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GUAIANOLIDES AND OTHER CONSTITUENTS OF *HELIANTHUS MICROCEPHALUS*

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Key Word Index—*Helianthus microcephalus*; Compositae; Heliantheae; guaianolides; sesquiterpene lactones; flavonoids; flavan; eudesmane.

Abstract—The chloroform extract of the aerial parts of *Helianthus microcephalus* afforded three guaianolides, the flavones hymenoxin and nevadensin, (2*S*)-5,7-dimethoxy-4′-hydroxyflavan and 1β,6α-dihydroxy-4(15)-eudesmene.

INTRODUCTION

In continuation of our earlier work on *Helianthus* species [1, 2], we have examined a South Carolina collection of *Helianthus microcephalus* T. & G. (*Helianthus* sec. Divaricati, ser. Microcephali). Isolated were threee closely related guaianolides **1**, **2** and **3**, a new flavan **4a**, the flavonoids nevadensin (**5a**) and hymenoxin (**5b**), and the eudesmane derivative **6**.

RESULTS AND DISCUSSION

The structure and relative stereochemistry of the main sesquiterpene lactone constituent **1**, mp 173–174°, were deduced from its MS (loss of a unit of *m/z* 143 representing $C_5H_7O_3$), IR (see Experimental) and ^{1}H and ^{13}C NMR data (Tables 1 and 2). The extensive decoupling experiments which formed part of the NMR study will not be discussed in detail. Due to superposition of several chromophores the CD curve which exhibited a relatively weak negative maximum at 268 nm ($[\theta]_{268} = -1210$) offered no secure basis for deducing the absolute stereochemistry of the guaianolide nucleus, although it is likely to be that shown in the formula (5*S*,6*R*,7*R*,8*R*). The epoxide ester side chain was derived from biological oxidation of an angelyl side chain and hence has 2′*R**, 3′*R** stereochemistry because of the coincidence of its carbon signals with those of authentic epoxyangelates [3–5]. Non-crystalline lactone **2**, obtained only in very small amount, was obviously the $\Delta^{10(14)}$-isomer of lactone **1** on the basis of its ^{1}H NMR spectrum (Table 1). We assign it the usual 1α(H)-stereochemistry because of the value of $J_{1,5}$(8 Hz).

Lactone **3** was the 3,4-epoxide of **1** on the basis of its ^{1}H and ^{13}C NMR spectra (Tables 1 and 2); the NOE data listed in Table 3 which show that H-6 is *trans* to H-5, H-7 and H-8 provide independent verification for the relative stereochemistry assigned to these compounds. The relative stereochemistry of the 3,4-epoxide linkage is not known, although it has been claimed [6] that the stereochemistry of such 3,4-epoxides can be assigned by taking into the consideration the chemical shift of H-6, with a β-oriented epoxide shifting H-6 to lower field. This, however, cannot be correct as inspection of the literature, [e.g. 7–11] shows that it is the presence or absence or the orientation of a substituent at C-8 which affects the chemical shift of H-6. If the C-8 substituent is α, H-6 is shifted downfield; if the substituent is β, H-6 is shifted upfield. If a 1,10-double bond is present, the shift of H-6 is not affected significantly regardless of the orientation of the epoxide.

That compound **4a** was a 5,7,4′-dimethoxymonohydroxyflavan was apparent from the ^{1}H and ^{13}C NMR spectra listed in Tables 4 and 5.* The location of the hydroxyl group at C-4′ could be deduced from the MS [14] and the paramagnetic shifts of H-2′,6′ and H-3′,5′ in the ^{1}H NMR spectrum of the derived acetate (Table 4). Finally, comparison of the CD curve of **4a** (see Experimental) with those of flavans of known absolute configuration [15] indicated that the new compound had the *S* configuration at C-2.

Analysis of the ^{1}H NMR spectrum of a fifth substance from *H. microcephalus* (see Experimental) showed that it was 1β,6α-dihydroxy-4(15)-eudesmene (**6**). A search of the literature revealed that the substance had been isolated earlier [16] from a *Senecio* species. Its previously unreported ^{13}C NMR spectrum is listed in Table 2; assignments are based in part on comparison with those of related compounds [17, 18].

