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Conformational analysis of three germacranolides by the PM3 semi-empirical method*

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Abstract: The conformations of the C-6 lactonized germacranolides **1–3** were calculated by applying the PM3 semi-empirical method. The low-temperature ^{1}H-NMR spectra of parthenolide (**1**) were also measured. The relations between the calculated and the experimentally determined geometries were established using a modified Karplus equation. The applied quantum-chemical calculations proved to be an efficient and easy-to-use tool for the elucidation and prediction of the properties of germacranolide-type sesquiterpene lactones.

Keywords: germacranolides, parthenolide, ridentin, 1β,10α-epoxy-3β,9β-diacetoxy-11α,13-dihydrocostunolide, conformations, PM3 semi-empirical calculations.

INTRODUCTION

Among the natural sesquiterpene lactones, germacranolides represent one of the most abundant groups. Due to their prominent and diverse physiological activity, there is a wide interest in the elucidation of the conformational properties of these compounds. The conformational studies reported for these ten-membered lactones[1] are based mainly on NMR spectroscopy, namely, on the torsional angle dependence of the vicinal coupling constants and on the interatomic distances dependence of NOE.[2] Considering the computational calculations, most are based on molecular mechanics.[1(e),1h),1(i),1(k–m)]

The isolation and structure determination of the germacranolides **1–3** (Fig. 1) have been reported earlier.[3–5] Parthenolide (**1**), known for a long time as the main bioactive constituent of extracts of *Tanacetum parthenium* (feverfew) used for centuries in the UK for treatment of migraine and painful periods, is still very actual.[3] Some preliminary attained investigations also suggested a promising role of **1** in increasing the efficacy of some common anti-cancer drugs.[6] Lactone **2** was isolated from the plant species *Achillea critmifolia*

* Dedicated to Professor Miroslav Gašić on the occasion of his 70th birthday.

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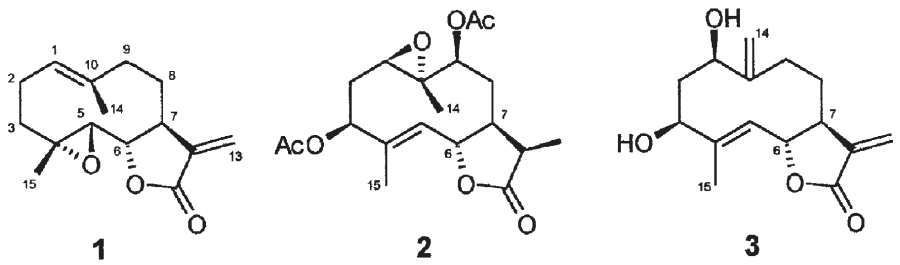

Fig. 1. Sesquiterpene lactones **1–3**.

and identified as a new compound.[4] The structure and conformation of ridentin (**3**) were originally proposed by Irwin *et al.*[5]; they pointed out its precursor-role in the biosynthetic pathway of eudesmanolides and guaianolides of the genus. Since germacranolides **1** and **3** were also isolated from plant material collected in Serbia and Montenegro,[7,8] this paper continues the long lasting, systematic investigation of medicinal plants (*i.e.* natural sesquiterpene lactones) in our faculty.

Herein the results of the conformational analysis of germacranolides **1–3** (Fig. 1) by the PM3 semi-empirical method are presented.

EXPERIMENTAL

NMR studies. The ^{1}H-NMR spectra of parthenolide (**1**) (isolated from *Tanacetum larvatum*)[7] were measured with a Bruker AMX500 spectrometer (operating frequency 500.13 MHz), in CD_2Cl_2 in the temperature range ≈180–298 K. For temperatures below ≈180 K, the solvent was a deuterated freon mixture $CDClF_2/CDF_3$ and the samples were prepared in a high vacuum line which was connected with a turbo molecular pump TPH 060 from Pfeiffer/Balzers, using thick-walled sample tubes purchased from Spintec (VNMR/5). The low temperatures were achieved by controlled flow of cold, gaseous nitrogen using an evaporator immersed in a container with liquid nitrogen and connected to the probehead. A '5 mm double resonance probehead ^{1}H/^{13}C' was used which was kept at room temperature by an external thermostat, so only the sample tube was cooled down to the required temperatures. The temperatures were adjusted by a Eurotherm Variable Temperature Unit using a 'Pt-100-thermo' external device. All temperatures were measured to an accuracy of ± 1 ºC and are reported in Kelvins. The synthesis of the freon mixture and 3',5'-bis(triisopropylsilyl)-2'-deoxyguanosine, used in experiments with compound **1**, are given in the literature.[9]

