ISSN : 0974 - 7508 Volume 10 Issue 3

Natural Products

An Indian Journal

Full Paper

NPAIJ, 10(3), 2014 [61-68]

Triterpenoid saponins from *Eryngium agavifolium*

Cristina B.Colloca[1]*, Luis Ariza Espinar[2], Virginia E.Sosa[3]

[1]Instituto de Ciencias Polares, Ambiente y Recursos Naturales. Universidad Nacional de Tierra del Fuego, Antártida e Islas del Atlántico Sur. 9410 Ushuaia, (TIERRA DEL FUEGO)

[2]Instituto Multidisciplinario de Biología Vegetal - IMBIV, (CONICET-UNC)

[3]Departamento de Química Orgánica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Pabellón de Ciencias II. Ciudad Universitaria, 5000 Córdoba, (ARGENTINA)

E-mail: ccolloca@untdf.edu.ar

ABSTRACT

Aerial parts of *E. agavifolium* G. yielded 3-O-(β-D-glucopyranosyl) betulinic acid 28-O-(β-D-glucopyranosyl) ester, 3-O-(β-D-glucuronopyranosyl) betulinic acid 28-O-(β-D-glucopyranosyl) ester, 3-O-(β-D-galactopyranosyl) betulinic acid 28-O-[β-D-glucopyranosyl (1→ 3)-β-D- glucopyranosyl] ester, 3-O-[3-β-D-glucopyranosyl(1→2)-α-L-arabinopyranosyl] betulinic acid 28-O-(β-D-glucopyranosyl) ester and the already known 3-O-β-D-glucopyranosyl betulinic acid. The structures of the isolated compounds were determined using spectroscopic methods.

KEYWORDS

Eryngiumagavifolium;
Apiaceae;
Saponins;
Betulinic acid glycosides.

INTRODUCTION

Eryngium L. is a complex genus with *ca.* 250 species, approximately 29 species growing in Argentina[30]. The delimitation of the species is frequently laborious, particularly for the Sec. Areata ser. Platyphylla from the South of Brazil, Paraguay and East of Argentina (coastal zone of the Rio de la Plata).

Eryngium (subfamily Saniculoideae) is known to contain acetylenes[2,6,29], flavonoids and coumarins[4,12,33,28] and ciclohexenone derivatives[7]. Several classes of terpenoids have been described, such as essential oils[32,35,36], sesquiterpenes[1], phytosterols[10], sapogenins[14,16,17,20,21] and triterpenesaponins[5,13,15,18-20,22,23,25,27].

In this paper we report the isolation and structure elucidation of five triterpene saponins, 1 - 5 from *Eryngium agavifolium* Griseb,

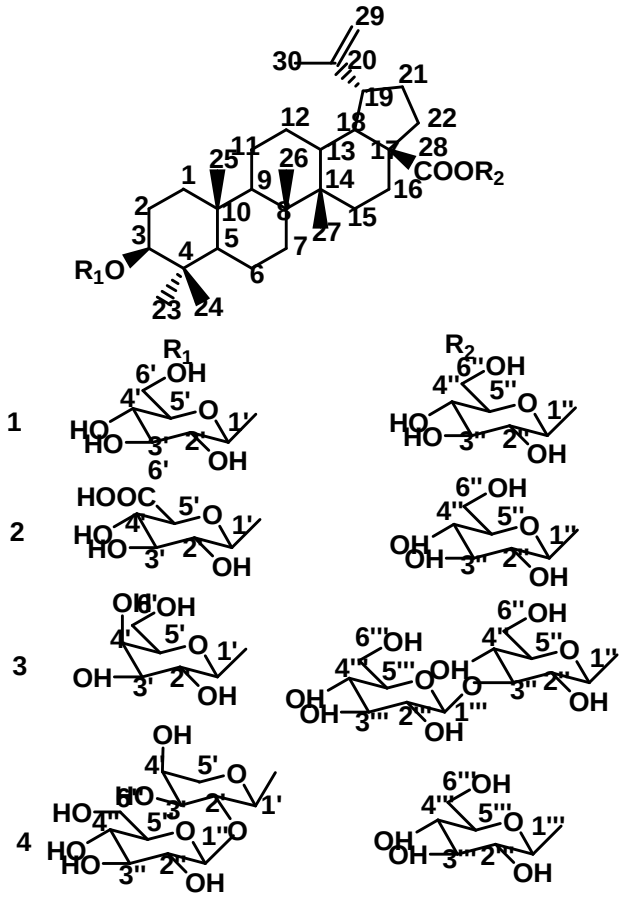
RESULTS AND DISCUSSION

The n-butanol soluble fraction of the EtOH extract of the fresh aerial parts of *E. agavifolium* G. gave saponins1 – 4 and the already known 3-O-β-D-glucopyranosylbetulinic acid 5[8,9].

Five triterpene saponins (1-5) were isolated, which were purified by successive chromatographic steps, and its structure was mainly determined by NMR analysis, including 1D and 2DNMR (^{1}H -^{1}HCOSY, TOCSY, NOESY, HSQC, HMBC), and mass spectrometry

Compound 1 was obtained as an amorphous white powder. The IR spectrum showed a broad absorption due to hydroxyl groups near 3388 cm^{-1}, as well as absorption attributable to a carbonyl group of the ester at 1731 cm^{-1}. Compound 1 exhibited in HRFABMS a quasi-molecular ion peak at *m /z* 803.9716, consistent

Full Paper



with a molecular formula of $C_{42}H_{68}O_{13}Na$. Also, we observed other significant peaks in the spectrum at *m/z* 641 [M +Na-162], and 479 [M+Na- 2 x162] indicating the loss of two hexose residues.

