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

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Received April 7, 2005

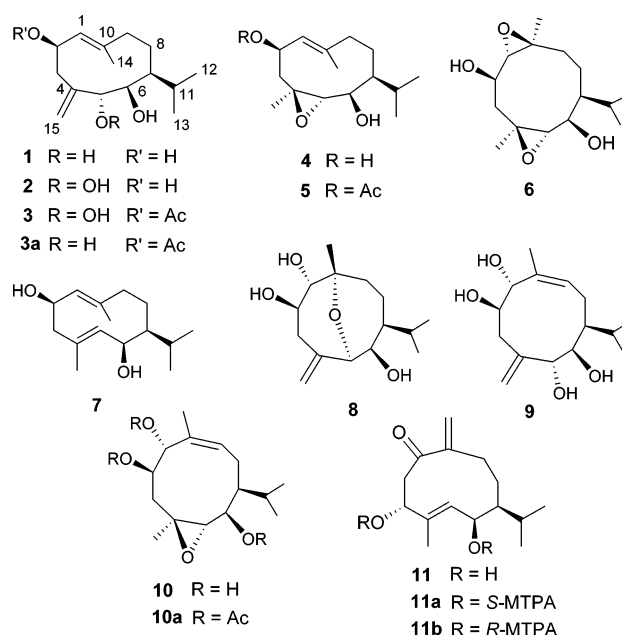
Chemical investigation of *Santolina insularis* afforded 11 germacrane sesquiterpenes (**1–11**), four of which (**2**, **3**, **10**, and **11**) are new. The stereostructures of these compounds have been established by a combination of spectroscopic techniques (mainly NMR), chemical transformations, 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 germacrane (**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 C₁₅H₂₆O₄, implying three unsaturation degrees, was assigned to compound **2** by HRMS. Analysis of ¹H and ¹³C NMR data of **2** (CDCl₃, Table 1), assisted by 2D HMQC experiment, indicated the presence of two double bonds, one trisubstituted (δ_C 121.6, δ_H 4.95; δ_C 139.7) and the other one geminally disubstituted (δ_C



117.5, δ_H 5.20 and 5.02; δ_C 138.8), and of three oxymethines (δ_C 69.5, 90.6, and 70.0; δ_H 4.59; 4.34, and 4.16, respectively). The remaining eight carbons were assigned to three methyls, three sp³ methylenes, and two additional sp³ methines. Therefore, **2** should be monocyclic.

The 2D ¹H–¹H 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 H₂-3 through an oxymethine at C-2 and the second one connecting H-5 to H₂-9 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, H₂-15 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 H₃-14 (δ_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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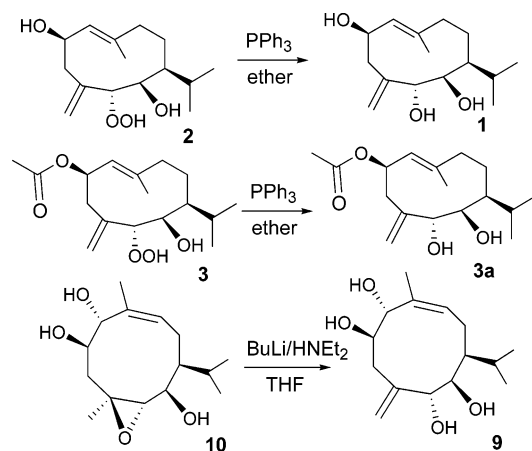
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Table 1. ^{13}C (125 MHz) and ^1H (500 MHz) NMR Data of Compounds **2** and **3**^a

pos.	2		3	
	δ_{C} (mult.)	δ_{H} (mult., J in Hz)	δ_{C} (mult.)	δ_{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 CDCl₃. ^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 (δ_{C} 90.6), leading us to draw the planar structure of compound **2**.

The structure of compound **3** ($\text{C}_{17}\text{H}_{28}\text{O}_5$ 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 $\text{C}_2\text{H}_2\text{O}$ unit) and confirmed by inspection of the ^1H (δ_{H} 2.05, singlet) and ^{13}C NMR spectra (δ_{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 (δ 5.53) located the acetyl at this carbon.

