Phytochemistry, Vol. 29, No 10, pp 3213–3216, 1990
Printed in Great Britain.

0031-9422/90 $3.00+0.00

BISABOLENE DERIVATIVES AND OTHER CONSTITUENTS FROM *ACHILLEA ODORATA*

ALEJANDRO F. BARRERO, E. J. ALVAREZ-MANZANEDA R. and R. ALVAREZ-MANZANEDA R.

Department of Organic Chemistry, Faculty of Sciences of the University of Granada, Granada, Spain

(*Received in revised form* 5 *March* 1990)

Key Word Index—*Achillea odorata*; Compositae; bisabolene derivatives; nerolidol derivatives; flavones.

Abstract—From a hexane extract of the aerial parts of *Achillea odorata*, two nerolidol derivatives, (6*E*)-5,9-diacetoxynerolidol and (6*E*)-9-acetoxy-5-hydroxynerolidol, six bisabolene derivatives, 7,10-dihydroxy-bisabol-2,11-diene, 7,11-dihydroxy-bisabol-2,9*E*-diene, 1,10-diacetoxybisabol-2,11-diene, 7-hydroxy-11-hydroperoxybisabol-2,9*E*-diene, 7-hydroxy-10-hydroperoxybisabol-2,11-diene and (−)-α-bisabolol, as well as two flavones, 3,6,7,4′-tetramethoxy-5-hydroxyflavone and 3,6,7,3′,4′-pentamethoxy-5-hydroxyflavone, have been isolated. Structures of the first three new terpenoids were established from their spectroscopic properties and by the use of some chemical correlations.

INTRODUCTION

Achillea odorata L. (Compositae) grows in the mountains of the Mediterranean area. In a previous paper [1] we have reported on the isolation and the characterization of a triterpene alcohol with a new monocyclic skeleton. Following now with a chemical study of the secondary metabolites of this species, we describe herein the isolation of some sesquiterpenoid derivatives of nerolidol and bisabolene, unusual structures in *Achillea*, and flavonoids.

RESULTS AND DISCUSSION

Aerial parts of *A. odorata* gave the nerolidol derivatives **1** and **2**, which have been previously found in *Santolina oblongifolia* [2], (−)-α-bisabolol and some other derivatives of bisabolene, **3–6** and **9**, together with 3,6,7,4′-tetramethoxy-5-hydroxyflavone [3] and 3,6,7,3′,4′-pentamethoxy-5-hydroxyflavone [3].

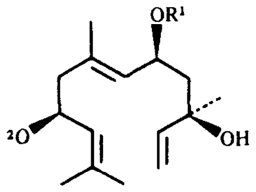
The unstable hydroperoxides **3** and **5** have been isolated previously from *Schistostephium crataegifolium* [4], but were poorly described. Chloroform solutions of these substances are unstable, presenting difficulties for the study of their ^{13}C NMR. Compound **5** was isolated as a mixture of epimers at C-10, as could be demonstrated by ^{13}C NMR of its reduction product **6** (Table 1). Reduction of **3** and **5** with $NaBH_4$ afforded the corresponding alcohols **4** and **6**, which were identical to those isolated from the plant.

The mass spectrum of **4** showed no $[M]^+$, but fragmentations at *m/z* 220 and 202, proceeding from the loss of one and two molecules of water, respectively, could be observed. The IR spectrum showed absorption bands at 3390 cm^{-1} (OH) and 1675 and 976 cm^{-1} (C=C). The ^{1}H NMR spectrum (Table 2) exhibited differences with respect to that of (−)-α-bisabolol, due to the change in the functionality at the end of the chain. Whereas, (−)-α-bisabolol showed the presence of three methyl groups on a carbon–carbon double bond (*s* at δ1.62 and 1.66), methyls geminal to an hydroxyl group (*s* at δ1.10) and a broad triplet at 5.12 ppm of H-10, the spectrum of **4** contained signals for one methyl group on a carbon–carbon double bond (*s* at δ1.63) and three methyls geminal to an hydroxyl group (1.07, *s*, 3H and 1.30, *s*, 6H). In addition, **4** also showed three olefinic protons: H-2 appearing as a broad singlet at δ5.36, as in (−)-α-bisabolol, and H-9 and H-10 as a multiplet centred at δ5.68.

