# Cytotoxic Germacrane Sesquiterpenes from the Aerial Parts of *Santolina insularis*

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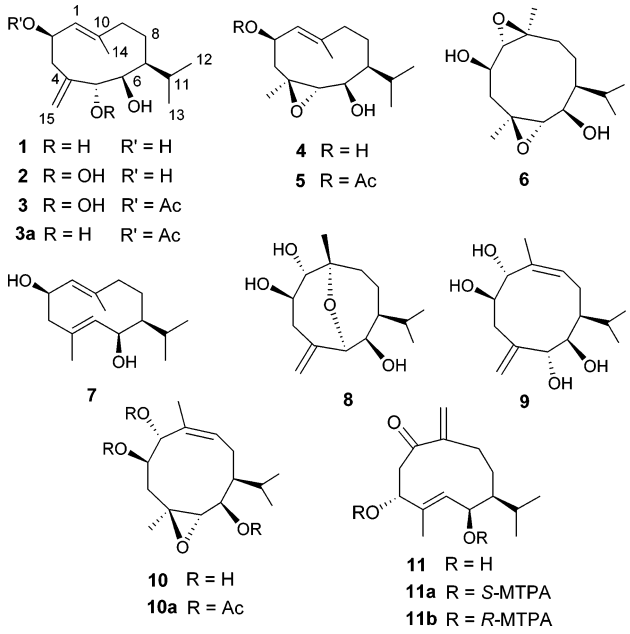
Chemical investigation of *Santolina insularis* afforded 11 germacrane sesquiterpenes (**1**−**11**), four of which (**2**, **3**, **10**, and **11**) are new. The stereostructures of these compounds have been established by a combination of spectroscopic techniques (mainly NMR), chemical transformations, and application of the modified Mosher method. Compounds **8** and **10** showed a potent and selective cytotoxic activity against the human colon carcinoma cell line.

Plants belonging to the genus *Santolina* (Asteraceae, tribe Anthemideae) have been intensely investigated because of their rich ethnopharmacology, which includes both medicinal (antispasmodic, antiseptic, anti-inflammatory, antihelmintic) and insecticidal uses.[1,2] As part of an ongoing program aimed at the isolation of bioactive compounds from Mediterranean plants, we have undertaken the chemical investigation of *S. insularis* (Genn. ex Fiori) Arrigoni, a bush endemic to Central and Southern Sardinia (Italy), where its aerial parts are traditionally used as vermifuge and to repel insects.[3] The plant revealed to be a prolific producer of terpenoids, and in a previous investigation, we characterized several eudesmane sesquiterpenoids, chrysanthemane monoterpenoids, and a mosquito-repellent *p*-menthane derivative from the least polar fractions of the terpenoid mixture.[4] Remarkably, the most polar fractions contained compounds of a different type, and in this paper we describe the stereostructural characterization and the cytotoxic activity of 11 polyoxygenated germacranes (**1**−**11**), four of which (**2**, **3**, **10**, and **11**) are new molecules.




## Results and Discussion

The aerial parts of *S. insularis*, collected in Sardinia (Italy) during blossom in 2001, were sun-dried, pulverized, and sequentially extracted with *n*-hexane and acetone. The acetone extract was subjected to medium-pressure liquid chromatography (MPLC) over a column packed with silica gel, affording a series of germacrane-containing fractions, which were further purified by HPLC to eventually afford 11 pure compounds (**1**−**11**). The structures of compounds **1** and **4**−**9** were assessed by comparison of their spectroscopic data with those reported in the literature.[5−7] The remaining compounds are new.

The molecular formula $C_{15}H_{26}O_4$, implying three unsaturation degrees, was assigned to compound **2** by HRMS. Analysis of $^1$H and $^{13}$C NMR data of **2** ($CDCl_3$, Table 1), assisted by 2D HMQC experiment, indicated the presence of two double bonds, one trisubstituted ($\delta_C$ 121.6, $\delta_H$ 4.95; $\delta_C$ 139.7) and the other one geminally disubstituted ($\delta_C$ 117.5, $\delta_H$ 5.20 and 5.02; $\delta_C$ 138.8), and of three oxymethines ($\delta_C$ 69.5, 90.6, and 70.0; $\delta_H$ 4.59; 4.34, and 4.16, respectively). The remaining eight carbons were assigned to three methyls, three sp$^3$ methylenes, and two additional sp$^3$ methines. Therefore, **2** should be monocyclic.

