasound during 10 min. A final volume of 0.5 ml was left to which a small amount of TMS in $CDCl_3$ was added, and all measurements were performed at room temperature. Those of **1–8** were recorded on a JEOL ECA 500 spectrometer (JEOL Ltd., Tokyo, Japan), at 500 MHz with number of scans = 16, acquisition time = 6.98 s, relaxation time = 1.0 s, 90° pulse width (P1) = 12.8 ms, spectral width = 9384 Hz, and FT size = 65536. Measurements of **9** and **10** were carried out on a Bruker ASCEND spectrometer (Bruker Corp.,

Karlsruhe, Germany) at 750.12 MHz with number of scans = 32, acquisition time = 4.35 s, relaxation time = 1.0 s, 90° pulse width (P1) = 12.8 ms, spectral width = 15000 Hz, and FT size = 32768.

^{1}H NMR full spin analysis

Complete ^{1}H NMR spectra analysis of compounds **1–10** was performed by iterative full spin analysis using the PERCH v.2011.1 software. The ^{1}H NMR experimental data at 500 and 750 MHz were imported into the PERCH shell and using the PAC module for baseline correction, peak picking, and signals integration. Construction of molecular models for cedranolides **1–10** was performed in the MMS module and after their geometry optimization and Monte Carlo analysis, the minimum energy conformer was selected for obtaining the initial ^{1}H NMR δ and J_{H-H} values. The subsequent optimization of spectral parameters was made by setting the known values in the spectral parameter editor of the PMS module and subsequent iterations using the integral-transform (D) and total-line-shape-fitting modes in the PERCHit. Iterative optimizations were performed until the experimental and calculated spectra showed excellent concordance and the total intensity root mean square deviations were below 0.1%.

Conformational analysis

Molecular models of cedranolides **1–10** were constructed and subjected to a full minimization routine employing molecular mechanics in the Spartan'04 W package (Wavefunction, Irvine, CA, USA). The conformational searching was made with the Monte Carlo protocol. The minimum energy conformers below 2 kcal/mol for each compound were then submitted to geometry optimization by density functional theory calculations at the B3LYP/6-31G(d) level of theory, followed by re-optimization using the CBS-4M method in the Gaussian 03 W program (Gaussian Inc., Wallingford, CT, USA), providing the Boltzmann distribution used for the estimation of the vicinal coupling constants, which were calculated using the Altona software.[17]

Acknowledgement

Partial financial support from Conacyt-México, grant 152994 is acknowledged.

