Transannular Cyclization in Cyclodecenes: The Case Study of Melampolides

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The structures of products formed by electrophilic transannular cyclization (ETC) in medium-ring compounds are affected by several parameters. The melampolides, (*cis*,*trans*)-cyclodeca-1(10),5-diene terpenes, undergo ring closure only under Lewis acid conditions. The transannular distance proves to be a key factor. Molecular mechanics calculations suggest that the *cis* ring junction of products is formed through a less populated conformer. ETC has chemical significance because it may play an important role in the pharmacological action of sesquiterpene lactones.

Introduction

The term "sesquiterpene lactones" covers a plethora of widespread natural compounds that share the α-methylene-γ-lactone functionality but differ in the arrangements of the fifteen carbon atoms. Among them, the cyclodecadiene skeleton is characteristic of the germacranolide group, which is divided into four subgroups depending on the configurations of the two double bonds.

We have recently proposed an approach aimed at unravelling the relationships between the chemical structure and the reactivity of the parthenolide **1** (germacrolide subgroup, Scheme 1) and its biological activity.[1] The 1(10)-(*trans*) double bond dictates a molecular conformation with a C1−C5 transannular distance of 292 pm (Figure 1); this system can undergo facile electrophilic transannular cyclization [ETC], which produces mainly guaiane derivatives (Scheme 2).

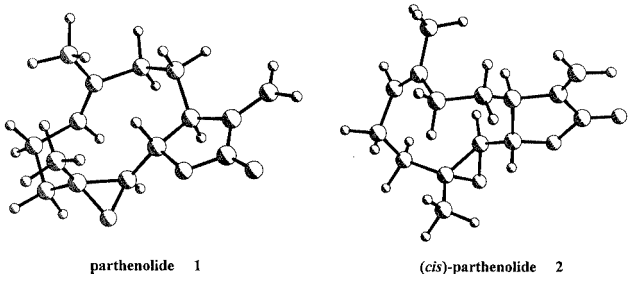
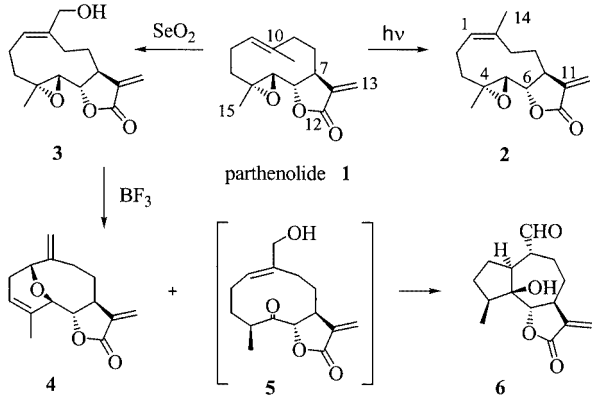
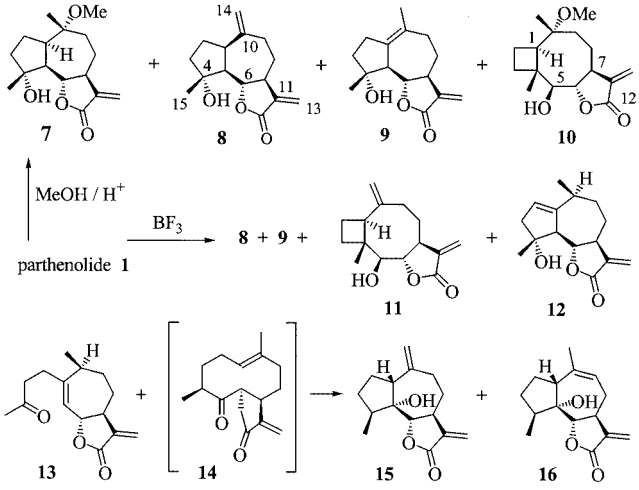


Figure 1. MM-calculated lowest-energy conformers of **1** and **2**



Scheme 1



Scheme 2

The 1(10)-*cis*-parthenolide **2** (melampolide subgroup, Scheme 1) has for the first time been synthesized,[1] spectroscopically characterised and investigated with respect to its chemical properties. Its preferred conformation (Figure 1) has a C1−C5 distance of 333 pm, preventing the occurrence

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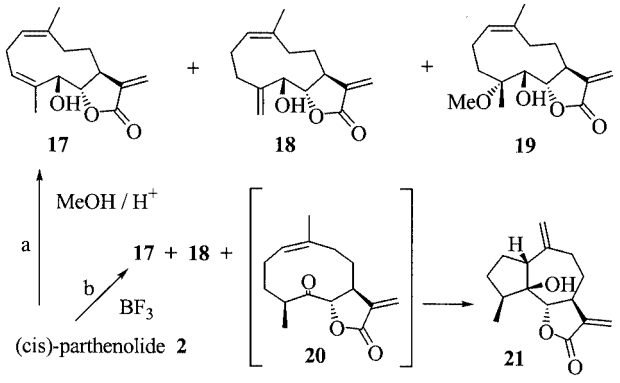
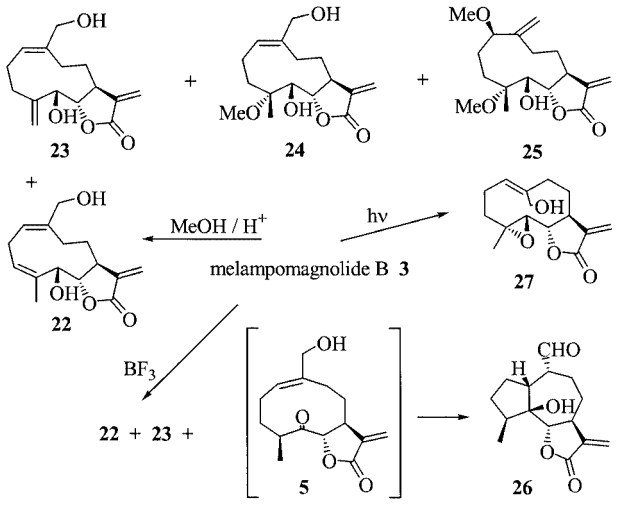


