

Triterpenoid Saponins from Leaves of *Hedera pastuchowii*

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Five new triterpenoid saponins, pastuchoside A (1), B (3), C (5), D (7) and E (9), were isolated from the leaves of *Hedera pastuchowii*. They have oleanolic acid or hederagenin as aglycone. The structures were established by NMR spectroscopy including gs (gradient selected)-COSY, gs-HSQC, gs-HSQC-TOCSY and gs-HMBC experiments, and mass spectrometry (ESI-HR-MS). Heptaoside saponins, compounds 1 and 3, are described for the first time in the genus *Hedera*.

Key words *Hedera pastuchowii*; Araliaceae; triterpenoid saponin; NMR; MS; pastuchoside

In the course of our phytochemical investigation of *Hedera* genus growing in Georgia,¹⁾ we have previously reported the isolation of triterpene saponins from leaves and berries of *Hedera colchica*^{2,3)} and their biological activities *in vitro*. α -Hederin and hederacolchiside A were the more active against *Candida glabra* (LD₁₀₀=6.25 μ g/ml) and dermatophytes sp. (LD₁₀₀=12.5 μ g/ml).⁴⁾ The antileishmanial activity of α -hederin, β -hederin and hederacolchiside A₁ in association with Pentamidine and Amphotericin B showed that subtoxic concentrations of these saponins enhance the efficiency of Pentamidine and Amphotericin B on the promastigote and the amastigote forms of the parasites.^{5,6)}

Hederacolchiside A₁ was strongly cytotoxic against malignant melanoma M₄ Beu (IC₅₀=5 μ M).⁷⁾ This saponin exhibits a preferential cytotoxicity on pigmented melanoma cells and interacts specifically with melanin.⁸⁾ *Hedera helix* saponins were found to have acute and chronic anti-inflammatory effects in rats.⁹⁾

In this paper we describe the isolation and structure determination of five new triterpene saponins, named pastuchosides A (1), B (3), C (5), D (7) and E (9), from the microwave dried leaves of *H. pastuchowii* (Fig. 1). Their structures were established on the basis of various 2D-NMR experiments (COSY, HSQC, HSQC-TOCSY and HMBC). Saponins with seven sugars, compounds 1 and 3, are reported for the first time in the genus *Hedera*. In addition, four known saponins, hederacolchiside F (2),¹⁰⁾ hederacolchiside E (4),¹¹⁾ hederasaponin C (6)¹²⁾ and hederasaponin B (8),¹³⁾ were isolated from this plant.

Pastuchoside B (3) was assigned the molecular formula C₇₁H₁₁₆O₃₄ by electrospray ionisation high-resolution mass spectrometry (ESI-HR-MS) ([M+Na]⁺ quasi-molecular ion at *m/z* 1535.7280; C₇₁H₁₁₆NaO₃₄ requires *m/z* 1535.7246). Acid hydrolysis of 3 yielded arabinose, glucose and rhamnose as sugars identified by TLC and oleanolic acid as a genin moiety. The ¹³C-NMR spectrum exhibited seven anomeric carbons located at δ 105.7, 105.2, 104.3, 102.9, 102.9, 102.0 and 95.8 (Table 1). The resonances of C-3 at δ 90.6 and C-28 at δ 178.1 for pastuchoside B (3) were characteristic of a bisdesmoside. Interglycosidic and sugar-agly-

cone linkages as well as signal and structural assignments of the sugars were deduced on the basis of the following arguments. First of all, the gs (gradient selected)-HSQC¹⁴⁾ spectral analysis displayed the connectivities network for the anomeric atoms. Then, a gs-HSQC-TOCSY¹⁵⁾ experiment showed for each sugar residue the intra-correlated peaks between anomeric proton and sugar carbons (Table 1). To illustrate this strategy, only four carbon signals at δ 79.1 (CH), 77.0 (CH), 73.4 (CH) and 64.1 (CH₂) showed correlations with the anomeric proton at δ 4.49 (*J*=6.0 Hz). The above evidence (number and nature of carbons, chemical shifts and coupling constants) indicated that this sugar was an α -L-arabinopyranosyl residue. Subsequent examination of the HSQC-TOCSY diagram also indicated the occurrence of three glucose and three rhamnose units (Table 1). It should be noted that the HSQC-TOCSY sequence is more helpful than normal TOCSY for structural elucidation of numerous sugar chains, since severe crowding of sugar resonances occurs in the ¹H-NMR spectrum with increasing monosaccha-

