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SESQUITERPENES FROM *SANTOLINA CHAMAECYPARISSUS* SUBSP. *SQUARROSA*

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Key Word Index—*Santolina chamaecyparissus* subsp. *squarrosa*; Compositae; sesquiterpenes; conformation.

Abstract—Four new sesquiterpenes, (*E*)-6β-acetoxy-7αH-germacra-4,10(14)-diene-1α,2β-diol (**1**), (4*E*,9*Z*)-6β-acetoxy-7αH-germacra-4,9-diene-1α,2β-diol (**2**), (*E*)-6β-acetoxy-7αH-germacra-1(10),4-diene-2β-ol (**3**) and 6β-acetoxy-5βH,7αH,10βMe-eudesm-4(15)-ene-1α,2β-dol (**4**), were identified in the ether extract from the aerial parts of *Santolina chamaecyparissus* subsp. *squarrosa*. Their structures and preferred conformation in solution were determined by spectroscopic methods and molecular mechanics calculations.

INTRODUCTION

The genus *Santolina* (tribe Anthemideae) is constituted by a taxonomically complex group of species whose botanical classification is periodically revised [1–3]. Some members of this group are used widely in popular medicine. Following our studies [4, 5] on the chemical composition of the species of *Santolina* which grow in the south east of Spain, here, we report the results obtained from the study of the chemical composition of *Santolina chamaecyparissus* subsp. *squarrosa*. The plant was collected in the Sierra Nevada mountains where it is used in popular medicine as a substitute for the "manzanilla real" (*Artemisia granatensis*). This species has been studied before [6], being described as having major germacrenediol derivative components.

RESULTS AND DISCUSSION

After repeated chromatography of the ether extract of the aerial parts of *S. chamaecyparissus* subsp. *squarrosa* and in some cases after derivation, four sesquiterpenes were isolated: (*E*)-6β-acetoxy-7αH-germacra-4,10(14)-diene-1α,2β-diol (**1**) isolated as its acetonide derivative (**1a**), (4*E*,9*Z*)-6β-acetoxy-7αH-germacra-4,9-diene-1α,2β-diol (**2**) isolated as 2-TBDMS derivative (**2a**), (*E*)-6β-acetoxy-7αH-germacra-1(10),4-diene-2β-ol (**3**) and 6β-acetoxy-5βH,7αH,10βMe-eudesm-4(15)-ene-1α,2β-diol (**4**) isolated as its acetonide derivative (**4a**). The known compounds (−)-borneol and its acetate, oplopanone and kaempherol 3-methyl ether were also isolated. They were identified by comparison of their spectroscopic data with those of standards and with published data [7, 8].

In its mass spectrum the acetonide **1a** showed $[M]^+$ at *m/z* 336 which, together with the ^1H and ^{13}C NMR data allowed the proposal of the molecular formula $C_{20}H_{32}O_4$. The IR spectrum showed absorption bands due to an acetate group (1728 and 1241 cm^{-1}) and a disubstituted terminal double bond (3070, 1647 and 891 cm^{-1}). In addition to the signals corresponding to the methyls of the isopropylidenedioxy group, the ^1H NMR spectrum (Table 1) contained those of an isopropyl group at δ 0.91 (*d*, *J* = 6.6 Hz) and 0.95 (*d*, *J* = 6.7 Hz), three geminal protons to oxygenated function at δ 3.95 (*d*, *J* = 8.7 Hz), 4.06 (*ddd*, J_1 = 10.8 Hz, J_2 = 8.8 Hz, J_3 = 4.4 Hz) and 5.54 (*br s*, *J* = 5.7 Hz), a proton on a trisubstituted double bond at δ 5.32 (*br d*, *J* = 5.7 Hz), together with those of an exocyclic methylene at δ 5.31 and 5.10, a methyl on a double bond at δ 1.59 and an acetate group at δ 2.04. Combined analysis of its COSY and HETCOR spectra (Table 3) allowed the determination of the presence of the connectivities I, II and III (Fig. 1) in the molecule. Study of the long-range correlations observed in the HMBC spectrum (Table 3) established a structure of 6-acetoxy-1,2-isopropylidenedioxygermacra-4,10(14)-diene for **1a**. The relative configuration of C-1, C-2, C-6 and C-7 together with