Theoretical calculations. The computational analysis of lactones **1–3** was performed by the semi-empirical PM3 method comprised in the HyperChem 4.0 program package. All energies were minimized to RMS gradient less than 0.2 kJ/Å mol applying the Polak-Ribiere minimization algorithm. The input geometries for these calculations were prepared by simultaneously varying the dihedral angles of the carbocyclic skeleton in steps of 20º, while the dihedral angles in the OR substitutents of lactones **2** (Φ = H–C*–O–C(Ac) and Φ = C*–O–C=O, where C* = C(3), *i.e.* C* = C(9)) and **3** (Φ = H–C*–O–H, where C* = C(1), *i.e.*, C* = C(3)) were changed by 60º, *i.e.*, all combinations of Φ = 60, 120, 180, – 120, – 60º were taken into account. Each of these starting geometries was preoptimized by molecular mechanics (MM+, HyperChem 4.0), then by the semi-empirical (PM3) method. During the geometry optimization many of the starting rotamers[10] passed to another rotamer arrangement (for lactones **2** and **3**), or to another conformation of the carbocyclic skeleton; hence, the large number of starting geometries[11] was reduced to a very few resulting structures.

For calculating the coupling constants of the resulting conformations, the Karplus equation reparametrized by Haasnoot[12] was used. All coupling constants (*J*) are reported in Hz.

The distributions of the conformers were calculated according to the Boltzmann distribution law: $n_i/n_1 = e^{-\Delta E/RT}$ (n_1 is the contribution of the most stable conformer, n_i the contribution of the i^{th} conformer), $\Delta E = H_{fl} - H_{fi}$ (H_f, heat of formation, is the output of the PM3 calculation), R = 8.31 J/K mol, T = 298 K.

RESULTS AND DISCUSSION

Parthenolide (**1**). Optimization of the geometry of lactone **1** by the PM3 semi-empirical method revealed four distinctive conformations (Fig. 2).

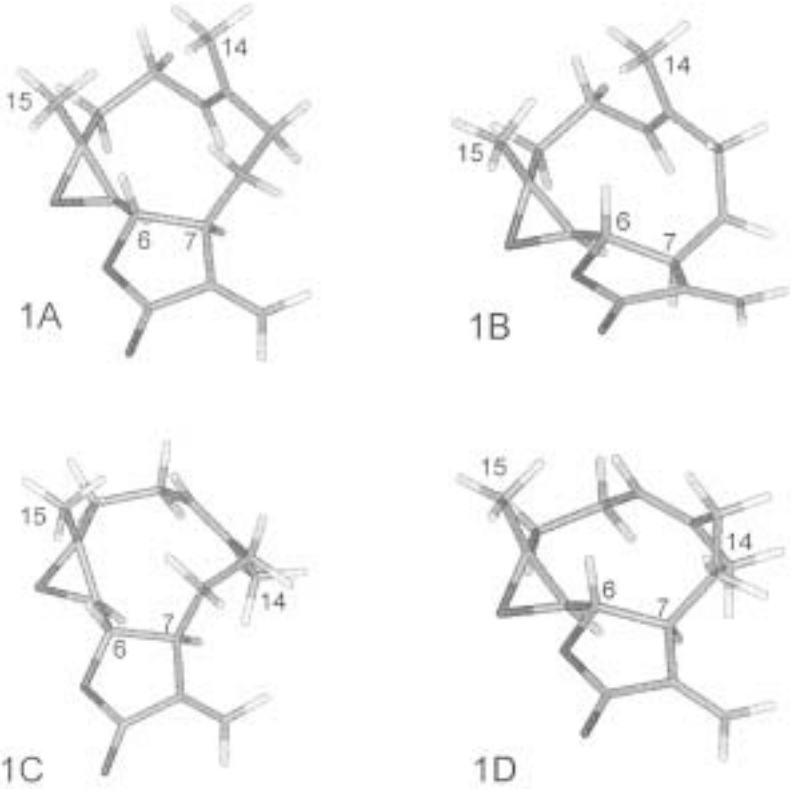

Fig. 2. Calculated geometries of lactone **1**.

