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BISABOLENES FROM *ACHILLEA CRETICA*

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Key Word Index—*Achillea cretica*; Anthemideae; Compositae; bisabolenes; sesquiterpenes; lignans.

Abstract—Aerial parts of *Achillea cretica* furnished six new 4-oxobisabol-2-enes and the lignans sesartemin B and syringaresinol dimethyl ether.

INTRODUCTION

Previous chemical studies of *Achillea cretica* L., a species found in the Aegean region [1], dealt with identification of glycosylflavones in the leaf [2–4]. We now report isolation of seven new 4-oxobisabol-2-enes (**1–5** and **7a,b**) from aerial parts of plant material collected in Cyprus. The lignans **9a** and **9b** were also found.

RESULTS AND DISCUSSION

Compounds **2** and **3** were obtained only in the form of a binary mixture and while **5** was isolated as such, **7a** was only obtained in admixture with **5**. Structure assignments are based on the ^{1}H NMR data listed in Table 1. All compounds were α-methyl-δ-alkylated cyclohexenones, as shown by sequential decoupling, with the α-methyl group (H-15) allylically coupled to H-2 and the latter further coupled, in the case of **1–4**, to two mutually coupled protons (H-1eq, ax) and, in the case of compounds **5** and **7a,b**, to a single proton (H-1eq) that was obviously under a hydroxyl group because of its chemical shift near δ 4.5 and because of the shift in the ^{13}C NMR spectrum of **5** (Table 2) of the C-1 frequency from near δ 27 to δ 64.9. The two H-1 protons (or H-1eq in the case of **5** and **7a,b**) were further coupled to a single proton (H-6) which was in turn coupled to the two protons of a methylene group (H-5a,b) whose chemical shifts and coupling constants indicated that they were α to a cyclohexenone carbonyl.

Side-chains of the type encountered in **1**, **5** and **7a,b** and the 13-nor side chain of **4** are common in naturally occurring bisabolenes [5–19]. Thus, the nature of the side-chains in these substances was readily inferred from the ^{1}H NMR spectra and decoupling. That compound **1** in which the 7-hydroxyl group was acetylated was the *E*-isomer depicted in the formula was established by NOE spectrometry, irradiation at the frequency of H-10 causing an 8.4% enhancement of the H-12a,b signal and *vice versa*. Bisabolenes **7a** and **7b** were C-10 epimers, as shown by differences in the chemical shifts of H-10 and H-12a,b, while **6**, not originally found in the plant extract, was formed, together with minor contaminants, from **5** on prolonged standing, possibly under the influence of acid. Bisabolenes containing the side-chains present in **2** and **3** have not been isolated previously; their structures were evident from the mass spectra and from the ^{1}H NMR data in Table 1.

We now deal with the stereochemistry of the new compounds at C-6, C-7 and in the case of **5a** and **7a,b** also with the stereochemistry at C-1. It should be noted that in all instances one of the H-5 protons, i.e. H-5eq, in the case of **1–4** at lower field than H-5ax and in the case of **5–7a,b** at higher field than H-5ax, is long-range coupled, presumably through W-coupling, to H-1eq in **1–4** or to the single H-1 proton in **5–7a,b**. The magnitudes of $J_{5,6}$ (≈3 Hz), $J_{5ax,6}$ (≈14 Hz), $J_{1eq,6}$ (≈4 Hz) (3 Hz in the case of **5–7a,b**) and $J_{1ax,6}$ in the case of **1–4** (11 Hz), indicated that H-5 and H-6, and H-1$_{ax}$ and H-6 were diaxially related and led to the relative stereochemistry of the six-membered ring shown in the formulae. This might also account for the inversion of the chemical shifts of H-5eq and H-5ax when a quasiaxial hydroxyl group in introduced at C-1. The NOE data for **7b** in Table 2 confirm that H-1eq, H-5ax and H-6 are *cis* with respect to each other, i.e. the side-chain is attached to C-6 is syn with respect to

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Table 1. ^{1}H NMR spectra of compounds **1–7a,b** (500 MHz, $CDCl_3$)