The ^1H NMR spectrum of 1 (TABLE 1) clearly showed presence of five tertary methyl groups at δ_H0.96, 0.93, 0.86, 0.79 and 0.75 (each 3H, s, H-23, H- 27, H- 26, H- 23 and H- 24), one secondary methyl at δ_H 1.62 (3H, br s, H-30) and one exo-methylene group at δ_H 4.62 and 4.75 (each 1H, br s, H-29a and H-29b). Moreover, displayed signals for two anomeric protons at δ_H 4.24 (*d*, *J* = 7.2 Hz) and 5.37 (*d*, *J* = 8.2 Hz), which gave correlations, in the HSQC spectrum, with anomeric carbon signals at δ_C 105.2 and 95.5, respectively.

The comparison of ^1H and ^{13}C NMR data (TABLE 1) with of data of literature, permitted assignment the structure of the betulinic acid for the aglycon moiety, in relation to report for Janeczko*et al.*1990.

The ring protons of the monosaccharide residues were assigned starting from the readily identifiable anomeric protons by means of $^1H-^1H$ COSY, TOCSY, HSQC and HMBC spectroscopic experiments (TABLE 2).

The anomeric protons atδ4.24 (*d*, *J* = 7.2 Hz) and 5.37 (*d*, *J* = 8.2 Hz) correlated with the carbon signals at δ87.9 and 177.0, respectively, in the HMBC spectrum (TABLE 3). In pyranosides, the six-membered ring generally forms a chair of fixed conformation providing a classification of the protons as axial or equatorial. Therefore, the coupling patterns are characteristic of the stereochemistry of the type of the carbohydrate. For example, if the H-2 is axial, as it is for *gluco* and *galacto* stereochemistry, then a small coupling constant ($^3J_{HH}$) of *ca* 2-4 Hz is observed as resulted of the *gauche* conformation of H-1 and H-2 following the Karplus relation (dihedral angle *ca* 60º) The *trans* diaxial relationship of H-1 and H-2 in *β*-anomers of sugars with a *gluco* and *galacto* configuration leads to larger (7-9 Hz) coupling constants (dihedral angle *ca* 180º). The anomeric coupling constants obtained for both sugar units were indicative of a β-configuration. The protons sequence in each glycosyl residue was deduced from $^1H-^1H$ COSY experiment and the HMQC correlated all proton resonances with those of the corresponding carbons and revealed the presence of two terminal glucopyranosyl units. The ring protons of the glucosyl residues were assigned starting from the anomeric protons by means of the COSY, HMQC, HMBC (TABLE 3) and NOESY spectra.

All carbon signals due to these sugar moieties were assigned by comparison with literature data[8,9].

On the basis of the above results, the structure of 1 was determined as 3-O-(β-D-glucopyranosyl) betulinic acid 28-O-(β-D-glucopyranosyl) ester, a new natural compound.

Compound 2 was obtained as an amorphous white powder. The IR spectrum showed a broad absorption due to hydroxyl groups near 3388 cm^{-1} as well as two absorptions attributable to an ester carbonyl group and a carboxilic acid group at 1731 and 1710 cm^{-1}, respectively. The FABMS of compound 2 showed a quasimolecular ion peak at *m/z*: 817 $[M + Na]^+$. Other significant peaks in the spectrum were at 655 [M + Na – 162], and 479 [M + Na - 162 - 176] that indicated the loss of one hexose and a glucuronic acid residue. The positive HRFABMS showed a clustered molecular ion peak at *m/z* 817.9551 that accounted for the

TABLE 1 : ^{1}H and ^{13}C NMR spectroscopic data foraglycone moieties of compounds 1-4[a]