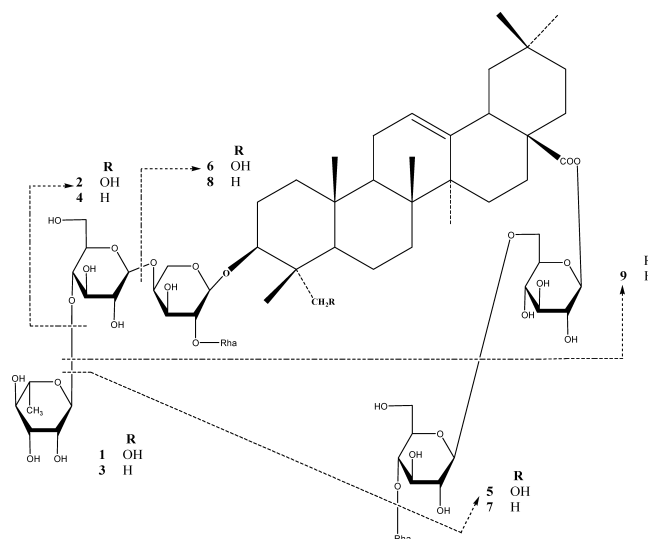


Fig. 1. Triterpenoid Glycosides Isolated from the Leaves of *Hedera pastuchowii*

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ride groups. The sequence and linkage site of the sugar moieties were established using long-range correlation peaks observed in the gs-HMBC¹⁶⁾ diagram (Fig. 2). The HMBC spectrum showed correlations between C-3 (δ 90.6) of oleanolic acid and H-1 (δ 4.49) of the arabinose, between C-2 (δ 77.1) and C-4 (δ 79.1) of the arabinose and H-1 (δ 5.20) of the first rhamnose and H-1 (δ 4.48) of the first glucose, respectively, and between C-4 (δ 79.3) of the first glucose and H-1 (δ 4.87) of the second rhamnose. Similarly, the sequence of the trisaccharide chain at C-28 was indicated by the cross peaks between C-6 (δ 69.4) of the second glucose and H-1 (δ 4.42) of the third glucose, C-4 (δ 79.5) of the third glucose and H-1 (δ 4.86) of the third rhamnose. A cross peak between H-1 (δ 5.38) of the second glucose and the ¹³C resonance of the aglycone carboxy group (δ 178.1) provided definitive evidence for an ester linkage between this trisaccharide chain and the genin. The complementary data from the gs-COSY spectrum was used to obtain a full assignment of the proton resonances (Table 2). Finally, the anomeric configuration for individual monosaccharides

was deduced from the magnitude of the coupling constants of anomeric protons and ¹³C-NMR data. Based upon the above observations, the structure of **3** was elucidated as 3 β -O- $\{\alpha$ -L-rhamnopyranosyl-(1 \rightarrow 2)-[α -L-rhamnopyranosyl-(1 \rightarrow 4)- β -D-glucopyranosyl-(1 \rightarrow 4)]- α -L-arabinopyranosyl}-28-O-[α -L-rhamnopyranosyl-(1 \rightarrow 4)- β -D-glucopyranosyl-(1 \rightarrow 6)- β -D-glucopyranosyl]-oleanolate.

