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Phenolic compounds and rare polyhydroxylated triterpenoid saponins from *Eryngium yuccifolium*

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ABSTRACT

Phytochemical investigation on the whole plant of *Eryngium yuccifolium* resulted in the isolation and identification of three phenolic compounds (**1**–**3**) and 12 polyhydroxylated triterpenoid saponins, named eryngiosides A–L (**4**–**15**), together with four known compounds kaempferol-3-*O*-(2,6-di-*O*-*trans*-*p*-coumaroyl)-β-D-glucopyranoside (**16**), caffeic acid (**17**), 21β-angeloyloxy-3β-[β-D-glucopyranosyl-(1→2)]-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosyloxyolean-12-ene-15α,16α,22α,28-tetrol (**18**), and saniculasaponin III (**19**). This study reports the isolation of these compounds and their structural elucidation by extensive spectroscopic analyses and chemical degradation.

1. Introduction

Eryngium L. is a cosmopolitan genus (Apiaceae) of about 317 taxa (Wörz, 1999). Some species, such as *E. maritimun* L. (Lisciani et al., 1984), *E. campestre* L. (Baytop, 1999), and *E. foetidum* L. (Editorial Committee of Zhonghua Bencao, 1999), have been used in folk medicine. *Eryngium yuccifolium* Michx., known as "rattlesnake master", "button eryngo", or "button snakeroot", is a perennial species naturally distributed in eastern North America. Traditionally, the poultice, infusion or tincture made from the roots of this species was used for snakebites, fevers, or female reproductive disorders (Weiner, 1980; Foster and Duke, 1990; Moerman, 1998). However, there is no report available on the chemical and bioactive investigation on this species.

Previous phytochemical investigations on the *Eryngium* genus indicated the presence of flavonoids (Ikramov et al., 1973; Zarnack et al., 1979; Hiller et al., 1981; Kartnig and Wolf, 1993; Hohmann et al., 1997), essential oil (Brophy et al., 2003; Ayoub et al., 2003, 2006; Pala-Paul et al., 2005a,b, 2006), coumarins (Erdelmerier and Sticher, 1985), a rosmarinic acid derivative (Le Claire et al., 2005), and saponins (Hiller et al., 1972, 1974a,b, 1975, 1976a,b, 1977, 1978; Ikramov et al., 1974; Anam, 2002; Kartal et al., 2005, 2006). The main saponins from this genus belong to polyhydroxylated triterpenoid glycosides with ester functions. This class of saponins has been found in various groups of plants such as *Aesculus chinensis* L. (Hippocastanaceae) (Yang et al., 1999; Zhang et al., 1999a; Zhao et al., 2001; Zhao and Yang, 2003; Wei et al., 2004), *Pittosporum tobira* (Thunb.) Ait. (Pittosporaceae) (D'Acquarica et al., 2002), *Sanicula elata* var. *chinensis* Makino (Apiaceae) (Matsushita et al., 2004), and *Harpullia austro-caledonica* Baill. (Sapindaceae) (Voutquenne et al., 2005). Some saponins have been shown to possess anti-inflammatory properties (Matsuda et al., 1997; Sirtori, 2001; Wei et al., 2004), anti-HIV-1 protease activity (Yang et al., 1999), and cytotoxicity for tumor cells (D'Acquarica et al., 2002; Fu et al., 2006; Chan, 2007; Zhang and Li, 2007). Recently, we isolated and identified 25 new polyhydroxylated triterpenoid saponins from North American *Aesculus pavia* L. (Zhang et al., 2006; Zhang and Li, 2007). The saponins with two acyl groups at C-21 and C-22 had cytotoxic activity against 60 cell lines from nine different human cancers (Zhang and Li, 2007). Our interests in identification of novel bioactive agents from native plants in Texas prompted us to conduct a chemical investigation on *E. yuccifolium*. In this paper, we report the isolation and structural elucidation of 15 new compounds (**1**–**15**).

2. Results and discussion

The *n*-butanol-soluble part partitioned from the ethanol extract of *E. yuccifolium* was fractionated by a silica gel column to give three fractions A, B and C. Fraction C was subfractionated by an octadecyl-functionalized silica gel (ODS) column to afford fractions

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C_1, C_2 and C_3. Further separation of these fractions was achieved by preparative HPLC to furnish compounds **1**–**19** (see Section 4 for details).

Compounds **16**–**19** are known compounds and were identified by their spectroscopic data as kaempferol-3-*O*-(2,6-di-*O*-*trans*-*p*-coumaroyl)-β-D-glucopyranoside (**16**) (Yamashita et al., 1989), caffeic acid (**17**), 21β-angeloyloxy-3β-[β-D-glucopyranosyl-(1→2)]-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosyloxyolean-12-ene-15α, 16α, 22α, 28-tetrol (**18**) (Lavaud et al., 1992), and 21β-angeloyloxy-22α-acetyloxy-3β-[β-D-glucopyranosyl-(1→2)]-[α-L-arabinopyranosyl-(1→3)]-β-D-glucuronopyranosyloxyolean-12-ene-15α, 16α, 28-triol (saniculasaponin III, **19**) (Matsushita et al., 2004).

Compounds **1** and **2** had molecular formulae of $C_{15}H_{12}O_6$ and $C_{21}H_{22}O_{11}$, respectively, deduced from their $[M+H]^+$ HRESIMS and NMR spectroscopic data. Both compounds contained characteristic NMR signals of caffeic acid as **17**. Alkaline hydrolysis of both **1** and **2** liberated caffeic acid, which was confirmed by co-HPLC analysis with a reference sample. The NMR spectra of **1** exhibited 15 carbons and 8 aromatic protons, of which nine carbons and five protons were assigned to the caffeic acid unit and the remaining six carbons and three protons to an oxygenation-aromatic ring (B). HMQC and HMBC correlations established the 1′,3′,4′-oxygenation pattern of ring B. The linkage between the caffeic acid unit and the ring B was deduced from the downfield shifts of δ 167.3 (caffeic acid unit C-9) and δ 129.4 (ring B C-1′). The full assignments of the protons and carbons were achieved by a combination of ^{1}H, ^{13}C, COSY, HMQC, and HMBC experiments. The structure of **1** was determined as 3,4-dihydroxyphenyl caffeate.

Compared to compound **1**, the ^{13}C NMR spectrum of **2** displayed additional six carbons (δ 102.6, 77.2, 76.3, 73.6, 70.0, 61.0). This coupled with an anomeric proton at δ 5.08 (1H, *d*, *J* = 7.3 Hz) and a protonated carbon at δ 102.6 observed in the HMQC spectrum indicated **2** contained a β-glucopyranosyl moiety. Acidic hydrolysis of **2** produced **1** and D-glucose, which were both confirmed by co-HPLC analysis with authentic samples and by measurement of optical activity after separation by HPLC. A key HMBC correlation between δ 5.08 (glc H-1″) and δ 144.3 (ring B C-4′) established that the glucose was attached to the assigned position. The full assignments of the protons and carbons were achieved by a combination of ^{1}H, ^{13}C, COSY, HMQC, and HMBC experiments and their analyses. The structure of **2** was determined as (4-β-D-glucopyranosyloxy)-3-hydroxyphenyl caffeate.

Compound **3** was obtained as yellow powder and had a positive reaction to $FeCl_3$. The molecular formula $C_{40}H_{34}O_{15}$ for **3**, deduced from its HRMADILMS, was 14 mass units higher than **16**. Compared to **16**, the NMR spectra of **3** displayed additional signals (δ_C 55.7 and δ_H 3.82), which corresponded to a methoxy group. Detailed NMR spectroscopic analysis concluded that **3** also had two acyl moieties as **16**, which were assigned to *trans*-*p*-coumaroyl and *trans*-*p*-methoxycoumaroyl, respectively. The *trans*-configuration for the two-acyl functions was deduced from the larger coupling constant (*J* = 15.9 Hz) for the olefinic protons, while a *cis*-configuration had a smaller coupling constant (*J* = 13.0 Hz) (Budzianowski and Skrzypczak, 1995). Alkaline hydrolysis of **3** furnished kaempferol-3-*O*-β-D-glucopyranoside (**3a**) identified by co-HPLC with an authentic sample (Zhang et al., 2004) and *trans*-*p*-coumaric acid (**3b**) and *trans*-*p*-methoxycoumaric acid (**3c**) identified by their NMR spectroscopic data. The two-acyl groups of glucose at C-2 and C-6 were established through the following HMBC long-range correlations: δ 4.99 (1H, *t*, *J* = 8.0 Hz, glc H-2) with δ 165.8 (*trans*-*p*-methoxycoumaroyl, C-9), and δ 4.07 and 4.31 (each 1H, glc H-6) with δ 166.3 (*trans*-*p*-coumaroyl, C-9). The full structural assignment of **3** was confirmed by a combinational NMR spectroscopic interpretation including analyses of the ^{1}H, ^{13}C, COSY, HMQC, and HMBC spectra. The structure of **3** was elucidated as kaempferol-3-*O*-(2-*O*-*trans*-*p*-methoxycoumaroyl-6-*O*-*trans*-*p*-coumaroyl)-β-D-glucopyranoside.