Structures **1** and **2** have previously been ascribed to two non-crystalline guaianolides from *Trichogonia santosii* [19]. Since our lactone **1** has a relatively high mp it is possible that the *Trichogonia* lactones contain enantiomeric angelate-derived ester side chains. As we have pointed out the ^{1}H NMR spectra of such isomers cannot be differentiated easily [4, 5]. More recently a lactone, mp 176–177°, apparently identical with our **1**, has been isolated from *Helianthus glaucophyllus* and an Indiana collection of *H. microcephalus* [20]. Reference was made to an X-ray analysis which established the 2*R*,3*R* config-

*Assignments in Table 5 are based on those for similar compounds reported previously [12, 13] with the exception of assignments for C-2 and C-3 which differ from those in the literature [13] and are based on single frequency decoupling experiments.

Table 1. ^{1}H NMR spectra of compounds **1–3** (270 MHz)

H	1*	1†	2*	3*	3†
1	—	—	3.35 *brd* (8)	—	—
3	6.20 *m*	5.96*m*	6.18 *t* (1)	3.44 *s*	3.06 *s*
5	3.50 *br d* (10)	2.62 *br d*	3.19*m*	3.46 *br d*	2.84 *br d* (10.5)
6	4.08 *t* (10)	3.62 *t*	4.53 *dd* (10.9)	4.12 *t* (10)	3.51 *t* (10.5)
7	3.14 *dddd* (10,3,3,2)	obsc.	3.22 *m*	3.14 *dddd* (10,3,3,1.5)	1.96 *dddd* (10.5,3,3,2)
8	5.74 *dt* (5.5,2)	5.16 *br d*	5.71 *m*	5.78 *dt* (6,1.5)	5.11 *br d*
9a	2.85 *dd* (15,5.5)	2.28 *dd* (15,6)	2.60§	2.86 *dd* (15,6)	2.11 *dd*
9b	2.77 *br d* (15)	1.86 *br d*		2.71 *br d* (15)	1.71 *br d*
13a	6.2 4*d* (3)	6.00 *d*	6.35 *d* (3.5)	6.26 *d* (3)	6.04 *d*
13b	5.48 *d* (3)	5.00	5.62*d* (3.5)	5.48 *d* (3)	5.01 *d*
14	2.40 *br* ‡	2.29 *br* ‡	5.15 *br* 5.01*s*	2.33 *br* ‡	2.09 *br* ‡
15	2.34 *br* ‡	1.94 *br* ‡	2.39 *br* ‡	1.82 *s* ‡	1.59 *s* ‡
3′	299 *q* (5.5)	2.40 *q*	3.01 *q*	3.03 *q*	2.51 *q*
4′‡	1.18 *d* (5.5)	0.92 *d*	1.19 *d*	1.24 *d*	1.00 *d*
5′‡	1.44 *s*	1.12 *s*	1.49 *s*	1.46 *s*	1.23 *s*

* Run in $CDCl_3$.
† Run in C_6D_6.
‡ Intensity 3 protons.
§ Centre of AB part of ABX system where X = H-8.

uration for the angelate-derived ester side chain, presumably on the assumption that the absolute configuration of the guaianolide nucleus is 5*S*,6*R*,7*R*,8*R*. However, the ^{13}C NMR spectrum reported in this publication differed significantly from that given in Table 2 in the chemical shifts allocated to C-1, C-5, C-7, C-11, C-12 and C-1′. The Indiana collection did not yield lactones **2** and **3**, but several more highly- unsaturated lactones resulting from elimination of the ester function; and instead of **4**, **5a,b** and **6** 7,4′-dimethoxy-5-hydroxy flavan and the flavones ladanetin, eupatin, casticin and mikanin.

EXPERIMENTAL

Helianthus microcephalus T. & G. was collected by Dr John B. Nelson on 5 Sept. 1986 in Chester Co., South Carolina (voucher Nelson 4945 on deposit in herbaria of Florida State University and University of South Carolina). The dried aerial parts (3 kg) were extracted with $CHCl_3$ and worked-up in the usual fashion [21] to give 35 g of crude gum which was adsorbed on 60 g of silica gel (Merck, particle size 0.063–0.200 mm) and chromatographed over 600 g of the same adsorbent packed in a *n*-hexane, 100 ml fractions being collected as follows: 1–6 (hexane), 7–16 (hexane–EtOAc, 9:1), 17–26 (hexane–EtOAc, 4:1), 27–36 (hexane–EtOAc, 7:3), 37–46 (hexane–EtOAc, 7:3), 47–56 (hexane–EtOAc, 3:2), 57–66 (hexane–EtOAc, 2:3), 67–76 (hexane–EtOAc, 3:7), 77–86 (hexane–EtOAc, 1:4), and 87–96 (EtOH).

