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Further constituents of *Achillea ageratum*

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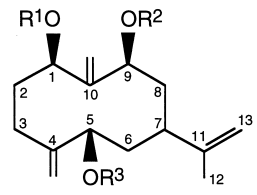
Abstract

Further study of the aerial parts of *Achillea ageratum* yielded the new ageratriol derivative 1-*O*-acetylageratriol, a new oplopane 7β-hydroxyoplop-11-enone and the phenoxychromone 6-demethoxycapillarisin.

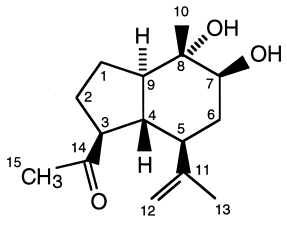
Keywords: *Achillea ageratum*; Anthemideae; Compositae; Ageratriol derivative; Germacrane; Oplopanone derivative; Phenoxychromone

1. Introduction

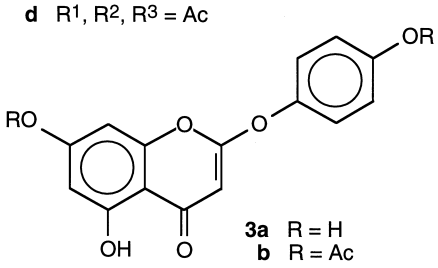
In an earlier article (Vieira, Kijjoa, Pereira, Gedris, & Herz, 1997), we described isolation of several new germacranes related to ageratriol (**1a**) and known flavonoids from the herb of a Portuguese collection of *Achillea ageratum* L. One of the new germacranes was 9-*O*-acetylageratriol (**1b**). Further study of the extract has now led to isolation of a new ageratriol derivative 1-*O*-acetylageratriol (**1c**), a new oplopane 7β-hydroxyoplop-11-enone (**2**), the chromone 6-demethoxycapillarisin (**3a**) (Komiya, Naruse, & Oshio, 1976; Hashidoko, Tahara, & Mizutani, 1991) and the β-D-glucoside of β-sitosterol.



1a R1, R2, R3 = H
b R1, R3 = H, R2 = Ac
c R1 = Ac, R2, R3 = H
d R1, R2, R3 = Ac



2a
b 11, 12 - dihydro



3a R = H
b R = Ac

^{1}H and ^{13}C NMR spectra of **1b** and synthetic triacetate **1d** were redetermined and are listed in Tables 1 and 2 for comparison with those of the new monoacetate **1c**[1]. Table 2 also includes revised assignments for **1a** and **1b**, namely an interchange of the frequencies formerly (Vieira et al., 1997) assigned to C-1 and C-5 based on the following experiments. HETCOR showed that H-5 of **1a** at δ 3.64 (Vieira et al., 1997) was

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[1] In Table 2 of Vieira et al. (1997), there are two misprints. In column 1 the chemical shift of the signal assigned provisionally to C-1 should have been δ 74.6, not 76.6, and the third column should have been headed **1b**†; i.e. the data in column 3 were those of 9-*O*-acetylageratriol in $CDCl_3$

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Table 1
^{1}H NMR spectra of compounds **1b**–**d** ($CDCl_3$, 300 MHz)

H	1b	1b[a]	1c	1 d
1α	4.23 *dd* (11, 4)	4.00 *m*	5.30 *m*	5.58 *dd* (10.5, 2)
2α	2.0–2.3 *c*	1.99 *c*	2.05–2.3 *c*	2.05–2.3 *c*
2β	2.0–2.3 *c*	1.99 *c*	2.05–2.3 *c*	2.05–2.3 *c*
3α	2.0–2.3 *c*	1.99 *c*	2.05–2.3 *c*	2.05–2.3 *c*
3β	2.0–2.3 *c*	1.99 *c*	2.05–2.3 *c*	2.05–2.3 *c*
5α	3.91 *dd* (11, 4)	3.67 *dt* (11, 3.5)	3.96 *dd* (11, 4)	5.01 *dd* (11, 4)
6α	1.62–1.8 *c*	1.46 *ddd* (14, 11, 3.5)	1.55–1.7 *c*	1.7–1.8 *c*
6β	1.62–1.8 *c*	1.67 *c*	1.55–1.7 *c*	1.7–1.8 *c*
7α	1.62–1.8 *c*	1.67 *c*	1.75–1.95 *c*	2.0 *c*
8α	1.89 *dd* (12, 5.5)	1.67 *c*	1.95 *ddd* (13, 5, 1)	1.89 *ddd* (13, 5, 1)
8β	1.62–1.8 *c*	1.67 *c*	1.51 *ddd* (13, 10.5, 9)	1.58 *ddd* (13, 11, 9)
9α	4.71 *dd* (11.5, 5.5)	4.93 *dd* (11.5, 5)	4.05 *dd* (11.5, 5.5)	4.98 *ddd* (11, 5.5)
12[b]	1.69 *s*	1.63 *s*	1.69 *s*	1.69 *s*
13a	4.68 *brs*	4.64 *brs*	4.68 *brs*	4.77 *brs*
13b	4.66 *brs*	4.64 *brs*	4.68 *brs*	4.74 *brs*
14a	5.45 *s*	5.38 *s*	5.74 *s*	5.60 *s*
14b	5.39 *s*	5.36 *s*	5.51 *s*	5.51 *s*
15a	5.18 *s*	4.98 *s*	5.21 *s*	5.54 *s*
15b	5.14 *s*	4.96 *s*	5.19 *s*	5.32 *s*
OAc[b]	2.04 *s*	1.97 *s*	2.05 *s*	2.09 *s*, 2.01 *s* 1.98 *s*