Scheme 3



Scheme 4

of ETC (Scheme 3, a). The chemical properties of **1** and **2** fitted nicely with the results from chemotaxis index assays that pointed to intense bioactivity with **1** and its guaianolide derivatives in contrast with the marginal effect of **2**.

To the best of our knowledge, the sole literature report on a melampolide compound that has been subjected to acidic medium and checked for ETC is that on melampomagnolide B **3** (Scheme 1).[2] Gonzales et al. treated **3** with BF_3 in CH_2Cl_2 or benzene to obtain the germacranolide **4** and, after pinacol-like rearrangement of the 4,5-epoxide functionality to the 5-ketone **5**, the *trans*-guaianolide aldehyde **6**. This result is quite different from that observed by us on treatment of **2** with *p*TSA (Scheme 3, a).[1] Two possible factors may account for such a different outcome: the use of Lewis acid instead of a protic acid catalyst and the presence of the hydroxy functionality at C14.

Comparison of the treatment of **1** with *p*TSA[1] or BF_3 [3] demonstrated that the catalyst does undoubtedly play a role (Scheme 2).[4] In fact, use of *p*TSA gave rise to compounds **7**, **8**, **9** and **10**, whereas that of BF_3 furnished the compounds **8**, **9**, **11**, **12** and **13** and induced rearrangement at the 4,5-epoxide moiety. The intermediate **14** generated the guaianes **15** and **16**, which each possess a *trans* ring junction, as established by Fisher. All previously known results are displayed in Schemes 1, 2 and 3 (a).

We obtained the germacranolides **17**, **18** and **19** by protic acid treatment of 1(10)-*cis*-parthenolide (Scheme 3, a). Our interest then was in subjection of **2** to the BF_3-induced rearrangement (Scheme 3, b) and of **3** to protic and Lewis acid conditions (Scheme 4), in order to determine their complete reaction schemes as for **1** *(Question 1)*. Our new results are depicted in Schemes 3 (b) and Scheme 4.

Despite the availability of a reasonable amount of reaction product **6**, the authors proposed a *trans*-(1αH,5βOH) stereochemistry (Scheme 1) on the basis of the reaction mechanism rather than of experimental data. This motivated us to examine the junction of the fused rings in **6** experimentally *(Question 2)*.

Careful reading of literature data revealed a discrepancy basically arising from molecular mechanics (MM) calculations. The lowest-energy conformer of **5** was found to have an *anti* relationship between the 5-carbonyl and the 14-hydroxymethyl ("**Oo14d**" conformer type, Figure 2), implying **6**, with a *trans* ring junction.[2] In a study on ETC of 5-cyclodecenones,[5] however, the (*Z*)-isomers gave only *cis*-fused hydronaphthalenols. This *cis* ring junction product formed through a high-energy conformer with the carbonyl oxygen and the olefinic protons in a *syn* orientation ("**Ou14u**" conformer type, Figure 2). This motivated us to investigate how the stereochemistries of the obtained products depended on the conformations of the ketones **5** and **20** and whether the substituents could affect the regiochemistry of cyclization. *(Question 3)*.

In our previous work, a strict relationship had emerged between the chemotaxis indices of **1** and **2** and their configurations at the 1(10)-double bond; we thought that the influence of **3** and its 1(10)-*trans* counterpart on cellular migration might provide additional hints to point to the active centre of these molecules *(Question 4)*.

This work deals with definite answers to the above questions.

Results and Discussion

1(10)-*cis*-Parthenolide **2** was treated with BF_3 in benzene for 10 min at room temp. (Scheme 3); the reaction furnished the new guaiane derivative **21** in 20% yield, along with our previously described[1] compounds **17** (20%) and **18** (11%). The structure **21** was inferred from MS and NMR spectroscopic data. The HRMS value at *m*/*z* = 248.14124 corresponds to $C_{15}H_{20}O_3$, which implies six sites of unsaturation in total. The ^{13}C NMR signals indicate one methyl group, three shielded and one deshielded tertiary carbons, one quaternary carbon bound to oxygen, two exo-methylene moieties, one lactone carbonyl group and hence two carbocycles. The COSY maps showed two fragments: the first from H1 to H4 and H_3C15, and the second from H6 to H9 and to H13. The proton spectrum allowed the chemical shift assignments at δ = 1.31 ppm of Me15 as a d coupled with H4, at δ = 3.13 ppm of H1 as a dd in an allylic position, and at δ = 4.48 ppm of H6 as a d coupled only to