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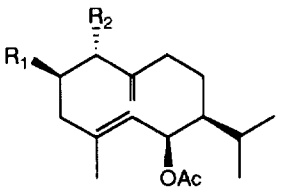
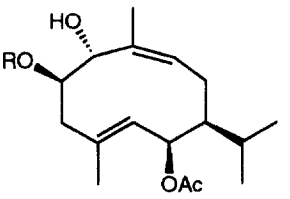


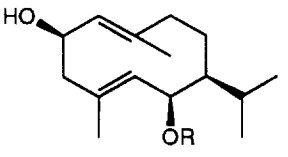
1 $R_1 = R_2 = OH$

1a $R_1 = R_2 =$ O(Me)(Me)C



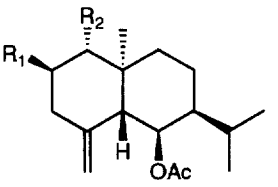
2 R = H

2a R = TBDMS



3 R = Ac

5 R = H



4 $R_1 = R_2 = OH$

4a $R_1 = R_2 =$ O(Me)(Me)C

Table 1. ^{1}H NMR (400 MHz) data for **1**, **1a**, **3**, **4a**

H	**1a**	**1***	**3**	**4a**
1	3.95 *d* (8.7)	3.47 *d* (9.1)	5.04 *d* (10.0)	3.27 *d* (9.1)
2	4.06 *ddd* (10.8, 8.8, 4.4)	3.76 *dt* (10.8, 5.4)	4.66 *dt* (10.0, 5.5)	3.54 *ddd* (13.7, 9.2, 4.7)
3α	2.58 *dd* (11.8, 4.3)	2.51 *dd* (12.2, 4.0)	2.55 *dd* (11.1, 5.5)	2.67 *dd* (11.2, 4.6)
3β	2.16 *t* (11.3)	2.15 *t* (11.5)		2.20 *br t* (12.0)
5	5.32 *br d* (5.7)	5.22 *br d* (5.8)	5.07 *d* (7.1)	2.37 *d* (11.9)
6	5.54 *br d* (5.7)	5.89 *br d* (5.8)	5.55 *dd* (7.1, 1.8)	5.21 *dd* (12.0, 5.0)
7	1.25 *m*	0.82 *m*		
8α	1.51 *m*	1.52 *m*		
8β	1.87 *m*	1.96 *dt* (13.5, 2.0)		
9α	2.07 *ddd* (14.5, 10.4, 3.0)			
9β	2.40 *ddd* (14.8, 7.5, 4.5)	2.37 *ddd* (13.8, 9.1, 4.3)		
11	1.53 *m*			
12	0.91 *d* (6.6)	0.94 *d* (6.9)	0.92 *d* (7.6)	0.95 *d* (6.9)
13	0.95 *d* (6.7)	0.99 *d* (6.6)	0.96 *d* (7.8)	0.99 *d* (6.6)
14	5.31 *s*	4.76 *s*	1.71 *s*	0.89 *s*
14	5.10 *s*	4.98 *s*		
15	1.59 *br s*	1.40 *br s*	1.55 *s*	4.48 *br s*
				5.06 *br s*
17	1.40 *s*†			1.38 *s*‡
18	1.44 *s*†			1.45 *s*‡
OAc	2.04 *s*	2.02 *s*	2.02 *s*	2.00 *s*

* Spectrum recorded in C_6D_6 (300 MHz).

†–‡ Assignments with the same symbol may be interchanged.

Values in parentheses are coupling constants in Hz.