[a] In DMSO-d_6.
[b] Intensity three protons.

coupled to the carbon doublet at δ 74.6, which therefore represents C-5 rather than C-1, and that the two proton multiplet of H-1 and H-9 at δ 3.87 was coupled to the carbon signals at δ 69.3 and 72.0, which therefore arose from C-1 and C-9, respectively. Similarly for **1b** in DMSO, H-5 at δ 3.67 was coupled to the doublet at δ 74.5 which could therefore be attributed to C-5, H-9 at δ 4.93 was coupled to the doublet at δ 74.1, which therefore arose from C-9, and H-1 at δ 4.00 was coupled to the doublet at δ 69.7 (C-1) while long range COSY for **1b** in DMSO showed that H-14a, b were allylically coupled to the protons at δ 4.0 (H-1) and 4.93 (H-9), thus confirming these earlier assignments. The changed assignments for C-1 and C-5 in **1a,b** also required interchanges in the earlier assignments (Vieira et al., 1997) for C-1, C-5 and C-7

Table 2
^{13}C NMR spectra of compounds **1a**–**1 d** (75 MHz)

C	1a[a]	1b[a]	1b[b]	1c[b]	1 d[b]	1 d[a]
1	69.3 *d*	69.7 *d*	70.6 *d*	73.5 *d*	71.1 *d*	70.4 *d*
2	32.1 *t*	31.6 *t*	29.9 *t*	28.4 *t*	28.4 *t*	28.0 *t*
3	22.5 *t*	21.6 *t*	21.8 *t*	22.2 *t*	22.8 *t*	22.6 *t*
4	150.4 *s*	149.5 *s*	147.8 *s*	148.0 *s*	143.0 *s*	143.4 *s*
5	74.6 *d*	74.5 *d*	76.2 d	76.3 d	77.9 d	77.1 d
6	36.9 t	36.5 t	36.2 t	36.6 *t*	34.3 *t*	33.8 *t*
7	37.7 *t*	37.6 *t*	37.9 *d*	38.3 *d*	37.3 *d*	37.1 *d*
8	44.4 *t*	39.8 *t*	40.0 *t*	43.2 *t*	40.5 *t*	39.6 *t*
9	72.0 *d*	74.1 *d*	77.2 *d*	73.8 *d*	74.1 *d*	73.2 *d*
10	154.4 *s*	150.4 *s*	150.0 *s*	150.2 *s*	146.4 *s*	145.7 *s*
11	148.7 *s*	148.0 *s*	147.3 *s*	147.5 *s*	146.5 *s*	146.9 *s*
12	18.4 *q*	18.2 *q*	18.0 *q*	18.2 *q*	18.2 *q*	18.0 *q*
13	110.6 t	110.1 *t*	111.0 *t*	110.7 *t*	111.7 *t*	111.0 *t*
14	109.7 *t*	113.6 *t*	114.7 *t*	116.8 *t*	117.0 *t*	117.5 *t*
15	112.8 *t*	113.1 *t*	115.4 *t*	115.6 *t*	118.1 *t*	117.5 *t*
Ac		169.5 s	171.7 *s*	170.9 *s*	171.0, 169.8, 169.4 *s*	170.1, 169.2, 168.9 *s*
		21.0 *q*	21.3 *q*	21.3 *q*	21.9, 21.3, 21.3 *q*	21.0, 20.9, 20.9 *q*

[a] In DMSO-d_6.
[b] In $CDCl_3$.

in triacetate **1d** whose revised ^{13}C NMR spectrum in $CDCl_3$ is included in Table 2, while in the ^1H NMR spectrum of **1d** the earlier assignments (Vieira et al., 1997) of H-1 and H-5 had to be interchanged to align them with the signals of H-1 and H-5 in the spectra of **1a** (Vieira et al., 1997), **1b** and **1d**.