Eryngioside A (**4**) had a molecular formula of $C_{54}H_{90}O_{24}$ as deduced from its $[M+Na]^+$ ion at *m*/*z* 1145.5762 and its $[M+K]^+$ ion at *m*/*z* 1161.5483 in the positive HRMALDIMS. The NMR spectrum of **4** displayed 54 carbon signals, of which 30 were assigned to the aglycone, and the remaining 24 to the sugar moieties. This suggests that compound **4** has four sugar units, which was supported by four anomeric protons at δ 4.89 (1H, *d*, *J* = 7.7 Hz), 5.09 (1H, *d*, *J* = 7.8 Hz), 5.16 (1H, *d*, *J* = 7.7 Hz), 5.38 (1H, d, *J* = 7.6 Hz), and four protonated carbons at δ 103.9, 104.3, 104.8, 101.8 observed in the HMQC spectrum. Acid hydrolysis of **4** produced camelliagenin A (**4a**), identified by NMR spectroscopic analysis with the reference data (Chakravarty et al., 1987), and with the D-glucose identified by co-HPLC analysis with authentic sugar and by measurement of optical activity after separation by HPLC. All monosaccharides were determined to be in the pyranose form from analysis of their ^{13}C NMR spectroscopic data. The β-anomeric configuration of glucose was defined from their $^3J_{H1,H2}$ and $^1J_{CH}$ coupling constants as well as from NOE information (Jia et al., 1998; Zhang et al., 1999b). The linkage of the trisaccharidic chain at C-3 of aglycone was established from the following HMBC correlations: Glc′ H-1 (δ 4.89) with aglycone C-3 (δ 89.7), Glc″ H-1 (δ 5.38) with Glc′ C-2 (δ 80.4), and Glc‴ H-1 (δ 5.16) with Glc″ C-2 (δ 84.0). The fourth Glc⁗ attached at C-22 was confirmed by a HMBC correlation between Glc⁗ H-1 (δ 5.09) and aglycone C-22 (δ 80.1). The full assignments of the protons and carbons of **4** were achieved by a combination of ^{1}H, ^{13}C, DEPT, COSY, HMQC, HMBC, and ROESY experiments. The structure of eryngioside A (**4**) was established as 3β-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyloxy-22α-β-D-glucopyranosyloxyolean-12-ene-16α, 28-diol.

Eryngioside B (**5**) had the same molecular formula of $C_{54}H_{90}O_{24}$ as **4**, deduced from its HRMALDIMS. Detailed NMR spectroscopic analyses indicated that compounds **5** and **4** were structurally different only in the trisaccharidic chain at the C-3 functionality of the aglycone. In compound **4**, the third glucose (Glc‴) of the trisaccharidic chain was replaced by a galactose in **5**, as indicated by their different chemical shifts (Table 2) (Yoshikawa et al., 1998; Zhang et al., 1999a). Acid hydrolysis of **5** yielded camelliagenin A (**4a**), D-glucose and D-galactose (Gal), which were identified by co-HPLC analysis with reference compounds and by measurement of optical rotation values after separation by HPLC. The structure of eryngioside B (**5**) was assigned as 3β-[β-D-galactopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyloxy-22α-β-D-glucopyranosyloxyolean-12-ene-16α,28-diol.

The molecular formula of $C_{54}H_{88}O_{24}$ for **6**, established by positive-ion HRMALDIMS, was two mass units (2 × H) lower than **4**. Detailed comparison of the NMR spectroscopic data of **6** with those of **4** concluded that both compounds shared a common sugar-substitution pattern, with a difference for the aglycone moieties. In the ^{13}C NMR spectrum of **4**, the signal at δ 65.9 for oxymethine at C-16 was replaced by a resonance at δ 211.5 in **6**. This, together with the MS data, clearly indicated that compound **6** had a carbonyl carbon. This carbonyl carbon was assigned to the C-16 position based on HMBC correlations. The significant chemical shift differences (Table 1) for C-14 (Δ 6.0 ppm), C-15 (Δ 11.1 ppm), C-17 (Δ 12.4 ppm), C-18 (Δ 4.5 ppm), and C-22 (Δ 6.0 ppm) between **6** and **4** also supported this assignment. The structure of eryngioside C (**6**) was thus determined as 3β-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyloxy-22α-β-D-glucopyranosyloxyolean-12-ene-16-oxo-28-ol.

Eryngioside D (**7**) had the same molecular formula of $C_{54}H_{90}O_{24}$ as **4**, as indicated by its HRMALDIMS and ^{13}C NMR spectroscopic data. The NMR data comparison of compounds **7** and **4** concluded that both compounds had the same aglycone of camelliagenin A

Table 1
^{13}C NMR spectroscopic data for the aglycone moieties of saponins **4**–**15** (150 MHz in pyridine-d_5)[a]

Carbon	4	5	6	7	8	9	10	11	12	13	14	15
1	38.3	38.1	38.1	38.3	38.8	38.7	38.8	38.9	38.9	38.7	38.6	38.6
2	25.7	25.6	26.0	26.2	26.5	26.4	26.5	26.6	26.6	26.5	26.6	26.6
3	89.7	90.2	89.6	89.3	89.4	89.5	89.6	89.4	89.4	89.5	89.6	89.6
4	39.0	38.9	39.0	39.2	39.4	39.6	39.5	39.5	39.5	39.6	39.5	39.5
5	55.1	55.0	54.9	55.8	55.3	55.6	55.5	55.4	55.4	55.3	55.5	55.5
6	17.7	17.7	17.6	18.3	18.6	18.3	18.7	18.7	18.7	18.7	18.8	18.8
7	32.1	32.6	31.9	32.6	36.6	32.8	36.6	36.6	36.6	36.7	32.9	32.9
8	39.6	39.5	39.6	39.6	41.3	40.0	40.9	40.9	40.9	41.0	40.6	40.6
9	46.4	46.3	46.0	47.2	47.0	46.8	47.0	47.1	47.1	47.0	46.8	46.8
10	36.0	35.9	36.0	36.6	37.0	36.9	36.9	36.9	36.9	36.9	37.0	37.0
11	23.2	23.1	23.2	23.1	23.9	23.7	23.7	23.9	23.9	23.8	23.8	23.8
12	123.8	123.5	124.3	121.6	125.0	123.5	125.4	124.7	124.7	125.0	123.5	123.5
13	141.6	141.4	139.7	142.8	144.0	142.7	143.6	144.4	144.4	143.1	142.7	142.7
14	41.8	41.7	47.8	42.9	47.6	41.7	47.7	47.7	47.7	47.6	41.8	41.8
15	34.5	34.4	45.6	32.5	67.4	34.7	67.4	67.4	67.4	67.4	34.2	34.2
16	65.9	65.9	211.5	67.2	74.2	67.8	74.9	74.9	74.9	74.6	67.6	67.6
17	45.2	45.1	57.6	41.9	48.0	48.0	48.3	45.1	45.1	48.1	48.0	48.0
18	42.7	42.6	47.2	42.3	41.2	40.1	41.4	41.4	41.4	40.7	40.1	40.1
19	45.6	45.6	46.3	45.4	47.2	47.0	46.8	47.0	47.0	46.8	47.0	47.0
20	31.4	31.4	31.3	33.4	36.8	36.2	36.2	31.9	31.9	36.0	36.0	36.0
21	41.1	41.1	42.6	41.7	76.4	76.3	79.1	41.7	41.7	79.1	79.1	79.1
22	80.1	79.9	86.1	77.7	76.8	76.8	73.3	72.6	72.6	73.6	73.8	73.8
23	27.4	27.2	27.3	27.8	27.7	27.7	27.8	27.8	27.8	27.6	27.6	27.6
24	16.1	15.9	16.1	16.5	16.6	16.4	16.7	16.7	16.7	16.8	16.6	16.6
25	14.9	14.8	14.8	15.3	15.8	15.5	15.8	15.7	15.7	15.6	15.7	15.7
26	16.2	16.1	16.2	17.2	17.4	16.7	17.5	17.5	17.5	17.6	16.8	16.8
27	26.9	27.1	26.9	28.1	21.1	27.3	21.0	20.8	20.8	20.3	27.5	27.5
28	58.0	57.8	68.0	66.7	63.7	63.8	63.0	62.9	62.9	62.9	63.2	63.2
29	32.6	32.8	32.6	28.2	30.2	30.1	29.9	33.4	33.4	29.5	29.6	29.6
30	24.2	24.2	23.8	27.9	19.2	19.9	20.0	25.1	25.1	20.1	20.0	20.0

[a] Assignments were based on COSY, HMQC, and HMBC experiments.

(**4a**), and the same trisaccharidic chain at the C-3 functionality of the aglycone, but a different position for the fourth glucose (Glc$^{''''}$). As observed in the HMBC spectrum, the long-range correlations of Glc$^{''''}$ H-1 (δ 4.87, 1H, *d*, 7.8 Hz) with C-28 (δ 66.7) and H-28 (δ 3.85, 4.40, each 1H, *d*, 10.5 Hz) with Glc$^{''''}$ C-1 (δ 104.3) established that Glc$^{''''}$ was linked to the C-28 of the aglycone. Therefore, the structure of eryngioside D (**7**) was elucidated as 3β-[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyloxy-28-β-D-glucopyranosyloxyolean-12-ene-16α,22α-diol.