Table 2. ^{13}C NMR (75 MHz) data for **1**, **1a**, **3**, **4a**

C	1a	1*	3	4a
1	81.6	77.8	131.7**	88.6
2	79.3	73.1§	72.1	74.8
3	43.7	47.1	47.8	40.5
4	130.1	130.1	137.3††	141.5
5	130.8	131.9	131.8**	47.6
6	72.8	72.8§	66.5	72.5
7	44.4	43.7	47.6	43.0
8	24.2	24.3	29.9	22.2
9	36.8	36.3	35.2	31.5
10	144.5	150.1	137.3††	39.1
11	31.3	31.7	31.1	27.2
12	20.9†	21.1¶	20.4‡‡	22.2
13	21.0†	21.2§	22.2‡‡	24.1
14	116.3	113.1	17.3	12.6
15	18.0	17.9	17.2	113.2
16	108.7			109.9
17	27.2‡			25.4
18	27.3‡			26.7
OCOMe	170.8	170.5	20.7	170.8
OCO*Me*	21.2	21.0§	170.4	21.5

* Spectrum recorded in $(CD_3)_2CO$ (100 MHz).

†–‡‡ Assignments with the same symbol may be interchanged.

the preferred conformation of **1a** in solution were determined on the basis of the correlations observed in its NOESY spectrum (Fig. 2). The molecular mechanics calculations made for **1a** showed close agreement with the experimental results in the determination of the conformation of the molecule in solution. Treatment of **1a** with *p*-TsOH yielded the natural compound **1**, whose mass spectrum did not show $[M]^+$ and whose highest peak appeared at *m/z* 236 corresponding to $[M-HOAc]^+$. In the IR spectrum, absorptions of a hydroxyl group (3402 cm^{-1}) were observed and the remaining spectroscopic data (Tables 1 and 2) were in agreement with the proposed structure. The similarity of the $^3J_{HH}$ values in the ^1H NMR spectrum made it clear that **1** must possess a similar preferred conformation to that of its acetonide **1a**.

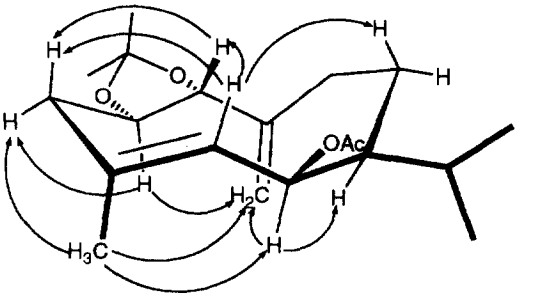
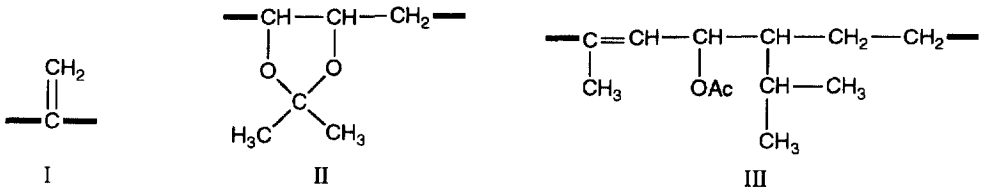


Fig. 2. NOESY correlations for **1a**.

The mass spectrum of **2a** did not contain a $[M]^+$, but showed the highest peak at *m/z* 336 corresponding to $[M-H_2O]^+$, which together with the ^1H NMR

Table 3. COSY, HETCOR and HMBC correlations of compound **1a**

COSY	HETCOR	HMBC
H-1—H-2, H-14a	C-1—H-1	H-1—C-2, C-9, C-14
H-2—H-1, H-3α, H-3β	C-2—H-2	H-3α—C-1, C-2, C-4, C-5
H-3α—H-2, H-3β	C-3—H3α, H-3β	H-3β—C-1, C-2, C-4, C-5, C-15
H-3β—H-2, H-3α	C-5—H-5	H-9α—C-10
H-5—H-6	C-6—H-6	H-9β—C-10
H-6—H-5, H-15	C-7—H-7	H-14a—C-1, C-9
H-8β—H-9α	C-8—H-8α, H-8β	H-14b—C-1, C-9
H-9α—H-8β	C-9—H-9α, H-9β	
H-12—H-15	C-11—H-11	
H-13—H-15	C-12—H-12	
H-14a—H-1, H-14b	C-13—H-13	
H-14b—H-14a, H-15	C-14—H-14a, H-14b	
H-15—H-6, H-12, H-13, H-14b	C-15—H-15	



I II III

Fig. 1. Connectivities determined by COSY and HETCOR NMR measurements for **1a**.

