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Bioactive sesquiterpenes from *Santolina rosmarinifolia* subsp. *Canescens*. A conformational analysis of the germacrane ring

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Abstract

The hexane extract of aerial parts of *Santolina rosmarinifolia* subsp. *canescens* afforded eight new sesquiterpenes in addition to known compounds. Their structures were determined by spectroscopic methods and chemical transformations. The conformational analysis of the germacrane constituents was carried out by spectroscopic methods, including NMR at varying temperature and by molecular mechanics calculations. The antifeedant, antibacterial and antitumoral activity of selected compounds has been tested. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Santolina rosmarinifolia* subsp. *canescens*; Compositae; Sesquiterpenes; Conformational study; Biological activities; Antifeedants

1. Introduction

As part of an ongoing chemical study on plants from the genus *Santolina* (Barrero, Sánchez, & Arana, 1988; Barrero, Herrador, Molina Molina, Quílez, & Quiros, 1994; Barrero, Alvarez-Manzaneda, Quílez, & Herrador, 1998) (Fam. *Compositae*, tribe *Anthemideae*) growing in Southern of Spain and used in folk medicine, we investigated an hexane extract from the aerial parts of *Santolina rosmarinifolia* subsp. *canescens*. 33 compounds were identified, including 8 new sesquiterpenes, 6 of which with a germacrane skeleton. We report here their structure elucidation and conformational analysis. Selected constituents have also been investigated for their antifeedant, antibacterial and antitumoral activity.

2. Results and discussion

Compounds **7** and **8** were dereplicated by comparison of their spectroscopic data with those reported in the literature (Wada, Enomoto, & Munakata, 1970; Stahl-Biskup & Laakso, 1990). In chloroform solution at 25°C, **7** and **8** are a mixture of interconvertible conformers, as shown by the broad shape of several ^{1}H NMR signals, and the poor resolution of the ^{13}C NMR spectra. A conformational study was carried out varying the temperature. The ^{1}H NMR spectra at −30°C showed two groups of signals in a 1:1 ratio for **7** and 3:1 ratio for **8**. The values of J_{5-6} and J_{6-7} for the conformers of **7** are similar ($J_{5-6}=7.0$ Hz and

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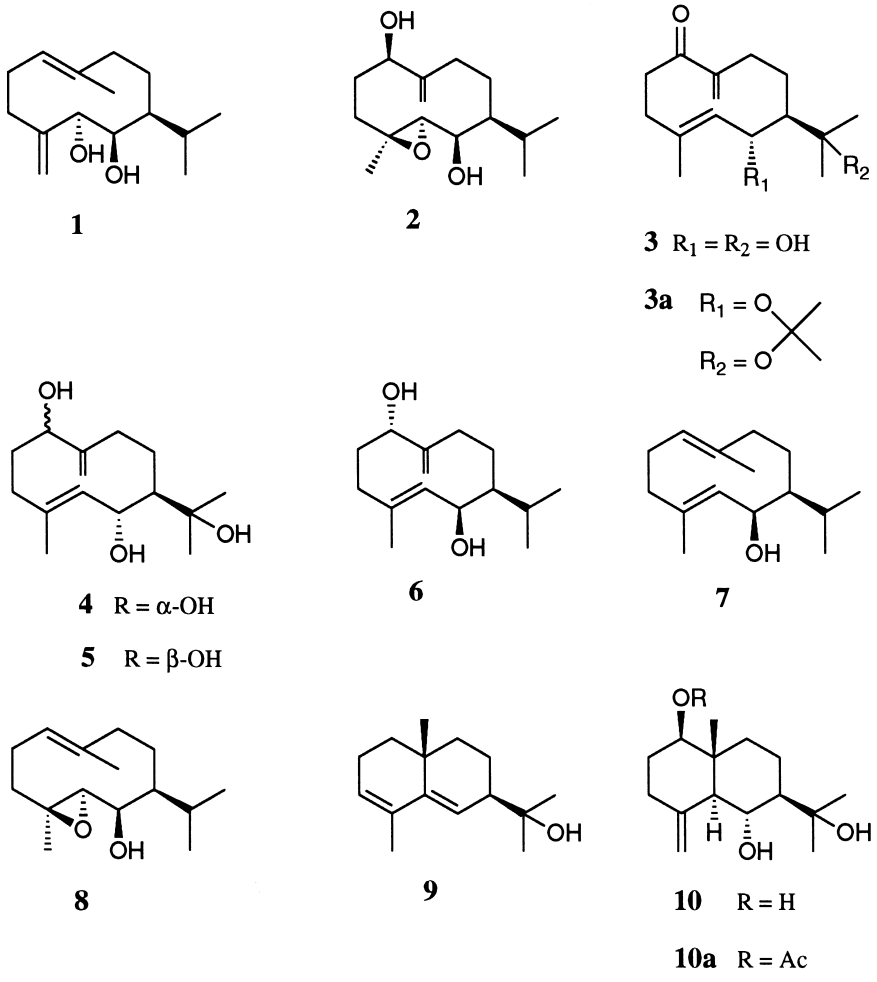


$J_{6-7}=0.0$ Hz), showing an *anti* arrangement between H-5 and H-6 and an orthogonal one between H-6 and H-7. This suggests that bonds C-3–C-4–C-5–C-6–C-7 remain unchanged in both conformers and that the conformational equilibrium involves inversion of the endocyclic double bond 1–10 across the ring. This is in accordance with the observation that the value of J_{5-6} was not affected by heating to 57°C and the multiplicity presented for H-1 (t, $J=7.0$ Hz) to that temperature. Therefore **7** presents a conformational equilibrium between conformations 'C/α,α/N' and 'P/β,α/N' (Ugliengo, Appendino, Chiari, & Viterbo, 1990) (Fig. 1) which are analogous to those described for shiromodiol (Appendino & Tettamazi, 1990).

Compound **8** showed a similar conformational behaviour.

The IR spectrum of **1** showed absorption bands due to a hydroxyl group (3366 cm^{-1}) and terminal exocyclic and trisubstituted double bonds (3076, 1663, 1645, 902 and 842 cm^{-1}). Its mass spectrum showed $[M]^+$ at *m/z* 238, which together with the ^1H NMR and ^{13}C NMR data allowed a molecular formula $C_{15}H_{26}O_2$ to be established. NMR spectra carried out at 25°C presented poor resolution owing to the existence of a conformational equilibrium. Heating at 57°C sharpened the signals and enabled their analysis. The main difference between the spectra of **1** and **8** is the presence in **1** of an exocyclic methylene (δ 4.79 and

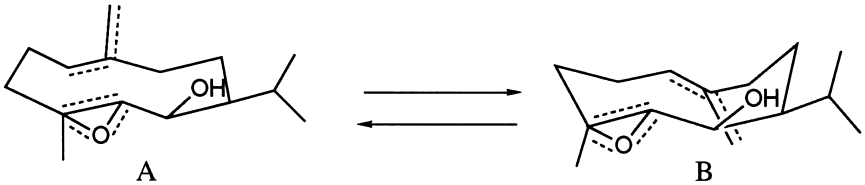
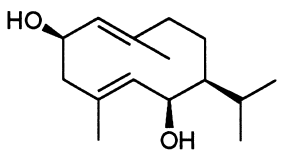


Fig. 1. Conformational equilibrium of **1**, **7** and **8**: (A) 'P/β,α/N' conformation; (B) 'C/α,α/N' conformation.



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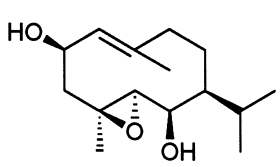

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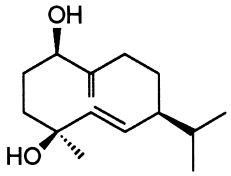

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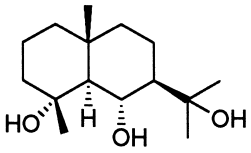

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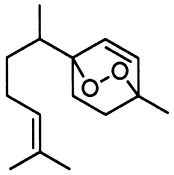

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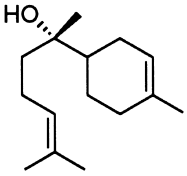

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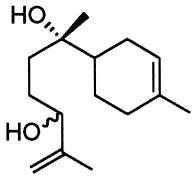

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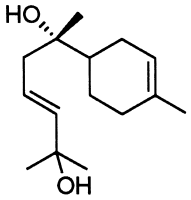

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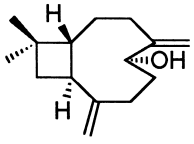

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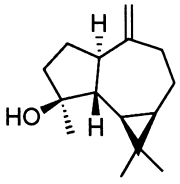

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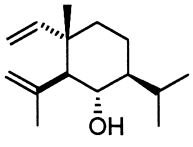

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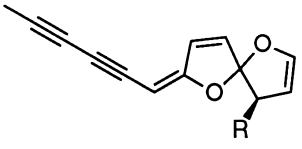
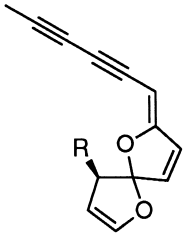


25 R = H
26 R = OH
27 R = OAc



28 R = H
29 R = OAc

4.75) and an hydroxylated allylic methine (δ 3.78, d, $J = 9.8$ Hz) replacing the epoxy group and the methyl on oxygenated carbon in **8**. The relative stereochemistry of C-5, C-6 and C-7 was assessed by an analysis of the vicinal interproton couplings. Thus, J_{5-6} (9.9 Hz) points to an *anti* type-coplanar arrangement between H-5 and H-6, while the value of $J_{6-7} = 0$ Hz indicates an orthogonal arrangement of the dihedral angle H-6–C-6–C-7–H-7, as observed in **8**. The structure and stereochemistry of **1** was confirmed by chemical correlation with **8** (isomerization with Et_2NH and *n*BuLi in THF).

