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Investigación

Two New Oleanolic Acid Saponins from the Roots of *Viguiera hypargyrea*

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Dedicated to Professor Alfonso Romo de Vivar

Abstract. Two new triterpene saponins whose aglycons are based on the oleanane skeleton (**1-2**), were isolated from the roots of *Viguiera hypargyrea*, together with two known triterpene saponins (**3** and **4**) as well as the triterpenes friedelin, friedelan 3β-ol and oleanolic acid. The structures of the new compounds were established mainly by 2D NMR techniques of their peracetylated derivatives as 3-*O*-[α-L-rhamnopyranosyl (1 → 3)-β-D-xylopyranosyl (1 → 4)]-β-D-glucopyranosyl-oleanolic acid-28-*O*-β-D-glucopyranoside and 3-*O*-[α-L-rhamnopyranosyl (1 → 3)-β-D-xylopyranosyl (1 → 4)]-β-D-glucopyranosyl oleanolic acid respectively.
Keywords: *Viguiera hypargyrea*, Asteraceae, roots, oleanolic acid saponins, bisdesmosides.

Resumen. De las raíces de *Viguiera hypargyrea* se aislaron dos nuevas saponinas triterpénicas (**1-2**), cuyas agliconas corresponden al esqueleto del oleanano, junto con dos saponinas triterpénicas conocidas (**3** y **4**), así como los triterpenos friedelina, friedelan-3β-ol y ácido oleanólico. Las estructuras de los compuestos novedosos fueron establecidas principalmente por medio de técnicas de RMN 2D de sus derivados peracetilados como 3-*O*-[α-L-rhamnopiranosil (1 → 3)-β-D-xilopiranosil (1 → 4)]-β-D-glucopiranosil-ácido oleanólico-28-*O*-β-D-glucopiranósido y ácido 3-*O*-[α-L-ramnopiranosil (1 → 3)-β-D-xilopiranosil (1 → 4)]-β-D-glucopiranosil oleanólico respectivamente.
Palabras clave: *Viguiera hypargyrea*, Asteraceae, raíces, saponinas del ácido olanólico, bisdesmósidos.

Introduction

Viguiera hypargyrea Blake (Asteraceae) is a perennial herb distributed on Northern Mexico [1]. The roots of this plant are used for gastrointestinal disorders in Mexican traditional medicine and it is commonly known as "plateada" [2]. Diterpenic acids and sesquiterpene lactones have been reported from the leaves [3]. We have recently reported that the *n*-hexane and ethyl acetate-soluble portions and their principal diterpenic acid components *ent*-beyer-15-en-19-oic and *ent*-kaur-16-en-19-oic acids showed antispasmodic and antimicrobial effects [4]. Although the methanol-soluble portion did not exhibit apparent antispasmodic and antimicrobial activity at a sample concentration of 25 μg/mL and 10 mg/mL respectively, we had interest in the chemical constituents of this fraction, and here we report the results.

Results and discussion

Chromatographic separations of the methanol soluble fraction have resulted in the isolation of the known triterpenes friedelin, friedelan-3-β-ol and oleanolic acid, which were identified by direct comparison with authentic samples. Two new triterpene saponins based on the oleanane skeleton (**1,2**), which were characterized as their peracetate derivatives (**1a,2a**) were also isolated, together with the known saponins β-D-glucopyranosyl olean-12-en-28-oate (**3**) and 3-*O*-[methyl-β-D-glucuronopyranosiduronoate]-28-*O*-β-D-glucopyranosyl oleanolate (**4**), which were identified by comparison of their spectroscopic data with those previously described [5, 6].

In this paper, we report the structural determination of the new saponins on the basis of spectroscopic analysis and acid-catalyzed hydrolysis.

Compound **1a** was obtained as an oil after acetylation of the natural product **1**. In the positive-ion FABMS of **1a**, quasi-molecular ion peaks were observed at m/z 1600 $[M + K + H]^+$, 1584 $[M + Na + H]^+$, and 1561 $[M + H]^+$, and HRFABMS analysis revealed the molecular formula to be $C_{77}H_{108}O_{33}$. Other significant peaks visible at m / z 1254 $[M + K - C_{14}H_{19}O_{10}]^+$, 1068 $[M - C_{12}H_{17}O_7 - C_{11}H_{15}O_7]^+$, and 777 $[M - C_{33}H_{45}O_{21}]^+$, indicated the successive loss of one hexosyl, one deoxyhexosyl, one pentosyl and one hexosyl moieties. Another fragment ion at m/z 437 corresponded to the pseudo-molecular ion of the aglycon. On acid hydrolysis, **1a** liberated oleanolic acid as the genin, and glucose, rhamnose and xylose, which were identified by comparison with authentic samples by co-TLC, IR and NMR. On alkaline hydrolysis, only glucose was detected by co-TLC with an authentic sample, indicating that the glucose was bound to the genin by a glycosidic ester linkage at C-28 [7]. The ^1H and ^{13}C NMR spectra of **1a**, which are presented in Table 1, showed that most of the signals of the aglycon were in good agreement with literature data for oleanolic acid [8]. Glycosylation shifts were observed