Table 4. ^1H and ^{13}C NMR (400 MHz) data for **2**

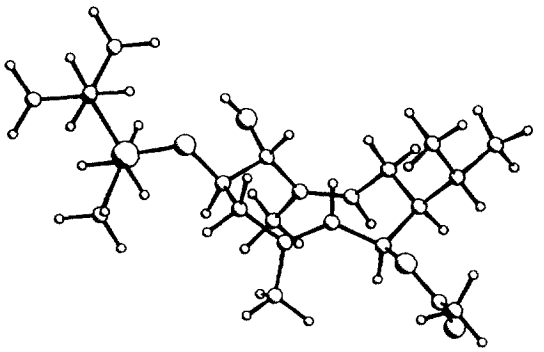
H	2*	2†	2‡	C	2‡
1	4.36 *d* (7.8)	4.69 *d* (8.0)	4.62 *d* (8.2)	1	73.4
2	3.67 *br dt*	4.05 *m*	3.67 *br dt*	2	67.9
	(8.2, 5.2)		(8.2, 5.2)	3	47.5
3			2.43 *dd*	4	§
			(12.4, 4.7)	5	126.6
5	5.23 *br s*	5.55 *br s*	5.39 *br s*	6	72.9
6	5.52 *br t* (6.3)	5.88 *t* (5.5)	5.89 *br t* (6.4)	7	29.7¶
8	2.43 *ddd*	2.71 *dd*	2.68 *ddd*	8	26.8
	(14.0, 12.5, 7.7)	(12.5, 7.7)	(14.1, 12.4, 7.9)	9	129.8
9	5.12 *dd*	5.18 *dd*	5.10 *dd*	10	§
	(12.7, 4.7)	(12.3, 5.0)	(12.3, 4.7)	11	30.1¶
12	0.89 *d* (6.7)	0.98 *d* (6.7)	0.99 *d* (6.9)	12	20.1
13	0.98 *d* (6.7)	1.07 *d* (6.7)	1.03 *d* (6.9)	13	20.7
14	1.63 *br s*	1.82 *br s*	1.73 *t* (1.5)	14	18.2
15	1.57 *s*	§	1.69 *br s*	15	17.4
OAc	2.07 *s*	2.11 *s*	1.85 *s*	OCO*Me*	21.3
*Me*SiMe	0.03 *s*	0.22 *s*	0.15 *s*	OCOMe	189.4
MeSi*Me*	0.10 *s*	0.25 *s*	0.20 *s*	*Me*SiMe	−4.6
$(Me)_3C$	0.90 *s*	1.01 *s*	1.01 *s*	MeSi*Me*	−4.9
				$(Me)_3C$	§
				$(Me)_3C$	25.8

* Spectrum recorded at 25°C.
† Spectrum recorded in C_5D_5N at 25°C.
‡ Spectrum recorded at 57°C in C_6D_6.
§: not observable.
¶, ‡ Assignments with the same symbol may be interchanged.

and ^{13}C NMR data, suggests the molecular formula $C_{23}H_{42}O_4Si$. Absorption bands at 3406 and 1736 cm^{-1} in its IR spectrum confirmed the presence of hydroxyl and acetate groups. The room temperature NMR spectra of **2a** showed poorly resolved signals, some of which were close to coalescence. However, the NMR spectra measured at 57° (Table 4) showed better resolution, and were closely analogous to those of **1**. The main difference lay in the presence of a trisubstituted double bond (1H: δ 5.10, *dd*, $J_1 = 12.3$ Hz, $J_2 = 4.7$ Hz; 3H: δ 1.73, *t*, $J = 1.5$ Hz) in place of the exocyclic double bond present in **1** whose location at positions 9 and 10 was conformed by COSY and HMQC experiments. The NOE effect observed between H-9 and H-14 established the *Z* geometry of this double bond. Location of the silyloxy group at C-2 was determined on the basis of the unsheilding experienced by H-14 in the ^1H NMR spectrum in pyridine-d_5 ($\Delta\delta = \delta C_5D_5N - \delta CDCl_3 = 0.19$ ppm) together with the invariability of the chemical shift value of H-3 in the same spectrum [9]. The molecular mechanics calculations made for **2a** confirmed the *Z* geometry of the C-9/C-10 double bond and showed that **2a** must exist as a mixture of three conformers in solution (Fig. 3), which explains the bad resolution observed in their NMR spectra performed at room temperature.