The 2D $^1$H−$^1$H COSY spectrum of compound **2** proved to be very informative since only two spin systems were detected, the first fragment running from H-1 to $H_2$-3 through an oxymethine at C-2 and the second one connecting H-5 to $H_2$-9 and encompassing the two remaining oxymethines (C-5 and C-6) as well as an isopropyl branching at C-7. Analysis of the HMBC spectrum revealed that these two fragments are mutually joined, with eventual merging into the 10-membered carbocycle typical of the germacrane skeleton. In particular, $H_2$-15 showed $^2J_{H-C}$ correlation with the non-proton-bearing C-4 and $^3J_{H-C}$ correlations with both C-3 and C-5. Similarly, the allylic methyl protons $H_3$-14 ($\delta_H$ 1.79) showed $^2J_{H-C}$ correlation with the non-proton-bearing C-10 and $^3J_{H-C}$ correlations with both C-1 and C-9. To deduce the gross structure of compound **2**, the precise nature of groups linked at C-2, C-5, and C-6 remained to be defined. Indeed, since these

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**Table 1.** $^{13}C$ (125 MHz) and $^{1}H$ (500 MHz) NMR Data of Compounds **2** and **3**[a]

| | **2** | | **3** | |
|---|---|---|---|---|
| pos. | $\delta_C$ (mult.) | $\delta_H$ (mult., $J$ in Hz) | $\delta_C$ (mult.) | $\delta_H$ (mult., $J$ in Hz) |
| 1 | 121.6 (CH) | 4.95 (d, 10.7) | 123.8 (CH) | 5.22 (d, 10.7) |
| 2 | 69.5 (CH) | 4.59 (ddd, 10.7, 9.8, 5.8) | 71.9 (CH) | 5.53 (ddd, 10.7, 9.8, 5.8) |
| 3a | 43.4 ($CH_2$) | 2.80 (dd, 11.5, 5.8) | 41.0 ($CH_2$) | 2.80 (dd, 12.1, 5.8) |
| 3b | | 2.20 (dd, 11.5, 9.8) | | 2.26 (dd, 12.1, 9.8) |
| 4 | 138.8 (C) | | 138.8 (C) | |
| 5 | 90.6 (CH) | 4.34 (d, 10.2) | 90.7 (CH) | 4.35 (d, 10.2) |
| 5-OOH | | 8.20 (s) | | 8.26 (s) |
| 6 | 70.0 (CH) | 4.16 (bd, 10.2) | 70.1 (CH) | 4.14 (bd, 10.2) |
| 7 | 42.0 (CH) | 1.03[b] | 42.0 (CH) | 1.03[b] |
| 8a | 29.5 ($CH_2$) | 1.72[b] | 29.7 ($CH_2$) | 1.72[b] |
| 8b | | 1.50 (m) | | 1.50 (m) |
| 9a | 35.5 ($CH_2$) | 2.43 (m) | 35.6 ($CH_2$) | 2.46 (m) |
| 9b | | 1.71[b] | | 1.71[b] |
| 10 | 139.7 (C) | | 142.5 (C) | |
| 11 | 31.3 (CH) | 1.78[b] | 31.6 (CH) | 1.78[b] |
| 12 | 21.2 ($CH_3$) | 1.00 (d, 7.3) | 21.2 ($CH_3$) | 1.00 (d, 7.3) |
| 13 | 21.3 ($CH_3$) | 1.00 (d, 7.3) | 21.3 ($CH_3$) | 1.00 (d, 7.3) |
| 14 | 21.4 ($CH_3$) | 1.79 (s) | 21.4 ($CH_3$) | 1.77 (s) |
| 15a | 117.5 ($CH_2$) | 5.20 (bs) | 119.0 ($CH_2$) | 5.29 (bs) |
| 15b | | 5.02 (bs) | | 5.00 (bs) |
| 2-OAc | | | 170.5 (C) | |
| | | | 21.1 ($CH_3$) | 2.05 (s) |

[a] Recorded in $CDCl_3$. [b] Overlapped with other signals.

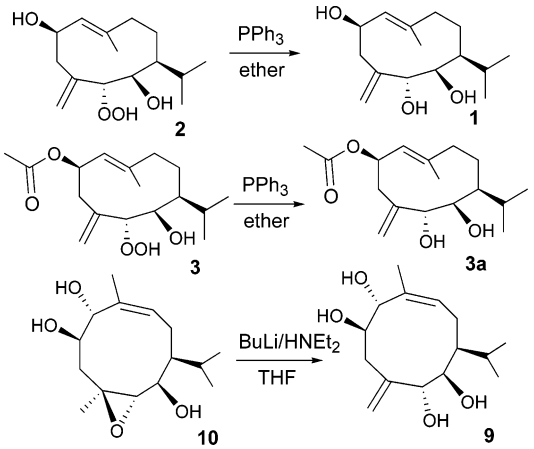


**Figure 1.** Chemical transformations of germacrenes **2**, **3**, and **10**.

three oxygenated carbons had to accommodate the four oxygen atoms of the molecular formula, one of them had to bear a hydroperoxyl group. This carbon was easily identified as C-5 on the basis of its low-field $^{13}C$ NMR resonance ($\delta_C$ 90.6), leading us to draw the planar structure of compound **2**.

