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DFT molecular modeling and NMR conformational analysis of a new longipinenetriolone diester

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Abstract

The structure and conformational behavior of the new natural compound (4*R*,5*S*,7*S*,8*R*,9*S*,10*R*,11*R*)-longipin-2-en-7,8,9-triol-1-one 7-angelate-9-isovalerate (1) isolated from *Stevia eupatoria*, were studied by molecular modeling and NMR spectroscopy. A Monte Carlo search followed by DFT calculations at the B3LYP/6-31G* level provided the theoretical conformations of the sesquiterpene framework, which were in full agreement with results derived from the ^1H–^1H coupling constant analysis.

Keywords: DFT calculations; Molecular modeling; Conformation; ^1H–^1H coupling constants; Longipinene derivatives; *Stevia eupatoria*; NMR

1. Introduction

Remarkable developments in NMR spectroscopy combined with molecular modeling at the density functional level of theory can nowadays provide an accurate description of the chemical structure and conformational behavior of organic substances. Much of this information can be very useful to establish correlations between the geometry and the biological activity of new substances, a situation of particular relevance in the case of natural products. In this work, we applied a molecular modeling protocol to a new natural substance which possesses a highly oxygenated tricyclic framework. The protocol includes a Monte Carlo random search [1] followed by geometry optimization by DFT calculations [2,3] at the B3LYP/6-31G* level of theory [4,5], and comparison of the calculated conformational parameters with those obtained from NMR spectral data, as achieved in a recent work [6]. The new substance, a longipin-2-en-7,8,9-triol-1-one derivative (**1**), belongs to the sesquiterpenoid class of natural products which can be obtained from *Stevia eupatoria* (Asteraceae). This species grows in humid conifer forests between 2300 and 2900 m over the sea level [7,8] and is used in the traditional medicine of Mexico as a diuretic and antimalarial agent, and to treat digestive disorders in mammals [9]. Previous studies on the aerial parts of this plant, collected in different locations, afforded two kaurene derivatives [10], three guaianolides and two longipinene derivatives [11], while studies from the roots yielded the first *seco*-C oleanane isolated from nature [12].

Polyoxygenated longipinenes are representative secondary metabolites of the genus *Stevia* [13,14] which have been versatile starting materials for the preparation of novel carbocyclic sesquiterpenic structures [15–20]. Most longipin-2-en-7,8,9-triol-1-one derivatives have been found as 7,8-diesters, and only three 7,9-diesters are known [21,22].

2. Results and discussion

The hexane extracts of the roots of *S. eupatoria*, collected in the State of Mexico, were subjected to column chromatography on silica gel. The ^1H NMR spectrum of fractions eluted with hexane-EtOAc 4:1 revealed the presence of a complex mixture of longipinene derivatives. This spectrum showed at δ 4.10 a multiplet for a proton geminal to oxygen, thus indicating the presence of a –C_7(OR)–C_8(OH)–C_9(OR)– fragment where R are acyl groups. As mentioned, longipinene derivatives with this functionality are rare in nature and therefore, it was relevant to carry out a detailed chemical study of the sesquiterpene fraction. HPLC purification of this mixture afforded compound **1**, whose structure corresponded to (4*R*,5*S*,7*S*,8*R*,9*S*,10*R*,11*R*)-longipin-2-en-7,8,9-triol-1-one