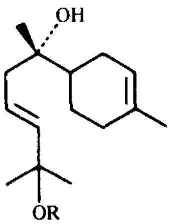
The ^{13}C NMR spectrum of **4** (Table 1), which also reflected differences with respect to that of (−)-α-bisabolol, confirmed the assigned structure, the most characteristic signal relating to C-7 and C-11, appearing at δ74.2 and 70.8, respectively.

The mass spectrum of **6** contained peaks for the $[M]^+$ (*m/z* 238), as well as those for the loss of a methyl group (*m/z* 223) and one or two molecules of water (*m/z* 220 and 202, respectively). The IR spectrum showed absorption bands for an hydroxyl group (3372 cm^{-1}), trisubstituted (1673 cm^{-1}) and terminally disubstituted carbon–carbon double bonds (1647 and 899 cm^{-1}). The structural change in the branched chain could be observed from the ^{1}H NMR spectrum (Table 2). Thus, H-10 appeared at δ4.01 as a double doublet ($J = 10.0$ and 4.4 Hz), whereas H-12 and H-12′ appeared at δ4.80 and 4.92, as broad singlets. An important difference from (−)-α-bisabolol was the presence of signals for only two methyl groups (*s* at δ1.61 and 1.70) on a carbon–carbon double bond. The ^{13}C NMR spectrum of **6** (Table 1) was in agreement with the proposed structure and reflected the epimerization at C-10, as some of the carbons near to this chiral centre appeared as double signals. Finally, the oxygenated carbon atoms, C-7 and C-10, gave signals at δ74.0, 76.1 and 76.2, respectively.

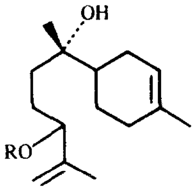
Whereas **4** was not acetylated on treatment with acetic anhydride in pyridine, compound **6** yielded the expected acetyl derivative **7**, whose spectroscopic properties were in agreement with the proposed structure. Thus, its mass



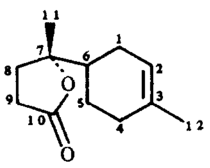
1 R¹ = R² = Ac
2 R¹ = H, R² = Ac



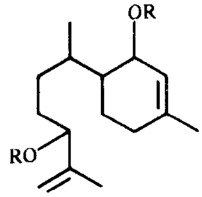
3 R = OH
4 R = H



5 R = OH
6 R = H
7 R = Ac



8



9 R = H
10 R = Ac

Table 1 ^{13}C NMR chemical shifts of compounds **4**, **6**–**8** and **10** (75 MHz, $CDCl_3$, TMS as int standard)

C	**4**	**6**	**7**	**8**	**10**
1	27.0	27.0, 27.1	26.9	23.8	72.1
2	120.4	120.5, 120.6	120.5, 120.4	31.5	121.3, 121.4
3	134.3	134.2, 134.3	134.2	134.4	139.4, 139.5
4	31.0	31.1	31.0	119.5	30.4, 30.5
5	23.4	23.4	23.4	26.3	20.9
6	43.2	42.9, 43.4	42.0, 43.0	43.5	42.4
7	74.2	74.0	73.9	89.1	31.4
8	43.0	35.8, 36.0	35.5, 35.6	29.1	21.0
9	122.0	28.6, 28.7	26.3	30.4	29.8, 30.0
10	142.4	76.1, 76.2	77.7, 77.8	176.8	77.4, 77.6
11	70.8	147.6	143.0	22.3	143.1, 143.2
12	29.9	110.9	112.9, 113.0	23.4	112.9, 113.1
13	29.9	17.9	18.1	–	18.0, 18.2
14	23.1	23.2	23.4	-	14.7
15	23.4	23.4	23.4		23.2
$-\underline{C}OMe$	-	-	170.4	–	170.3, 171.3
$-CO\underline{Me}$	—	—	21.3		21.3, 21.5