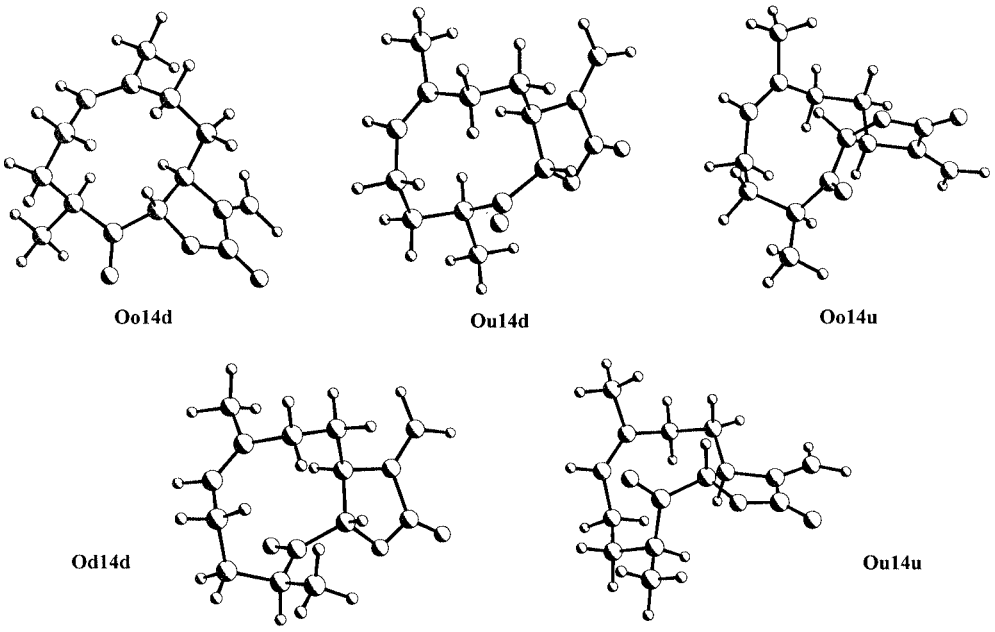


Figure 2. MM-calculated lowest-energy conformers of **20**; **O** stands for the 5-carbonyl oxygen and **14** for the 14-methyl; **o** means outwards-up the ideal plane of the medium ring, **d** means down and **u** up-inwards

H7 and suggests the C1−C5 linkage. The above resonances displayed low-field shifts [$\Delta\delta = \delta(CDCl_3) - \delta(C_5D_5N) = -0.24, -0.28$, and -0.23, respectively] when the proton spectrum of **21** was recorded in C_5D_5N. From the well established rules for pyridine-induced shifts in the NMR spectra of hydroxy compounds,[6] the extents of the deshielding effects of pyridine coordinated to C5−OH on the vicinal protons requires that the magnitude of dihedral angles between H_3C15, H1 or H6 and the alcohol function be ≤ 60 °. A positive NOE at H4 on irradiation of H7 confirmed the C1−C5 *cis* ring junction in **21**.

Melampomagnolide B **3** was stirred with *p*TSA in MeOH for 15 min at room temp.; four compounds were isolated after repeated HPLC analyses of the reaction mixture (Scheme 4). These consisted of germacrane lactones formed through epoxide opening and different modes of neutralisation of the intermediate carbocation. Their molecular formulas were deduced from HRMS measurements on the molecular ions, and the structural features were established from their 1D NMR spectra and 2D correlations. Each compound possessed a secondary alcohol at the C5 position, arising from regioselective opening of the epoxide, forming a C4 tertiary cation. This evolved in various ways, to produce a trisubstituted C3−C4 double bond in compound **22** or a C4−C15 exomethylene group in compound **23**, whereas it added a methoxy group in compounds **24** and **25**. Compounds **22**, **23** and **24** conserved the *cis* geometry of the 1(10) double bond, which in compound **25** rearranged to a methyl ether, allylic with respect to the C10-exo-methylene.

Melampomagnolide B was also subjected to Lewis acid conditions; it was dissolved in benzene, $BF_3{\cdot}Et_2O$ was added, and the system was allowed to react for 15 min at room temp. Chromatographic separation of the mixture gave compounds **22**, **23** and the aldehyde **26** as pure products. The HRMS measurement at $m/z = 264.13728$ established the molecular formula as $C_{15}H_{20}O_4$, implying three sites of unsaturation and three cycles for **26**. Its gross structure was determined: i) by the chemical shifts and multiplicities of its carbon atoms, ii) by a COSY experiment that showed the couplings H4-H_3C15, H7-H13 and the sequence from H6 through H10 and H1 to H4, and iii) by positive NOE enhancement between H1, H10 and H14. Its relative configuration was determined first by the low-field shifts of H1, H6 and Me15 in C_5D_5N, which indicate their close proximity to the 5-hydroxy group, and then by the positive NOE of H10 on irradiation at H6.

The formation of **21** and **26** probably takes place through a BF_3-mediated, pinacol-like rearrangement at the 4,5-epoxide moiety and might imply the intermediacy of the ketones **20** and **5**. Their conformations determine the stereochemistry of the annulation products. With **20**, MM calculations suggested the existence of five conformers. In the lowest-energy conformer ("**Oo14d**", 88.5%, Figure 2), the 5-carbonyl and the 14-methyl groups adopt an *anti* arrangement with the oxygen above and outwards and the methyl below the ideal plane of the medium ring. Second in energy is a very similar conformer ("**Ou14d**", 9.5%, Figure 2), which differs because the oxygen points inside the cyclodecene ring (O5−C5−C6−H6 = 7.8 °), in comparison with the outward direction for the "**Oo14d**" conformer (O5−C5−C6−H6 = −106.0 °). The third conformer found originates from drastic motions of C9, C10, C1 and C2, orienting the C14 above the mean plane ("**Oo14u**", 0.8%, Figure 2) concordant with O5, which is outwards (O5−C5−C6−H6 = −106.2 °). The next form can again be imagined to derive from the dominant conformer, through flipping of the 5-carbonyl group, which points downwards