With this background the structure of the new monoacetate could be considered. Comparison of its ^1H NMR spectrum with the ^1H NMR spectra of **1a**, **1b** and **1d** (Table 1) showed that the acetate was clearly not located on C-5. The unlikely possibility that it was a C-9 epimer of **1b** was ruled out as follows. In the new isomer, the chemical shifts of two multiplets at δ 1.95 and 1.51 were essentially identical with the shifts of H-8α and H-8β in **1b** and **1d**, hence these signals could presumably be attributed to H-8α,β as well. Irradiation of the signal at δ 1.51 collapsed the signal of δ 4.05 to a doublet while irradiation of the signal at δ 5.30 had no effect on the signals of H-8α and H-8β. Conversely, COSY showed that the signal of the methine proton under the hydroxyl at δ 4.05 was coupled to the signals of H-8α and H-8β at δ 1.95 and 1.51 while the signal at δ 5.30 due to the methine proton under the acetate was coupled to signals (for 2-H_2) centered at δ 2.17. Hence the new derivative was 1-*O*-acetylageratriol.

Compound **2a** whose ^1H and ^{13}C NMR spectral data are listed in Table 3 was the 11,13-dehydro analog of an oplopane derivative **2b** previously reported from *Artemisia sieberi* Bess. (Marco et al., 1993). Although H-H decoupling and COSY were only partially useful due to overlap of pairs of CH_2 and CH signals (e.g. H-1 and H-6, H-2 and H-9 and H-4 and H-5), HETCOR established the relationship of the carbon multiplets to the ^1H signals while the HMBC spectrum (Table 4) allowed expansion to formula **2a** with the stereochemistry based on NOE data and on the similarity of the spectoscopic data of H-2a and H-2b. Oplopanes are most frequently found in members of the Senecioneae although a few have been isolated from members of the Anthemideae (Marco et al., 1993), Heliantheae (Jakupovic et al., 1988), Eupatoricae (Tamayo-Castillo, Jakupovic, Bohlmann, Rojas, Castro, & King, 1988) and Inuleae (Ahmed, Jakupovic, & Bohlmann, 1990).

6-Demethoxycapillarisin (**3a**) (Hashidoko, Tahara, & Mizutani, 1991; Komiya, Naruse, & Oshio, 1976) is one of a small group of 2-phenoxychromones (Adesogan, & Okunade, 1978; Ibewuike, Ogudaini, Ogunghamila, Martin, Gaillard, Bohlin et al., 1996) whose biogenesis probably involves oxidative rearrangement of the corresponding flavone (Crombie, 1984). It was characterized by MS, ^1H and ^{13}C NMR spectroscopic analyses and conversion to the diacetate **3b**.

Table 3
^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra of **2a** ($CDCl_3$) (multiplicities of ^{13}C signals by DEPT)

	H	C
1	1.75 *c*[a]	24.1 *t*
2	150 *c*[a]	29.0 *t*
3	2.53 *m*	54.8 *d*
4	1.95 *c*	46.8 *d*
5	1.95 *c*	49.9 *d*
6	1.80 *c*[a]	36.3 *t*
7	3.52 *dd* (11.7, 4.5)	78.2 *d*
8	–	75.7 *s*
9	1.47 *m*	54.1 *d*
10	1.45 *s*[b]	14.3 *q*
11	–	147.6 *s*
12	4.65 *brs*, 4.54 *brs*	111.9 *t*
13	1.50 *s*[b]	18.09 *q*
14	–	211.4 *s*
15	2.04 *s*[b]	30.7 *q*

[a] Intensity two protons.
[b] Intensity three protons.

Table 4
^1H ^{13}C HMBC spectral correlations of **2a** (75 MHz, $CDCl_3$)

^{13}C	^1H
C-3	H-15
C-5	H-6, H-12a,b, H-13
C-7	H-10
C-8	H-7, H-10
C-9	H-10
C-10	H-7
C-11	H-6, H-13
C-12	H-6, H-13
C-13	H-12,a,b
C-14	H-15

2. Experimental

Extraction and fractionation of the extract of the aerial parts of *Achillea ageratum* were described earlier (Vieira et al., 1997). Frs. 88–94 (1.9 g) were rechromatographed (Si gel 60), 50 ml subfrs. being collected as follows; subfrs. 1–25 (petrol–$CHCl_3$, 4:1), 25–91 (petrol–$CHCl_3$, 3:2). Purification of subfrs. 68–91 by RP-2 TLC (Si gel 60 F_{254}, Merck 7730, toluene–EtOAc–Me_2CO–HCO_2H, 60:35:5:1) gave **1c** (18 mg). Frs. 127–136 were combined and recrystallized from MeOH to give hispidulin (20 mg). The mother liquor was purified by TLC (Si gel, toluene–EtOAc – Me_2CO–HCO_2H, 60:35:5:1) to give the 2:1 mixture of 5,9-dihydroxy-1-oxogermacra-4(15),10(14),11(13)-triene and 5,9-dihydroxy-1(10)-epoxygermacra-4(15),11(13)-diene described previously (Vieira et al., 1997). After separation of this mixture the more polar components