The structure of compound **3** ($C_{17}H_{28}O_5$ by HRMS) was mainly derived by comparing its spectroscopic data with those of **2**. The presence of an additional acetyl unit in **3** was suggested by the molecular formula (additional $C_2H_2O$ unit) and confirmed by inspection of the $^{1}H$ ($\delta_H$ 2.05, singlet) and $^{13}C$ NMR spectra ($\delta_C$ 21.1 and 170.5) (Table 1). Analysis of 2D NMR data (COSY, HMQC, HMBC) led to the complete assignment of NMR signals, while the low-field resonance of the C-2 proton ($\delta$ 5.53) located the acetyl at this carbon.

Stereochemical details for compounds **2** and **3** were assigned taking advantage of the easy conversion of a hydroperoxide group into the corresponding alcohol using triphenylphosphine in ether (Figure 1). In particular, reduction of **2** yielded a compound identical ($[\alpha]_D$ and NMR data) to **1**,[5] a compound also present in extracts from *S. insularis*, while reduction of **3** afforded a compound identical ($[\alpha]_D$ and NMR data) to **3a**, not detected in *S. insularis*, but a constituent of *S. chamaecyparissus* L.[5]

The presence of the hydroperoxide analogues of two oxygenated germacranes is worthy of note, and considering their stereochemical purity (epimers at C-5 are not detectable in the extract of *S. insularis*), most likely compounds **2** and **3** are genuine natural products. From a biogenetic standpoint, the hydroperoxides **2** and **3** might derive from the formal photo-oxygenation of the corresponding $\Delta^{4,5}$ olefin, a reaction well precedented in medium-sized olefins.[8]

The molecular formula $C_{15}H_{26}O_4$, implying three unsaturation degrees, was assigned to compound **10** on the basis of HREIMS analysis. The $^{1}H$ and $^{13}C$ NMR spectra of **10** ($CDCl_3$, Table 2) were analyzed with the aid of a 2D HMQC experiment, revealing the presence of six $sp^3$ methines (four of which are oxygenated), a trisubstituted double bond, four methyls, and two methylenes. A further non-proton-bearing oxygenated carbon at $\delta$ 59.5 in the $^{13}C$ NMR spectrum of **10** required the presence of an ether linkage to account for the presence of five oxymethines but only four oxygen atoms in the molecular formula of **10**. The COSY spectrum of **10** disclosed the sequential arrangement of proton resonances revealing the presence of two spin systems. The first one comprises a vicinal diol group ($\delta_H$ 4.51 and 3.86) and a diastereotopic methylene, while the second spin system connects two mutually coupled oxygenated methines ($\delta_H$ 3.15 and 3.65) to the $sp^2$ methine H-9 ($\delta_H$ 5.42) spanning an isopropyl branched methine and a second methylene group. As for compounds **2** and **3**, the 2D HMBC spectrum of compound **10** was of pivotal importance to solve the structure, with the $^{2,3}J_{CH}$ correlations of methyl protons at C-14 and C-15 proving critical. Thus, $H_3$-14 correlated with the non-proton-bearing double-bond carbon at C-10 ($\delta_C$ 133.3) and with both C-1 ($\delta_C$ 70.7) and C-9 ($\delta_C$ 130.3), thus connecting the above two spin systems. A second link was established on the basis of $^{3}J_{CH}$ correlations of $H_3$-15 with both C-3 ($\delta_C$ 45.7) and C-5 ($\delta_C$ 64.7), indicating the presence of the germacrane macrocycle, while the $^{2}J_{CH}$ correlation with the signal resonating at $\delta$ 59.5 revealed that C-4 is oxygenated. This carbon must be indeed involved in an epoxide ring with C-5, as suggested by the relatively low-field resonance of H-5 ($\delta$ 3.15) and confirmed by acetylation of **10**, which gave the triacetyl derivative **10a**.