Compound **3** gave a $[M]^+$ at *m/z* 280 which, together with the ^{13}C and ^1H NMR data (Tables 1 and 2) allowed the establishment of the molecular formula $C_{17}H_{28}O_3$. The IR spectrum presented absorption bands of hydroxyl (3421 cm^{-1}) and acetate groups (1736 and 1241 cm^{-1}). The ^1H and ^{13}C NMR spectra were very similar to those published for **5** [6], except for the presence of an acetate group in place of one of the hydroxyls present in **5**. The location of this group in position 6 was based on the chemical shift value for H-6 (δ 5.55). The value of the coupling constants $^3J_{HH}$ observed allowed the proposal of the same relative stereochemistry and preferred conformation "C/α,α/N" for **3** as for **5** (Fig. 4).

The mass spectrum of **4a** contained a $[M]^+$ at *m/z* 336 which, together with the ^1H and ^{13}C NMR data, established the molecular formula $C_{20}H_{32}O_4$. In the IR spectrum, absorption bands due to an acetate group (1733 and 1253 cm^{-1}) and terminal exocyclic double bond (3087, 1647 and 890 cm^{-1}) were seen. The ^1H NMR and ^{13}C NMR spectra (Tables 1 and 2) established the presence of a eudesm-4(15)-ene structure with an acetate group and an acetonide group. The location of these oxygenated functions in the 1, 2 (acetonide group) and 6 (acetate group) positions was based on the chemical shifts and the multiplicity of H-1 (δ 3.27, *d*), H-2 (δ 3.54, *ddd*) and H-6 (δ 5.21, *dd*). The stereochemistry shown in **4a** for these functions was determined on the basis of the $^3J_{HH}$ values ($J_{1,2} = 9.1$ Hz, $J_{2,3\beta} = 13.7$ Hz, $J_{2,3\alpha} = 4.7$ Hz, $J_{5,6} = 12.0$ Hz, $J_{6,7} = 5.0$ Hz). The NOESY spectrum allowed the establishment of the conformation and



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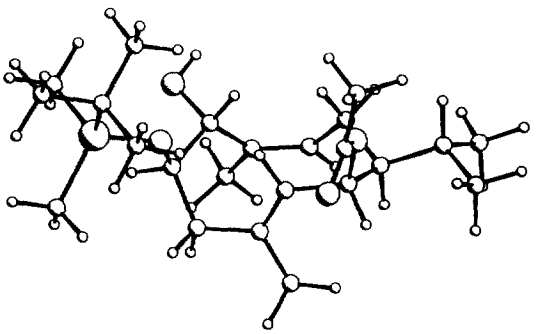
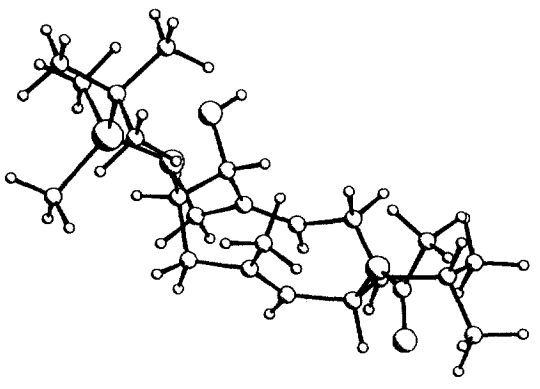


II



III

Fig. 3. Main conformation in equilibrium of **2a** obtained by molecular mechanics calculations: **II** is the lowest energy conformation.

the confirmation of the stereochemistry of **4a**. The main correlations are shown in Fig. 5, where the axial orientation of the isopropyl group is seen.

