Triterpene glycosides from *Pulsatilla chinensis*

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Four triterpene glycosides were isolated from the roots of *Pulsatilla chinensis* (Bunge) Regel (Ranunculaceae). Two new glycosides, chinensiosides A (**1a**) and B (**2**), were identified as 3-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]-28-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]-3β,23-dihydroxylup-20(29)-en-28-oic acid and 3-*O*-{α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-α-L-arabinopyrano-syl}-28-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]-3β,23-dihydroxylup-20(29)-en-28-oic acid. The other two glycosides were identified as previously known hederasaponin C (**3**) from *Hedera helix* and glycoside III (**4**) from *Pulsatilla cernua*.

Key words: *Pulsatilla chinensis,* Ranunculaceae, triterpene glycosides, glycosides of 23-hydroxybetulinic acid, chinensiosides A and B, ^{1}H and ^{13}C NMR spectra, MALDI-TOF mass spectra.
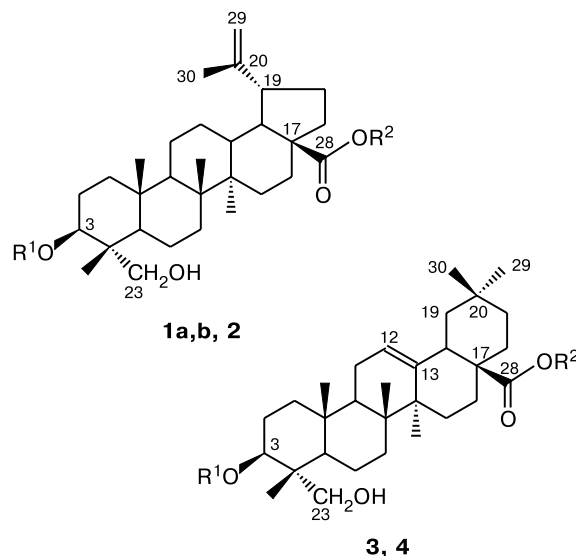
Chinese anemone *Pulsatilla chinensis* (Bunge) Regel (Ranunculaceae) is one of the most well-known means of alternative Oriental medicine. The roots of this plant are used to treat amebic dysentery, malaria, vaginal trichomoniasis, and bacterial infections. During previous studies of *P. chinensis*, a new lupanic triterpenoid, *viz.*, pulsatillic acid, 23-hydroxybetulinic acid, and three new lupane-type triterpene glycosides, *viz.*, pulsatillosides A, B, and C, were isolated from plants that grow in China,[1,2] and two new and several known oleanane-type glycosides and two known lignanes were isolated from plants that grow in Japan.[3] The glycoside fraction of the methanol extract from the roots of *P. chinensis* exhibits a cytotoxic activity against the HL-60-cells of human leukemia.[3]

To continue the studies of triterpene metabolites of the *Pulsatilla Mill.* plants growing in the Russian Far East,[4—6] we studied the triterpene composition of the roots of *P. chinensis*.

Results and Discussion

Usual techniques including ethanol extraction and chromatographic separation on silica gel columns using gradient elution with CHCl$_3$—EtOH and CHCl$_3$—MeOH solvent mixtures resulted in the isolation of two glycoside fractions A and B, which were first considered to be individual compounds. Examination of the ^{13}C NMR spectra showed that each fraction is a mixture of two glycosides that are difficult to separate. The ultimate isolation and purification of individual compounds were performed by

HPLC using the RP-18 reversed phase, aqueous MeCN as the eluent, and refractometric detection. As a result, a new glycoside called chinensioside A (**1a**) and known hederasaponin C [7] (**3**) were isolated from fraction A and a new glycoside called chinensioside B (**2**) and previously known[8] glycoside III (**4**) were isolated from fraction B. A brief communication on the structures of these compounds has been published.[9]

