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Triterpene saponins and iridoid glucosides from *Galium rivale*

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Abstract

Three new glycosides of the oleanene-type triterpenes, rivalosides C–E (**1**–**3**), along with three known triterpene saponins, momordin IIb (**4**) and rivalosides A–B (**5**–**6**), and five known iridoid glucosides: monotropein, scandoside, deacetylasperulosidic acid, geniposidic acid and asperulosidic acid, were isolated from aerial parts of *Galium rivale*. The structures of the new compounds were elucidated by spectral methods and chemical means as 2α-acetoxy-3α,19α-dihydroxy-olean-12-en-28-oic acid 28-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside, 2α,3α,19α-trihydroxy-olean-12-en-28-oic acid 28-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside and 3-*O*-β-D-glucuronopyranosyl-24-hydroxy-olean-12-en-28-oic acid 28-*O*-β-D-glucopyranoside, for rivalosides C–E, respectively. The taxonomic significance of the rivalosides in *G. rivale* was discussed.

Keywords: *Galium rivale*; Rubiaceae; Rivalosides C–E; Triterpene saponin; Iridoid glycoside

1. Introduction

Galium rivale (Sibth. and Sm.) Griseb. (Rubiaceae) is a very variable species and represents an interesting taxonomic problem. The populations in the western and northern parts of its European range differ from the southern and eastern parts in morphology of flowers and leaves. On this ground, Pobedimova (1958) distinguishes two species, while Ehrendorfer and Krendl (1976) and Ehrendorfer and Schönbeck-Temesy (1982) consider no obvious justification for recognizing two species, because of the occurrence of transitional populations with intermediate characteristics in the Balkan Peninsula. To our knowledge in *G. rivale*, only tannins (Buckova et al., 1970) and phenols (TLC) (Borissov and Zoz, 1975) were found, and recently, we reported the isolation of two 19-oxo triterpenoid saponins, rivalosides A and B (de Rosa et al., 2000). In continuation of the chemical investigation on genus *Galium* (Handjieva et al., 1996; Mitova et al., 1996, 1999) herein, we report the isolation of triterpene saponins and iridoid glucosides. Till now, β-amyrine was reported in *G. aparine* (Tzakou et al., 1990), oleanolic acid in *G. boreale* (Corrigan et al., 1978) and unidentified saponins in *G. verum* (Alimbaeva et al., 1983), *G. mollugo* (Kohlmuenzer, 1965), *G. sylvaticum* (Wagner, 1941) and *G. aparine* (Roberg, 1937).

2. Results and discussion

The aerial parts of two populations of *G. rivale* (Struma valley and Slavyanka mountain) were examined. Both the populations contained similar main constituents and differ in the minor compounds. The methanol extract of *G. rivale* was partitioned between dichloroethane and water. The water-soluble part,

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Table 1
^{13}C-NMR spectral data of compounds **1**–**4** and **7**, in C_5D_5N (δ ppm/TMS)[a]