**Table 2.** $^{13}C$ (125 MHz) and $^{1}H$ (500 MHz) NMR Data of Compounds **10** and **11**[a]

| | **10** | | **11** | |
|---|---|---|---|---|
| pos. | $\delta_C$ (mult.) | $\delta_H$ (mult., $J$ in Hz) | $\delta_C$ (mult.) | $\delta_H$ (mult., $J$ in Hz) |
| 1 | 70.7 (CH) | 4.51 (bd, 9.2) | 200.2 (C) | |
| 2a | 67.8 (CH) | 3.86 (m) | 42.3 ($CH_2$) | 3.32 (dd, 11.8, 11.0) |
| 2b | | | | 2.54 (dd, 11.8, 7.5) |
| 3a | 45.7 ($CH_2$) | 2.53[b] | 75.6 (CH) | 4.48 (dd, 11.0, 7.5) |
| 3b | | 1.30 (m) | | |
| 4 | 59.5 (C) | | 133.2 (C) | |
| 5 | 64.7 (CH) | 3.15 (d, 8.5) | 132.7 (CH) | 5.26 (d, 6.8) |
| 6 | 68.5 (CH) | 3.65 (dd, 8.5, 1.7) | 68.0 (CH) | 4.56 (dd, 6.8, 1.5) |
| 7 | 45.3 (CH) | 1.86 (m) | 47.5 (CH) | 0.78 (m) |
| 8a | 27.2 ($CH_2$) | 2.53[b] | 25.9 ($CH_2$) | 1.70 (m) |
| 8b | | 1.98 (m) | | 1.24 (m) |
| 9a | 130.3 (CH) | 5.42 (dd, 10.5, 3.5) | 33.1 ($CH_2$) | 2.93 (ddd, 13.0, 2.5, 1.5) |
| 9b | | | | 1.87 (ddd, 13.0, 11.2, 3.0) |
| 10 | 133.3 (C) | | 150.6 (C) | |
| 11 | 26.7 (CH) | 2.21 (m) | 30.9 (CH) | 1.66 (m) |
| 12 | 21.4 ($CH_3$) | 1.00 (d, 7.0) | 20.6 ($CH_3$) | 0.99 (d, 7.0) |
| 13 | 18.5 ($CH_3$) | 1.05 (d, 7.0) | 20.6 ($CH_3$) | 1.00 (d, 7.0) |
| 14a | 17.8 ($CH_3$) | 1.80 (s) | 126.2 ($CH_2$) | 5.83 (bs) |
| 14b | | | | 5.63 (bs) |
| 15 | 17.5 ($CH_3$) | 1.19 (s) | 10.6 ($CH_3$) | 1.58 (bs) |

[a] Recorded in $CDCl_3$. [b] Overlapped with other signals.

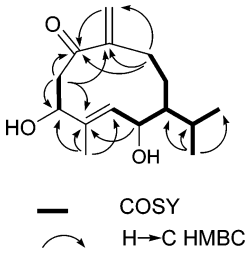


**Figure 2.** COSY and $^{2,3}J_{H\rightarrow C}$ HMBC correlations of compound **11**.

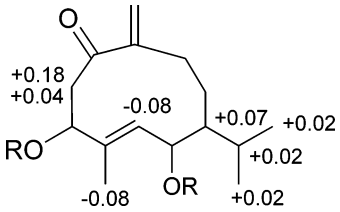


**Figure 3.** Application of the modified Mosher's method to determine the absolute configuration at C-3 and C-6 of compound **11**. $\Delta\delta(S-R)$ values are given in ppm.

Treatment of the epoxyalcohol **10** with *n*-butyllithium/diethylamine in THF (Figure 1),[5,9] which afforded the corresponding allylic alcohol **9** (30% yields), unambiguously indicated that compounds **9**[5] and **10** share the same configuration at the common stereogenic centers, thus establishing the configuration of all the chiral centers of **10** apart from C-4. The configuration at the epoxidic carbon C-4 was deduced from the spatial proximity (evidenced through a ROESY spectrum) of H-6 with $H_3$-15.

The structure of the fourth new germacrene derivative **11** ($C_{15}H_{24}O_3$ by HREIMS) followed from interpretation of its $^{1}H$ and $^{13}C$ NMR spectra (Table 2) guided by 2D NMR data. The HMQC spectrum of **11** revealed 10 proton-bearing $sp^3$ carbons (resonating in the $^{13}C$ NMR spectrum between $\delta_C$ 10.6 and 75.6), including four methines (two of which are oxymethines), three methylenes, and three methyls. On the other hand, four of the five $sp^2$ carbons could be combined into two double bonds, one trisubstituted ($\delta_H$ 5.26) and one geminally disubsituted ($\delta_H$ 5.63 and 5.83, both broad singlets), while the remaining $sp^2$ carbon, resonating at $\delta_C$ 200.2, could be assigned to a conjugated ketone carbonyl, as suggested also by the IR absorption band at $\nu_{max}$ 1680 $cm^{-1}$.