Table 1. MM-calculated relative populations of conformers; ΔE is the difference of strain energies between the local minima and the global minimum

Conformer type	Ketone	ΔE (kcal/mol)	Population (%)	C5−C10 (pm)	C5−C1 (pm)
Oo14d	**20**	−	88.5	423	384
	5	−	77.7	423	384
Ou14d	**20**	1.316	9.5	366	351
	5	1.272	9.1	366	351
Oo14u	**20**	2.773	0.8	415	372
	5	2.899	0.6	416	372
Od14d	**20**	2.947	0.6	329	313
	5	2.575	1.0	323	305
Ou14u	**20**	2.959	0.6	319	288
	5	1.124	11.6	317	288

(O5−C5−C6−H6 = −167.0 °) along the medium ring and parallel to the 14-methyl group ("**Od14d**", 0.6%, Figure 2). The least abundant conformer ("**Ou14u**", 0.6%, Figure 2) looks like the "**Oo14u**" form, but the O5 now adopts the inward position (O5−C5−C6−H6 = 5.7 °). The C5−C1 and C5−C10 transannular distances in these five conformers are listed in Table 1.

The outcome of MM calculations for **5** proves to be similar to that obtained with **20**, but computations are complicated by the presence of a hydroxy functionality at C14 and mostly by a hydrogen bond between C14−OH and O5, which stabilises the "**Ou14u**" conformer. The distribution of energy, the relative populations and the transannular C5−C1 and C5−C10 distances in the conformers of **5** are listed in Table 1.

A solution of **3** in benzene was irradiated with UV light and the double bond was cleanly isomerized to give **27** (Scheme 4). The anti-inflammatory activities of compounds **3** and **27** were evaluated by in vitro chemotaxis index towards human neutrophiles, the bioassay being performed as described elsewhere.[1]

We have been able to gather, and have expounded satisfactorily on, the data necessary to answer the four questions posed in the introduction.

The different outcomes of electrophilic rearrangements induced by protic or Lewis acids are now clearly demonstrated: under protic conditions neither of the melampolides **2** and **3** undergoes ETC reaction; this may occur only after the Lewis acid has catalysed pinacol-like rearrangement of the 4,5-epoxide. The hydroxy functionality at C14 plays no role in ETC reactions, it causes the formation of 1-methoxy and 14-aldehyde groups in compounds **25** and **26**, respectively *(Answer 1)*.

The *cis*-(1βH,5βOH) stereochemistry at the ring junction in compounds **21** and **26** was then established. The assignment of proton resonances for H1 and H6 allowed the unequivocal evaluation of pyridine-induced NMR shifts. The configuration previously reported for **6** must be now corrected and changed to that in **26** *(Answer 2)*.

Both the 5-cyclodecenone and the melampolide substrates can exist in several conformations. Independently of their stability and population, ETC can take place only in those conformations in which the distance between the C5 and the olefinic carbons is shorter than roughly 310 pm. The conformational findings reached in the ETC study of the (*Z*)-5-cyclodecenone[5] need to be adjusted for melampolides. In the sesquiterpene lactones **20** and **5**, the distance C5−C10 goes beyond that value and does not allow the formation of products with the hydronaphthalene ring system. The stabilization of the tertiary carbocation at C10 by the alkyl substituents may also contribute to shift the reaction toward the hydroazulene ring system in the products. Moreover, the long C5−C1 distance prevents the conformers "**Oo14d**", "**Ou14d**" and "**Oo14u**" from undergoing transannular cyclization, despite their higher concentrations. The conformers susceptible to undergoing ETC are the scarcely populated "**Ou14u**" and, to a minor extent, "**Od14d**". Therefore, the transannular distance between the C5 and the olefinic carbons, as given by molecular conformations, is the main cause of regio- and stereoselectivity *(Answer 3)*.

We observed only the products derived from "**Ou14u**". This result needs a few comments: 1) we have conducted a rough approximation by choosing the ketones **5** and **20**, which are not the actual transition states, 2) computations have been performed with the arbitrary imposition of a value of 2.2 to the dielectric constant, 3) the averaged value of 310 pm as a cutoff distance might be shortened to about 300 pm in order that ETC rearrangement takes place, 4) the reactions catalysed by BF_3 were experimentally carried out on a small amount of substrate so that the products derived from "**Od14d**" were lost, 5) MM calculations gave a lower heat of formation for **21** than for its "α-*cis*" ring junction counterpart, and 6) the conformation "**Ou14u**" appeared very similar to the lowest energy conformer of **21**, the same did not occur with the conformation "**Od14d**". Complete determination of the causes of selectivity between "**Od14d**" and "**Ou14u**" requires a specific investigation and will be the object of future works.