Table 1. ^{13}C (125 MHz) and ^{1}H (500 MHz) NMR Spectral Data for the Aglycon Part of Compounds **1a**, **2a** and **2b** ($CDCl_3$, δ in ppm).

	1a		**2a**		**2b**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	38.50	0.82, 1.59	38.46	0.89, 1.56	38.6	0.85, 1.60
2	27.69	1.07, 1.67	27.65	1.07, 1.67	27.1	1.07, 1.66
3	90.41	2.97 dd(11.7, 5)	90.44	2.95 dd(13.5, 4.5)	90.41	2.97 dd (13.0, 5)
4	38.81		38.84		38.80	
5	55.56	0.64	55.59	0.67	54.9	0.67
6	18.15	1.32, 1.46	18.13	1.31, 1.46	18.13	1.31, 1.46
7	31.74	1.26, 1.61	32.37	1.59, 1.74 dd(13.5, 4.5)	32.5	1.55,
8	39.33		39.30		39.30	
9	47.58	1.46	47.65	1.49 dd(11, 6.5)	47.60	1.47 dd (11, 6.5)
10	36.68		36.75		36.74	
11	23.41	1.86	23.38	1.86	23.30	1.83
12	122.92	5.31 t(3)	122.64	5.26 t(3.5)	122.59	5.25 t(3.5)
13	142.85		143.60		143.61	
14	41.02		40.96		41.00	
15	25.62	1.1, 1.72	25.69	1.64, 1.74	25.65	1.60, 1.73
16	22.82	1.28, 1.54	2.97	1.60, 1.90	22.95	1.60, 1.92
17	46.81		46.49		46.52	
18	41.68	2.81 dd(14, 4)	1.60	2.80 dd(14, 4)	41.65	2.80 dd (13.5, 5)
19	45.79	1.14, 1.62	45.95	1.13, 1.60	45.93	1.14, 1.62
20	30.58		30.63		30.60	
21	33.74	1.20, 1.32	33.80	1.20, 1.33	33.79	1.21, 1.32
22	32.96	1.27, 1.36	32.63	1.27, 1.36	32.59	1.27, 1.36
23	27.69	0.90	27.72	0.86	27.70	0.88
24	16.27	0.70	16.25	0.69	16.25	0.69
25	15.22	0.86	15.19	0.87	15.20	0.88
26	16.92	0.72	17.01	0.73	17.10	0.73
27	25.62	1.10	25.85	1.11	25.80	1.11
28	175.59		183.05		179.80	
29	32.96	0.89	33.02	0.89	32.90	0.89
30	23.41	0.88	23.54	0.91	23.50	0.90
OMe					51.82	3.61

at C-3 and C-28 of the aglycon, indicating that the saccharide units were attached at these positions (*i.e.*, signals at δ 90.44 and 175.56 represented a downfield shift by 10.6 ppm and an upfield shift by 3.6 ppm, respectively, when compared with the analogous data for oleanolic acid). Compound **1a** was shown to contain four sugar residues in a HMQC NMR experiment, which revealed the correlations between anomeric carbons in the δ 105-90 range and anomeric proton signals resonating between δ 4.0 and 6.1. Thus, the anomeric ^{13}C signals at δ 103.0, 100.89, 95.98 and 91.58 gave cross-peaks with anomeric protons at δ 4.35 d (J = 8.0 Hz), 4.44 d (J = 8.0 Hz), 5.07 d (J = 1.5 Hz), and 5.58 d (J = 8.0 Hz) respectively. The sugar moieties of **1a** were assigned mainly from the ^{1}H-^{1}H COSY, HMQC, and HMBC NMR spectra. Evaluation of spin-spin couplings and chemical shifts allowed the identification of one β-xylopyranose unit with the anomeric proton at δ 4.44, one α-rhamnopyranose unit with the anomeric proton at δ 5.07, and two β-glucopyranose units with the anomeric protons resonating at δ 5.58 and δ 4.35 respectively, with the former linked to the carboxylic group of the aglycon through an ester linkage, and the latter being linked to C-3 of the aglycon [9,10]. The common D-configuration for xylose and glucose and the L-configuration for rhamnose were assumed to be those of the most commonly encountered analogues in the plant kingdom [11]. The sequence of the sugar moieties in **1a** was determined from the HMBC and NOESY NMR spectra. In the HMBC spectrum, long-range ^{13}C-^{1}H correlations were observed between the signals at δ_C 175.56 and δ_H 5.58, δ_C 72.65 and δ_H 5.07, δ_C 74.87 and δ_H 4.44, and δ_C 90.44 and δ_H 4.35. Accordingly, the glucopyranose unit with the anomeric proton at δ 4.35 was linked to C-3 of the aglycon, and the rhamnose and xylose units were linked to C-3 and C-4 positions of this glucose unit. The other glucose unit (δ 5.58) was linked to C-28 of the aglycon. On the basis of all evidence, the natural product (**1**) was identified as 3-*O*-[α-L-rhamnopyranosyl (1 → 3)-β-D-xylopyranosyl (1 → 4)]-β-D-glucopyranosyl-oleanolic acid-28-*O*-β-D-glucopyranoside.