	1	2	3	4	7
Aglycone part					
1	38.6 *t*	42.8 *t*	38.6 *t*	38.6 *t*	42.2 *t*
2	71.9 *d*	66.3 *d*	27.0 *t*	26.5 *t*	65.9 *d*
3	76.3 *d*	79.6 *d*	89.1 *d*	89.0 *d*	79.1 *d*
4	39.6 *s*	39.0 *s*	44.5 *s*	39.4 *s*	38.6 *s*
5	49.0 *d*	49.0 *d*	56.2 *d*	55.7 *d*	49.4 *d*
6	18.7 *t*	18.8 *t*	19.0 *t*	18.4 *t*	18.3 *t*
7	33.2 *t*	33.2 *t*	33.5 *t*	33.0 *t*	33.4 *t*
8	40.6 *s*	40.6 *s*	40.0 *s*	39.8 *s*	40.05 *s*
9	48.4 *d*	48.4 *d*	48.0 *d*	47.9 *d*	48.6 *d*
10	39.2 *s*	39.0 *s*	36.8 *s*	36.9 *s*	38.6 *s*
11	24.4 *t*	24.4 *t*	24.2 *t*	23.7 *t*	23.9 *t*
12	123.2 *d*	122.8 *d*	123.0 *d*	122.8 *d*	123.0 *d*
13	144.7 *s*	144.5 *s*	144.2 *s*	144.0 *s*	144.7 *s*
14	42.3 *s*	42.3 *s*	42.3 *s*	42.0 *s*	41.9 *s*
15	29.0 *t*	29.0 *t*	28.5 *t*	28.1 *t*	29.7 *t*
16	28.8 *t*	28.1 *t*	23.5 *t*	23.3 *t*	28.8 *t*
17	46.6 *s*	46.6 *s*	47.2 *s*	46.9 *s*	45.8 *s*
18	44.6 *d*	44.6 *d*	41.9 *d*	41.6 *d*	44.5 *d*
19	81.2 *d*	81.2 *d*	46.3 *t*	46.1 *t*	81.0 *d*
20	35.7 *s*	35.7 *s*	30.9 *s*	30.7 *s*	35.4 *s*
21	29.1 *t*	29.1 *t*	34.1 *t*	33.9 *t*	29.7 *t*
22	33.2 *t*	33.3 *t*	32.7 *t*	32.4 *t*	33.0 *t*
23	29.5 *q*	29.6 *q*	23.5 *q*	28.1 *q*	29.2 *q*
24	22.4 *q*	22.4 *q*	63.4 *t*	16.9 *q*	21.9 *q*
25	16.7 *q*	16.8 *q*	15.5 *q*	15.5 *q*	16.2 *q*
26	17.9 *q*	17.9 *q*	17.5 *q*	17.3 *q*	17.3 *q*
27	25.0 *q*	25.0 *q*	26.2 *q*	26.0 *q*	24.5 *q*
28	177.5 *s*	177.5 *s*	176.6 *s*	176.6 *s*	181.4 *s*
29	28.3 *q*	28.9 *q*	33.3 *q*	33.1 *q*	29.2 *q*
30	24.8 *q*	24.8 *q*	23.8 *q*	23.6 *q*	24.5 *q*
Glucoronic acid					
1′			106.6 *d*	107.0 *d*	
2′			75.4 *d*	75.3 *d*	
3′			78.3 *d*	77.9 *d*	
4′			73.7 *d*	73.2 *d*	
5′			78.2 *d*	77.5 *d*	
6′			173.0 *s*	172.7 *s*	
Glucose					
1″	95.9 *d*	95.9 *d*	95.6 *d*	95.5 *d*	
2″	74.0 *d*	74.0 *d*	73.8 *d*	73.9 *d*	
3″	78.5 *d*	78.5 *d*	78.6 *d*	78.6 *d*	
4″	71.0 *d*	71.0 *d*	71.0 *d*	71.0 *d*	
5″	78.9 *d*	78.9 *d*	78.2 *d*	79.0 *d*	
6″	69.5 *t*	69.5 *t*	62.4 *t*	62.1 *t*	
Glucose					
1‴	105.5 *d*	105.5 *d*			
2‴	75.3 *d*	75.1 *d*			
3‴	78.6 *d*	78.6 *d*			
4‴	71.6 *d*	71.6 *d*			
5‴	78.1 *d*	78.1 *d*			
6‴	62.8 *t*	62.8 *t*			
$\underline{C}OCH_3$	170.7 *s*				
$CO\underline{C}H_3$	21.5 *q*				

[a] Assignments were established by DEPT, HETCOR and HMBC spectra.

upon repeated charcoal chromatography, DCCC and reverse phase LPLC, afforded nine pure compounds. By means of spectroscopic data and published information, five of them were identified as the known iridoid glucosides: monotropein, scandoside, deacetylasperulosidic acid, asperulosidic acid and geniposidic acid (Boros and Stermitz, 1990). The other six compounds appeared as triterpene saponins: the known momordin IIb (**4**) (Kawamura et al., 1988), rivalosides A (**5**) and B (**6**) (de Rosa et al., 2000), and three new compounds named rivalosides C (**1**), D (**2**) and E (**3**). The major constituents in both examined samples were rivalosides A (**5**) and C (**1**) and monotropein, followed by scandoside and deacetylasperulosidic acid. Minor components were asperulosidic acid and geniposidic acid. The sample from Slavyanka contained rivalosides B (**6**) and D (**2**), while sample from Struma valley contained low concentrations of rivaloside E (**3**) and momordin IIb (**4**). An initial examination of the spectroscopic data revealed that the six triterpenoids were three pairs of very similar compounds (**1**–**2**, **3**–**4** and **5**–**6**).