Atom	1[b]		2[c]		3[d]		4[b]	
	δ ^{1}H	δ ^{13}C	δ ^{1}H	δ ^{13}C	δ ^{1}H	δ ^{13}C	δ ^{1}H	δ ^{13}C
1	1.45 m, 0,70 m	39.1	0.97 m, 1.67 m	39.1	1.50m, 0.82 m	39.3	1.39 m, 0.69 m	39.1
2	1.95 m, 1.75 m	25.8	2.00 m, 1.62 m	26.1	1.90 m, 1.60 m	26.0	2.00 m, 1.80 m	25.8
3	3.29 d (10.3 Hz)	88.9	3.20 *dd* (10.2, 4.5)	89.5	3.20 m	89.1	3.21 m	89.3
4		39.3		39.3		39.6		39.6
5	0,62 d (9 Hz)	55.2	0.75 d (8.5 Hz)	56.3	0.65 d (8.0)	55.9	0.60 d (9 Hz)	55.6
6	1.50 m, 1,40 m	18.7	1.53 m, 1.43 m	18.4	1.50 – 1.40 m	18.1	1.50 m, 1.30 m	18.6
7	1.40 m	34.3	1.42 m	34.6	1.40 m	33.1	1.45 m	34.5
8		41.4		41.0		41.2		41.1
9	1.26 m	50.8	1.28 m	51.2	1.25 m	50.5	1.20 m	50.9
10		38.9		37.1		37.1		38.9
11	1.30 m, 1.15 m	20.9	1.20 m, 1.10 m	21.0	1.30 – 1.10 m	21.5	1.25 m, 1.07 m	21.1
12	1.76 m, 1.20 m	26.1	1.65 m, 1.23 m	25.9	1.62, 1.26	25.9	1.70 m, 1.10 m	26.1
13	2.55 m	37.9	2.34 m	38.5	2.20 m	38.4	2.58 m	38.3
14		42.9		42.5		43.1		42.3
15	1.20-1.00 m	30.4	1.32 m, 1.20 m	29.7	1.35 – 1.15 m	29.5	1.25 – 1.05 m	30.3
16	2.57 m, 1,41 m	31.7	2.50 m, 1.48 m	31.9	2.20 m, 1.30 – 1.15 m	32.3	2.56 m, 1.46 m	32.1
17		56.9		56.8		56.4		57.1
18	1.64 m	49.3	1.68 m	49.6	1.56 m	49.4	1.69 m	49.8
19	3.41 m	47.3	3.40 m	47.2	3.25 m	47.2	3.30 m	47.6
20		150,6		150.8		150.7		150.7
21	2.10 m, 1.30 m	30.5	1.95 m, 1.40 m	30.4	2.00 m, 1.46 m	30.2	1.95 m, 1.40 m	30.4
22	2.13 m, 1.40 m	36.4	2.00m, 1.45 m	36.5	2.00 m, 1.35-1.20 m	35.9	2.11 m, 1.42 m	36.6
23	1.10*s*	27.8	1.04*s*	27.6	0.97*s*	28.2	1.08*s*	27.9
24	0.95 *s*	15.9	0.84*s*	16.1	0.75*s*	16.9	0.98 *s*	16.0
25	0.67 *s*	16.0	0.88*s*	16.0	0.78*s*	16.8	0.65*s*	15.8
26	1.00*s*	16.0	0.97*s*	15.5	0.85*s*	15.9	1.00*s*	16.1
27	0.87*s*	16.8	1.02*s*	15.1	0.92*s*	14.9	0.85*s*	15.9
28		174.9		175.6		175.1		175.2
29a	4.62 *br s*	110.2	4.65*br s*	109.3	4.56*br s*	110.7	4.65*br s*	110.0
29b	4.75 *br s*		4.73*br s*		4.68*br s*		4.81*br s*	
30	1.61brs	19.0	1.72 brs	18.5	1.68brs	19.1	1.67brs	19.2

[a]At 500 MHz. Assignments based on COSY and HMQC spectra. J in Hz in between parenthesis; [b] in pyridine-d_5, [c]in DMSO-d_6, [d]in MeOD

molecular formula $C_{42}H_{66}O_{14}$. The ^{1}H NMR spectrum of 2 (TABLE 1) displayed signals for two anomeric protons at δ 4.44 (*d*, *J* = 7.6 Hz) and 5.42 (*d*, *J* = 8.2 Hz) which correlated with the carbon signals at δ 103.8 and 95.2, respectively, in the HMQC spectrum. The anomeric coupling constants obtained for both sugar units were indicative of a β-configuration for both monosaccharides.

Comparison of the ^{13}C NMR spectral data of 2 with thosereported in literature[11,34] indicated that the structure for 2 was a betulinic acid glycoside.

The cross-peak in the HMBC spectrum (TABLE 3) between δ 4.44 and δ 89.7 showed that a monosaccharide moiety was linked to the betulinic acid at C-3 while the cross-peak between δ 5.42 and δ 176.2 showed that the second monosaccharide moiety was linked to the aglycon at C-28. The ring protons of the sugar residues were assigned starting from the

Full Paper

TABLE 2 : ^{1}H and ^{13}C NMR spectroscopic data for sugar moieties of compounds 1-4[a]

Atom	1[b]		2[c]		3[d]		4[b]	
	δ ^{1}H	δ ^{13}C	δ ^{1}H	δ ^{13}C	δ ^{1}H	δ ^{13}C	δ ^{1}H	δ ^{13}C
1'	4.92 *d* (7.2)	105.0	4.35 *d* (7.6)	105.8	4.57 *d* (7.0)		4.81*br s*	104.9
2'	3.91 *m*	74.8	3.20 (dd, J = 10, 2.7)	74.6	3.26 *m*		4.23 *m*	82.2
3'	4.11 *m*	78.0	3.40 *m*	77.1	3.19 *m*		4.30 *m*	71.2
4'	4.24 *m*	70.0	3.46 *(t 9)*	72.8	3.43 *m*		4.48 *m*	74.5
5'	4.03 *m*	77.7	3.56 *d* (11)	75.6	*m*		*m* 4.09 *m*	62.4
6a'	4.40 *m*	62.2		176.3	3.39 *m*			
6b'	4.27 *m*				3.52 *m*			
1"	6.26 *d* (7.8)	95.1	5.52 *d* (8.2)		5.37 *d* (8.0)		6.20 d (8.0)	105.6
2"	4.01 *m*	75.0	3.26 *m*	73.2	3.20 *m*		4.02 *m*	76.6
3"	4.21 *m*	78.0	3.35 *m*	70.8	3.30 *m*		4.18 *m*	78.0
4"	4.50 *m*	71.2	3.46 t (8.8)	73.0	3.21 *m*		3.90*m*	71.7
5"	4.27 *m*	78.2	3.40 *m*	77.8	3.13 *m*		4.32*m*	76.3
6a"	4.27 *m*	*62.2*	3.73*dd* (12.1, 4.2)	61.4	3.39 *m*		4.39 *m*	62.2
6b"	4.38 *m*		3.86 *d* (12.1)		3.62 *m*		4.10 *m*	
1"'					4.14 *d* (8.0)		6.33 *d* (7.8)	
2"'					3.05 *m*		4.14 *m*	
3"'					3.10 *m*		4.24 *m*	
4"'					3.35 *m*		4.31 *m*	
5"'					3.07 *m*		3.98 *m*	
6a"'					3.49 *m*		3.86 *m*	62.3
6b"'					3.72 *m*		4.28 *m*	