The mass spectrum of **2** showed $[M]^+$ at *m/z* 254 corresponding to the molecular formula $C_{15}H_{26}O_3$. The IR spectrum showed absorptions due to a hydroxyl group (3340 cm^{-1}) and one terminal exocyclic double bond (3075, 1640 and 902 cm^{-1}). The unambiguous assignment of the ^1H and ^{13}C NMR signals by COSY and HETCOR experiments confirmed the structural relationship between **2** and **8**, which differed for

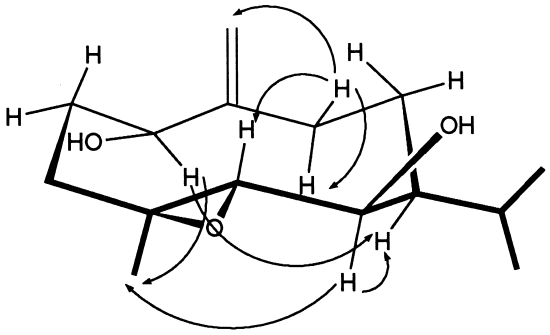


Fig. 2. NOE effects observed in **2** and major stable conformation ('P/β,α/N').

the oxidation of the double bond of **8**. The stereochemistry at C-1 and the preferred conformation by the molecule ('P/β,α/N') followed from considerations of NOE effects (NOE diff. experiments) (Fig. 2). The oxidation of **8** by singlet oxygen in *i*PrOH, followed by reduction with $NaBH_4$ of the corresponding hydroperoxide yielded **2**, confirmed structure and stereochemistry of this compound. The stereoselectivity of the reaction implies attack by oxygen on the external face of the double bond C-1–C-10 in the 'P/β,α/N'-conformation of **8**.

Compound **3** was isolated as its acetonide derivative **3a**. The mass spectrum of **3a** showed $[M]^+$ at *m/z* 292. This, together with the ^1H and ^{13}C NMR data, led to molecular formula $C_{18}H_{30}O_3$. Its IR spectrum had absorption bands of α,β-unsaturated ketone (1680 cm^{-1}) and double bond (3070 and 1638 cm^{-1}). Its ^1H NMR spectrum revealed, in addition to signals due to exocyclic methylene group conjugated with ketone at δ 5.62 (1H, d, $J=0.7$ Hz) and 5.67 (1H, s) and a proton on a trisubstituted double bond at δ 5.09 (1H, d, $J=9.6$ Hz), signals corresponding to a proton on an oxygenated carbon at δ 4.22 (1H, t, $J=9.7$ Hz) and to five methyls: two of these were due to the protective group (δ 1.36 and 1.48), two were bound to an oxygenated carbon (δ 1.23 and 1.29), and the last one was an allylic methyl at δ 1.68 (d, $J=0.9$ Hz). The combined analysis of the COSY and HETCOR spectra identified three spin systems, then assembled on the basis of the correlations observed in COLOC experiments.

The relative configuration at C-6 and C-7 was assigned on the basis of the observed values of $J_{5-6}=J_{6-7}=9.7$ Hz, which indicate a double *anti* arrangement of H-6 with respect to H-5 and H-7. NOESY experiment established a 'P/α,β/N' conformation for **3a**, with the most significant correlations being shown in Fig. 3.

The hydrolysis of **3a** with *p*-TsOH yielded the natural product **3**, whose spectroscopic data (see Section 3) are in agreement with the proposed structure.

The IR spectrum of **4** showed absorption bands due to a hydroxyl group (3333 cm^{-1}) and to trisubstituted and terminal exocyclic double bonds (3078, 1660, 1638, 909 and 825 cm^{-1}). The mass spectrum featured $[M]^+$ at *m/z* 254 corresponding to the molecular formula $C_{15}H_{26}O_3$. The NMR spectra of **3** and **4** were remarkably similar, except for the presence in **3** of a hydroxylated methine (^1H NMR: δ 3.83, dd, $J_1=9.7$ Hz, $J_2=4.6$ Hz; ^{13}C NMR: δ 72.6) which replaced the ketone group of **3**. The β orientation of H-1 was suggested by NOE experiments, showing correlations between H-1 and H-8β, H-15 and H-6 and H-8β, H-15. These NOE effects also established a 'P/α,β/N' conformation for **4**.

Compound **5** was isolated as an unseparable mixture with **4**. The ^1H NMR spectrum of the mixture indicated that **4** and **5** were stereoisomers, differing for the configuration at C-1. Thus, the major differences were the chemical shift and multiplicity of the H-1 signal (δ 3.89, d, $J=7.9$ Hz versus 3.87, dd, $J_1=9.4$ Hz, $J_2=4.2$ Hz for **4**). Reduction of **3** with $NaBH_4$ yielded a 3:2 mixture of **5** and **4**, confirming the structural assignment of the two natural products.

Compound **6** showed $[M]^+$ at *m/z* 258 corresponding to the molecular formula $C_{15}H_{26}O_2$. The ^{13}C NMR spectrum showed the signals of three methyl groups, five methylene groups (one olefinic), five methine groups (two oxygenated and one olefinic) and two olefinic quaternary carbons. This pattern of carbon multiplicities is in accordance with the presence of a germacradiene skeleton and showed a great similarity with that of **11** (Sanz, Garcia-Sarrión, & Marco, 1991), the major difference being the presence in **6** of an exocyclic methylene group (δ 112.3) in place of one of the trisubstituted double bonds of **11**. The ^1H NMR spectrum of **6** (see Section 3) confirmed its structure. The relative configuration at C-6 and C-7 shown in **6** was determined on the basis of the values of J_{5-6} (6.4 Hz) and J_{6-7} (0 Hz). NOE difference experiments confirmed the location of the hydroxyl group at C-1 and the assignment of its configuration and established the preferred conformation in solution ('C/α,α/N'-type).

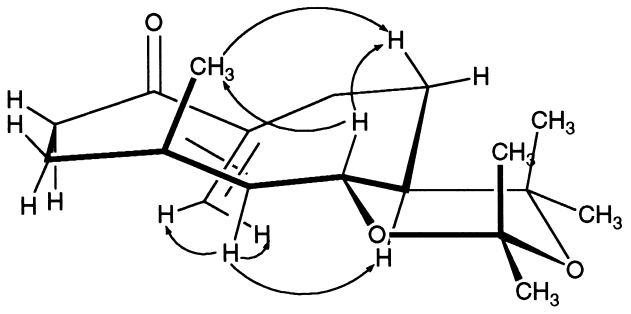


Fig. 3. NOESY correlations for **3a** and major stable conformation ('P/α,β/N').

The conformational analysis of the germacranes **1**, **2**, **3a**, **4**–**8** was complemented by molecular mechanics calculations (MMX forcefield). The results are shown in Fig. 4 and in Table 1–3.

Table 1 shows the values of the steric energy and population for different conformations of the ring and of the side chain of the compounds **1**, **2**, **3a**, **4**–**8**, which represent over 1% of the conformational mixture. A close examination of Table 1 reveals that compounds **2** and **6** are ananchomeric (conformational types 'P/β,α/N' and 'C/α,α/N', respectively). Compounds **4**, **5** and **7** display instead several ring conformations, one of which, however, makes up for more than 90% of the population ('P/α,β/N', 'C/β,β/N' and 'C/α,α/N', respectively). For compounds **1**, **3a** and **8**, the major rotamer ('C/α,α/N', 'P/α,β/N' and 'P/β,α/N', respectively) made up any 70% of the mixture. These results are in line with those obtained experimentally, except for compound **7**. For this compound molecular mechanics calculations indicate a major conformation ('C/α,α/N', 93.6%) at equilibrium, whereas a 1:1 mixture of rotamers was observed experimentally.