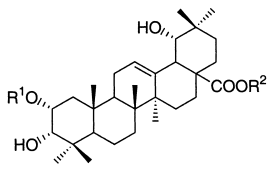
Rivaloside C (**1**) and D (**2**), isolated as amorphous solids, were assigned the molecular formula $C_{44}H_{70}O_{16}$ and $C_{42}H_{68}O_{15}$, respectively, as determined from FABMS and NMR data (Table 1). Compound **1** possessed hydroxyl and two ester groups, as shown by its IR spectrum; whereas **2** exhibited the IR absorption of hydroxyl and an ester function. On treatment with alkali, **1** and **2** gave the same aglycone **7**. Acid hydrolysis of **1** and **2** both afforded glucose (TLC; GC; alditol acetate) and aglycone **7**. The ^{13}C-NMR spectra of **1** and **2** both showed two olefinic carbon signals [δ 123.2 (*d*), 144.7 (*s*) in **1** and δ 122.8 (*d*), 144.5 (*s*) in **2**] which were in good agreement with those of C-12 and C-13 of olean-12-ene derivatives (Mahato and Kundu, 1994). The difference observed in the molecular formula of **1** and **2** and the presence of an acetyl group [δ 1.94, (3H, *s*); 21.5 (*q*) and 170.7 (*s*)] in the NMR spectra of **1** showed that **1** is the acetyl derivative of **2**. The FABMS of both **1** and **2** exhibited the fragment peak (*m*/*z* 531 and 489, for **1** and **2**, respectively) due to the loss of two linked hexose units. The signals of the anomeric protons at δ 6.30 (*d*, $J = 8.1$ Hz) and 5.02 (*d*, $J = 7.7$ Hz), in the ^{1}H-NMR spectra of both compounds, showed that both glucose had the β-configuration. A combination of 2D-NMR experiments (COSY, TOCSY, HETCOR and HMBC) allowed us to assign all the chemical shifts in the ^{1}H- and ^{13}C-NMR spectra, and to define the 1–6 connection between the two glucose units. The chemical shift of one anomeric carbon atom at δ 95.9 confirmed the presence of an ester-linked sugar unit. HMBC correlation observed between the anomeric proton (δ 6.30) and the carboxyl group (δ 177.5) defined the connection between the aglycone and the sugar units. Its pos-

ition at C-28 was further confirmed by alkaline hydrolysis. The ^{1}H- and ^{13}C-NMR spectra of both **1** and **2** (Section 3 and Table 1) displayed in addition to the trisubstituted double bond and seven tertiary methyl groups, characteristic of a Δ^{12} oleanene skeleton, three oxymethines [δ 5.43, 3.80, 3.51 (each 1H, *bs*), 71.9, 76.3, 81.2 (each *d*) in **1**, δ 4.25, 3.72, 3.51 (each 1H, *bs*), 66.3, 79.6, 81.2 (each *d*) in **2**]. The COSY-45 spectrum of rivaloside C indicated that the oxymethine proton at δ 3.80 is coupled with the second oxymethine at δ 5.43, which, in turn, is coupled to nonequivalent methylene protons (δ 1.80, 1.65). These data located the two hydroxyl groups in ring A. The deshielded C-2 signal (δ 71.9) and shielded C-3 signal (δ 76.3) and the chemical shift of H-3 (δ 5.43) observed in the NMR spectra of **1** determined the positions of the acetoxy and hydroxyl groups at C-2 and C-3 position, respectively. The position of third hydroxyl group and the relative stereochemistry of these groups, in rivaloside C (**1**) and D (**2**), were determined from the examination of the aglycone **7**.