[a] At 500 MHz. Assignments based on COSY and HMQC spectra. J in Hz in between parenthesis, [b]in pyridine-d_5, [c]in DMSO-d_6, [d]in MeOD; [a] Multiplicities assigned from DEPT spectra. [b] The assignments were based on HMBC and HMQC experiments (125 MHz for ^{13}C and 500 MHz for ^{1}H NMR)

anomeric protons by means of the COSY, HMQC, HMBC (TABLE 3) and NOESY spectra, thus the hexosyl moiety at C-3 was identified as a β-D-glucuronopyranosil unit, while that at C-28 was coincident with a β-D-glucopyranosil moiety. All these data allowed us to assign compound 2 as the new natural saponin 3-O-(β-D-glucuronopyranosyl) betulinic acid 28-O-(β-D-glucopyranosyl) ester.

The IR spectrum of compound 3 showed a broad absorption due to hydroxyl groups near 3388 cm^{-1} as well as one strong absorption peak attributable to carbonyl ester group at 1730. The FABMS of compound 3 showed a quasimolecular ion peak at *m/z*: 965 $[M + Na]^{+}$. Other significant peaks in the spectrum were at 803 [M + Na – 162], and 641 [M + Na - 162 - 162] due to sequential losses of hexosyl units. The positive HRFABMS showed a clustered molecular ion peak at *m/z* 966.1120 giving a molecular formula of $C_{48}H_{78}O_{18}$ and consistent with a triterpene glycoside containing three hexosyl moieties.

The signals identified in the ^{1}HNMR spectra and (TABLES 1 and 2) allowed us to establish the structure of compound 3 as 3-O-(β-D-galactopyranosyl) betulinic acid 28-O-[β-D-glucopyranosyl (1→3)-β-D-glucopyranosyl] ester. The ^{1}HNMR spectrum of 3 (TABLE 1) showed three anomeric proton signals at δ 4.57 (*d*, *J* = 7.0 Hz), 5.37 (*d*, *J* = 8.0 Hz) and 4.14 (*d*, J= 8.0 Hz) that correlated with the signals a δ 102.5, 93.5 and 103.4, respectively, in the HMQC spectrum. The cross-peaks in the HMBC spectrum (TABLE 3) between the signals at δ 4.57 and δ87.7 showed that the β-D-galactopyranosyl moiety was linked to the aglycon at C-3, between δ 5.37 and δ177.3 showed that a β-D-glucopyranosyl moiety was linked to the aglycon at C-28 while the cross-peak between δ 4.14 and δ81.3 showed that a β-D-glucopyranosyl moiety

Full Paper

TABLE 3 : Selected cross peaks (δ Values) in the ^{1}H-Detected Long-Range ^{1}H-^{13}C (HMBC) NMR spectra used for defining the sugar units attached to the aglycon for saponins 1-4