spectrum showed the $[M]^+$ at *m/z* 280, together with other significant fragmentations, such as that at *m/z* 220, resulting from the loss of acetic acid. The IR spectrum showed absorption bands for OH (3510 cm^{-1}), acetate (1737 cm^{-1}), carbon–carbon trisubstituted (1678 cm^{-1}) and terminally disubstituted double bonds (1649 and 704 cm^{-1}). The ^{1}H NMR spectrum had resonances for an acetoxyl group (δ2.03) and the proton geminal to this group at δ5.12 as a double triplet ($J = 6.6$ and 2.1 Hz). The ^{13}C NMR spectrum (Table 1) was very similar to that of **6**, but the carbon atom at C-10 was shifted to low field (δ77.7 and 77.8).

In order to confirm that **6** was a epimeric mixture at C-10, we attempted to oxidize the hydroxyl group at this position Thus, **6** was treated with pyridinium dichromate. The result was degradation of the lateral chain to produce the γ-lactone **8**, whose IR spectrum had absorption bands for carbonyl and a trisubstituted carbon–carbon double bond at 1771 and 1677 cm^{-1}, respectively The olefinic proton appeared as a broad singlet at δ5.36, whereas the protons of the lactone ring were revealed as four double double doublets between δ2.13 and 2.63 In addition, in the ^{13}C NMR spectrum (Table 1) a signal for a lactone carbonyl carbon (δ176.8) could be observed

Configurational assignments of the above compounds were demonstrated by chemical correlation with (−)-α-bisabolol. In this manner, photooxidation of (−)-α-

Table 2 ^{1}H NMR spectral data of compounds **4**, **6**–**8** and **10** (300 MHz, $CDCl_3$, TMS as int standard)

H	4	6	7	8	10
1	—	—	—	—	5.26 *m*
2	5.36 *s*	5.33 *s*	5.34 *s*	5.36 *s*	5.29 *s*
8	2.16 *ddd* (24.7, 3.8, 2.2)* 2.21 *ddd* (24.7, 4.8, 1.7)	—	—	1.89 *ddd* (13.0, 9.5, 4.8) 2.13 *ddd* (13.0, 9.5, 9.5)	—
9	5.68 *m*			2.63 *ddd* (18.0, 9.5, 9.5) 2.53 *ddd* (18.0, 9.5, 4.8)	—
10	5.68 *m*	4.01 *dd* (10.0, 4.4)	5.12 *dt* (6.6, 2.1)	—	5.13 *t* (6.9)
11	—	—	—	1.31 *s*	—
12	1.30 *s*	4.80 *s* 4.92 *s*	4.86 *dq* (1.5, 1.5) 4.91 *s*	1.63 *s*	4.92 *s* 4.87 *q* (1.5)
13	1.30 *s*	1.70 *s*	1.69 *s*	—	1.70 *s*
14	1.07 *s*	1.07 *s*	1.07 *s*	—	0.79 *d* (6.7)
15	1.63 *s*	1.61 *s*	1.62 *s*	—	1.66 *s*
OAc	—	—	2.03 *s*	—	2.05 *s*

* Coupling constants (*J* in Hz) are given in parentheses.

bisabolol, using Rose Bengal as sensitizer, gave a mixture of hydroperoxides **3** and **5**, which were not further investigated, but reduced ($NaBH_4$) to alcohols **4** and **6**.