Finally, we were interested in relating the chemical properties of SL to their biological activities. By our previous hypothesis, if the bioactivity were to depend on the generation of active products through ETC, the melampomagnolide B would have to display a high index in the chemotaxis assays and the 1(10)-(*trans*)-melampomagnolide B a low one. Actually, compounds **3** and **27** reduced the

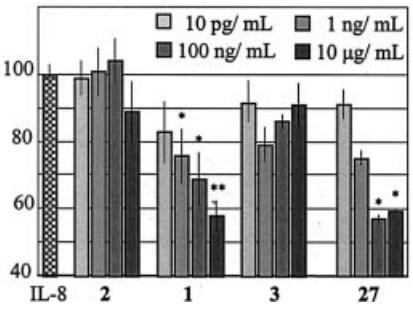


Figure 3. Chemotactic response of **1**, **2**, **3** and **27** at four different concentrations; data are given as percentage values ± standard error of the mean (SEM); statistical analysis: Mann−Whitney *U* (* $P < 0.05$; ** $P < 0.001$) after Kruskal−Wallis ($P < 0.01$); $n = 6$ for **1**, and **2,** $n = 3$ for **3** and **27**

migration of neutrophils toward IL-8 as much as 1(10)-*cis*-parthenolide **2** and parthenolide **1** *(Answer 4*, Figure 3).

Conclusion

The ETC reaction depends on the following parameters:

a) the choice of the electrophilic catalyst,

b) the position of the carbon atom at which the positive charge is initially formed,

c) the configuration of the endocyclic double bond,

d) the transannular distance between the positively charged carbon and the olefinic atoms,

e) the conformation of the carbocation intermediate before annelation, and

f) the stability of carbon atom (2^{ary}, 3^{ary}) on which the positive charge becomes located after annelation.

All these factors must be taken into account in the choice of substrates, reaction conditions and the prediction of obtainable products.

In this study we have particularly highlighted the role of points a), d) and e) in the ETC of melampolides.

SLs display numerous and diverse pharmacological properties, these effects seeming to derive from the potential to undergo a metabolic transformation into the active binders. This suggestion stems from the indices of the natural parthenolide and of their semisynthetic derivatives in diminishing the chemotactic response that is a mark of anti-inflammatory properties.

Experimental Section

General Remarks: All chemicals were analytical grade and used without further purification. TLC: Merck Kieselgel 60 PF_{254} (0.5 mm). HPLC: Reinin Dynamax 60A CN (8 µm); 25 × 1 cm column, solvent flux 3 mL/min. Machery-Nagel Nucleosil 100−5 C18; 25 × 1 cm column, solvent flux 3 mL/min. Optical rotation: JASCO-DIP-181 polarimeter, $[\alpha]_D$ in deg·mL·dm^{-1}·g^{-1}. NMR: Varian XL-300 (^{1}H at 299.94 MHz, ^{13}C at 75.4 MHz), δ in ppm with use of residual solvent signals as internal standard ($CDCl_3$: δ_H = 7.26, δ_C = 77.0 ppm; C_6D_6: δ_H = 7.16, δ_C = 128.7 ppm; C_5D_5N: δ_H = 7.21 ppm), *J* values in Hz, multiplicities and peak assignments from DEPT, ^{1}H,^{1}H-COSY, $^1J_{C,H}$- and $^nJ_{C,H}$-COSY. MS: Kratos MS80 with home-built acquisition system. Molecular mechanics (MM) calculations were carried out with the aid of the PCMODEL V7.5 computer program, searching for the global energy minimum. *, ** may be interchanged; # missing.

BF_3·Et_2O (excess, 10 µL) was added at room temperature to a solution of **2** (9.0 mg, 0.037 mmol) in benzene (1 mL). After 10 min the reaction mixture was treated with a saturated solution of $NaHCO_3$. After filtration the crude products were purified by HPLC- CN with hexane/ethanol, 9:1, with three main products being obtained: **21** (1.8 mg, 20%), **17** (1.8 mg, 20%) and **18** (1.0 mg, 11%).

(+)(1*R*,4*S*,5*S*,6*S*,7*S*)-5-Hydroxyguaiana-10(14),11(13)-dien-12,6-olide (21): $[\alpha]_D$ = +9 (*c* = 0.9, EtOH). ^{1}H NMR ($CDCl_3$): δ = 1.27 (dtd, *J* = 13.0, 12.0, 6.5 Hz, 1 H, H-8β), 1.31 (d, *J* = 6.6 Hz, 3 H, H-15), 1.51−1.69 (m, 3 H, 2 H-2, H-3β), 1.80−1.88 (m, 1 H, H-3α), 1.96−2.15 (m, 2 H, H-4, H-8α), 2.16−2.25 (m, 2 H, H-9), 2.86 (ddtd, *J* = 12.0, 9.3, 3.5, 3.5, 2.8 Hz, 1 H, H-7), 3.13 (dd, *J* = 11.8, 7.0 Hz, 1 H, H-1), 4.48 (d, *J* = 9.3 Hz, 1 H, H-6), 4.93 (d, *J* = 2.8 Hz, 1 H, H-14), 5.01 ,(d, *J* = 2.8 Hz, 1 H, H-14), 5.53 (d, *J* = 3.5 Hz, 1 H, H-13), 6.34 (d, *J* = 3.5 Hz, 1 H, H-13) ppm. ^{13}C NMR (C_5D_5N): δ = 15.9 (q, C-15), 31.0* (t, C-2), 31.4* (t, C-3), 32.5* (t, C-8), 34.3 (t, C-9), 39.6** (d, C-7), 40.9** (d, C-4), 62.4 (d, C-1), 82.0 (s, C-5), 91.9 (d, C-6), 116.4 (t, C-14), 120.3 (t, C-13), 140.7 (s, C-11), 148.0 (s, C-10), 170.1 (s, C-12) ppm. Irradiation at δ = 2.86 ppm (H-7) gives a positive NOE for the signal at δ = 1.9−2.1 ppm (H-4, 1.2%).