Eryngioside E (**8**) was assigned a molecular formula of $C_{52}H_{82}O_{22}$ based on its HRMALDIMS data. The HMQC spectrum of **8** displayed three anomeric protons at δ 4.97, 5.44, 5.73, and three protonated carbons at δ 105.3, 104.8, and 103.6, indicating that **8** has three sugar units, which were identified from their NMR spectroscopic data as glucuronic acid (GlcA), xylose (Xyl), and glucose. Alkaline hydrolysis of **8** liberated prosapogenin (**8b**). Further acidic hydrolysis of **8b** yielded R_1-barrigenol (**8a**) (Konoshima and Lee, 1986; Kartal et al., 2005; Zhang et al., 2006) and three sugars (D-glucuronic acid, D-glucose, and D-xylose), which were identified by co-HPLC with authentic compounds and by measurement of optical rotation values after separation by HPLC. The prosapogenin (**8b**) was assigned as 3β-[β-D-glucopyranosyl-(1→2)]-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosyloxyolean-12-ene-15α, 16α, 21β, 22α, 28-pentaol by its extensive NMR spectroscopic analysis. The linkage and sequence of the trisaccharidic chain of **8b** was confirmed by the following key HMBC correlations of GlcA H-1 (δ 4.87, 1H, *d*, 7.9 Hz) with aglycone C-3 (δ 89.8), Glc H-1 (δ 5.59, 1H, *d*, 7.6 Hz) with GlcA C-2 (δ 78.5), Xyl H-1 (δ 5.24, 1H, *d*, 7.6 Hz) with GlcA C-3 (δ 85.5), aglycone H-3 (δ 3.23) with GlcA C-1 (δ 105.0), GlcA H-2 (δ 4.34) with Glc C-1 (δ 103.1), and GlcA H-3 (δ 4.30) with Xyl C-1 (δ 104.6). In addition to the prosapogenin **8b**, compound **8** contained an angeloyl group as indicated by its characteristic NMR signals (Tables 2 and 3) (Yoshikawa et al., 1998; Zhang et al., 1999a). As observed in the HMBC spectrum of **8**, a long-range HMBC correlation of H-22 (δ 6.22, 1H, *d*, 9.8 Hz) with angeloyl C-1 (δ 169.3) established that C-22 was the acyl position. The downfield shift of H-22 also supported this conclusion. The full assignments of the protons and carbons were achieved by a combination of ^{1}H, ^{13}C, COSY, DEPT, HMQC, and HMBC experiments. The structure of eryngioside E (**8**) was identified as 22α-angeloyloxy-3β-[β-D-glucopyranosyl-(1→2)]-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosyloxyolean-12-ene-15α, 16α, 21β,28-tetrol.

Eryngioside F (**9**) gave a $[M+Na]^+$ ion at *m*/*z* 1065.5288 in the positive HRMALDIMS, 16 mass units lower than that of **8**, implying the absence of an oxygen-bearing function in **9**. Extensive NMR spectroscopic analyses indicated that **9** and **8** are structurally different only in the substitutent at C-15 of aglycone. In compound **8**, the oxymethine (δ 67.4, C-15) was replaced by a methylene (δ 34.7, C-15) in **9**. Accordingly, the structure of eryngioside F (**9**) was identified as 22α-angeloyloxy-3β-[β-D-glucopyranosyl-(1→2)]-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosyloxyolean-12-ene-16α, 21β,28-triol.

Eryngioside G (**10**) had the same molecular formula of $C_{52}H_{82}O_{22}$ as **8**. The anomeric protons (δ 4.90, 5.38, 5.67) and the protonated carbons (δ 105.3, 105.0, 103.5) observed in the HMQC spectrum implied that **10** contained three sugar units. These three sugar units were identified as β-D-glucuronopyranosyl (GlcA), β-D-glucopyranosyl (Glc) and α-L-arabinopyranosyl (Ara) based on their NMR spectroscopic data and their optical activity after separation from acidic hydrolysate by HPLC. HMBC correlations established the linkage and the sequence of the trisaccharidic chain at C-3 position. In addition to the trisaccharidic unit, compound **10** also exhibited characteristic NMR signals of an angeloyl group (Tables 2 and 5) (Yoshikawa et al., 1998; Zhang et al., 1999a). A key HMBC correlation between H-21 (δ 6.58, 1H, *d*, 10.1 Hz) and angeloyl C-1 (δ 167.9) indicated that the angeloyl group in **10** was acylated at C-21, instead of C-22, in **8**. The structure of eryngioside G (**10**) was elucidated as 21β-angeloyloxy-3β-[β-D-glucopyranosyl-(1→2)]-

Table 2
^{13}C NMR spectroscopic data for the sugar and acyl groups of saponins **4**–**15** (150 MHz in pyridine-d_5)[a]

Carbon	4	5	6	7	8	9	10	11	12	13	14	15
C-3	Glc′	Glc′	Glc′	Glc′	GlcA	GlcA	GlcA	GlcA	GlcA	GlcA	GlcA	GlcA
1	103.9 (160)[b]	104.1	103.9	104.5	105.3	105.1	105.3	105.4	105.4	105.1	105.2	105.2
2	80.4	80.1	80.3	82.3	79.1	79.1	79.2	79.2	79.2	79.2	79.3	79.3
3	76.3[c]	76.2[b]	76.3[b]	76.2[b]	85.5	85.6	85.6	85.8	85.9	85.6	85.9	85.9
4	69.7[d]	69.0[c]	69.6[c]	69.2[c]	71.6	71.8	71.6	71.5	71.5	71.6	71.6	71.6
5	77.1[e]	76.9[d]	77.5[d]	77.9[d]	77.2	77.2	77.3	77.2	77.2	77.3	77.3	77.3
6	61.3[f]	61.2[e]	61.2[e]	62.1[e]	172.1	171.8	171.5	171.9	171.9	171.8	171.9	171.9
	Glc″ (161)[b]	Glc″	Glc″	Glc″	Glc	Glc	Glc	Glc	Glc	Glc	Glc	Glc
1	101.8	102.1	101.8	102.9	103.6	103.4	103.5	103.7	103.7	103.3	103.6	103.6
2	84.0	86.1	84.0	85.2	76.3	76.1	76.3	76.4	76.4	76.3	76.5	76.5
3	76.4[c]	76.2[b]	76.4[b]	77.0[b]	78.3	77.8	78.3	78.4	78.4	78.5	78.5	78.5
4	69.9[d]	69.9[c]	70.0[c]	70.4[c]	72.2	71.6	72.3	72.3	72.3	72.3	72.3	72.3
5	77.1e	77.4[d]	77.8[d]	77.9[d]	78.0	77.6	77.9	77.9	77.8	77.9	77.9	77.9
6	61.3[f]	61.3[e]	61.3[e]	62.3[e]	63.1	62.9	63.2	63.2	63.2	62.8	62.9	62.9
	Glc‴	Gal	Glc‴	Glc‴	Xyl	Xyl	Ara	Xyl	Ara	Xyl	Xyl	Ara
1	104.8 (160)[b]	103.5	104.8	105.9	104.8	104.6	105.0	104.9	105.1	104.9	105.0	105.0
2	75.1	73.8	75.1	75.2	75.2	75.0	72.9	75.1	72.8	75.2	75.1	72.9
3	76.9[c]	75.1	77.0[d]	77.1[b]	78.3	77.8	74.5	78.4	74.6	78.5	78.5	74.7
4	69.9[d]	71.2[c]	70.3[c]	70.7[c]	70.7	69.6	69.7	70.8	69.6	70.7	70.6	69.6
5	77.1[e]	76.9[d]	77.1[d]	78.1[d]	67.3	67.6	67.5	67.3	67.7	67.3	67.4	67.5
6	61.5[f]	61.3[e]	61.3[e]	62.4[e]								
C-21							Ang			Ang	Ang	Ang
1							167.9			167.7	167.9	167.9
2							129.0			129.5	129.3	129.3
3							136.5			136.9	136.8	136.8
4							15.7			15.8	15.8	15.8
5							20.6			20.7	20.7	20.7
C-22/28	Glc⁗	Glc‴	Glc⁗	Glc⁗	Ang	Ang		Ang	Ang	Ac	Ac	Ac
1	104.3 (159)[b]	104.2	106.4	104.3	169.3	169.0		167.9	167.9	170.6	170.7	170.7
2	74.4	74.3	73.9	74.8	129.5	129.6		129.4	129.4	20.3	20.7	20.6
3	77.0[c]	77.0[d]	77.2[d]	77.7[b]	136.1	136.8		136.4	136.4			
4	70.7[d]	70.3[c]	70.7[c]	71.3[c]	15.7	15.6		15.7	15.7			
5	77.8[e]	77.4[d]	77.3[d]	78.8[d]	20.8	20.7		21.2	21.2			
6	62.0[f]	62.2[e]	61.9[e]	62.5[e]								

[a] Assignments were based on COSY, HMQC, and HMBC experiments.
[b] The number in the parentheses is the $^1J_{CH}$ coupling constant (Hz).
[c–f] The data with the same labels in each column may be interchanged.

[α-L-arabinopyranosyl-(1→3)]-β-D-glucuronopyranosyloxyolean-12-ene-15α,16α, 22α,28-tetrol.

Eryngiosides H (**11**) and I (**12**) had the same molecular formula $C_{52}H_{82}O_{21}$ and the angeloyl group in the same position as **9** on the basis of HRMALDIMS data and HMBC correlations. The difference in the structures of these compounds was located in the aglycone and the trisaccharidic chain. Extensive NMR analysis suggested that **11** and **12** possessed a hydroxyl group at C-15, instead of the hydroxyl group at C-21 in **9**. Therefore, the aglycone of **11** and **12** was characterized as A_1-barrigenol (**11a**) (Konoshima and Lee, 1986; Kartal et al., 2005). Alkaline hydrolysis of **11** and **12** yielded **11b** and **12b**, respectively. Further acidic hydrolysis of **11b** and **12b** gave **11a**. Compound **11** has the same trisaccharidic chain as **9**, but **12** had a difference in the components of the trisaccharidic chain. In compounds **9** and**11**, the β-D-xylopyranose of the trisaccharidic chain was replaced by a α-L-arabinopyranose in **12**, as indicated by their NMR chemical shifts (Table 2) and optical rotation values. On the basis of the foregoing evidence, the structures of eryngiosides H (**11**) and I (**12**) were assigned as 22α-angeloyloxy-3β-[β-D-glucopyranosyl-(1→2)]-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosyloxyolean-12-ene-15α,16α,28-triol and 22α-angeloyloxy-3β-[β-D-glucopyranosyl-(1→2)]-[α-L-arabinopyranosyl-(1→3)]-β-D-glucuronopyranosyloxyolean-12-ene-15α,16α,28-triol, respectively.