Compound **2a** displayed a ^{1}H NMR spectrum very similar to that of compound **1a**, with a triplet at δ_H 5.20 (J = 3.0 Hz) and seven methyl singlets in the high-field region. A significant difference was the absence of the anomeric doublet at δ_H 5.58. The ^{13}C NMR spectrum contained six carbon less (47

Table 2. ^{13}C (125 MHz) and ^{1}H (500 MHz) NMR Spectral Data for the Sugar Moieties of Compounds **1a**, **2a** and **2b** ($CDCl_3$, δ in ppm; *J* in Hz).

	1a		**2a**		**2b**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
C-3-glucose						
1	103.0	4.35 d(8)	103.05	4.36 d(8)	103.05	4.36 d(8)
2	73.91	5.03 dd(9.5, 8)	73.92	5.02 dd(9.5, 8)	73.90	5.02 dd(9.5, 8)
3	72.65	3.87 t(9.5)	72.65	3.87 t(9.5)	72.65	3.87 t(9.5)
4	74.82	3.79 t(9.5)	74.87	3.79 t(9.5)	74.85	3.78 t(9.5)
5	72.87	3.47 ddd(9.5, 5, 2)	72.86	3.47 ddd(9.5, 5, 2)	72.86	3.47 ddd(9.5, 5, 2)
6	61.70	4.01 dd(12, 5)	61.73	4.01 dd(12. 5)	61.72	4.01 dd(12,5)
		4.60 dd(12, 2)		4.60 dd(12, 2)		4.60 dd(12,2)
xylose						
1	100.89	4.44 d(8)	100.91	4.44 d(8)	100.90	4.44 d(8)
2	71.33	4.96 dd(9.5, 8)	71.36	4.96 dd (9.5, 8)	71.35	4.96 dd(9.5, 8)
3	73.0	5.14 t(9.5)	73.05	5.14 t(9.5)	73.02	5.14 t(9.5)
4	68.03	5.09 ddd(10.5, 9.5, 5)	68.30	5.07 ddd(10.5, 9.5, 5)	68.30	5.07 ddd(10.5, 9.5, 5)
5	62.82	3.28 t(10.5)	62.83	3.28 t(10.5)	62.84	3.28 t (10.5)
		4.35 dd(10.5, 5)		4.36 dd(10.5, 5)		4.36 dd (10.5, 5)
rhamnose						
1	95.98	5.07 d(1.5)	96.02	5.07 d(1.5)	96.05	5.06 d(1.5)
2	70.95	5.01 dd(3.5, 1.5)	70.93	5.01 dd(3.5, 1.5)	70.95	5.01 dd(3.5, 1.5)
3	67.85	5.68 dd(10.5, 3.5)	67.87	5.68 dd(10.5, 3.5)	67.87	5.68 dd (10.5, 3.5)
4	71.49	5.13 t(9.5)	71.53	5.13 t (9.5)	71.52	5.13 t(9.5)
5	66.26	4.53 dd(9.5, 6.5)	66.27	4.53 dd(9.5, 6.5)	66.25	4.53 dd (9.5, 6.5)
6	17.13	1.25 d(6.5)	17.13	1.25 d(6.5)	17.13	1.25 d(6.5)
C-28-glucose						
1	91.58	5.58 d(8)				
2	68.28	5.10 dd(9.5,8)				
3	72.87	5.24 t(9.5)				
4	69.96	5.17 t(9.5)				
5	72.46	3.79 ddd(9.5, 5, 2)				
6	61.54	4.04 dd(12, 5)				
		4.27 dd(12, 2)				

