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Further Sesquiterpene Lactones from *Eupatorium semialatum**

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Abstract

Six new sesquiterpene lactones were isolated from *Eupatorium semialatum* (Asteraceae). Their structures were elucidated as esters of the eudesmanolides 8β-hydroxyreynosin (**1** and **2**), 8β-hydroxybalchanin (**3** and **4**) and 8β-hydroxymagnolialide (**5** and **6**) with the isomeric 4-hydroxy-5-(5-hydroxytigloyloxy)tiglic acid (**1**, **3** and **5**) and 5-hydroxy-4-(5-hydroxytigloyloxy)tiglic acid (**2**, **4** and **6**), respectively. The chemotaxonomic importance of these findings is discussed.

Keywords: *Eupatorium semialatum*, Asteraceae, sesquiterpene lactones, eudesmanolides.

Introduction

The Guatemalan Asteraceae *Eupatorium semialatum* Benth., which is a popular medicinal plant in the Alta Verapaz (Cáceres, 1996; Morton, 1981; Medinilla, 1978), was described by Nash and Williams (1976) to be quite different from the South Mexican *E. ligustrinum*, but King and Robinson (1987) treated both plants as one species. Moreover, as a result of their studies, this species was suggested to be a *Ageratina* (*A. ligustrina*) and not *Eupatorium* species (King & Robinson, 1970, 1987).

In a recent paper, we reported the isolation and identification of derivatives of the eudesmanolide sesquiterpene lactones 8β-hydroxyreynosin and 8β-hydroxybalchanin as well as one oplopanone sesquiterpene (Lang et al., 2000). Besides oplopanone, none of the eudesmanolides isolated from *E. semialatum* was previously found in *A. ligustrina* or *E. ligustrinum*, where germacranolides and guaianolides occur (Rojas et al., 1988; Tamayo-Castillo et al., 1988). In continuation of our studies on the sesquiterpene lactones of *E. semialatum*, we now report the isolation and structure elucidation of six further eudesmanolides.

Materials and methods

Plant Material. Leaves of *Eupatorium semialatum* Benth. (725 g, voucher no. JC2541), collected near Purulhá, Baja Verapaz, Guatemala, were identified by Juan José Castillo (Facultad de Agronomia, Universidad de San Carlos de Guatemala) and extracted with CH_2Cl_2. The extract (81 g) was then dissolved in MeOH, which yielded 36.86 g methanol soluble parts. This residue was divided in six portions of 6.14 g each, five of which were chromatographed on equal columns, containing 500 g Sephadex LH-20 each, using MeOH as eluent. Fractions obtained using a LKB fraction collector were built separately from eluates (15 ml each) of all columns after monitoring by TLC [silica gel 60 F_{254} (Merck No. 5554), CH_2Cl_2/MeOH (2 : 1)]. Elutes from different columns containing compounds in the same R_f range were finally combined to five fractions (D-I to D-V). Fraction D-II (8.8 g), containing all sesquiterpene lactones, was further purified using silica gel 60 with CH_2Cl_2/MeOH (9 : 1) as eluent. The resulting elutes were combined to five fractions (D-II-1 to D-II-5). Fraction D-II-4 (730 mg) was then purified by CC on silica gel 60 with CH_2Cl_2/acetone 2 : 1 as mobile phase, which gave three fractions (D-II-4-1 to D-II-4-3). Compounds **1**–**6** were isolated from fraction D-II-4-2 (74 mg) by HPLC using an isocratic mixture of H_2O/ace-

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tonitrile 1 : 3, which gave 29 mg of **1**, 4.6 mg of **2**, 3.5 mg of **3**, 2.3 mg of **4**, 1.2 mg of **5** and 1 mg of **6**.

NMR: Bruker ARX 500, 500 MHz, $CDCl_3$.

GC-MS: EI (70 eV) HP MSD 5972 with GC 5890 plus (HP); Optima-1 (MN), 25 m × 0.25 mm, 150 °C (3 min) to 280 °C at 10° min^{-1}. R_t (min.): **1**: 13.7; **2**: 13.7; **3**: 13.5; **4**: 13.5; **5**: 13.6; **6**: 13.6.

D-MS: CI (NH_3) Finnigan MAT INCOS 50.

UV: All spectra were recorded on a Beckman DV-G photometer in MeOH (Spectranal, Merck).