Eryngioside J (**13**) was assigned a molecular formula of $C_{54}H_{84}O_{23}$ from its HRMALDIMS. The NMR spectroscopic comparison of compounds **13** and **8** demonstrated that both compounds shared a common aglycone R_1-barrigenol (**8a**) and an identical trisaccharidic chain at the C-3 of the aglycone. In addition to the characteristic signals of an angeloyl (Yoshikawa et al., 1998; Zhang et al., 1999a), the NMR spectra of **13** displayed an acetyl group (δ_C 170.6, 20.3 and δ_H 1.80). The presence of the acetyl group was also supported by the MS data of **13**, which showed 42 amu supplementary compared to **8**. The HMBC correlations of H-21 (δ 6.61, 1H, *d*, 10.2 Hz) with angeloyl C-1 (δ 167.7), and H-22 (δ 6.21, 1H, *d*, 10.2 Hz) with acetyl C-1 (δ 170.6) established that the angeloyl and the acetyl were attached at C-21 and C-22, respectively. The structure of eryngiosides J (**13**) was thus elucidated as 21β-angeloyloxy-22α-acetyloxy-3β-[β-D-glucopyranosyl-(1→2)]-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosyloxyolean-12-ene-15α,16α,28-triol.

Eryngioside K (**14**) exhibited an intense $[M+Na]^+$ peak at *m*/*z* 1107.5370 and a $[M+K]^+$ peak at *m*/*z* 1123.5101 in the HRMALDIMS, 16 mass units lower than that of **13**, suggesting **14** was a derivative of **13** with the absence of an oxygen-bearing function. Detailed NMR spectroscopic analyses indicated that **14** was structurally different from **13** only in the substituent at C-15. In the ^{13}C NMR spectra, the oxymethine (δ 67.4, C-15) in **13** was replaced by a methylene (δ 34.2, C-15) in **14**. Therefore, the structure of eryngioside K (**14**) was determined as 21β-angeloyloxy-22α-acetyloxy-3β-[β-D-glucopyranosyl-(1→2)]-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosyloxyolean-12-ene-16α,28-diol.

Eryngioside L (**15**) had the same molecular formula as **14** based on the HRMALDIMS data. The NMR spectroscopic resonances of **15**

Table 3
^{1}H NMR spectroscopic data for the aglycone moieties of **4**–**9** (600 MHz in pyridine-d_5)[a]

Proton	4	5	6	7	8	9
1	0.77, 1.38	0.76, 1.33	0.79, 1.41	0.79, 1.38	0.90, 1.40	0.76, 1.38
2	1.85, 2.16	1.86, 2.12	1.88, 2.13	1.78, 2.15	1.84, 2.21	1.78, 2.15
3	3.25 (1H, *dd*, 12.0/ 4.3 Hz)	3.20 (1H, *dd*, 12.3/ 4.2 Hz)	3.26 (1H, *dd*, 12.1/ 4.3 Hz)	3.30 (1H, *dd*, 12.3/ 4.0 Hz)	3.29 (1H, *dd*, 12.6/ 4.5 Hz)	3.23 (1H, *dd*, 12.7/ 4.4 Hz)
5	0.59	0.55	0.58	0.69	0.80	0.70
6	1.13, 1.35	1.14, 1.33	1.16, 1.36	1.31, 1.53	1.56, 1.57	1.22, 1.43
7	1.20, 1.37	1.21, 1.41	1.23, 1.41	1.35, 1.41	2.06, 2.19	1.25, 1.54
9	1.39	1.38	1.38	1.50	1.72	1.68
11	1.66	1.64	1.76	1.73	1.78,1.91	1.75, 1.85
12	5.31 (1H, *brs*)	5.30 (1H, *brs*)	5.38 (1H, *brs*)	5.50 (1H, *brs*)	5.56 (1H, *brs*)	5.39 (1H, *brs*)
15	1.54, 1.87	1.55, 1.87	1.90, 3.06	1.81, 2.45	4.27 (1H, *d*, 2.7Hz)	1.55, 1.80
16	4.87	4.86	–	5.12	4.47 (1H, *d*, 2.7 Hz)	4.30
18	2.86	2.85	2.72	2.85	3.09	3.02
19	1.06, 1.81	1.06, 1.80	1.19, 1.65	1.55, 1.75	1.49 3.02	1.41, 2.93
21	1.89, 2.17	1.90, 2.16	2.20, 2.43	1.90, 2.02	5.04 (1H, *d*, 9.8 Hz)	4.90 (1H, *d*, 9.8 Hz)
22	4.88	4.87	4.12	4.48	6.22 (1H, *d*, 9.8 Hz)	6.02 (1H, *d*, 9.8 Hz)
23	1.19 (3H, *s*)	1.10 (3H, *s*)	1.20 (3H, *s*)	1.28 (3H, *s*)	1.22 (3H, *s*)	1.15 (3H, *s*)
24	1.01 (3H, *s*)	0.94 (3H, *s*)	1.02 (3H, *s*)	1.10 (3H, *s*)	1.10 (3H, *s*)	0.99 (3H, *s*)
25	0.71 (3H, *s*)	0.67 (3H, *s*)	0.75 (3H, *s*)	0.71 (3H, *s*)	0.85 (3H, *s*)	0.78 (3H, *s*)
26	0.79 (3H, *s*)	0.77 (3H, *s*)	0.95 (3H, *s*)	0.53 (3H, *s*)	1.03 (3H, *s*)	0.85 (3H, *s*)
27	1.22 (3H, *s*)	1.21 (3H, *s*)	1.24 (3H, *s*)	1.33 (3H, *s*)	1.90 (3H, *s*)	1.75 (3H, *s*)
28	3.98, 4.56 (each, 1H, *d*, 10.6 Hz)	3.91, 4.55 (each, 1H, *d*, 10.5 Hz)	4.16, 4.55 (each, 1H, *d*, 10.1 Hz)	3.85, 4.40 (each, 1H, *d*, 10.5 Hz)	3.53, 3.78 (each, 1H, *d*, 10.6 Hz)	3.29, 3.55 (each, 1H, *d*, 10.0 Hz)
29	0.89 (3H, *s*)	0.87 (3H, *s*)	0.79 (3H, *s*)	1.03 (3H, *s*)	1.34 (3H, *s*)	1.30 (3H, *s*)
30	1.09 (3H, *s*)	1.09 (3H, s)	1.05 (3H, *s*)	1.09 (3H, *s*)	1.43 (3H, *s*)	1.32 (3H, *s*)
C_{22}					Ang	Ang
3					5.80 (1H, *q*, 7.0 Hz)	5.90 (1H, *q*, 7.0 Hz)
4					2.02 (3H, *d*, 7.0 Hz)	2.03 (3H, *d*, 7.0 Hz)
5					1.89 (3H, *s*)	1.95 (3H, *s*)

[a] Assignments were based on COSY, HMQC, and HMBC experiments. Due to severe overlapping in the ^{1}H spectrum only detectable relative *J* (Hz) are reported.

due to the aglycone and two acyl groups at C-21 and C-22 were superimposable with those of **14**, implying **15** had the same aglycone and substituents at C-21 and C-22 as **14** did. The anomeric proton and protonated carbon signals observed in HMQC spectra indicated **15** also contained three sugar units as **14**. Detailed NMR spectroscopic interpretation showed that **15** contained an arabinose, instead of the xylose in **14**. The structure of eryngioside L (**15**) was elucidated as 21β-angeloyloxy-22α-acetyloxy-3β-[β-D-glucopyranosyl-(1→2)]-[α-L-arabinopyranosyl-(1→3)]-β-D-glucuronopyranosyloxyolean-12-ene-16α,28-diol.

3. Conclusions

Recent studies (Kartal et al., 2005, 2006) indicated that *E. campestre* contained R_1- and A_1-barrigenol-based polyhydroxylated triterpenoid glycosides (saponins) with two or three sugar units. This study shows that the saponins in *E. yuccifolium* are more variable both in their aglycone and oligosaccharidic chain moieties with different components and sequences. The type of polyhydroxylated triterpenoid glycosides usually possessed acyl functions at C-21, C-22, and C-28 positions, and had glucuronic acid attached to C-3 position of polyhydroxylated aglycone (Yang et al., 1999; Zhang et al., 1999a; D'Acquarica et al., 2002; Matsushita et al., 2004; Voutquenne et al., 2005; Kartal et al., 2005, 2006). Interestingly, several bisdesmosidic polyhydroxylated triterpenoid glycosides without ester chains (**4**–**7**) were first found in *E. yuccifolium*. These new compounds had glucose, but not glucuronic acid, attached to the C-3 of the aglycone. To the best our knowledge, this type of polyhydroxylated triterpenoid glycosides is rare. It is also worthwhile to mention that C-28 (δ 57–58 ppm, Table 1) of compounds **4** and **5** experienced an unusual upfield shift from the influence of the glycosylation at C-22. Saponins **11**–**15** and **19** are the main compounds in the saponin fraction.

4. Experimental

4.1. General experimental procedures

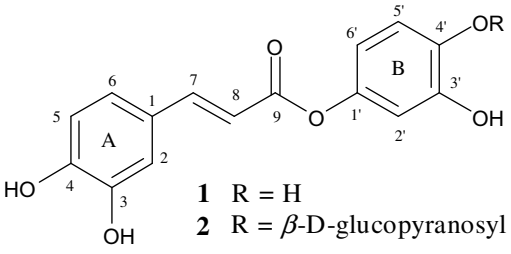
NMR experiments were performed on a Bruker 600 MHz NMR instrument, with NMR spectroscopic data reported as δ (ppm) values and referenced to the solvent used. HRMALDIMS were acquired on a MALDI TOF instrument (Applied Biosystems Voyager STR), where HRESIMS were obtained using an Electrospray instrument (MDS Sciex Pulsar Qstar). UV spectra were recorded in MeOH with a μ Quant spectrophotometer (Bio-Tek Instruments Inc.). Optical rotation values were measured on a JASCO P-1010 polarimeter. Octadecyl-functionalized silica gel (ODS, Aldrich) was used for low-pressure chromatography. HPLC analysis was performed on an Agilent 1100 HPLC system with an Agilent 1100 diode array detector or an Agilent 1100 refractive index detector using a Hypersil ODS column (column A, 250 × 4.6 mm, 5 μm, Supelco) or a SUPEICOGEL CA column (column B, 300 × 7.8 mm, Supelco). Preparative HPLC was performed with an Acuflow Series III pump connected with an Acutect 500 UV/VIS detector using an Econosil ODS column (column C, 250 × 22 mm, 10 μm, Alltech). D-glucose, D-galactose, L-arabinose, D-xylose, and D-glucuronic acid were purchased from Aldrich.