Furthermore, all compounds, except **3a**, presented several conformations of the side chains. In this context, compound **3a** is ananchomeric because of the conformational constraint imposed by the dioxolane ring. On the other hand, this is the only compound in which three ring conformations with significant populations 'C/β,β/N', 'P/β,β/N' and 'P/α,β/N', were calculated. Conformation 'P/β,β/N' appears distorted, as may be seen (Table 2) from the value of the angles around C-1–C-10 and C-5–C-4, which corresponds to a relatively high value (14.7°). Moreover, the exocyclic methylene on C-10, though identified as β, is located slightly above the middle plane of the ring.

The isopropyl group on C-7 tends to adopt a pseudoequatorial position due to its bulk. All the ring conformations obtained present this orientation, as is shown by the negative value of the torsion angle around C-7–C-8 (see Tables 1 and 2).

Table 3 displays the theoretical coupling constants calculated from the whole of the conformational mixt., obtained for each compound. A good agreement with the experimental data was observed.

Some general trends emerged: in compounds with a *cis* orientation between H-6 and H-7 a conformational equilibrium between the 'P/β,α/N' and the 'C/α,α/N' conformation is present. Conversely, in compounds with a *trans* orientation between these protons a conformational equilibrium of the 'P/α,β/N' ⇄ 'C/β,β/N' is present. In 1,6-dihydroxy-germacra-4,10(14)-dienes the configuration of C-1 determines the conformation of the rest of the molecule notwithstanding the configuration at C-6.

In addition to the germacranes **1**–**8**, the eudesmanes **9** and **10** were also isolated.

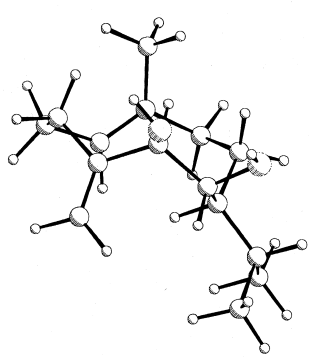
The mass spectrum of **9** ($[M]^+$ at m/z 220) suggested a molecular formula $C_{15}H_{24}O$. The IR, ^1H and ^{13}C NMR spectra (see Section 3) showed the presence of a conjugated diene system and a 2-hydroxy-2-propyl group. On the basis of these data, the structure of eudesma-3,5-dien-11-ol was established for **9**. The relative stereochemistry at C-7 and C-10 was established by analogy with that of other eudesmanes (cryptomeridiol, pigmol and **10** (see infra)) isolated from the plant in study.

Compound **10** was isolated as its monoacetate derivative **10a**. The mass spectrum did not reveal $[M]^+$, but showed the highest peak at m/z 281 corresponding to the loss of methyl group $[M–CH_3]^+$. Its IR spectrum showed absortion bands due to a hydroxyl group (3370 cm^{-1}), an acetate group (1731 and 1243 cm^{-1}) and a terminal exocyclic double bond (3072, 1648 and 905 cm^{-1}). In addition to an acetate methyl, the ^1H NMR spectrum revealed the presence of three additional methyl singlets (δ 0.77, 1.19 and 1.25), together with the signals of a hydroxymethine at δ 4.02 (t, $J=9.8$ Hz), an acyloxymethine at δ 4.66 (dd, $J_1=11.6$ Hz, $J_2=4.8$ Hz) and an exocyclic olefinic methylene at δ 4.78 and 5.04. These data suggested that **10** was a β-eudesmol derivative bearing a hydroxyl and an acetoxyl. The ^{13}C NMR spectrum (see Section 3) supported this proposal. The multiplicity and the value of coupling constants of the hydroxymethine located the hydroxyl group at C-6 (δ 4.02, t, $J=9.8$ Hz). The acetoxyl group could instead be located either at C-1 or at C-9. The location on C-1 being chosen on the basis of HMBC correlations (H-1/C-2; H-1/C9 and H-1/C-14).

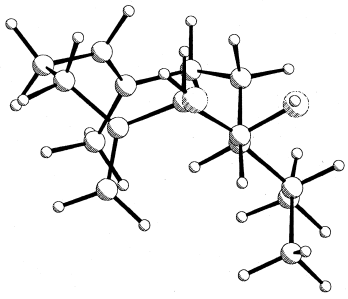
Compared to two previous studies (Barrero et al., 1988; Maqua et al., 1988), our results expand considerably the portfolio of constituents of *S. rosmarinifolia* subsp. *canescens*.

The antimicrobial activity of the sesquiterpenes **2**, **8**, **10**, **12**, **15**, **17**, **18**, **21**, **23** and the polyacetylenes **25**–**29** was investigated against several gram-positive (*Enterococcus faecalis* OGIX, *Bacillus subtilis* CECT 397 and *Staphylococcus aureus* ATCC 8), gram-negative bacteria (*Salmonella tiphymurium* LT 2, *Escherichia coli* U 9 and *Proteus* sp) and yeasts (*Sacharomyces cerevisiae* S 3, *Candida albicans* CECT 1394 and *Cryptococcus neoformans* CECT 1075) was studied. Only compounds **17** and **21** showed significant activity against yeasts (MIC = 6.25 and 12.5 μg/ml, respectively). None of the compounds tested showed significant activity against *Gram*-positive and *Gram*-negative bacteria.

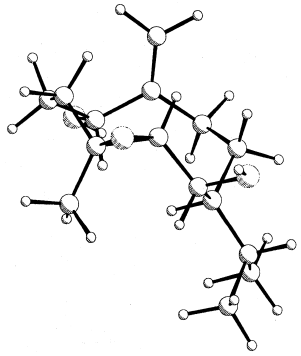
The cytotoxicity of the sesquiterpenes **3**, **7**–**10**, **12**, **13**, **15**, **18**–**21**, **23**, **24** and the polyacetylenes **25**, **27**–**29** was tested against the cell lines P-388 (murine leukemia), A-549 (human non-small cell lung cancer), HT-29 (human colon cancer) and MEL-28 (human mela-



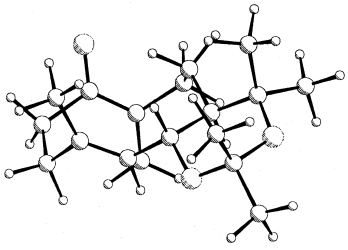
1 "P/β,α/N" (28.6%)



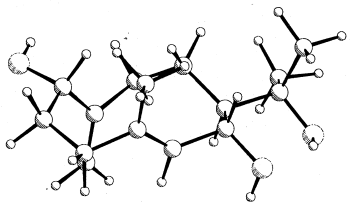
1 "C/α,α/N" (71.0%)



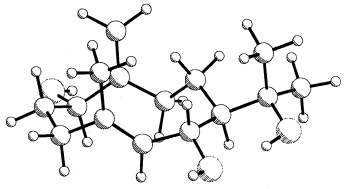
2 "P/β,α/N" (99.9%)



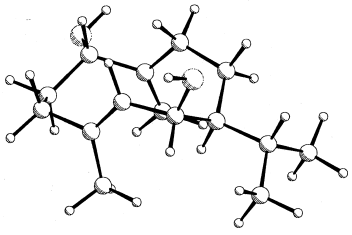
3a "P/α,β/N" (72.9%)



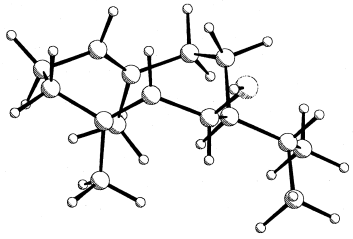
4 "P/α,β/N" (96.7%)



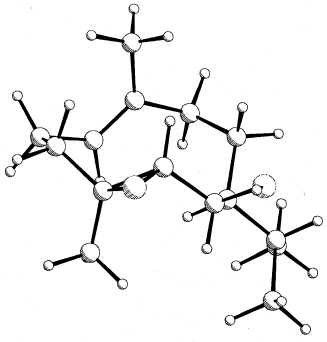
5 "C/β,β/N" (97.6%)



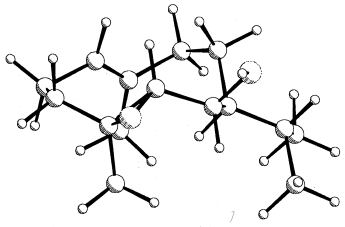
6 "C/β,β/N" (99.8%)



7 "C/α,α/N" (90.7%)



8 "P/β,α/N" (74.5%)



8"C/α,α/N" (25.3%)

Fig. 4. Most stable conformations in equilibrium of **1**, **2**, **3a** and **4**–**8** obtained by molecular mechanics calculations.