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7-angelate-9-isovalerate, as determined from its spectroscopic and optical rotation data. The IR spectrum showed typical absorptions for a hydroxyl group (3682 and 3550 cm^{-1}), a saturated ester group (1726 cm^{-1}), an α,β-unsaturated ester group (1710 and 1642 cm^{-1}), and an α,β-unsaturated ketone (1672 and 1620 cm^{-1}). The molecular formula was determined as $C_{25}H_{36}O_6$ by HREIMS which showed exact mass 432.2515 (calcd 432.2512). The ^{1}H NMR spectrum showed a vinylic hydrogen signal at δ 6.12 and two vinylic methyl groups at δ 2.00 and δ 1.94, characteristic of an angelate ester. The presence of an isovalerate ester was evidenced by a complex multiplet located at δ 2.21, a methylene multiplet centered at δ 2.37 and two doublets for two secondary methyl groups at δ 1.00 and δ 0.99. The signals for the three hydrogen atoms geminal to oxygen atoms appeared at δ 5.46 (d, $J_{8,9}=3.4$ Hz, H-9), 5.20 (d, $J_{7,8}=11.0$ Hz, H-7) and 4.10 (br m, which collapses to a dd upon addition of D_2O, $J_{7,8}=11.0$ and $J_{8,9}=3.4$ Hz, H-8). Three signals for hydrogens attached to the cyclobutane ring were observed at δ 3.10 (br d, $J_{4,11}=7.0$ Hz, H-11), 2.66 (br d, $J_{4,11}=7.0$ Hz, H-4) and 2.34 (br s, H-5). It is interesting to remark that the cyclobutane ring exhibits a fixed geometry in which both H_4–C_4–C_5–H_5 and H_5–C_5–C_{11}–H_{11} dihedral angles are close to 90°, thus providing very small coupling constants between H-4 and H-11 and between H-5 and H-11, but a large long-range coupling constant between H-4 and H-11 [23]. The ^{1}H and ^{13}C NMR spectral data were assigned on the basis of gHSQC and gHMBC spectra, whose correlations are summarized in Table 1. The assignments were in full agreement with the proposed structure for **1**. In particular, the positions for the angelate and isovalerate groups were secured from *g*HMBC correlations. The H-7 signal at δ 5.20 showed a cross-peak with the ^{13}C signal at δ 169.0, corresponding to the angelate carbonyl group C-1′. The H-9 signal at δ 5.46 displayed a correlation with the ^{13}C signal at δ 69.6, corresponding to C-8, and with that at δ 74.2, corresponding to C-7. Additionally, the ^{1}H–^{13}C gHSQC experiment was very useful to confirm the assignment of the C-8 signal, which correlated with the hydrogen geminal to the hydroxyl group at δ 4.10. The optical rotation for compound **1** was dextrorotatory at the sodium D-line, showing $[\alpha]_{589}=+57°$. This value was within the characteristic range for natural (+)-longipin-2-en-1-one derivatives of known absolute configuration [21,22,24,25].

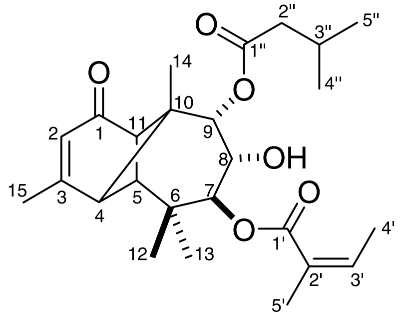
Longipinene derivative **1** represents an attractive case for conformational studies because some parts of the molecular structure are rigid (i.e. the fused four- and six-membered rings) and others show flexibility (i.e. the seven-membered ring and the short chain ester moieties). According to a Dreiding model, the seven-membered ring may exist in two main conformations, which, in conjunction with the wide rotameric possibilities of the ester groups, generate a complex conformational scenario to be solved.

Table 1
NMR data for longipinene derivative **1** measured at 300 MHz in $CDCl_3$. Chemical shifts δ are in ppm and coupling constants (J) are in Hz