MS: *m*/*z* (%) = 248 (40.9) $[M]^+$, 41 (100). HRMS calcd. for $C_{15}H_{20}O_3$ $[M^+]$, 248.14124; found, 248.14146.

TSA (excess, 67 mg, 5 mmol) was added at room temperature to a solution of **3** (21.0 mg, 0.078 mmol) in MeOH (2 mL). After 15 days the reaction mixture was neutralised with Na_2HPO_4. HPLC purification (CN, hexane/EtOH, 75:25, λ = 213 nm) gave 2 fractions, **A** and **B**. Further chromatography of fraction **A** (R_t = 17.5 min, 19.5 mg) by HPLC (Nucleosil with MeOH/H_2O, 40:60) gave pure **22** (2.5 mg, 11%) and **23** (1.5 mg, 6.5%), fraction **B** (R_t = 13.6 min, 2.8 mg) by HPLC (Nucleosil with MeOH/H_2O, 6:4) gave pure **24** (1.2 mg, 5.2%) and **25** (1.0 mg, 4.3%).

(−)-(1*E*,3*Z*,5*R*,6*S*,7*S*)-5,14-Dihydroxygermacra-1(10),3,11(13)-trien-12,6-olide (22): $[\alpha]_D$ = −28.0 (*c* = 1.7, EtOH). ^{1}H NMR ($CDCl_3$): δ = 1.66 (dddd, *J* = 14.5, 11.5, 6.0, 3.0 Hz, 1 H, H-8β), 1.80 (br. s, 3 H, H-15), 1.91 (ddt, *J* = 14.5, 12.5, 3.1 Hz, 1 H, H-8α), 2.20 (ddd, *J* = 14.1, 6.0, 3.1 Hz, 1 H, H-9α), 2.73 (dddd, *J* = 14.1, 12.5, 3.0, 1.0 Hz, 1 H, H-9β), 2.81−2.93 (m, 3 H, 2H-2, H-7), 4.05 (br. s, 2 H, H-14), 4.39 (t, *J* = 5.3 Hz, 1 H, H-6), 4.49 (d, *J* = 5.3 Hz, 1 H, H-5), 5.52−5.54 (m, 1 H, H-3), 5.57 (d, *J* = 2.6 Hz, 1 H, H-13), 5.59−5.63 (m, 1 H, H-1), 6.27 (d, *J* = 3.0 Hz, 1 H, H-13) ppm. ^{13}C NMR ($CDCl_3$): δ = 20.3 (q, C-15), 23.9 (t, C-9), 28.3 (t, C-8), 32.0 (t, C-2), 40.5 (d, C-7), 67.6 (t, C-14), 74.3 (d, C-5), 84.6 (d, C-6), 122.6 (t, C-13), 125.2 (d, C-1), 125.7 (d, C-3), 134.8 (s, C-4), 137.5 (s, C-10), 139.5 (s, C-11), 169.5 (s, C-12) ppm. MS: *m*/*z* (%) = 264 (1.3) $[M]^+$, 28 (100). HRMS calcd. for $C_{15}H_{20}O_4$ $[M^+]$, 264.13616; found, 264.13535.

(−)-(1*E*,5*R*,6*S*,7*S*)-5,14-Dihydroxygermacra-1(10),4(15),11(13)-trien-12,6-olide (23): $[\alpha]_D$ = −127.0 (*c* = 1.0, EtOH). ^{1}H NMR ($CDCl_3$): δ = 1.58−1.78 (m, 2 H, H-8β, H-9α), 2.15−2.23 (m, 3 H, H-2α, H-3β, H-8α), 2.32−2.46 (m, 1 H, H-2β), 2.56 (ddd, *J* =

14.0, 12.0, 6.0 Hz, 1 H, H-9β), 2.76−2.85 (m, 2 H, H-3α, H-7), 3.89 (d, *J* = 9.5 Hz, 1 H, H-6), 4.05 (d, *J* = 13.0 Hz, 1 H, H-14), 4.10 (d, *J* = 13.0 Hz, 1 H, H-14), 4.57 (d, *J* = 9.5 Hz, 1 H, H-5), 4.92 (d, *J* = 1.1 Hz, 1 H, H-15), 4.96 (d, *J* = 1.1 Hz, 1 H, H-15), 5.44 (dd, *J* = 12.1, 4.2 Hz, 1 H, H-1), 5.57 (d, *J* = 1.5 Hz, 1 H, H-13), 6.21 (d, *J* = 1.2 Hz, 1 H, H-13) ppm. ^{13}C NMR ($CDCl_3$): δ = 23.6 (t, C-9), 25.7 (t, C-8), 30.4* (t, C-2), 31.8* (t, C-3), 38.2 (d, C-7), 66.4 (t, C-14), 80.9 (d, C-5), 82.5 (d, C-6), 120.6 (t, C-15), 122.9 (t, C-13), 128.4 (d, C-1), 136.3 (s, C-10), 139.0 (s, C-11), 144.3 (s, C-4), 169.7 (s, C-12) ppm. MS: *m*/*z* (%) = 264 (1.7) $[M]^+$, 28 (100). HRMS calcd. for $C_{15}H_{20}O_4$ [M^+], 264.13616; found, 264.13543.

