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Conformational analysis and DFT calculations of 8α-hydroxy-germacradiene-6,12-olide derivatives

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ABSTRACT: The Systematic Conformational Search Analysis (*SCSA*) code was employed to study the conformational properties of the 8α-hydroxy-germacradiene-6,12-olide isomers. This procedure was extended to the *trans,cis*- and *trans,trans*-1(10),4-isomers of 8α,15-dihydroxy-germacradiene-6,12-olides and 8α-hydroxy-15-oxo-germacradiene-6,12-olides, to investigate structural and energetic analogies between these and the 8α-hydroxy-germacradiene-6,12-olide species. The calculated ^{13}C NMR spectra, at the hybrid DFT mPW1PW91 level, showed a sound correlation with the corresponding experimental spectra, providing a valid support to the reliability of the calculated structures and to the consistence of our conformational analysis. The results presented here allowed us to support our previous mechanistic interpretation on the oxidation of 8α,15-dihydroxy-*trans*,*trans*-1(10),4-germacradiene-6,12-olides to 8α-hydroxy-15-oxo-*trans*,*cis*-1(10),4-germacradiene-6,12-olides. *Supplementary electronic material for this paper is available in Wiley Interscience at http://www.interscience.wiley.com/jpages/0894-3230/suppmat/*

KEYWORDS: natural products; germacranolides; systematic conformational analysis; DFT calculations

INTRODUCTION

The oxidative reaction of 8α,15-dihydroxy-*trans,trans*-1(10),4-diene-germacra-6,12-olides, i.e. compound **1a** and its derivative **1b** (Scheme 1), to give the elemanes **3a** and **3b** presents an important side-reaction, improved in acidic medium up to 100% yield, leading to the unexpected heliangolides **4a** and **4b**.[1]

These findings induced us to hypothesize that compounds **2a** and **2b** are the kinetically controlled products that, in the presence of non-acidic oxidizing agents (e.g. MnO_2), give compounds **3a** and **3b** in high yields by Cope rearrangement.[1] Conversely, species **4** should be the thermodynamically controlled product, obtained through **2** by *trans–cis* interconversion[1] of the C4—C5 bond after the acid-catalysed keto-enolic equilibrium.

Compounds **1a** and **1b** are derivatives of the natural product cnicin, a metabolite isolated from some species of *Centaurea* (Asteraceae)[2] that shows interesting antibacterial,[3] cytotoxic and antifungal properties. Germacradiene sesquiterpenoids have been investigated widely for their biological activities and their central role in the biosynthesis of other sesquiterpenes.[2] Because of the presence of two double bonds between C1—C10 and C4—C5, it is possible to group them in four types of isomers: *trans*,*trans*-germacradienes, *cis*,*trans*-germacradienes (melampodienes), *trans*,*cis*-germacradienes (heliangodienes) and *cis*,*cis*-germacradienes.

Owing to its flexibility, the ten-membered ring of the germacradiene species can adopt several conformations. This feature could play a fundamental role in the reactivity of these compounds and in the biosynthesis of other sesquiterpene skeletons.[4] Concerning this, quantum chemistry (QC) calculations could provide structural and energetic suggestions on the mechanistic effects caused by the conformational rearrangements of the germacradiene isomers occurring in solution.

Indeed, involvement of the solvent in QC calculations is a difficult computational challenge that strongly limits the molecular size that is possible to investigate.[5] For this reason, when possible, as in the case of chemical systems formed by low-polar organic molecules in non-polar solvent, the approximation to compute properties of gas-phase instead of solution-phase species is justified to save computational time and is performed without loss of critical details.[6]

It is possible to define the chemical properties of a molecule characterized by different conformations having small interconversion energies as the conformational average of the same properties.[7] An example of this approach was given recently by mimicking, at the DFT

