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A GUAIANOLIDE AND A GERMACRANOLIDE FROM *ACHILLEA SANTOLINA*

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Key Word Index—*Achillea santolina*; Compositae; Anthemideae; guaianolide; germacranolide; flavonoid; santoflavone; X-ray analysis.

Abstract—An extract of whole plants of *Achillea santolina* afforded a new guaianolide and a new germacranolide in addition to two known guaianolides and three flavonoids. Structures were elucidated by spectroscopic methods, and the structure of the germacranolide was confirmed by a single crystal X-ray analysis. © 1997 Elsevier Science Ltd

INTRODUCTION

The genus *Achillea* comprises over 100 species distributed worldwide. *Achillea* species exhibit pharmacological activities such as anti-bacterial [1], anti-inflammatory [2] and antiallergic [3] properties.

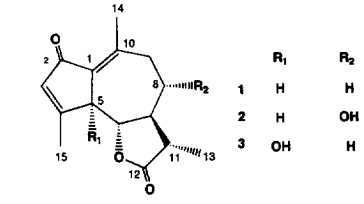
Many species, including *A. santolina* have been studied, and in addition to taxonomic studies on the distribution of flavonoids [4, 5], attention is still being paid to the sesquiterpene lactones [6–9]. Most species contain guaianolides [10], germacranolides [11], and less frequently eudesmanolides [12].

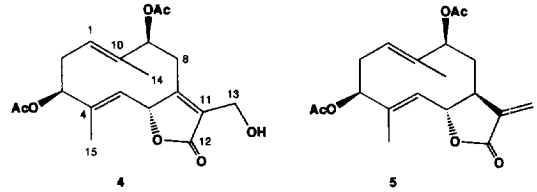
Phytochemical reinvestigation of *A. santolina* collected in north Egypt afforded in addition to the known compounds leucodin (**1**) [13], desacetylmatricarin (**2**) [13], and three flavonoids (**6–8**), a new guaianolide and a new germacranolide, **3** and **4**, respectively.

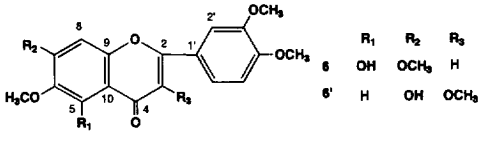
RESULTS AND DISCUSSION

The compounds were purified by various types of chromatography (see experimental), and the structures of known compounds, **1**, **2**, **7** and **8**, were elucidated by comparison with reported physical data.

The elemental composition of **3** was $C_{15}H_{18}O_4$ by HR-FAB-mass spectroscopy. The ^1H NMR data







(Table 1) showed the presence of a derivative of leucodin [13]. From the missing coupling $J_{5,6}$, the position of an additional hydroxyl group was deduced to be at C-5. Its α-orientation was confirmed by the downfield shift of the H-7 and H-9α signals, compared with those in leucodin (Table 1). The large coupling constant ($J_{6,7}$ = 10 Hz) confirmed the *trans*-arrangement of H-6 and 7. Therefore, the structure of **3** was 5-hydroxyleukodin.

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Table 1. ^{1}H NMR data of compounds **1** and **3–5** (400 MHz, $CDCl_3$)

	1	**3**	**4**	**5***
H-1	—	—	5.08, *ddd*, 2/3/12	5.25, *br dd*, 4/12
H-2α	—	—	2.55, *m*	2.58, *ddd*, 4/5/13
H-2β	—	—	2.36, *dt*, 10/12	2.35, *ddd*, 11/12/13
H-3	6.14, *qui*, 1	6.11, *d*, 0.5	5.17, *dd*, 6/10	5.19, *dd*, 5/11
H-5	3.48, *br d*, 10	—	4.42, *brd*, 10	4.81, *brd*, 9
H-6	3.60, *t*, 10	3.86, *d*, 10	5.41, *d*, 10	4.58, *dd*, 9/10
H-7	1.94, *dtd*, 3/10/13	2.72, *dtd*, 3/10/13	—	2.70, *dddd*, 3/3.5/10/10
H-8α	1.98, *m*	2.01, *ddd*, 3/6/13	3.30, *dd*, 1/13	2.18, *dd*, 3/13
H-8β	1.35, *q*, 13	1.39, *q*, 13	2.58, *dd*, 10/13	1.93, *ddd* 10/10/13
H-9α	2.31, *ddd*, 2/13/13	2.90, *br t*, 13	5.14, *dd*, 1/10	5.16, *dd*, 3/10
H-9β	†	2.17, *dd*, 6/13	—	—
H-11	2.23, *dq*, 7/13	2.20, *qd*, 6/13	—	—
H-13	1.24 (3H), *d*, 7	1.26 (3H), *d*, 6	4.46, *d*, 14	5.58, *d*, 3
H-13′	—	—	4.51, *d*, 14	6.25, *d*, 3.5
H-14	2.41, *s*	2.39, *s*	1.67, *s*	1.50, *br s*
H-15	2.27, *d*, 1	2.33, *s*	1.74, *d*, 1	1.73, *br s*
MeCO			2.09, *s*	2.06, *s*
			2.10, *s*	2.11, *s*