References

[1] a) P. Walter. *Ann. Chem.* **1841**, *39*, 247; b) P. Walter. *Ann. Chem.* **1843**, *48*, 35.
[2] A. M. Clark, J. D. McChesney, R. P. Adams. *Phytother. Res.* **1990**, *4*, 15.
[3] E. Nibret, M. Wink. *Phytomedicine* **2010**, *17*, 911.
[4] a) J. Oda, N. Ando, Y. Nakajima, Y. Inouye. *Agric. Biol. Chem.* **1977**, *41*, 201; b) R. Adams, C. A. McDaniel, F. L. Carter. *Biochem. Syst. Ecol.* **1988**, *16*, 453.
[5] J. Vinholes, A. Rudnitskaya, P. Gonçalves, F. Martel, M. A. Coimbra, S. M. Rocha. *Food Chem.* **2014**, *146*, 78.
[6] S. P. Bhatia, D. McGinty, C. S. Letizia, A. M. Api. *Food Chem. Toxicol.* **2008**, *46*, S100.
[7] a) E. Hori, H. Shojaku, N. Watanabe, Y. Kawasaki, M. Suzuki, M. F. P. De Araujo, Y. Nagashima, Y. Yada, T. Ono, H. Nishijo. *Auton. Neurosci. Basic Clin.* **2012**, *168*, 88; b) M. H. Jin, S. G. Park, Y. Hwang, M. Lee, N. Jeong, S. Roh, Y. Lee, C. D. Kim, J. Lee. *Ann. Dermatol.* **2012**, *24*, 16.
[8] a) G. Stork. *F. H. Clarke. J. Am. Chem. Soc.* **1955**, *77*, 1072; b) G. Stork, F. H. Clarke. *J. Am. Chem. Soc.* **1961**, *83*, 3114.
[9] a) D. Chappell. *A. T. Russell. Org. Biomol. Chem.* **2006**, *4*, 4409; b) J. C. Green, T. R. R. Pettus. *J. Am. Chem. Soc.* **2011**, *133*, 1603; c) T. Bach, J. Hehn. *Angew. Chem. Int. Ed.* **2011**, *50*, 1000; d) T. Hudlicky, J. W. Reed, *The Way of Synthesis*, Wiley-VCH, Weinheim, **2007**.
[10] P. A. Wender, J. J. Howbert. *J. Am. Chem. Soc.* **1981**, *103*, 688.
[11] a) P. Joseph-Nathan, M. E. Garibay, R. L. Santillan. *J. Org. Chem.* **1987**, *52*, 759; b) P. Joseph-Nathan, A. Gutiérrez, J. D. Hernández, L. U. Román, R. L. Santillan. *J. Nat. Prod.* **1986**, *49*, 79.
[12] a) G. D. Brown, G. Liang, L. Sy. *Phytochemistry* **2003**, *64*, 303; b) P. Joseph-Nathan. *Rev. Soc. Quím. Mex.* **1988**, *32*, 106.
[13] a) P. Diehl, S. Sykora, J. Vogt. *J. Magn. Reson.* **1975**, *19*, 67; b) R. Laatikainen, M. Niemitz, U. Weber, J. Sundelin, T. Hassinen. *L. Vepsalainen. J. Magn. Reson.* **1996**, *120*, 1; c) R. Laatikainen, M. Tiainen, S. P. Korhonen, M. Niemitz, *Encyclopedia of Magnetic Resonance, Wiley; Chichester*, **2011**.
[14] a) J. G. Napolitano, D. C. Lankin, J. B. McAlpine, M. Niemitz, S. Korhonen, S. Chen, G. F. Pauli. *J. Org. Chem.* **2013**, *78*, 9963; b) L. G. Zepeda, E. Burgueño-Tapia, N. Pérez-Hernández, G. Cuevas, P. Joseph-Nathan. *Magn. Reson. Chem.* **2013**, *51*, 245; c) M. A. Muñoz, N. Pérez-Hernández, M. W. Pertino, G. Schmeda-Hirschmann, P. Joseph-Nathan. *J. Nat. Prod.* **2012**, *75*, 779; d) C. Villanueva-Cañongo, N. Pérez-Hernández, B. Hernández-Carlos, E. Cedillo-Portugal, P. Joseph-Nathan, E. Burgueño-Tapia. *Magn. Reson. Chem.* **2014**, *52*, 251.
[15] a) R. Laatikainen. *J. Magn. Reson.* **1991**, *92*, 1; b) R. Laatikainen. *QCPE Bull.* **1992**, *12*, 23.
[16] a) A. D. Becke. *J. Chem. Phys.* **1993**, *98*, 1372; b) B. Gordillo-Román, J. Camacho-Ruiz, M. A. Bucio, P. Joseph-Nathan. *Chirality* **2013**, *25*, 939; c) B. Gordillo-Román, J. Camacho-Ruiz, M. A. Bucio, P. Joseph-Nathan. *Chirality* **2012**, *24*, 147.
[17] a) C. A. G. Haasnoot, F. A. A. M. De Leeuw, C. Altona. *Tetrahedron* **1980**, *36*, 2783; b) C. M. Cerda-García-Rojas, L. G. Zepeda, P. Joseph-Nathan. *Tetrahedron Comput. Methodol.* **1990**, *3*, 113.
[18] a) J. W. Ochterski, G. A. Petersson, J. A. Montgomery Jr. *J. Chem. Phys.* **1996**, *104*, 2598; b) J. A. Montgomery Jr., M. J. Frisch, J. W. Ochterski, A. Petersson. *J. Chem. Phys.* **2000**, *112*, 6532.
[19] E. L. Eliel, S. H. Wilen, *Stereochemistry of Organic Compounds, Wiley; New York*, **1994**.

Supporting information

Additional supporting information may be found in the online version of this article at the publisher's web site.