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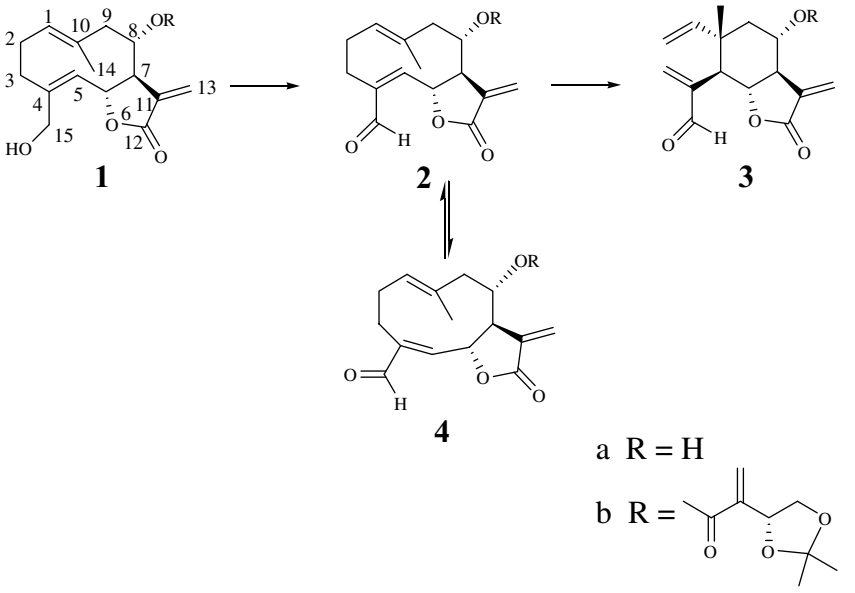


Scheme 1

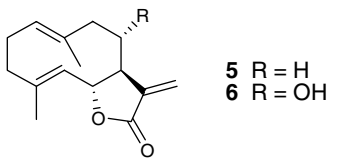
level, the ^{13}C NMR spectra of flexible organic compounds using Boltzmann distributions.[8] In any case, it is generally of great consequence to analyse the conformational space of a given species because this knowledge can be of pivotal importance in molecular recognition and perhaps in understanding the origin of a possible catalytic effect[9–11] and biological activity.[12,13]

The energies of the conformers of the *trans*,*trans*-germacradiene-6,12-olides (species **5** in Scheme 2) and 8,12-olides have been calculated already by molecular mechanics (MM) methods[14–18] and it has been shown that four distinct conformations of the ten-membered ring can be adopted.

These conformers can be described as up–up (**UU**), up–down (**UD**), down–up (**DU**) and down–down (**DD**), depending on the orientation of the C14 and C15 methyl groups[4] (see also the Results and discussion section).

The MM studies pointed out that the most stable conformation of molecule **5**[14,18] presents crossed double bonds and a **UU** arrangement of the two ring methyl groups. Only recently has conformational analysis of the other isomers of **5** been reported at MM level.[15–17]

In order to deepen the computational level on the germacradiene studies and, in particular, to get information on the conformational space of germacrane **6** of Scheme 2 and of the **1a**, **2a** and **4a** derivatives occurring in the side-reaction path of the mechanism in Scheme 1, the multi-step Systematic Conformational Search Analysis (*SCSA*)[19] has been employed.



Scheme 2

In detail, the conformational space of the four geometrical isomers of 8α-hydroxy-costunolide (germacrane **6**, which differs with respect to compound **5**[14,18] by the α-group on C8) and the conformational space of the *trans,trans*- and *trans,cis*-isomers of compounds **1a** and **2a** have been analysed at DFT level.

Owing to the peculiarity[19] of the *SCSA* approach, conformational analysis of species **2a** allowed us to fix accurate conformational information also on species **4a**.

The calculated ^{13}C NMR spectra of the conformers supported the reliability of the *SCSA* procedure, whereas conformer energetic analysis allowed us to find mechanistic details on the side-reaction path of Scheme 1.

In the next section a short description of the *SCSA* approach and references on the method to calculate the GIAO ^{13}C chemical shifts are given. The *SCSA* treatment, as well as the calculated and experimental NMR comparison and our conclusions, are presented later. Finally, detailed energetic aspects that are useful to understand the mechanistic aspects of the side-reaction path of Scheme 1, are available in supplementary material.