Given the co-existence of **4** and **1** in *S. chamaecyparissus* subsp. *squarrosa* and supposing the same absolute configuration at C-6 and C-7 for both molecules (the absolute configuration has not been established), it appears probable that the former originates biosynthetically from the latter, via biomimetic cyclization initiated by the protonation of the terminal exocyclic double bond of **1**. The stereochemistry of **4a**, and therefore the natural product **4**, differs from the structural type of eudesmanes usually found in the Compositae and, contrary to the hypothesis of Holub *et al.* [10, 11], coincides with that found in eudesmanes of the Umbelliferae. This circumstance, together with the presence of eudesmanolides in *Melanoselinum decipiens* possessing structural characteristics of both families [12], would question Holub *et al.*'s proposal of the separation of the biosynthetic pathways in both families.

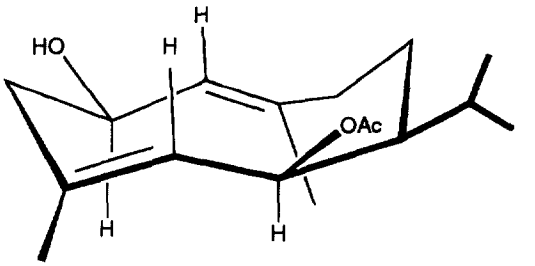


Fig. 4. "C/α,α/N" conformation for **3**.

EXPERIMENTAL

General

IR: Perkin-Elmer 983 G Spectrometer; EIMS: Hewlett-Packard 5988A mass spectrometer; NMR: Bruker ARX 400 or a Bruker AMX 300 spectrometer (δ values given in ppm relative to int. Me_4Si and J values in Hz); 2D NMR: Bruker ARX 400 spectrometer using the sequences of the Pulse Program Brucker Library. Assignments of ^{13}C NMR signals were achieved with the aid of additivity rules, DEPT and bidimensional experiments. CC: Merck 60 (70–230 mesh) silica gel, eluting with mixts of hexane–*t*-

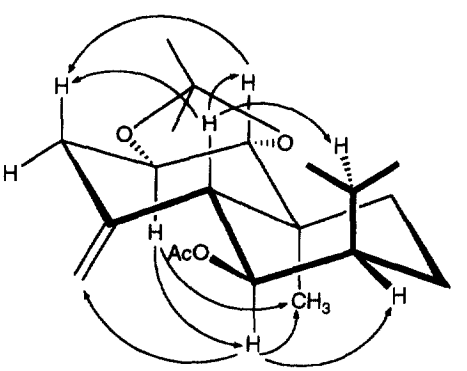


Fig. 5. NOESY correlations for **4a**.

butylmethyl ether–EtOAc of increasing polarity; Analytical TLC: Merck 60 G silica gel (0.25 mm thickness) using a 7% phosphomolybdic acid soln (EtOH) for visualization.

Plant material

S. chamaecyparissus subsp. *squarrosa* Nyman was collected in the Sierra Nevada (Granada, Spain) in June 1994 and identified by Prof. G. Blanca, Professor of the Department of Plant Biology, University of Granada. A voucher specimen (GDAC 25729) is available for inspection at the Herbarium of the Faculty of Sciences of the University of Granada.