The molecular formula of pastuchoside A (**1**) was deduced to be C₇₁H₁₁₆O₃₅ from ESI-HR-MS (measured mass: 1551.7191; theoretical mass for C₇₁H₁₁₆NaO₃₅: 1551.7195). TLC analysis of acid hydrolysis yielded glucose, rhamnose and arabinose as sugars and hederagenin as an aglycone. The ¹³C-NMR spectrum of the sugar moiety (Table 3) were similar to the data previously determined for pastuchoside B. Sugar arrangement was, consequently, the same for both

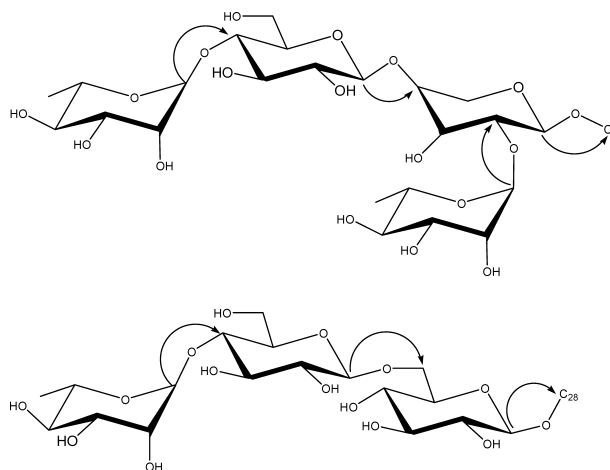


Fig. 2. Selected gs-HMBC Correlations (H \rightarrow C) Showing Interglycosidic Sugar Linkage for Pastuchoside B (**3**)

Table 1. Anomeric Proton Connectivities from gs-HSQC and gs-HSQC-TOCSY for Pastuchoside B (**3**) (δ in ppm, CD₃OD)

¹ H	¹³ C	
	HSQC	HSQC-TOCSY
5.38, d, <i>J</i> =8.1 Hz	95.8	78.2; 78.1; 73.8; 70.9; 69.4
5.20, d, <i>J</i> =1.2 Hz	102.0	73.9; 72.1; 72.1; 70.2; 18.0
4.87, d, <i>J</i> =1.5 Hz	102.9	73.7; 72.4; 72.2; 70.6; 17.9
4.86, d, <i>J</i> =1.5 Hz	102.9	73.7; 72.4; 72.2; 70.6; 17.9
4.49, d, <i>J</i> =6.0 Hz	105.2	79.1; 77.0; 73.4; 64.1
4.48, d, <i>J</i> =7.8 Hz	105.7	79.3; 76.9; 76.5; 75.4; 61.9
4.42, d, <i>J</i> =7.5 Hz	104.3	79.5; 76.8; 76.7; 75.2; 61.9

Table 2. ¹H-NMR Data for Sugar Moieties of Saponins **1** and **3** (CD₃OD)

Sugar on C-3 ^{a)}		1	Sugar on C-28 ^{a)}	3	1	3	
ara	1	4.49	4.47	glc	1	5.38	5.38
	2	3.78	3.77		2	3.35	3.36
	3	3.83	3.83		3	3.43	3.42
	4	3.91	3.93		4	3.41	3.41
	5	4.14; 3.58	4.15; 3.57		5	3.55	3.55
rham	1	5.20	5.19	glc	6	4.11; 3.82	4.12; 3.83
	2	3.93	3.94		1	4.42	4.43
	3	3.72	3.71		2	3.26	3.25
	4	3.40	3.40		3	3.48	3.48
	5	3.89	3.89		4	3.55	3.56
	6	1.20	1.22		5	3.38	3.38
glc	1	4.48	4.46	rham	6	3.85; 3.67	3.85; 3.66
	2	3.34	3.33		1	4.86	4.84
	3	3.50	3.48		2	3.66	3.67
	4	3.59	3.59		3	3.85	3.85
	5	3.32	3.34		4	3.41	3.41
	6	3.85; 3.67	3.86; 3.66		5	4.00	4.02
rham	1	4.87	4.87	6	1.21	1.20	
	2	3.64	3.64				
	3	3.85	3.86				
	4	3.41	3.40				
	5	3.98	3.99				
	6	1.21	1.21				

a) ara = α -L-arabinopyranosyl; glc = β -D-glucopyranosyl; rham = α -L-rhamnopyranosyl.