The acronym **n-XX-YY-m** is used to denote the **m**th conformer of compound **n**. In the acronym **XX**, i.e. **TT** or **CT** or **TC** or **CC**, defines the *trans,trans*-, *cis,trans*-, *trans,cis*- and *cis,cis*-isomers as determined by the presence of the two double bonds between C1–C10 and C4–C5 (see schemes), whereas **YY** defines one of the possible orientations (**UU**, **UD**, **DU** or **DD**) of the C14 and C15 methyl groups in the isomers.[4]

METHOD AND COMPUTATIONAL ASPECTS

The species discussed in this paper are low-polarity molecules soluble in $CHCl_3$ or CH_2Cl_2. The final discussion and conclusions on these species rely on a hybrid

DFT *in vacuo* conformational analysis performed at the MPW1PW91/6-31G(d)[20] level. The approach has been chosen in the hypothesis that the solute–solvent interactions and the temperature changes should slightly affect the relative energy of the conformers.[6]

To test solvent and temperature effects,[21] self-consistent reaction field (SCRF)[22] single-point calculations on the *in vacuo* optimized structures of given conformers of the **1a**, **2a** and **4a** derivatives (namely **1-TC-UD-1**, **1-TT-UU-1**, **2-TT-UU-1** and **2-TC-UD-1**) were performed by applying the polarized continuum model [23] in the cosmo approach (CPCM) [24] of the solvent, namely chloroform at 298.15 K.

The ΔE values between homonymous compounds *in vacuo* at 0 K and in chloroform at 298.15 K differed on average by ca. 5%, confirming the hypothesis above.

The conformational analysis on the geometrical isomers was performed by the *SCSA* algorithm.[19] In the following, the main steps involved in the *SCSA* to study a given isomer among the 8α-hydroxy-germacradiene-6,12-olide derivatives are reported as an example:

(i) *Preliminary systematic search on ca. 2×10^6 molecular geometries obtained from one parent structure*:[19]—this procedure, by employing bump and distance matrix checks, reduced the number of possible geometries to ca. 2×10^2 starting structures.

(ii) *Low-level geometry optimization on the starting structures and energy and distance matrix checks on the corresponding low-level minima*:[19]—in this step, after the AM1[25] optimizations, analogous conformers were eliminated by the distance and energy filter and the most stable ones (ca. 3×10^1 in an energy range of 80 kJ mol^{-1}) were collected.

(iii) *Energy refinement on the low-level optimized conformers*:[19]—here, high-level geometry optimizations were performed on the already low-level optimized conformers. The high-level optimizations in this work were accomplished by the MPW1PW91/6-31G(d) hybrid DFT method.[20] The DFT optimizations were followed by energy and distance matrix checks. Thus, analogous structures were eliminated and the final ones (ca. 2×10^1 in an energy range of 80 kJ mol^{-1}) were selected.

In the protocol above, each dihedral angle in the first step was incremented by a fixed value of 60° or 180° for the saturated and unsaturated bonds, respectively. All the possible combinations of the dihedral angles were considered throughout the analysis of the conformational space. Interatomic distance monitoring was performed by bump check and distance matrix procedures in order to verify that the atoms of a given conformer were not falling in non-physical neighbouring spots[26] and to impose structural constraints such as those useful to preserve the bicyclic starting structure.

The conformations of the **TT** and **TC** isomers of **1a** and **2a** were obtained by geometry optimizations, at MPW1PW91/6-31G(d) level, of derivatives obtained by substitutions of one or two hydrogen atoms with one hydroxy or one oxo group on the corresponding conformers of compound **6** and, allowing three more rotations (120°) along the C—CH_2OH bond and two more rotations (180°) along the C—CO bond. This procedure allowed us to characterize the **4a** conformers by, analysing the **2a** conformational space.

The GIAO ^{13}C chemical shift (δ) of the carbon atoms, to be compared with the experimental ones in order to monitor the consistence of our conformational search results and to re-verify the starting hypothesis on the environment effects, were obtained following a procedure described in Ref. 6.

All the QC calculations were performed using the Gaussian 98W program package.[27]

RESULTS AND DISCUSSION

The energetics concerning all the species discussed in this section rely on the hybrid DFT *in vacuo* calculations performed by step (iii) of the *SCSA* protocol[19] at the MPW1PW91/6-31G(d) level.[20] The energy difference values corresponding to the conformer structures found within each of the four families of isomers **6** are shown in Fig. 1. In these geometrical isomers–1(10)-*trans*,4-*cis* (**TC**), 1(10)-*cis*,4-*cis* (**CC**), 1(10)-*cis*,4-*trans* (**CT**) and 1(10)-*trans*,4-*trans* (**TT**)–the *trans* and *cis* notation was referred to the relative position of the ring carbons C3 and C6 and C2 and C9 bound to the two double bonds C4—C5 and C1—C10, respectively.

