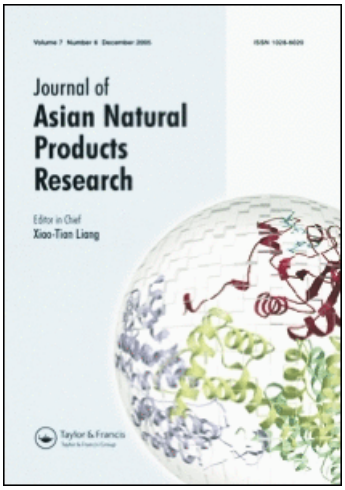

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Triterpene constituents from the seedling of *Aronia melanocarpa*

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Triterpene constituents from the seedling of *Aronia melanocarpa*

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Two new triterpene saponins, 16-*O*-acetyl-21-*O*-angeloyltheasapogenol A 3-*O*-[β-D-galactopyranosyl(1 → 2)][β-D-xylopyranosyl(1 → 2)-α-L-arabinopyranosyl (1 → 3)]-β-D-glucopyranosiduronic acid (**1**) and 16,28-*O*-diacetyl-21-*O*-tigloyltheasapogenol A 3-*O*-[β-D-galactopyranosyl(1 → 2)][β-D-xylopyranosyl(1 → 2)-α-L-arabinopyranosyl (1 → 3)]-β-D-glucopyranosiduronic acid (**2**), together with four known triterpenenes, have been isolated from the dried seedling of *Aronia melanocarpa*, and their structures established by spectroscopic and chemical evidence.

Keywords: *Aronia melanocarpa*; Roseceae; Triterpene saponin; Triterpene

1. Introduction

Aronia melanocarpa Elliot (Roseceae) is a low wood originating in North America. It was introduced as a new kind of economic tree into China in 1991. The fruits of *A. melanocarpa* are rich in anthocyanins and have antioxidant, anti-inflammatory, antimicrobial, hepatoprotective and gastroprotective activities [1]. Previous phytochemical studies on this plant revealed the presence of triterpenes, flavonoids and organic acids [2–4]. In the present investigation, we obtained compounds **1**–**6** from the seedling of the plant, including two new triterpene saponins, 16-*O*-acetyl-21-*O*-angeloyltheasapogenol A 3-*O*-[β-D-galactopyranosyl(1 → 2)][β-D-xylopyranosyl(1 → 2)-α-L-arabinopyranosyl(1 → 3)]-β-D-glucopyranosiduronic acid (**1**) and 16,28-*O*-diacetyl-21-*O*-tigloylltheasapogenol A 3-*O*-[β-D-galactopyranosyl(1 → 2)][β-D-xylopyranosyl(1 → 2)-α-L-arabinopyranosyl (1 → 3)]-β-D-glucopyranosiduronic acid (**2**), along with four known triterpenes, 23-hydroxybetulinic acid (**3**), betulinic acid 3β-caffeate (**4**), 2α-hydroxyoleanolic acid (**5**) and 23 -hydroxybetulinic acid 3β -caffeate (**6**). Compounds **3**–**6** were isolated from the genus for the first time. This paper describes the structural elucidation of compounds **1** and **2**.