Table 1
Steric energy (kcal/mol) and population of the conformers which are over 1% in the conformational mixture

Compound	Conformer	Energy	Population (%)
1	P/β,α/N (1)	25.64	18.8
	P/β,α/N (2)	26.27	6.5
	P/β,α/N (3)	26.67	3.3
	C/α,α/N (1)	26.69	3.2
	C/α,α/N (2)	25.06	50.0
	C/α,α/N (3)	25.67	17.8
2	P/β,α/N (1)	27.21	4.3
	P/β,α/N (2)	26.30	20.2
	P/β,α/N (3)	25.64	61.7
	P/β,α/N (4)	26.53	13.7
3a	C/β,β/N	48.57	12.0
	P/β,β/N	48.48	15.1
	P/α,β/N	47.5	72.9
4	C/β,β/N	27.38	2.5
	P/α,β/N (1)	26.69	7.9
	P/α,β/N (2)	25.53	58.2
	P/α,β/N (3)	25.89	30.6
5	C/β,β/N (1)	26.54	5.4
	C/β,β/N (2)	26.21	9.4
	C/β,β/N (3)	25.06	70.0
	C/β,β/N (4)	26.65	4.9
	C/β,β/N (5)	27.16	1.9
	P/α,β/N	26.51	5.8
6	C/α,α/N (1)	25.04	15.9
	C/α,α/N (2)	26.23	2.1
	C/α,α/N (3)	24.06	81.8
7	C/α,α/N (1)	28.39	4.2
	C/α,α/N (2)	26.97	49.2
	C/α,α/N (3)	27.74	12.5
	C/α,α/N (4)	27.37	23.4
	C/α,α/N (5)	29.02	1.4
	P/β,α/N (1)	28.84	2.0
	P/β,α/N (2)	28.30	4.4
8	P/β,α/N (1)	23.70	18.1
	P/β,α/N (2)	23.78	15.8
	P/β,α/N (3)	23.35	32.7
	P/β,α/N (4)	24.19	7.9
	C/α,α/N (1)	25.17	1.6
	C/α,α/N (2)	23.54	23.7

noma). Compounds **9**, **10**, **12** and **20** showed significant cytotoxic activity ($IC_{50} \leqslant 5$ μg/ml) against the four cell lines tested. Of these, **12** was the most potent showing selectivity for the human cell lines ($IC_{50} = 0.25$ μg/ml).

The antifeedant activity of the sesquiterpenes **2**, **3a**, **7, 8**, **11**–**13** and the polyacetylenes **27**, **29** was tested against *Spodoptera littoralis* (final larvae stadium). Compounds **2**, **8** and **29** showed significant antifeedant activity at 100 ppm (AI = 46 for **2** and **29**, 45 for **8**), while **13** showed significant phagostimulatory activity at that concentration (AI = −67). An analysis of the results obtained from the antifeedant activity of these compounds suggests some preliminary structure–activity relationships. Thus, the presence of an epoxide group at C-4, C-5 increases the activity of compounds with a germacrane skeleton (**7** (AI = 12) versus **8** (AI = 45)). The introduction of a second hydroxyl group has a variable influence, generally leading to a decrease of activity; thus, the mixture **6** + **11** (AI = −2) showed slight phagostimulatory activity, while **2** (AI = 46) had an activity similar to that of **8**. A comparison of the antifeedant activity of the polyacetylenes **27** (AI = 29) and **29** (AI = 46) showed the influence of geometric stereoisomerism on the activity of these types of compounds, since the *Z* stereoisomer was the most active one.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured on a 141 Perkin-Elmer polarimeter. IR spectra were recorded on a 983 G Perkin-Elmer spectrometer. MS were determined on a 5988 A Hewlett-Packard mass spectrometer. NMR spectra were recorded on a Bruker AM 300, a Bruker ARX 400 spectrometers and a Bruker AMX 400 spectrometer with an inverse detection probe broadband and a variable temp. unit B-VT 200 (δ values given in ppm relative to internal TMS and J values in Hz). GC–MS analyses were carried out in a Hewlett-Packard 5890 A using an ionization voltage of 70 eV. The GC conditions were: HP-1 capillary column (25 m × 0.32 mm) packed with methyl silicone, temp. programmed from 120 to 220°C at 5°C min^{-1}, 220 to 280°C at 3°C min^{-1} and 10 min hold at 280°C, injector temp. 260°C, detector temp. 180°C, He at 25 ml min^{-1}. HPLC was done on a Spherisorb ODS-2® column (250 × 10 mm) elution with CH_3CN–MeOH and CH_3CN–MeOH–H_2O mixts. Column chromatographies were carried out using silica gel Merck 60 (70–230 mesh), eluting with mixts hexane/*t*-butylmethyl ether/ethyl acetate/methanol of increasing polarity. Analytical TLC was performed on layers of silica gel Merck 60G 0.25 mm thick, using a 7% phosphomolybdic acid soln (EtOH) to visualize the spots.

3.2. Plant material

Santolina rosmarinifolia subsp. *canescens* was collected in the Fuente del Hervidero and the collado de la Sabina (Sierra Nevada, Granada, Spain) in June 1994 and identified by Professor G. Blanca (Department of Plant Biology, University of

Table 2
Selected torsion angles for the compounds **1**–**8**. φ_1 = C-1–C-2–C-3–C-4; φ_2 = C-2–C-3–C-4–C-5; φ_3 = C-3–C-4–C-5–C-6; φ_4 = C-4–C-5–C-6–C-7; φ_5 = C-5–C-6–C-7–C-8; φ_6 = C-6–C-7–C-8–C-9; φ_7 = C-7–C-8–C-9–C-10; φ_8 = C-8–C-9–C-10–C-1; φ_9 = C-9–C-10–C-1–C-2; φ_{10} = C-10–C-1–C-2–C-3; φ_{11} = C-1–C-10–C-5–C-4

Comp.	Conf.	φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_7	φ_8	φ_9	φ_{10}	φ_{11}
1	PβαN	−39.4	107.9	−143.8	63.5	66.5	−121.6	70.7	91.2	169.7	108.8	−12.3
	CααN	−47.3	101.2	−148.7	58.1	62.2	−130.6	51.5	61.3	−169.6	112.9	70.9
2	PβαN	−44.8	100.7	−150.8	84.0	56.7	−115.7	75.4	−100.4	153.3	−52.3	−17.2
3a	CββN	62.7	−103.3	174.7	−113.4	55.0	−88.3	123.6	−127.9	125.8	−9.1	−64.9
	PββN	−47.3	−64.2	169.9	−109.1	48.9	−95.8	142.3	−69.0	−58.5	126.9	−14.7
	PαβN	34.2	−104.1	171.6	111.2	57.3	−100.8	82.1	62.9	−151.0	52.7	9.9
4	CββN	63.7	−112.2	176.1	−95.8	40.1	−80.1	140.9	−135.5	104.4	−74.7	−63.9
	PαβN	31.4	103.0	172.7	−112.0	57.8	−108.7	78.3	70.7	−148.0	4.97	7.63
5	CββN	63.6	−113.2	175.2	−99.5	46.5	−83.6	139.3	−137.5	107.8	−75.0	−64.5
	PαβN	81.2	−94.9	168.8	−127.6	65.4	−44.5	−58.2	164.7	−51.7	−54.6	−26.5
6	CααN	60.4	101.2	−172.9	67.2	62.8	−54.4	−54.1	152.8	−124.5	72.6	62.2
7	CααN	−42.8	92.8	−168.8	77.9	54.8	−122.7	55.6	62.1	−168.9	108.3	70.1
	PβαN	−29.4	96.4	−113.9	83.2	60.5	−110.6	19.6	69.2	169.7	165.2	−12.2
8	PβαN	−37.5	92.5	−151.4	86.5	56.6	−112.9	72.6	−92.2	172.2	−74.2	−16.8
	CααN	−42.8	90.3	−153.2	75.7	55.5	−126.5	52.3	60.2	−171.0	117.0	67.2

Granada). A voucher specimen (GDAC 40148 and GDAC 40149) is available at the herbarium of the Faculty of Sciences of the University of Granada.