* Data taken from ref. [15].

† Could not be assigned due to the overlapping of many signals.

Table 2. ^{13}C NMR data of compounds **1**, **3** and **4** (100 MHz, $CDCl_3$)

	1	**3**	**4**
C-1	131.8	134.2	127.9
C-2	195.8	194.1	30.2
C-3	135.4	135.2	77.6
C-4	169.9	171.5	137.3
C-5	52.5	79.5	122.7
C-6	84.1	86.2	80.2
C-7	56.3	47.6	159.8
C-8	25.9	27.3	32.5
C-9	37.5	35.1	79.5
C-10	152.1	155.4	135.3
C-11	41.1	41.1	131.8
C-12	177.5	177.5	172.9
C-13	12.2	12.3	54.6
C-14	21.5	22.3	11.4
C-15	19.7	15.6	11.7
MeCO			21.2, 21.0
MeCO			170.7, 169.8

The elemental composition of **4** was $C_{19}H_{24}O_7$ by HR-FAB-mass spectrometry. The ^{1}H NMR spectral data of 4 (Table 1) showed that it was a sesquiterpene lactone of the *trans*, *trans*-germacranolide type. The ^{1}H NMR spectrum exhibited a methyl singlet at δ_H 1.67 (H_3-14), a doublet at δ_H 1.74 (H_3-15), and two acetyl singlets at δ_H 2.09 and 2.10. The stereochemistries at C-3 and C-9 were tentatively deduced from the couplings ($J_{9\alpha,8\beta}$ = 10 Hz, and $J_{2,3}$ = 6 Hz) [14], and by comparison with haageanolide acetate (**5**) [15]. From the ^{13}H NMR and DEPT spectra, a carbon signal with two hydrogen atoms [δ_C 54.9 with δ_H 4.46 and 4.51] could be ascribed to be the primary alcohol attached to C-11 position. Finally, a crystal suitable for X-ray analysis was grown from methanol and the structure was solved by the direct method. A computer-generated perspective drawing and bond lengths are shown in Fig. 1 and Table 3, respectively. As expected, two acetoxyl groups are β and the C-6 oxygen at C-6 is α.

Although the ^{1}H NMR and ^{13}C NMR spectral data of **6** in $CDCl_3$ were essentially identical with those reported for a flavone, isolated from the same species collected in Pakistan [16] and ascribed structure 6′, in the ^{1}H NMR spectrum in DMSO-d_6, one D_2O exchangeable proton was observed in a low field region (δ_H 12.89) together with five aromatic protons, as for the presumed 6′. The UV spectrum also showed some discrepancy in that on addition of $AlCl_3$ to the methanol solution, a significant bathochromic shift was observed in absorption band I (338→372 nm),

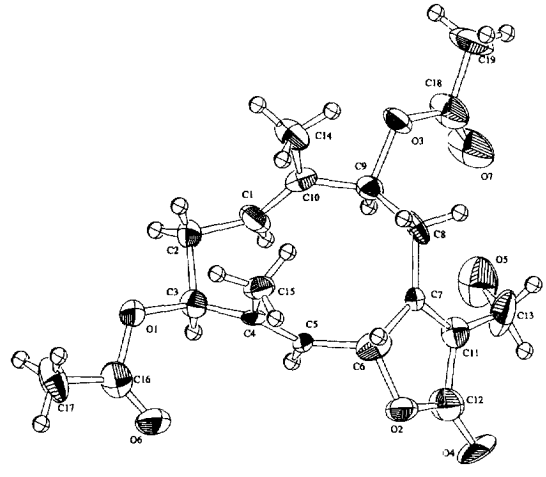

Fig. 1. Computer-generated perspective drawing of compound **4**.

