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SESQUITERPENES FROM *RUDBECKIA GRANDIFLORA*

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Key Word Index—*Rudbeckia grandiflora*; Asteraceae; Heliantheae; sesquiterpene esters; eudesmanes; sesquiterpene lactones; pseudoguaianolides.

Abstract—Chemical analysis of *Rudbeckia grandiflora* afforded two new sesquiterpene esters, 6α-hydroxycostic acid, methyl ester and arbusculin E, methyl ester as well as two new pseudoguaianolide type sesquiterpene lactones, desacylligulatin C and rudbeckin A. Their structures were elucidated by spectroscopic methods and chemical transformations.

INTRODUCTION

The genus *Rudbeckia* L. consists of *ca* 18 species and 30 taxa of annual and perennial herbs divided into two subgenera, *Rudbeckia* and *Macrocline*. *Rudbeckia grandiflora* is very abundant along roadsides and in pastures in Arkansas, Oklahoma and Louisiana. Previous chemical studies of members of this genus included *R. mollis* [1] and *R. laciniata* [2]. Sesquiterpene lactones isolated from *R. mollis* exhibited antitumour-activity [1].

As part of a biochemical systematic study within the tribe Heliantheae combined with a search for bioactive plant products we have analysed the previously uninvestigated species *R. grandiflora*. The structure of the four new compounds which we named 6α-hydroxycostic acid methyl ester (**1**), arbusculin E methyl ester (**2**), desacylligulatin C (**3**) and rudbeckin A (**4**), was established by chemical and spectroscopic methods.

RESULTS AND DISCUSSION

The dichloromethane extract of floral parts of *R. grandiflora* afforded, after column chromatography and further purification by preparative TLC, four new compounds. 6α-Hydroxycostic acid methyl ester (**1**), $C_{16}H_{24}O_3$, exhibited in the ^{1}H NMR spectrum a three-proton singlet at δ0.82 suggesting an angular methyl in a eudesmane skeleton. Two one-proton broadened singlets at δ6.28 and 5.22 together with a three-proton singlet at δ3.79 indicated an α-methylene methyl ester of the costic acid type [3]. Two broadened singlets at δ5.00 and 4.67 were in agreement with an olefinic methylene group which had to be on C-15 on biogenetic grounds. A doublet of doublets at δ3.92 was assigned to a proton on a carbon bearing a hydroxyl group which showed a coupling to H-5, a doublet at δ1.91 ($J = 10.1$ Hz), and to H-7 (*ddd*, δ2.60) as determined by 2D COSY experiments. This finding unambigeously established the position of the hydroxyl group at C-6. The stereochemistry at C-6 was derived from the couplings between H-5, H-6 and H-7 ($J_{5,6} = J_{6,7} = 10.1$ Hz) which were in agreement with a *trans*-diaxial orientation of these three protons. Assuming an H-7α as in all sesquiterpenes from higher plants [4] the hydrogens at C-5 and C-6 had to be α and β, respectively. This was further substantiated by NOE difference experiments which showed effects between H-5 and H-7 but had no effect on H-6. *In situ* acylation of the hydroxyl group with trichloroacetyl isocyanate (TAI) [5] corroborated the above findings. The ^{1}H NMR spectrum of the trichloroacetylcarbamate (TAC) derivative (**1a**) showed one NH signal at δ8.20, providing further evidence for the presence of one hydroxyl group in compound **1**. The paramagnetic acylation shift of the doublet of doublet at δ3.92 (H-6) in **1** to δ5.5 in **1a** ($\Delta\delta = 1.58$) was consistent with a geminal position of H-6 and the hydroxyl group. The signal of the C-10 methyl group at δ0.82 showed no paramagnetic shift, implying that the C-10 β-methyl is oriented away from H-6, which is in agreement with the relative stereochemistry of the two groups, that is, C-6 α-hydroxyl and C-10 β-methyl.