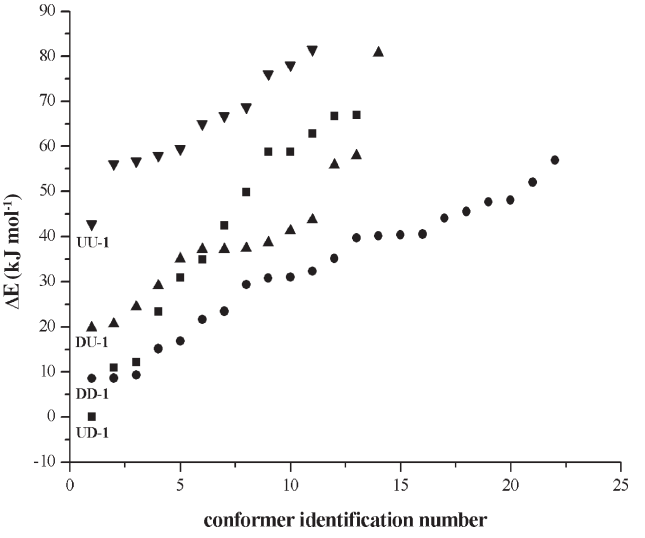


Figure 1. Energy difference values at DFT level (see also Table S1) of the 60 isomers found for compound **6** determined by step (iii) of the *SCSA* protocol: (■) **TC**, (●) **CC**, (▲) **CT** and (▼) **TT** isomer families