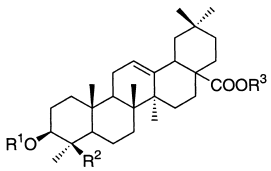
The ^{13}C-NMR spectrum of the aglycone **7** resembled that of the triterpene thomandertriol (2α,3α,19α-trihydroxyolean-12-ene) (Ngadjui et al., 1994), regarding the chemical shifts of the C-2, C-3 and C-19 signals, thus indicating position of the three hydroxyl groups at C-2, C-3 and C-19 with α-configuration. The chemical shift and the coupling constant of H-3 (δ 3.75, *d*, $J = 2.8$ Hz) and H-19 (δ 3.51, *d*, $J = 3.0$ Hz) signals showed their equatorial position. The magnitude of the coupling constants associated with H-2 (δ 4.25, *ddd*, $J = 11.4$, 3.0, 2.8 Hz) indicated that the proton had a trans–diaxial relationship with one H-1 proton, and therefore, the hydroxyl group at C-2 was equatorial. The presence of NOEs between 24β-Me with H-2 and H-3 and between 23α-Me and H-3, and on the other hand of 29α-Me and 30β-Me with H-19 gave further support to the proposed relative strereochemistry. Thus, the structure of rivaloside C (**1**) and rivaloside D (**2**) were elucidated as 2α-acetoxy-3α,19α-dihydroxy-olean-12-en-28-oic acid 28-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside and 2α,3α,19α-trihydroxy-olean-12-en-28-oic acid 28-*O*-β-D-glucopyranosyl-(1 → 6)-β-d-glucopyrano-side, respectively.

Rivaloside E (**3**) was obtained as an amorphous solid. Its FABMS (positive-ion mode) exhibited peaks at *m/z* 849 $[M + K]^+$, 833 $[M + Na]^+$ and 811 $[M + H]^+$ indicating molecular weight of 810, compatible with the molecular formula $C_{42}H_{66}O_{15}$. Other fragment ion peaks at *m/z* 649 $[MH - 162]^+$ and 455 $[MH - 162 - 176 - 18]^+$ indicated the loss of two hexose moieties. On acid hydrolysis, **3** gave epihederagenin (3β,24-dihydroxy-olean-12-en-28-oic acid), identified by its spectral data (Kizu and Tomimori, 1982), glucose (TLC, GC) and glucuronolactone (TLC). The ^{1}H-NMR spectrum of **3** showed the signals of two sugar anomeric protons, a glucuronide at δ 5.10 (*d*, $J = 7.8$ Hz) and a glucosyl ester at δ 6.31 (*d*, $J = 8.1$ Hz). In the ^{13}C-NMR (Table 1), one anomeric carbon atom signal appeared at δ 95.6 suggesting the presence of an ester-linked sugar unit. HMBC correlation observed between the anomeric proton (δ 6.31) and the carboxyl group (δ 176.6) defined the connection between the aglycone and the glucose unit. The ^{1}H- and ^{13}C-NMR spectra (Section 3 and Table 1) indicated the presence of a triterpene possessing an olefinic bond, two carbonyl groups (δ 173.0 and 176.6) and two sugar residues. The presence of two sp^2 carbons at δ 123.0 (*d*) and 144.2 (*s*) confirmed that the aglycone possessed an olean-12-ene skeleton. Instead of seven methyl groups, six methyls and one hydroxymethyl group [δ 4.50, 3.67 (each 1H, *d*, $J = 10.7$ Hz), 63.4 (*t*)] were observed. A comparison of the ^{1}H- and ^{13}C-NMR data of **3** with that of momordin IIb (**4**) indicated that they differ on the C-24 substituent and that **3** was glycosylated at the same positions, C-3 and C-28. Alkaline hydrolysis of rivaloside E afforded the prosapogenin **3a**, identical to the known 4-epihederagenin-3-*O*-β-D-glucuronopyranoside (Srivastava and Jain, 1989), which confirmed the assignment of a glucuronic acid residue at C-3. From the above evidence, rivaloside E (**3**) was characterized as 3-*O*-β-D-glucuronopyranosyl-24-hydroxy-olean-12-en-28-oic acid 28-*O*-β-D-glucopyranoside.