3.3. Extraction and isolation

The air dried and powdered aerial parts (1.2 kg) of *Santolina rosmarinifolia* subsp. *canescens* were extracted in a Soxhlet with hexane for 12 h. The soln was allowed to reach room temp. Removal of the solvent under vacuum gave 55 g of residue. The residue was dissolved in $CHCl_3$ (125 ml). This mixt. was slowly added to 600 ml of MeOH at 50°C and allowed to cool to room temp. The mixt. was then further cooled at −10°C for 24 h, yielding an insoluble fraction (18 g). The defatted hexane extract (18 g) was then purified by CC (hexane/*t*-butylmethyl ether/ethyl acetate/methanol mixts of increasing polarity). Six main fractions were collected (A–F). Fraction A (hexane/*t*-butylmethyl ether 7:3) was saponified (5% KOH–MeOH) and subjected to repeated separations with silica gel chromatography (hexane/*t*-butylmethyl ether mixts of increasing polarity) and HPLC (CH_3CN–MeOH 4:1), affording ar-curcumene (**16**) (48 mg) (Hall, McEnroe, & Shue, 1975), (*E*)-7-(2,4-hexadiynilidene)-1,6-dioxaspiro[4.4]nona-2, 8-diene (**25**) (688 mg) (Bohlmann & Zdero, 1968), 3,6-epidioxybisabola-1,10-diene (**17**) (140 mg) (Metwally & Dawidar, 1986), caryophyllene oxide (**22**) (76 mg) (Barrero et al., 1995), (*Z*)-7-(2,4-hexadiynilidene)-1,6-dioxaspiro[4.4]-nona-2,8-diene (**28**) (116 mg) (Bohlmann & Zdero, 1968), cycloartenol (**30**) (88 mg) (Milon, Nakatani, Kintzinger, & Ourisson, 1989), (−)-α-bisabolol (**18**) (552 mg) (Bohlmann, Jakupovic, Ahmed, & Schuster, 1983), (*E*)-7αH-germacra-1(10),4-dien-6β-ol (**7**) (86 mg) (Stahl-Biskup & Laakso, 1990), spathulenol (**23**) (342 mg) (Krebs, Rakotoarimanga, & Habermehl, 1990), shiromool (**8**) (162 mg) (Wada et al., 1970), 20-taraxastene-3β,16β-diol (**31**) (32 mg) (Pyrek & Baranowska, 1973), 20(29)-lupene-3β,16β-diol (**32**) (24 mg) (Wenkert, Baddeley, Burfitt, & Moreno, 1978), 20(30)-taraxastene-3β,16β-diol (**33**) (54 mg) (Pyrek & Baranowska, 1973), (*E*)-4-acetoxi-7-(2,4-hexadiynilidene)-1,6-dioxaspiro[4.4]nona-2,8-diene (**27**) (6172 mg)

Table 3
Calculated coupling constants for compounds **1**–**8**

Comp.	$J_{1,2}$		$J_{2,3}$				$J_{5,6}$	$J_{6,7}$	$J_{7,8}$		$J_{8,9}$			
1	11.3	4.5	11.3	5.1	5.1	1.8	9.7	1.4	3.1	6.8	12.3	3.0	4.0	3.1
2	5.3	10.0	11.5	5.6	5.4	2.2	7.3	1.8	4.7	4.6	12.5	2.1	2.7	5.4
3a	–	–	9.6	5.6	5.4	2.9	9.3	10.5	2.7	7.3	7.0	3.2	3.6	9.7
4	9.4	5.6	1.7	6.7	6.8	9.9	9.3	10.6	6.4	3.3	12.7	2.4	2.2	5.9
5	3.9	10.7	3.5	3.7	3.3	12.4	9.2	10.7	11.9	3.2	9.7	3.2	2.4	7.7
6	10.8	3.4	12.3	3.6	4.1	3.2	6.2	1.1	11.0	4.2	4.7	4.4	4.3	10.7
7	11.8	5.4	11.0	5.3	4.7	1.6	4.6	1.6	3.8	5.8	12.3	3.2	4.0	3.2
8	10.0	3.9	7.7	9.1	2.8	4.6	7.6	1.9	4.6	4.8	5.7	4.4	9.7	2.3

(Maqua et al., 1988), (*Z*)-4-acetoxi-7-(2,4-hexadiynilidene)-1,6-dioxaspiro[4.4]nona-2,8-diene (**29**) (2762 mg) (Maqua et al., 1988), **1** (22 mg), bisabola-2,11-diene-7α,10ζ-diol (**19**) (30 mg) (Barrero, Alvarez-Manzaneda, & Alvarez-Manzaneda, 1990) and caryophyllodienol (**21**) (430 mg) (Maqua et al., 1988). Fraction B (hexane/*t*-butylmethyl ether 1:1) was rechromatographed with hexane and increasing amounts of *t*-butylmethyl ether to give (*E*)-4-hydroxy-7-(2,4-hexadiynilidene)-1,6-dioxaspiro[4.4]nona-2,8-diene (**26**) (32 mg) (Bohlmann & Zdero, 1968) and 6α-hydroxyelemol (**24**) (676 mg) (De Pascual Teresa et al., 1981). Fraction C (100 mg) eluted with hexane/*t*-butylmethyl ether 3:7 was dissolved in 4 ml Me_2CO. To the resulting soln, 0.1 ml of 1-methoxypropene, 2 mg of *p*-TsOH and 4 Å molecular sieves were added. After 2 h, the usual work-up gave 90 mg of a mixt. of compounds, which was column chromatographed using mixts of hexane/*t*-butylmethyl ether of increasing polarity as eluents. 60 mg of **3a** were then obtained. The treatment of **3a** with *p*-TsOH gave the corresponding natural product **3** (42 mg). Fraction D (hexane/*t*-butylmethyl ether 1:4) afforded pigmol (**15**) (6410 mg) (Maqua et al., 1988). Fraction E (*t*-butylmethyl ether) was subjected to repeated separations with silica gel chromatography (hexane/*t*-butylmethyl ether of increasing polarity) and HPLC (CH_3CN–MeOH–H_2O 78:20:2) to give (*E*)-7αH-germacra-1(10),4-diene-2β,6β-diol (**11**) (54 mg) (Sanz et al., 1991), **2** (20 mg), (*E*)-7αH-germacra-5,10(14)-diene-1β,4β-diol (**13**) (70 mg) (Kitagawa, Ciu, Son, Kobayashi, & Kyogoku, 1987), (*E*)-4β,5α-epoxy-7αH-germacr-1(10)-ene-2β,6β-diol (**12**) (94 mg) (Sanz et al., 1991), **6** (1 mg), bisabola-2,9-diene-7α,11-diol (**20**) (222 mg) (Barrero et al., 1990). Fraction F (MeOH) yielded, after further chromatography (hexane/*t*-butylmethyl ether/ethyl acetate of increasing polarity), **9** (244 mg), **4** (2 mg), **4**+**5** (6 mg), **10a** (520 mg), cryptomeridiol (**14**) (1040 mg) (Evans, Miller, Cairns, Baddeley, & Wenkert, 1982). Acetyl derivatives of some of these fractions were prepared by acetylation with Ac_2O in pyridine.

*3.4. (E)-7αH-Germacra-1(10),4(15)-diene-5α,6β-diol (**1**)*

Colourless syrup. $[\alpha]_D^{20}$ +14.2° ($CHCl_3$; *c* 1). IR ν_{max}^{film} cm^{-1}: 3376 (OH), 3076, 2930, 2861, 1663 (C=C), 1645 (C=C), 1460, 1383, 1309, 1260, 1141, 1096, 1064, 1017, 902, 842, 803. ^1H NMR (300 MHz, $CDCl_3$, 25°C): δ 5.20 (1H, br t, *J*=7.2 Hz, H-1), 5.04 (1H, s, H-15a), 5.00 (1H, s, H-15b), 3.96 (1H, d, *J*=9.9 Hz, H-5), 3.51 (1H, br d, *J*=9.9 Hz, H-6), 1.66 (3H, br s, Me-14), 1.01 (6H, d, *J*=6.7 Hz, Me-12, Me-13). ^1H NMR (400 MHz, C_6D_6, 57°C): δ 5.08 (1H, br t, *J*=7.3 Hz, H-1), 4.79 (1H, s, H-15a), 4.74 (1H, s, H-15b), 3.78 (1H, d, *J*=9.8 Hz, H-5), 3.40 (1H, d, *J*=9.8 Hz, H-6) 1.52 (3H, br s, Me-14), 1.03, 1.02 (6H, 2 d, *J*=6.7 Hz, Me-12, Me-13). ^{13}C NMR (75 MHz, $CDCl_3$, 25°C): δ 150.3 (s, C-4, C-10), 124.4 (d, C-1), 113.2 (t, C-15), 76.8 (d, C-5), 73.1 (d, C-6), 42.9 (d, C-7), 34.4 (t, C-3), 32.6 (d, C-11), 21.2, 21.1 (2q, C-12, C-13), 19.9 (q, C-4). ^{13}C NMR (100 MHz, C_6D_6, 57°C): δ 151.3 (s, C-4, C-10), 124.5 (d, C-1), 112.5 (t, C-15), 76.8 (d, C-5), 73.3 (d, C-6), 43.4 (d, C-7), 38.4 (t, C-9), 34.7 (t, C-3), 33.2 (d, C-11), 27.4 (t, C-2) 26.3 (t, C-8), 21.2 (q, C-12, C-13), 19.0 (q, C-14). EIMS (probe) 70 eV *m*/*z* (rel. int.): 238 $[M]^+$ (3), 22 (3), 205 (4), 177 (12), 153 (30), 125 (46), 107 (39), 82 (33), 81 (62), 67 (56), 55 (60), 41 (100).