Frs 43–50 (1.25 g) were rechromatographed over 50 g of silica gel. Elution with C_6H_6 gave 0.165 g of (2*S*)-5,7-dimethoxy-4′-hydroxyflavan (**4a**) as a yellow powder. Purification of frs 51–55 by CC (C_6H_6–Me_2CO, 19:1) followed by PTLC (*n*-hexane–EtOAc, 3:2) afforded 21.5 mg of 1β,6α-dihydroxy-4(15)-eudesmene (**6**) as a colourless gum. Trituration of frs 56–64 with hexane–EtOAc (1:1) gave 54 mg of nevadensin (**5a**), mp 191–193°, identical (NMR, MS, TLC) with an authentic sample. The mother liquors after concentration *in vacuo* afforded 2.01 g of gum which after CC (silica gel, C_6H_6 and increasing amounts of Me_2CO) gave impure **3**. Rechromatography (silica gel, C_6H_6–EtOAc, 4:1) furnished 445 mg of pure **3** as a gum which on standing overnight in Et_2O deposited crystalline material, mp 71–73° (dec).

Frs 65–96 on standing in Et_2O–Me_2CO deposited yellow crystalline hymenoxin (**5b**), 215 mg, mp 214–216°, identical (NMR, MS, UV) with an authentic sample. The mother liquors were combined and rechromatographed (silica gel, C_6H_6–Me_2CO 9:1). Fr. 10 on trituration with hexane–EtOAc deposited 1.4 g of **1**, mp 173–174°. Purification of frs 11–17 by a combination of CC (silica gel) and TLC (C_6H_6–Me_2CO, 17:3, multiple developments) furnished an additional 1.03 g of **1** and 6 mg of **2** as a gum.

(5S,6R,7R,8R,2′R*,3R*)-2-*Oxo*-8-(2-*methyl*-2,3-*epoxybutan*-

Table 2. ^{13}C NMR data of compounds **1**, **3** and **6** (67.89 MHz, $CDCl_3$)

C	1	3	3‖	6
1	132.63 *s*‡	131.99 *s*‡	132.42 *s*‡	79.06 *d**
2	194.91 *s*	196.64 *s*	196.57 *s*	31.96 *t*
3	135.87 *d**	63.06 *d*	63.14 *d**	35.16 *t*
4	169.06 *s*†	62.30 *s*	62.13 *s*	146.25 *s*
5	53.0;*d**	50.70 *d*	50.51 *d**	55.95 *d*
6	78.60 *d**	74.91 *d*	74.58 *d**	67.03 d*
7	55.06 *d**	56.36 *d*	55.99 *d**	49.40 *d*
8	65.58 *d**	66.15 *d*	65.99 *d**	18.27 *t*
9	41.06 *t*	41.07 *t*	40.60 *d*	36.33 *t*
10	145.77 *s*	154.54 *s*	151.76 *s*	41.70 *s*
11	134.28 *s*‡	134.16 *s*‡	135.10 *s*‡	26.07 *d*
12	167.82 *s*	167.55 *s*†	167.22 *s*†	21.0 *q**
13	120.40 *t*	120.16 *t*	119.01 *t*	16.24 *q**
14	23.15 *q*§	24.44 *q*§	24.02 *q*§	11.58 *q*
15	19.69 *q*§	18.60 *q*§	18.64 *q*§	107.78 *s*
1′	168.95 *s*†	168.86 *s*†	168.79 *s*†	
2′	59.32 *s*	59.26 *s*	59.21 *s*	
3′	59.81 *d**	59.90 *d*	59.59 *d**	
4′	13.66 *q**	13.60 *q*	13.88 *q**	
5′	19.00 *q*§	19.02 *q*§	19.21 *q*§	

* Assignments by selective decoupling.

‡†§ Assignments with the same sign in each column may be interchanged.

‖ Run in C_6D_6.

¶Assignments by comparison with spectra of eudesmanediols in refs [14, 15].