According to the relative energies, conformations **1A** and **1C** are the most probable, so if they would constitute a conformational mixture the ratio should be **1A/1C**=3/2 (Table I). Moreover, the experimental coupling constants agree even better with those calculated for conformer **1C** (Table I). On the other hand, the differences between these two geometries are substantial: both Me-groups are β-oriented (UU)[13] and the C(10)–C(1) and C(4)–C(5) bonds are 'crossed' in conformer **1A**, since in conformer **1C** C(14) and C(15) are α- and β-oriented, respectively (DU), while the C(10)–C(1) and C(4)–C(5) bonds are 'parallel' (Fig 2). Hence, the conformational exchange between conformations **1A** and **1C** must occur over a high potential barrier and this exchange is of a low probability at room temperature.[14]

The only difference between conformers **1A** and **1B** is due to a rotation around the C(8)–C(9) bond. Anyway, regarding their fairly large relative energies, as well as the calculated coupling constants (Table I), conformations **1B** and **1D** certainly could be ignored.

TABLE I. Experimental $^3J_{HH}$ (CD_2Cl_2, r.t.), relative energies and calculated $^3J_{HH}$ for the theoretical conformers of lactone **1**.

Conformer	ΔE (kJ/mol)	*J*calc. (Hz)		Orientation of	
		$J_{5,6}$	$J_{6,7}$	C(14)	C(15)
1A	0.00	8.04	6.44	U	U
1B	34.37	4.40	9.48		
1C	1.3	9.93	8.21	D	U
1D	16.99	6.71	5.37		
*J*exp. (Hz)		8.9	8.6		

The sharp lines in the room-temperature ^{1}H- and ^{13}C-NMR spectra of germacranolide **1** indicate its rigid conformation.[1(i),4(d)] In the NOESY (r.t.) spectrum there are only negative cross-peaks,[1(m)] as well. The measured low-temperature ^{1}H-NMR spectra of lactone **1** till 133 K gave additional proof that, even at really low temperatures, parthenolide (**1**) adopts one stable conformation in solution.[15] In this conformation, typical for *trans*, *trans*-germacra-1(10),4-dien-6,12-olides,[16] ten-membered ring has a double chair[17] geometry with both Me-groups *syn*-oriented on the β-side of the molecule. The UU conformation appears to be the most stable for this type of germacranolides and they undergo a conformational exchange only if the stereochemistry