All other proton absorptions of ester **1** were assigned on the basis of 2D COSY, spin decoupling and NOE difference experiments (Table 1). The ^{13}C NMR spectrum of **1** (Table 2) was assigned with the aid of the heteronuclear multipulse DEPT experiments; the data of this new sesquiterpene supported the ^{1}H NMR assignments of **1**, which was named 6α-hydroxycostic acid methyl ester.

Arbusculin E methyl ester (**2**), $C_{16}H_{24}O_4$. The ^{1}H NMR spectrum (Table 1) values are very similar to those of 6α-hydroxycostic acid methyl ester (**1**), the major differences between **1** and **2** residing in the proton signals of C-4/C-15. Instead of the two olefinic protons at C-15 in **1** compound **2** exhibited a three-protons singlet at δ1.32, which is characteristic of a methyl group on a carbon bearing a hydroxyl group. The chemical shift of H-5 in **2** was also shifted upfield by about 0.5 ppm suggesting that it was in a non-allylic position. Therefore, the methylene group at C-4 in **1** had to be changed to a methyl group at a carbon bearing a hydroxyl group in **2**. The ^{13}C NMR spectrum of **2** (Table 2) corroborated the proposed structure with signals at δ106.92 (C-15) and

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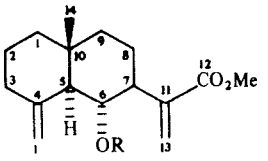
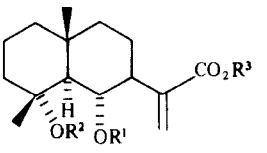
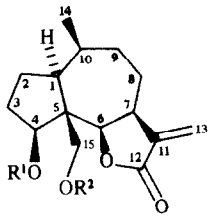


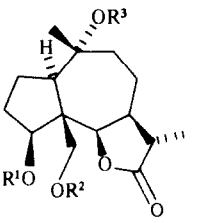
1 R = H
1a R = $CONHCOCCl_3$



2 $R^1 = R^2 = H$, $R^3 = Me$
2a $R^1 = R^2 = R^3 = H$
2b $R^1 = R^2 = CONHCOCCl_3$, $R^3 = Me$



3 $R^1 = R^2 = H$
3a $R^1 = R^2 = CONHCOCCl_3$



4 $R^1 = R^2 = R^3 = H$
4a $R^1 = R^2 = R^3 = CONHCOCCl_3$

14.3 (C-4) in the spectrum of **1** being replaced by signals at δ23.78 (C-15) and 73.47 (C-4) in compound **2**.

The stereochemistry at the chiral centres was proposed on the basis of the results of the TAI experiments and by examination of stereo models. The ^{1}H NMR spectrum of the trichloroacetylcarbamate **2b** showed NH signals at δ8.20 and 8.30 indicating two hydroxyl groups in compound **2**. The signal due to H-6 at δ4.10 in the spectrum of compound **2** was shifted to δ5.28 in that of **2b** ($\Delta\delta$ = 1.18) due to the strong deshielding effect of the TAC acyl group. Also, the C-4 methyl group was shifted from δ1.32 in the spectrum of **2** to δ1.60 in that of **2b** ($\Delta\delta$ = 0.28). These shifts strongly suggested an α-orientation of the two hydroxy groups in the molecule as well as the α-orientation of H-5. The structure of **2** was unambiguously established by its transformation to 6α-hydroxycostic acid (**2a**) with NMR spectral data being in complete agreement with data reported in the literature [3]. Compound **2** which was not previously reported as a natural product, represents the methyl ester of the known arbusculin E [7].