Extraction and isolation

The air-dried aerial parts (0.5 kg) of *S. chamaecyparissus* subsp. *squarrosa* were macerated in *t*-butylmethyl ether for 15 min, resulting in 50 g of extract. A 10 g portion was defatted by precipitation in MeOH at low temp. The defatted extract (6.17 g, 61.7%) was subjected to CC, eluting with hexane–*t*-butylmethyl ether–EtOAc mixts of increasing polarity. Twelve major frs were collected (F1–F12). F1 consisted of 572 mg of (−)-borneol. F3 (124 mg) consisted of (−)-borneol acetate. F5 (132 mg) was dissolved in 2 ml of DMF and treated with 125 mg of TDMSCl and 118 mg of imidazole. Usual work-up after 24 h yielded 102 mg, which were rechromatographed using hexane–*t*-butylmethyl ether mixts of increasing polarity to afford 4 mg of pure **2a**. F6 (336 mg) was rechromatographed using hexane–*t*-butylmethyl ether mixts of increasing polarity. 132 mg of **3** were obtained. F10 consisted of 91 mg of oplopanone. F12 (428 mg) was dissolved in *t*-butylmethyl ether and extracted with 1 M NaOH. The aq. fraction was then acidified to give 72 mg of kaempherol 3-methyl ether. The organic fraction (256 mg) was dissolved in 8 ml of Me_2CO. To the resulting soln, 0.2 ml of 1-methoxypropene, 5 mg of *p*-TsOH and 4 Å molecular sieves were added. After 2 h, the usual work up gave 245 mg of a mixt. of compounds, which on CC with mixts of hexane–*t*-butylmethyl ether of increasing polarity give 51 mg **1a** and 12 mg **4a**. Treatment of **1a** with *p*-TsOH gave the corresponding natural product **1**.

(*E*)-6*β*-*acetoxy*-7-*αH*-*germacra*-4,10(14)-*diene*-1*α*,2*β*-*diol* (**1**). Colorless syrup, $[\alpha]_D^{20}$ −37.5 ($CHCl_3$; *c* 1). IR ν_{max}^{film} cm^{-1}: 3402, 2959, 2925, 1737, 1441, 1368, 1259, 1241, 1023, 801; EIMS (probe) 70 eV *m/z* (rel. int.): 236 $[M]^+$ (24), 218 (21), 203 (12), 193 (21), 189 (7), 175 (45), 157 (17), 147 (21), 119 (16), 109 (16), 91 (21), 79 (14), 69 (20), 55 (19), 43 (100).

(*E*)-6*β*-*Acetoxy*-1*α*2*β*-*isopropylidenedioxy*-7*αH*-*germacra*-4,10(14)-*diene* (**1a**). Colorless syrup, $[\alpha]_D^{20}$ −53.0° ($CHCl_3$; *c* 1). IR ν_{max}^{film} cm^{-1}: 3070, 2958, 2937, 1728, 1647, 1449, 1368, 1241, 1167, 1108, 1030, 980, 891, 818; EIMS (probe) 70 eV *m/z* (rel. int.): 336 $[M]^+$ (4), 276 (2), 261 (1), 218 (9), 201 (8), 175 (25), 163 (7), 147 (15), 140 (16), 121 (15), 109 (26), 95 (23), 81 (21), 69 (20), 55 (17), 43 (100).

(4*E*,9*Z*)-6*β*-*Acetoxy*-2*β*-*tert*-*butyldimethylsilyloxy*-7*Hα*-*germacra*-4,9-*dien*-1*α*-*ol* (**2a**). Colorless syrup, $[\alpha]_D^{20}$ +24.3° ($CHCl_3$; *c* 1). IR ν_{max}^{film} cm^{-1}: 3406, 2958, 2930, 1736, 1460, 1368, 1259, 1023, 836, 801; EIMS (probe) 70 eV *m/z* (rel. int.): 392 $[M-18]^+$ (1), 307 (5), 293 (18), 279 (7), 253 (7), 219 (6), 198 (94), 175 (16), 159 (22), 141 (85), 119 (26), 105 (20), 75 (100), 43 (62).

(*E*)-6*β*-*Acetoxy*-7*αH*-*germacra*-1(10),4-*dien*-2*β*-*ol* (**3**). Colorless syrup, $[\alpha]_D^{20}$ −15.9 ($CHCl_3$; *c* 1). IR ν_{max}^{film} cm^{-1}: 3421, 2933, 1736, 1632, 1437, 1368, 1241, 1138, 1101, 1020, 974, 865, 802; EIMS (probe) 70 eV *m/z* (rel. int.): 280 $[M^+]$ (1), 265 (1), 220 (7), 205 (6), 176 (47), 161 (12), 149 (9), 133 (36), 123 (28), 107 (42), 93 (38), 81 (19), 69 (16), 55 (23), 43 (100).