Table 3. ^{13}C -NMR Data for Sugar Moieties of Saponins **1**, **3**, **5**, **7** and **9** (CD_3OD)

		1		3		5		7		9	
Sugar on C-3 ^{a)}											
ara	1	105.2	ara	1	105.2	ara	1	105.2	ara	1	105.2
	2	77.1		2	77.0		2	77.1		2	77.0
	3	73.4		3	73.4		3	73.3		3	73.4
	4	79.1		4	79.1		4	77.8 ^{b)}		4	77.9 ^{b)}
	5	64.2		5	64.1		5	64.2		5	64.1
rham	1	102.0	rham	1	102.0	rham	1	101.9	rham	1	102.0
	2	72.1		2	72.1		2	72.1		2	72.1
	3	72.1		3	72.1		3	72.1		3	72.1
	4	73.9		4	73.9		4	73.9		4	73.9
	5	70.2		5	70.2		5	70.1		5	70.2
	6	18.0		6	18.0		6	17.9		6	17.9
glc	1	105.7	glc	1	105.7	glc	1	106.1	glc	1	105.9
	2	75.4		2	75.4		2	75.3		2	75.3
	3	76.5		3	76.5		3	77.9 ^{b)}		3	77.8 ^{b)}
	4	79.3		4	79.3		4	71.3		4	71.5
	5	76.9		5	76.9		5	78.0 ^{b)}		5	78.0 ^{b)}
	6	61.9		6	61.9		6	62.6		6	62.7
rham	1	102.9	rham	1	102.9						
	2	72.2		2	72.2						
	3	72.4		3	72.4						
	4	73.8		4	73.7						
	5	70.7		5	70.6						
	6	17.9		6	17.9						
Sugar on C-28 ^{a)}											
glc	1	95.8	glc	1	95.8	glc	1	95.7	glc	1	95.7
	2	73.8		2	73.8		2	73.8		2	73.9
	3	78.2		3	78.2		3	78.1 ^{b)}		3	78.2
	4	70.9		4	70.9		4	70.9		4	71.1
	5	78.1		5	78.1		5	78.1 ^{b)}		5	78.6
	6	69.4		6	69.4		6	69.5		6	62.9
glc	1	104.3	glc	1	104.3	glc	1	104.6	glc	1	104.7
	2	75.3		2	75.3		2	75.1		2	75.1
	3	76.8		3	76.8		3	79.7		3	78.9
	4	79.5		4	79.5		4	71.5		4	71.5
	5	76.7		5	76.7		5	78.0 ^{b)}		5	78.0 ^{b)}
	6	61.9		6	61.9		6	62.7		6	62.7
rham	1	102.9	rham	1	102.9						
	2	72.2		2	72.2						
	3	72.4		3	72.4						
	4	73.7		4	73.7						
	5	70.6		5	70.6						
	6	17.9		6	17.9						

a) ara = α -L-arabinopyranosyl; glc = β -D-glucopyranosyl; rham = α -L-rhamnopyranosyl. b) May be reversed.

saponins. Thus, the structure of pastuchoside A (**1**) was established as 3β -O- $\{\alpha$ -L-rhamnopyranosyl-(1 \rightarrow 2)- $[\alpha$ -L-rhamnopyranosyl-(1 \rightarrow 4)- β -D-glucopyranosyl-(1 \rightarrow 4)]- α -L-arabinopyranosyl $\}$ -28-O- $[\alpha$ -L-rhamnopyranosyl-(1 \rightarrow 4)- β -D-glucopyranosyl-(1 \rightarrow 6)]-hederagenin.

The molecular formula of pastuchoside C (**5**) was found to be $\text{C}_{59}\text{H}_{96}\text{O}_{27}$ by ESI-HR-MS (quasi-molecular $[\text{M}+\text{Na}]^+$ ion m/z 1259.5994; calculated m/z for $\text{C}_{59}\text{H}_{96}\text{NaO}_{27}$: 1259.6037). Acid hydrolysis of **5** gave arabinose, glucose, rhamnose and hederagenin. ^{13}C -NMR chemical shifts of C-3 and C-28 indicated that saponin was a bisdesmoside. Moreover, this spectrum showed five anomeric carbons at δ 106.1, 105.2, 104.6, 101.9 and 95.7. Further analysis of the ^{13}C -NMR data showed for sugar chains linked at C-3 similar chemical shifts with previously reported data of hederacolchiside A' = hederacolchiside A₁.²⁾ It can be concluded that the structure of pastuchoside C was 3β -O- $\{\alpha$ -L-rhamnopyranosyl-(1 \rightarrow 2)- $[\beta$ -D-glucopyranosyl-(1 \rightarrow 4)]- α -L-

arabinopyranosyl $\}$ -28-O- $[\beta$ -D-glucopyranosyl-(1 \rightarrow 6)]- β -D-glucopyranosyl]-hederagenin.

