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TWO SAPONINS FROM *ZEXMENIA BUPHTHALMIFLORA*

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Key Word Index—*Zexmenia buphthalmiflora*; *Wedelia buphthalmiflora*; Heliantheae; Compositae; structure elucidation; saponins; oleanolic acid 3-*O*-(3′-*O*-α-L-rhamnopyranosyl)-β-D-glucuronopyranoside; oleanolic acid 3-*O*-(3′-*O*-α-L-rhamnopyranosyl)-β-D-glucuronopyranoside-28-*O*-β-D-glucopyranoside.

Abstract—Oleanolic acid 3-*O*-(3′-*O*-α-L-rhamnopyranosyl)-β-D-glucuronopyranoside and its 28-*O*-β-D-glucopyranosyl ester have been isolated from aerial parts of *Zexmenia buphthalmiflora*. The chemotaxonomical significance of the transfer of *Wedelia buphthalmiflora* to *Zexmenia buphthalmiflora* is discussed.

INTRODUCTION

As a part of our investigations on *Wedelia* species we have previously reported on the isolation and identification of di- and triterpenoids from the aerial parts of *W. glauca* [1] and *W. buphthalmiflora* [2]. In the meantime *W. buphthalmiflora* Lorentz has been reclassified as *Zexmenia buphthalmiflora* (Lorentz) Ariza [3]. Both genera are closely related (family: Compositae, tribe: Heliantheae) although *Wedelia* belongs to the subtribe Ecliptinae and *Zexmenia* to Verbesininae [4].

This paper deals with the structure elucidation of two saponins: oleanolic acid 3-*O*-(3′-*O*-α-L-rhamnopyranosyl)-β-D-glucuronopyranoside (**1**) and oleanolic acid 3-*O*-(3′-*O*-α-L-rhamnopyranosyl)-β-D-glucuronopyranoside-28-*O*-β-D-glucopyranoside (**2**) isolated from *Z. buphthalmiflora* and identified by spectral and chemical methods.

RESULTS AND DISCUSSION

Column chromatography of the ethanolic extract of aerial parts of *Z. buphthalmiflora* yielded three fractions. Fractions 2 and 3 contained the acidic terpenic glycosides **1** and **2**, respectively, which were purified by chromatography of their methyl derivatives (**3** and **4**, respectively).

Upon acid hydrolysis of **3**, methyl oleanolate, rhamnose and an uronic acid were obtained whereas **4** afforded oleanolic acid, rhamnose, glucose and an uronic acid. Alkaline hydrolysis of **4** yielded glucose and a glycoside that on methylation with diazomethane led to **3**. This fact showed that **4** was a glucoside ester of **3**.

Reduction of **4** provided **5** which was hydrolysed to give erythrodiol, rhamnose and glucose and thus providing evidence for the presence of glucuronic acid in **3** and **4**. The ^{1}H NMR spectrum of the TMSi derivative of **3** showed, among other signals, a singlet at δ5.11 ascribed to the anomeric proton of one moiety of rhamnose [5], a doublet (J = 6 Hz) at δ4.52 of the anomeric proton of one glucuronic acid moiety with β-configuration [6], two singlets at 3.80 and 3.64 assigned to the methyl esters of the glucuronic acid and the aglycone, respectively. In the ^{1}H NMR spectrum of the TMSi derivative of **4** a new doublet (J = 6 Hz) appeared at δ5.60 assigned to the anomeric proton of one β-D-glucose [7] while the singlet at 3.64 was absent. Consequently the anomeric hydroxyl of this β-D-glucose was attached to the carboxyl group of the oleanolic acid.

The mass spectrum of the TMSi derivative of **3** showed the presence of a very intense ion at m/z 363 (TMSi rhamnose oxonium ion) indicating that rhamnose was the terminal moiety [8] of the disaccharide attached to the HO-3 of the aglycone.