Desacylligulatin C (**3**), $C_{15}H_{22}O_4$, is a gum with ^{1}H NMR spectral signals typical for an α-methylene-γ-lactone moiety with two one-proton doublets at δ6.20 (H-13b) and 5.49 (H-13a), both coupled to a one-proton multiplet at δ3.45 (H-7). Strong IR absorptions at 1757 and 3429 cm^{-1} corroborated the presence of a γ-lactone and hydroxyl(s), respectively. 2D COSY studies indicated that H-7 was coupled to a doublet at δ4.51 ($J_{6,7}$ = 9.5 Hz) which was assigned to the lactonic proton at C-6. The multiplicity of H-6 suggested a pseudoguaianolide-type skeleton for lactone **3** [4]. A three-proton doublet at δ0.99 (J = 7.5 Hz) was assigned to the methyl group at C-10. However, the common methyl signal due to an angular methyl at C-5 was missing in compound **3**. Instead, a pair of geminally coupled doublets (J = 12.2 Hz) at δ4.05 and 3.90 suggested the presence of a $-CH_2OH$ group at C-5, which was supported by a ^{13}C NMR triplet at δ61.82. Compound **3** lacked a ^{13}C NMR absorption for a cyclopentanone carbonyl but exhibited a doublet at δ83.91 typical of a carbon bearing an oxygen. The ^{1}H NMR spectrum supported the above findings by the presence of a doublet of a doublet at δ4.35 (J = 8.8 and 9.0 Hz). The chemical shift together with the multiplicity of this proton suggested the presence of a hydroxyl group at C-4. All other proton signals were assigned on the basis of spin decoupling experiments, 2D COSY and 2D ^{1}H–^{13}C correlations (Table 1). The ^{13}C NMR spectral signals (Table 2) were assigned on the basis of 2D COSY, ^{1}H–^{13}C correlation and DEPT experiments. The spectroscopic data were consistent with data reported for a synthetic derivative of ligulatin C [6]. To our best knowledge this is the first report of desacylligulatin C (**3**) as a natural product.

Rudbeckin A (**4**), $C_{15}H_{24}O_5$, showed an IR band at 1765 cm^{-1} (γ-lactone) and the ^{1}H NMR (Table 1) also suggested a lactone which had to be closely related to desacylligulatin C (**3**). Compound **4** differed from **3** in that the two doublets characteristic of an α-methylene-γ-lactone were missing in the spectrum of compound **4**. Instead, a three-proton doublet at δ1.35 (J = 7.8 Hz) appeared which was assigned to the C-11 methyl group. The doublet at δ0.99 (C-10-Me) in the spectrum of **3** was replaced by a singlet at δ1.3 characteristic of a methyl group at a carbon bearing a hydroxyl and H-7 at δ2.25 appeared to be non-allylic. The ^{13}C NMR spectrum (Table 2) was assigned by comparison of **4** with lactone **3**. Application of heteronuclear multipulse DEPT experiments and 2D COSY allowed assignment of all ^{1}H NMR spectral signals.

The above spectral data were in agreement with structure **4** exclusive of its stereochemistry, which was determined with the aid of NOE difference spectral data and TAI experiments. Irradiations of the C-11 methyl signal from a NOE difference experiment showed an effect on H-6 and H-7, indicating an α-orientation of the C-11 methyl group. The ^{1}H NMR spectrum of the TAC derivative (**4a**) exhibited paramagnetic acylation shifts of H-

Table 1. ^{1}H NMR spectral data of compounds **1**–**4** (400 MHz, $CDCl_3$, TMS as internal standard)*