H	1*	2*	3*	4	5†	6	7a‡	7b
1eq	2.38 *dddd* (18,5,5,1)	2.39 *dddd*		2.40 *dddd* (18,6,4,1.5)	4.67 *brdd* (6,2.5,1)	4.50 *brdd* (6,2.5,1	4.68 *m*	4.69 *brdd* (5.5,2.5,1)
1ax	2.24 *dddd* (18,11,2.5,2.5)	2.25 *m*		2.24 *ddd* (18,11,2.5)	–	–	–	–
2	6.74 *ddq* (6,2.5,1)	6.74 *ddq*		6.74 *ddq* (6,2.5,1)	6.75 *dq* (6,1.5)	6.75 *dq* (6,1.5)	6.73 *dq* (6,1)	6.74 *dq* (6,1)
5eq	2.60 *ddd* (16,3,1.5)	2.64 *brdd*		2.63 *ddd* (16,3,1.5)	2.57 *brdd* (16,3.5,1)	2.37 *brdd* (6,2.5,1)	2.51 *brdd* (17,3.5,1)	2.54 *brdd* 17,3,1)
5ax	2.26 *dd* (16,14)	2.27 *m*		2.29 *dd* (16,14)	2.88 *dd* (16.5,13.5)	2.57 *dd* (16,14)	2.88 *dd* (17,3.5)	2.84 *dd* (17,14)
6	2.08 *m*	2.1 *m*		2.11 *dddd* (14,11,4.3)	1.97 *dt* (13.5,3)	1.90 *dt* (14.5,3)	1.94 *dt* (14,3)	1.95 *dt* (17,14)
8a	1.54 *dd* (9.5,6.5)	2.27 *m*	Obsc.	2.45 *brdd* (14.5,7.5,1)	2.27 *dd* (14,7)	1.58 *dd* (14.5,11)	Obsc.	1.76 *ddd* (14,8,6.5)
8b		2.23 *m*	Obsc.	2.38 *ddd* (14.5,7.5,1)	2.24 *dd* (14,7)	1.46 *brd* (14.5)	Obsc.	1.49 *ddd* (14,8,6)
9a	2.09 *m*	5.77 *dt* (16,7)	4.26 *dt* (13,7)	6.85 *dt* (16,7.5)	5.59 *dt* (15.5,7)	4.56 *ddd* (11,8,2)	Obsc.	1.88 *m*
9b		–	–	–	–	–	Obsc.	1.60 *m*
10	5.44 *brt* (7)	5.61 *dd* (16,5.5)	4.18 *brdd* (13,2)	6.15 *dt* (16,1)	5.73 *d* (15.5)	5.15 *brd* (8)	4.27 *dd* (6.5,6)	4.10 *t* (5.5)
11	–	2.23 *m*	–	–	–	–	–	–
12a	4.44 *brs*	3.49 *dd* (11,5)	5.14 *brs*	2.26 *s*§	1.31 *s*§	1.73 *brs*	5.14 *brs*	4.97 *brs*
12b	–	3.44 *dd* (11,15)	5.12 *brs*	–	–	–	–	–
13§	1.66 *brs*	–	–	–	1.31 *s*	1.73 *brs*	1.74 *brs*	1.71 *brs*
14§	1.19 *s*	1.17 *s*	1.17 *s*	1.22 *s*	1.40 *s*	1.24 *s*	1.39 *s*	1.40 *s*
15§	1.77 *brs*	1.77 *brs*	1.77 *brs*	1.77 *brs*	1.83 *brs*	1.80 *brs*	1.82 *brs*	1.81 *brs*
Ac§	2.07 *s*	–	–	–				

*From mixture of **2** and **3**
†At 250 MHz.
‡From mixture with **5**.
§Intensity three protons.
††Intensity two protons.

the hydroxyl group on C-1. The significant NOEs between H-5 or H-6 and H-14 could theoretically accommodate either configuration at C-7, depending on the conformation of the side-chain.