Evidence of the attachment of the glucose moiety in **4** to the carboxyl group of the oleanolic acid and not to the uronic acid was provided by the ^{13}C NMR spectrum of **4** which showed the presence of a signal for a COOMe at δ52.1 ascribed to a COOMe of the uronic acid (COOMe of methyl oleanolate: 51.6; COOMe of methyl glucuronate: 52.1). Additionally, the C-28 (C=O) signal of the triterpenoid was affected in **4** due to the glycosylation of this carbon. (Compare C-28 in **3** and **4**, Table 1; 178.0 and 176.4, respectively). The latter (176.4) is in agreement with the C-28 chemical shift of other glucosyl esters of oleanolic acid derivatives [9].

On the contrary, C-6′ (COOMe of the methyl glucuronate) in **3** and **4** have the same value, 170.5 and 170.3, respectively. The ^{1}H NMR spectrum of **3** showed signals at δ3.80 and 3.64 assigned to COOMe of methyl glucuronate and methyl oleanolate, respectively. The ^{1}H NMR spectrum of **4** gave a signal at 3.80 due to the methyl glucuronate. Also acid hydrolysis of **4** afforded oleanolic acid. If the glucose moiety was attached to the COOH of the glucuronic acid, methyl oleanolate would have been the hydrolysis product.

The ^{13}C NMR spectra of **3**, **4** and **5** (Table 1) showed that all the sugars were in the pyranose form [10, 11] and that rhamnose was α-linked [5] to C-3′ of glucuronic acid. Comparison of the ^{13}C NMR spectrum of **5** with that of the 3-*O*-β-D-glucopyranoside of methyl oleanolate [12, 13] showed deshielding of C-3′ (5.3 ppm) and shielding of

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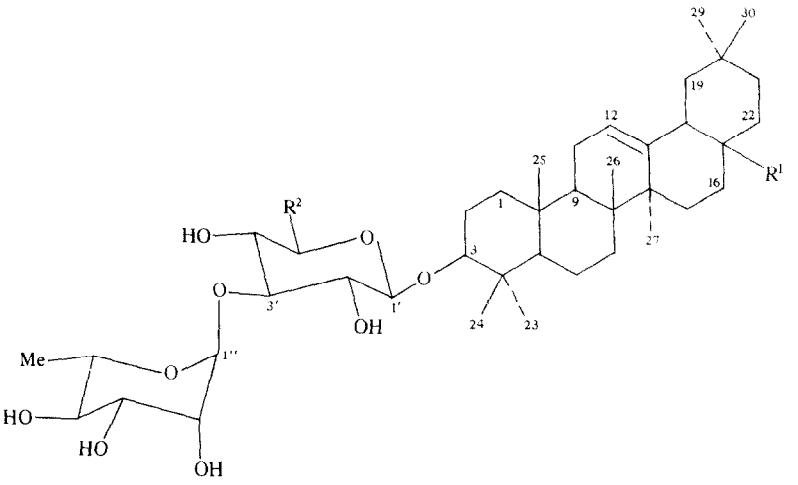
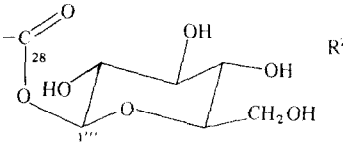
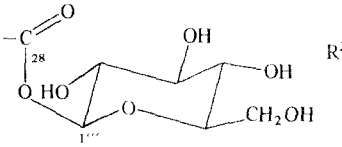


1 $R^1 = R^2 = COOH$

2 $R^1 =$  $R^2 = COOH$

3 $R^1 = R^2 = COOMe$

4 $R^1 =$  $R^2 = COOMe$

5 $R^1 = R^2 = CH_2OH$

C-4′ (2.3 ppm) while C-2′ was not affected. Glycosidation of either C-2′ or C-4′ was not in agreement with the observed upfield shift of C-4′.

Taking into account the acylation effect of secondary hydroxyl groups [14] C-1‴ is expected to appear downfield to the signal of the anomeric carbon of β-D-glucopyranose (δ98.9, C_5D_5N). However, in **4** C-1‴ is observed at 95.4 in accordance with data reported for other glucopyranosyl esters, e.g. arjunglucosides [9], steviosides and paniculosides [15].