Table 3. NOE spectrum of compound **3**

Irradiated	Observed
H-3	H-15 (13)
H-5	H-7 (10)
	H-9b (5)
	H-15 (10)
H-6	none
H-7	H-5 (8)
	H-8 (10)
H-9a	H-8 (8)
	H-14 (2.5)
H-9b	H-8 (20)
H-14	H-9a (3.5)
H-15	H-3 and H-5 (22)

Table 5. ^{13}C NMR spectrum of compound of compound **4a** ($CDCl_3$, 270 MHz)

C	δ
2	77.55 *d*
3	29.35 *d**
4	19.35 *t**
5	158.57 *s*
6	93.57 *d*
7	155.32 *s*
8	91.45 *d*
9	156.40 *s*
10	103.49 *s*
11	133.93 *s*
2′,6′	127.66 *d*
3′,5′	115.40 *d*
4′	159.32 *d*
OMe	55.44 *q*
	53.34 *q*

* Assignments by selective decoupling.

Table 4. ^{1}H NMR spectra of compounds **4a,b** (270 MHz, $CDCl_3$)

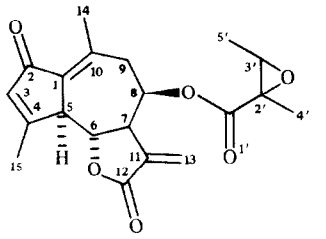
H	4a	4b
2ax	4.90 *dd* (10.5,2.5)	4.98 *dd*
3ax	1.98 *dddd* (13.5,11,10.5,3)	2.00 *dddd*
3eq	2.15 *dddd* (13.5,6,3,2.5)	2.15 *dddd*
4ax	2.61 *ddd* (16.5,11,3)	2.62 *ddd*
4eq	2.74 *ddd* (16.5,6,3)	2.74 *ddd*
6	6.07 *d* (2.5)	6.08 *d*(2)
8	6.12 *d* (2.5)	6.11 *d*(2)
2′,6′	7.30 *brd* (9)	7.42 *d*
3′,5′	6.84 *brd* (8.5)	7.10 *d*
O'Me	3.79	3.79
	3.72	3.74
Ac	—	2.29

oyl)-guaia-1(10),3,11(13)-*trien*-6,12-*olide* (**1**). Mp 173–174°; IR ($CHCl_3$) 1769, 1745, 1683, 1636 cm^{-1}; CD curve (MeOH) $[\theta]_{268}$-1210 (min), $[\theta]_{249}$ 0(max), $[\theta]_{215}$-828 (last reading); ^{1}H and ^{13}C NMR: Tables 1 and 2; (Calc. for $C_{20}H_{22}O_6$: m_r, 358.1416. Found: MW (MS), 358.1434). Low resoln MS *m/z* (rel. int): 358 (100), 243 (14.8), 242 (19.2), 241 (15.4) 227 (18.7), 214 (12.4), 213 (13.2), 199 (16.6), 197 (16.4), 196 (14.7), 186 (17.5), 185 (13.1), 171 (15.7), 169 (10.9), 146 (17.0), 109 (13.9), 91 (26.7).

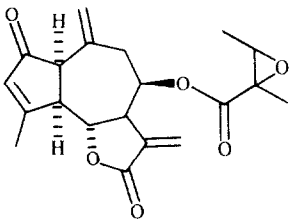
(5R,6R,7R,8R,2′R*,3′R*)-2-*Oxo*-8(2-*methyl*-2,3-*epoxybutanoyl*)-*guaia*-3,10(14),11(13)-*trien*-6,12-*olide* (**2**). Gum, IR ($CHCl_3$) 1760, 1748, 1699, 1633, 1618 cm^{-1}; ^{1}H NMR Table 1; MS *m/z* (rel. int.): 358 $[M]^+$ (14.1), 243 (20.2), 242 (33.2), 199 (10.3), 197 (13.8), 196 (20.2), 178 (23.3), 163 (19.5) 153 (18.2), 149 (35.8).

(5S,6S,7R,8R2′R*,3′R*)-2-*Oxo*-3,4-*epoxy*-8(2-*methyl*-2,3-*epoxybutanoyl*)-*guaia*-10 (14),11(13)-*dien*-6,12-*olide* (**3**). Mp 71–73° (dec); IR ($CHCl_3$) 1763, 1748, 1718, 1626 cm^{-1}; CD curve (MeOH) $[\theta]_{361}$-747 (min), $[\theta]_{327}$, 0, $[\theta]_{298}$+300 (sh), $[\theta]_{255}$ +672 (max), $[\theta]_{232}$ 0, $[\theta]_{212}$-3810 (last reading); MS *m/z* (rel. int.): 374 $[M]^+$ (0.4), 258 (26.4), 201 (6.8), 187 (10.4), 151 (9.8), 111 (5.4), 109 (8.1) 91 (8.1); ^{1}H- and ^{13}C-NMR: Tables 1 and 2.