*3.5. Base-catalysed ring opening of epoxide **8** to diol **1***

A 1.6 M soln of BuLi in hexane (2.27 ml) was added under Ar to a soln. of diethylamine (0.52 ml) in dry THF (18 ml). The resulting soln was stirred at room temp. for 30 min. Compound **8** (230 mg, 0.97 mmol) dissolved in a mixt. of dry THF (5.4 ml) and dry HMPT (0.4 ml) was then added. The reaction mixt. was kept at room temp. for 2.5 h. Glacial HOAc (0.2 ml) was then added and the solvents were evaporated *in vacuo*. CC of the residue (200 mg) on silica gel afforded 153 mg of unreacted **8** (hexane/*t*-butylmethyl ether 3:2) and 38 mg of **1** (hexane/*t*-butylmethyl ether 1:1).

*3.6. 4β,5α-Epoxy-7αH-germacr-10(14)-ene-1β,6β-diol (**2**)*

Colourless syrup. $[\alpha]_D^{20}$ +37.5° ($CHCl_3$; *c* 1). IR ν_{max}^{film} cm^{-1}: 3406 (OH), 3075, 2954. 2869, 1645 (C=C), 1457, 1385, 1260, 1195, 1147, 1013, 905, 820, 755. ^1H NMR (400 MHz, $CDCl_3$): δ 5.06 (1H, s, H-14a), 4.99 (1H, s, H-14b), 4.13 (1H, t, *J*=7.4 Hz, H-1), 3.57 (1H, dd, J_1=8.1 Hz, J_2=1.8 Hz, H-6), 2.97 (1H, d, *J*=8.1 Hz, H-5), 2.53 (1H, ddd, J_1=14.6 Hz, J_2=7.9 Hz, J_3=2.4 Hz, H-9β), 2.07 (1H, ddd, J_1=14.5 Hz, J_2=10.4 Hz, J_3=3.0 Hz, H-9α), 1.91 (3H, m, H-2β, H-3α, H-8β), 1.64 (2H, m, H-8α, H-11), 1.25 (3H, s, Me-15), 1.25 (1H, m, H-2α), 1.12 (1H, m, H-7), 0.97, 0.91 (6H, 2d, *J*=6.6 Hz, Me-12, Me-13). ^{13}C NMR (100 MHz, $CDCl_3$): δ 112.3 (t, C-14), 72.2 (d, C-1), 69.7 (d, C-6), 67.8 (d, C-5), 47.3 (d, C-7), 33.1 (t, C-2), 32.5 (t, C-9), 30.7 (d, C-11), 26.2 (t, C-8), 20.4, 19.8 (2q, C-13, C-12), 16.1 (q, C-15). EIMS (probe) 70 eV *m*/*z* (rel. int.): 254 $[M]^+$ (1), 236 (1), 218 (1), 208 (4), 193 (4), 179 (12), 165 (27), 120 (33), 109 (43), 95 (90), 81 (100), 69 (44), 55 (61), 43 (70).

*3.7. Photooxidation of compound **8**: synthesis of compound **2***

Rose Bengal (10 mg) was added to a soln of **8** (130

mg, 0.55 mmol) in *i*-PrOH (10 ml) and the soln exposed to sunshine for 3 h. The solvent was removed, yielding a residue, which was dissolved in MeOH (10 ml) and treated with $NaBH_4$ (113 mg, 3.0 mmol) at room temp. for 1 h. After the usual work-up 23 mg of unreacted **8** and 81 mg of **2** were isolated by silica gel CC (hexane/*t*-butylmethyl ether of increasing polarity).

3.8. (*E*)-6α,11-Dihydroxy-7αH-germacra-4,10(14)-dien-1-one (*3*)

Colourless syrup. $[\alpha]_D^{20}$ −66.4° ($CHCl_3$; *c* 1.2). UV λ_{max}^{MeOH} (log ε): 205.2 (3.50), 222.5 (3.34). IR ν_{max}^{film} cm^{-1}: 3314 (OH), 2970, 2714, 1673 (CO), 1625, 1441, 1382, 1260, 1165, 1086, 960, 878, 755. ^1H NMR (300 MHz, $CDCl_3$): δ 5.66 (1H, br s, H-14a), 5.61 (1H, s, H-14b), 5.11 (1H, br d, $J=9.7$ Hz, H-5), 4.19 (1H, t, $J=9.8$ Hz, H-6), 3.23 (1H, m, H-2α), 2.88 (1H, ddq, $J_1=13.7$ Hz, $J_2=6.0$ Hz, $J_3=1.1$ Hz, H-9β), 2.41 (3H, m, H-2β, H-3), 1.83 (1H, dt, $J_1=13.9$ Hz, $J_2=2.2$ Hz, H-9α), 1.68 (3H, d, $J=1.2$ Hz, Me-15), 1.33 (2H, m, H-7, H-8α), 1.25 (3H, s, Me-13), 1.21 (3H, s, Me-12), 0.79 (1H, m, H-8β). ^{13}C NMR (100 MHz, $CDCl_3$): δ 204.9 (s, C-1), 151.2 (s, C-10), 134.6 (s, C-4), 132.7 (d, C-5), 122.0 (t, C-14), 75.8 (s, C-11), 71.6 (d, C-6), 53.5 (d, C-7), 36.5 (t, C-2), 35.5 (t, C-3), 34.4 (t, C-9), 29.6 (q, C-12), 26.1 (t, C-8), 23.6 (q, C-13), 17.5 (q, C-15). EIMS (probe) 70 eV *m/z* (rel. int.): 252 $[M]^+$ (1), 234 (9), 219 (4), 216 (4), 201 (6), 176 (37), 161 (36), 150 (39), 135 (41), 133 (72), 119 (40), 107 (43), 93 (44), 91 (44), 79 (33), 69 (85), 59 (53), 43 (100), 41 (77).

3.9. Compound *3a*

Crystals (hexane–*t*-butylmethyl ether): m.p. 113–115°C. $[\alpha]_D^{20}$ −76.2°C ($CHCl_3$; *c* 1). IR ν_{max}^{film} cm^{-1}: 2982, 2937, 1668 (CO), 1624, 1430, 1367, 1257, 1216, 1182, 1156, 1122, 1092, 1049, 1022, 964, 930, 896, 859, 803. ^1H NMR (400 MHz, $CDCl_3$): δ 5.67 (1H, br s, H-14a), 5.62 (1H, d, $J=0.7$ Hz, H-14b), 5.09 (1H, br d, $J=9.6$ Hz, H-5), 4.22 (1H, t, $J=9.7$ Hz, H-6), 3.18 (1H, m, H-2α), 2.89 (1H, ddq, $J_1=13.9$ Hz, $J_2=7.0$ Hz, $J_3=1.2$ Hz, H-9β), 2.56 (2H, m, H-2β, H-3β), 2.35 (1H, m, H-3α), 1.86 (1H, dt, $J_1=14.1$ Hz, $J_2=2.1$ Hz, H-9α), 1.68 (3H, d, $J=0.9$ Hz, Me-15), 1.36, 1.48 (6H, 2s, $(CH_3)_2CO$), 1.29 (3H, s, Me-13), 1.23 (3H, s, Me-12), 1.22 (2H, m, H-7, H-8α), 0.94 (1H, m, H-8β). ^{13}C NMR (100 MHz, $CDCl_3$): δ 204.7 (s, C-1), 151.2 (s, C-10), 135.7 (s, C-4), 130.4 (d, C-5), 121.8 (t, C-14), 97.8 (s, $(CH_3)_2\mathbf{C}O$), 75.2 (s, C-11), 67.2 (d, C-6), 49.8 (d, C-7), 36.9 (t, C-2), 36.1 (t, C-3), 34.4 (t, C-9), 32.1, 25.0 (2s, $(\mathbf{C}H_3)_2CO$), 30.9 (q, C-12), 24.1 (t, C-8), 23.6 (q, C-13), 17.2 (q, C-15). EIMS (probe) 70 eV *m/z* (rel. int.): 292 $[M]^+$ (1), 277 (74), 234 (14), 217 (21), 199 (27), 175 (14), 161 (27), 150 (27), 135 (29), 119 (23), 109 (31), 91 (35), 79 (28), 69 (55), 55 (25), 43 (100), 41 (56).