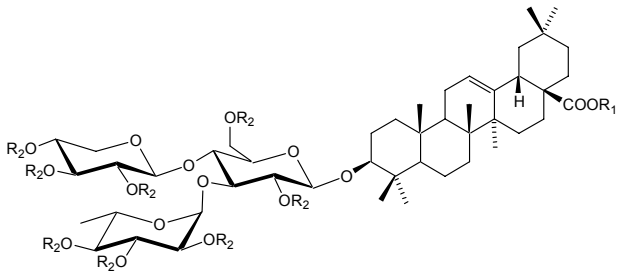
singlets) than **1a**, and the chemical shifts values of the carbons were within the range of 1 ppm of those found for compound **1a**, with the exception of C-28, which appeared at δ_C 183.05 (cf. δ_C 175.56 for **1a**, Tables 1 and 2). These observations indicated the absence of the glucopyranose unit at the carboxyl group. As additional proof, compound **1** was hydrolyzed with KOH giving compound **2** and glucose. Moreover, methylation of **2a** with diazomethane afforded the methyl ester derivative (**2b**). The position of the methyl ester in **2b** was determined from the HMBC correlation between the methyl ester proton δ_H 3.61 (s, $-OCH_3$) and C-28 (δ_C 179.80).

All those data allowed compound **2** to be assigned the structure 3-*O*-[α-L-rhamnopyranosyl (1 → 3)-β-D-xylopyranosyl (1 → 4)]-β-D-glucopyranosyl oleanolic acid.

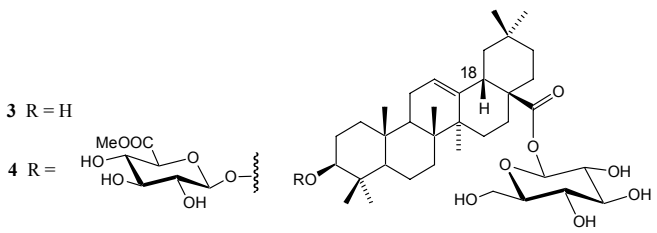
Although the methanol extract from the roots of *Viguiera hypargyrea* did not show any antispasmodic and antimicrobial activities [4], the presence of mono and bisdesmoside saponins in this extract is noteworthy, since various triterpene saponins structurally related to those isolated in this work, have shown important biological activities such as inhibitory effects on ethanol absorption [12], as well as hypoglycemic activity [13].

Experimental

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 digital polarimeter at 25 °C. IR spectra were recorded on a Bruker Vector 22 FTIR. All NMR spectra were recorded on a Varian Unity Plus-500 at 500 MHz for ^{1}H NMR, ^{1}H-^{1}H COSY, HMQC, HMBC and ^{1}H-^{1}H TOCSY and 125 MHz for ^{13}C NMR and ^{13}C DEPT in $CDCl_3$. Chemical shifts are reported in ppm relative to TMS. FABMS and HRFABMS were performed using a Hewlett Packard 5985-B and a JEOL-AX 505 HA mass spectrometer, respectively.



	R_1	R_2
1	Glcp	H
1a	Glcp	Ac
2	H	H
2a	H	Ac
2b	Me	Ac



3 R = H

4 R =

Plant Material. The roots of *V. hypargyrea* were collected near to Durango City, on September 15th of 1997 and identified by Dr. Robert Bye (Instituto de Biología de la UNAM). A Botanical sample was prepared and deposited for reference at the National Herbarium of Mexico (MEXU) with the code number MEXU961417.