1a,b, 2

3, 4

R^1 = α-L-Rha*p*-(1→2)-α-L-Ara*p*→ (**1a,b, 3**),
R^2 = α-L-Rha*p*-(1→4)-β-D-Glc*p*-(1→6)-β-D-Glc*p*→ (**1a, 3**),
R^2 = H (**1b**);
R^1 = α-L-Rha*p*-(1→2)-[β-D-Glc*p*-(1→4)]-α-L-Ara*p*→,
R^2 = α-L-Rha*p*-(1→4)-β-D-Glc*p*-(1→6)-β-D-Glc*p*→ (**2, 4**)

Compound **1a** is a white amorphous powder whose IR spectrum exhibits absorption bands due to an exo methylene group (1643, 881 cm^{-1}), an ester carbonyl group (1732 cm^{-1}), and hydroxy groups (3419 cm^{-1}). Total acid hydrolysis of compound **1a** yielded 23-hydroxybetulinic acid as the aglycon; this product was identified by comparing the IR, ^{1}H NMR, and ^{13}C NMR spectra with the corresponding data for an authentic sample.[1] Using a carbohydrate analyzer, the aqueous fraction of the hydrolysate produced from **1a** was found to contain arabinose, rhamnose, and glucose in 1 : 2 : 2 ratio. The MALDI-TOF(+) mass spectrum (detection of positive ions) of compound **1a** exhibts a pseudo-molecular-ion peak [M + Na]$^+$ with *m/z* 1243.6071, which is in good agreement with the molecular formula $C_{59}H_{96}O_{26}$ + Na (calculated, 1243.6083). In addition, this mass spectrum contains a clear peak with *m/z* 493 corresponding to the [23-hydroxybetulinic acid + Na − 2 H]$^+$ ion.

The ^{1}H NMR spectrum of the aglycon moiety of compound **1a** exhibits singlets at δ_H 0.89, 0.96, 1.06, 1.16, and 1.69, due to the protons of five tertiary methyl groups, and at δ_H 4.67 (C(29)H$_B$) and 4.84 (C(29)H$_A$) for two olefinic protons of the exo mehylene group and also a multiplet at δ_H 3.39 for the methine proton (C(29)H). The chemical shifts of the olefinic protons, the methine proton, and the (C(30)H$_3$) protons completely coincide with the corresponding values in the spectra of known lupane-type glycosides.[1,2]

The ^{13}C NMR spectrum of **1a** confirmed the presence of five C atoms of the tertiary methyl groups (δ_C 13.32, 14.58, 16.12, 16.61, and 19.12), two C atoms of the exo methylene group (δ_C 109.70 and 150.50), the methine-group C atom (δ_C 49.55), and the ester carbonyl group C atom (δ_C 174.60). In the ^{13}C NMR spectrum of **1** recorded without proton decoupling (gated decoupling, GD spectrum), the C signal at δ_C 109.70 is a triplet with the spin-spin coupling constant $J_{C,H}$ = 151 Hz; according to

published data,[10] this signal corresponds to the olefinic C(29) atom.

The chemical shift of the signal of the aglycon C(3) atom in the ^{13}C NMR spectrum of **1a** is shifted downfield (+7.25 ppm) relative to a similar signal in the spectrum of C(3)-unsubstituted 23-hydroxybetulinic acid. The substantial β- (−2.23 ppm) and γ-effects (−3.24 ppm) of glycosylation are also observed on the aglycon C(2) and C(23) atoms; these effects are comparable in magnitude with and have the same size as those in the spectrum of pulsatilloside A.[1] The signal of the C(28) atom in this spectrum is shifted upfield (−4.39 ppm) with respect to the corresponding signal for the free acid. A similar effect of glycosylation of a carboxy group has been found previously for pulsatilloside C.[2] The presence of a carbohydrate chain at C(28) is confirmed additionally by the presence of an absorption band for the ester group (1732 cm^{-1}) in the IR spectrum of **1a**. On the basis of these results, we concluded that compound **1a** is the 3,28-*O*-bis(glycoside) of 23-hydroxybetulinic acid.