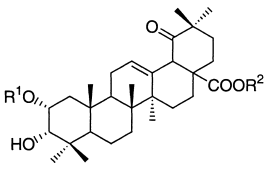
Our studies of more than 20 *Galium* species showed a lack of triterpene saponins. The presence of triterpene saponins in *G. rivale*, moreover in considerable concentrations, appear to be a promising chemotaxonomic marker for this species.



	R^1	R^2
1	Ac	Glc(1→6)Glc
2	H	Glc(1→6)Glc
7	H	H



	R^1	R^2	R^3
3	GlcUA	CH_2OH	Glc
3a	GlcUA	CH_2OH	H
4	GlcUA	CH_3	Glc



	R^1	R^2
5	Ac	Glc(1→6)Glc
6	H	Glc(1→6)Glc

3. Experimental

3.1. General

Melting points were measured on a Kofler apparatus and were uncorrected. IR spectra were recorded on a Bio-Rad FTS-7 FT-IR spectrometer. Optical rotations were measured on a Jasco DIP 370 polarimeter, using a 10-cm microcell. FABMS were obtained on a VG-ZAB instrument, using glycerol as matrix. ^{1}H- and ^{13}C-NMR spectra were recorded at 500 and 125 MHz, respectively, with TMS as internal standard on a Bruker AM 500 instrument, under Aspect X32 control. The 2D-NMR spectra were obtained using Bruker's microprograms. GC was carried out using Hewlett-Packard 5890A gas chromatograph equipped with a flame-ionization detector. Droplet counter current chromatography was performed on a Büchi 670 apparatus by ascending mode. Aluminium sheets silica-gel 60 F_{254} were used for TLC. Reverse phase LPLC was carried out with a Merck Lobar C-18 column size B with H_2O–MeOH mixtures as eluent.

3.2. Plant material

Aerial parts of *G. rivale* (sample 1: Struma valley, July 1992, A9297; sample 2: Slavyanka mountain, August 1994, A94101) were collected at florescence and dried in shade at room temperature. The voucher specimens are deposited in the herbarium of the Institute of Botany, Bulgarian Academy of Sciences (SOM).

3.3. Extraction and isolation

Dried above-ground parts of sample 1 (145 g) and sample 2 (60 g) were extracted twice with MeOH and the concentrated extracts (10.2 g; 6.0 g) partitioned between $Cl(CH_2)_2Cl$ and H_2O. The aqueous phases (7.7 g; 4.8 g) were treated with charcoal and eluted with H_2O (1 l; 600 ml), 5% MeOH (500 ml; 300 ml), 30% MeOH (500 ml; 300 ml), 50% MeOH (500 ml; 300 ml), MeOH (500 ml; 300 ml), MeOH–Me_2CO (1:1) (500 ml; 300 ml), MeOH–$Cl(CH_2)_2$ Cl (1:1) (500 ml; 300 ml).

3.3.1. Sample 1

The combined MeOH–Me_2CO (0.6 g) and MeOH–$Cl(CH_2)_2Cl$ (1.5 g) fractions were separated by ascending DCCC with $CHCl_3$–MeOH–H_2O–*n*-PrOH (9:12:8:2) to give geniposidic acid (frs. 13–14, 46 mg), asperulosidic acid (frs. 17–30, 341 mg), rivaloside E (**3**, frs. 60–75, 164 mg) and momordin IIb (**4**, frs. 80–92, 82 mg). Pure monotropein (36 mg), deacetylasperulosidic acid (6 mg) and scandoside (5 mg) were obtained after additional purification of frs. 9–11 (143 mg) using Lobar RP-18 column and elution with MeOH–H_2O mixtures. The DCCC stationary phase was collected in fractions of 100 ml; frs. 3–4 (70 mg) contained rivaloside A (**5**), fr. 7 (34 mg) contained rivaloside C (**1**).