(−)-(1*E*,4*R*,5*S*,6*S*,7*S*)-5,14-Dihydroxy-4-methoxygermacra-1(10),11(13)-dien-12,6-olide (24): $[\alpha]_D$ = −62.5 (*c* = 0.8, EtOH). ^{1}H NMR ($CDCl_3$): δ = 1.23 (s, 3 H, H-15), 1.60−1.72 (m, 2 H), 1.73−1.87 (m, 1 H), 1.95−2.06 (m, 1 H), 2.10−2.23 (m, 1 H), 2.30−2.47 (m, 3 H), 3.19 (s, 3 H, CH_3O), 3.50−3.58 (overlapped, 1 H, H-7), 3.53 (d, *J* = 6.5 Hz, 1 H, H-5), 4.09 (br. s, 2 H, H-14), 4.78 (dd, *J* = 6.5, 2.1 Hz, 1 H, H-6), 5.58 (d, *J* = 1.8 Hz, 1 H, H-13), 5.57−5.63 (m, overlapped, 1 H, H-1), 6.19 (d, *J* = 2.0 Hz, 1 H, H-13) ppm. ^{13}C NMR ($CDCl_3$): δ = 24.5 (C-15), 22.2* (C-2), 24.5* (C-9), 31.0* (C-8), 32.1* (C-3), 49.0 (C-7, MeO), 66.6 (C-14), 76.2 (C-5), 79.8 (C-4), 82.1 (C-6), 122.1 (C-13), 129.0 (C-1), # (C-10), 139.9 (C-11), # (C-12) ppm; Irradiation at δ = 3.19 ppm (MeO) gives a positive NOE for the signal at δ = 1.23 ppm (H-15, 0.6%). MS: *m*/*z* (%) = 296 (0.3) $[M]^+$, 72 (100). HRMS calcd. for $C_{16}H_{24}O_5$ [M^+], 296.16237; found, 296.16112.

(−)-(4*R*,5*S*,6*S*,7*S*)-5-Hydroxy-1,4-dimethoxygermacra-10(14),11(13)-dien-12,6-olide (25): $[\alpha]_D$ = −43.5 (*c* = 0.7, EtOH). ^{1}H NMR ($CDCl_3$): δ = 1.11 (s, 3 H, H-15), 1.32−1.45 (m, 1 H, H-3β), 1.68−1.95 (m, 3 H), 1.96−2.06 (m, 1 H, H-8β), 2.17−2.27 (m, 2 H, H-8α, H-9), 2.42−2.53 (m, 1 H, H-9), 2.99 (ddddd, *J* = 11.5, 6.0, 3.2, 2.8, 2.5 Hz, 1 H, H-7), 3.20 (s, 3 H, MeO−C4), 3.29 (s, 3 H, MeO−C1), 3.65 (br. s, 1 H, H-5), 3.70 (m, 1 H, H-1), 4.66 (d, *J* = 6.0 Hz, 1 H, H-6), 5.29 (bd, *J* = 2.0 Hz, 1 H, H-14), 5.32 (m, 1 H, H-14), 5.59 (d, *J* = 2.8 Hz, 1 H, H-13), 6.28 (d, *J* = 3.2 Hz, 1 H, H-13) ppm. ^{13}C NMR ($CDCl_3$): δ = 16.7 (q, C-15), 22.9* (t, C-2), 25.4* (t, C-9), 28.3* (t, C-8), 29.9* (t, C-3), 44.5 (q, MeO−C1), 49.0 (d, C-7), 56.9 (q, MeO−C4), 76.1** (d, C-5), 76.6** (d, C-1), 78.6 (s, C-4), 82.9 (d, C-6), 112.5 (t, C-14), 121.9 (t, C-13), 139.1 (s, C-11), 145.3 (s, C-10), 170.28 (s, C-12) ppm; Irradiation at δ = 1.11 ppm (H-15) gives a positive NOE for the signals at δ = 3.20 ppm (MeO−C-4, 3%) and at δ = 4.66 ppm (H-6, 3.7%); irradiation at δ = 3.29 ppm (MeO−C1) gives a positive NOE for the signals at δ = 3.70 (H-1, 3.7%) and 5.32 ppm (H-14, 2.8%); irradiation at δ = 3.65 ppm (H-5) gives a positive NOE for the signal at δ = 2.99 ppm (H-7, 6.6%); irradiation at δ = 4.66 ppm (H-6) gives a positive NOE for the signals at δ = 1.32−1.45 (H-3β, 7.1%) and 5.29 ppm (H-14, 2.6%).

BF_3·Et_2O (excess, 10 μL) was added at room temperature to a solution of **3** (15.0 mg, 0.056 mmol) in benzene (1 mL). After 15 min the reaction mixture was treated with a saturated solution of $NaHCO_3$. Purification of the crude products on TLC (EtOAc/2-propanol, 1:1) as mobile phase gave two fractions, **A** and **B**. Further chromatography of fraction **A** (R_f = 0.61−0.80, 6.9 mg) by HPLC (Nucleosil, MeOH/H_2O, 36:64) gave pure **22** (0.7 mg, 4.6%) and **23** (0.4 mg, 2.7%), fraction **B** (R_f = 0.80−0.95, 5.0 mg) by HPLC (Nucleosil, CH_3CN/H_2O, 4:6 and MeOH/H_2O, 6:4) gave pure **26** (1.7 mg, 11.4%).