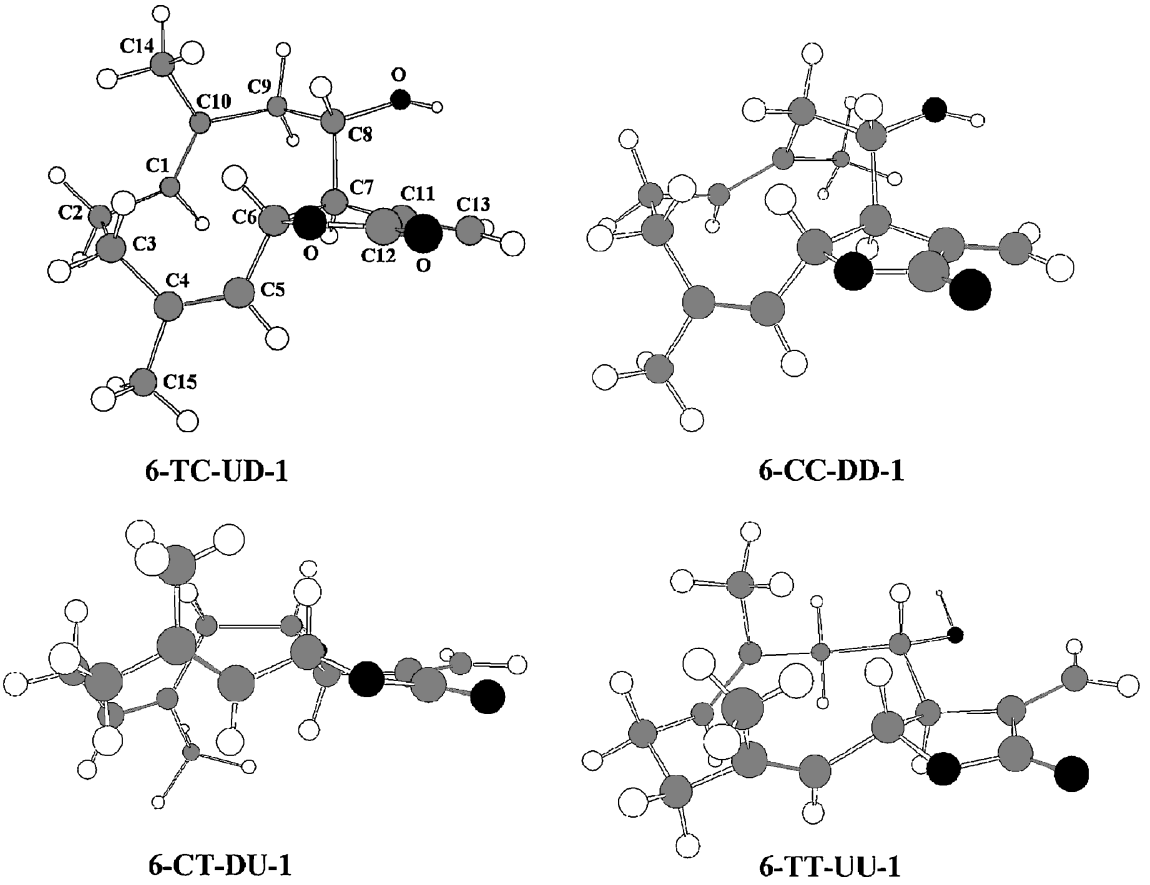


Figure 2. Structure of the conformers corresponding to the absolute energy DFT minima, determined by step (iii) of the *SCSA* protocol, of the different isomers of compound **6**: **6-TC-UD-1**, **6-CC-DD-1**, **6-CT-DU-1** and **6-TT-UU-1**

Tables S1–S3 (see Supplementary material) show the values of the angles (α**1** and α**2**) that the C10—C14 and C4—C15 bonds set with the C10—C4—C7 plane (see Scheme 1). It is recalled here that the values of the angles above characterize, by a previously introduced notation,[4] the nomenclature of the conformers, employing **U** or **D** when α**1** and α**2** are larger than 20° or smaller than −20°, respectively. We have also introduced the notation plane (**P**) when α**1** or α**2** were within the range −20° and 20°.

Sixty minima of compound **6** were found within 85 kJ mol^{-1}. Figure 2 shows the absolute energy minimum structures of the four isomers of compound **6**. Conformer **TC-UD-1** shows the lowest energy value (its two double bonds are in a parallel orientation and the structure is relaxed in a kind of chair conformation where the steric repulsions are minimized) and it is stabilized by >10 kJ mol^{-1} with respect to the other conformations of the **TC** isomers. Thus, referring to the Boltzmann distribution of the **6-TC** species,[8,19] we could thoroughly assume that at room temperature the **6-TC-UD-1** conformer should be almost the only species in solution.

Similar considerations, supported by the data of Table S1, allow us to conclude that the **TT** isomer essentially should exist as the **6-TT-UU-1** conformer, whereas the **CC** isomer should be represented mainly by a mixture of the **6-CC-DD-1**, **6-CC-DD-2** and **6-CC-UD-1** species, and the **CT** isomer by a mixture of the **6-CT-DU-1** and **6-CT-DU-2** species.

The structure of **6-TT-UU-1** (Fig. 2) is in good agreement with the MM results on compound **5**,[14–18] the two double bonds being in the same crossed orientation. From the considerations above, it is possible to conclude that the presence of the hydroxyl group in C8 does not induce appreciable distortions in the geometry minima.

Although the lowest energy minima found here at DFT level are in agreement with the results of the conformational analyses performed at MM level,[14–18] some differences concern the stability order of the higher energy species within a given family of isomers.

However, we are confident that the *SCSA* results of the present work are more consistent than the MM results,[14–18] either because they have been performed at higher theory level or because a larger number of local energy minima have been found.

The structures of the whole energy minima and the difference energy values of the conformers belonging to the **TC** and **TT** isomer families of **1a**, **2a** and **4a** are shown in Figs 3–6. The number of conformers is increased directly by the presence of the hydroxy or the oxo group.