3.10. (*E*)-7αH-Germacra-4,10(14)-diene-1α,6α,11-triol (*4*)

Colourless syrup. IR ν_{max}^{film} cm^{-1}: 3398 (OH), 3078, 2929, 1660, 1638, 1466, 1382, 1260, 1107, 1019, 973, 909, 825. ^1H NMR (300 MHz, $CDCl_3$): δ 5.16 (1H, d, $J=9.9$ Hz, H-5), 5.00 (1H, s, H-14a), 4.87 (1H, s, H-14b), 4.37 (1H, t, $J=9.7$ Hz, H-6), 3.87 (1H, dd, $J_1=9.4$ Hz, $J_2=4.2$ Hz, H-1), 1.68 (3H, d, $J=1.0$ Hz, Me-15), 1.30, 1.24 (6H, 2s, Me-13, Me-12). ^1H NMR (400 MHz, $(CD_3)_2CO$): δ 5.08 (1H, d, $J=9.7$ Hz, H-5), 4.88 (1H, s, H-14a), 4.73 (1H, s, H-14b), 4.29 (1H, t, $J=9.7$ Hz, H-6), 3.83 (1H, dd, $J_1=9.7$ Hz, $J_2=4.6$ Hz, H-1), 1.63 (3H, d, $J=1.2$ Hz, Me-15), 1.45 (1H, m, H-7), 1.22, 1.17 (6H, 2s, Me-13, Me-12), 0.84 (1H, m, H-8β). ^{13}C NMR (75 MHz, $CDCl_3$): δ 151.9 (s, C-10), 135.9 (s, C-4), 129.1 (d, C-5), 112.2 (t, C-14), 75.6 (s, C-11), 72.6, 72.0 (2d, C-1, C-6), 56.2 (d, C-7), 36.4 (t, C-9), 33.8 (t, C-8), 31.8 (t, C-3), 30.1, 23.8 (2q, C-12, C-13), 26.9 (t, C-2), 18.2 (q, C-15); GC-MS (probe) 70 eV *m/z* (rel. int.): 239 $[M–Me]^+$ (2), 221 (2), 203 (2), 178 (15), 160 (95), 145 (100), 131 (24), 118 (17), 105 (16), 93 (15), 79 (15), 67 (10), 59 (33).

3.11. (*E*)-7αH-Germacra-4,10(14)-diene-1β,6α,11-triol (*5*)

^1H NMR (300 MHz, $CDCl_3$): δ 5.29 (1H, d, $J=8.9$ Hz, H-5), 5.25 (1H, s, H-14a), 4.83 (1H, s, H-14b), 4.50 (1H, t, $J=9.5$ Hz, H-6), 3.89 (1H, d, $J=7.9$ Hz, H-1), 1.63 (3H, d, $J=1.0$ Hz, Me-15), 1.31, 1.22 (6H, 2s, Me-13, Me-12). ^{13}C NMR (75 MHz, $CDCl_3$): δ 157.5 (s, C-10), 134.1 (s, C-4), 130.8 (d, C-5), 111.0 (t, C-14), 77.0 (d, C-1), 75.3 (s, C-11), 72.2 (d, C-6), 56.0 (d, C-7), 36.8 (t, C-3), 33.9 (t, C-8), 30.7, 23.9 (2q, C-13, C-12), 29.7 (t, C-9), 29.2 (t, C-2), 17.6 (q, C-15). GC–MS (probe) 70 eV *m/z* (rel. int.): 254 $[M]^+$ (1), 239 (16), 230 (1), 221 (4), 203 (5), 193 (3), 178 (25), 160 (97), 145 (100), 119 (35), 107 (37), 93 (30), 79 (279, 69 (20), 59 (66).

3.12. Reduction of compound *3*: Synthesis of compounds *4* and *5*

$NaBH_4$ (5 mg, 0.13 mmol) was added to a soln of **3** (8 mg, 0.03 mmol) in MeOH (2.5 ml) cooled at −10°C. The reaction mixt. was stirried at room temp. for 1 h. After the usual work-up 7 mg of a 3:2 mixt. of **4** and **5** was obtained.

3.13. (*E*)-7αH-Germacra-4,10(14)-diene-1α,6β-diol (*6*)

Colourless syrup. IR ν_{max}^{film} cm^{-1}: 3352 (OH), 2920,

1673,1441, 1382,1261, 1015, 902. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.26 (1H, d, $J=6.4$ Hz, H-5), 5.19 (1H, s, H-14a), 4.83 (1H, s, H-14b), 4.58 (1H, br s, $J=6.2$ Hz, H-6), 3.90 (1H, br d, $J=8.8$ Hz, H-1), 1.45 (3H, br s, Me-15), 1.00, 0.98 (6H, 2d, $J=6.6$ Hz, Me-12, Me-13). ^{13}C NMR (400 MHz, $CDCl_3$): δ 131.9 (d, C-5), 112.3 (t, C-14), 72.9 (d, C-1), 69.5 (d, C-6), 46.2 (d, C-7), 37.6, 36.5 (2t, C-2, C-9), 32.8 (t, C-3), 31.1 (d, C-11), 23.7 (t, C-8), 22.7, 21.4 (2q, C-12, C-13), 17.3 (q, C-15). EIMS (probe) 70 eV m/z (rel. int.): 238 $[M]^+$ (1), 220 (4), 205 (5), 191 (4), 177 (18), 159 (17), 135 (15), 121 (20), 107 (26), 97 (100), 84 (92), 69 (43), 55 (51), 41 (66).

3.14. (1E,4E)-7αH-Germacra-1(10),4-dien-6β-ol (7)

Colourless syrup. $[\alpha]_D^{20}$ −14.8° ($CHCl_3$; c 1). IR ν_{max}^{film} cm^{-1}: 3370 (OH), 2919, 2850, 1642 (C=C), 1382, 1368, 1183, 1134, 1060, 1015, 991, 888, 847, 664. ^{1}H NMR (300 MHz, $CDCl_3$, 25°C): δ 4.95 (2H, m, H-1, H-5), 4.59 (1H, br d, $J=7.4$ Hz, H-6), 1.42 (6H, br s, Me-14, Me-15), 0.99, 0.97 (6H, d, $J=6.8$ Hz, Me-13, Me-12). ^{1}H NMR (400 MHz, C_6D_6, 57°C): δ 4.98 (1H, d, $J=7.4$ Hz, H-5), 4.87 (1H, br t, $J=7.0$ Hz, H-1), 4.52 (1H, br d, $J=7.4$ Hz, H-6), 1.48 (3H, br s, Me-14), 1.30 (3H, br s, Me-15), 0.97, 0.95 (6H, 2d, $J=6.7$ Hz, Me-13, Me-12). ^{1}H NMR (400 MHz, $CDCl_3$, −30°C): δ 5.06 (1H, d, $J=7.0$ Hz, H-5), 4.94 (1H, d, $J=7.0$ Hz, H-5′), 4.91 (2H, m, H-1, H-1′), 4.62 (1H, br d, $J=7.0$ Hz, H-6′), 4.59 (1H, br d, $J=7.0$ Hz, H-6), 1.62 (3H, br s, Me-14′), 1.55 (3H, br s, Me-14), 1.44 (6H, br s, Me-15, Me-15′), 0.98, 0.96 (6H, d, $J=6.5$ Hz, Me-12′, Me-13′), 0.97, 0.94 (6H, d, $J=6.5$ Hz, Me-12, Me-13), prime numbers have been given to the 'P/β,α/N' conformer. ^{13}C NMR (75 MHz, $CDCl_3$, 25°C): δ 138.9, 133.3 (2s, C-10, C-4), 135.7, 133.0 (2s, C-10′, C-4′), 133.5, 128.7 (2d, C-5, C-1), 131.4 (d, C-5′), 121.3 (d, C-1′), 68.8 (d, C-6), 68.6 (d, C-6′), 52.2 (d, C-7), 49.3 (d, C-7′), 41.3 (t, C-9), 39.0 (t, C-9′), 37.2 (t, C-3), 35.7 (t, C-3′), 32.1 (d, C-11), 31.7 (d, C-11′), 30.3 (t, C-2), 25.2, 24.3 (2t, C-2′, C-8′), 22.1, 21.3, 21.0 (3q, C-12, C-13, C-14), 21.5, 21.2 (2q, C-12′, C-13′), 17.0 (q, C-14′), 16.5 (q, C-15), 16.4 (q, C-15′), prime numbers have been given to a 'P/β,α/N' conformer. EIMS (probe) 70 eV m/z (rel. int.): 222 $[M]^+$ (5), 207 (4), 189 (10), 161 (35), 136 (36), 121 (72), 109 (51), 93 (69), 81 (100), 69 (51), 55 (51), 41 (65).