(−)-(1*R*,4*S*,5*S*,6*S*,7*S*,10*R*)-5-Hydroxy-14-oxoguaiana-11(13)-en-12,6-olide (26): $[\alpha]_D$ = −8.0 (*c* = 1.13, EtOH). ^{1}H NMR (C_6D_6): δ = 0.54 (dddd, *J* = 12.0, 11.6, 10.5, 3.5 Hz, 1 H, H-8β), 0.56−0.65 (m, 1 H, H-2β), 0.91 (d, *J* = 6.4 Hz, 3 H, H-15), 0.92 (dddd, *J* = 13.0, 11.5, 10.5, 3.5 Hz, 1 H, H-9α), 1.02−1.20 (m, 2 H, H-2α, H-3β), 1.26−1.42 (m, 3 H, H-3α, H-4, H-8α), 1.62−1.71 (m, 1 H, H-9β), 1.86 (ddddd, *J* = 11.6, 9.5, 3.6, 3.1, 3.0 Hz, 1 H, H-7), 1.91 (ddd, *J* = 11.5, 2.5, 2.0 Hz, 1 H, H-10), 2.41 (ddd, *J* = 13.0, 7.0, 1.9 Hz, 1 H, H-1), 3.66 (d, *J* = 9.5 Hz, 1 H, H-6), 4.86 (d, *J* = 3.1 Hz, 1 H, H-13), 6.13 (d, *J* = 3.1 Hz, 1 H, H-13), 9.06 (s, 1 H, H-14) ppm. ^{13}C NMR (C_6D_6): δ = 15.6 (q, C-15), 21.7* (t, C-8), 26.7* (t, C-2), 30.5* (t, C-3), 33.7* (t, C-9), 39.6** (d, C-1), 40.9** (d, C-4), 50.6 (d, 2C, C-7, C-9), 81.9 (s, C-5), 91.4 (d, C-6), 120.9 (t, C-13), 140.4 (s, C-11), 169.9 (s, C-12), 201.6 (d, C-14) ppm; irradiation at δ = 2.41 ppm(H-1) gives a positive NOE for the signals at δ = 1.02−1.20 (H-3β, 0.5%), 1.91 (H-1, 0.5%) and 9.06 ppm (H-14, 4.0%); irradiation at δ = 3.66 ppm (H-6) gives a positive NOE for the signals at δ = 0.54 (H-8β, 0.9%) and 1.91 ppm (H-1, 4.8%); irradiation at δ = 4.86 ppm (H-13) gives a positive NOE for the signals at δ = 1.35−1.42 (H-8α, 3.5%) and 6.13 ppm (H-13, 17%);). MS: *m*/*z* (%) = 264 (8.9) $[M]^+$, 235 (100). HRMS calcd. for $C_{15}H_{20}O_4$ [M^+], 264.13616; found, 264.13728.

A solution of **3** (7.5 mg, 0.029 mmol) in benzene (3 mL) was degassed for 30 min by bubbling with N_2 and then irradiated with UV light (λ = 254 nm) for 24 h, and the solvent was removed. The crude reaction product was purified by HPLC (CN, λ = 213 nm, hexane/ethanol, 75:25) to provide unchanged **3** (5.0 mg, 0.019 mmol, 66%) and **27** (1.8 mg, 0.007 mmol, 24%).

(−)-(1*Z*,4*R*,5*S*,6*S*,7*S*)-4,5−Epoxy-14-hydroxygermacra-1(10),11(13)-dien-12,6-olide (27): $[\alpha]_D$ = −39.0 (*c* = 1.0, EtOH). ^{1}H NMR ($CDCl_3$): δ = 1.25 (s, 3 H, H-15), 1.28 (td, *J* = 13.0, 5.0 Hz, 1 H, H-3α), 1.81 (dddd, *J* = 15.0, 12.5, 8.5, 1.7 Hz, 1 H, H-8β), 2.07 (ddd, *J* = 13.5, 12.5, 1.5 Hz, 1 H, H-9α), 2.13−2.28 (m, 3 H, H-2α, H-3β, H-8α), 2.48 (dddd, *J* = 14.0, 13.0, 12.5, 5.5 Hz, 1 H, H-2β), 2.75−2.87 (m, 2 H, H-7, H-9β), 2.78 (d, *J* = 8.6 Hz, 1 H, H-5), 3.85 (d, *J* = 8.6 Hz, 1 H, H-6), 4.09 (d, *J* = 11.6 Hz, 1 H, H-14), 4.41 (d, *J* = 11.6 Hz, 1 H, H-14), 5.37 (dd, *J* = 12.5, 4.0 Hz, 1 H, H-1), 5.63 (d, *J* = 3.3 Hz, 1 H, H-13), 6.34 (d, *J* = 3.7 Hz, 1 H, H-13) ppm. ^{13}C NMR ($CDCl_3$): δ = 16.9 (q, C-15), 23.7 (t, C-2), 31.3 (t, C-8), 36.3 (t, 2 C, C-3, C-9), 47.3 (d, C-7), 59.7 (t, C-14), 60.1 (s, C-4), 66.2 (d, C-5), 82.4 (d, C-6), 121.4 (t, C-13), 129.0 (d, C-1), 137.7 (s, C-10), 139.1 (s, C-11), 169.2 (s, C-12) ppm; irradiation at δ = 1.25 ppm (H-15) gives a positive NOE for the signals at δ = 2.20 (H-3β, 7.9%) and at δ = 3.85 ppm (H-6, 5.0%); irradiation at δ = 5.37 ppm (H-1) gives a positive NOE for the signals at δ = 2.07 (H-9α, 1.3%), at δ = 2.2 (H-2α, 0.5%) and at δ = 2.78 ppm (H-5, 1.3%). MS: *m*/*z* (%) = 264 (1.2) $[M]^+$, 28 (100). HRMS calcd. for $C_{15}H_{20}O_4$ [M^+], 264.13616; found, 264.13562.

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