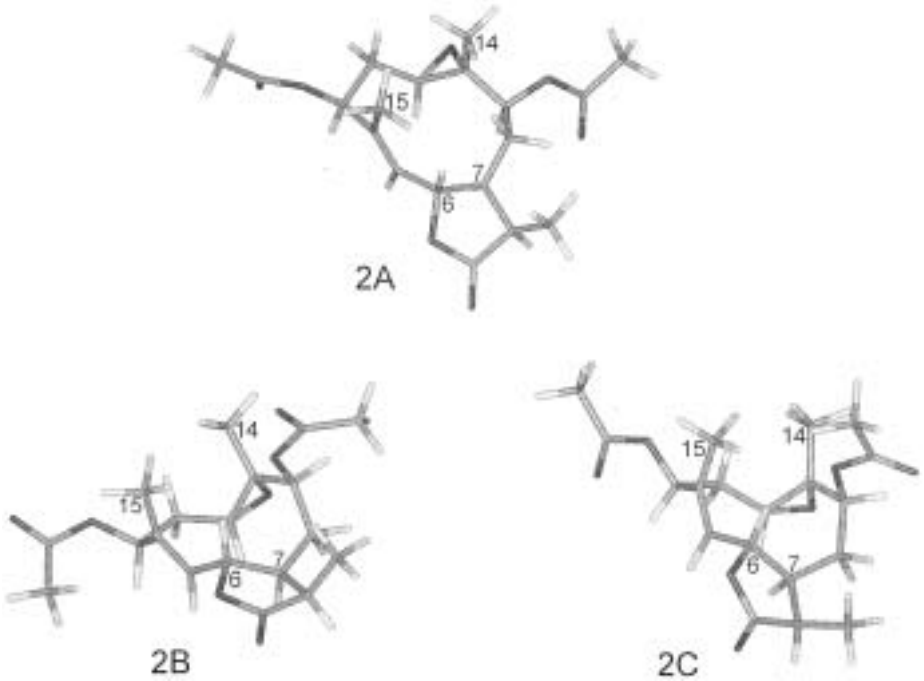

Fig. 3. Calculated geometries of lactone **2**.

and/or substituents destabilize the molecule.[1(d–h),1(j–m)] Obviously, in the parthenolide molecule such destabilizing factors do not exist.

Anticipating that lactone **1** would form an intermolecular hydrogen bond with a suitable proton-donor and therefore change conformation, the low-temperature ^{1}H-NMR spectra of a mixture of an equimolar ratio of compound **1** with 3',5'-bis(triisopropylsilyl)-2'-deoxyguanosine (pK_a = 10), and compound **1** with acetic acid (pK_a = 4.75), were recorded. However, in these spectra there was no evidence of any hydrogen bonds.

The results obtained by theoretical and experimental (NMR) methods applied in the conformational analysis of parthenolide (**1**) are in full accordance. The conformation **1A** (Fig. 2) is the most stable and it could be considered as being solely significant.

1β,10α-Epoxy-3β,9β-diacetoxy-11α,13-dihydrocostunolide (**2**). The applied semi-empirical calculations predicted three conformations for compound **2** (Fig. 3).

The obtained conformations differ in the geometry of the carbocyclic skeleton, hence in the orientation of the OAc moieties, although the Me-groups are β-oriented (UU) in all of them (Fig. 3). Variation of the dihedral angles in the OAc substituents (see Experimental) did not significantly influence the stability of the conformers.[18] The calculations also revealed that conformer **2A**, with a 'double chair' geometry of the ten-membered ring and equatorial orientations of both OAc groups, is by far the most stable and that the calculated coupling constants of this conformer have the best concordance with the experimental ones[4] (Table II).

TABLE II. Experimental $^3J_{HH}$, relative energies and calculated $^3J_{HH}$ for the theoretical conformers of lactone **2**.

Conformer	Δ*E* (kJ/mol)	*J*calc. (Hz)					
		$J_{3,2\alpha}$	$J_{3,2\beta}$	$J_{5,6}$	$J_{6,7}$	$J_{9,8\alpha}$	$J_{9,8\beta}$
2A	0.00	3.57	9.92	10.06	8.81	1.67	7.71
2B	61.55	5.90	8.14	10.12	9.03	2.18	4.7
2C	62.38	6.34	7.63	10.12	6.79	3.46	3.19
	*J*exp. (Hz)[4]	5.6	11.3	10	10	2.1	10.5

Obviously, the results of the theoretical calculations for germacranolide **2** are in excellent agreement with experimentally established conformation based on correlations in the PS NOESY spectrum.[4]

Ridentine (**3**). As a result of exomethylene double bond, ridentine (**3**) should exhibit more conformational flexibility than lactones **1** and **2** (Fig. 1). Accordingly, the PM3 method predicted six distinctive conformations (Fig. 4).