The ^{1}H NMR spectrum of the carbohydrate fragment of compound **1a** exhibits doublets for five anomeric protons with δ_H 4.98, 5.13, 5.88, 6.28, and 6.37. In addition, in the high field, proton signals occur at δ_H 1.64 and 1.72. Undoubtedly, these belong to the secondary methyl groups of two rhamnose residues (Table 1). Correspondingly, the ^{13}C NMR spectrum of **1a** contains signals for the five anomeric C atoms at δ_C 94.97, 101.31, 102.40, 103.73, and 104.72 ppm and a combined signal at δ_C 18.15 corresponding to two C atoms of the secondary methyl groups in the two rhamnose residues (Table 2); this confirms the presence of five monosaccharide residues in **1a**.

By analogy with other triterpene glycosides of the plant origin, one can suggest that the arabinose and rhamnose residues in **1a** belong to the L-series, while glucose residues correspond to the D-series.

Table 1. ^{1}H NMR data for anomeric and methyl protons of the carbohydrate fragments of compounds **1a,b** and **2** (in pyridine-d$_5$)

Proton	Monosaccharide residue	δ_H (*J*/Hz)		
		1a	**1b**	**2**
	at the aglycon C(3) atom			
C(1)H	Ara*p*	5.13 (d, *J* = 6.2)	5.12 (d, *J* = 6.2)	5.00 (d, *J* = 6.2)
C(1)H	Rha*p* (1→2)	6.28 (d, *J* = 1.2)	6.25 (d, *J* = 1.2)	6.27 (d, *J* = 1.5)
C(6)H$_3$	Rha*p* (1→2)	1.64 (d, *J* = 6.2)	1.64 (d, *J* = 6.2)	1.65 (d, *J* = 6.3)
C(1)H	Glc*p* (1→4)	—	—	5.13 (d, *J* = 7.8)
	at the aglycon C(28) atom			
C(1)H	Glc*p* (intern.)	6.37 (d, *J* = 8.1)	—	6.36 (d, *J* = 8.1)
C(1)H	Glc*p* (1→6)	4.98 (d, *J* = 7.8)	—	4.95 (d, *J* = 7.5)
C(1)H	Rha*p* (1→4)	5.88 (d, *J* = 1.0)	—	5.87 (d, *J* = 1.5)
C(6)H$_3$	Rha*p* (1→4)	1.72 (d, *J* = 6.1)	—	1.71 (d, *J* = 6.3)

Table 2. ^{13}C NMR data for carbohydrate parts (δ_C and direct spin-spin coupling constants ($J_{C(1),H(1)}$/Hz) for compounds **1** and **2** (in pyridine-d$_5$)

Atom C	1a		2	
	δ_C	J	δ_C	J
Ara*p*				
C(1)	103.73	160.1	103.90	160.3
C(2)	75.63		76.12	
C(3)	73.92		74.26	
C(4)	68.67		79.66	
C(5)	63.73		63.69	
Rha*p* (1→2)				
C(1)	101.31	171.1	101.36	172.6
C(2)	71.97		71.89	
C(3)	72.19		72.21	
C(4)	73.80		73.85	
C(5)	69.41		69.36	
C(6)H$_3$	18.15		18.25	
Glc*p* (1→4)				
C(1)			106.24	159.3
C(2)			75.16	
C(3)			78.23	
C(4)			71.09	
C(5)			78.40	
C(6)			62.31	
Glc*p* (intern.)				
C(1)	94.97	163.7	94.99	164.9
C(2)	73.80		73.75	
C(3)	78.35		78.40	
C(4)	70.68		70.76	
C(5)	77.71		77.69	
C(6)	69.22		69.26	
Glc*p* (1→6)				
C(1)	104.72	159.1	104.77	159.3
C(2)	74.94		74.96	
C(3)	76.18		76.22	
C(4)	78.19		78.23	
C(5)	76.79		76.83	
C(6)	61.11		61.11	
Rha*p* (1→4)				
C(1)	102.40	168.3	102.44	168.6
C(2)	72.19		72.15	
C(3)	72.41		72.45	
C(4)	73.63		73.67	
C(5)	70.01		70.04	
C(6)H$_3$	18.15		18.15	

The direct spin-spin coupling constants $J_{C(1),H(1)}$ of the doublet signals of the anomeric C atoms of the monosaccharide residues in the GD spectrum of **1a** (see Table 2) attest unambiguously, according to published data,[11−13] to the α-configuration of the anomeric centers of arabinopyranose and rhamnopyranoses and to the β-configuration of the anomeric centers of glucopyranoses.