Extraction and Isolation. 200 g of the methanol extract obtained previously [4] were fractionated by percolation using a gradient of CH_2Cl_2-MeOH yielding six fractions: Fr. I (9:1, 16.0 g); Fr. II (85:15, 2.4 g); Fr. III (4:1, 4.0 g); Fr. IV (7:3, 7.3 g); Fr. V (1:1, 18.2 g) and Fr. VI (3:7, 22.4 g). Fr. I was purified by column chromatography using mixtures of *n*-hexane-EtOAc; fractions eluted with 95:5 (*n*-hexane-EtOAc) afforded 325 mg of friedelin (0.010 %, mp 242-245 °C); from fractions eluted with *n*-hexane-EtOAc 9:1 crystallized 83 mg of friedelan-3-β-ol (0.0027 %, mp 249-253 °C), fractions eluted with *n*-hexane-EtOAc (85:15) yielded 54 mg of the mixture of β-sitosterol and stigmasterol. Fr. II was applied to a silica gel column using a gradient system of CH_2Cl_2-MeOH to yield 1.7 g of oleanolic acid (0.056 %, mp 196-198 °C). Chromatographic analyses of Fr. III showed that this was mainly composed by the saponins glucopyranosyl oleanolate (**3**), found previously in the ethyl acetate extract [4], and 3-*O*-(methyl-β-D-glucuronopyranosiduronoate)-28-O-β-D-glucopyranosyl oleanolate (**4**, mp 217-218 °C) isolated previously from *V. decurrens* [5]. Fr. IV was applied to a silica gel column using EtOAc-MeOH-AcOH-H_2O (11:2:2:1) as isocratic elution mixture. Fractions 8-12 afforded 50 mg of **4**; fractions 17-26 yielded 12 mg of **1** (mp 129-132 °C) and 9 mg of **2** (mp 276-278 °C), and fractions 31-43 afforded 820 mg of glucose. Fr. V was mainly composed by sucrose, identified by direct comparison with authentic sample. Fr. VI was ground with acetone to yield a mixture of saponins which was acetylated with Ac_2O-Py and the residue was chromatographed on silica gel column using mixtures of CH_2Cl_2-Acetone: Fractions eluted with 9: 1 (CH_2Cl_2-acetone) yielded 843 mg of 1a (0.028 % of dry plant). Fractions eluted with CH_2Cl_2-acetone (8:2) afforded sucrose acetylated and 632 mg of **2a** (0.021 % of dry plant).

Friedelin, friedelan-3-β-ol and oleanolic acid, were identified by direct comparison (IR, TLC) with authentic samples, while compounds **1a**, **2a** and **3**-**4** were characterized by means of physicochemical evidence.

Compound 1a. Oil, $[\alpha]_D^{25}$ – 4.4° (c 0.05, MeOH); IR ($CHCl_3$) ν_{max} 2922, 1757, 1452, 1376, 1050 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) see Tables 1 and 2; ^{13}C NMR ($CDCl_3$, 125 MHz) see Tables 1 and 2; FABMS m/z 1600 $[M + K + H]^+$ and 1584 $[M + Na + H]^+$, 1254 $[M + K – Glc]^+$, 1068 $[M – rham – xyl]^+$, 785 $[M – glc – rham – xyl]^+$, 437 $[C_{30}H_{46}O_2]^+$; HRFABMS m/z 1561.6983 (calcd for $C_{77}H_{108}O_{33}$, 1561. 6995).

Compound 2a. Colorless powder, mp 101-102 °C, α_D^{25} + 6° (c 0.05, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3500-3400, 2922, 1757, 1452, 1376, 1050 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) see Tables 1 and 2 ^{13}C NMR ($CDCl_3$, 125 MHz) see Tables 1 and 2; FABMS m/z 1271 $[M + K]^+$, 1255 $[M + Na]^+$, 1233 $[M]^+$, 958 $[M – xyl]^+$, 954 $[M – rham]^+$, 669 $[M – rham – xyl]^+$, 467 $[M – rham – xyl – glc]^+$; HRFABMS m/z 1233.4209 (calcd for $C_{63}H_{92}O_{24}$, 1233.4212).

Compound 2b. Compound **2a** (20 mg) was treated with diazomethane in diethyl ether to yield 20 mg of **2b**: Oil, $[\alpha]_D^{25}$ + 2.5° (c 0.2, $CHCl_3$), IR ($CHCl_3$) ν_{max} 2958, 1728, 1462, 1377, 1072 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) see Tables 1 and 2; ^{13}C NMR ($CDCl_3$, 125 MHz) see Tables 1 and 2.

Acid hydrolysis of compounds 1 and 2. Saponins **1** (5 mg), and **2** (5 mg) in 0.5 M HCl (dioxane-H_2O, 1:1; 5 ml) were refluxed on a water bath at 100 °C for 2 h. After cooling, the nonpolar reaction product was separated by precipitation with ice (3 g) and filtration. The aqueous layer was neutralized with NH_4OH and reduced to dryness by lyophilization. The sugars were analyzed by silica gel TLC [EtOAc-MeOH-H_2O-AcOH (11:2:2:2)] by comparison with standard sugars.

Alkaline hydrolysis of compound 1. The saponin (12 mg) in KOH 10 % (4 mL) was heated at 100 °C for 75 min. After acidification with HCl (pH 5), the monodesmoside was extracted with *n*-BuOH. Comparison with compound **2** demonstrated that both compounds were identical. The aqueous solution contained glucose was identified by TLC comparison with an authentic sample.

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