Position	δ_C, multiplicity	δ_H, multiplicity (J)	HMBC (H→C)	COSY (H→H)
1	202.5 s	–		
2	122.8 d	5.82, ddq (1.5)	4	4, 11, 15
3	169.9 s	–		
4	48.2 d	2.66, br d (7.0)	2, 3, 6, 11, 15	5
5	65.4 d	2.34, br s	3, 4, 6, 7, 10, 11, 12	12, 13
6	36.3 s	–		
7	74.2 d	5.20, d (11.0)	6, 8, 12, 13, 1′	5, 8
8	69.6 d	4.10, dd (11.0, 3.4)	6, 7, 9, 10	7, 9
9	77.4 d	5.46, d (3.4)	7, 8, 11, 14	8
10	54.9 s	–		
11	53.1 d	3.10, d (7.0)	2, 4	4, 5
12	26.7 q	0.95, s	5, 6, 7, 13	
13	19.8 q	1.11, s	5, 6, 7, 12	12
14	21.3 q	1.03, s	4, 9, 10,11	
15	23.4 q	2.06, d (1.5)	2, 3, 4	
1′	169.0 s	–		
2′	127.5 s	–		
3′	139.2 d	6.12, qq (7.5, 1.5)	5′	4′, 5′
4′	15.8 q	2.00, dq (7.5, 1.5)	2′, 3′	
5′	20.6 q	1.94, quint (1.5)	1′, 2′, 3′	
1″	173.3 s	–		
2″	43.4 t	2.37, m		
3″	25.6 d	2.21, m		
4″	22.5 q	0.99, d (6.3)	2″, 3″	
5″	22.4 q	1.00, d (6.8)	2″, 3″	

A randomized conformational search using the Monte Carlo method [1] with a search limit of 200 structures, and employing the Merck molecular force field as implemented in the Spartan'04 software package [26] gave a total of 131 minimum energy structures. A geometry restriction for the C=C–C=O torsion angle of the angelate ester was set at 0° to emulate the π-electron conjugation effect. X-ray diffraction analyses of angelate esters have shown that the *s*-cis arrangement is the preferred conformation for this moiety over the *s*-trans arrangement [27–29]. Our molecular modeling calculations confirmed this observation. The molecular mechanics structures were geometry analyzed to eliminate those which appeared as duplicated and sorted according to their energy. A total of 38 structures within the range of 0.0–3.3 kcal/mol were selected for geometry optimization by density functional theory calculations, employing the B3LYP method and the 6-31G* basis set [4,5], without any geometrical restrictions. After DFT optimization, a set of 21 conformations were considered for the Boltzmann population analysis. The remaining 17 conformations were discarded because they were either duplicated or of high energy, mainly those which did not show the most favorable *anticlinal* conformation for the acyloxy moieties [30]. It is relevant to comment that the order, according to the energy of each conformer, as well as the relative population, obtained from the Boltzmann distribution, strongly differ when comparing molecular mechanics results with density functional theory calculations. This difference is explained due to electronic interactions present in the structure of **1**, which are not considered in the molecular mechanics calculations.

The DFT global minimum (Fig. 1) was located at $E_{\text{DFT}} = -894616.593$ kcal/mol which, together with the following nine conformations (Fig. 2(a)) accounted for 99.8% of the conformational population (Table 2). According to analysis using the Cremer and Pople parameters [31], carried out with the RICON program [32], the seven membered ring of the global minimum structure exists in a single hybrid conformation, composed by the canonical twist-chair, boat and twist-boat conformations, as quantified in Table 3. The angelate group at C-7 and the hydroxyl group at C-8 remain in pseudo-equatorial orientations, while the isovalerate group is in a pseudo-axial orientation. The six-membered ring exists in a conformation intermediate between envelope and half-chair, and the four-membered ring adopts a puckered conformation (Table 3). This conformation of the sesquiterpene framework was sustained for a total of ten calculated minima (Table 2 and Fig. 2(a)), within a relative energy range up to 2.014 kcal/mol, followed by an energetic gap up to 3.836 kcal/mol where inversion of the seven-membered ring occurred at conformation number eleven. This conformation, together with the following ten conformations (i.e. conformations 11–21), corresponded to a second conformational group (Fig. 2(b)) where the angelate group at C-7, and the hydroxyl group at C-8 remain in pseudo-axial orientations, and the isovalerate group at C-9 remains in a pseudo-equatorial orientation. According to molecular mechanics calculations, this second conformational array could be more stable than the group depicted in Fig. 2(a). However, an analysis of the ^{1}H–^{1}H NMR coupling constants for the hydrogen atoms of the seven-membered ring $J_{7,8}$ and $J_{8,9}$ indicates that, experimentally, the predominant conformations are those represented in Fig. 2(a), as properly modeled by the DFT calculations. A comparative analysis for the calculated and observed ^{1}H–^{1}H coupling constants is listed in Table 4. The calculated coupling constant values were obtained from theoretical H–C–C–H DFT dihedral angles using a generalized Karplus-type equation [33,34], and are in agreement with the observed $^3J_{\text{H–H}}$ coupling constant values, thus validating the theoretical results for longipinene derivative **1**.