	1			**2**			**3**		**4**	
H	$CDCl_3$		C_6D_6†	$CDCl_3$		C_6D_6†	$CDCl_3$		$CDCl_3$	
1α	1.58–1.66	*m*‡	1.51–1.59‡	1.70	*ddd*	1.55–1.46‡	1.65	*ddd*	2.05	*dd*
1β	1.38	*ddd*	1.10	1.40	*ddd*	1.33	—	—	—	—
2α	1.48	*ddddd*	1.24	1.51	*ddddd*	0.99	1.51	*dddd*	1.55	*dddd*
2β	1.58–1.66	*m*‡	1.45	1.38	*m*‡	1.10	1.76–1.92	*m*‡	1.91–1.97	*m*‡
3α	2.00	*ddd*	1.77	2.26	*ddd*	1.55–1.46‡	1.76–1.92	*m*‡	1.89	*dddd*
3β	2.34	*ddd*	2.15	2.01	*ddd*	1.75	2.20	*dddd*	1.87	*dddd*
4	—	—	—	—	—	—	4.35	*dd*	4.24	*dd*
5α	1.91	*d*	1.67	1.45	*d*	1.37	—	—	—	—
6	3.92	*dd*	4.00	4.10	*dd*	4.16	4.51	*d*	4.75	*d*
7	2.60	*ddd*	2.70	2.60	*ddd*	2.63	3.45	*ddd*	2.25	*ddd*
8α	1.58–1.66	*m*‡	1.51–1.59‡	1.78	*dddd*	1.68	1.90	*dddd*	1.54	*dddd*
8β	1.72	*dddd*	1.68	1.61	*dddd*	1.47	2.09	*dddd*	2.10	*dddd*
9α	1.41	*ddd*	1.17	1.73	*ddd*	1.08	1.58	*ddd*	1.68	*ddd*
9β	1.32	*ddd*	1.05	1.79	*ddd*	1.72	1.75	*dddd*	1.91–1.97	*m*‡
10	—	—	—	—	—	—	2.02	*ddq*		—
11β	—	—	—	—	—	—	—	—	2.35	*dq*
13b	6.28	*br s*	6.30	6.28	*br s*	6.20	6.20	*d*	1.35	*d*
13*a*	5.22	*br s*	5.48	5.22	*br s*	5.45	5.49	*d*	—	—
14	0.82	*s*	0.78	0.98	*s*	0.79	0.99	*d*	1.27	*s*
15	5.00	*br s*	4.82	1.32	*s*	1.31	4.05	*d*	4.17	*d*
15′	4.67	*br s*	4.62	—	—	—	3.90	*d*	4.05	*d*
OMe	3.79	*s*	3.4	3.79	*s*	3.38	—	—	—	—

Compound **1** *J*(Hz): 1α,2α, = 1β,2α = 7,8α = 8β,9β = 3.5; 1β,1α = 2α,2β = 8α,8β = 13.1; 1β,2β = 4.6; 2α,3α = 8α,9β = 6.4; 2α,3β = 1.9; 3β,2β = 8α,9α = 4.0; 2β,3α = 9α,9β = 12.0; 3α,3β = 12.8; 5,6 = 6,7 = 10.1; 7,8β = 9; 8β,9α = 11. Compound **2** *J* (Hz): 1α,2α = 3.5; 1α,2β = 2.30; 1α,1β = 12; 1β,2β = 1β,2α = 7,8α = 2α,3α = 3.30; 2α,2β = 17; 2β,3α = 12; 3α,3β = 9.7; 3β,2β = 2α,3β = 8α,9α = 4.0; 5,6 = 6,5 = 6,7 = 7,8β = 10.4; 7,13 = 0.8; 8α,8β = 5; 8α,9β = 5.8. Compound **3** *J*(Hz): 1,10 = 8.9; 1,2α = 10.5; 1.2β = 5.9; 2α,2β = 12.5; 2α,3β = 8.9; 2α,3α = 4.5; 3β,4 = 9.0; 3α,3β = 14.3; 3β,2β = 6.0; 4,3β = 9.0; 4,3α = 8.8; 6,7 = 9.5; 7,8β = 7,8α = 8α,9β = 13.3; 8α,9α = 3; 8α,8β = 17.8; 8β,9α = 8β,9β = 7.4; 9α,9β = 11.8; 9α,10 = 0.0; 9β,10 = 11; 10,14 = 7.5; 13a,7 = 3.8; 13b,7 = 3.4; 15,15′ = 12.2. Compound **4** *J*(Hz): 1,2α = 4.5; 1,2β = 10; 2α,2β = 12.5; 2α,3α = 4.5; 2α,3β = 10.5; 3α,3β = 12.6; 3α,2β = 3α,4 = 7.8; 3β,4 = 5.6; 3β,2β = 6.0; 6,7 = 6.7; 7,8α = 0.0; 7,11 = 1.8; 7,8β = 12.7; 8α,9α = 3; 8α,8β = 17.8; 8α,9β = 13.3; 8β,9α = 8β,9β = 9α,8β = 7.4; 9α,8α = 3; 9α,9β = 11.8; 9α,10 = 0.0; 9β,8α = 13.3; 9β,10 = 11; 13,11 = 3.8; 15,15′ = 12.2.