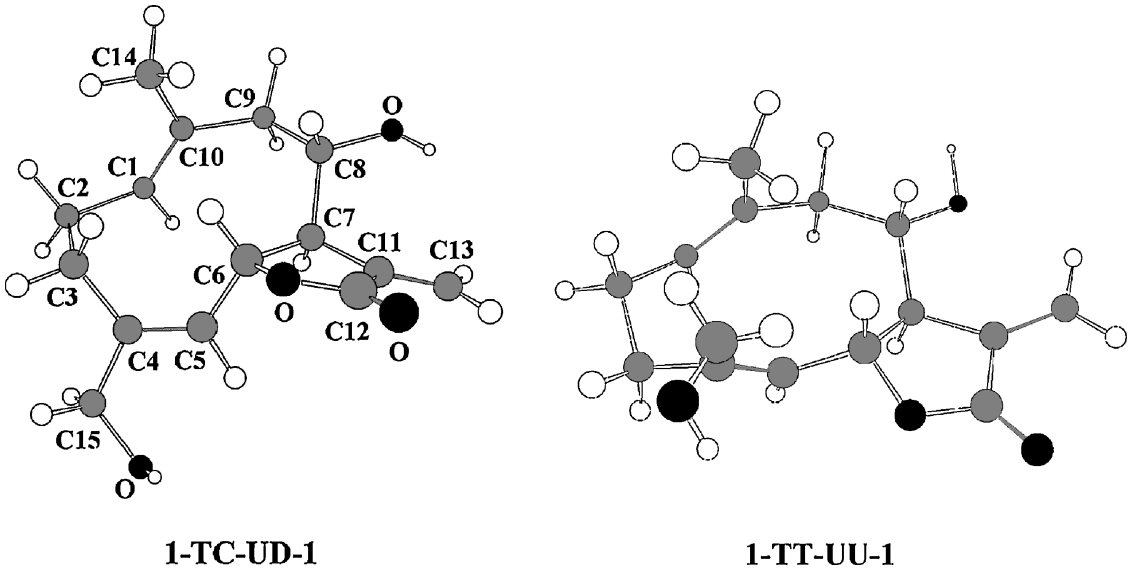


Figure 3. Structures of the conformers corresponding to the absolute energy DFT minima, determined by step (iii) of the *SCSA* protocol, of the isomers of compound **1a**: **1-TC-UD-1** and **1-TT-UU-1**

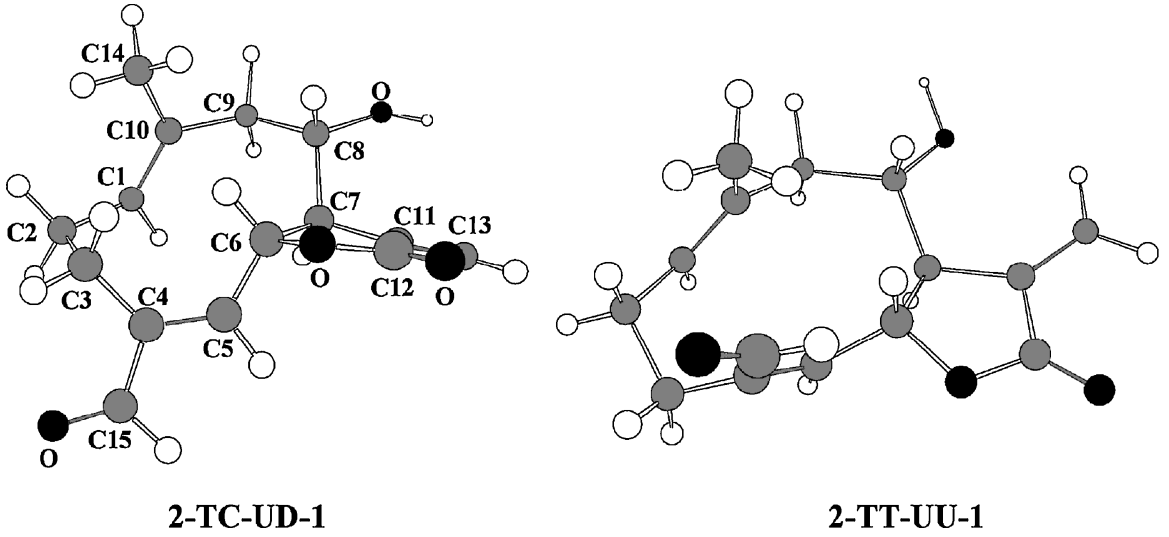


Figure 4. Structures of the conformers corresponding to the absolute energy DFT minima, determined by step (iii) of the *SCSA* protocol, of the isomers of compound **2**: **2-TC-UD-1** (identifying the most stable among the **4a** conformers) and **2-TT-UU-1** (identifying the most stable among the **2a** conformers)

Several conformations show the same germacrane skeleton, the only difference being the relative position of the hydroxyl or oxo groups on C15. This occurs for the **1-TC-UD-1** and **1-TC-UD-2** species (see also Table S2), whose energy difference is 2.3 kJ mol^{-1}, suggesting that both conformers should be present simultaneously in solution at room temperature.