Proton resonance	Carbon resonances
	1
3.01 (H-3) →	C-2 (24.9), C-4 (39.0), C-5 (54.9), C-1' (105.2)
4.24 (H-1') →	C-3 (87.9), C-2' (73.7)
3.00 (H-2') →	C-1' (105.2), C-3' (76.7), C-4' (69.4)
3.26 (H-3') →	C-2' (73.7), C-4' (69.4)
3.08 (H-4') →	C-2' (73.7), C-3' (76.7), C-5' (76.5)
3.01 (H-5') →	C-4' (69.4), C-6' (60.5)
3.37 (H-6a') →	C-4' (69.4), C-5' (76.5)
3.65 (H-6b') →	C-4' (69.4), C-5' (76.5)
5.37 (H-1") →	C-28 (177.0), C-2" (72.3)
3.10 (H-2") →	C-1" (95.5), C-3" (74.2), C-4" (69.6)
3.24 (H-3") →	C-2" (72.3), C-4" (69.6)
3.16 (H-4") →	C-2" (72.3), C-3" (74.2), C-5" (77.6)
3.11 (H-5") →	C-4" (69.6), C-6" (60.5)
3.47 (H-6a") →	C-4" (69.6), C-5" (77.6)
3.65 (H-6b") →	C-4" (69.6), C-5" (77.6)
	2
3.20 (H-3) →	C-2 (29.8), C-4 (40.1), C-5 (56.1), C-1' (103.8)
4.44 (H-1') →	C-3 (89.7), C-2' (78.3)
3.45 (H-2') →	C-1' (103.8), C-3' (77.1), C-4' (72.8)
3.40 (H-3') →	C-2' (78.3), C-4' (72.8)
3.46 (H-4') →	C-2' (78.3), C-3' (77.1), C-5' (78.5)
3.56 (H-5') →	C-4' (72.8), C-6' (176.3)
5.42 (H-1") →	C-28 (176.2), C-2" (73.8)
3.26 (H-2") →	C-1" (95.2), C-3" (78.3), C-4" (72.9)
3.35 (H-3") →	C-2" (73.8), C-4" (72.9)
3.46 (H-4") →	C-2" (73.8), C-3" (78.3), C-5" (77.8)
3.40 (H-5") →	C-4" (72.9), C-6" (62.0)
3.73 (H-6a") →	C-4" (72.9), C-5" (77.8)
3.86 (H-6b") →	C-4" (72.9), C-5" (77.8)
	3
3.02 (H-3) →	C-2 (24.9), C-4 (39.2), C-5 (54.9), C-1' (102.5)
4.57 (H-1') →	C-3 (87.7), C-2' (71.6)
3.26 (H-2') →	C-1' (102.5), C-3' (74.9), C-4' (69.8)
3.19 (H-3') →	C-2' (71.6), C-4' (69.8)
3.43 (H-4') →	C-2' (71.6), C-3' (74.9), C-5' (73.5)
3.29 (H-5') →	C-4' (69.8), C-6' (60.0)
3.39 (H-6a') →	C-4' (69.8), C-5' (73.5)
3.52 (H-6b') →	C-4' (69.8), C-5' (73.5)
5.37 (H-1") →	C-28 (177.3), C-2" (74.8)
3.20 (H-2") →	C-1" (93.5), C-3" (81.3), C-4" (71.3)
3.30 (H-3") →	C-2" (74.8), C-4" (71.3), C-1'" (103.4)
3.21 (H-4") →	C-2" (74.8), C-3" (81.3); C-5" (77.5)
3.13 (H-5") →	C-4" (71.3), C-6" (60.5)
3.39 (H-6a") →	C-4" (71.3), C-5" (77.5)
3.62 (H-6b") →	C-4" (71.3), C-5" (77.5)
4.14 (H-1'") →	C-3" (81.3), C-2'" (73.3)
3.05 (H-2'") →	C-1'" (103.4), C-3'" (76.8), C-4'" (70.5)
3.10 (H-3'") →	C-2'" (73.3), C-4'" (70.5)
3.35 (H-4'") →	C-2'" (73.3), C-3'" (76.8), C-5'" (76.3)
3.07 (H-5'") →	C-4'" (70.5), C-6'" (61.2)
3.49 (H-6a'") →	C-4'" (70.5), C-5'" (76.3)
3.72 (H-6b'") →	C-4'" (70.5), C-5'" (76.3)
	4
3.40 (H-3) →	C-2 (29.8), C-4 (39.6), C-5 (55.6), C-1' (104.9)
4.81 (H-1') →	C-3 (89.6), C-2' (82.2)
4.23 (H-2') →	C-1' (104.9), C-3' (73.2), C-4' (67.5), C-1" (105.6)
4.30 (H-3') →	C-2' (82.2), C-4' (67.5)
4.48 (H-4') →	C-2' (82.2), C-3' (73.2), C-5' (64.4)
3.95 (H-5a') →	C-4' (67.5), C-1' (104.9)
4.09 (H-5b') →	C-4' (67.5), C-1' (104.9)
5.30 (H-1") →	C-2' (82.2), C-2" (76.6)
4.02 (H-2") →	C-1" (105.6), C-3" (78.0), C-4" (71.7)
4.18 (H-3") →	C-2" (76.6), C-4" (71.7)
4.00 (H-4") →	C-2" (76.6), C-3" (78.0), C-5" (78.3)
3.98 (H-5") →	C-4" (71.7), C-6" (62.2)
4.32 (H-6a") →	C-4" (71.7), C-5" (78.3)
4.39 (H-6b") →	C-4" (71.7), C-5" (78.3)
6.33 (H-1'") →	C-28 (175.2), C-2'" (74.1)
4.14 (H-2'") →	C-1'" (95.4), C-3'" (78.6), C-4'" (71.2)
4.24 (H-3'") →	C-2'" (74.1), C-4'" (71.2)
4.31 (H-4'") →	C-2'" (74.1), C-3'" (78.6), C-5'" (79.2)
3.98 (H-5'") →	C-4'" (71.2), C-6'" (62.3)
3.86 (H-6a'") →	C-4'" (71.2), C-5'" (79.2)
4.28 (H-6b'") →	C-4'" (71.2), C-5'" (79.2)

was linked to C-3''.

The structure of the chain sugar was confirmed from the observed NOEs across the glycosidic linkages. All the carbon signals due to these sugar moieties were in good agreement with literature data[8,9].

According to the analysis of both HMQC and HMBC spectra all the proton sugar units were assigned (TABLE 2).

Full Paper

Compound 4 showed a quasimolecular ion peak at *m/z* 935 $[M + Na]^+$, and the molecular formula $C_{47}H_{76}O_{17}$ was determined by HRFABMS. Furthermore, fragment ion peaks at m/z 773 $[M + Na – 162]^+$, 641 $[M + Na – 162 – 132]^+$, 611 $[M + Na – 162 – 162]^+$, in the positive FABMS indicated the loss of two hexose units and one pentose unit. Spectral analysis (^{1}HNMR, ^{13}CNMR, ^{1}H-^{1}HCOSY, HMQC and HMBC) of 4 revealed similar data to those chemical shifts reported for betulinic-type aglycone[3].

^{1}H NMR spectrum showed three signals assigned to anomeric protons in δ4.81 (d, *J*= 5.5Hz), 5.30 (d, *J*= 8.0Hz) and 6.33 (d, *J* =7.8Hz), where as the ^{13}C NMR spectrum showed three signs of δ anomeric carbons at 104.9, 105.6 and 95.4 (TABLE 2). From the analysis of the HMQC and HMBC spectrums (TABLE 3), the presence of two β-glucopyranosyl units and terminal-substituted-2 arabinopyranosyl β unit was determined.