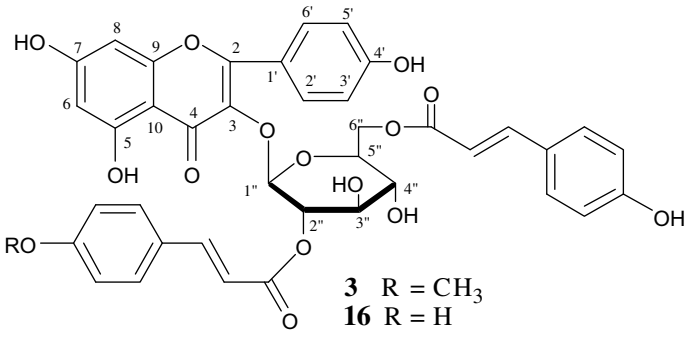
4.2. Plant material

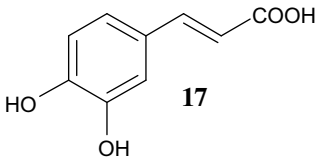
Whole plants of *E. yuccifolium* were collected in Nacogdoches, Texas, on June 28, 2006, and were identified by Shiyou Li. A voucher specimen (Tax-Nac-SFAEF-20060628-#0002-WP) and a sample of the experimental plant material (CMPR-EY20060628) were deposited at the National Center for Pharmaceutical Crops at Stephen F. Austin State University, USA.

4.3. Extraction and isolation

Air-dried plant materials (1 kg) were ground to a coarse powder and percolated three times with 95% ethanol at room temperature (each 3000 ml, 8 h). The extract was concentrated in vacuo to give a residue (70.5 g), which was suspended in MeOH–H_2O (800 ml, 1:1, v/v), and then partitioned successively with hexanes and *n*-butanol. The *n*-butanol-soluble fraction (25.0 g) was applied to a column of silica gel eluting with a mixture of $CHCl_3$:MeOH (9:1, 4:1, 2:1, each 2000 ml) to give three column fractions A, B, and C, respectively. Compounds **3**, **16**, and **17** were obtained from fraction A by preparative HPLC (column C, MeOH/0.5% HoAc in H_2O: 75/25, detection 254 nm). Fraction B was isolated by preparative HPLC (column C, MeCN/0.5% HoAc in H_2O: 47/53, detection 210 nm) to give **11** (t_R 56 min), **12** (t_R 51 min), **13** (t_R 69 min), **19** (t_R 63 min), **14** (t_R 81 min) and **15** (t_R 76 min). Fraction C was further fractionated by an ODS column eluting with a mixture of MeOH and H_2O (30:70, 45:55, 60:40, each 2000 ml) into three subfractions C_1, C_2 and C_3, respectively. Compounds **1** (t_R 40 min) and **2** (t_R 52 min) were purified from subfraction C_1 by preparative HPLC (column C, MeOH/0.5% HoAc in H_2O: 43/57, detection 254 nm). Subfraction C_2 was isolated by preparative HPLC (column C, $CNCH_3$/0.5% HoAc in H_2O: 30/70, detection 210 nm) to yield compounds **4** (t_R 52 min), **5** (t_R 57 min), **6** (t_R 59 min) and **7** (t_R 63 min). While **8** (t_R 31 min), **9** (t_R 48 min), **18** (t_R 58 min), and **10** (t_R 51 min) were obtained from subfraction C_3 by preparative HPLC (column C, $MeCN_3$/0.5% HoAc in H_2O: 42/58, detection 210 nm).







	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
4	H	OH	H	Glc	H	H	CH_2OH	Glc
5	H	OH	H	Glc	H	H	CH_2OH	Gal
6	H	=O	H	Glc	H	H	CH_2OH	Glc
7	H	OH	H	H	Glc	H	CH_2OH	Glc
8	OH	OH	OH	Ang	H	Xyl	COOH	H
9	H	OH	OH	Ang	H	Xyl	COOH	H
18	OH	OH	O-Ang	H	H	Xyl	COOH	H
10	OH	OH	O-Ang	H	H	Ara	COOH	H
11	OH	OH	H	Ang	H	Xyl	COOH	H
12	OH	OH	H	Ang	H	Ara	COOH	H
13	OH	OH	O-Ang	Ac	H	Xyl	COOH	H
19	OH	OH	O-Ang	Ac	H	Ara	COOH	H
14	H	OH	O-Ang	Ac	H	Xyl	COOH	H
15	H	OH	O-Ang	Ac	H	Ara	COOH	H

4.4. Compound **1**

Colorless powder, UV λ_{max}^{MeOH} nm (log ε): 216 (4.65), 248 (4.00), 283 (4.26), 326 (4.50). ^1H NMR (600 MHz, pyridine-d_5): δ 6.56 (1H, *d*, *J* = 15.8 Hz, H-8), 6.97 (1H, *d*, *J* = 7.6 Hz, H-6), 7.00 (1H, *d*, *J* = 8.0 Hz, H-6′), 7.09 (1H, *d*, *J* = 8.0 Hz, H-5′), 7.12 (1H, *d*, *J* = 7.6 Hz, H-5), 7.40 (1H, *brs*, H-2), 7.42 (1H, *brs*, H-2′), 7.89 (1H, *d*, *J* = 15.8 Hz, H-7); ^{13}C NMR (150 MHz, pyridine-d_5): δ 114.7 (*d*, C-8), 115.5 (*d*, C-2), 116.2 (*d*, C-5′), 116.3 (*d*, C-5), 117.6 (*d*, C-2′), 120.9 (*d*, C-6′), 122.0 (*d*, C-6), 126.7 (*s*, C-1), 129.4 (*s*, C-1′), 145.5 (*s*, C-3′), 146.1 (*s*, C-3), 146.1 (*d*, C-7), 146.6 (*s*, C-4′), 147.1

(*s*, C-4), 167.3 (*s*, C-9). HRESIMS: *m*/*z* 289.0798 $[M+H]^+$ (calcd for $C_{15}H_{13}O_6$, 289.0712).

4.5. Compound **2**

Colorless powder, $[\alpha]_D^{25} + 43.2$ (*c* 0.1, MeOH). UV λ_{max}^{MeOH} nm (log ε): 213 (4.59), 246 (3.98), 280 (4.22), 325 (4.43). ^{1}H NMR spectral data (600 MHz, pyridine-d_5): δ 3.80 (1H, *m*, glc H-5″), 4.02 (1H, *m*, glc H-4″), 4.08 (1H, *m*, glc H-2″), 4.13 (1H, *m*, glc H-6a″), 4.15 (1H, *m*, glc H-3″), 4.23 (1H, *d*, *J* = 11.0 Hz, glc H-6b"), 5.08 (1H, *d*, *J* = 7.3 Hz, glc H-1″), 6.51 (1H, *d*, *J* = 15.2 Hz, H-8), 6.88 (1H, *d*, *J* = 7.8 Hz, H-6), 6.93 (1H, *d*, *J* = 7.7 Hz, H-6′), 7.10 (1H, *d*, *J* = 7.8 Hz, H-5), 7.27 (1H, *d*, *J* = 7.7 Hz, H-5′), 7.33 (1H, *brs*, H-2′), 7.37 (1H, *brs*, H-2), 7.72 (1H, *d*, *J* = 15.2 Hz, H-7); ^{13}C NMR (150 MHz, pyridine-d_5): δ 61.0 (*t*, glc C-6″), 70.0 (*d*, glc C-4″), 73.6 (*d*, glc C-2″), 76.3 (*d*, glc C-3″), 77.2 (*d*, glc C-5″), 102.6 (*d*, glc C-1″), 114.6 (*d*, C-8), 115.0 (*d*, C-2), 115.9 (*d*, C-5), 117.0 (*d*, C-5′), 117.4 (*d*, C-2′), 120.7 (*d*, C-6′), 121.7 (*d*, C-6), 126.3 (*s*, C-1), 133.9 (*s*, C-1′), 144.3 (*s*, C-4′), 145.5 (*d*, C-7), 145.9 (*s*, C-3), 146.8 (*s*, C-3′), 148.8 (*s*, C-4), 167.8 (*s*, C-9). HRESIMS: *m*/*z* 451.1226 $[M+H]^+$ (calcd for $C_{21}H_{23}O_{11}$, 451.1240).

