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PSEUDOGUAIANOLIDES FROM *GAILLARDIA GRANDIFLORA*

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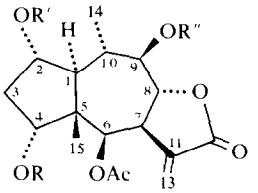
Key Word Index—*Gaillardia grandiflora*; Compositae; new pseudoguaianolides; spathulin derivatives.

Abstract—The aerial parts of *Gaillardia grandiflora* afforded two new pseudoguaianolides, 9-*O*-desacetylspathulin-2-*O*-angelate and 9-*O*-desacetylspathulin-2-*O*-isovalerate. The structures were elucidated by ^{1}H NMR spectroscopic investigations and by some chemical transformations.

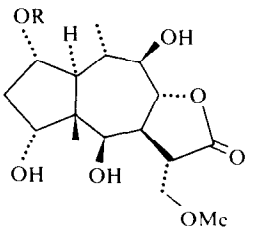
The aerial parts of *Gaillardia grandiflora* Hort. have been investigated before, and as with many other species of the subtribe Gaillardiinae, they contained a pseudoguaianolide, spathulin (**1**) [1, 2]. The structure of **1** was elucidated unambigously by X-ray analysis [3]. A re-investigation of this species afforded a crystalline compound, which, however, turned out to be a mixture of two different esters. Intensive ^{1}H NMR studies, especially with the triacetates obtained by acetylation, clearly showed that we were dealing with pseudoguaianolides **2** and **3** (Table 1). The esters were subsequently separated by means of HPLC in the recycle mode and all physical chemical data of pure **2** and **3** were recorded.

The nature of the ester residues clearly followed from the ^{1}H NMR spectra, but their relative positions could not be determined directly. The oxygenation pattern and the stereochemistry, however, were elucidated by decoupling experiments. Irradiation of the signal at δ 4.64 in the triacetate **4** collapsed the triplet at 4.91 to a doublet and the complex signal at 3.32 was changed to a three-fold doublet. Irradiation of the latter signal collapsed the doublet at 5.83 and the methylene proton signals to singlets. Therefore, the signal at 3.32 must be assigned to 7-H and consequently also the signals at 4.64, 4.91 and 5.83 can be assigned for 8-H, 9-H and 6-H. The signal of 9-H showed a drastic downfield shift on acetylation and therefore the natural compounds must have a free 9- hydroxy group. Irradiation of this signal allowed the assignment of an overlapped multiplet at 2.08 to 10-H. Consequently, irradiation of this signal collapsed the methyl doublet to a singlet and a signal at 2.48 to a doublet. The latter signal also coupled with a three-fold doublet at 5.13. Irradiation of this signal simplified the signals at 2.70 and 1.63 to a double doublet and a doublet with geminal coupling. The signal at 2.70 was further coupled with the doublet at 4.85. This signal was also shifted downfield upon acetylation. These correlations clearly show that the triacetate must have structure **4**. Inspection of models showed that only the given stereochemistry is in accordance with the observed coupling constants.

The relative position of the different ester residues was elucidated by partial hydrolysis with potassium carbonate in methanol–water [4]. At room temperature, apart from addition of methanol to the 11,13-double bond, only the acetate was saponified as revealed by inspection of the ^{1}H NMR spectrum (Table 1). The upfield shift of the



	1	2	3	4	5
R	H	H	H	Ac	Ac
R'	H	Ang	*i*-Val	Ang	*i*-Val
R''	Ac	H	H	Ac	Ac



6 R = Ang
7 R = *i*-Val

Table 1. ^{1}H NMR spectral data of compounds **2**, **4**, **5** and **6** (270 MHz, $CDCl_3$, TMS as internal standard)

	2*	4	5	6*
1α-H	2.40 *dd*	2.48 *dd*	2.48 *dd*	2.23 *dd*
2β-H	5.06 *ddd*	5.13 *ddd*	5.05 *ddd*	5.08 *ddd*
3α-H	2.65 *ddd*	2.70 *ddd*	2.70 *ddd*	2.65 *ddd*
3β-H	1.65 *dd*	1.63 *dd*	1.63 *dd*	1.62 *dd*
4β-H	3.85 *d*	4.85 *d*	4.84 *d*	3.86 *d*
6α-H	6.04 *d*	5.83 *d*	5.82 *d*	4.69 *d*
7α-H	3.17 *dddd*	3.32 *dddd*	3.32 *dddd*	2.44 *dddd*
8β-H	4.58 *dd*	4.64 *dd*	4.63 *dd*	4.56 *dd*
9α-H	3.34 *t*(*br*)	4.91 *t*	4.91 *t*	3.19 *t*(*br*)
10β-H	1.9 *m*	2.08 *m*	2.08 *m*	1.9 *m*
11β-H	—	—	—	2.17 *m*
13-H	6.33 *d*	6.32 *d*	6.32 *d*	3.59 *d*
13′-H	5.51 *d*	5.50 *d*	5.50 *d*	3.28 *m*
14-H	1.18 *d*	1.00 *d*	0.99 *d*	1.17 *d*
15-H	0.83 *s*	0.94 *s*	0.95 *s*	0.94 *s*
OAc	2.05 *s*	2.03 *s*	2.03 *s*	—
		2.13 *s*	2.14 *s*	
		2.17 *s*	2.17 *s*	
OCOR	6.09 *qq*	6.11 *qq*	0.93 *d*	6.08 *dd*
	2.00 *dq*	1.99 *dq*		2.00 *dq*
	1.89 *dq*	1.87 *dq*		1.89 *dq*