3.3.2. Sample 2

The combined 50% MeOH (0.3 g), MeOH (0.3 g), MeOH–Me_2CO (0.3 g) and MeOH–$Cl(CH_2)_2Cl$ (0.5 g) were separated by ascending DCCC with $CHCl_3$–MeOH–H_2O–*i*-PrOH (5:6:4:1) to give geniposidic acid (frs. 12–13, 26 mg), asperulosidic acid (frs. 19–25, 89 mg), rivaloside D (**2**, frs. 38–45, 30 mg) and rivaloside B (**6**, frs. 46–56, 31 mg). Frs. 2–8 (431 mg) contained a mixture of monotropein, deacetylasperulosidic acid and scandoside. The DCCC stationary phase was collected in fractions of 100 ml; frs. 3–4 (243 mg) contained rivaloside A (**5**), frs. 5–7 (151 mg) contained rivaloside C (**1**).

3.4. Rivaloside C (*1*)

Amorphous solid, $[\alpha]_D = -3.6^\circ$ (MeOH; *c* 0.2); IR ν_{max} (Nujol) cm^{-1}: 3600–3400, 1740, 1730, 1240, 1090 and 1045; FABMS (positive) *m/z*: 855 $[M + H]^+$, 531 $[M + H - 324]^+$; ^{1}H-NMR (pyridine-d_5): δ 6.30 (1H, *d*, $J = 8.1$ Hz, H-1″, inner glucose), 5.49 (1H, *bs*, H-12), 5.43 (1H, *bs*, H-2), 5.02 (1H, *d*, $J = 7.7$ Hz, H-1′, outer glucose), 3.80 (1H, *bs*, H-3), 3.51 (1H, *bs*, H-19), 2.15 (1H, *bs*, H-18), 1.94 (3H, *s*, Ac), 1.80 (1H, *m*, H-1a), 1.65 (1H, *m*, H-1b), 1.49, 1.20, 1.15, 1.10, 1.08, 0.97, 0.96 (each 3H, *s*, Me × 7); ^{13}C-NMR: see Table 1.

3.5. Rivaloside D (*2*)

Amorphous solid, $[\alpha]_D = -25.9^\circ$ (MeOH; *c* 0.1); IR ν_{max} (Nujol) cm^{-1}: 3600–3400, 1730, 1090 and 1045; FABMS (positive) *m/z*: 813 $[M + H]^+$,$^+$, 489 $[M + H - 324]^+$; ^{1}H-NMR (pyridine-d_5): δ 6.29 (1H, *d*, $J =$ 7.9 Hz, H-1″, inner glucose), 5.45 (1H, *bs*, H-12), 5.02 (1H, *d*, $J = 7.7$ Hz, H-1′, outer glucose), 4.25 (1H, *bs*, H-2), 3.72 (1H, *bs*, H-3), 3.51 (1H, *bs*, H-19), 2.13 (1H, *bs*, H-18), 1.51, 1.24, 1.16, 1.11, 1.04, 0.98, 0.91 (each 3H, *s*, Me × 7); ^{13}C-NMR: see Table 1.

3.6. Rivaloside E (*3*)

Amorphous solid, $[\alpha]_D = -7.6^\circ$ (MeOH; *c* 0.2); IR ν_{max} (Nujol) cm^{-1}: 3600–3400, 1725, 1695, 1090 and 1045; FABMS (positive) *m/z*: 849 $[M + K]^+$, 833 $[M + Na]^+$, 811 $[M + H]^+$, 649 $[M + H - 162]^+$, 455 $[M + H - 162 - 176 - 18]^+$; ^{1}H-NMR (pyridine-$d_5$): δ 6.31 (1H, *d*, $J = 8.1$ Hz, H-1″, glucose), 5.38 (1H, *bs*, H-12), 5.10 (1H, *d*, $J = 7.8$ Hz, H-1′, glucuronic acid), 4.50 (1H, *d*, $J = 10.7$ Hz, H-24a), 3.67 (1H, *d*, $J = 10.7$ Hz, H-24b), 3.25 (1H, *bs*, H-3), 1.24 (3H, *s*, Me), 1.05

(3H, *s*, Me), 0.89 (3H, *s*, Me), 0.86 (6H, *s*, Me × 2), 0.74 (3H, *s*, Me); ^{13}C-NMR: see Table 1.