Table 3. Bond lengths (Å) for compound **4** with their e.s.d.'s in parentheses

Atom	Atom	Distance
O1	C3	1.48 (2)
O1	C16	1.41 (2)
O2	C6	1.44 (2)
O2	C12	1.36 (2)
O3	C9	1.50 (1)
O4	C12	1.17 (2)
O5	C13	1.38 (2)
O6	C16	1.15 (2)
O7	C18	1.15 (2)
C1	C2	1.49 (2)
C1	C10	1.34 (2)
C2	C3	1.53 (2)
C3	C4	1.49 (2)
C4	C5	1.33 (2)
C4	C15	1.50 (2)
C5	C6	1.49 (2)
C6	C7	1.53 (2)
C7	C8	1.51 (2)
C7	C11	1.33 (2)
C8	C9	1.54 (2)
C9	C10	1.50 (2)
C10	C14	1.51 (2)
C11	C12	1.49 (2)
C11	C13	1.47 (2)
C18	C19	1.51 (2)

while no shift was observed on the addition of sodium acetate, as was the case for 6′. This evidence strongly indicated that the free hydroxyl group must be located at the 5-position. Therefore, the structure of the flavone is most probably that shown as **6**, which has been isolated from several sources as eupatilin 7-methyl ether (=5-hydroxy-6,7,3′,4′-tetramethoxyflavone). Contrary to the melting point reported for santoflavone (150°), that of **6** (182°) was close to that reported for eupatilin 7-methyl ether (187–188° [17] and 188–189° [18]), and there was no optical rotation, as expected from the structure. With some revision of ^{13}C NMR signal assignments, almost all HMBC correlations in the literature can be explained on the basis of structure **6**.

EXPERIMENTAL

General. Mps: uncorr.; ^1H and ^{13}C NMR: 400 MHz and 100 MHz, respectively, with TMS as an int. standard; Prep. HPLC: ODS [Inertsil (GL Science, Tokyo): 20 mm × 250 mm with H_2O–MeOH at 6 ml min^{-1} (A) or 6 mm × 250 mm with H_2O–MeOH at 1.6 ml min^{-1} (B), detection: UV at 254 nm].

Plant material. Whole plants of *Achillea santolina* L. were collected from the West Desert, north of Egypt, in 1992. A voucher specimen was deposited in the Herbarium of the Faculty of Science, Minia University, El-Minia, Egypt.

Extraction and Isolation. The air-dried aerial parts of *A. santolina* (2.5 kg) were extracted with $CHCl_3$–MeOH (1:1, 3 l), ×3 each for 48 hr. The combined extract was concd *in vacuo* and the residue was dissolved in 95% MeOH (1 l). The soln was washed with *n*-hexane (1 l) and the aq. MeOH layer was concd *in vacuo*. The residue was suspended in H_2O (1 l), and then the suspension was extracted with EtOAc (1 l) and *n*-BuOH (1 l) successively. The EtOAc layer was concd to give 55 g of residue.

The EtOAc Fr. was chromatographed over silica gel (200 g) with mixts of $CHCl_3$ and MeOH with increasing MeOH contents [$CHCl_3$ (2 l), $CHCl_3$–MeOH (199:1, 2 l), $CHCl_3$–MeOH (99:1, 2 l), $CHCl_3$–MeOH (49:1, 1.5 l), $CHCl_3$–MeOH (24:1, 1.5 l), $CHCl_3$–MeOH (47:3, 1.5 l), $CHCl_3$–MeOH (9:1, 1.5 l), $CHCl_3$–MeOH (7:1, 1.5 l), $CHCl_3$–MeOH (17:3, 1.5 l), $CHCl_3$–MeOH (7:3, 1.5 l) and $CHCl_3$–MeOH–H_2O (700:300:1, 1.5 l)] successively; 250 ml fr. were collected.

Frs 6–10 were combined and evapd *in vacuo* to give a residue (1.3 g), from which a mixt. of two crystalline materials was obtained by filtration. The mixt. was recrystallised from MeOH to give compound **6** as pale yellow crystals. While compound **1** was obtained in the mother liquid as colourless crystals, which were collected by filtration.