4.6. Compound **3**

Yellow powder, $[\alpha]_D^{25} - 63.9$ (*c* 0.5, MeOH). UV λ_{max}^{MeOH} nm (log ε): 218 (4.76), 267 (4.59), 316 (4.43). ^{1}H NMR (600 MHz, MeOH-d_4): aglycone δ 6.10 (1H, *brs*, H-6), 6.30 (1H, *brs*, H-8), 6.86 (2H, *d*, *J* = 8.0 Hz, H-3′, 5′), 7.96 (2H, *d*, *J* = 8.0 Hz, H-2′, 6′); glucose δ 3.37 (1H, *m*, H-4), 3.53 (1H, *m*, H-5), 3.57 (1H, *m*, H-3), 4.07 (1H, *m*, H-6a), 4.31 (1H, *d*, *J* = 10.6 Hz, H-6b), 4.99 (1H, *t*, *J* = 8.0 Hz, H-2), 5.74 (1H, *d*, *J* = 7.8 Hz, H-1); *p*-methoxycoumaroyl δ 3.82 (3H, *s*, OCH_3-4), 6.42 (1H, *d*, *J* = 15.9 Hz, H-8), 6.81 (2H, *d*, *J* = 7.7 Hz, H-3, 5), 7.54 (2H, *d*, *J* = 7.7 Hz, H-2, 6), 7.59 (1H, *d*, *J* = 15.9 Hz, H-7); *p*-coumaroyl δ 6.17 (1H, *d*, *J* = 15.9 Hz, H-8), 6.79 (2H, *d*, *J* = 7.6 Hz, H-3, 5), 7.31 (1H, *d*, *J* = 15.9 Hz, H-7), 7.37 (2H, *d*, *J* = 7.6 Hz, H-2, 6); ^{13}C NMR (150 MHz, MeOH-d_4): aglycone δ 93.6 (*d*, C-8), 99.0 (*d*, C-6), 103.6 (*s*, C-10), 115.2 (*d*, C-3′, 5′), 120.8 (*s*, C-1′), 130.8 (*d*, C-2′, 6′), 132.7 (*s*, C-3), 159.9 (*s*, C-2), 159.9 (*s*, C-9), 160.1(*s*, C-4′), 161.1 (*s*, C-5), 165.0 (*s*, C-7), 177.5 (*s*, C-4); glucose δ 62.9 (*t*, C-6), 70.1 (*d*, C-4), 74.0 (*d*, C-2), 74.1 (*d*, C-3), 74.5 (*d*, C-5), 98.3 (*d*, C-1), *p*-methoxycoumaroyl δ 55.7 (*q*, OCH_3-4), 114.3 (*d*, C-8), 115.8 (*d*, C-3, 5), 125.2 (*s*, C-1), 130.3 (*d*, C-2, 6), 145.0 (*d*, C-7), 148.8 (*s*, C-4), 165.8 (*s*, C-9), *p*-coumaroyl acid δ 113.7 (*d*, C-8), 115.8 (*d*, C-3, 5), 124.9 (*s*, C-1), 130.2 (*d*, C-2, 6), 144.7 (*d*, C-7), 145.1 (*s*, C-4), 166.3 (*s*, C-9). HRMALDIMS: *m*/*z* 777.1771 $[M+Na]^+$ (calcd for $C_{40}H_{34}NaO_{15}$, 777.1795).

4.7. Eryngioside A (**4**)

Colorless powder, $[\alpha]_D^{25} + 17.4$ (*c* 0.1, MeOH). For ^{13}C and ^{1}H NMR spectroscopic data, see Tables 1–4. HRMALDIMS: *m*/*z* 1145.5762 $[M+Na]^+$ (calcd for $C_{54}H_{90}NaO_{24}$, 1145.5720), and 1161.5483 $[M+K]^+$ (calcd for $C_{54}H_{90}KO_{24}$, 1161.5459).

4.8. Eryngioside B (**5**)

Colorless powder, $[\alpha]_D^{25} + 6.2$ (*c* 0.1, MeOH). For ^{13}C and ^{1}H NMR spectroscopic data, see Tables 1–4. HRMALDIMS: *m*/*z* 1145.5741 $[M+Na]^+$ (calcd for $C_{54}H_{90}NaO_{24}$, 1145.5720), and 1161.5475 $[M+K]^+$ (calcd for $C_{54}H_{90}KO_{24}$, 1161.5459).

4.9. Eryngioside C (**6**)

Colorless powder, $[\alpha]_D^{25} + 5.2$ (*c* 0.1, MeOH). For ^{13}C and ^{1}H NMR spectroscopic data, see Tables 1–4. HRMALDIMS: *m*/*z* 1143.5590 $[M+Na]^+$ (calcd for $C_{54}H_{88}NaO_{24}$, 1143.5563), and 1159.5342 $[M+K]^+$ (calcd for $C_{54}H_{88}KO_{24}$, 1159.5303).

Table 4
^{1}H NMR spectroscopic data for the sugar moieties of **4**–**9** (600 MHz in pyridine-d_5)[a]

Proton	**4**	**5**	**6**	**7**	**8**	**9**
C-**3**	Glc′	Glc′	Glc′	Glc′	GlcA	GlcA
1	4.89 (1H, *d*, 7.7 Hz)	4.79(1H, *d*, 7.2 Hz)	4.89(1H, *d*, 7.7 Hz)	4.95(1H, *d*, 7.7 Hz)	4.97(1H, *d*, 7.4 Hz)	4.80(1H, *d*, 7.6 Hz)
2	4.18	4.16	4.16	4.17	4.53	4.35
3	4.14	4.02	4.15	4.06	4.40	4.27
4	4.18	4.17	3.98	4.17	4.47	4.30
5	4.37	4.18	4.37	4.23	4.55	4.29
6	4.18, 4.40	4.16, 4.29	4.15, 4.35	4.20, 4.45	–	–
	Glc″	Glc″	Glc″	Glc″	Glc	Glc
1	5.38 (1H, *d*,7.6 Hz)	5.54 (1H, *d*, 7.6 Hz)	5.39 (1H, *d*, 7.9 Hz)	5.49 (1H, *d*, 7.3 Hz)	5.73 (1H, *d*, 7.3 Hz)	5.56 (1H, *d*, 7.6 Hz)
2	4.01	4.29	4.00	4.12	4.09	3.98
3	4.08	4.02	4.08	4.03	4.27	4.08
4	4.13	4.17	4.02	4.10	4.13	3.99
5	3.91	3.92	3.89	3.90	3.93	3.80
6	4.18, 4.40	4.16, 4.29	4.15, 4.35	4.20, 4.45	4.34, 4.50	4.12, 4.35
	Glc‴	Gal	Glc‴	Glc‴	Xyl	Xyl
1	5.16 (1H, *d*, 7.7 Hz)	5.21 (1H, *d*,7.6 Hz)	5.17 (1H, *d*, 7.7 Hz)	5.28 (1H, *d*, 7.5 Hz)	5.44 (1H, *d*, 7.8 Hz)	5.28 (1H, *d*, 7.3 Hz)
2	3.92	4.03	3.92	4.00	4.01	3.99
3	4.14	3.84	4.16	4.15	4.15	4.10
4	4.17	4.18	4.02	4.11	4.15	4.16
5	3.83	3.84	3.87	3.86	3.73, 4.30	3.72, 4.25
6	4.16, 4.32	4.16, 4.29	4.15, 4.35	4.20, 4.45		
C-22/28	Glc⁗	Glc‴	Glc⁗	Glc⁗		
1	5.09 (1H, *d*, 7.8 Hz)	5.09 (1H, *d*, 7.2 Hz)	4.96 (1H, *d*, 7.7 Hz)	4.87 (1H, *d*, 7.8 Hz)		
2	3.85	3.85	3.89	3.98		
3	4.15	4.16	4.16	4.18		
4	3.84	3.85	3.83	3.99		
5	3.86	3.87	3.92	3.88		
6	4.07, 4.44	4.03, 4.40	4.10, 4.40	4.20, 4.45		

[a] Assignments were based on COSY, HMQC, and HMBC experiments. Due to severe overlapping in the ^{1}H spectrum only detectable relative *J* (Hz) are reported.

4.10. Eryngioside D (**7**)

Colorless powder, $[\alpha]_D^{25} + 11.6$ (*c* 0.1, MeOH). For ^{13}C and ^{1}H NMR spectroscopic data, see Tables 1–4. HRMALDIMS: *m*/*z* 1145.5791 $[M+Na]^+$ (calcd for $C_{54}H_{90}NaO_{24}$, 1145.5720), and 1161.5477 $[M+K]^+$ (calcd for $C_{54}H_{90}KO_{24}$, 1161.5459).

4.11. Eryngioside E (**8**)

Colorless powder, $[\alpha]_D^{25} - 27.1$ (*c* 0.3, MeOH). For ^{13}C and ^{1}H NMR spectroscopic data, see Tables 1–4. HRMALDIMS: *m*/*z* 1081.5209 $[M+Na]^+$ (calcd for $C_{52}H_{82}NaO_{22}$, 1081.5195), and 1097.4948 $[M+K]^+$ (calcd for $C_{52}H_{82}KO_{22}$, 1097.4935).