Therefore **1** is oleanolic acid 3-*O*-(3′-*O*-α-L-rhamnopyranosyl)-β-D-glucuronopyranoside and **2** is oleanolic acid 3-*O*-(3′-*O*-α-L-rhamnopyranosyl)-β-D-glucuronopyranoside-28-*O*-β-D-glucopyranoside. The Klyne rule [16] confirmed the proposed configurations.

The structures were confirmed by chemical methods using total methylation of **3** and **5**. Hakomori methylation [17] and further hydrolysis of **5** afforded 2,3,4-tri-*O*-methylrhamnose and 2,4,6-tri-*O*-methylglucose which were identified by comparison with standards. Hakomori methylation of **3** followed by $LiAlH_4$ reduction and hydrolysis gave 2,3,4-tri-*O*-methylrhamnose and 2,4-di-*O*-methylglucose.

Compounds **1** and **2** have been isolated from seedcoats of *Putranjiva roxburghii* (Euphorbiaceae) [18] but they were only identified by chemical methods. The present paper is the first report on the spectral data (^1H NMR, ^{13}C NMR and MS) of these glycosides and on their occurrence in the family Compositae.

In spite of the similarity of the petrol extracts of *W. glauca* [1] and *Z. buphthalmiflora* [2] the methanolic extracts of both differ considerably since **1** and **2** were not found in *W. glauca* that contains instead atractyloside, which is responsible for its high toxicity to cattle [C. D. Schteingart and A. B. Pomilio, unpublished results].

Sesquiterpene lactones [19–21], sesquiterpenes [21], tricyclic diterpenes [20], kaurenic acid and grandiflorenic acid [20] have been reported in *Zexmenia* species. On the other hand, esters of 15α-hydroxykaur-16-en-19-oic acid have been frequently found in *Wedelia* species but only

Table 1. ^{13}C NMR spectral data of compounds **3**, **4** and **5** (δ, C_5D_5N, 20.15 MHz)

Carbon	3	4	5	Carbon	3	4	5
1	39.3	39.1	39.3	1′	106.7	106.5	106.4
2	26.3	25.9	26.4	2′	75.4	75.2	75.8
3	89.3	89.3	89.0	3′	82.0	81.9	83.8
4	38.4	38.5	38.7*	4′	71.2	71.0	69.8
5	55.6	55.6	55.6	5′	76.8	76.6	77.9
6	18.3	18.1	18.5	6′	170.5	170.3	62.6
7	33.0	32.9	32.8				
8	39.5	39.6	40.0*	1″	102.6	102.3	102.8
9	47.7	47.7	47.8	2″	72.1	72.0	72.4
10	36.8	36.6	36.7	3″	72.4	72.0	72.4
				4″	73.8	73.5	73.9
11	23.5	23.4	23.8	5″	69.6	69.5	69.8
12	—	—	—	6″	18.3	18.1	18.2
13	144.0	143.8	145.0				
14	41.8	41.8*	41.9	1‴		95.4	
15	27.9	27.9	26.1	2‴		73.5	
16	23.5	23.4	22.6	3‴		78.2*	
17	46.8	46.7	37.5	4‴		70.7	
18	41.8	41.5*	42.7	5‴		78.6*	
19	46.0	46.7	47.0	6‴		61.8	
20	30.6	30.5	31.1				
				6′-Me	52.1	52.1	
21	33.8	32.9	34.5				
22	33.0	31.9	31.7				
23	27.9	27.9	28.0				
24	16.7	16.6	16.3				
25	15.3	15.3	15.6				
26	17.0	17.2	16.3				
27	26.0	25.9	26.1				
28	178.0	176.4	68.6				
29	33.0	32.9	33.3				
30	23.5	23.4	23.8				
28-Me	51.6						

—, Signal masked by solvent.
*Assignments may be interchanged in each vertical column.

once in *Zexmenia* [2]. Further chemotaxonomic studies on species of both genera would clear up the actual relationship between them.

EXPERIMENTAL

Mps are uncorr. ^1H NMR spectra were recorded at 100 MHz in $CDCl_3$, ^{13}C NMR spectra at 20.15 MHz in C_5D_5N and MS at 70 eV by direct inlet.

Plant material. Zexmenia buphthalmiflora (Lorentz) Ariza was collected in Padre Buodo (Utracán, Province of La Pampa, Argentina). A voucher specimen Steibel 6105 is deposited in University of La Pampa, Faculty of Agronomy.