An unambiguous determination of the sites of the sequence and the linkage is obtained from the HMBC correlations. HMBC spectra (TABLE 3) indicated that 4 β-glucopyranosyl unit was attached to the carboxyl group at C-28. Correlation with δ 4.81 (H-1, b-arabinose) and δ 89.6 (C-3, aglycon) allowed to establish the position of attachment of said residue with the aglycone, while the correlation between δ 5 30 (H-1" terminal glucopyranosyl unit) and δ 82.2 (C-2', arabinopyranosyl unit), allowed us to assign the binding between the two sugar units. The ring protons of the sugars units were assigned starting from the anomeric proton by means of its COSY, HMQC, HMBC and NOESY spectra (TABLE 2).

The common D-configuration for glucose and the L-configuration for arabinose were assumed, according to those most encountered among the plant glycosides in each case[31]. On the basis of these evidences, 4 was established as the new saponin3-O-[3-β-D-glucopyranosyl(1→2)-α-L-arabinopyranosyl] betulinic acid 28-O-(β-D-glucopyranosyl) ester.

EXPERIMENTAL

General

IR spectra were recorded on a NIICOLET FT IR on AgCl disks. Optical rotation was determined on a Jasco P-1010 polarimeter. NMR spectra were recorded on Bruker AVANCE DRX-500 with TMS as internal standard. Mass spectra were obtained with a ZAB-SEQ4F (V6). Prep. TLC were carried out on 0.5 mm thickness pre-coated silica gel sheets. MeOH was used to recover the compounds.

Plant material

Eryngiumagavifolium G. were collected in Córdoba Province, Argentina, and identified by L. Ariza Espinar. Voucher specimens are deposited in the Museo Botánico Córdoba (CORD 3222).

Extraction and isolation

Finely cut whole fresh aerial parts (1605 g) of *E. agavifolium* G. were extracted three times with EtOH at room temp., 48 h each. The combined EtOH extracts were evaporated to give 30.24 g of a gummy residue. This residue was suspended in EtOH:H_2O (7:3) mixture, and partitioned successively with Hexane (10.6 g), Cl_2CH_2 (0.75 g), EtOAc(6.3 g) and *n*-BuOH (3.1 g). The *n*-BuOH extract was subjected to CC on silica gel, eluting with gradient mixtures of Cl_2CH_2-EtOH (9:1 to 7:3) to give four fractions, 1 through 4.

Fraction 1 was purified by successively CC on silica gel, eluting with gradient mixtures of Cl_2CH_2-MeOH of increasing polarity and Cl_2CH_2-EtOH (1:0.5 to 7:3). Further purification by preparative TLC with Cl_2CH_2-EtOH (4:1) yielded 20 mg of 1 and 5.3 mg of 2.

Fraction 2 was purified by repeated CC on silica gel and eluted with a gradient of increasing polarity with Cl_2CH_2-MeOH and Cl_2CH_2-EtOH (1:0.5 to 7:3), and preparative TLC with Cl_2CH_2-EtOH (9:1) to yield compound 7.2 mg of 5.

Fraction 3 was purified by repeated CC on silica gel and eluted with a gradient of increasing polarity with Cl_2CH_2-MeOH and Cl_2CH_2-EtOH (1:0.5 to 7:3), and preparative TLC with Cl_2CH_2-EtOH (7:3) to yield 1.4 mg of 3,and 4.7 mg of 4.

3-O-(β-D-glucopyranosyl) betulinic acid 28-O-(β-D-glucopyranosyl) ester (1)

Amorphous white powder; $[\alpha]_D^{23.5^\circ C}$– 16.71 (MeOH; *c* 0.78); IR $\nu_{max.}^{film}$ cm^{-1}: 3388 (OH), 2939 (CH), 1731 (C=O). HR-FAB-MS, *m/z*803.9716 $[M + Na]^+$, calcd for $C_{42}H_{68}O_{13}$ + Na 803.9715, FABS *m/z*: 803 $[M + Na]^+$, 641, 479; for ^{1}H NMR and ^{13}C

NMR data see TABLES 1 and 2.

3-O-(β-D-glucuronopyranosyl) betulinic 28-O-(β-D-glucopyranosyl) ester (2)

Amorphous white powder; $[\alpha]_D^{23.5°C}$ – 1.94 (MeOH; *c* 0.35); IR $\nu_{max.}^{film}$ cm^{-1}: 3388 (OH), 2939 (CH), 1731 (C=O, ester group), 1710 (C=O, carboxylic acid). HR FAB-MS, *m/z* 817.9551 $[M + Na]^+$, calcdfor $C_{42}H_{66}O_{14}$ + Na 817.9548. FABMS *m/z*: 817 $[M + Na]^+$, 655 $[M + Na – 162]^+$, 479 [M + Na - 162- 176]$^+$; for ^1H NMR and ^{13}C NMR data see TABLES 1 and 2.

3-O-β-D-(galactopyranosyl) betulinic 28-O-[β-D-glucopyranosyl (1→3)-β-D- glucopyranosyl] ester (3)

Amorphous white powder; $[\alpha]_D^{23.5°C}$– 3.36 (MeOH; *c* 0.10); IR $\nu_{max.}^{film}$ cm^{-1}: 3388 (OH), 2939 (CH), 1730 (C=O). HRFAB-MS, *m/z* 966.1120 [M + Na]$^+$, calcdfor $[C_{48}H_{78}O_{18} + Na]^+$ 966.1119. FABS *m/z*: 965 $[M + Na]^+$, 803 $[M + Na – 162]^+$, 641 [M + Na – 162 - 162]$^+$; for ^1H NMR and ^{13}C NMR data see TABLES 1 and 2.