1

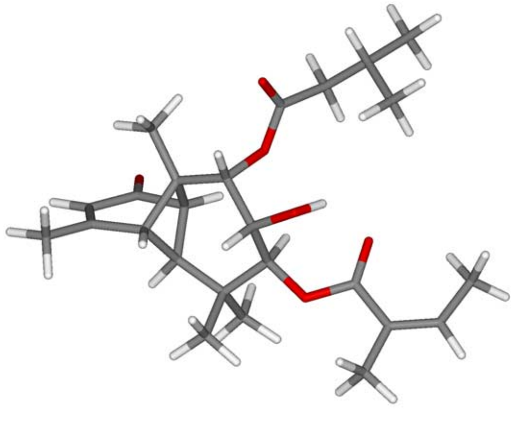


Fig. 1. Molecular formula and global minimum energy structure of (4*R*,5*S*,7*S*,8*R*,9*S*,10*R*,11*R*)-longipin-2-en-7,8,9-triol-1-one 7-angelate-9-isovalerate (**1**).

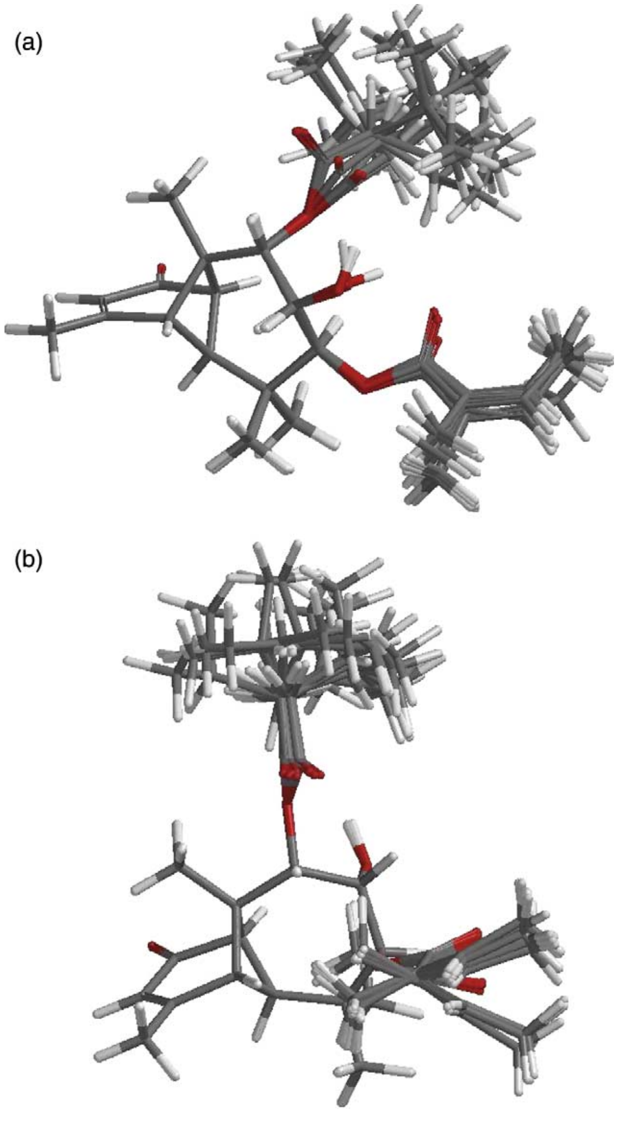


Fig. 2. Superimposed minimum energy structures of longipinene derivative **1** (a) with a DFT relative energy range from 0 to 2.014 kcal/mol and (b) the higher energy conformational group (DFT relative energy range 3.836–6.507 kcal/mol), showing the inversion of the seven-membered ring.