Table 2. ^{13}C NMR spectra of compounds **1**, **4** and **5** ($CDCl_3$, 67.89 MHz)

C	1	4	5*
1	27.3 *t*	27.1 *t*	64.9
2	144.6 *d*	144.1 *d*	142.7
3	135.4 *s*	135.5 *s*	137.3
4	199.1 *s*	199.3 *s*	200.3
5	39.2 *t*†	38.9 *t*	33.3
6	44.6 *t*	44.8 *d*	44.1
7	73.1 *s*	73.1 *s*	74.3
8	39.1 *t*†	42.8 *t*	42.7
9	25.3 *t*	142.3 *d*	120.8
10	128.8 *d*	134.3 *d*	141.7
11	130.7 *s*	197.7 *s*	70.7
12	13.9 *q*	29.7 *q*	29.8
13	69.9 *t*	–	29.8
14	24.4 *q*	24.4 1	25.3
15	15.5 *q*	15.4 *q*	15.8
16	170.8 *s*		
17	20.7 *q*		

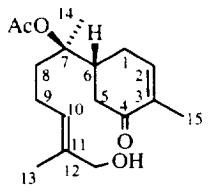
*At 62.9 MHz; multiplicities not established.
†Assignments may be interchanged.

Table 3. NOE difference spectra of compounds **4** and **7b**

Compound	Irr.	Obs. (% enhancement)
4	H-6	H-5eq (1.2), H-8,b (1.2), H-14 (1.0)
	H-14	H-5eq (2.9), H-8a,b (3.5), H-6 (2.2)
7b	H-1	H-2 (14.5), H-6 (8.4), H-14 (6.3)
	H-6	H-1eq (13.1), H-5ax (14.0), H-14 (7.6)

In all 7-hydroxybisabolenes isolated to date [5–21] the ^{1}H NMR frequency (in $CDCl_3$) of the methyl group attached to C-7 is reported to lie between δ 1.07 and δ 1.20. This is true of **1–4** as well as **6**, the rearrangement product of **5**. It is also true of hydroxydelobanone from *Lindera triloba* to which relative configuration **8** has been assigned [5]. Conversely, the frequency of the C-7 methyl group in **5** and **7a,b** is δ 1.40, which indicates deshielding by the hydroxyl group on C-1. On this basis we suggest the relative stereochemistries shown in formula **5** and **7a,b**. The formation of **6** from **5** might involve epimerization at C-1 or C-7 in addition to the allylic rearrangement of the hydroxyl group originally on C-11. The congeners **1–4** possess the same C-7 stereochemistry as **5** and **7a,b** is not only suggested by their co-occurrence but is also indicated by the NOEs exhibited by **4** (Table 3).

Terpenoids are relative common in the large genus



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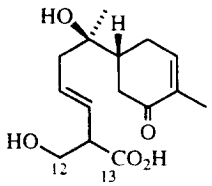

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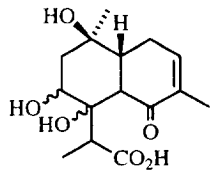

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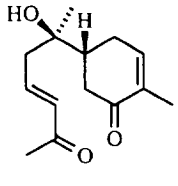

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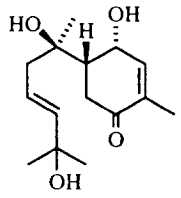

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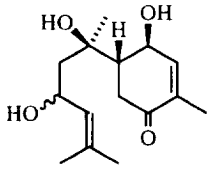

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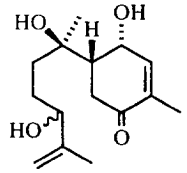
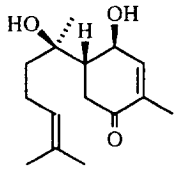


7a isomer A
b isomer B



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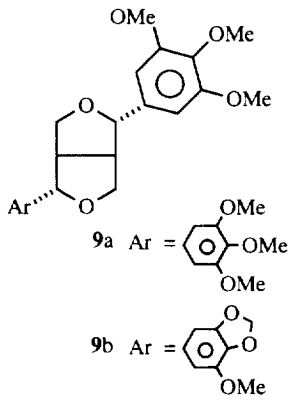

Achillea but bisabolenes have so far been reported only from *A. odorata* [14].

EXPERIMENTAL

Plant material. Achillea cretica L. was collected at Kato Pyrgos (Cyprus) on volcanic soil in May 1991. A voucher specimen #91/216 is on deposit in the Instituto di Botanica Farmaceutica dell'Università di Camerino, Italy.