Harsh alkaline hydrolysis of **1a** afforded progenin **1b**, whose ^{1}H NMR spectrum exhibits (in the aglycon part) signals at δ_H 0.88, 1.02, 1.06, 1.07, and 1.77 due to the

protons of five tertiary methyl groups and signals at δ_H 4.93 (C(29)H$_B$) and 4.84 (C(29)H$_A$) for two protons of the exo methylene group and contain a multiplet at δ_H 3.55 for the methine proton of the C(19)H group.

The carbohydrate part of the ^{1}H NMR spectrum of **1b** was found to contain doublets for the anomeric protons of the arabinopyranose residues (δ_H 5.12) and rhamno-pyranose (δ_H 6.25) and a signal for the protons of the secondary methyl group of the rhamnopyranosyl residues at δ_H 1.64 (see Table 1). The data obtained for progenin **1b** proved that the initial glycoside contains a disaccha-ride fragment consisting of the arabinopyranose and rhamnopyranose residues.

The ^{13}C NMR spectrum confirmed that compound **1b** contains five C atoms of tertiary methyl groups (δ_C 13.53, 14.64, 16.19, 16.68, and 19.19), the C(29) and C(20) atoms of the exo methylene group (δ_C 109.73 and 151.02), the C(19) atom of the methine group (δ_C 49.50), and the C(28) atom of the free carboxy group (δ_C 178.62). In the GD spectrum of **1b**, the signal at δ_C 109.73 for C(29) is a triplet with the spin-spin coupling constant $J_{C,H}$ = 157 Hz.

The signal of the aglycon C(3) atom in the ^{13}C NMR spectrum of **1b** is shifted downfield (+7.32 ppm) rela-tive to the corresponding signal of C(3)-unsubstituted 23-hydroxybetulinic acid. Upfield β-shifts were noted for the C(2) (−2.15 ppm) and C(4) (−0.37 ppm) atoms and γ-shifts were found for C(5) (−1.28 ppm) and C(23) (−2.67 ppm) atoms. These data indicate that the disac-charide fragment in **1b** is localized at the aglycon C(3) atom. Comparison of the carbohydrate region of the ^{13}C NMR spectrum of **1b** with the published data[14] for hederasaponin C showed them to be fully identical.

In the GD spectrum of **1b**, the direct spin-spin cou-pling constants $J_{C(1),H(1)}$ are 172 Hz for the rhamno-pyranosyl residue and 163 Hz for the arabinopyranosyl residue, which proves unambiguously the axial and equa-torial positions of the oxygen atoms, respectively.

The downfield shift of the signal for C(2) of the arabinopyranosyl residue (+3.44 ppm) relative to the cor-responding signal of methyl α-L-arabinopyranoside[15] con-firms the presence of the 1→2 bond between the rhamno-pyranosyl and arabinopyranosyl residues.

It follows from the obtained data that progenin **1b** is 3-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyrano-syl]-3β,23-dihydroxylup-20(29)-en-28-oic acid.

The remaining three signals of anomeric protons and anomeric C atoms in the ^{1}H and ^{13}C NMR spectra of glycoside **1a**, which belong to the α-L-rhamnopyranose residue and two β-D-glucopyranose residues, point to the presence of an oligosaccharide chain at the aglycon C(28) atom. Comparison of the spectral data for this chain with the data for the α-L-Rha*p*-(1→4)-β-D-Glc*p*-(1→6)-β-D-Glc*p* fragment in pulsatilloside C [2] demonstrated that they are fully identical. The MALDI-TOF(+) mass spec-trum of **1a** exhibits clear peaks with *m/z* 965 and 773,

corresponding to the [M + Na − Rha − Ara]+ and [M + Na − Rha − 2 Glc]+ ions. On the basis of the results obtained, glycoside **1a**, called chinensioside A, was identified as 3-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]-28-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]-3β,23-dihydroxylup-20(29)-en-28-oic acid.