Finally, its is relevant to mention that according to Fig. 2(a), the longipinene structure **1** shows a wide mobility of

Table 2
DFT relative energy, population according to the Boltzmann distribution, and isovalerate dihedral angles for the most relevant conformers of longipinene derivative **1**

Conformer	E_{DFT}[a]	Population[b]	C_8–C_9–O_9–$C_{1''}$[c]	C_9–O_9–$C_{1''}$–$C_{2''}$[c]	O_9–$C_{1''}$–$C_{2''}$–$C_{3''}$[c]	$C_{1''}$–$C_{2''}$–$C_{3''}$–$C_{4''}$[c]
1	0.000	29.19	−115.3	172.8	−138.7	63.6
2	0.294	17.79	−118.0	175.0	−75.4	178.8
3	0.537	11.80	−88.1	179.6	−80.6	178.4
4	0.562	11.32	−80.5	−167.7	−157.8	71.1
5	0.632	10.05	−79.0	178.7	165.0	167.7
6	0.696	9.03	−114.0	174.9	73.4	63.8
7	0.950	5.88	−117.0	175.7	−125.8	−70.2
8	1.573	2.05	−80.0	−179.5	53.2	62.1
9	1.673	1.74	−117.6	171.9	93.1	−66.7
10	2.014	0.98	−78.1	179.3	179.1	−65.1

[a] In kcal/mol.
[b] In percent.
[c] In degrees.

Table 3
Conformational parameters of the four, six and seven-membered rings at the DFT global minimum structure of longipinene derivative **1**

Parameter	$C_4C_5C_{10}C_{11}$	$C_1C_2C_3C_4C_5C_{11}$	$C_1C_2C_3C_4C_{10}C_{11}$	$C_4C_5C_6C_7C_8C_9C_{10}$	$C_5C_6C_7C_8C_9C_{10}C_{11}$
Q	0.373	0.851	0.800	1.010	0.974
Q_2	–	–	–	0.716	0.636
Q_3	–	–	–	0.712	0.738
θ	–	49.6	57.2	–	–
Ψ_2	–	7.6	5.0	30.4	44.1
Ψ_3	–	–	–	323.4	61.6
Conformation	Puckered	Between envelope and half-chair	Between envelope and screw-boat	Combination of twist-chair, boat and twist-boat	Combination of twist-chair, boat and twist-boat

the isovalerate ester side chain at C-9, while the angelate ester remains essentially rigid with a certain degree of molecular vibration. The dihedral angles for the global minimum of **1**, which define the conformation of the angelate moiety are C_6–C_7–O_7–$C_{1'} = +149.5°$, C_7–O_7–$C_{1'}$–$C_{2'} = -178.0°$, O_7–$C_{1'}$–$C_{2'}$–$C_{3'} = +169.9°$ and $C_{1'}$–$C_{2'}$–$C_{3'}$–$C_{4'} = -1.2°$, while the representative dihedral angles for the rotamers of the isovalerate ester are listed in Table 2.

3. Experimental

3.1. General experimental procedures

Column chromatography was carried out on Merck silica gel 60 (70–230 mesh ASTM). HPLC separations were done on a Varian ProStar chromatographic equipment. Optical rotations were measured in $CHCl_3$ on a Perkin–Elmer 341 polarimeter. The UV spectrum was measured in EtOH on a Perkin–Elmer Lambda 12 spectrophotometer. The IR spectrum was obtained in $CHCl_3$ on a Perkin–Elmer 16F PC FT spectrophotometer. 1D and 2D NMR spectra were measured at 300 MHz for ^1H and 75.4 MHz for ^{13}C from $CDCl_3$ solutions containing TMS as the internal standard on a Varian Mercury 300 spectrometer. The low-resolution mass spectrum was recorded at 20 eV on a Hewlett-Packard 5989A spectrometer, while the HRMS was measured on a VG 7070 high-resolution mass spectrometer at the UCR Mass Spectrometry Facility, University of California, Riverside.