J(Hz): 1α,2β = 7.5; 1α,10β = 11.5; 2β,3α = 9; 2β,3β = 2; 3α,3β = 16.5; 3α,4β = 4.5; 6α,7α = 3.5; 7α,8β = 9; 7α,13 = 3.5; 7α,13′ = 3.2; 8β,9α = 10; 9α,10β = 10; 10β,14 = 6.5; OAng: 3′,4′ = 7; 3′,5′ = 4′,5′ = 1.5; **6**: 7α,11β = 11.

* Signals of **3** and **7** showed the same small differences in comparison with those of **4**, respectively **6** as **4** and **5** (*Oi*-Val: 2.18 *d*, (2 H, *J* = 7), 2.12 (1 H, *m*), 0.96 (6 H, *d*, *J* = 7).

doublet for 6-H indicated that the acetate must be located at this position and consequently the angelate or the isovalerate must be at C-2. The stereochemistry at C-11 followed from the observed large coupling constant for $J_{7,11}$. Therefore, the two new lactones are 9-desacetylspathulin angelate (**2**) and 9- desacetylspathulin isovalerate (**3**).

EXPERIMENTAL

9-O-Desacetylspathulin-2-O-angelate and 9-O-desacetylspathulin-isovalerate (**2** *and* **3**). The $CHCl_3$ extract (128.1 g) obtained from the dried plant (2.75 kg) was partitioned between 95% MeOH and petrol. The MeOH layer was diluted with H_2O (1:1) and extracted with a mixture of $CHCl_3$–petrol (1:1). The $CHCl_3$–petrol extract (28.9 g) was subjected to CC on Si gel (300 g) which was eluted first with pure C_6H_6 then C_6H_6–$CHCl_3$ mixtures (9:1, 7:3, 1:1). Fractions eluted with C_6H_6–$CHCl_3$ (7:1) mixture were combined and evapd. The residue recrystallized from MeOH–Me_2CO gave **2** and **3** (1.76 g) as colourless crystals, mp 215°, IR: ν_{max} cm^{-1}: OH 3500; C=O lactone 1765; C=O ester 1710. MS *m/e* (rel. int.): 422.194 (M^+, 3) and 424.210 (M^+, 03) ($C_{22}H_{30}O_8$ and $C_{22}H_{32}O_8$); 322 (M − RCO_2H, 32); 262 (322 − HOAc, 70); 83 ($C_4H_7CO^+$, 100).

$$[\alpha]_{20}^{\lambda} = \frac{589}{+32.4}\ \frac{578}{+34.3}\ \frac{546}{+38.7}\ \frac{436\text{ nm}}{+62.7^\circ} \quad (c = 1.2).$$

Esters **2** and **3** were separated by HPLC using a series of four 9 mm × 30 cm columns packed with Lichrosorb 10. The columns were eluted with EtOAc–hexane (3:7) flow rate 50 ml/min. To obtain complete separation the solute (20 mg) had to be recycled twice. A refractive index detector was used for monitoring of the chromatography. *9-O-Desacetylspathulin-2-O*-angelate (**2**, 13 mg) had mp 226°.

$$[\alpha]_{20}^{\lambda} = \frac{589}{-39.6}\ \frac{578}{-41.4}\ \frac{546}{-46.6}\ \frac{436\text{ nm}}{+75.2^\circ} \quad (c = 1.1).$$

9-O-Desacetylspathulin-2-O-isovalerate (**3**, 6 mg) had mp 185°.

$$[\alpha]_{20}^{\lambda} = \frac{589}{+26.0}\ \frac{578}{+27.3}\ \frac{546}{+30.8}\ \frac{436\text{ nm}}{+49.8^\circ} \quad (c = 0.97).$$