Of the theoretical conformations, in conformers **3A** and **3D** both C(14) and C(15) are β-oriented (UU), whereas in conformers **3C**, **3E** and **3F** C(14) and C(15) are α- and β-oriented, respectively, (DU). In conformer **3B** C(15) is β-oriented, while the C(10)–C(14) and C(4)–C(15) bonds are perpendicular. Changing the dihedral angles in the OH substituents (see Experimental) had a considerable influence on the stability of the resulting ro-

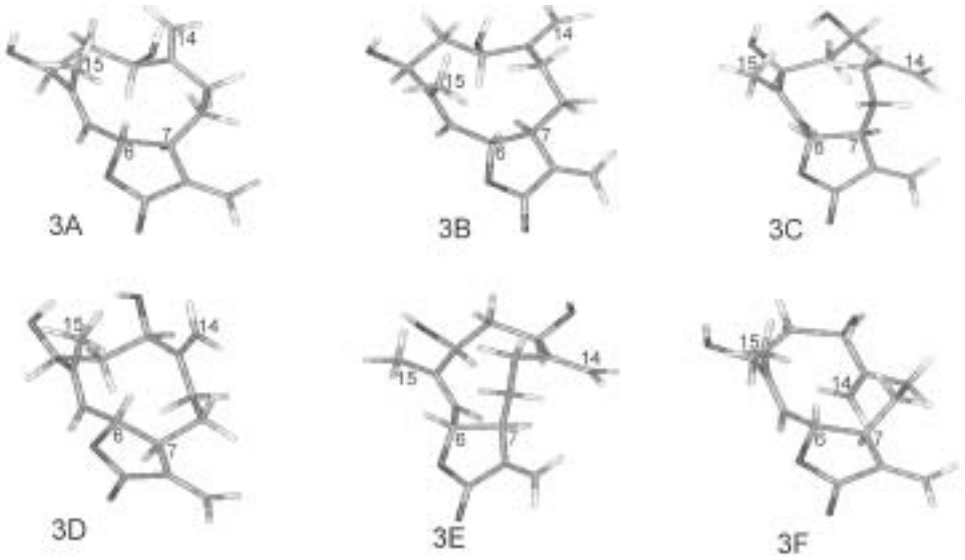

Fig. 4. Calculated geometries of lactone **3**.

tamers. The relative energies of the rotamers often exceed 12 kJ/mol, favoring those with antiperiplanar orientation of O-H and geminal C-H in every conformational family.[19] If the conformers **3A–3F** are compared, it is obvious that the only, but essential, difference between them is the geometry of the C(2)–C(1)–C(10)–C(9) fragment of the carbocyclic skeleton. The calculated relevant couplings illustrate these differences (Table III).

TABLE III. Experimental $^3J_{HH}$, relative energies and calculated $^3J_{HH}$ for the theoretical conformers of lactone **3**.

Conformer	ΔE (kJ/mol)	*J*calc (Hz)			
		$J_{1,2\alpha}$	$J_{1,2\beta}$	$J_{3,2\alpha}$	$J_{3,2\beta}$
3A	0.00	2.55	8.03	3.63	9.75
3B	3.89	4.57	3.72	2.42	10.15
3C	7.54	1.4	6.66	4.27	2.28
3D	17.50	1.84	4.92	3.75	2.59
3E	22.57	1.76	7.51	1.38	9.3
3F	26.50	5.19	1.82	5.66	7.96
*J*exp. (Hz)[5]		5	10	4	10

Regarding the relative energies, conformers **3A**, **3B** and **3C** could be considered as significant (Table III), so their ratio in the hypothetical conformational mixture would be **3A:3B:3C** = 80:16:4. The suggested conformation of ridentine (**3**)[20] matches with the calculated conformation **3A** (Fig. 4): the ten-membered ring adopts a 'double chair' geometry, C(14) and C(15) are β-oriented (UU) and both OH groups are equatorial. Again, the applied PM3 method showed very good agreement with the experimental results.

CONCLUSION

The geometry optimization of the germacranolides **1–3** by the PM3 semi-empirical method was undertaken with two goals: to gain a better insight into the conformational properties of these compounds and to evaluate the applicability of the method. The experimental results suggest that all three lactones adopt a rigid conformation in solution,[21] namely that already assigned as the most stable for *trans*,*trans*-germacra-1(10),4-dien-6,12-olides:[1(d–m),2(a),2(f)] the ten-membered ring is in the 'double chair' geometry and C(14) and C(15) are β-orientated. The theoretical results are in full concordance with the experimental ones. The PM3 method predicts that the conformations **1A**, **2A** and **3A** are the most probable ones for lactones **1**, **2** and **3**, respectively (Fig. 5).