Compound **2** was prepared as a white amorphous powder. The IR spectrum of compound **2** exhibits the same absorption bands as the IR spectrum of glycoside **1a**. The MALDI-TOF(+) spectrum of compound **2** contains a pseudo-molecular-ion peak [M + Na]+ with *m/z* 1405.6643, which is consistent with the molecular formula $C_{65}H_{106}O_{31}$ + Na (calculated, 1405.6612). The peak with *m/z* 493 occurring in this spectrum implies the presence of the [23-hydroxybetulinic acid + Na − 2 H]+ ion in **2**. Comparison of the ^{1}H and ^{13}C NMR spectra of compounds **2** and **1a** revealed the full identity of their aglycon fragments. These results lead to the conclusion that the aglycon in compound **2**, as in glycoside **1a**, is C(3)- and C(28)-glycosylated 23-hydroxybetulinic acid.

The ^{1}H NMR spectrum of glycoside **2** contain six doublets for anomeric protons at δ_H 4.95, 5.00, 5.13, 5.87, 6.27, and 6.36 and signals for the protons of the secondary methyl groups of two rhamnose residues with δ_H 1.65 and 1.71 (see Table 1). Correspondingly, the ^{13}C NMR spectrum of **2** contains signals for six anomeric C atoms and signals for two C atoms of the secondary methyl groups of two rhamnose residues (see Table 2). Comparative examination of the ^{1}H and ^{13}C NMR spectra of compounds **2** and **1a** showed that these compounds contain an identical trisaccharide fragment at the aglycon C(28) atom and that **2**, unlike **1a**, contains an additional monosaccharide residue (glucose). In the ^{13}C NMR spectrum of compound **2**, the signal for the arabinose C(4) atom occurs in a lower field than the corresponding arabinose signal in the spectrum of **1a**. This means that the additional monosaccharide residue is attached to the arabinose C(4) atom.

The fact that the proton chemical shifts and the corresponding spin-spin coupling constants as well as the carbon chemical shifts in the NMR spectra of **2** coincide almost completely with analogous data for the α-L-Rha*p*-(1→2)-β-D-Glc*p*-(1→4)-α-L-Ara*p* fragment in pulsatille saponin D [16] confirmed the structure of the branched trisaccharide chain at the aglycon C(3) atom of compound **2**.

The direct spin-spin coupling constants $J_{C(1),H(1)}$ for the doublets of the anomeric C atoms of monosaccharide residues in the GD spectrum of **2** (see Table 2) are in good agreement with the analogous data reported in the literature[11−13] and point to the α-configuration of the anomeric centers of arabinopyranose and rhamnopyranose and to the β-configuration of the anomeric centers of glucopyranose. By analogy with **1a**, the arabinose and rhamnose

residues were attributed to the L-series and the glucose residues were ascribed to the D-series.

The MALDI-TOF(+) mass spectrum of compound **2**, like the mass spectrum of **1a**, exhibits a peak with *m/z* 935 for the [M + Na − Rha − 2 Glc]+ ion, resulting from elimination of the trisaccharide fragment. In addition, this spectrum contains peaks with *m/z* 1259 and 1244 for the [M + Na − Rha]+ and [M + Na − Glc]+ fragments, resulting from elimination of rhamnose or glucose residues. Based on examination of the results, the structure of compound **2**, called chinensioside B, was identified as 3-*O*-{α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyl}-28-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]-3β,23-dihydroxylup-20(29)-en-28-oic acid.

The MALDI-TOF(+) mass spectrum of compound **3** exhibits a peak for the pseudo-molecular ion [M + Na]+ with *m/z* 1243.6097, which corresponds to the molecular formula $C_{59}H_{96}O_{26}$ + Na (calculated, 1243.6083). Comparison of the spectroscopic data for glycosides **3** and **1a** revealed differences in the triterpenoid parts of these molecules and full identity of the carbohydrate parts. The ^{1}H and ^{13}C NMR spectra of compound **3** are in good agreement with published data[7,14] for hederasaponin C from the *Hedera helix* plant. Thus, glycoside **3** was identified as hederasaponin C.