3.2. Plant material

Specimens of *S. eupatoria* (Spreng.) Willd. were collected at 40 km of the Atlacomulco-Jilotepec state road No. 10, near Timilpan, State of Mexico, Mexico, in October 2002. A voucher specimen (number 136) is deposited in the Herbarium of Instituto de Ecología A.C., Pátzcuaro, Michoacán, Mexico, where Prof Jerzy Rzedowski kindly identified the plant.

3.3. Extraction and isolation

The air-dried roots of *S. eupatoria* (900 g) were extracted (×3) with hexane (3 L) under reflux. After vacuum evaporation of the solvent, the crude extract (9 g) was suspended in MeOH (500 mL) at 0 °C, to remove waxy materials. Filtration and evaporation of the extract afforded a dark-yellow syrup (4.5 g) which was subjected to column chromatography on

Table 4
Observed and calculated ^1H–^1H NMR vicinal coupling constants (in Hz) and calculated dihedral angles (in degrees) for longipinene derivative **1**

Fragment	J_{obs}	J_{calc}	Dihedral angle
H_4–C_4–C_5–H_5	1.0[a]	0.8	98.4
H_5–C_5–C_{11}–H_{11}	1.0[a]	0.8	−98.9
H_7–C_7–C_8–H_8	11.0	10.2	165.8
H_8–C_8–C_9–H_9	3.4	2.4	60.6

[a] Estimated from the width at half-height of H-4, H-5 and H-11.

silica gel. Elution with hexane-EtOAc, 4:1, yielded 10 fractions (268 mg) containing compound **1** admixed with several other longipinene derivatives. These fractions were combined and an aliquot was purified by normal phase HPLC. The optimal chromatographic conditions to obtain pure **1** were: an aliquot of 3.5 mg of the longipinene fraction in 40 μL of hexane–CH_2Cl_2–EtOAc (3:3:1) was injected to a Si-10 column (Varian), i.d. 4 mm, length 30 cm, eluting with the same solvent mixture at 1 mL/min and monitoring by UV detection at 254 nm. The process was repeated 16 times to accumulate 6 mg of **1** ($R_t = 7.6$ min). *S. eupatoria* also afforded known stigmasterol [35], friedelin [36], taraxerol [37], 5-acetyl-2-[2′-hydroxyisopropyl]-benzofuran [38] and the longipinene derivatives rastevione [29], dehydrorastevione [39] and longipin-2-en-7,8,9-triol-1-one 7-angelate-8-isovalerate [25].

3.4. (4R,5S,7S,8R,9S,10R,11R)-Longipin-2-en-7,8,9-triol-1-one 7-angelate-9-isovalerate (*1*)

Colourless oil. $[\alpha]_{589} = +57°$, $[\alpha]_{578} = +61°$, $[\alpha]_{546} = +70°$, $[\alpha]_{436} = +135°$, $[\alpha]_{365} = +356°$ (*c* 1.0, $CHCl_3$). IR ($CHCl_3$) ν_{max}: 3682, 3550, 1726, 1710, 1672, 1642, 1620, 1234 cm^{-1}. UV (EtOH) λ_{max} (log ε): 216 nm (3.94). ^1H NMR ($CDCl_3$, 300 MHz): see Table 1. ^{13}C NMR ($CDCl_3$, 75.4 MHz): see Table 1. EIMS *m/z* 432 $[M]^+$(2), 330 (8), 248 (6), 230 (9), 201 (24), 135 (9), 83 (100), 57 (22), 55 (50). HREIMS *m/z* 432.2515 (calcd for $C_{25}H_{36}O_6$, 432.2512).