Optical Rotation: The compounds were dissolved in ethanol and diluted to a concentration of 1%. [α] values were recorded on a Perkin Elmer 341 LC polarimeter using a microcell (0.35 ml, 1 dm) at 20 °C and 589 nm.

IR: All spectra were recorded on a Perkin Elmer 881 in KBr.

HPLC: HP 1050, DAD. 215 and 260 nm, Hibar RP 18 LiChrosorb (7 µm, 25.0 × 7 mm), flow 2.0 ml min^{-1}. MeOH-H_2O (50 : 50) to MeOH/H_2O (56.5 : 43.5), 32.5 min. R_t (min.): **1**: 16.94; **2**: 20.71; **3**: 22.60; **4**: 28.80; **5**: 24.88; **6**: 30.31.

TLC: Silica gel 60 F_{254} (Merck), CH_2Cl_2/MeOH (2 : 1); anisaldehyde/H_2SO_4. R_f **1**: 0.53; **2**: 0.53; **3**: 0.53; **4**: 0.53; **5**: 0.53; **6**: 0.53.

8β-[4-Hydroxy-5-(5-hydroxytigloyloxy)tigloyloxy] reynosin (**1**): λ_{max} MeOH/H_2O (1 : 1): 209 nm, ε = 5678. $[\alpha]_D^{20}$ (ethanol): +27.2° (c = 1.923 g/100 ml). MS (*m/z*, rel. int.): 264 (<1, [M-acyl]$^+$); 246 (3); 228 (4); 213 (3); 202 (6); 179 (18); 161 (12); 157 (10); 133 (13); 117 (12); 106 (28); 91 (43); 79 (36); 77 (36); 69 (48); 55 (37); 43 (50); 41 (100). IR: ν_{max}^{KBr} (cm^{-1}): 3411 br (OH), 2944 (CH), 1765 (γ-lactone), 1709 (unsaturated ester), 1650 (double bond).

8β-[5-Hydroxy-4-(5-hydroxytigloyloxy)tigloyloxy] reynosin (**2**): λ_{max} MeOH/H_2O (1 : 1): 209 nm, ε = 5813. $[\alpha]_D^{20}$ (ethanol) = +15.6° (c = 0.311 g/100 ml). MS (*m/z*, rel. int.): 228 (2); 202 (2); 179 (16); 161 (10); 157 (10); 145 (6); 133 (12); 117 (13); 106 (24); 91 (45); 79 (35); 77 (36); 69 (48); 55 (37); 43 (50); 41 (100). IR: ν_{max}^{KBr} (cm^{-1}): 3514 (OH), 2956 (CH), 1764 (γ-lactone), 1710 (unsaturated ester), 1650 (double bond).

8β-[4-Hydroxy-5-(5-hydroxytigloyloxy)tigloyloxy] balchanin (**3**): λ_{max} MeOH/H_2O (1 : 1): 209 nm, ε = 6645. $[\alpha]^{20}{}_D$ (ethanol) = +0.30° (c = 8.871 g/100 ml). MS (*m/z*, rel. int.): 264 (5) [M-acyl]$^+$; 217 (4); 207 (4); 189 (8); 173 (5); 164 (12); 150 (9); 133 (9); 119 (23); 105 (21); 97 (23); 91 (42); 77 (34); 69 (51); 55 (43); 43 (50); 41 (100). IR: ν_{max}^{KBr} (cm^{-1}): 3497 (OH), 2933 (CH), 1760 (γ-lactone), 1710 (unsaturated ester), 1643 (double bond).

8β-[5-Hydroxy-4-(5-hydroxytigloyloxy)tigloyloxy] balchanin (**4**): λ_{max} MeOH/H_2O (1 : 1): 209 nm, ε = 6497. $[\alpha]_D^{20}$ (ethanol) = +0.1° (c = 0.2 g/100 ml). MS (*m/z*, rel. int.): 264 (6) [M-acyl]$^+$; 246 (<1); 228 (<1); 217 (3); 202 (2); 189 (8); 175 (5); 164 (13); 150 (10); 145 (7); 133 (9); 119 (24); 105 (20); 91 (43); 79 (33); 77 (35); 69 (52); 55 (43); 43 (54); 41 (100). IR: ν_{max}^{KBr} cm^{-1}: 3460 (OH), 2939 (CH), 1763 (γ-lactone), 1711 (unsaturated ester), 1647 (double bond).