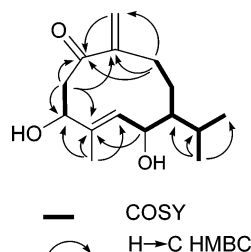
Stereochemical details for compounds **2** and **3** were assigned taking advantage of the easy conversion of a hydroperoxide group into the corresponding alcohol using triphenylphosphine in ether (Figure 1). In particular, reduction of **2** yielded a compound identical ($[\alpha]_{\text{D}}$ and NMR data) to **1**,⁵ a compound also present in extracts from *S. insularis*, while reduction of **3** afforded a compound identical ($[\alpha]_{\text{D}}$ 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 preceded in medium-sized olefins.⁸

The molecular formula $\text{C}_{15}\text{H}_{26}\text{O}_4$, implying three unsaturation degrees, was assigned to compound **10** on the basis of HREIMS analysis. The ^1H 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 δ 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 (δ_{H} 4.51 and 3.86) and a diastereotopic methylene, while the second spin system connects two mutually coupled oxygenated methines (δ_{H} 3.15 and 3.65) to the sp^2 methine H-9 (δ_{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 $^2J_{\text{CH}}$ correlations of methyl protons at C-14 and C-15 proving critical. Thus, H₃-14 correlated with the non-proton-bearing double-bond carbon at C-10 (δ_{C} 133.3) and with both C-1 (δ_{C} 70.7) and C-9 (δ_{C} 130.3), thus connecting the above two spin systems. A second link was established on the basis of $^3J_{\text{CH}}$ correlations of H₃-15 with both C-3 (δ_{C} 45.7) and C-5 (δ_{C} 64.7), indicating the presence of the germacrene macrocycle, while the $^2J_{\text{CH}}$ correlation with the signal resonating at δ 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 (δ 3.15) and confirmed by acetylation of **10**, which gave the triacetyl derivative **10a**.

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

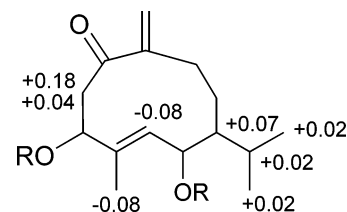
pos.	10		11	
	δ_{C} (mult.)	δ_{H} (mult., J in Hz)	δ_{C} (mult.)	δ_{H} (mult., J in Hz)
1	70.7 (CH)	4.51 (bd, 9.2)	200.2 (C)	
2a	67.8 (CH)	3.86 (m)	42.3 (CH_2)	3.32 (dd, 11.8, 11.0)
2b				2.54 (dd, 11.8, 7.5)
3a	45.7 (CH_2)	2.53 ^b	75.6 (CH)	4.48 (dd, 11.0, 7.5)
3b		1.30 (m)		
4	59.5 (C)		133.2 (C)	
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 $^{2,3}J_{\text{H}-\text{C}}$ HMBC correlations of compound **11**.

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 H₃-15.

The structure of the fourth new germacrene derivative **11** ($\text{C}_{15}\text{H}_{24}\text{O}_3$ by HREIMS) followed from interpretation of its ^1H 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 δ_{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 (δ_{H} 5.26) and one geminally disubstituted (δ_{H} 5.63 and 5.83, both broad singlets), while the remaining sp^2 carbon, resonating at δ_{C} 200.2, could be assigned to a conjugated ketone carbonyl, as suggested also by the IR absorption band at ν_{max} 1680 cm^{-1} .

Inspection of the 2D COSY spectrum of **11** revealed that one of the two oxymethines (δ_{H} 4.48) is coupled only with a deshielded methylene (δ_{H} 2.54 and 3.32), while the second oxymethine proton (δ_{H} 4.56) is coupled both with the double bond proton at δ_{H} 5.26 and with a methine proton at δ_{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 (δ_{H} 1.87 and 2.93) of the latter methylene showed HMBC correlations with the non-proton-bearing carbon C-10 (δ_{C} 150.6), with the sp^2 methylene carbon (δ_{C} 126.2), and with the ketone

**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.

carbon (C-1). The HMBC couplings of both C-1 and C-10 with the protons at C-2, and those of H₃-15 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 H₃-15, 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 germacrene 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 ($\text{IC}_{50} \geq 10 \mu\text{g/mL}$), compounds **8** and **10** proved to be potently active against the first cell line, with $\text{IC}_{50} = 1.0 \mu\text{g/mL}$ (3.7 μM) and 0.3 $\mu\text{g/mL}$ (1.1 μM), 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 anti-feedant *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 γ -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- γ -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 ($\lambda_{\text{max}} = 589 \text{ nm}$) 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. ^1H (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 : $\delta_{\text{H}} = 7.26$, $\delta_{\text{C}} = 77.0$). The multiplicities of ^{13}C NMR resonances were determined by DEPT experiments. One-bond heteronuclear ^1H – ^{13}C connectivities were determined with the HMQC experiment. Two- and three-bond ^1H – ^{13}C connectivities were determined by HMBC experiments optimized for a 2,3J 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 \times 10 mm and 250 \times 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 \times 2 L), and then extracted with acetone (4 \times 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.

(2R,5R,6R,7S)-Germacra-1(10E),4(15)-dien-5-hydroperoxy-2,6-diol (2): colorless amorphous solid; $[\alpha]_{\text{D}}^{25} -20.5^\circ$ (*c* 0.02, CHCl_3); IR (KBr) ν_{max} 3350, 1450, 1365 cm^{-1} ; ^1H (CDCl_3 , 500 MHz) and ^{13}C (CDCl_3 , 125 MHz) NMR spectra, Table 1; EIMS (70 eV) m/z 270 $[\text{M}]^+$ (5), 253 (20), 252 (40), 97 (100); HREIMS m/z 270.1868 (calcd for $\text{C}_{15}\text{H}_{26}\text{O}_4$, 270.1831).