Inspection of the 2D COSY spectrum of **11** revealed that one of the two oxymethines ($\delta_H$ 4.48) is coupled only with a deshielded methylene ($\delta_H$ 2.54 and 3.32), while the second oxymethine proton ($\delta_H$ 4.56) is coupled both with the double bond proton at $\delta_H$ 5.26 and with a methine proton at $\delta_H$ 0.78 that links an isopropyl residue and a methylene, which, in turn, showed coupling with another methylene group (Figure 2). The deshielded protons ($\delta_H$ 1.87 and 2.93) of the latter methylene showed HMBC correlations with the non-proton-bearing carbon C-10 ($\delta_C$ 150.6), with the $sp^2$ methylene carbon ($\delta_C$ 126.2), and with the ketone carbon (C-1). The HMBC couplings of both C-1 and C-10 with the protons at C-2, and those of $H_3$-15 with C-5 and C-3 (Figure 2), unambiguously defined the planar structure of compound **11**.

The configuration of **11** was assessed by inspection of scalar coupling constants and dipolar correlations (ROESY spectrum). In particular, the *E* configuration of the endocyclic double bond was suggested by the spatial proximity of H-6 with $H_3$-15, while the large value of $J_{5,6}$ (6.8 Hz) and the small value of $J_{6,7}$ (1.5 Hz) are diagnostic, in $\Delta^{4(5)}$ germacrene derivatives, of a *cis*-orientation of the 6-hydroxyl and the 7-isopropyl groups (as in compound **7**).[6,10−12] This kind of analysis gave no clue to the configuration at C-3, and, thus, to solve this structural ambiguity, we applied the modified Mosher method for the configurational assignment of secondary alcohols.[13] Treatment of two aliquots of **11** with (−)- and (+)-MTPA chloride in dry pyridine gave the corresponding diesters **11a** and **11b**, respectively. The pattern of $\Delta\delta(S-R)$ values (see Figure 3) allowed us to establish the absolute configuration at C-3 and to upgrade the above determined relative configuration at C-6/C-7 to the absolute one. Thus, the complete stereostructure of compound **11** was defined as reported in the figure.

The isolated germacrane sesquiterpenes were evaluated for cytotoxic activity against two different cell lines: Caco-2 (human colon carcinoma) and peritoneal macrophages. Remarkably, while all the tested compounds were inactive against the second cell line ($IC_{50} \geq 10$ $\mu$g/mL), compounds **8** and **10** proved to be potently active against the first cell line, with $IC_{50} = 1.0$ $\mu$g/mL (3.7 $\mu$M) and 0.3 $\mu$g/mL (1.1 $\mu$M), respectively. This selective activity against a human tumor cell line is worthy of note and of further investigation.

## Conclusions

The results of the present study complement previous data on the occurrence of various types of lipophilic terpenoids in *S. insularis*. With the phytochemical pattern of this species now established, several issues are worth noting. First, the plant contains, in addition to an antifeedant *p*-menthane, a remarkable array of compounds structurally related to known insecticides and insect pheromones (chrysanthemane monoterpenoids and periplanones, respectively).[14] Since *S. insularis* is extensively used in traditional veterinary and human medicine to repel and kill insects, it qualifies as a source of potentially insecticidal compounds, and it does not seem unconceivable that extracts from the plant might be developed for household use. Second, the exuberance of natural product elaboration by *S. insularis* is remarkable, since secondary metabolism is not funneled into a few major compounds, as often occurs in Asteraceae plants, but is spread over a host of structurally related compounds, none of which, however, prevails over the others in terms of abundance. Recent studies have shown that this pattern of secondary metabolites production is a very efficient way to convey a broad-band defense against pests.[15] Finally, the cytotoxicity of the germacrane derivatives **8** and **10** should spur investigations aimed at establishing the mechanism(s) of this activity. An extensive study on cytotoxic sesquiterpene exomethylene $\gamma$-lactones has evidenced a significant role for the terpenoid core.[16] The activity of compounds **8** and **10** testifies that the sesquiterpene moiety can per se show cytotoxic activity even in the absence of the electrophilic exomethylene-$\gamma$-lactone moiety. Furthermore, the close similarity between the active compound **8** and the inactive compound **9** and, analogously, between the active compound **10** and the inactive compound **6** suggests that subtle structural changes can dramatically influence the cytotoxic activity within this class of compounds.