Extraction and isolation. Above-ground parts (370 g) were extracted with Me_2CO (3 × 5 l) for 1 week. The crude gum (27 g) was adsorbed on 50 g of silica gel (Merck No. 7734 deactivated with 15% H_2O) and chromatographed over 400 g of the same adsorbent, 500-ml frs were collected as follows: frs 1–5 (petrol), 6–9 (petrol–EtOAc, 9:1), 10–14 (petrol–EtOAc, 4:1), 15–22 (petrol–EtOAc, 7:3), 23–28 (petrol–EtOAc, 3:2), 29–36 (petrol–EtOAc, 1:1), 37–44 (petrol–EtOAc, 2:3), 45–50 (petrol–EtOAc, 3:7), 51–57 (petrol–EtOAc, 1:4), 58–59 (EtOAc), 60 (EtOAc–MeOH, 19:1). Frs 9–14 were rechromatographed over a silica gel column and then by radial chromatography ($CHCl_3$–MeOH, 49:1) to give 48 mg of **9a**. Frs 15–22 were rechromatographed over a silica gel column and then by radial chromatography ($CHCl_3$–MeOH 97:3) to give 10 mg of a mixture of bisabolones. Frs 23–28 were also rechromatographed over silica gel (petrol–EtOAc, 4:1, 7:3 and 3:2) and then by radial chromatography ($CHCl_3$–MeOH, 24:1) to give, in order of polarity, sesartemin (**9b**, 18 mg), **1** (27 mg), mixts of **5** and **7a** (27 and 4 mg), complex mixtures of bisabolones (2 × 3 mg), **4** (11 mg), **7b** (14 mg), and **5** (12 mg). Frs 37–50 of the original chromatogram were resubjected to CC (petrol–EtOAc, 1:4 and 1:9) and then to radial chromatography to give 6 mg of impure **7b** and 8 mg of the mixture of **2** and **3**. Frs 51–57 were purified by radial chromatography to give an additional 9 mg of **5**.

7-*Acetoxy*-12-*hydroxy*-4-*oxobisabol*-2,10*E*-*diene* (**1**). Gum; MS PCI (isobutane) *m/z* (rel. int.) 295 $[M + H]^+$ (63.1), 251 (16.4), 235 (100); ^1H NMR Table 1; ^{13}C NMR Table 2.

Mixture of 7,12-*dihydroxy*-4-*oxobisabol*-2,9*E*-*dien*-13-*oic acid and* 7,9,10-*trihydroxy*-4-*oxobisabol*-2,11-*dien*-13-*oic acid* (**2** *and* **3**). Gum; MS PCI (isobutane) *m/z* (rel. int.) 501 (dimer of M^1-3 Hz, 71.5), 299 $[M^1 + H]^+$ (7.7), 283 $[M^2 + H]^+$, (6.1), 281 (10.1), 265 (36.6), 251 (100), 233 (94), 121 (45.3), ^1H NMR Table 1.

13-*Nor*-4,11-*dioxo*-7-*hydroxybisabol*-2,9*E*-*diene* (**4**). Gum; MS PCI *m/z* (rel. int.) 237 $[M + H]^+$ (64.9), 219

(15.7), 117 (100); ^{1}H NMR Table 1; ^{13}C NMR Table 2.

4-*Oxo*-1,7,11-*trihydroxybisabol*-2,9*E*-*diene* (**5**). Gum; MS PCI *m/z* (rel. int) 269 $[M+H]^+$ (9.8), 251 (29.4), 233 (100), 215 (18.6), 125 (61)' ^{1}H NMR (250 MHz) in Table 1; ^{13}C NMR in Table 2. On keeping for some months this substance had rearranged to a mixt. whose major component was **6**. ^{1}H NMR Table 1.

The mixture of **5** and **7a** was a gum; MS PCI *m/z* (rel. int.) 269 $[M+H]^+$ (13), 251 (40.2), 233 (100), 125 (80.1); ^{1}H NMR of **7a** in Table 1.

4-*Oxo*-1,7,10-*trihydroxybisabol*-2,11-*diene* (**7b**). Gum; MS PCI *m/z* (rel. int) 537, dimer of $[M+H]^+$ (40.2), 269 $[M+H]^+$ (32), 125 (100); ^{1}H NMR Table 1.

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