were combined, extracted and subjected again to TLC (Si gel, toluene–EtOAc–Me_2CO–HCO_2H, 60:30:5:1, 3 runs). This afforded **2a** (6 mg). Fr. 156 (2.6 g) was rechromatographed (Si gel 60), 100 ml subfrs. being collected as follows: subfrs. 1–3 (petrol–$CHCl_3$, 7:3), subfrs. 4–7 (petrol–$CHCl_3$, 3:2), 8–12 (petrol–CHCl, 2:3), 13–30 (petrol–$CHCl_3$, 4:1), 31–55 ($CHCl_3$), 56–65 ($CHCl_3$–Me_2CO, 9:1), 66–72 ($CHCl_3$–Me_2CO, 7:3), subfrs. ($CHCl_3$–Me_2CO, 1:1). Subfrs. 40–45 were combined and recrystallized from $CHCl_3$ to give **3a** (43 mg). Frs. 187–200 were combined and precipitated from Me_2CO to give 3β-D-glucosyl-β-sitosterol (37 mg) identified by MS and NMR spectrometry and conversion to the tetraacetate.

2.1. 1-O-Acetylageratriol (*1b*)

Viscous liquid; PCI MS *m/z* (rel. int.) 295 ([M + H$^+$], 63), 277 (38), 235 (46), 217 (32), 199 (23); IR (film) γ_{max} cm^{-1}: 3600–3200 (OH), 3077, 3069, 2930, 2874, 2856, 1730, 1644, 1463, 1454, 1445, 1433, 1373, 1241, 1077, 1024, 960, 924, 895, 803; $[\alpha]_D^{20} + 31^\circ$ ($CHCl_3$, 0.16 g/100 ml); ^{1}H NMR in Table 1; ^{13}C NMR spectrum in Table 2.

2.2. 7β-hydroxyoplop-11-enone (*2a*)

Viscous yellow liquid; EI MS *m/z* (rel. int.) 252 ([M$^+$], 20), 219 (15), 183 (18), 167 (35), 149 (100), 141 (35), 135 (70); $[\alpha]_0^{25}$ -13.0° (c. 4×10^{-4} g/ml, $CHCl_3$); ^{1}H and ^{13}C NMR spectra in Table 3, HMBC spectrum in Table 4.

2.3. 6-Demethoxycapillarisin (*3a*)

EI MS *m/z* (rel. int.) 286 ([M$^+$], 100), 257 (10), 229 (5), 194 (10), 165 (5), 153 (37), 134 (30), 121 (17), 106 (20); ^{1}H NMR (DMSO-d_6) δ 12.80 (*s*, 5-OH), 7.19 (*dd*, *J* = 9 Hz, 2H, H-2′, 6′), 6.87 (*d*, *J* = 9 Hz, 2H, H-3′, 5′), 6.36 (*d*, *J* = 2 Hz, H-6), 5.02 (*s*, H-3); ^{13}C NMR (DMSO-d_6) δ 183.0 (C-4), 168.0 *s* (C-2), 163.9 *s* (C-7), 161.4 *s* (C-5), 156.1 *s* (C-4′), 154.9 *s* (C-8a), 143.1 *s* (C-1′), 121.7 *d* (C-2′,6′), 116.5 *d* (C-3′,5′), 102.0 *s* (C-4a), 99.3 *d* (C-3), 94.0 *d* (C-6), 86.6 *d* (C-8). Acetylation of **3a** (20 mg) in the usual manner furnished the diacetate **3b** (14 mg); ^{1}H NMR (DMSO-d_6) δ 12.96 (*s*, 5-OH), 7.49 (*d*, *J* = 9 Hz, 2H, H-2′, 6′), 7.32 (*d*, *J* = 9 Hz, 2H, H-3′, 5′), 6.97 (*d*, *J* = 2 Hz, H-8), 6.69 (*d*, *J* = 2 Hz, H-6), 5.35 (*s*, H-3), 2.30 and 2.29 (both *s* and 3H, Ac); ^{13}C NMR (DMSO-d_6) δ 183.6 *s* (C-4), 169.1 and 168.4 (both *s*, C═O of acetates), 167.4 (*s*, C-2), 160.7, 155.5, 153.7, 148.8, 148.3 (all *s*, C-7, 5, 4′, 8a, 1′), 123.9 *d* (C-2′, 6′), 121.9 *d* (C-3′, 5′), 106.6 *s* (C-4a), 106.2 and 101.4, (both *d*, C-6, C-3), 88.8 *d* (C-8), 20.9 and 20.8 (both acetate methyls).

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