(2S)-5,7-*Dimethoxy*-4′-*hydroxyflavan* (**4a**). Amorphous yellow powder, mp 119–120°; IR ($CHCl_3$) 1610, 1590, 1200, 1120 cm^{-1}; $[\alpha]_D^{24}$ +10.66° (*c* 1.57 g/100 ml $CHCl_3$). $[\alpha]_{Hg}^{25}$ 86.83° (*c* 1.13 g/100 ml $CHCl_3$); CD curve (MeOH) $[\theta]_{274}$ −1960 (min), $[\theta]_{273}$-1780, $[\theta]_{268}$-1980 (min), $[\theta]_{249}$-257 (max), $[\theta]_{241}$-824 (last reading); MS *m/z* (rel. int.); 286 $[M]^+$ (100), 179 (ion *d*, 72) [11], 167 (ion *a*, 52.3), 166 (26.8), 165 (7.8), 154 (7.0), 138 (13.7), 133 (14.0), 120 (ion **b**, 10.4), 105 (ion **c**, 1.7); Cl MS *m/z* (rel. int.) 287 $[M+1]^+$ (100), 167 (ion, **a**, 48.6); ^{1}H and ^{13}C NMR: Tables 4 and 5. Acetylation (Ac_2O–Py overnight) gave **4b** as a yellow amorphous solid, mp 92–94° (dec); IR ($CHCl_3$) 1754, 1615,



1



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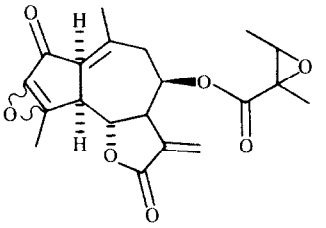

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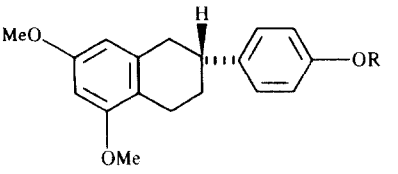
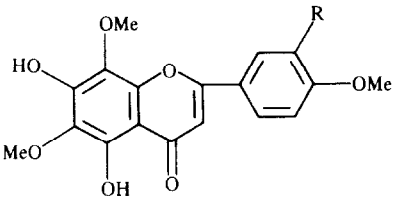
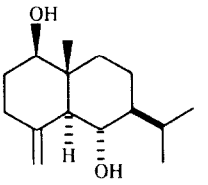


4a R = H
4b R = Ac



5a R = H
5b R = OMe



6

1592 cm^{-1}; MS *m/z* (rel. int.): 328 $[M]^+$ (64.3), 283 (53.2), 285 (9.2), 179 (20.6, ion, **d**), 167 (100, ion **a**); ^1H NMR: Table 4.

1β,6α-Dihydroxy-4(15)-eudesmene (**6**) Gum; IR ($CHCl_3$) 3140; MS *m/z* (rel. int.) 238 $[M]^+$ (15.8), 220 (100), 205 (26.0), 201 (3.2), 177 (94.6); ^1H NMR (270 MHz, $CHCl_3$): δ3.44 (*dd*, $J = 12$, 5 Hz, H-1α), 2.10 (*m*, H-3a), 1.75 *br d*, ($J = 9$ Hz, H-5α), 3.7 (4*t*, $J = 9$ Hz, H-6β), 2.73 (*m*, $J = 7$, 3 Hz, H-11), 0.88 and 0.96 (each *d*, 3H, $J = 7$ Hz, H-12 and H-13), 0.70 (*s*, 3H, H-14), 5.04 and 4.76 (each *br*, H-15a,b); (C_6D_6) δ3.40 (*dd*, $J = 11.5$, 5 Hz, H-α), 1.65 (*br d*, $J = 9$ Hz, H-5α), 3.68 (*t*, $J = 9.5$ Hz, H-6β), 2.51 (*m*, $J = 7.2$ Hz, H-11), 0.94*d* and 0.99*d* ($J = 7$ Hz, H-12 and H-13), 0.68 (*s*, H-14), 4.78 and 4.62 (each *br d* $J = 2$ Hz, H-15a,b); ^{13}C NMR: Table 2.

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