3-O-[3-β-D-glucopyranosyl(1→2)-α-L-arabinopyranosyl] betulinic 28-O-(β-D-glucopyranosyl) ester(4)

Amorphous white powder; $[\alpha]_D^{18.9°C}$ – 1.05 (MeOH; *c* 0.31). IR $\nu_{max.}^{film}$ cm^{-1}:3388 (OH), 2939 (CH), 1730 (C=O) cm^{-1}. FABMS *m/z*: 935 $[M + Na]^+$, 773 $[M + Na – 162]^+$, 641 $[M + Na – 162 – 132]^+$, 611 $[M + Na – 162 – 162]^+$. HRFABMS, *m/z* 936.0829 $[M + Na]^+$, calcd for $[C_{47}H_{76}O_{17} + Na]$ 936.0859; for ^1H NMR and ^{13}C NMR data see TABLES 1 and 2.

3-O-β-D-glucopyranosylbetulinicacid (5)

Amorphous solid; ^1H NMR and ^{13}C NMR data and FABMS were in good agreement with those reported in lit.[8,9].

Acidhydrolysis of 1,2,3,4

The saponin1 (20 mg in 5 mL of MeOH) was refluid in 5 mL of 2N HCl for 3.5 h; H_2O was added to the reaction mixture, and this was extracted with $CHCl_3$ (3 x15 mL). The $CHCl_3$ extract was purified on a Sphadex LH-20 column eluted with MeOH to afford a crop of betulinic acid (8.3 mg), which was identified by TLC, NMR and IR by comparison with an authentic sample. The aqueous layer of the hydrolysate was neutralized with Ag_2CO_3, and the neutral hydrolysate revealed the presence of glucose on high-perfomance TLC when compared with authentic sample. By the same method, high-perfomance TLC analyses showed the monosaccharides of 2 to be glucose and glucuronic acid; that of 3galactose and glucose and that 4 to be glucose and arabinose.

ACKNOWLEDGEMENTS

Thanks are dueto CONICET, Agencia Córdoba Ciencia, Agencia Nacional de Promoción Científica y Técnica andSECyT – UNC for financial support.

REFERENCES

[1] N.A.Ayoub, K.H.Kubeczka, M.A.Nawwar; An unique n-propyl sesquiterpene from *Eryngiumcreticum* L. (Apiaceae). Pharmazie, **58**, 674-676 **(2003)**.

[2] F.Bohlman, C.Zdero; Uberweitere Terpenaldehydester aus *Eryngium*- Arten. Chem.Ber. **104**, 1957-1961 **(1971)**.

[3] P.Chatterjee, J.M.Pezzuto, S.A.Kouzi; Glucosidation of betulinic acid by *Cunninghamella* species. J.Nat.Prod., **62**, 761-63 **(1999)**.

[4] R.K.Crowden, J.B.Harborne, V.H.Heywood; Chemosystematics of the Umbelliferae-A general survey. Phytochemistry, **8**, 1963-1984 **(1969)**.

[5] I.D'Acquarica, M.C.Di Giovanni, F.Gasparrini, D.Misiti, C.D'Arrigo, N.Fagnano, D.Guarnieri, G.Iacono, G.Bifulco, R.Riccio; Isolation and structure elucidation of four new triterpenoid estersaponins from fruits of *Pittosporum tobira*ait. *Tetrahedron*, **58(51)**, 10127–10136 **(2002)**.

[6] D.Drake, J.Lam; Seseli acetylene from *Eryngiumbourgatti*. Phytochemistry, **11**, 2651-2652 **(1972)**.

[7] C.A.J.Erdelmeier, O.Sticher; A cyclohexenone and a cyclohexadienone glycoside from *Eryngiumcampestre*. Phytochemistry, **25,** 741-743 **(1986)**.

[8] G.Gaidi, T.Miyamoto, A.Rustaiyan, V.Laurens, M.A.Lacaille-Dubois; Two new biologically active triterpenesaponins from *Acantophyllum squarrosum*. J.Nat.Prod., **63(11)**, 1497-1502 **(2000)**.

[9] G.Gaidi, T.Miyamoto, A.Rustaiyan, M.A.Lacaille-

Full Paper

Dubois; Three new acylatedtriterpenesaponins from *Acanthophyllum squarrosum*. Journal Nat.Prod., **64(7)**, 920-4 **(2001)**.

[10] M.D.Garcia, M.T.Saenz, M.A.Gomez, M.A.Fernández; Topycalantiinflamatory activity of phytosterols isolated from *Eryngiumfoetidum* on chronic and acute inflammation models. Phytother.Res., **13**, 78-80 **(1999)**.

[11] M.Glensk, V.Wray, M.Nimtz, T.Schöpke; Silenosides A-C triterpenoidssaponins from *Silene vulgaris*. J.Nat.Prod., **62**, 717-21 **(1999)**.

[12] J.B.Harborne, C.A.Williams; Flavonoid patterns in the fruits of the Umbelliferae. Phytochemistry, **11**, 1741-1750 **(1972)**.

[13] A.J.Herlt, L.N.Mander, E.Pongoh, R.J.Rumampuk, P.Tarigan; Two Major Saponins from Seeds of *Barringtoniaasiatica*: Putative Antifeedants toward *Epilachna* sp. Larvae J.Nat.Prod., **65(2)**, 115–120 **(2002)**.