The MALDI-TOF(+) mass spectrum of compound **4** contains a pseudo-molecular-ion peak, [M + Na]+ with *m/z* 1405.6637, consistent with the formula $C_{65}H_{106}O_{31}$ + Na (calculated, 1405.6612). Analysis of the spectroscopic data of glycosides **4** and **2** showed that the compounds being compared have identical carbohydrate chains and differ only in the structure. The ^{1}H and ^{13}C NMR spectra of compound **4** are in good agreement with published data[8] for glycoside III, isolated previously from *P. cernua*. As a result, compound **4** was identified as glycoside III.

Since the previous publication[8] reports only partly the spectroscopic data for glycoside III, we considered it pertinent to report the NMR spectra of compound **4** in the Experimental.

Thus, compounds **1a,b** and **2** can be considered to be new glycosides of 23-hydroxybetulinic acid. Compounds **1a**, **2—4**, which were not found previously in *P. chinensis*, were found to be the major glycosides in the roots of this plant.

Experimental

^{1}H and ^{13}C NMR spectra were recorded on Bruker WM-250, Bruker DPX-300, and Bruker DRX-500 spectrometers for solutions of compounds in pyridine-d_5 with Me_4Si as the internal standard; the chemical shifts are given in the δ-scale (ppm) and spin-spin coupling constants are in Hertz. IR spectra (KBr) were recorded on a Bruker 240 spectrometer; MALDI-TOF(+) mass spectra were run on a Bruker Biflex-III mass spectrometer

30.59 (C(15)), 31.96 (C(16)), 56.69 (C(17)), 47.54 (C(18)), 49.55 (C(19)), 150.50 (C(20)), 29.88 (C(21)), 36.73 (C(22)), 64.84 (C(23)), 13.32 (C(24)), 16.61 (C(25)), 16.12 (C(26)), 14.58 (C(27)), 174.60 (C(28)), 109.70 (C(29)), 19.12 (C(30)); the spectrum of the carbohydrate part is given in Table 2.

Acid hydrolysis of glycoside 1a. Glycoside **1a** (20 mg) in 3 mL of MeOH was treated with 2 *M* HCl (10 mL) for 2 h at 100 °C. The hydrolysate was diluted with water and extracted with AcOEt, and the extract was washed with water, dried, and concentrated. The residue was crystallized from MeOH to give 6 mg of a compound. By comparing the IR and NMR spectra with published data,[1] the product was identified as 23-hydroxybetulinic acid. Using a carbohydrate analyzer, arabinose, rhamnose, and glucose in 1 : 2 : 2 ratio were detected in the aqueous fraction.

Alkaline hydrolysis of glycoside 1a. Glycoside **1a** (10 mg) was hydrolyzed with a 5% solution of KOH (10 mL) at ~20 °C for 24 h. The hydrolysate was neutralized by the KU-2 cation exchanger in the H^{+} form to pH 5, the resin was separated on a filter, the solution was extracted with *n*-butanol, the extract was concentrated to dryness, and the residue was chromatographed on a column with silica gel using the $CHCl_3$—MeOH solvent system (40 : 5→40 : 35) to give progenin **1b** (3.7 g).

Progenin 1b. ^{1}H NMR, aglycon part: 0.88, 1.02, 1.06, 1.07, 1.77 (all s, each 3 H, C(24)H_3, C(25)H_3, C(26)H_3, C(27)H_3, C(30)H_3); 3.55 (m, 1 H, C(19)H), 4.93 and 4.75 (both br.s, each 1 H, C(29)H_B, C(29)H_A); the spectrum of the carbohydrate part is presented in Table 1. ^{13}C NMR, aglycon part: 39.01 (C(1)), 25.85 (C(2)), 80.93 C((3)), 42.60 (C(4)), 47.67 (C(5)), 17.93 (C(6)), 34.19 (C(7)), 40.84 (C(8)), 50.73 (C(9)), 36.79 (C(10)), 21.01 (C(11)), 26.18 (C(12)), 38.35 (C(13)), 43.40 (C(14)), 30.96 (C(15)), 32.69 (C(16)), 56.37 (C(17)), 47.54 (C(18)), 49.50 (C(19)), 151.02 (C(20)), 30.47 (C(21)), 37.32 (C(22)), 65.41 (C(23)), 13.53 (C(24)), 16.68 (C(25)), 16.19 (C(26)), 14.64 (C(27)), 178.62 (C(28)), 109.73 (C(29)), 19.19 (C(30)); carbohydrate part: Ara: 104.14 (C(1)), 75.64 (C(2)), 74.48 C((3)), 69.10 (C(4)), 63.71 (C(5)); Rha: 101.48 (C(1)), 72.16 (C(2)), 72.32 C((3)), 73.92 (C(4)), 69.50 (C(5)), 18.33 (C(6)).