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2. Results and discussion

Compound **1** was obtained as an amorphous white powder, mp 221–223°C, $[\alpha]_D^{20}$ − 3.56 (*c* 0.02, MeOH). It showed positive Liebermann–Burchard and Molish reactions. The molecular formula $C_{59}H_{92}O_{27}$ was determined from negative and positive-ion FAB-MS at *m/z* 1231 $[M - H]^-$ and *m/z* 1255 $[M + Na]^+$ and HR-MS measurement. Additionally, fragment ion peaks at *m/z* 1099 $[M - C_5H_9O_4]^-$ and 1069 $[M - C_6H_{11}O_5]^-$were observed in the negative-ion FAB-MS, indicating the potential presence of a terminal pentose and a terminal hexose. The ^{13}C NMR spectrum of **1** showed four anomeric carbon signals at δ 107.0, 104.3, 101.7 and 103.2, which revealed the existence of tetrasaccharide. In the ^1H NMR and ^{13}C NMR spectra, six methyl proton signals (δ 1.42, 1.29, 1.12, 1.06, 0.85 and 0.77, 3H each, all s), an olefinic proton (δ 5.34, 1H, br s), together with six methyl carbon signals (δ 13.5, 16.1, 16.8, 20.1, 27.1 and 30.0) and two olefinic carbons (δ 124.4 and 141.8) suggested that **1** was an oleanene-type triterpene saponin. In the ^1H NMR spectrum**,** the signals at δ 1.94 (3H, s), 2.02 (3H, d, $J = 7.0$ Hz), 5.91 (1H, dq-like) could be assigned to α-Me, β-Me and β-H of angeloyl function [5], which was also supported by the ^{13}C NMR spectrum at δ 168.3, 136.8 and 129.2. Additionally, the signal at δ 170.0 attributed to an ester carbonyl and δ 22.2 assigned to a methyl group indicated the existence of an acetyl group in **1**; this was supported by the ^1H NMR signal at δ 2.50 (3H, s). The above data revealed that compound **1** was similar to the acylated oleanene-type triterpene oligoglycoside [6]. Furthermore, the ^{13}C NMR data of **1** were very close to those of the known compound assamsaponin D [6]. In particular, the signals of A, B, C rings and the saccharides moiety at C-3 were fully in agreement. The differences between **1** and assamsaponin D were as following. The signals of C-15, C-22 in D and E rings of **1** were shifted upfield by 3.7 and 3.8 ppm, respectively, while the signal of C-16 in D ring of **1** was shifted downfield by 3.4 ppm, suggesting that the acetyl group must be at C-16. The stereochemistry of H-16 and H-21 were determined by NOESY spectrum, in which the correlations of H-18 with H-16 and H-22 were shown. In the HMBC spectrum (figure 1), the long-range correlations between H-21 at δ 5.96 and the angeloyl carbonyl carbon (δ 168.3) as well as H-16 at δ 5.92 and the acetyl carbonyl carbon (δ 170.0) confirmed that the angeloyl function and the acetyl group were connected to C-21 and C-16, respectively. Additionally, the correlations between the following protons and carbons: H-22 at δ 4.78 and C-21 at δ 80.4, C-17 at 47.6*;* H-23 at δ 3.76, 4.42 and C-3 at δ 82.9, C-5 at 48.0; H-28 at δ 3.66, 3.92 and C-16 at 71.6, C-22 at δ 70.8 were also observed, further indicating the presence of hydroxyl groups on C-22, 23 and 28.

On acid hydrolysis with sulfuric acid-1,4-dioxane, compound **1** gave D-glucuronic acid, D-galactose, L-arabinose and D-xylose, which were identified by GC [7]. The ^1H NMR and ^{13}C NMR spectra of **1**, which were assigned with the aid of TOCSY, $^1H-^1H$ COSY and HMQC experiments, showed the presence of a β-glucuronic acid moiety [δ 5.07 (1H, d, $J = 7.7$ Hz)], a β-galactopyranosyl moiety [δ 5.87 (1H, d, $J = 7.7$ Hz)], an α-arabinopyranosyl moiety [δ 5.78 (1H, d, $J = 5.8$ Hz)], and a β-xylopyranosyl moiety [δ 5.02 (1H, d, $J = 7.3$ Hz)].The sugar sequences and linkage position of the saccharide chain were confirmed by HMBC (figure 1). In the HMBC spectrum, correlations between the H-1 (δ 5.07) of GluA and C-3 (δ 82.9) of the aglycone, H-1 (δ 5.87) of Gal and C-2 (δ 78.6) of GluA, H-1 (δ 5.78) of Ara and C-3 (δ 84.5) of GluA, and H-1 (δ 5.02) of Xyl and C-2 (δ 82.3) of Ara were easily observed, indicating the sugar sequences of the saccharide chain