Compound **9** was characterized through its acetate **10**, because it was isolated, after several chromatographic procedures, from the acetylation fraction in which **6** was also present. Although the mass spectrum of **10** did not show the $[M]^+$, peaks at *m/z* 262 and 202, corresponding to the loss of one and two molecules of acetic acid, respectively, were present. The IR spectrum had absorption bands for acetate (1735, 1241 and 1020 cm^{-1}), trisubstituted (1675 cm^{-1}) and terminal disubstituted carbon-carbon double bonds (1649 and 890 cm^{-1}). The ^{1}H NMR spectrum of **10** (Table 2) also confirmed the existence of a bisabolene skeleton, because it was very similar to that of **6**, the most significant signal was a methyl doublet at δ0.79 ($J = 6.7$ Hz). The protons geminal to the acetoxyl groups appeared at δ5.13 and 5.26 as a triplet ($J = 6.9$ Hz) and a broad doublet ($J = 8.0$ Hz). The ^{13}C NMR spectrum (Table 1) also supported the proposed structure, as well as demonstrating the presence of epimers at C-10, due to the fact that some of the carbon resonances appeared as double signals; C-10 appeared at δ77.4 and 77.6. No epimerizations at C-1 and C-7 seem to occur, because both carbons resonances gave signals at δ77.1 and 31.4, respectively.

EXPERIMENTAL

IR: film. ^{1}H (300 MHz) and ^{13}C NMR (75 TMS) $CDCl_3$ using TMS as int. std. Carbon substitution degrees were established by DEPT pulse sequence. EIMS at 70 eV, direct inlet. Analytical TLC: silica gel G (Merck 7331); CC silica gel 60 (Merck 7734); flash CC: silica gel (Merck 7729).

The plant was collected in May 1987 in Sierra Nevada 1700 m altitude (Granada, Spain) and was identified by Professor G. G. Blanca (Department of Botany, University of Granada). A voucher specimen is deposited in the Herbarium of the Faculty of Sciences of the University of Granada.

Dried above-ground parts (1580 g) were extracted with hexane (6 l) in a Soxhlet to yield an extract (67 g, 4.2% from dried plant), which was defatted to afford a residue (36.4 g). This residue was chromatographed under pressure on a silica gel column (140 g) eluting with hexane–Et_2O mixts of increasing polarity. (−)-α-Bisabolol (573 mg) was isolated from the less polar fr. eluted with hexane–Et_2O (19 : 1) and purified after a second CC, eluting with hexane–Et_2O (9 : 1). From the fr. eluted with hexane–Et_2O (1 : 1) were obtained, after successive CC, **1** (113 mg), **2** (148 mg) and a mixt. of **3** and **5** (150 mg). Acetylation of the fr. eluted with hexane–Et_2O (2 : 3) yielded, by means of further chromatography, **7** (152 mg) and **10** (33 mg). From the more polar fr. eluted with Et_2O, was isolated in the same way **4** (24 mg) and the flavonoids, 3,6,7,4′-tetramethoxy-5-hydroxyflavone (59 mg) and 3,6,7,3′,4′-pentamethoxy-5-hydroxyflavone (260 mg), by fractioned crystallization. Acetylation of **2** with Ac_2O–pyridine yielded **1**. Compound **6** was prepd by saponification of **7** with MeOH–KOH at room temp.

Compound **4**. Oil. Eluted with hexane–Et_2O (3 : 7). $[\alpha]_D$ −44.4° ($CHCl_3$; *c* 1.0). IR ν_{max}^{film} cm^{-1}: 3390 (OH), 1675 (C=C), 976, 800. MS *m/z* (rel. int): 220 $[M-H_2O]^+$ (2), 205 $[M-Me]^+$ (1), 202 $[M-2H_2O]^+$ (4), 139 (35), 95 (41), 82 (100), 67 (23), 43 (46).