## Experimental Section

**General Experimental Procedures.** Optical rotations were measured in $CHCl_3$ on a Perkin-Elmer 192 polarimeter equipped with a sodium lamp ($\lambda_{max}$ = 589 nm) and a 10 cm microcell. IR (KBr) spectra were recorded on a Bruker model IFS-48 spectrophotometer. Low-resolution FAB (CsI ions, glycerol matrix) and low- and high-resolution EI mass spectra (70 eV, direct inlet) were performed on a VG Prospec (FISONS) mass spectrometer. $^1$H (500 MHz) and $^{13}$C (125 MHz) NMR spectra were measured on a Bruker AMX-500 spectrometer; chemical shifts are referenced to the residual solvent signal ($CDCl_3$: $\delta_H = 7.26$, $\delta_C = 77.0$). The multiplicities of $^{13}$C NMR resonances were determined by DEPT experiments. One-bond heteronuclear $^1$H−$^{13}$C connectivities were determined with the HMQC experiment. Two- and three-bond $^1$H−$^{13}$C connectivities were determined by HMBC experiments optimized for a $^{2,3}J$ of 8 Hz. Nuclear Overhauser effect (NOE) measurements were obtained from 2D ROESY experiments. Medium-pressure liquid chromatography (MPLC) was performed using a Büchi 861 apparatus using Merck SI60 (230−400 mesh) stationary phase. High-performance liquid chromatography (HPLC) separations in isocratic mode were achieved on a Beckmann apparatus equipped with refractive index detector and with Phenomenex LUNA SI60 (250 × 10 mm and 250 × 4 mm) columns.

**Plant Material.** Specimens of *Santolina insularis* (Genn. ex Fiori) Arrigoni were collected near Arzana (Nu), Sardinia (Italy), in June 2001. The plant was identified by M.B., and a voucher specimen (ref. no. 732) is held in CAG (Dipartimento di Scienze Botaniche dell'Università), Cagliari (Italy).

**Extraction and Isolation.** Dried aerial parts of *Santolina insularis* (stems and leaves, 250 g) were pulverized, defatted with *n*-hexane (4 × 2 L), and then extracted with acetone (4 × 2 L) at room temperature, affording 12 g of a dark gum. This was fractionated by MPLC column chromatography (silica gel) using a solvent gradient system from *n*-hexane/EtOAc, 9:1, to EtOAc, and affording 10 main fractions (A−J). Fraction E (430 mg), obtained by elution with *n*-hexane/EtOAc, 1:1, was further purified by column chromatography over silica gel (eluant $CHCl_3$/*n*-hexane/MeOH, 78:28:2), to obtain compound **6** (25 mg) in the pure state and a crude fraction that was subjected to a second silica gel column chromatography (eluant *n*-hexane/EtOAc, 1:1), yielding pure compound **7** (18 mg) and a fraction that was finally subjected to HPLC (*n*-hexane/EtOAc, 55:45, as eluant), affording 32 mg of compound **5**. Fraction G (125 mg), obtained by elution with EtOAc/*n*-hexane, 65:35, was purified by HPLC (eluant *n*-hexane/EtOAc, 1:1), affording compound **8** (13 mg) and the new compounds **3** (6.5 mg), **10** (15.5 mg), and **11** (2.8 mg). Fraction H (1.1 g), obtained by elution with EtOAc/*n*-hexane, 75:25, was purified by column chromatography (eluant EtOAc/*n*-hexane, 65:35), which yielded pure compounds **1** (190 mg) and **4** (85 mg) and a fraction that was further purified by HPLC (eluant EtOAc/*n*-hexane, 6:4), affording compounds **2** (2.5 mg) and **9** (11.5 mg) in the pure state.

**(2*R*,5*R*,6*R*,7*S*)-Germacra-1(10)*E*,4(15)-dien-5-hydroperoxy-2,6-diol (2):** colorless amorphous solid; $[\alpha]_D^{25}$ −20.5° (*c* 0.02, $CHCl_3$); IR (KBr) $\nu_{max}$ 3350, 1450, 1365 cm$^{-1}$; $^1$H ($CDCl_3$, 500 MHz) and $^{13}$C ($CDCl_3$, 125 MHz) NMR spectra, Table 1; EIMS (70 eV) *m/z* 270 $[M]^+$ (5), 253 (20), 252 (40), 97 (100); HREIMS *m/z* 270.1868 (calcd for $C_{15}H_{26}O_4$, 270.1831).

**(2*R*,5*R*,6*R*,7*S*)-Germacra-1(10)*E*,4(15)-dien-5-hydroperoxy-2,6-diol-2-acetate (3):** colorless amorphous solid; $[\alpha]_D^{25}$ −8.1° (*c* 0.03, $CHCl_3$); IR (KBr) $\nu_{max}$ 3350, 1730, 1435, 1365 cm$^{-1}$; $^1$H ($CDCl_3$, 500 MHz) and $^{13}$C ($CDCl_3$, 125 MHz) NMR spectra, Table 1; EIMS (70 eV) *m/z* 312 $[M]^+$ (15), 295 (20), 294 (40), 252 (40), 97 (100); HREIMS *m/z* 312.1949 (calcd for $C_{17}H_{28}O_5$, 312.1937).