The agreement of both the stability order and the structure geometry of the energy minimum conformers found between the two isomers of 8α,15-di-hydroxy-germacradiene-6,12-olide and 8α-hydroxy-15-oxo-germacradiene-6,12-olide and the corresponding conformers of compounds **6** induces us to hypothesize that the hetero-atom substitution on C15 and the intra-molecular interactions should affect the structural stability of these species less than the germacrane ring tension. This hypothesis seems to be confirmed, observing that some conformers of salonitenolides (**TT** isomers of species **1**) present a hydrogen bond between the hydroxyl on C15 and the oxygen atom of the lactone ring, e.g. **1-TT-DD-1*** in Table S2, is less stable than the **1-TT-UU-1** and **1-TT-UU-2** species.

Concerning 8α,15-di-hydroxy-germacra-*trans,trans*-1(10),4-diene-6,12-olide (**1a** in Scheme 1), the results of the conformational search are supported by ROESY NMR spectroscopy measurements, indicating the existence of such a compound in **UU** conformation. Both lowest energy minimum structures (**1-TT-UU-1** or **1-TT-UU-2**) separated by 5.6 kJ mol^{-1} are hence congruent with the experimental results.

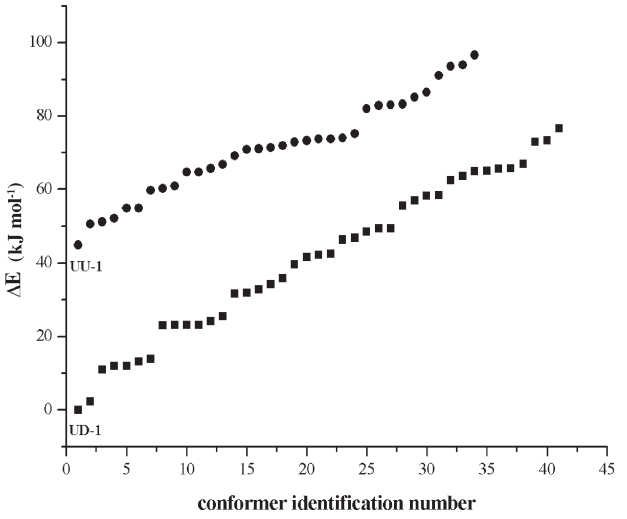


Figure 5. Energy difference values of the 75 isomeric species found for compound **1a** at the DFT level by step (iii) of the *SCSA* protocol: (■) **TC** and (●) **TT** isomer families

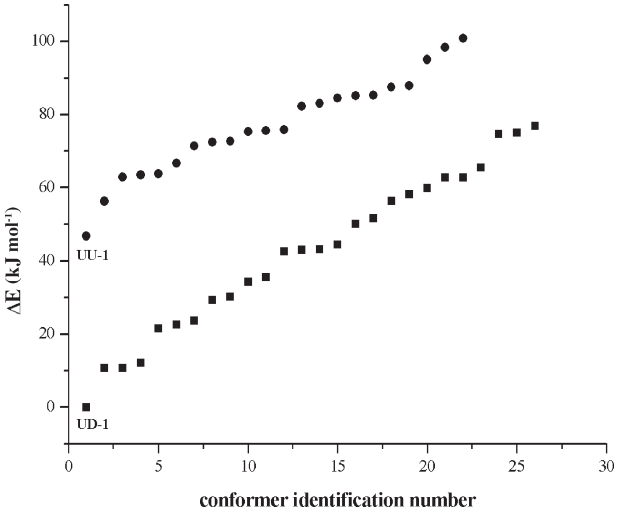


Figure 6. Energy difference values of the 48 species found, at DFT level by step (iii) of the *SCSA* protocol, for compounds **2a** (●), corresponding to the **TT** isomer, and **4a** (■), corresponding to the **TC** isomer