3.7. Sugar identification

The glycosides (5 mg) were heated with 2 M CF_3COOH (1 ml containing 0.9 mg of myo-inositol as an internal standard) at 100°C for 8 h. The sugars released were converted into acetylated alditols by reduction with $NaBH_4$ followed by acetylation with acetic anhydride–pyridine mixture. The alditol acetates obtained were analyzed by GC, using an HP Ultra-2 capillary column, temperature programmed to hold for 1 min at 175°C, increase at 10°C/min to 290°C and hold for 3 min. Individual alditol acetates were identified by comparison of their retention times with those of authentic samples.

3.8. Acid hydrolysis

The glycosides (5 mg) were added into 5% HCl (1 ml) and refluxed for 3 h. The solutions were neutralized and processed as usual to obtain aglycon and sugars.

Rivaloside C (**1**) and D (**2**) gave glucose and aglycon **7**.

2α,3α,19α-Trihydroxy-olean-12-en-28-oic acid (**7**). Colorless needles (from MeOH), mp 166–168°C, $[\alpha]_D = -11.4^\circ$ (MeOH; *c* 0.02); IR ν_{max} (Nujol) cm^{-1}: 3600–3200, 1680, 1615; EIMS *m/z* 488 $[M]^+$ (5), 470 $[M - H_2O]^+$ (30), 452 $[M - 2H_2O]^+$ (100), 434 (35), 407 (28), 389 (45), 246 (25), 223 (11), 217 (82), 205 (65), 201 (35); ^{1}H-NMR (pyridine-d_5): δ 5.54 (1H, *bs*, H-12), 4.25 (1H, *ddd*, *J* = 11.4, 3.0, 2.8 Hz, H-2), 3.75 (1H, *d*, *J* = 2.8 Hz, H-3), 3.51 (1H, *d*, *J* = 3.0 Hz, H-19) 2.10 (1H, *d*, *J* = 3.0 Hz, H-18), 1.49 (3H, *s*, Me), 1.20 (3H, *s*, Me), 1.15 (3H, *s*, Me), 1.09 (6H, *s*, Me × 2), 0.97 (3H, *s*, Me), 0.96 (3H, s, Me); ^{13}C-NMR: see Table 1.

Rivaloside E (**3**) gave glucose, glucuronolactone (TLC: mobile phase CH_2Cl_2–MeOH–H_2O (6.5:4:0.8)) and epihederagenin: EIMS *m/z* %: 472 $[M]^+$, (10), 248 (60), 205 (24), 203 (100); ^{1}H-NMR (pyridine-d_5): δ 5.17 (1H, *t*, *J* = 3.2 Hz), 4.44, 3.63 (each 1H, *d*, *J* = 10.7 Hz), 1.13, 1.05, 0.84, 0.81, 0.79, 0.68 (each 3H, *s*, Me × 6).

3.9. Alkaline hydrolysis

The glycosides (5 mg) were dissolved in 10% methanolic KOH (1 ml) and refluxed for 1 h. After neutralization, the reaction mixtures were extracted with *n*-BuOH.

Rivaloside C (**1**) and D (**2**) gave aglycon **7**.

Rivaloside E (**3**) yielded 4-epihederagenin-3-*O*-β-D-glucuronopyranoside-6′-*O*-methyl ester, identified by NMR spectra.^{1}H-NMR (pyridine-d_5): δ 5.28 (1H, *bs*), 5.05 (1H, *d*, *J* = 7.8 Hz), 4.48, 3.65 (each 1H, *d*, *J* = 10.7 Hz), 3.53 (3H, *s*), 1.22, 1.06, 0.88, 0.85, 0.80, 0.74 (each 3H, *s*, Me × 6).

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