Frs 17–19 of the EtOAc fr. were combined and evapd *in vacuo* to give a residue (0.8 g), which was chromatographed over silica gel (50 g) with a solvent system [$CHCl_3$–*n*-hexane (9:1, 500 ml)], and was collected as one fr., and then with a gradient solvent system of $CHCl_3$–MeOH (199:1, 1 l → 99:1, 1 l), frs of 8 g were collected. From frs 121–176, 400 mg of residue was obtained. On further sepn by HPLC (A) with a solvent system of 45% MeOH in H_2O, compound **3** (20.0 mg) was obtained (R_t = 29 min).

Frs 25–57 of the same EtOAC fr. were combined and evapd *in vacuo* to give a residue (1.0 g), which was chromatographed over silica gel (50 g) with a solvent system [$CHCl_3$–*n*-hexane (9:1, 500 ml)], 500 ml, $CHCl_3$ and a gradient system $CHCl_3$–MeOH (199:1, 1 l) and $CHCl_3$–MeOH (9:1, 1 l)], which was collected as four frs. Fr. no. 4 (350 mg) was rechromatographed over silica gel (50 g) with a gradient solvent system [$CHCl_3$–MeOH (199:1, 500 ml → 99:1, 500 ml)], frs of 8 g were collected. From frs 44–70, 366 mg of residue was obtained, which was sepd by HPLC (A) using a solvent system of 40% MeOH in H_2O to give compound **2** (40 mg) (R_t = 28 min).

Frs 20–24 were combined and evapd *in vacuo* to give a residue (2.1 g), which was chromatographed over silica gel (50 g) with the same solvent system as for the previous fr., four frs were collected. Fr. nos. 3 and 4 were combined and evapd *in vacuo* to give a residue (1.1 g), which was rechromatographed over silica gel with a gradient solvent system [$CHCl_3$–MeOH (199:1, 1 l → 49:1, 1 l)], frs of 15 g were collected. From frs 34–50, compound **4** was obtained by HPLC (A) sepn using 50% MeOH in H_2O [R_t = 29

min]. While compound **8** (15 mg) was obtained as a yellow crystalline material from frs 57–76, which was sepd by filtration.

Frs 13–15 of the EtOAc fr. were combined and evapd *in vacuo* ti give a residue (1.5 g) which gave compound **7** (30 mg) as a yellow crystalline material.

Known compounds isolated. Leucodin (**1**), colourless crystals, mp 199–201°, $[\alpha]_D$ +19.1° ($CHCl_3$, *c* 0.62), ^1H and ^{13}C NMR ($CDCl_3$): see Tables 1 and 2 [13]. Desacetylmatricarinin (**2**), colourless needles, mp 108°, $[\alpha]_D$ +20° ($CHCl_3$, *c* 0.17) [13]. Eupatolin (5,3′-dihydroxy-6,7,4′-trimethoxy flavone) (**7**), pale yellow crystals, mp 190–192° [17, 18] Cirsimartin (5,4′-dihydroxy-6,7-dimethoxy flavone) (**8**), pale yellow crystals, mp 262° [19].

5-*Hydroxyleucodin* (**3**). $[\alpha]_D$ +33.3° ($CHCl_3$, *c* 0.60), IR ν_{max}^{KBr} cm^{-1}: 3375, 2900, 1770, 1665, 1620, 1605, 1425, 1280, 1160, 985, 880; UV λ_{max}^{MeOH} nm (log ε): 245 (3.97), 262sh (3.91); ^1H and ^{13}C NMR ($CDCl_3$): Tables 1 and 2; HR-FAB-MS (negative centroid) *m/z*: 261.1136 $[M-H]^-$ ($C_{15}H_{17}O_4$ requires 261.1127).

3,9-*Diacetoxy*, 13-*hydroxy*-1(10),4,7(11)-*germacratrien*-12,6-*olide* (**4**). Colourless crystals, mp 153–155°, $[\alpha]_D$ +51.5° ($CHCl_3$, *c* 0.33), IR ν_{max}^{KBr} cm^{-1}: 3450, 2900, 1745, 1725, 1705, 1435, 1385, 1370, 1260, 1225, 1075, 1015, 990, 970; UV λ_{max}^{MeOH} nm (log ε): 209 (4.23), 240sh (3.47); ^1H and ^{13}C NMR ($CDCl_3$): see Tables 1 and 2; HR-FAB-MS (negative centroid) *m/z*: 363.1445 $[M-H]^-$ ($C_{19}H_{23}O_7$ requires 363.1444).