8β-[4-Hydroxy-5-(5-hydroxytigloyloxy)tigloyloxy]magnolialide (**5**): λ_{max} MeOH/H_2O (1 : 1): 209 nm, ε = 4815. $[\alpha]_D^{20}$ (ethanol) = −18.0° (c = 0.69 g/100 ml). MS (*m/z*, rel. int.): 246 (14); 228 (5); 213 (6); 202 (9); 185 (7); 153 (32); 135 (44); 128 (27); 115 (39); 107 (52); 93 (100); 91 (94); 77 (73); 65 (38); 55 (52); 43 (99); 41 (88). IR: ν_{max}^{KBr} cm^{-1}: 3502 br (OH), 2927 (CH), 1766 (γ-lactone), 1718 (unsaturated ester), 1637 (double bond).

8β-[5-Hydroxy-4-(5-hydroxytigloyloxy)tigloyloxy]magnolialide (**6**): λ_{max} MeOH/H_2O (1 : 1): 209 nm, ε = 4466. $[\alpha]_D^{20}$ (ethanol) = −14.0° (c = 0.02 g/100 ml). MS (*m/z*, rel. int.): 246 (13); 228 (4); 213 (6); 202 (8); 185 (7); 167 (9); 157 (15); 153 (28); 135 (42); 128 (29); 115 (41); 107 (49); 93 (92); 91 (99); 77 (74); 65 (38); 55 (53); 43 (100); 41 (89). IR: ν_{max}^{KBr} cm^{-1}: 3485 br (OH), 2932 (CH), 1764 (γ-lactone), 1715 (unsaturated ester), 1645 (double bond).

Results and discussion

Separation of the methanol soluble part of the dichloromethanie extract afforded one fraction rich in sesquiterpene lactones, from which the compounds **1**–**6** were isolated using CC, preparative TLC and HPLC. The NMR spectra of **1**–**4** clearly indicated the presence of ester derivatives of the eudesmanolide sesquiterpene lactones alcohols 8β-hydroxyreynosin and 8β-hydroxybalchanin as partial structures of **1** and **2**, and **3** and **4**, respectively. Ester derivatives with the enantiomeric (+)- and (−)-(2-hydroxy-1-methoxyethyl)acrylic acid of both basic structures were previously found in the same plant (Lang et al., 2000). The spectroscopic data (see Tables 1 and 2), including two-dimensional experiments (COSY, HMQC, HMBC, and NOESY), revealed the esterification of the hydroxyl group at C-8 in **1**–**4**, which is clearly indicated by the shift values of the carbons C-1 and C-8 in comparison to the free sesquiterpene lactone alcohol (Budesinsky & Saman, 1995). Overall, the signals found for the acid moiety clearly suggested the presence of an esterified 4,5-dihydroxytiglic acid, which contains a 5-hydroxytiglic acid as side chain (Bohlmann et al., 1985; Budesinsky & Saman, 1987). The position of the ester group at the 4,5-dihydroxytiglic acid varies from C-4′ (**2** and **4**) to C-5′ (**1** and **3**), which was deducible from characteristic downfield shifts of C-4′ in **2** and C-5′ in **1** and **3**, together with upfield shifts of C-2′ in **1** and **3** and C-3′ in **2**, respectively (see Tables 1 and 2). As expected, the corresponding signals were found in the ^{1}H NMR spectrum, so the acids could be identified as 4-hydroxy-5-(5-hydroxytigloyloxy) tiglic acid in **1** and **3**, and 5-hydroxy-4-(5-hydroxytigloyloxy)tiglic acid in **2** and **4**, respectively. Both acids were previously found in nature (Budesinsky & Saman, 1987, 1995), but all isolated ester derivatives are new natural compounds.

The sesquiterpene lactone moiety in **5** and **6** differed from **1**–**4** by the position of the double bond. Whereas **1** and **2** are containing a Δ4,15 double bond, and a Δ3,4 double bond is