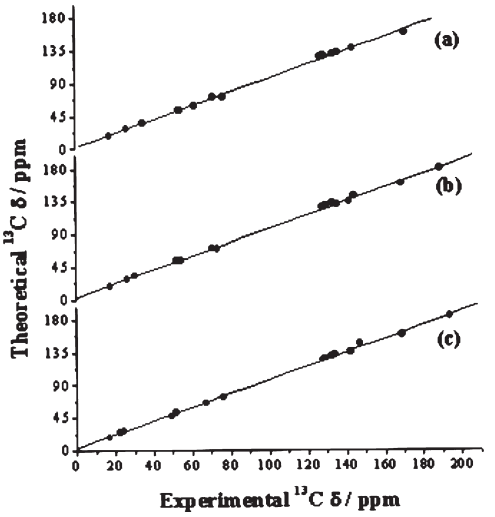


Figure 7. Correlation plots of calculated versus experimental ^{13}C NMR chemical shifts.[1] compound **1-TT-UU-1** (line a, species **1a**), compound **2-TT-UU-1** (line b, species **2a**) and compound **2-TC-UD-1** (line c, species **4a**)

We have found a good linear correlation between the calculated and experimental values[1] of the ^{13}C chemical shift (δ) of compounds **1a**, **2a** and **4a** of Scheme 1 (see Table S4 and Fig. 7). This result supports the calculated *in vacuo* conformational structures (**1-TT-UU-1**, species **1a** of Fig. 3; **2-TT-UU-1** and **2-TC-UD-1**, species **2a** and **4a** of Fig. 4) being consistent with the experimental solution structures.

Finally it has to be underlined that a careful analysis of Figs 1–3 provides outstanding support to the side-reaction path of the mechanism in Scheme 1 (route **1a** → **2a** → **4a**). In fact, independently from the functional group in C15, i.e. for all the germacrane compounds **6**, **1** and **2** investigated, the **TC-DU-1** isomer is ca. 40 kJ mol^{-1} more stable than the **TT-UU-1** isomer (see also Figs 1, 5 and 6).

Owing to this occurrence, when isomerization of the C4—C5 double bond is possible, e.g. by the acid-catalysed keto-enolic equilibrium as in the case of compound **2a**, the **TC** isomer on an energetic basis should be the only product observed.[1]

The energetics findings of this paper can be utilized also for rationalizing mechanistic evidence and for designing a synthetic route to the title derivatives. For example, it can be framed in the energetic context above that it is possible to obtain the 15-oxo-*trans,trans*-1(10),4-diene-germacra-6,12-olides by using no acidic oxidating agent and, conversely, that it is not possible to isolate natural products such as the **2a** and **2b** derivatives. Regarding the latter findings, it is probable that the **2a** and **2b** species, on the basis of biogenetic considerations,[28] can exist just as plant metabolites because of their easy isomerization to the more stable thermodynamic isomers.

CONCLUSIONS

The *SCSA* approach employed to investigate the conformational space of the four geometrical isomers of 8α-hydroxy-germacradiene-6,12-olide, (compounds **6**), furnishes a greater number of conformers than that obtained previously at MM level for the analogous germacradiene compound **5**.

The occurrence of the side-reaction leading to the 15-oxo-*trans,cis*-1(10),4-diene-germacra-6,12-olides (compounds **4a** and **4b**), which takes place by oxidation of 15-hydroxy-*trans,trans*-1(10),4-diene-germacra-6,12-olides (compounds **1a** and **1b**) to the elemanes (compounds **3a** and **3b**), could be explained by considering the

energetics involved in the heliangolide conformers and in the conformers of those derivatives that in the present work were denoted as species **2a** and **2b**.

Supplementary material

Tables S1–S3 report, for the **TC**, **TT**, **CC** and **CT** isomers of compounds **6** and for the **TC** and **TT** isomers of compounds **1a** and **2a**, the list of the energy differences and the values of the angles α**1** and α**2** formed, respectively, by the C10—C14 and C4—C15 bonds with the C10—C7—C4 plane. It has to be stressed that the **TC** isomer of the **2a** conformers is the species here denoted as **4a**. Table S4 reports the intercept, the slope and the correlation coefficient, obtained by least-squares linear fits, of the calculated versus experimental ^{13}C NMR chemical shifts relative to compounds **1a** (conformer **1-TT-UU-1**), **2a** (conformer **2-TT-UU-1**) and **4a** (conformer **2-TC-UD-1**). These tables are available in Wiley Interscience.

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