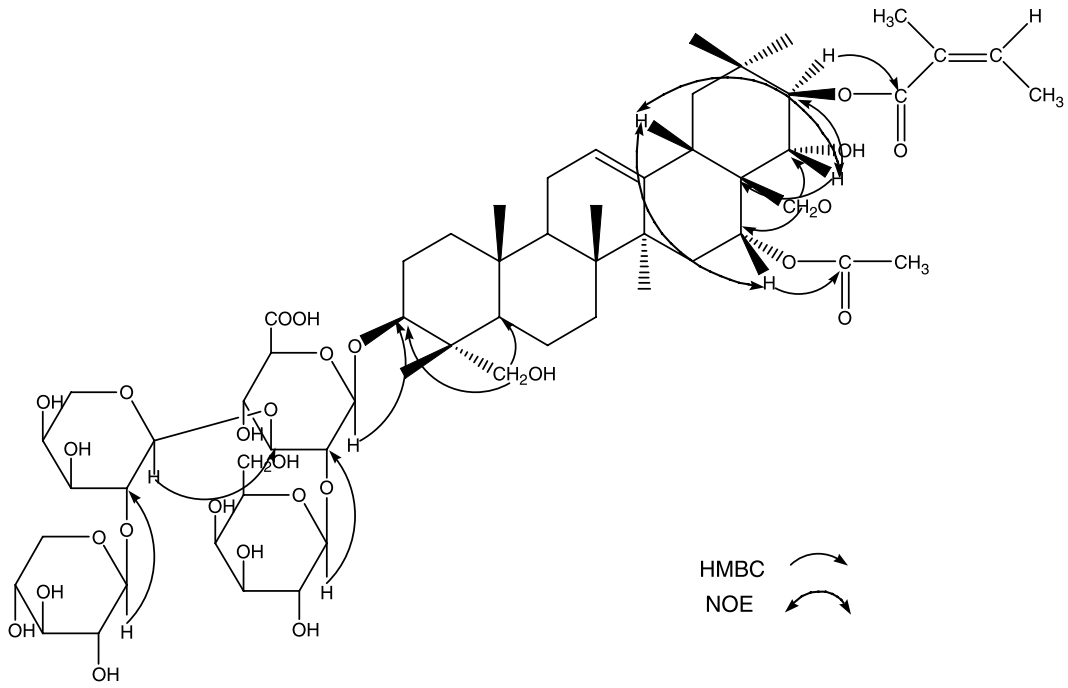

Figure 1. Structure and key HMBC and NOE correlations of **1**.

shown in figure 1. The structure of compound **1** was thus established as 16-*O*-acetyl-21-*O*-angeloyl theasapogenol A 3-*O*-[β-D-galactopyranosyl(1 → 2)][α-L-xylopyranosyl(1 → 2)-β-L-arabinopyranosyl(1 → 3)]-β-D-glucopyranosiduronic acid.

Compound **2** was obtained as an amorphous white powder, mp 219–221°C, $[\alpha]_D^{20}$ + 12.07 (*c* 0.01, MeOH). It showed positive Liebermann–Burchard and Molish reactions. The molecular formula $C_{61}H_{94}O_{28}$ was determined from negative and positive-ion FAB-MS *m/z* 1273 $[M - H]^-$ and *m/z* 1297 $[M + Na]^+$ and HR-MS measurement. The negative-ion FAB-MS showed fragment ion peaks at *m/z* 1141 $[M - C_5H_9O_4]^-$and 1111 $[M - C_6H_{11}O_5]^-$. The ^{1}H NMR and ^{13}C NMR spectra (tables 1 and 2) and the MS

Table 1. ^{1}H NMR and ^{13}C NMR data for the aglycone moieties of compounds **1** and **2** (in pyridine-d_5).