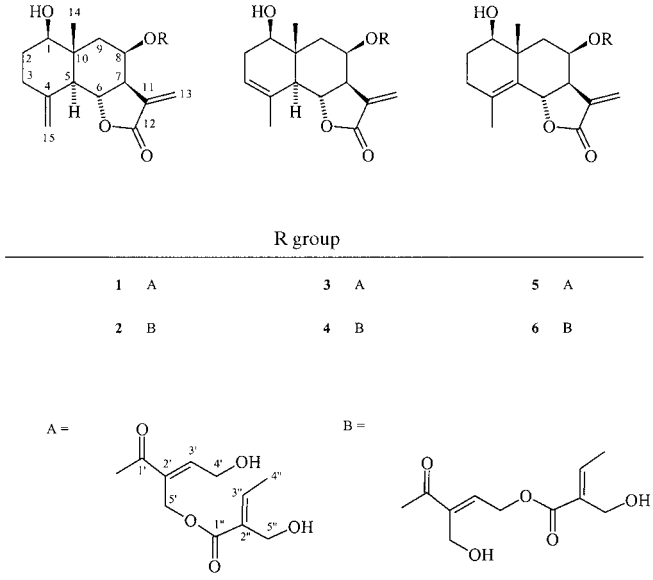
Table 1. ^{13}C NMR data of compounds **1**–**3** and **5** (125 MHz, $CDCl_3$).

carbon	**1**	**2**	**3**	**5**	carbon	**1**	**2**	**3**	**5**
1	78.36	78.47	75.67	77.98	1′	165.47	165.77	165.38	165.38
2	30.93	30.88	32.93	26.75	2′	126.97	127.30	127.19	127.13
3	33.32	33.30	121.54	33.16	3′	147.62	138.22	147.11	147.20
4	141.89	141.66	133.07	127.19	4′	59.28	60.32	59.52	59.56
5	53.32	53.35	51.34	128.23	5′	58.12	57.35	58.37	58.33
6	75.11	74.99	77.17	77.54	1″	167.16	167.25	167.24	167.27
7	51.92	51.90	53.49	51.72	2″	131.49	131.59	131.58	131.56
8	67.14	67.23	67.02	67.55	3″	142.52	142.31	142.19	142.23
9	40.26	40.29	39.30	42.83	4″	14.36	14.39	14.30	14.32
10	42.67	42.62	40.63	41.78	5″	56.42	56.69	56.65	56.67
11	134.23	134.22	133.92	133.82					
12	169.78	169.61	169.72	169.32					
13	119.74	119.77	119.54	121.17					
14	13.73	13.67	12.94	20.88					
15	110.76	111.01	23.27	19.51					

Table 2. ^{1}H NMR data of compounds **1**–**6** (500 MHz, $CDCl_3$).

Proton	**1**	**2**	**3**	**4**	**5**	**6**
1	3.52 dd	3.53 dd	3.68 dd	3.69 m	3.56 dd	3.56 dd
2 eq	1.83 m	1.86 m	2.42 m	2.41 m	2.06 m	2.06 m
ax	1.59 ddd	1.60 m	1.96 m	1.98 m	1.76 ddd	1.76 ddd
3 eq	2.35 m	2.37 m	5.37 bs	5.37 bs	2.23 ddd	2.23 m
ax	2.13 m	2.14 m			1.70 m	1.70 m
5	2.27 d	2.28 d	2.45 d	2.47 d		
6	4.54 t	4.54 t	4.45 t	4.46 t	5.13 d	5.13 d
7	2.88 ddd	2.88 ddd	2.82 ddd	2.83 ddd	2.95 ddd	2.96 ddd
8	5.85 dd	5.84 dd	5.88 dd	5.89 dd	5.89 dd	5.86 dd
9 eq	2.38 dd	2.43 dd	2.38 dd	2.41 dd	2.41 dd	2.44 dd
ax	1.64 dd	1.65 dd	1.60 dd	1.60 dd	1.65 dd	1.65 dd
13	6.10 d	6.10 d	6.11 d	6.17 d	6.18 d	6.24 d
	5.47 d	5.48 d	5.46 d	5.45 d	5.54 d	5.54 d
14	0.96 s	0.97 s	1.05 s	1.05 s	1.24 s	1.23 bs
15	5.03 s	5.04 s	1.89 bs	1.90 bs	1.89 bs	1.90 bs
	4.93 s	4.97 s				
3′	7.06 q	6.80 t	7.05 t	6.79 t	7.06 t	6.79 t
4′	4.48 d	4.95 d	4.50 d	4.95 d	4.51 dd	4.95 d
5′	4.96 s	4.41 s	5.00 d	4.37 s	5.03 d	4.40 s
					4.99 d	
3″	6.92 q	7.05 m	6.93 q	7.05 t	6.92 dd	7.05 t
4″	1.91 d	1.94 d	1.91 d	1.94 d	1.91 d	1.94 d
5″	4.30 s	4.37 s	4.32 s	4.36 s	4.32 s	4.37 s