**Conversion of the Hydroperoxides 2 and 3 to the Corresponding Alcohols.** Compound **2** (1.0 mg) and triphenylphosphine (3.1 mg) were dissolved in ether (800 $\mu$L) and stirred at room temperature for 3 h. Then the solvent was removed under reduced pressure and the obtained residue was purified by silica gel HPLC (SI60 250 × 4 column, EtOAc/*n*-hexane, 6:4, as eluant) to give pure compound **1** (0.9 mg). Compound **3** (1.5 mg) was allowed to react with triphenylphosphine under the same conditions as above and afforded compound **3a** (1.2 mg).

**(1*R*,2*R*,4*S*,5*S*,6*R*,7*S*)-4,5-Epoxygermacra-9*Z*-en-1,2,6-triol (10):** colorless amorphous solid; $[\alpha]_D^{25}$ +4.0° (*c* 0.08, $CHCl_3$); IR (KBr) $\nu_{max}$ 3480, 1450, 1360, 1120, 1070 cm$^{-1}$; $^1$H (500 MHz, $CDCl_3$) and $^{13}$C (125 MHz, $CDCl_3$) NMR spectra, Table 2; EIMS (70 eV) *m/z* 270 $[M]^+$ (15), 252 (22), 236 (67), 125 (100); HREIMS *m/z* 270.1866 (calcd for $C_{15}H_{26}O_4$, 270.1831).

**Acetylation of Compound 10.** Compound **10** (2.5 mg) was dissolved in dry pyridine (0.5 mL) and treated with $Ac_2O$ (0.5 mL). After standing overnight, the reaction was worked up by addition of a few drops of methanol to destroy the excess $Ac_2O$, water (ca. 1 mL), and EtOAc (ca. 3 mL). The organic phase was washed sequentially with 2 N $H_2SO_4$, saturated $NaHCO_3$, and brine. After drying ($Na_2SO_4$) and removal of the solvent, the residue was purified by HPLC (*n*-hexane/EtOAc, 8:2) to afford 2.2 mg of the triacetate **10a**.

**Compound 10a:** amorphous solid; $[\alpha]_D^{25}$ +2.5 (*c* 0.02, MeOH); $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 6.12 (H-1, d, $J$ = 10.4 Hz), 5.53 (H-9, dd, $J$ = 12.5, 3.5 Hz), 5.37 (H-2, ddd, $J$ = 10.4, 6.0, 3.0 Hz), 5.04 (H-6, dd, $J$ = 8.5, 3.0 Hz), 3.08 (H-5, d, $J$ = 8.5 Hz), 2.58 (H-3a, dd, $J$ = 11.0, 6.0 Hz), 2.45 (H-8a, m), 2.15 (H-8b, m), 2.05 (OCOC$H_3$), 2.01 (OCOC$H_3$), 2.00 (H-11, overlapped), 1.99 (OCOC$H_3$), 1.75 ($H_3$-14, bs), 1.70 (H-7, m), 1.43 (H-3b, m), 1.37 ($H_3$-15, s), 1.09 ($H_3$-12, d, $J$ = 7.3 Hz), 0.99 ($H_3$-13, d, $J$ = 7.3 Hz); EIMS *m/z* 396.

**Base-Catalyzed Epoxide Ring Opening of Compound 10.** A 1.7 M solution of BuLi in hexane (0.6 mL, ca. 0.1 mmol) was added under Ar atmosphere to a solution of diethylamine (0.02 mL, ca. 0.1 mmol) in dry THF (1 mL). The resulting solution was then stirred at room temperature for about 30

min. Compound **10** (6 mg, ca. 0.025 mmol) was dissolved in a mixture of dry THF (1 mL) and dry HMPT (0.05 mL) and added to the lithium diethylamide solution. The reaction mixture was then heated at reflux for 4 h. After this time, 10 $\mu$L of glacial acetic acid was added and the solvents were evaporated. HPLC on silica gel (eluent EtOAc/*n*-hexane, 55:45) afforded 1 mg of unreacted **10**, some degradation products that were not further characterized, and 1.6 mg of compound **9**.

**(3*R*,6*R*,7*S*)-3,6-Dihydroxygermacra-4(5)*E*,10(14)-dien-1-one (11):** colorless amorphous solid; $[\alpha]_D^{25}$ −22.0° (*c* 0.02, $CHCl_3$); IR (KBr) $\nu_{max}$ 3610, 1680, 1635 $cm^{-1}$; $^1H$ (500 MHz, $CDCl_3$) and $^{13}C$ (125 MHz, $CDCl_3$) NMR spectra, Table 2; EIMS (70 eV) *m/z* 252 $[M]^+$ (55), 210 (40), 109 (100); HREIMS *m/z* 252.1750 (calcd for $C_{15}H_{24}O_3$, *m/z* 252.1725).