3.5. Computational details

The molecular modeling calculations started with a systematic conformational search for the seven-membered ring and the ester side chains of longipinene derivative **1**. This procedure was carried out with the aid of Dreiding models considering torsion angle rotations of ≈20° for those bonds which allowed such movements. All conformational species derived from the hydroxyl group rotation were also examined. Geometry optimizations were carried out using the Merck molecular force-field calculations [40]. The E_{MMFF} values were used as the convergence criterion and a further search with the Monte Carlo protocol [1] was carried out. The selected conformers were submitted to density functional theory calculations (B3LYP/6-31G*) without bond rotation restrictions. The MMFF calculations, the Monte Carlo random searching, the density functional theory B3LYP/6-31G* quantum calculations and the Boltzmann distribution calculations were achieved using Spartan’04 software from Wavefunction, Inc. (Irvine, California, USA). Conversions of dihedral angles into vicinal coupling constants ($^3J_{H–H}$) for each conformer were done using the Altona equation [33,34]. The population-weighted average coupling constants for the H–C–C–H dihedral fragments were calculated using the equation

$$^3J_{calc} = n_1J_1 + n_2J_2 + \ldots + \Sigma n_iJ_i.$$

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References

[1] G. Chang, W.C. Guida, W.C. Still, J. Am. Chem. Soc. 111 (1989) 4379.
[2] J.P. Perdew, Phys. Rev. B 33 (1986) 8822.
[3] A.D. Becke, Phys. Rev. A 38 (1988) 3098.
[4] A.D. Becke, J. Chem. Phys. 98 (1993) 5648.
[5] C. Lee, W. Yang, R.G. Parr, Phys. Rev. B 37 (1988) 78.
[6] C.M. Cerda-García-Rojas, A.C. Coronel, M.E.P. de Lampasona, C.A.N. Catalán, P. Joseph-Nathan, J. Nat. Prod. 68 (2005) 659.
[7] L.S. Rodríguez-Jiménez, J. Espinoza-Garduño, Flora del Bajío y Regiones Adyacentes, Listado Florístico del Estado de Michoacán. Sección I. 4 (1995) 210.
[8] J. Espinoza-Garduño, L.S. Rodríguez-Jiménez, Flora del Bajío y Regiones Adyacentes, Listado Florístico del Estado de Michoacán. Sección II. 7 (1995) 240.
[9] R. Bye, E. Linares, in: T.P. Lyons, K.A. Jaques (Eds.), Biotechnology in the Feed Industry, Nottingham University Press, Nottingham, UK, 1999, pp. 265–294.
[10] A. Ortega, F.J. Morales, M. Salmón, Phytochemistry 24 (1985) 1850.
[11] C. Zdero, F. Bohlmann, H.M. Niemaeyer, Phytochemistry 30 (1991) 693.
[12] L.U. Román, D. Guerra-Ramírez, G. Morán, I. Martínez, J.D. Hernández, C.M. Cerda-García-Rojas, J.M. Torres-Valencia, P. Joseph-Nathan, Org. Lett. 6 (2004) 173.
[13] L.R. Hernández, C.A.N. Catalán, P. Joseph-Nathan, Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales 22 (1998) 229.
[14] C.M. Cerda-García-Rojas, R. Pereda-Miranda, Stevia the Genus Stevia, in: A.D. Kinghorn (Ed.), Medicinal and Aromatic Plants Industrial Profiles, Taylor and Francis, London, 2002, pp. 86–118 (Chapter 5).
[15] L.U. Román, J.D. Hernández, R.E. del Río, M.A. Bucio, C.M. Cerda-García-Rojas, P. Joseph-Nathan, J. Org. Chem. 56 (1991) 1938.
[16] L.U. Román, L.G. Zepeda, N.R. Morales, J.D. Hernández, C.M. Cerda-García-Rojas, P. Joseph-Nathan, J. Nat. Prod. 59 (1996) 391.
[17] L.U. Román, N.R. Morales, J.D. Hernández, C.M. Cerda-García-Rojas, L.G. Zepeda, C.A. Flores-Sandoval, P. Joseph-Nathan, Tetrahedron 57 (2001) 7269.
[18] C.M. Cerda-García-Rojas, C.A. Flores-Sandoval, L.U. Román, J.D. Hernández, P. Joseph-Nathan, Tetrahedron 58 (2002) 1061–1068.
[19] M. Meléndez-Rodríguez, C.M. Cerda-García-Rojas, C.A.N. Catalán, P. Joseph-Nathan, Tetrahedron 58 (2002) 2331–2338.
[20] L.U. Román, C.M. Cerda-García-Rojas, R. Guzmán, C. Armenta, J.D. Hernández, P. Joseph-Nathan, J. Nat. Prod. 65 (2002) 1540.
[21] E. Sánchez-Arreola, C.M. Cerda-García-Rojas, P. Joseph-Nathan, L.U. Román, J.D. Hernández, Phytochemistry 39 (1995) 853.
[22] E. Sánchez-Arreola, C.M. Cerda-García-Rojas, L.U. Román, J.D. Hernández, P. Joseph-Nathan, J. Nat. Prod. 63 (2000) 12.
[23] C.M. Cerda-García-Rojas, R.E. del Río, P. Joseph-Nathan, L.U. Román, J.D. Hernández, J. Nat. Prod. 57 (1994) 369.
[24] C.M. Cerda-García-Rojas, E. Sánchez-Arreola, P. Joseph-Nathan, L.U. Román, J.D. Hernández, Phytochemistry 32 (1993) 1219–1223.
[25] E. Sánchez-Arreola, C.M. Cerda-García-Rojas, L.U. Román, J.D. Hernández, P. Joseph-Nathan, Phytochemistry 52 (1999) 473–477.
[26] J. Kong, C.A. White, A.I. Krylov, C.D. Sherrill, R.D. Adamson, T.R. Furlani, et al., J. Comput. Chem. 21 (2000) 1532.