Acetylation. The mixture of **2** and **3** (50 mg) was dissolved in a mixture of Py (5 ml) and Ac_2O (2 ml) and left at room temp. overnight. Standard work-up of the reaction mixture gave **4** and **5** (57 mg; 95%) as colourless needles, mp 172–177°. IR ν_{max}^{Nujol} cm^{-1}: (C=O) 1770, 1740, 1715. MS *m/e* (rel. int.): 446.194 (M − HOAc, 1%) ($C_{24}H_{30}O_8$); 347 (M − $^\cdot$OCOR, 9); 287 (347 − HOAc, 21); 227 (287 − HOAc, 3); 83 ($C_4H_7CO^+$, 100); 43 ($MeCO^+$, 95). CI (isobutane): M + 1 not observed; 447 (M − HOAc, 13); 407 (RCO_2H, 8); 347 (447 − RCO_2H, 62); 287 (347 − HOAc, 54) 227 (287 − HOAc, 100).

$$[\alpha]_{20}^{\lambda} = \frac{589}{-27.0}\ \frac{578}{-28.4}\ \frac{546}{-32.6}\ \frac{436\text{ nm}}{-59.5^\circ} \quad (c = 2.0,\ CHCl_3).$$

Partial hydrolysis of **2** *and* **3**. To 10 mg **2** and **3** in 0.5 ml MeOH, 20 mg K_2CO_3 in H_2O were added. After 1 hr H_2O and dil H_2SO_4 were added. Extraction with $CHCl_3$ yielded a mixture which was separated by TLC ($CHCl_3$–Et_2O–MeOH, 13:6:1). In addition to unchanged **2** and **3**, 3 mg of **6** and **7** were isolated, colourless gum, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600 (OH); 1775 (lactone); 1725 (CO_2R); 1650 (C=C), ^1H NMR: see Table 1. MS *m/e* (rel. int.): 412.210 (M^+, 1%) and 414.225 (M^+, 0.3) ($C_{21}H_{32}O_8$ and $C_{21}H_{34}O_8$) 312 ($M - RCO_2H$, 31); 294 ($312 - H_2O$, 20); 85 ($C_4H_9CO^+$, 43); 83 ($C_4H_7CO^+$, 100); 57 ($85 - CO$, 66); 55 ($83 - CO$, 80).

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DITERPENES AND STEROLS FROM *WEDELIA GLAUCA*

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Key Word Index—*Wedelia glauca*; Compositae; diterpenes; sterols; kaur-16-en-19-oic acid; 15α-cinnamoyloxy-kaur-16-en-19-oic acid.

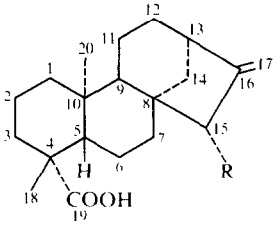
INTRODUCTION

Wedelia glauca (Ort.) Hoffmann ex Hicken (Compositae) is a perennial shrub widely distributed in Argentina, Brazil and Uruguay, and well known for its toxicity to cattle. Previous work on this species has not led to the isolation of any pure constituent, although Dominguez [1] and Burkart *et al.* [2] have reported the presence of a resin which could be responsible for the toxicity of the plant. Other species of the genus *Wedelia* have been studied previously, notably *W. calendulaceae* (L.) Less which has yielded wedelolactone [3].

The present work deals with the isolation and identification of higher alcohols, sterols, and the tetracyclic diterpenoids kaur-16-en-19-oic acid (**1**) and 15α-cinnamoyloxy-kaur-16-en-19-oic acid (**2**). **2** has been previously isolated from *Mikania oblongofolia* [4] and also reported as its methyl ester in *W. trilobata* [5].

RESULTS AND DISCUSSION

Chromatography of the petrol extract yielded fractions rich in higher alcohols, sterols, and tetracyclic diterpenes. One of the latter was characterized as (−)kaur-16-en-19-oic acid (**1**), from the ^1H NMR spectrum of the corresponding methyl ester [6]. From the same extract a diterpene ester was isolated and identified as the 15α-cinnamoyloxy-kaur-16-en-19-oic acid (**2**). The presence of the cinnamoyl group was evidenced by the ^1H NMR spectrum which showed an AB system at δ 6.45 and 7.70 (J_{AB} = 15 Hz) and a phenyl group at δ 7.38. The structure



1 R = H
2 R = OCOCH=CH–Ph

of **2** was confirmed by analysis of its alkaline hydrolysis products which were identified as 15α-hydroxy-kaur-16-en-19-oic acid [7] and cinnamic acid.

EXPERIMENTAL

Plant material. Whole plants of *W. glauca* were collected in Balcarce (province of Buenos Aires) during the flowering period. Voucher specimens were deposited in INTA (Balcarce) under No. 1768. Dried and milled plants (3 kg) were successively extracted with petrol (72 g of extract; 2.42% of dry plant) and EtOH (242 g

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