	1			***2***	
Carbon	^{13}C	^1H	*Carbon*	^{13}C	^1H
1	38.7		1	38.7	
2	25.5		2	25.6	
3	82.9	4.12 (1H, m)	3	82.8	4.12 (1H, m)
4	43.5		4	43.5	
5	48.0		5	48.0	
6	18.0		6	18.0	
7	32.7		7	32.7	
8	40.0		8	40.1	
9	47.0		9	47.0	
10	36.6		10	36.6	
11	23.8		11	23.9	
12	124.4	5.34(1H, br s)	12	125.2	5.45 (1H, br s)
13	141.8		13	141.0	
14	41.4		14	41.4	
15	30.9		15	31.0	
16	71.6	5.92 (1H, br s)	16	70.8	5.87 (1H, br s)
17	47.6		17	46.2	
18	39.8	2.94 (1H, dD-like)	18	40.1	2.81 (1H, dD-like)
19	47.2		19	47.1	
20	36.0		20	36.1	
21	80.4	5.96 (1H, d, J = 9.8 Hz)	21	80.4	5.93 (1H, d, J = 9.8 Hz)
22	70.8	4.78 (1H, d, J = 10.1 Hz)	22	69.8	4.45 (1H, d, J = 10.1 Hz)
23	64.8	3.76 (1H, d, J = 10.1 Hz)	23	64.6	3.77 (1H, m)
		4.42 (1H, d, J = 11.0 Hz)			4.42 (1H, m)
24	13.5	1.06 (3H, s)	24	13.5	1.06 (3H, s)
25	16.1	0.85 (3H, s)	25	16.1	0.88 (3H, s)
26	16.8	0.77 (3H, s)	26	16.9	0.94 (3H, s)
27	27.1	1.42 (3H, s)	27	27.1	1.42 (3H, s)
28	64.6	3.66 (1H, d, J = 10.7 Hz)	28	66.0	4.30 (2H, m)
		3.92 (1H, d, J = 10.1 Hz)			
29	30.0	1.12 (3H, s)	29	29.8	1.10 (3H, s)
30	20.1	1.29 (3H, s)	30	19.7	1.26 (3H, s)
16-OAc			16-OAc		
1	170.0		1	169.7	
2	22.2	2.50 (3H, s)	2	22.1	2.52 (3H, s)
21-OAng			28-OAc		
1	168.3		1	170.5	
2	129.2		2	20.7	2.02 (3H, s)
3	136.8	5.91 (1H, dq-like)	21-OTig		
4	16.0	2.02 (3H, d, J = 7.0 Hz)	1	168.1	
5	21.1	1.94 (3H, s)	2	129.6	
			3	136.4	6.96 (1H, dq-like)
			4	14.1	1.59 (3H, d, J = 7.0 Hz)
			5	12.4	1.82 (3H, s)

Table 2. ^{1}H NMR and ^{13}C NMR data for the sugar moieties of compounds **1** and **2** (in pyridine-d_5).

	1		***2***	
Carbon	^{13}C	^{1}H	^{13}C	^{1}H
GlcA				
1	104.3	5.07 (d, 7.7 Hz)	104.2	5.07 (d, 7.4 Hz)
2	78.6	4.61	78.6	4.62
3	84.5	4.27	84.4	4.28
4	71.0	4.46	71.0	4.48
5	77.4	4.43	77.4	4.50
6	171.9		171.9	
Xyl				
1	107.0	5.02 (d, 7.3 Hz)	107.0	5.03 (d, 7.6 Hz)
2	75.9	4.14	75.9	4.16
3	78.3	4.04	78.3	4.04
4	70.8	4.25	70.8	4.28
5	67.5	3.53, 4.43	67.5	3.53, 4.45
Ara				
1	101.7	5.78 (d, 5.8 Hz)	101.7	5.80 (d, 5.8 Hz)
2	82.3	4.57	82.2	4.59
3	73.3	4.38	73.3	4.40
4	68.3	4.34	68.3	4.37
5	66.0	3.73, 4.39	66.0	3.75, 4.40
Gal				
1	103.2	5.87 (d, 7.7 Hz)	103.3	5.87 (d, 7.7 Hz)
2	73.8	4.52	73.8	4.53
3	75.3	4.32	75.3	4.34
4	70.1	4.54	70.1	4.58
5	76.5	4.35	76.5	4.33
6	61.9	4.49 (2H)	61.9	4.50 (2H)