Table 5
^{1}H NMR spectroscopic data for the aglycone moieties of **10**–**15** (600 MHz in pyridine-d_5)[a]

Proton	**10**	**11**	**12**	**13**	**14**	**15**
1	0.86, 1.37	0.84, 1.39	0.84, 1.39	0.86, 1.39	0.85, 1.39	0.85, 1.39
2	1.80, 2.18	1.80, 2.15	1.80, 2.15	1.85, 2.15	1.90, 2.19	1.90, 2.19
3	3.28 (1H, *dd*, 12.6/ 4.3 Hz)	3.29 (1H, *dd*, 12.7/ 4.2 Hz)	3.20 (1H, *dd*, 12.8/ 4.5 Hz)	3.32 (1H, *dd*, 12.1/ 4.0 Hz)	3.31 (1H, *dd*, 12.2/ 4.3 Hz)	3.31 (1H, *dd*, 12.2/ 4.3 Hz)
5	0.73	0.79	0.79	0.80	0.73	0.73
6	1.38, 1.57	1.34, 1.56	1.34, 1.56	1.38, 1.60	1.50, 1.55	1.50, 1.55
7	2.06, 2.14	2.03, 2.12	2.03, 2.12	2.02, 2.17	1.35, 1.55	1.35, 1.55
9	1.70	1.70	1.70	1.70	1.70	1.70
11	1.79, 1.88	1.72, 1.89	1.72, 1.89	1.79, 1.87	1.78,1.85	1.78,1.85
12	5.53 (1H, *brs*)	5.48 (1H, *brs*)	5.48 (1H, *brs*)	5.51 (1H, *brs*)	5.42 (1H, *brs*)	5.42 (1H, *brs*)
15	4.22 (1H, *d*, 2.5 Hz)	4.26 (1H, *d*, 2.7 Hz)	4.26 (1H, *d*, 2.7 Hz)	4.21 (1H, *d*, 2.8 Hz)	1.65, 1.90	1.65, 1.90
16	4.51 (1H, *d*, 2.5 Hz)	4.54 (1H, *d*, 2.7 Hz)	4.54 (1H, *d*, 2.7 Hz)	4.53 (1H, *d*, 2.8 Hz)	4.48	4.48
18	3.07	2.78	2.78	3.07	3.07	3.07
19	1.41, 3.06	1.32, 2.88	1.32, 2.88	1.40, 3.05	1.36 3.10	1.36 3.10
21	6.58 (1H, *d*, 10.1 Hz)	2.06, 3.01	2.06, 3.01	6.61 (1H, *d*, 10.2 Hz)	6.61 (1H, *d*, 10.1Hz)	6.61 (1H, *d*, 10.1Hz)
22	4.98 (1H, *d*, 10.1 Hz)	6.17 (1H, *d*, 10.8 Hz)	6.17 (1H, *d*, 10.8 Hz)	6.21 (1H, *d*, 10.2 Hz)	6.21 (1H, *d*, 10.1 Hz)	6.21 (1H, *d*, 10.1 Hz)
23	1.21 (3H, *s*)	1.22 (3H, *s*)	1.22 (3H,*s*)	1.24 (3H, *s*)	1.25 (3H, *s*)	1.25 (3H, *s*)
24	1.09 (3H, *s*)	1.06 (3H, *s*)	1.06 (3H, *s*)	1.09 (3H, *s*)	1.11 (3H, *s*)	1.11 (3H, *s*)
25	0.85 (3H, *s*)	0.85 (3H, *s*)	0.85 (3H, *s*)	0.85 (3H, *s*)	0.83 (3H, *s*)	0.83 (3H, *s*)
26	1.01 (3H, *s*)	1.03 (3H, *s*)	1.03 (3H, *s*)	1.03 (3H, *s*)	0.88 (3H, *s*)	0.88 (3H, *s*)
27	1.89 (3H,*s*)	1.85 (3H, *s*)	1.85 (3H, *s*)	1.87 (3H, *s*)	1.85 (3H, *s*)	1.85 (3H, *s*)
28	3.50, 3.75 (each, 1H, *d*, 10.6 Hz)	3.63, 3.78 (each, 1H, *d*, 10.5 Hz)	3.63, 3.78 (each, 1H, *d*, 10.5 Hz)	3.45, 3.71 (each, 1H, *d*, 10.2 Hz)	3.39, 3.63 (each, 1H, *d*, 10.5 Hz)	3.39, 3.63 (each, 1H, *d*, 10.5 Hz)
29	1.09 (3H, *s*)	1.09 (3H, *s*)	1.09 (3H, *s*)	1.11 (3H, *s*)	1.09 (3H, *s*)	1.09 (3H, *s*)
30	1.32 (3H, *s*)	1.28 (3H, *s*)	1.28 (3H, *s*)	1.32 (3H, *s*)	1.32 (3H, *s*)	1.32 (3H, *s*)
C_{21}	Ang			Ang	Ang	Ang
3	5.82 (1H, *q*, 7.0 Hz)			5.98 (1H, *q*, 7.0 Hz)	5.99 (1H, *q*, 7.2 Hz)	5.99 (1H, *q*, 7.2 Hz)
4	2.02 (3H, *d*, 7.0 Hz)			2.12 (3H, d, 7.0 Hz)	2.11 (3H, d, 7.2 Hz)	2.11 (3H, *d*, 7.2 Hz)
5	1.87 (3H, *s*)			2.04 (3H, s)	2.03 (3H, s)	2.03 (3H, *s*)
C_{22}		Ang	Ang	Ac	Ac	Ac
2		–	–	1.80 (3H, *s*)	1.95 (3H, *s*)	1.95 (3H, *s*)
3		5.84 (1H, *q*, 7.2 Hz)	5.84 (1H, *q*, 7.2 Hz)	–	–	–
4		2.04 (3H, *d*, 7.2 Hz)	2.04 (3H, *d*, 7.2 Hz)	–	–	–
5		1.88 (3H, *s*)	1.88 (3H, *s*)	–	–	–

[a] Assignments were based on COSY, HMQC, and HMBC experiments. Due to severe overlapping in the ^{1}H spectrum only detectable relative *J* (Hz) are reported.

Table 6
^{1}H NMR spectroscopic data for the sugar moieties of **10**–**15** (600 MHz in pyridine-d_5)[a]

Proton	**10**	**11**	**12**	**13**	**14**	**15**
C-**3**	GlcA	GlcA	GlcA	GlcA	GlcA	GlcA
1	4.90 (1H, *d*, 7.8 Hz)	4.99(1H, *d*, 7.8 Hz)	4.98(1H, *d*, 7.8 Hz)	5.00(1H, *d*, 7.6Hz)	4.99(1H, *d*, 7.9 Hz)	4.98(1H, *d*, 7.8 Hz)
2	4.48	4.50	4.48	4.52	4.56	4.53
3	4.39	4.41	4.41	4.40	4.42	4.44
4	4.46	4.48	4.48	4.50	4.45	4.45
5	4.46	4.59	4.59	4.59	4.57	4.57
	Glc	Glc	Glc	Glc	Glc	Glc
1	5.67 (1H, *d*,7.6 Hz)	5.73 (1H, *d*, 7.6 Hz)	5.69 (1H, *d*, 7.6 Hz)	5.75 (1H, *d*, 7.7 Hz)	5.75 (1H, *d*, 7.7 Hz)	5.71 (1H, *d*, 7.8 Hz)
2	4.09	4.09	4.08	4.10	4.12	4.10
3	4.15	4.10	4.10	4.15	4.15	4.15
4	4.13	4.16	4.16	4.18	4.16	3.16
5	3.90	3.95	3.86	3.93	3.93	3.89
6	4.36, 4.48	4.32, 4.48	4.32, 4.48	4.35, 4.47	4.36, 4.52	4.36, 4.52
	Ara	Xyl	Ara	Xyl	Xyl	Ara
1	5.38 (1H, *d*, 7.5 Hz)	5.41 (1H, *d*,7.6 Hz)	5.32 (1H, *d*, 7.4 Hz)	5.43 (1H, *d*, 7.8 Hz)	5.42 (1H, *d*, 7.6 Hz)	5.36 (1H, *d*, 7.6 Hz)
2	4.45	3.98	4.46	4.00	4.00	4.45
3	4.10	4.25	4.12	4.27	4.28	4.13
4	4.23	4.13	4.25	4.14	4.13	4.26
5	3.77, 4.25	3.70, 4.28	3.79, 4.28	3.70, 4.29	3.73, 4.29	3.78, 4.29

[a] Assignments were based on COSY, HMQC, and HMBC experiments. Due to severe overlapping in the ^{1}H spectrum only detectable relative *J* (Hz) are reported.

4.12. Eryngioside F (**9**)

Colorless powder, $[\alpha]_D^{25}$ – 30.1 (*c* 0.3, MeOH). For ^{13}C and ^{1}H NMR spectroscopic data, see Tables 1–4. HRMALDIMS: *m*/*z* 1065.5288 $[M+Na]^+$ (calcd for $C_{52}H_{82}NaO_{21}$, 1065.5246), and 1081.5132 $[M+K]^+$ (calcd for $C_{52}H_{82}KO_{21}$, 1081.4986).

4.13. Eryngioside G (**10**)

Colorless powder, $[\alpha]_D^{25}$ – 56.1 (*c* 0.3, MeOH). For ^{13}C and ^{1}H NMR spectroscopic data, see Tables 1, 2, 5 and 6. HRMALDIMS: *m*/*z* 1081.5211 $[M+Na]^+$ (calcd for $C_{52}H_{82}NaO_{22}$, 1081.5195), and 1097.4965 $[M+K]^+$ (calcd for $C_{52}H_{82}KO_{22}$, 1097.4935).

4.14. Eryngioside H (**11**)

Colorless powder, $[\alpha]_D^{25}$ – 23.1 (*c* 0.3, MeOH). For ^{13}C and ^{1}H NMR spectroscopic data, see Tables 1, 2, 5 and 6. HRMALDIMS: *m*/*z* 1065.5299 $[M+Na]^+$ (calcd for $C_{52}H_{82}NaO_{21}$, 1065.5246), and 1081.5010 $[M+K]^+$ (calcd for $C_{52}H_{82}KO_{21}$, 1081.4986).

4.15. Eryngioside I (**12**)

Colorless powder, $[\alpha]_D^{25}$ – 53.6 (*c* 0.3, MeOH). For ^{13}C and ^{1}H NMR spectroscopic data, see Tables 1, 2, 5 and 6. HRMALDIMS: *m*/*z* 1065.5288 $[M+Na]^+$ (calcd for $C_{52}H_{82}NaO_{21}$, 1065.5246), and 1081.4991 $[M+K]^+$ (calcd for $C_{52}H_{82}KO_{21}$, 1081.4986).

4.16. Eryngioside J (**13**)

Colorless powder, $[\alpha]_D^{25}$ – 34.0 (*c* 0.3, MeOH). For ^{13}C and ^{1}H NMR spectroscopic data, see Tables 1, 2, 5 and 6. HRMALDIMS: *m*/*z* 1123.5355 $[M+Na]^+$ (calcd for $C_{54}H_{84}NaO_{23}$, 1123.5301), and 1139.5099 $[M+K]^+$ (calcd for $C_{54}H_{84}KO_{23}$, 1139.5041).