3.15. Shiromool (8)

Colourless syrup. $[\alpha]_D^{20}$ +34.2° ($CHCl_3$; c 1). IR ν_{max}^{film} cm^{-1}: 3436 (OH), 2929, 2866, 1664, 1474, 1385, 1255, 1200, 1136, 1114, 1064, 1011, 931, 859, 842, 824. ^{1}H NMR (300 MHz, $CDCl_3$, 25°C): δ 5.29 (1H, br t, $J=7.3$ Hz, H-1), 3.66 (1H, dd, $J_1=8.0$ Hz, $J_2=1.7$ Hz, H-6), 2.62 (1H, d, $J=8.0$ Hz, H-5), 1.68 (3H, br s, Me-14), 1.24 (3H, s, Me-15), 1.02, 0.96 (6H, 2d, $J=6.6$ Hz, Me-13, Me-12). ^{1}H NMR (400 MHz, C_6D_6, 57°C): δ 5.07 (1H, br t, $J=7.8$ Hz, H-1), 3.59 (1H, dd, $J_1=8.0$ Hz, $J_2=1.8$ Hz, H-6), 2.61 (1H, d, $J=8.0$ Hz, H-5), 1.43 (3H, br s, Me-14), 1.04 (3H, s, Me-15), 0.93, 0.92 (6H, 2d, $J=6.7$ Hz, Me-13, Me-12). ^{1}H NMR (400 MHz, $CDCl_3$, −30°C): δ 5.28 (1H, br t, $J=7.1$ Hz, H-1), 5.08 (1H, d, $J=11.7$, H-1′), 3.58 (2H, d, $J=8.0$ Hz, H-6, H-6′), 2.87 (1H, d, $J=6.8$ Hz, H-5′), 2.56 (1H, d, $J=8.2$ Hz, H-5), 1.70 (3H, s, Me-14′), 1.57 (3H, s, Me-14), 1.25 (3H, s, Me-15), 1.11 (3H, s, Me-15′), 0.93, 0,87 (12H, 2d, $J=6.7$ Hz, Me-13, Me-13′, Me-12, Me-12′), prime numbers have been given to the 'P/β,α/N' conformer. EIMS (probe) 70 eV m/z (rel. int.): 238 $[M]^+$ (1), 220 (4), 205 (1), 181 (9), 162 (16), 140 (10), 119 (199), 109 (22), 94 (50), 79 (47), 67 (19), 55 (28), 41 (100).

3.16. 7αH-10βMe-Eudesma-3,5-dien-11-ol (9)

Colourless syrup. $[\alpha]_D^{20}$ +17.5° ($CHCl_3$; c 1). IR ν_{max}^{film} cm^{-1}: 3412 (OH), 2961, 1742, 1642, 1460, 1368, 1260, 1023, 801. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.64 (1H, m, H-6), 5.55 (1H, m, H-3), 1.80 (3H, d, $J=1.3$ Hz, Me-15), 1.25, 1.19 (6H, 2s, Me-13, Me-12), 0.97 (3H, s, Me-14). ^{13}C NMR (75 MHz, $CDCl_3$): δ 144.1, 131.5 (2s, C-4, C-5), 124.9, 120.1 (2d, C-6, C-3), 73.3 (s, C-11), 48.5 (d, C-7), 38.2, 37.3 (2t, C-9, C-1), 32.3 (s, C-10), 28.1 (q, C-13), 25.9 (q, C-12), 23.4 (q, C-15), 23.0 (t, C-8), 20.6 (t, C-2), 20.3 (q, C-14). EIMS (probe) 70 eV m/z (rel. int.): 220 $[M]^+$ (1), 205 (1), 202 (2), 187 (3), 162 (98), 147 (100), 133 (169), 119 (16), 105 (25), 91 (22), 81 (12), 67 (7), 59 (62), 43 (11).

3.17. Compound 10a

Colourless syrup. $[\alpha]_D^{20}$ +29.2° ($CHCl_3$; c 1). IR ν_{max}^{film} cm^{-1}: 3370 (OH), 3072, 2934, 1731 (CO), 1648, 1457, 1368, 1243, 1178, 1025, 905, 874. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.04 (1H, s, H-15a), 4.78 (1H, s, H-15b), 4.66 (1H, dd, $J_1=11.6$ Hz, $J_2=4.8$ Hz, H-1), 4.02 (1H, t, $J=9.8$ Hz, H-6), 2.32 (1H, ddd, $J_1=13.1$ Hz, $J_2=4.8$ Hz, $J_3=1.8$ Hz, H-3β), 2.11 (1H, dt, $J_1=13.2$ Hz, $J_2=5.0$ Hz, H-3α), 2.00 (3H, s, $COCH_3$), 1.93 (1H, d, $J=9.9$ Hz, H-5), 1.25 (3H, s, Me-13), 1.19 (3H, s, Me-12), 0.77 (3H, s, Me-14). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.7 (s, $COCH_3$), 144.5 (s, C-4), 108.9 (t, C-15), 80.1 (d, C-1), 74.3 (s, C-11), 68.7 (d, C-6), 55.4 (d, C-5), 53.0 (d, C-7), 40.6 (s, C-10), 36.1 (t, C-9), 34.8 (t, C-3), 29.8, 24.2 (2q, C-13, C-12), 22.1 (t, C-8), 21.2 (q, $COCH_3$), 12.7 (q, C-14). EIMS (probe) 70 eV m/z (rel. int.): 281 $[M–Me]^+$ (1), 225 (3), 204 (5), 189 (12), 164 (42), 149 (100), 135 (14), 123 (33), 109 (38), 95 (24), 81 (28), 71 (41), 59 (85), 43 (70).

3.18. Computational aspects

The Allinger molecular mechanics methodology (Burket & Allinger, 1982) for theoretical calculations was used through the PC-Model software (Serena Sofware Programm) on an IBM-compatible-PC. Minimization was performed using the MMX force-field, which is a modification of the MM2 (Allinger, 1977) and MMP1 (Allinger & Sprague, 1973) Allinger's programs by Gajewski and Gilbert. The current version of MMX recognizes nearly 60 different atom types. Conformational analyses were performed using the MULTOR option of the PC-Model program, rotating all the possible side-chains of the structures considered. In some cases, a collection of conformers of minimum energies and their corresponding Cartesian coordinates could be obtained. The theoretical $^3J_{HH}$ coupling constants and the relative populations were calculated through the 3JHH2 PROGRAM (Imai & Osawa, 1990), which is set up in the use of an extended multiparametric Karplus equation (Imai & Osawa, 1989) mixing the above minimum energy Cartesian coordinates.

3.19. Antifeedant bioassays

An antifeedant choice bioassay was used to assess the activity of the compounds. The compounds were applied to glass-fiber discs (Whatman GF/A 2.1 cm diameter) made palatable by the addition of 100 ml of a sucrose soln. (50 mM). Control discs carried just sucrose, whereas the treatment discs carried in addition to sucrose, 100 ml of the test compound at 1, 10, 100 or 1000 ppm. The discs were left to dry and then weighed. Larvae of *S. littoralis* were placed individually in Petri dishes (8.5 cm diameter), each with a control and treatment disc. Each concentration of the compound was tested against 15–20 larvae. The bioassay terminated after 50% of either disc was eaten or after 18 h if the insects had not eaten 50% of either disc. The larvae were removed, the discs dried and reweighed. The antifeedant index $[(C-T)/(C+T)]*100$ was calculated, where C and T represent the mass eaten of control and treatment discs, respectively. The Wilcoxon matched pairs test was used to assess the significance of the activity and probit analysis was used to establish the concentration required to obtain an antifeedant index of 50% (AI_{50}).

3.20. Antitumoral assays

The antitumoral activity of the compounds were assayed against cells P-388 (murine leukemia), A-549 (human non-small cell lung cancer), HT-29 (human colon cancer) and MEL-28 (human melanoma), following the method reported by Bergeron, Davanangh, Kline, and Porter (1984).

3.21. Antimicrobial assays

The antimicrobial activity of the compounds was tested against gram-positive bacteria (*Enterococcus faecalis* OGIX, *Bacillus subtilis* CECT 397 and *Staphylococcus aureus* ATCC 8), gram-negative bacteria (*Salmonella tiphymurium*, *Escherichia coli* U 9 and *Proteus* sp) and yeasts (*Saccharomyces cerevisiae* S 3, *Candida albicans* CECT 1394 and *Cryptococcus neoformans* CECT 1075). The microorganisms were obtained from the Microbiology Department, Faculty of Sciences, University of Granada. The minimal inhibitory concentration (MIC) was measured in 1 ml of nutrient broth (tryptose broth ADSA-MICRO for bacteria and USP ADSA-MICRO Sabouraud medium for yeasts) containing the sample at the required concentration. The test tubes were inoculated with 10^4 cells of the microorganism and incubated at 28°C (24 h for bacteria and 48 h for fungi). The test tubes were then examined, taking as MIC the least concentration showing no turbidity.

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