Compound **6**. Oil. $[\alpha]_D$ −45.0° ($CHCl_3$; *c* 1.0). IR ν_{max}^{film} cm^{-1}:

3372 (OH), 3072, 1673 (C=C), 1647 (C=C), 899. MS *m/z* (rel. int.): 238 $[M]^+$ (1), 223 $[M-Me]^+$ (1), 220 $[M-H_2O]^+$ (17), 205 $[M-H_2O-Me]^+$ (9), 202 $[M-2H_2O]^+$ (8), 187 $[M-2H_2O-Me]^+$ (10), 134 (40), 132 (48), 125 (100), 119 (51), 107 (60), 43 (38)

Compound **7**. Oil Eluted with hexane–Et_2O (4:1). $[\alpha]_D$ −43.0° ($CHCl_3$, *c* 1.0). IR ν_{max}^{film} cm^{-1} 3510 (OH), 3079, 1737 (C=O), 1678 (C=C), 1649 (C=C), 904. MS *m/z* (rel int). 280 $[M]^+$ (0.1), 265 $[M-Me]^+$ (0.1), 262 $[M-H_2O]^+$ (0.7), 220 $[M-HOAc]^+$ (2), 205 $[M-HOAc-H_2O]$ (2), 202 $[M-H_2O-HOAc]^+$ (6), 187 $[M-H_2O-HOAc-Me]^+$ (6), 107 (29), 67 (26), 43 (100).

Reduction of hydroperoxides **3** *and* **5**. A mixt. (16 mg) of **3** and **5** in MeOH (3 ml) was treated with excess $NaBH_4$ at room temp. for 30 min The soln was neutralized with 2 M HCl, the MeOH evapd and the residue extracted with Et_2O (3 × 25 ml) The extracts were washed (3 × 25 ml), dried (Na_2SO_4) and then evapd to give a mixt. (10 mg) of alcohols **4** and **6**

Photoxidation of (−)-*α-bisabolol and further reduction.* (−)-α-Bisabolol (450 mg) and Rose Bengal (6 mg) in *iso*PrOH (25 ml) was stirred in sunlight for 30 min. The residue obtained after evapn was dissolved in MeOH (25 ml) and treated with $NaBH_4$ at room temp for 1 hr After removing solvent the residue was suspended in H_2O and extracted with Et_2O (3 × 50 ml). The extracts were dried (Na_2SO_4) and then evapd to yield a mixt (518 mg) of **4** and **6** in a ratio of 2 3

Oxidation of **6** *with pyridinium dichromate.* Compound **6** (50 mg) in CH_2Cl_2 (8 ml) was stirred with pyridinium dichromate (0.4 g) at room temp for 20 hr After filtration under vacuum a residue (35 mg) was obtained. Chromatography of this residue on silica gel with hexane–Et_2O mixts (9:1 and 4:1) allowed the isolation of **8** (15 mg), oil $[\alpha]_D$ −65 2° ($CHCl_3$, *c* 1 0). IR ν_{max}^{film} cm^{-1}. 1771 (C=O), 1677 (C=C), 863.

Compound **10**. Oil. Eluted with hexane–Et_2O (4 1) $[\alpha]_D$ 53 2° ($CHCl_3$, *c* 1.0) IR ν_{max}^{film} cm^{-1} 1735 (C=O), 1675 (C=C), 1649 (C=C), 905 MS *m/z* (rel int) 262 $[M-HOAc]^+$ (1), 202 $[M-2 \times HOAc]^+$ (3), 187 $[M-Me-2 \times HOAc]^+$ (3), 132 (57), 119 (41), 93 (100), 92 (52), 91 (56), 68 (64), 43 (41)

REFERENCES

1 Barrero, A. F, Manzaneda, E A and Manzaneda, R A. (1989) *Tetrahedron Letters* **30**, 3351

2. De Pascual Teresa, J, Bellido, J. S., González, M S and Vicente, S. (1986) *Phytochemistry* **25**, 185

3 Falk, A. J, Smolenski, S J, Baner, L. and Bell, C. L (1975) *J Pharm. Sci.* **64**, 1838.

4. Bohlmann, F, Jakupovic, J., Ahmed, M. and Schuster, A. (1983) *Phytochemistry* **22**, 1623