J (Hz): **1**: 1,2 eq: 4.5; 1,2 ax: 11.4; 2 ax,2 eq: 13.2; 2 ax,3 eq: 5.0; 2 ax,3 ax: 12.0; 5,6: 10.7; 6,7: 10.7; 7,8: 5.7; 7,13: 3.2; 8,9 eq: 2.5; 8,9 ax: 3.8; 9 eq,9 ax: 15.1; 3′,4′: 5.7; 3″,4″: 6.9. **2**: 1,2 eq: 4.5; 1,2 ax: 11.4; 5,6: 10.7; 6,7: 10.7; 7,8: 5.7; 7,13: 3.2; 8,9 eq: 2.5; 8,9 ax: 3.8; 9 eq,9 ax: 15.1; 3′,4′: 6.3; 3″,4″: 6.9. **3**: 1,2 eq: 6.9; 1,2 ax: 9.5; 5,6: 11.4; 6,7: 11.4; 7,8: 5.7; 7,13: 3.2; 8,9 eq: 2.5; 8,9 ax: 3.8; 9 eq,9 ax: 15.1; 3′,4′: 5.7; 3″,4″: 6.9. **4**: 5,6: 11.4; 6,7: 11.4; 7,8: 5.7; 7,13: 3.2; 8,9 eq: 2.5; 8,9 ax: 3.8; 9 eq,9 ax: 15.1; 3′,4′: 6.3; 3″,4″: 6.9. **5**: 1,2 eq: 3.8; 1,2 ax: 12.0; 2 ax, 2 eq: 13.2; 2 ax,3 eq: 5.0; 2 ax,3 ax: 12.0; 6,7: 12.0; 7,8: 5.7; 7,13: 3.2; 8,9 eq: 2.5; 8,9 ax: 3.8; 9 eq,9 ax: 15.1; 3′,4′: 5.7; 5′a,5′b: 12.0; 3″,4″: 6.9. **6**: 1,2 eq: 3.8; 1,2 ax: 12.0; 2 ax,eq: 13.2; 2 ax,3 eq: 5.0; 2 ax,3 ax: 12.0; 6,7: 12.0; 7,8: 5.7; 7,13: 3.2; 8,9 eq: 2.5; 8,9 ax: 3.8; 9 eq,9 ax: 15.1; 3′,4′: 6.3; 3″,4″: 6.9.



Structures of compounds **1-6**

present in **3** and **4**, the double bond in **5** and **6** was found between C-4 and C-5. This is evident from the absence of the signal for H-5 in the ^{1}H NMR and the corresponding signal for C-5 in the ^{13}C NMR spectrum, together with a downfield shift of H-6 in the ^{1}H-NMR (see Tables 1 and 2). Thus, the sesquiterpene lactone moiety in **5** and **6** was found to be 8β-hydroxymagnolialide (Budesinsky & Saman, 1995). In analogy to the other compounds, we found 8β-hydroxymagnolialide esterified with 4-hydroxy-5-(5-hytigloyloxy)tiglic acid in **5** and 5-hydroxy-4-(5-hytigloyloxy)tiglic acid in **6** (see Tables 1 and 2).

Compounds **1–6** are new ester derivatives of the known sesquiterpene lactone alcohols 8β-hydroxyreynosin, 8β-hydroxybalchanin and 8β-hydroxymagnolialide. We never observed an interchange of the 5-hydroxytiglic acid between C-4′ and C-5′ in our pure compounds, although this may generally be possible. This means that all isolated compounds are actually present in the plant.

Our chemical studies did not show much similarity between *E. semialatum* and *E. ligustrinum* or *A. ligustrina*, respectively. The only compound found in both species is the sesquiterpene cinnamoyloxyoplopanone (Tamayo-Castillo et al., 1988). Since all sesquiterpene lactones found in *A. ligustrina* (= *E. ligustrinum*) were guaianolides or germacranolides (Rojas et al., 1988; Tamayo-Castillo et al., 1988), and the compounds isolated from our collection were exclusively of the eudesmanolide type (Lang et al., 2000), *E. semialatum* seems to be a different species and may not be identical with *A. ligustrina*. Further investigations with other chemotaxonomic markers such as flavonoids, for example, are planned.

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