Extraction and isolation of **1** *and* **2**. Dried and ground aerial parts (3 kg) were successively extracted with petrol (69 g of extract, 2.4% of dry plant) and EtOH (151 g of extract, 5.2% of dry plant).

A portion of the ethanolic extract of *Z. buphthalmiflora* was chromatographed on a silica gel H (Merck) column using a gradient of MeOH in $CHCl_3$ as eluent. Three main fractions were obtained. Fraction 1 contained minor terpenoids while fractions 2 and 3 had mainly glycosides **1** and **2**, respectively; TLC (silica gel, **1**: R_f 0.51, **2**: R_f 0.24; $CHCl_3$–MeOH–HOAc–H_2O, 30:8:1.5:1; anisaldehyde–H_2SO_4). Fractions 2 and 3 were separately methylated with CH_2N_2 in Et_2O–MeOH and further purified by silica gel H column chromatography with $CHCl_3$–MeOH (30:2) to give the methyl derivative of **1** (**3**, 30 mg) and with a gradient of $CHCl_3$–MeOH (30:3 to 30:7) to yield the methyl derivative of **2** (**4**, 190 mg); TLC (silica gel, **3**: R_f 0.45, **4**: R_f 0.15; toluene–EtOH, 20:7; anisaldehyde–H_2SO_4).

*Identification of the glycosides. Dimethyl ester of oleanolic acid 3-O-(3′-O-α-*L*-rhamnopyranosyl)-β-*D*-glucuronopyranoside* (**3**). Mp 189–192° (MeOH–H_2O, 1:1); $[\alpha]_D^{20}$ −10°. (MeOH, *c* 0.20); IR ν_{max}^{KBr} cm^{-1}: 3370, 1740, 1630, 1000, 860, 760; ^1H NMR (TMSi deriv.): δ 5.30 (1H, *m*, H-12), 5.11 (1H, *s*, H-1″), 4.52 (1H, *d*, *J* = 6 Hz, H-1′), 4.1–3.5 (sugar protons), 3.80 (3H, *s*, 6′-COOMe), 3.64 (3H, *s*, 28-COOMe), 1.15 (3H, *s*, Me-27), 0.94 and 0.92 (12H, two *br s*, Me-23, 25, 29 and 30), 0.82 (3H, *s*, Me-24), 0.75 (3H, *s*, Me-26); ^{13}C NMR: see Table 1; MS *m/z* (rel. int.): 470 $[A]^+$ (6), 453 $[A-OH]^+$ (7), 452 $[A-H_2O]^+$ (9), 410 $[A-HCOOMe]^+$ (5), 262 (RDA in ring C) (70), 203 $[262-COOMe]^+$ (100), 189 $[203-14]^+$ (40); MS (TMSi deriv.) *m/z* (rel. int.): 527 $[A+TMS-H-15]^+$ (2), 470 $[A]^+$ (0.5), 453 $[A-OH]^+$ (43), 452 $[A-H_2O]^+$ (13), 363 (TMSi rhamnose oxonium ion) (74), 273 $[363-TMSiOH]^+$ (15), 262 (40), 204 (100), 203 (43), 189 (20); A = aglycone.

Acid hydrolysis of **3**. This was carried out in a sealed tube with 7% H_2SO_4 for 3 hr at 100°. The aglycone was extracted with

$CHCl_3$ and identified as methyl oleanolate by comparison with standards (TLC, mp, mmp, GC). The aq. layer was neutralized with $BaCO_3$, and successively treated with a strong cationic exchange resin (AG 50W-X8, Bio Rad, form: H^+) and a weak anionic exchange resin (AG 3-X4A, Bio Rad, form: $-NH_2$). Analysis of the eluate by TLC (Cellulose F, Merck, 100 μm; *n*-BuOH–C_5H_5N–H_2O, 6:4:3; $AgNO_3$–2% NaOH) with appropriate standards showed the presence of rhamnose. Elution of the anionic resin column with 7% H_2SO_4, neutralization with $BaCO_3$ and further treatment with a strong cationic resin (form: H^+) led to a soln that contained glucuronic acid (TLC, cellulose, as above).