data of **2** were very similar to those of **1.** The significant differences between both compounds were as following. The signals of the angeloyl function in the NMR spectra of **1** were absent in **2** instead of the signals for a tigloyl function at δ 1.59 (3H, d, $J = 7.0$ Hz, β-Me), 1.82 (3H, s, α-Me), 6.96 (1H, dq-like, β-H) [5]. Besides the signals of the acetyl group at C-16, the signals at δ 170.5, 20.7 and δ 2.02 (3H, s) assigned to another acetyl group in **2** were observed. In the HMBC spectrum (figure 2), the long-range correlations between H-21 at δ 5.93 and the tigloyl carbonyl carbon (δ 168.1), as well as H-28 at δ 4.30 and the acetyl carbonyl carbon (δ 170.5) confirmed that the tigloyl function and the acetyl group were connected to C-21 and C-28, respectively. Therefore, the structure of compound **2** was established as 16,28-*O*-diacetyl-21-*O*-tigloyl theasapogenol A 3-*O*-[β-D-galactopyranosyl(1 → 2)][β-D-xylopyranosyl(1 → 2)-α-L-arabinopyranosyl(1 → 3)]-β-D-glucopyranosiduronic acid.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a Perkin–Elmer 241 polarimeter. Melting points were measured on a Yanaco-hot-stage apparatus without correction. NMR spectra were recorded on ECP-500 and Brucker-ARX-300 instruments with TMS as an internal standard. FAB-MS

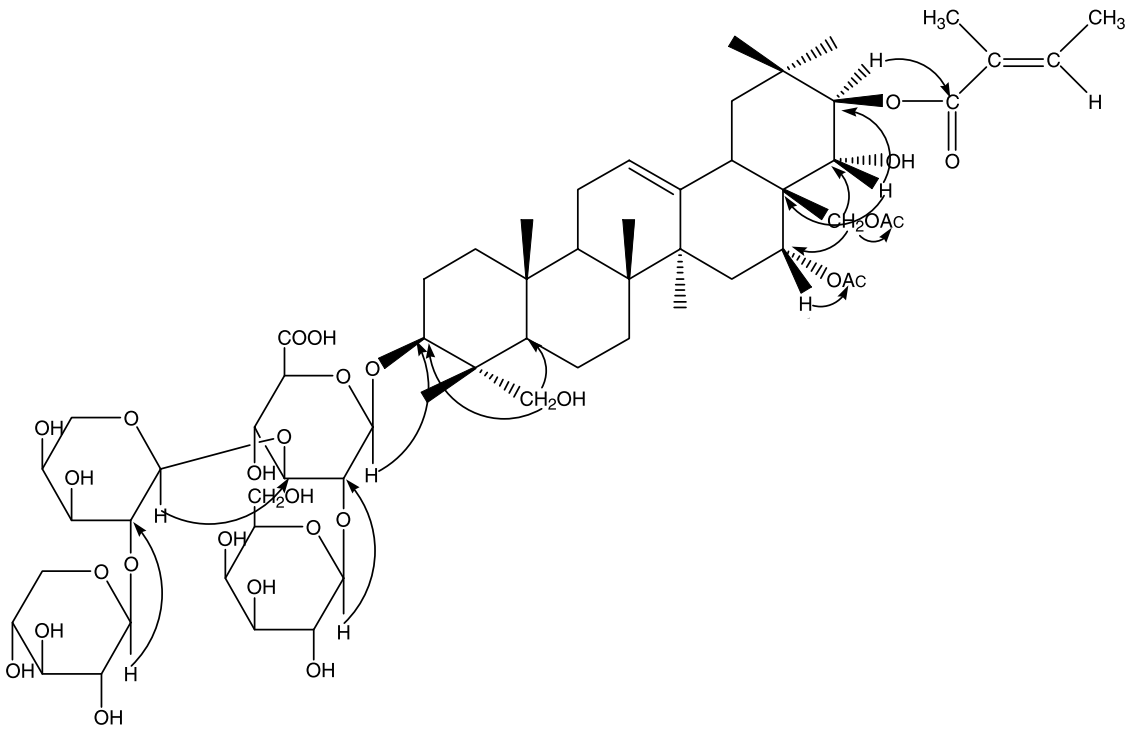

Figure 2. Structure and key HMBC correlations of **2**.

and HR FAB-MS were measured on a JEOL JMS-700 mass spectrometer. HPLC was performed using an *Hp* 1100 system. GC-MS was carried out on a Shimadzu-QP 5050A instrument. Silica gel (200–300 mesh, Qingdao Haiyang) and Sephadex LH-20 (Merck Co.) for column chromatography as well as silica gel GF_{254} (Qingdao Haiyang) for TLC were used.