*The signals due to hydroxyl groups are omitted.

†multiplicity and coupling constants are the same.

‡Obscured by other signals.

4 ($\Delta\delta = 1.16$), H-15 ($\Delta\delta = 1.35$) and H-1 ($\Delta\delta = 0.20$) establishing the presence of hydroxyl groups at C-4 and C-15 and the α-orientation for the hydroxyl group at C-10. An axial β-hydroxyl group should have caused a noticeable shift of the H-9α and H-2β signals, which were not observed. The C-11 methyl signal was not shifted significantly which is consistent with an α-orientation for the C-11 methyl group. Similarly, the H-6α signal was not affected suggesting a β-orientation of the CH_2OH group at C-5. This new pseudoguaianolide has not been previously reported and is named rudbeckin A (**4**).

EXPERIMENTAL

Rudbeckia grandiflora (Sweet) DC. was collected on 7 June 1986, in pinewoods and roadside of Louisiana Hwy 121, 3.3 miles west of Rapides Parish line east of La Camp. sec 16, T 2N, R5W. (P. Cox, L. E. Urbatsch and E. Harris; No. Cox 4889, voucher deposited at L.S.U., U.S.A.).

The air-dried flowers (50 g) were ground and extracted according to the general procedure [8] providing 1.3 g of the crude terpenoid extract. The crude extract (1.0 g) was separated by CC on silica gel using EtOAc–hexane mixtures of increasing polarity, 86 fractions of 50 ml each being collected. Upon further prep. TLC of the various fractions two sesquiterpenes (**1** and **2**) and two sesquiterpene lactones (**3** and **4**) were isolated. Fraction 12 provided 30 mg of **1** and fractions 24, 25 gave 34 mg of **2**. Fractions 29–31 afforded **3** (50 mg) and fractions 40, 41 provided **4** (35 mg).

6α-Hydroxycostic acid methyl ester (**1**). $C_{16}H_{24}O_3$, colourless gum; IR ν_{max}^{KBr} cm^{-1}: 3511 (OH), 1719 (ester), 1647 and 1626 (double bond); HRMS (FAB, probe) 70 eV, *m/z* (rel. int.): 264.1247 $[M]^+$ (4) (calc. for $C_{16}H_{24}O_3$:264.1249), 249.1330 $[M-Me]^+$ (2), 233.1145 $[M-OMe]^+$ (2), 186.0713 (64), 185.0781 (96), 93.0417 (100); ^{1}H NMR see Table 1; ^{13}C NMR see Table 2; TAC-derivative of **1**: ^{1}H NMR of H-6, δ3.92 ($\Delta\delta = +1.58$).

Arbusculin E methyl ester (**2**). $C_{16}H_{24}O_4$, colourless gum; IR ν_{max}^{KBr} cm^{-1}: 3347 (OH), 1719 (ester) 1626 and 1439 (double bond); HRMS (FAB, probe) 70 eV, *m/z*: 282.1541 $[M]^+$ (37) (calc. for $C_{16}H_{24}O_4$:282.1544), 265.1917 (75), 251.1692 $[M-OMe]^+$ (8), 247.1750 (37), 233.1542 (74), 161.1285 (48), 133.8546 (100), 109.0871 (45); ^{1}H NMR see Table 1; ^{13}C NMR see Table 2; TAC-derivative of **2**: ^{1}H NMR: H-6, δ4.10 ($\Delta\delta = +1.18$); H-4, δ1.32 ($\Delta\delta = +0.28$).