(2R,5R,6R,7S)-Germacra-1(10E),4(15)-dien-5-hydroperoxy-2,6-diol-2-acetate (3): colorless amorphous solid; $[\alpha]_{\text{D}}^{25} -8.1^\circ$ (*c* 0.03, CHCl_3); IR (KBr) ν_{max} 3350, 1730, 1435, 1365 cm^{-1} ; ^1H (CDCl_3 , 500 MHz) and ^{13}C (CDCl_3 , 125 MHz) NMR spectra, Table 1; EIMS (70 eV) m/z 312 $[\text{M}]^+$ (15), 295 (20), 294 (40), 252 (40), 97 (100); HREIMS m/z 312.1949 (calcd for $\text{C}_{17}\text{H}_{28}\text{O}_5$, 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 μL) 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 \times 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).

(1R,2R,4S,5S,6R,7S)-4,5-Epoxygermacra-9Z-en-1,2,6-triol (10): colorless amorphous solid; $[\alpha]_{\text{D}}^{25} +4.0^\circ$ (*c* 0.08, CHCl_3); IR (KBr) ν_{max} 3480, 1450, 1360, 1120, 1070 cm^{-1} ; ^1H (500 MHz, CDCl_3) and ^{13}C (125 MHz, CDCl_3) NMR spectra, Table 2; EIMS (70 eV) m/z 270 $[\text{M}]^+$ (15), 252 (22), 236 (67), 125 (100); HREIMS m/z 270.1866 (calcd for $\text{C}_{15}\text{H}_{26}\text{O}_4$, 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; $[\alpha]_{\text{D}}^{25} +2.5^\circ$ (*c* 0.02, MeOH); ^1H NMR (500 MHz, CDCl_3) δ 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 (OCOCH_3), 2.01 (OCOCH_3), 2.00 (H-11, overlapped), 1.99 (OCOCH_3), 1.75 (H-14, bs), 1.70 (H-7, m), 1.43 (H-3b, m), 1.37 (H-15, s), 1.09 (H-12, d, $J = 7.3$ Hz), 0.99 (H-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 μ 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**.

(3R,6R,7S)-3,6-Dihydroxygermacra-4(5)E,10(14)-dien-1-one (11): colorless amorphous solid; $[\alpha]_D^{25}$ -22.0° (c 0.02, CHCl₃); IR (KBr) ν_{\max} 3610, 1680, 1635 cm⁻¹; ¹H (500 MHz, CDCl₃) and ¹³C (125 MHz, CDCl₃) NMR spectra, Table 2; EIMS (70 eV) *m/z* 252 [M]⁺ (55), 210 (40), 109 (100); HREIMS *m/z* 252.1750 (calcd for C₁₅H₂₄O₃, *m/z* 252.1725).

Preparation of MTPA Ester Derivatives of Compound 11. Compound **11** (1.0 mg) was dissolved in 500 μ L of dry pyridine, treated with an excess of (–)-(*R*)-MTPA chloride (20 μ 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, CDCl₃) δ 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 OCH₃, 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 (H₃-15, bs); 1.42 (H-11, m); 1.29 (H-8b, m); 0.90 (H₃-12, d, *J* = 7.3 Hz); 0.90 (H₃-13, 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, CDCl₃) δ 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 OCH₃, 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 (H₃-15, bs); 1.40 (H-11, m); 1.29 (H-8b, m); 0.88 (H₃-12, d, *J* = 7.3 Hz); 0.88 (H₃-13, 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% CO₂/95% air. Culture medium was supplemented with 10% fetal bovine serum, 25 mM HEPES, 100 U/mL penicillin, and 100 μ g/mL streptomycin at 37 °C. Cells were plated onto 96-well culture plates (Falcon, Meylan, France) at a density of 1 \times 10⁴/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 10⁵/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 μ g/mL streptomycin, and 5 mM sodium pyruvate at 37 °C. Cell lines were treated with compounds **1–11** (0.1–10 μ 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 μ L). The extent of reduction of MTT to formazan within cells was quantified by the measurement of optical density (OD₄₉₀) with a microplate spectrophotometer (Titertek Multiskan MCC/340) equipped with a 620 nm filter.

Acknowledgment. This work was supported by MIUR (Progetto Sostanze Naturali ed Analoghi Sintetici ad Attività Antitumorale). Mass and NMR spectra were recorded at the "Centro di Ricerca Interdipartimentale di Analisi Strumentale" of the University of Naples "Federico II". The assistance of the staff is gratefully acknowledged.

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NP050120Q