[27] G. Appendino, M. Calleri, G. Chiari, J. Chem. Soc., Perkin Trans. 2 (1986) 205.

[28] M. Jiménez-E, K. Velázquez, A. Lira-Rocha, A. Ortega, E. Díaz, A. Aumelas, et al., Can. J. Chem. 67 (1989) 2071.

[29] L.U. Román, R.E. del Río, J.D. Hernández, P. Joseph-Nathan, V. Zabel, W.H. Watson, Tetrahedron 37 (1981) 2769.

[30] F. López-Calahorra, D. Velasco, J. Castells, C. Jaime, J. Org. Chem. 55 (1990) 3530.

[31] D. Cremer, J.A. Pople, J. Am. Chem. Soc. 97 (1975) 1354.

[32] A.Y. Zotov, V.A. Palyulin, N.S. Zefirov, J. Chem. Inf. Comput. Sci. 37 (1997) 766.

[33] C.A.G. Haasnoot, F.A.A.M. de Leeuw, C. Altona, Tetrahedron 36 (1980) 2783.

[34] C.M. Cerda-García-Rojas, L.G. Zepeda, P. Joseph-Nathan, Tetrahedron Comput. Methodol. 3 (1990) 113.

[35] J.L.C. Wrigh, A.G. McInnes, S. Shimizu, D.G. Smith, J.A. Walter, D. Idler, et al., Can. J. Chem. 56 (1978) 1898.

[36] A. Patra, S.K. Chauhuri, Magn. Reson. Chem. 25 (1987) 95.

[37] N. Sakurai, Y. Yaguchi, T. Inoue, Phytochemistry 26 (1987) 217.

[38] F. Bohlmann, N. Le Van, Phytochemistry 16 (1977) 1304.

[39] F. Bohlmann, C. Zdero, Liebigs Ann. Chem. (1985) 1764.

[40] T. Halgren, J. Comp. Chem. 17 (1996) 490; T. Halgren, J. Comp. Chem. 17 (1996) 520; T. Halgren, J. Comp. Chem. 17 (1996) 553; T. Halgren, R.B. Nachbar, J. Comp. Chem. 17 (1996) 587; T. Halgren, J. Comp. Chem. 17 (1996) 616.