[14] K.Hiller, M.Keipert, U.Missbach, G.Lehmann; Eryngiumgenin E -a new ester sapogenin. 23. The contents of some Saniculoideae. Pharmazie, **30,** 336 **(1975a)**.

[15] K.Hiller, M.Keipert, S.Pfeifer; Isolation of barringtogenol C-esters from *Eryngiumplanum* L. Pharmazie, **27**, 341-342 **(1972)**.

[16] K.Hiller, M.Keipert, S.Pfeifer, R.Kraft; The leaf sapogenin spectrum in *Eryngiumplanum* L. 20. Contribution on the content of several Saniculoideae. Pharmazie. **29**, 54-57 **(1974b)**.

[17] Hiller, K., Keipert, M., Pfeifer, S., Tokes, L., Nelson, J., 1973a. Erynginol A – a new triterpenesapogenin. 18. Information on the components of various saniculoideae. Pharmazie, **28,** 409 **(1973a)**.

[18] K.Hiller, K.Nguyen, P.Franke; Isolation of 3-O-β-D glucopyranosyloleanolic acid 28- O-D-xylopyranoside from *Eryngium bromeliifolim* Delar. 29. Constituents of some Saniculoideae. Pharmazie, **33**,78-80 **(1978)**.

[19] K.Hiller, K.Q.Nguyen, H.Dohnert, P.Franke; The saponine-saponinogen spectrum in *Eryngium amethystinum* L. 27. Contribution to chemicalscontained in various saniculoidae. Pharmazie, **32**, 184-185 **(1977)**.

[20] K.Hiller, K.Q.C.Nguyen, P.Franke, R.Hintsche; Isolation of betulic acid 3-O-β-D glucoside, a saponin of *Eryngium bromeliifolim*Delar. 26. The contents of Saniculoideae. Pharmazie, **31**, 891-893 **(1976a)**.

[21] K.Hiller, N.V.Thi, H.Dohnert, P.Franke; Isolation of new ester sapogenins from *Eryngium giganteum* M. B 22. Knowledge of various Saniculoideae components. Pharmazie, **30**, 105-109 **(1975b)**.

[22] K.Hiller, N.V.Thi, P.Franke; On saponin of *Eryngiumbro meliifoloium* Delar. 19. Information on the components of various Saniculoideae. Pharmazie, **28**, 546 **(1973b)**.

[23] K.Hiller, B.Von Mach, P.Franke; Saponins of *Eryngiummaritimum* L. 25. Contents of various saniculoideae. Pharmazie, **31**, 53 **(1976b)**.

[24] K.Hiller, N.von Thi, G.Lehmann, E.Grundemann; Betulinic acid - A sapogenin in *Eryngium bromeliifolium* Delar. 21. The contents of a saniculoidea. Pharmazie, **29**, 148-149 **(1974a)**.

[25] H.J.Jacker, K.Hiller; The antiexudative effect of saponin-5 from *Eryngiumplanum* L. and *Sanicular europaea* L. Pharmazie, **31**, 747-748 **(1976)**.

[26] Z.Janeczko, J.Sendra, K.Kmieć, C.H.Brieskorn; A triterpenoid glycoside from *Menyanthes trifoliata*. Phytochemistry, **29**, 3885-3887 **(1990)**.

[27] M.Kartal, A.C.Mitaine-Offer, T.Paululat, M.Abu-Asaker, H.Wagner, J.F.Mirjolet, N.Guilbaud, M.A.Lacaille-Dubois; TriterpeneSaponins from *Eryngium campestre*. J.Nat.Prod., **69(7)**, 1105–1108 **(2006)**.

[28] T.Karting, J.Wolf; Flavonoids from the aerial parts of *Eryngium campestre*. Planta Med., **59**, 285-286 **(1993)**.

[29] J.Lam, L.P.Christensen, T.Thomasen; Acetylenes from roots of *Eryngium bourgatii*. Phytochemistry, **31,** 2881-2882 **(1992)**.

[30] S.Martinez; Apiaceae, en F.Zoloaga, O.Morrone, (Eds); Catálogo de las Plantas Vasculares de la República Argentina II. Monogr.Sist.Bot., **74**, 46-64 **(1999)**.

[31] Y.Mimaki, A.Yokosuka, M.Kuroda, M.Hamanaka, C.Sakuma, Y.Sashida; New bisdesmosidic triterpene saponins from the roots of *Pulsatillachinensis*. J.Nat.Prod., **64,** 1226-1229 **(2001)**.

[32] J.Palá Paúl, M.J.Pérez Alonso, A.Velasco Negueruela, J.Vadaré, A.M.Villa, J.Sanz, J.J.Brophy; Essential oil composition of the different parts of *Eryngium bourgatii* Gouan from Spain. J.Chromat.A, **1074**, 235-239 **(2005)**.

[33] M.Pinar, M.P.Galan; Coumarins from *Eryngiu milicifolium*. J.Nat.Prod., **48,** 853-854 **(1985)**.

[34] O.Safir, S.Fkih-Tetouani, N.De Tommasi, R.Aquino; Saponins from *Zygophyllum gaetulum*. JNat.Prod., **61(1)**, 130-4 **(1998)**.

[35] G.F.Spencer; The effects of a terpene aldehyde-ester from *Eryngium paniculatum* and analogs on velvetleaf germination. J.Nat.Prod.**49,** 924-926 **(1986)**.

[36] K.C.Wong, M.C.Feng, T.W.Sam, G.L.Tan; Composition of the leaf and root oils of *Eryngium foetidum* L. J.Essent Oil.Res., **6**, 369-374 **(1994)**.