3.2 Plant material

The seedlings of *Aronia melanocarpa* were collected in Liaoning province, China, and identified by Professor Bai-Zhen Yang, Institute of Applied Ecology, Chinese Academy of Science. A voucher specimen (No. 040403) has been deposited in the Research Department of Natural Medicine, Shenyang Pharmaceutical University.

3.3 Extraction and isolation

Dried seedlings (3 kg) of *A. melanocarpa* were refluxed with 90% EtOH three times. The resultant extract was partitioned with petroleum ether (60–90°C), $CHCl_3$, EtOAc and n-BuOH successively. The n-BuOH extract (120 g) was subjected to column chromatography on silica gel eluted gradiently with $CHCl_3$/MeOH. The fraction (100:35) was chromatographed on Sephadex LH-20 eluted with MeOH and reverse-phase HPLC (CH_3CN/0.8% aq. HOAc 43:57) to obtain compounds **1** (25 mg) and **2** (17 mg). The $CHCl_3$ extract (30 g) was chromatographed on silica-gel column with gradient elution of petroleum ether/Me_2CO. Fraction 7 (100:14–18, 2 g) was rechromatographed on a silica-gel column eluted with petroleum ether/Me_2CO (from 10:1 to 2:1) to give the subfractions, which were further chromatographed on Sephadex LH-20 eluted with $CHCl_3$/MeOH to yield compounds **3** (56 mg), **4** (43 mg) and **5** (40 mg). Fraction 9 (100:22–26, 1.5 g) was chromatographed on a silica-gel column [petroleum ether/EtOAc/Me_2CO (100:8:8–100:16:16)] and with preparative thin-layer chromatography to obtain compound **6** (35 mg).

3.3.1 16-*O*-acetyl-21-*O*-angeloyltheasapogenol A 3-*O*-[β-D-galactopyranosyl(1 → 2)][β-D-xylopyranosyl(1 → 2)-α-L-arabinopyranosyl (1 → 3)]-β-D-glucopyranosiduronic acid (1). Amorphous white powder (MeOH/H_2O), mp 221–223°C, $[\alpha]_D^{20}$ − 3.56 (*c* 0.02, MeOH). ^{1}H NMR and ^{13}C NMR (pyridine-d_5, 500 and 125 MHz, respectively) data: see tables 1 and 2. FAB-MS: *m/z* 1255 $[M + Na]^+$, 1231 $[M - H]^-$, HR FAB-MS: *m/z* 1255.5731 (calcd for $C_{59}H_{92}O_{27}Na$, 1255.5724).

3.3.2 16,28-*O*-diacetyl-21-*O*-tigloyltheasapogenol A 3-*O*-[β-D-galactopyranosyl (1 → 2)][β-D-xylopyranosyl(1 → 2)-α-L-arabinopyranosyl(1 → 3)]-β-D-glucopyranosiduronic acid (2). Amorphous white powder (MeOH/H_2O), mp 219–221°C, $[\alpha]_D^{20}$ + 12.07 (*c* 0.01, MeOH). ^{1}H NMR and ^{13}C NMR (pyridine-d_5, 500 and 125 MHz, respectively) data: see tables 1 and 2. FABMS: *m/z* 1297 $[M + Na]^+$, 1273 $[M - H]^-$, HR FAB-MS: *m/z* 1297.5836 (calcd for $C_{61}H_{94}O_{28}Na$, 1297.5829).

3.3.3 Compounds 3–6. Identified as 23-hydroxybetulinic acid (**3**) [8], betulinic acid 3β-caffeate (**4**) [9], 2α-hydroxyoleanolic acid (**5**) [10] and 23-hydroxybetulinic acid 3β-caffeate (**6**) [9] by comparison of their spectral data with reported data from the literature.

Acknowledgements

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