The molecular formula of pastuchoside D (**7**) was established as $\text{C}_{59}\text{H}_{96}\text{O}_{26}$ by ESI-HR-MS (m/z 1243.6041 $[\text{M}+\text{Na}]^+$, theoretical mass for $\text{C}_{59}\text{H}_{96}\text{NaO}_{26}$, 1243.6088). Acid hydrolysis of **7** gave arabinose, glucose and rhamnose as sugars, and oleanolic acid as a genin. ^{13}C -NMR spectrum data showed that **7** was a bisdesmoside having the same sugar chains linked at C-3 and C-28 with pastuchoside C. Thus, the structure of **7** was determined as 3β -O- $\{\alpha$ -L-rhamnopyranosyl-(1 \rightarrow 2)- $[\beta$ -D-glucopyranosyl-(1 \rightarrow 4)]- α -L-arabinopyranosyl $\}$ -28-O- $[\beta$ -D-glucopyranosyl-(1 \rightarrow 6)]- β -D-glucopyranosyl]-oleanolate.

The ESI-HR-MS analysis of pastuchoside E (**9**) gave a quasi-molecular $[\text{M}+\text{Na}]^+$ ion at m/z 1081.5569 in agreement with a molecular formula of $\text{C}_{53}\text{H}_{86}\text{O}_{21}$ ($\text{C}_{53}\text{H}_{86}\text{NaO}_{21}$ requires 1081.5559 as theoretical mass). Acid hydrolysis of **9** yielded arabinose, glucose and rhamnose as sugars, and

Table 4. ^{13}C -NMR Data for Aglycone Moieties of Saponins **1**, **3**, **5**, **7** and **9** (CD_3OD)

C	1	3	5	7	9
1	39.8	40.0	39.8	40.0	40.0
2	26.5	27.0	26.5	27.0	27.1
3	82.3	90.6	82.3	90.5	90.6
4	44.0	40.7	44.0	40.8	40.7
5	48.2	57.2	48.3	57.2	57.2
6	18.8	19.4	18.8	19.4	19.4
7	33.2	33.3	33.2	33.2	33.2
8	40.7	40.3	40.7	40.3	40.3
9	49.2	49.0	49.2	49.1	49.1
10	37.7	37.9	37.6	37.9	38.0
11	24.6	24.6	24.6	24.6	24.6
12	123.8	123.8	123.7	123.8	123.8
13	144.9	144.9	144.9	144.9	144.9
14	43.0	42.9	43.0	43.0	43.0
15	28.9	28.9	28.9	28.9	28.9
16	24.1	24.0	24.1	24.0	24.0
17	48.1	48.1	48.1	48.1	48.1
18	42.5	42.5	42.6	42.8	42.6
19	47.2	47.2	47.2	47.3	47.3
20	31.6	31.6	31.5	31.6	31.6
21	34.9	34.9	34.9	34.9	34.9
22	33.4	33.9	33.3	33.9	34.0
23	65.1	28.6	65.0	28.7	28.7
24	13.2	17.2	13.2	17.2	17.2
25	16.6	16.2	16.6	16.2	16.2
26	18.0	17.7	18.0	17.8	17.9
27	26.4	26.3	26.4	26.3	26.4
28	178.1	178.1	178.1	178.1	178.1
29	33.5	33.5	33.5	33.5	33.6
30	24.1	24.1	24.1	24.1	24.1

oleanolic acid as a genin. The ^{13}C chemical shifts of C-3 and C-28 indicated that saponin **9** was a bisdesmoside. Moreover, ^{13}C shifts of sugars were indicative of similar sugar chains linked at C-3 with both saponins **5** and **7**. It can be concluded that the structure of pastuchoside E was $3\beta\text{-O-}\{\alpha\text{-L-rhamnopyranosyl-(1}\rightarrow\text{2)}\text{-}[\beta\text{-D-glucopyranosyl-(1}\rightarrow\text{4)}\text{-}\alpha\text{-L-arabinopyranosyl}\}\text{-28-O-}\beta\text{-D-glucopyranosyl-oleanolate}$.