6*β*-*Acetoxy*-1*α*,2*β*-*isopropylidenedioxy*-5*βH*,7*αH*,10*βMe*-*eudesm*-4(15)-*ene* (**4a**). Colorless syrup, $[\alpha]_D^{20}$ −38.1° ($CHCl_3$; *c* 1). IR ν_{max}^{film} cm^{-1}: 3087, 2982, 2934, 2871, 1733, 1647, 1455, 1367, 1253, 1138, 1072, 964, 890, 847, 791; EIMS (probe) 70 eV *m/z* (rel. int.): 336 $[M^+]$ (1), 321 (7), 276 (5), 261 (8), 219 (12), 201 (66), 175 (23), 157 (15), 145 (49), 131 (13), 119 (17), 105 (21), 91 (20), 69 (13), 55 (17), 43 (100).

Computational aspects

The Allinger molecular mechanics methodology [13] for theoretical calculations was used through the PC-Model software [14] on an IBM-PC-compatible computer. Minimization was performed using the MMX forcefield, which is a modification of the MM2 [15] and MMP1 [16] Allinger programs by J. J. Gajewski and K. E. Gilbert. The current version of MMX recognizes nearly 60 different atoms 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 were obtained. The theoretical $^3J_{HH}$ coupling constants and the relative populations were calculated through the 3JHH2 PROGRAM [17], which is set up in the use of an extended multiparametric Karplus equation [18] mixing the above minimum energy Cartesian coordinates.

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REFERENCES

1. Guinea, E., *An. Inst. Bot. A. S. Cavanilles*, 1970, **27**, 29.
2. Tutin, T. G., Heywood, V. H., Burges, N. A., Moore, D. M., Valentine, D. H., Walters, S. M. and Webb, D. A., *Flora Europea*, Vol. 4, Cambridge University Press, Cambridge, 1976.
3. Pérez-Alonso, M. J. and Velasco Negueruela, A., *Flavour Frag.*, 1988, **3**, 37.
4. Barrero, A. F., Fernández, J. F. and Arana, E., *Phytochemistry*, 1988, **27**, 3969.
5. Barrero, A. F., Herrador, M. M., Molina Molina, J., Quílez, J. F. and Quirós, M., *J. Nat. Prod.*, 1994, **57**, 873.
6. Sanz, J. F., Garcia-Sarrión, A. and Marco, J. A., *Phytochemistry*, 1991, **30**, 3339.
7. San Feliciano, A., Medarde, M., Gordaliza, M. and Lucas, M. J., *J. Nat. Prod.*, 1995, **58**, 1059.
8. Bacon, J. D., Urbatsch, L. E., Bragg, L. H., Mabry, T. J., Newman, P. and Jackson, D. W., *Phytochemistry*, 1978, **17**, 1939.
9. Demarco, P. V., Farkas, E., Doddrell, D., Banavara, L. M. and Wenkert, E., *J. Am. Chem. Soc.*, 1968, **90**, 5480.
10. Holub, M., Budesinki, M., Smitalova, Z., Saman, D. and Rychlewska, U., *Tetrahedron Letters*, 1984, 3755.
11. Holub, M. and Budesinki, M., *Phytochemistry*, 1986, **25**, 2015.
12. Massanet, G. M., Guerra, F. M., Dorado, J. M., Zacarías, D. J. and Valerga, P., *Phytochemistry*, 1995, **39**, 1123.
13. Burket, U., Allinger, N. L., *Molecular Mechanics*, American Chemical Society, Washington, D.C., 1982.
14. Serena Software Programm, P.O. Box 3076, Bloomington, IN 47402-3076.
15. Allinger, N. L., *J. Am. Chem. Soc.*, 1977, **99**, 8127.
16. Allinger, N. L. and Sprague, J. T., *J. Am. Chem. Soc.*, 1973, **95**, 3893.
17. Imai, K. and Osawa, E., *Q. C. P. E. Bull.*, 1990, **10**, 38 Program No. 591.
18. Imai, K. and Osawa, E., *Tetrahedron Lett.*, 1989, **30**, 4251.