Table 2. ^{13}C NMR data of compounds **1–4** (50.32 MHz, $CDCl_3$, TMS as internal standard)*

C	1	2	3†	4†
1	41.86*t*‡	42.92*t*‡	48.45*d*	46.89*d*
2	26.66*t*	19.53*t*	22.30*t*	22.69*t*
3	37.82*t*	43.59*t*	29.70*t*	27.16*t*
4	14.30*s*	73.47*s*	83.91*d*	82.74*d*
5	57.92*d*	57.92*d*	53.52*s*	60.23*s*
6	69.33*d*	73.26*d*	90.09*d*	85.71*d*
7	48.07*d*	50.25*d*	43.44*d*	43.99*d*
8	23.93*t*	26.80*t*	32.29*t*	31.86*t*
9	40.38*t*‡	42.63*t*‡	24.80*t*	42.41*t*
10	37.40*s*	36.31*s*	32.76*d*	85.31*s*
11	147.27*s*	142.27*s*	139.88*s*	53.00*d*
12	168.07*s*	168.33*s*	170.23*s*	179.45*s*
13	124.63*t*	125.75*t*	118.89*t*	16.64*q*
14	17.61*q*	19.70*q*	13.95*q*	24.32*q*
15	106.92*t*	23.78*q*	61.82*t*	68.91*t*
OMe	51.84*q*	52.01*q*	—	—

*Peak multiplicity was obtained by heteronuclear multipulse programs.

†Assignments for compounds **3** and **4** were confirmed by ^{13}C–^{1}H chemical shift correlation.

‡Assignments may be interchanged.

Saponification of compound **2**. A 10 mg sample of **2** was dissolved in 3 ml of 5% methanolic KOH. After 16 hr the soln was diluted with H_2O (10 ml) and neutralized with 1% HCl. Extraction with DCM gave 5 mg of **2a** which was purified by prep. TLC [3].

Desacylligulatin C (**3**). $C_{15}H_{22}O_4$, gum; IR ν_{max}^{KBr} cm^{-1}: 3429 (OH), 1757 (γ-lactone), 1663 (double bond). HRMS (FAB, probe) 70 eV *m/z*: 266.1436 $[M]^+$ (3), (calc. for $C_{15}H_{22}O_4$:266.1434), 248.1377 $[M-H_2O]^+$ (6) 235.1268 $[M-CH_2OH]^+$ (19), 230.1105 $[M-2H_2O]^+$ (17), 203.1301 (68), 159.1097 (100), 147.0913 (83), 145.0772 (94), 105.0594 (88), 95.0637 (84). ^1H NMR see Table 1; ^{13}C NMR see Table 2.

TAC-derivative of **3**: ^1H NMR: H-15′, δ3.90 ($\Delta\delta = +0.62$), H-15, δ4.05 ($\Delta\delta = +0.55$). H-4, δ4.35 ($\Delta\delta = +0.69$). H-6, δ4.51 ($\Delta\delta = +0.99$).

Rudbeckin A (**4**). $C_{15}H_{24}O_5$, gum; IR ν_{max}^{KBr} cm^{-1}: 3418 (OH), 1765 (γ-lactone); HRMS (FAB, probe) 70 eV *m/z* 266.1614 $[M-H_2O]^+$ (26) (calc. for $C_{15}H_{22}O_4[M-H_2O]^+$: 266.1616), 265.1161 (76), 251.1371 (74), 249.1264 (82), 231.1502 $[M-2H_2O]^+$ (30), 219.1437 $[M-3H_2O]^+$ (31). 203.1137 (62), 185.0731 (94), 93.0718 (100); ^1H NMR see Table 1. ^{13}C NMR see Table 2.

TAC-derivative of **4**: ^1H NMR: H-4, δ4.24 ($\Delta\delta = +1.16$); H-15, δ4.05 ($\Delta\delta + 1.35$), H-1, δ2.25 ($\Delta\delta = +0.20$).

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