Experimental

General Mass spectrometry analyses were performed on a Jeol JMS-700 (Jeol LTD, Akishima, Tokyo, Japan) double focusing mass spectrometer, equipped with an electrospray ionization (ESI) source operating under positive ion mode. Samples diluted in $\text{H}_2\text{O}/\text{CH}_3\text{OH}$ (50/50) were introduced into the ESI interface via a syringe pump (PHD 2000 infusion, Harvard Apparatus, Holliston, MA, U.S.A.) at a $30\ \mu\text{l}\cdot\text{min}^{-1}$ flow rate. A 5-kV acceleration voltage was applied and the elemental composition of ions was checked at a typical resolving power of 8000 (10% valley) using a mixture of PEGs as internal standard. ^1H - and ^{13}C -NMR spectra were recorded on a Bruker DRX-500 spectrometer in CD_3OD solutions. TMS was used as an internal standard in ^1H and ^{13}C measurements. Standard Bruker pulse sequences were used for two-dimensional experiments (gradient selected COSY, HSQC, HSQC-TOCSY and HMBC). Microwave Pr KS-22E (850 W, 2450 MHz) was used to dry the leaves. Melting points were determined on an Electrothermal IA 9300 apparatus. Optical rotations $[\alpha]_D^{25}$ were measured on a Perkin-Elmer model 341 Orot polarimeter. TLC analyses of saponins and sugars were performed on precoated silica gel plates (Kiesegel 60F254, Merck) using the following solvent systems: $\text{CHCl}_3\text{-MeOH-H}_2\text{O}$ (26 : 14 : 3) [system 1]; $n\text{-BuOH-HOAc-H}_2\text{O}$ (4 : 1 : 5) [system 2]; $\text{CHCl}_3\text{-MeOH}$ (20 : 1) [system 3]; $\text{CH}_2\text{Cl}_2\text{-MeOH-H}_2\text{O}$ (50 : 25 : 5) [system 4]. Spots were detected by spraying the plates with phosphoric acid naphthoresorcinol for sugars and H_2SO_4 for saponins followed by heating at $110\ ^\circ\text{C}$.

Extraction and Separation Plant material was collected in the Lagodekhi region of Georgia (September 1999) and dried by microwave oven. A voucher specimen is kept in the Department of Pharmacobotany, Institute of Pharmacology, Tbilisi, Georgia (leaves No. 97799). 500 g of

crushed leaves was extracted with 80% MeOH (2 l). After concentration, the aqueous layer was extracted by $n\text{-BuOH}$, to obtain a crude extract of saponins (100 g), which was subjected to column chromatography on silica gel (0.04–0.063 mm, Merck) and eluted with $\text{CHCl}_3\text{-MeOH-H}_2\text{O}$ (26 : 14 : 3) to afford 3 fractions. Fraction 3, containing the most polar triterpene saponins, was subjected repeatedly to low pressure liquid chromatography (ChromatoSPAC Prep 100, Lichroprep C-18, 15–25 μm) and eluted with $\text{MeOH-H}_2\text{O}$ (20% to 80% of MeOH) to give **1** (15 mg), **2** (180 mg), **3** (15 mg), **4** (150 mg), **5** (impure, 100 mg), **6** (110 mg), **7** (50 mg), mixture of **8** and **9** (250 mg).

A fraction containing a mixture of **8** and **9** was purified by CC on silica gel (0.04–0.063 mm, Merck) and eluted with $\text{CHCl}_3\text{-MeOH-H}_2\text{O}$ (26 : 14 : 3) to yield **8** (100 mg) and **9** (50 mg).

The purification of **5** was carried out on polyamide column (SC 6 0.07 mm, Macherey-Nagel), with 50% of MeOH to obtain 50 mg of **5**.