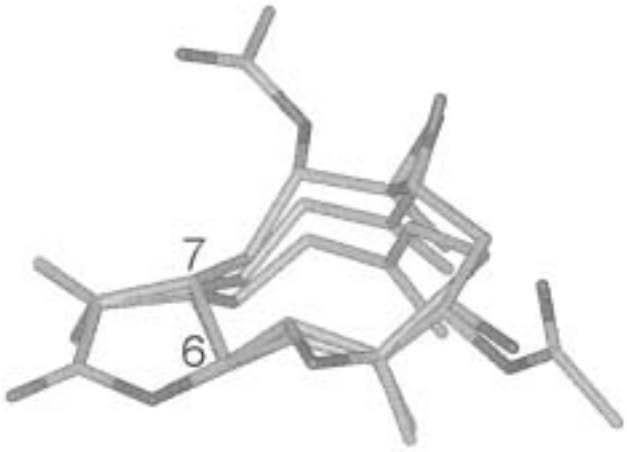

Fig. 5. Superimposed, the most stable conformations of lactones **1–3** (hydrogen atoms are not shown).

Thereafter, it is concluded that the PM3 method (contained in the HyperChem computer program), relatively fast and simple in use, represents a profoundly valuable tool in the conformational analysis of C-6 lactonized germacranolide molecules.

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ИЗВОД

КОНФОРМАЦИОНА АНАЛИЗА ТРИ ГЕРМАКРАНОЛИДА PM3 СЕМИ-ЕМПИРИЈСКОМ МЕТОДОМ

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Конформације гермакранолида **1–3**, лактонизованих у положају (6), израчунате су применом PM3 семи-емпиријске MO методе. Такође су снимљени протонски NMR спектри

партенолида (**1**) на ниским температурама. Помоћу модификоване Карплусове једначине је успостављена корелација између експериментално утврђених и израчунатих најстабилнијих конформација. Закључено је да примењена квантно-хемијска израчунавања брзо и поуздано могу предвидети и објаснити особине сесквитерпенских лактона типа гермакранолида.

(Примљено12. децембра 2002)

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10. Although the forms 'conformer' and 'rotamer' are synonymous, in this text the expression 'rotamer' is used in order to emphasize the conformation of the OR substituents
11. Molecular mechanics predicted 4 conformations of the carbocyclic skeleton, hence 400 possible rotamers of compound **2** and 9 conformations, *i.e.* 225 rotamers of compound **3**
12. C. A. G. Haasnoot, F. A. A. M. Leeuw, C. Altona, *Tetrahedron* **36** (1980) 2783, equation 5: $J = A\cos^2\Phi + B\cos\Phi + C$ (A = 7.76, B = –1.0, C = 1.40)
13. The four main conformations characteristic for *trans*,*trans*-germacradienolides are assigned as UU, UD, DU and DD, depending on the orientation 'up' or 'down' of C-14 and C-15, respectively; Ref. 1(h)
14. For costunolide-type germacranolides the barriers are > 84 kJ/mol, Ref. 1(h)
15. Already established for its solid state, Ref. 4(b)
16. An epoxide substituent and endocyclic double bond have a similar influence on the conformation of cyclic compounds; E. I. Eliel, N. L. Allinger, S. J. Angyal, G. A. Morrison, *Conformational Analysis*, Wiley, New York, 1966, p. 111, 226

17. Also 'extended chair' or 'crown'
18. For the resulting rotamers $\Delta E < 0.4$ kJ/mol; Fig. 3 presents the most stable rotamers of each conformational family
19. Fig. 4 shows the most stable rotamers of conformers **3A–3F**
20. This conformation is deduced only from ^{1}H-NMR spectral data (Ref. 5), so the alternative conformers **3B** and **3C** should perhaps not be disregarded; we are waiting for a new amount of ridentine to be isolated in order to check its conformational flexibility by NMR measurements at variable temperatures
21. C-8 lactonized germacranolides are frequently conformational mixtures, Refs. 1(a), 1(b), 2(c–e), 2(g), 2(h).