4.17. Eryngioside K (**14**)

Colorless powder, $[\alpha]_D^{25}$ – 18.5 (*c* 0.3, MeOH). For ^{13}C and ^{1}H NMR spectroscopic data see Tables 1, 2, 5 and 6. HRMALDIMS: *m*/*z* 1107.5370 $[M+Na]^+$ (calcd for $C_{54}H_{84}NaO_{22}$, 1107.5352), and 1123.5101 $[M+K]^+$ (calcd for $C_{54}H_{84}KO_{22}$, 1123.5091).

4.18. Eryngioside L (**15**)

Colorless powder, $[\alpha]_D^{25}$ – 27.8 (*c* 0.3, MeOH). For ^{13}C and ^{1}H NMR spectroscopic data, see Tables 1, 2, 5 and 6. HRMALDIMS: *m*/*z* 1107.5391 $[M+Na]^+$ (calcd for $C_{54}H_{84}NaO_{22}$, 1107.5352), and 1123.5109 $[M+K]^+$ (calcd for $C_{54}H_{84}KO_{22}$, 1123.5091).

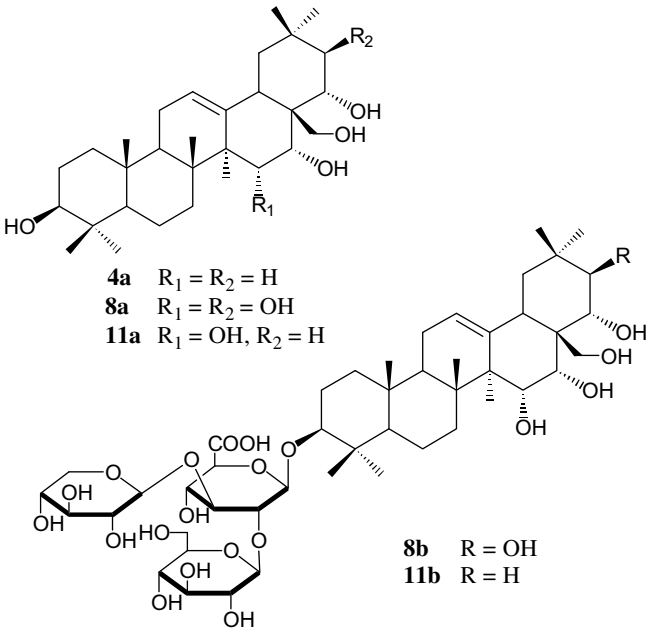
4.19. Alkaline hydrolysis of compounds **1**–**3**, **8**, **11**, and **12**

To each compound **1**–**3**, **8**, **11**, and **12** (3–15.0 mg), respectively, was added 5 ml of 0.8 M NaOH, with mixture heated to 80 °C until reflux began, thus being maintained for 4 h. After cooling, each reaction mixture was neutralized with 1 M HCl and then individually extracted with EtOAc for **1**–**3** or *n*-BuOH for **8**, **11**, and **12** (5 ml × 3). The organic layers were combined and then evaporated to dryness in vacuo. Each residue was subjected to HPLC purification. Compounds **1** and **2** both furnished **17**; **3** afforded **3a**, **3b**, and **3c**; **8** gave **8b**; **11** yielded **11b**; and **12** produced **12b**.

Compound **8b**: colorless powder, ^{1}H NMR (600 MHz, pyridine-d_5): Aglycone δ 0.82 (3H, *s*, H-25), 0.99 (3H, *s*, H-26), 1.00 (3H, *s*, H-24), 1.11 (3H, *s*, H-29), 1.14 (3H, *s*, H-23), 1.25 (3H, *s*, H-30), 1.31 (1H, *m*, H-19a), 1.73 (3H, *s*, H-27), 2.64 (1H, *m*, H-18), 2.85 (1H, *t*, *J* = 12.2 Hz, H-19b), 3.23 (1H, *dd*, *J* = 4.2/12.0 Hz, H-3), 3.63 (1H, *d*, *J* = 10.6 Hz, H-28a), 3.91 (1H, *d*, *J* = 10.6 Hz, H-28b), 4.26 (1H, *d*, *J* = 2.1 Hz, H-15), 4.41 (1H, *d*, *J* = 9.6 Hz, H-22), 4.61 (1H, *d*, *J* = 9.6 Hz, H-21), 4.72 (1H, *d*, *J* = 2.1 Hz, H-16), 5.43 (1H, *brs*, H-12); Sugars δ 4.30 (1H, *m*, GlcA H-3), 4.34 (1H, *m*, GlcA H-2), 4.87 (1H, *d*, *J* = 7.9 Hz, GlcA H-1), 5.24 (1H, *d*, *J* = 7.6 Hz, Xyl H-1), 5.59 (1H, *d*, *J* = 7.6 Hz, Glc H-1). ^{13}C NMR (150 MHz, pyridine-d_5): Aglycone δ 15.5 (*q*, C-25), 16.4 (*q*, C-24), 17.2 (*q*, C-26), 18.5 (*t*, C-6), 19.0 (*q*, C-30), 20.8 (*q*, C-27), 23.7 (*t*, C-11), 26.3 (*t*, C-2), 27.6 (*q*, C-23), 30.1 (*q*, C-29), 36.0 (*s*, C-20), 36.4 (*t*, C-7), 36.7 (*s*, C-10), 38.8 (*t*, C-1), 39.6 (*s*, C-4), 41.1 (*s*, C-8), 41.6 (*d*, C-18), 47.0 (*d*, C-9), 47.2 (*s*, C-14), 47.5 (*t*, C-19), 47.9 (*s*, C-17), 55.4 (*d*, C-5), 66.9 (*t*, C-28), 67.3 (*d*, C-15), 72.1 (*d*, C-16), 76.4 (*d*, C-22), 78.1 (*d*, C-21), 89.8 (*d*, C-3), 124.4 (*d*, C-12), 144.3 (*s*, C-13); Sugars GlcA δ 72.4 (*d*, C-4), 76.7 (*d*, C-5), 78.5 (*d*, C-2), 85.5 (*d*, C-3), 105.0 (*d*, C-1), 172.2 (*s*, C-6), Glc δ 62.9 (*t*, C-6), 72.2 (*d*, C-4), 75.9 (*d*, C-2), 77.7 (*d*, C-5), 78.0[a] (*d*, C-3), 103.1 (*d*, C-1), Xyl δ 67.1 (*t*, C-5), 70.4 (*d*, C-4), 74.9 (*d*, C-2), 78.1[a] (*d*, C-3), 104.6 (*d*, C-1) ([a]The data with the same label may be interchanged).

4.20. Acidic hydrolysis of compound **2**, **4**, **5**, **8b**, **11b**, and **12b**

Each compound **2**, **4**, **5**, **8b**, **11b**, and **12b** (2–7.0 mg), respectively, was added to 1 M HCl in dioxane – H_2O (1:1, 1 ml, v/v) with the whole then heated to 80 °C for 2 h. Following dioxane removal, each solution was extracted with $CHCl_3$–MeOH (7:3, 5 ml × 3). Each extraction was washed with H_2O and concentrated. Each residue was then applied to a small column of silica gel ($CHCl_3$/MeOH). Compound **2** gave **1**; **4** and **5** gave **4a**; **8b** gave **8a**; **11b** and **12b** gave **11a**. The monosaccharide portion was neutralized by passing through an ion-exchange resin (Amberlite MB-3) column, and then concentrated for analysis. Glucuronic acid (t_R 9.6 min), glucose (t_R 11.5 min), xylose (t_R 13.2 min), galactose (t_R 13.3 min), and arabinose (t_R 15.6 min) were determined by co-HPLC with reference sugars (Column B; Detector: refractive index; Mobile phase: deionized H_2O; Flow rate: 0.5 ml/min; Temperature: 78 °C). The monosaccharides of **2** and **4** were shown to be glucose; **5** to be glucose and galactose; **8** and **11** to be glucuronic acid, glucose and xylose; and **12** to be glucuronic acid, glucose and arabinose, respectively. The monosaccharide portion of **2** was purified by HPLC using above HPLC conditions and measured its optical activity (D-glucose, +53.9, *c* 0.5, H_2O).



4a $R_1 = R_2 = H$
8a $R_1 = R_2 = OH$
11a $R_1 = OH, R_2 = H$

8b R = OH
11b R = H

4.21. Isolation of individual sugars from hydrolysate of saponin fractions

Saponin fraction C_2 (100 mg, contained compounds **4**–**7**) was added 1 M HCl in dioxane – H_2O (1:1, 20 ml, v/v) and the whole was raised to 80 °C for 2 h. Following dioxane removal, the solution was extracted with $CHCl_3$–MeOH (7:3, 20 ml × 3), the aqueous portion (monosaccharide portion) neutralized by passing through an ion-exchange resin (Amberlite MB-3) column, and then freeze-dried to give a crude sugar mixture (61 mg). This mixture (30 mg) was separated by HPLC using the same chromatographic conditions described in Section 4.20 above to furnish glucose (22.6 mg) and galactose (2.1 mg). Both purified sugars had their optical rotation values measured (D-glucose, +56.8, *c* 0.5, H_2O; D-galactose, +99.9, *c* 0.5, H_2O). By using the same method, a mixture (100 mg, contained compounds **8**–**15**) of saponin fractions B and C_3 (1:1) was hydrolyzed to afford sugars D-glucuronic acid (18.0 mg, +31.2, *c* 0.5, H_2O), D-glucose (15.0 mg, +55.9, *c* 0.5, H_2O), D-xylose (10.1 mg, +22.9, *c* 0.5, H_2O), and L-arabinose (6.1 mg, −88.6, *c* 0.5, H_2O).

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