Eupatilin 7-methyl ether (=*santoflovone*) (**6**). Pale yellow crystals, mp 184–186°, UV λ_{max}^{MeOH} nm (log ε): 214 (4.48), 242 (4.23), 275 (4.23), 338 (4.38), $\lambda_{max}^{MeOH+AcONa}$ nm (log ε): 214 (4.51), 242 (4.24), 276 (4.24), 338 (4.29), $\lambda_{max}^{MeOH+AlCl_3}$ nm (log ε): 216 (4.52), 262 (4.19), 288 (4.24), 372 (4.42); ^1H NMR ($CDCl_3$): δ 7.52 (1H, *dd*, *J* = 2.5 and 8.5 Hz, H-6′), 7.34 (1H, *d*, *J* = 2.5 Hz, H-2′), 6.98 (1H, *d*, *J* = 8.5 Hz, H-5′), 6.60 (1H, *s*, H-8), 6.54 (1H, *s* H-3). 3.94, 3.89, 3.86 and 3.74 (each 3H, each *s*, OMe ×4); ^1H NMR (DMSO-d_6): δ 12.89 (1H, *s*, ex. with D_2O, 5-OH), 7.70 (1H, *dd*, *J* = 2.5 and 8.5 Hz, H-6′), 7.58 (1H, *d*, *J* = 2.5 Hz, H-2′), 7.13 (1H, *d*, *J* = 8.5 Hz, H-5′), 7.01 (1H, *s*, H-8), 6.95 (1H, *s*, H-3), 3.94, 3.90, 3.87, 3.75 (each 3H, each *s*, OMe ×4); ^{13}C NMR ($CDCl_3$): δ 182.6 (C-4), 164.0 (C-2), 158.9 (C-7), 153.3, 153.1, 152.4, 149.4 (C-3′), 132.7 (C-6), 123.8 (C-1′), 120.1 (C-6′), 111.3 (C-5′), 108.9 (C-2′), 106.2 (C-10), 104.5 (C-3), 90.6 (C-8), 60.9 (OMe on C-6), 56.4, 56.2, 56.1 (OMe on C-7, 3′ and 4′); ^{13}C NMR (DMSO-d_6): δ 182.5 (C-4), 163.9 (C-2), 158.9 (C-7), 152.9 (C-4′), 152.5 (C-5), 152.3 (C-9), 149.3 (C-3′), 132.2 (C-6), 123.1 (C-1′), 120.4 (C-6′), 111.9 (C-5′), 109.7 (C-2′), 105.4 (C-10), 103.9 (C-3), 91.9 (C-8), 60.3 (OMe on C-6), 56.8, 56.2, 56.0 (OMe on C-7, 3′ and 4′).

A single crystal X-ray analysis of **4**. A crystal (0.2 mm × 0.3 mm × 0.4 mm) was grown by slow evapn of the MeOH. All data were obtained with a Rigaku AFC-5S automated four circle diffractometer with graphite-monochromated Mo-Kα radiation. Crystal data: $C_{19}H_{24}O_7$, M_r = 364.4, orthorhombic, space group $P2_12_12_1$, *a* = 10.313 (7) Å, *b* = 18.224 (4) Å, *c* = 9.083 (8) Å, *V* = 1842 (2) $Å^3$, *Z* = 4, *Dx* = 1.314 g cm^{-3}, F(000) = 776, and μ (MoKα) = 0.935 cm^{-1}. The intensities were measured using the $\omega/2\theta$ scan mode up to 45°. Three standard reflections were monitored every 150 measurements. The data were corrected for the Lorentz and polarization factors. Absorption correction was applied, but decay correction was not applied. Of the 1436 independent reflections which were collected, 732 reflections with $I > 3.0\sigma(I)$] were used for the determination and refinement. The structure was solved by the direct method using a TEXSAN crystallographic software package [20]. All non-H atoms were found in a Fourier map. All H atoms were calculated at geometrical positions and not refined. The refinement of atomic parameters was carried out by the full matrix least squares refinement, using anisotropical temperature factors for all non-H atoms. The final refinement converged with *R* = 0.067 and R_w = 0.069 for 235 parameters. The minimum and maximum peaks in the final difference Fourier map were −0.19 and 0.26 $eÅ^{-3}$. Atomic scattering factors were taken from the 'International Tables for X-ray Crystallography' [21]. The final atom coordinates, and a list of the temperature factors and final structure factors have been deposited at the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB12 1EW, U.K.

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