Methyl ester of oleanolic acid 3-*O*-(3′-*O*-α-L-*rhamnopyranosyl*)-β-D-*glucuronopyranoside*-28-*O*-β-D-*glucopyranoside* (**4**). Mp 234–237° (MeOH–H_2O, 1:1); $[\alpha]_D^{20}$ −17°. (MeOH, *c* 0.25); ^{1}H NMR (TMSi deriv.): δ 5.60 (1H, *d*, *J* = 6 Hz, H-1‴), 5.33 (1H, *m*, H-12), 5.14 (1H, *s*, H-1″), 4.48 (1H, *d*, *J* = 6 Hz, H-1′), 4.1–3.4 (sugar protons), 3.80 (3H, *s*, 6′-COOMe), 1.12 (3H, *s*, Me-27), 0.92 (12H, *br s*, Me-23, 25, 29 and 30), 0.82 (3H, *s*, Me-24), 0.78 (3H, *s*, Me-26); ^{13}C NMR: see Table 1; MS *m/z* (rel. int.): 456 $[A]^+$ (4), 439 $[A-OH]^+$ (4), 438 $[A-H_2O]^+$ (5), 248 (RDA in ring C) (100), 207 (rings A and B) (48), 203 $[248-COOH]^+$ (85), 189 (42).

Acid hydrolysis of **4**. This was carried out as above yielding oleanolic acid (mp, TLC, GC, MS), rhamnose, glucose and glucuronic acid.

Alkaline hydrolysis of **4**. Compound **4** was heated in a sealed tube with NH_3 (c) for 2 hr at 100°. The soln was acidified with aq. H_2SO_4 and twice extracted with *n*-BuOH. The organic layer was evaporated to dryness, methylated with CH_2N_2 in Et_2O–MeOH and purified by silica gel CC (toluene–EtOH, 20:4) to give **3**. The aq. layer was neutralized, worked up and analysed as mentioned for **3**. Only glucose was detected.

Preparation of **5**. Compound **4** (100 mg) was treated with $LiAlH_4$ in excess under reflux in THF for 16 hr. The suspension was worked up in the usual way and the product was purified by silica gel H CC (toluene–EtOH, 20:4) yielding 47 mg of **5** (60%): TLC (silica gel, R_f 0.31, toluene–EtOH, 20:7, anisaldehyde–H_2SO_4); $[\alpha]_D^{20}$ +4°. (EtOH, *c* 0.43); ^{13}C NMR: see Table 1.

Acid hydrolysis of **5**. As above. Erythrodiol (TLC, GC with standards), glucose and rhamnose (TLC and GC of the acetate alditols with standards) were detected.

Total methylation of **5** *and characterization of the partially methylated sugars*. Compound **5** was methylated by the Hakomori method. The product was purified by silica gel H CC (toluene–EtOAc, 4:1) and hydrolysed in a sealed tube with Kiliani mixture (HOAc–HCl–H_2O, 35:15:50) for 16 hr at 80°. MeOH was added to the soln which was neutralized with the weak anion resin (form: $-NH_2$) in MeOH and analysed by TLC (silica gel; toluene–EtOH, 20:7) with partially methylated sugars as standards. 2,3,4-Tri-*O*-methylrhamnose (R_f 0.74) and 2,4,6-tri-*O*-methylglucose (R_f 0.35) were found.

Total methylation of **3**, *reduction and analysis of the partially methylated sugars*. Compound **3** was dissolved in DMSO and treated with dimsyl carbanion for 2 hr at room temp. MeI was added and the soln left standing for 1.5 hr at room temp. The product was isolated in the usual manner, reduced under reflux with $LiAlH_4$ in THF overnight and hydrolysed as described for **5**. Partially methylated sugars were analysed by HPTLC (silica gel, Merck, toluene–EtOH, 20:13) with standards. 2,3,4-tri-*O*-methylrhamnose (R_f 0.90) and 2,4-di-*O*-methylglucose (R_f 0.45) were detected. GC identification of the acetate alditols with authentic samples was also performed.

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