**Preparation of MTPA Ester Derivatives of Compound 11.** Compound **11** (1.0 mg) was dissolved in 500 $\mu$L of dry pyridine, treated with an excess of (−)-(*R*)-MTPA chloride (20 $\mu$L), and then stirred at room temperature overnight. After removal of the solvent, the reaction mixture was purified by HPLC on an SI60 column (eluent *n*-hexane/EtOAc, 85:15), affording (*S*)-MTPA diester **11a** in the pure state (1.1 mg). Using (+)-(*S*)-MTPA chloride, the same procedure afforded (*R*)-MTPA diester **11b** in the same yield.

**(*S*)-MTPA 3,6-diester (11a):** amorphous solid; $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ 7.35 and 7.45 (MTPA phenyl protons); 5.87 (H-14a, bs); 5.71 (H-6, overlapped); 5.70 (H-14b, bs); 5.56 (H-3, dd, $J$ = 10.5, 7.0 Hz); 5.18 (H-5, d, $J$ = 10.2 Hz); 3.53 (MTPA OC$H_3$, s); 3.44 (H-2a, t, $J$ = 10.5 Hz); 2.86 (H-9a, ddd, $J$ = 13.0, 2.5, 1.5 Hz); 2.68 (H-2b, dd, $J$ = 10.5, 7.0 Hz); 1.87 (H-9b, ddd, $J$ = 13.0, 11.0, 3.0 Hz); 1.68 (H-8a, m); 1.53 ($H_3$-15, bs); 1.42 (H-11, m); 1.29 (H-8b, m); 0.90 ($H_3$-12, d, $J$ = 7.3 Hz); 0.90 ($H_3$-13, d, $J$ = 7.3 Hz); 0.89 (H-7, overlapped); FABMS (glycerol matrix, positive ions) *m/z* 685 $[M + H]^+$.

**(*R*)-MTPA 3,6-diester (11b):** amorphous solid; $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ 7.32 and 7.55 (MTPA phenyl protons); 5.87 (H-14a, bs); 5.74 (H-6, overlapped); 5.72 (H-14b, overlapped); 5.62 (H-3, dd, $J$ = 10.5, 7.0 Hz); 5.26 (H-5, d, $J$ = 10.2 Hz); 3.49 (MTPA OC$H_3$, s); 3.40 (H-2a, t, $J$ = 10.5 Hz); 2.90 (H-9a, ddd, $J$ = 13.0, 2.5, 1.5 Hz); 2.50 (H-2b, dd, $J$ = 10.5, 7.0 Hz); 1.90 (H-9b, ddd, $J$ = 13.0, 11.0, 3.0 Hz); 1.70 (H-8a, m); 1.61 ($H_3$-15, bs); 1.40 (H-11, m); 1.29 (H-8b, m); 0.88 ($H_3$-12, d, $J$ = 7.3 Hz); 0.88 ($H_3$-13, d, $J$ = 7.3 Hz); 0.82 (H-7, overlapped); FABMS (glycerol matrix, positive ions) *m/z* 685 $[M + H]^+$.

**Cytotoxic Activity.** Tests were performed on Caco-2 (human colon carcinoma) and peritoneal macrophages cell lines. Caco-2 cells were grown in Dulbecco's modified Eagle's medium and cultured at 37 °C in humidified 5% $CO_2$/95% air. Culture medium was supplemented with 10% fetal bovine serum, 25 mM HEPES, 100 U/mL penicillin, and 100 $\mu$g/mL streptomycin at 37 °C. Cells were plated onto 96-well culture plates (Falcon, Meylan, France) at a density of $1 \times 10^4$/well for 48 h. Resident pleural cell macrophages were collected from mice 4 days after an intraperitoneal injection of a 10% thioglycollate solution. The cells, which were mainly macrophages (~70%), were plated onto 96-well culture plates at a density of $1 \times 10^5$/well. Macrophages were allowed to adhere for 3 h in DMEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 25 mM HEPES, 100 U/mL penicillin, 100 $\mu$g/mL streptomycin, and 5 mM sodium pyruvate at 37 °C. Cell lines were treated with compounds **1**−**11** (0.1−10 $\mu$g/mL) for 24 h. Cytotoxic activity has been evaluated by the measurement of mitochondrial respiration on the two cell lines. Cellular respiration has been assessed by measuring the mitochondrial-dependent reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan. Cell lines in 96-well plates were incubated at 37 °C with MTT (5 mg/mL) for 3 h. Culture medium was removed by aspiration, and the cells were treated with DMSO (100 $\mu$L). The extent of reduction of MTT to formazan within cells was quantified by the measurement of optical density ($OD_{490}$) with a microplate spectrophotometer (Titertek Multiskan MCC/340) equipped with a 620 nm filter.

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