Acid Hydrolysis of 1, 3, 7 and 9 The saponin (4 mg) was heated with aqueous 10% HCl (3 ml) in a sealed tube at $100\ ^\circ\text{C}$ for 4 h. The sapogenin was extracted with Et_2O and then the aqueous layer was neutralized with N,N -diethylmethylamine (10% in CHCl_3) and dried. The sapogenin and sugars were identified by TLC analyses with authentic samples in systems 3 and 4, respectively.

Alkaline Hydrolysis of 1, 3, 7 and 9 The saponin (5 mg) in 5% aqueous KOH (5 ml) was heated at $100\ ^\circ\text{C}$ in a sealed tube for 90 min. After neutralization with 10% HCl (pH 5) the prosapogenin was extracted with $n\text{-BuOH}$. TLC analyses were performed using systems 1 and 2.

Pastuchoside A (**1**): White powder; $R_f=0.09$ (in system 1). mp $198\ ^\circ\text{C}$; $[\alpha]_D^{20} -16^\circ$ ($c=0.1$, MeOH). ESI-HR-MS m/z : 1551.7191 $[\text{M}+\text{Na}]^+$ (Calcd for $\text{C}_{71}\text{H}_{116}\text{O}_{35}$). ^1H -NMR data for sugar part, see Table 2. ^{13}C -NMR data for sugar and aglycone parts see Tables 3 and 4.

Pastuchoside B (**3**): White powder; $R_f=0.13$ (in system 1). mp $212\ ^\circ\text{C}$; $[\alpha]_D^{20} -40^\circ$ ($c=0.1$, MeOH). ESI-HR-MS m/z : 1535.7280 $[\text{M}+\text{Na}]^+$ (Calcd for $\text{C}_{71}\text{H}_{116}\text{O}_{34}$). ^1H -NMR data for sugar part, see Table 2. ^{13}C -NMR data for sugar and aglycone parts see Tables 3 and 4.

Pastuchoside C (**5**): White powder; $R_f=0.12$ (in system 1). mp $201\ ^\circ\text{C}$; $[\alpha]_D^{20} -18^\circ$ ($c=0.1$, MeOH). ESI-HR-MS m/z : 1259.5994 $[\text{M}+\text{Na}]^+$ (Calcd for $\text{C}_{53}\text{H}_{96}\text{O}_{27}$). ^1H -NMR data for anomeric protons of sugar (CD_3OD) δ : 5.34 (d, $J=7.9$ Hz), 5.16 (br s), 4.47 (d, $J=5.3$ Hz), 4.43 (d, $J=7.6$ Hz), 4.32 (d, $J=7.8$ Hz). ^{13}C -NMR data for sugar and aglycone parts see Tables 3 and 4.

Pastuchoside D (**7**): White powder; $R_f=0.18$ (in system 1). mp $213\ ^\circ\text{C}$; $[\alpha]_D^{20} -30^\circ$ ($c=0.1$, MeOH). ESI-HR-MS m/z : 1243.6041 $[\text{M}+\text{Na}]^+$ (Calcd for $\text{C}_{53}\text{H}_{96}\text{O}_{26}$). ^1H -NMR data for anomeric protons of sugar (CD_3OD) δ : 5.34 (d, $J=7.9$ Hz), 5.16 (br s), 4.46 (d, $J=5.3$ Hz), 4.43 (d, $J=7.6$ Hz), 4.33 (d, $J=7.8$ Hz). ^{13}C -NMR data for sugar and aglycone parts see Tables 3 and 4.

Pastuchoside E (**9**): White powder; $R_f=0.36$ (in system 1). mp $205\ ^\circ\text{C}$; $[\alpha]_D^{20} +25^\circ$ ($c=0.1$, MeOH). ESI-HR-MS m/z : 1081.5569 $[\text{M}+\text{Na}]^+$ (Calcd for $\text{C}_{53}\text{H}_{96}\text{O}_{21}$). ^1H -NMR data for anomeric protons of sugar (CD_3OD) δ : 5.36 (d, $J=7.9$ Hz), 5.16 (br s), 4.48 (d, $J=5.3$ Hz), 4.33 (d, $J=7.8$ Hz). ^{13}C -NMR data for sugar and aglycone parts see Tables 3 and 4.

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