Chinensioside B (2), amorphous; $C_{65}H_{106}O_{31}$; $[\alpha]_D$ −9.0 (*c* 0.40, MeOH). IR (KBr), ν/cm[−1]: 3418 and 1053 (OH), 2932 (CH), 1745 (C(28)=O), 1693, 1646 and 883 (C(20)=C(29)H_2), 1455, 1381. MALDI-TOF(+) MS, *m/z* (I_{rel} (%)): 1405.6 [M + Na][+] (100), 1259 [M + Na − Rha][+] (8.7), 1244 [M + Na − Glc][+] (4.7), 1097 [M + Na − Rha − Glc][+] (6), 935 [M + Na − Rha − 2 Glc][+] (43), 493 [23-hydroxybetulinic acid + Na − 2 H][+] (57). ^{1}H NMR, aglycon part: 0.88, 0.97, 1.05, 1.16, 1.70 (all s, each 3 H, C(24)H_3, C(25)H_3, C(26)H_3, C(27)H_3, C(30)H_3); 3.40 (m, 1 H, C(19)H); 4.69 and 4.85 (both br.s, each 1 H, C(29)H_B, C(29)H_A); the spectrum of the carbohydrate part is presented in Table 1. ^{13}C NMR, aglycon part: 39.02 (C(1)), 25.81 (C(2)), 80.88 C((3)), 42.54 (C(4)), 47.60 (C(5)), 17.86 (C(6)), 34.05 (C(7)), 40.91 (C(8)), 50.74 (C(9)), 36.61 (C(10)), 20.92 (C(11)), 26.12 (C(12)), 38.12 (C(13)), 43.31 (C(14)), 30.62 (C(15)), 31.98 (C(16)), 56.72 (C(17)), 47.54 (C(18)), 49.59 (C(19)), 150.56 (C(20)), 29.92 (C(21)), 36.75 (C(22)), 64.73 (C(23)), 13.41 (C(24)), 16.65 (C(25)), 16.14 (C(26)), 14.60 (C(27)), 174.65 (C(28)), 109.71 (C(29)), 19.15 (C(30)); the spectrum of the carbohydrate part is presented in Table 2.

Glycoside 3, amorphous; $C_{59}H_{96}O_{26}$; $[\alpha]_D$ −15.1 (*c* 0.49, MeOH). IR (KBr), ν/cm[−1]: 3420 (OH), 1732 (C(28)=O), 1642

(Germany, N_2 laser, 337 nm); analyses were done with 1-μL samples of MeOH solutions (1 mg mL^{−1}) (with 2,5-dihydroxybenzoic acid as the matrix). The optical rotation was measured on a Perkin—Elmer 141 polarimeter. HPLC was performed using a GCP-100 chromatograph (Czechia), a column with Separon SGX (150×3.3 mm, 7 μm), and a refractometric detector. Monosaccharides were analyzed on a Biotronik-5000 carbohydrate analyzer (Germany).

Column chromatography was carried out using silica gel KSK (100—200 mesh, Russia) and silica gel L (40—100 μm, Chemapol, Czechia) as the sorbents. TLC was performed on glass plates (9×6 cm) with a layer of KSK silica gel fixed by a silicic acid sol.

Plant specimens were collected during the blossoming period (May and June) in the vicinity of the Chernyatino village, Oktyabr´sk region, Primorski Krai (Russia) and identified using references (No. 63997) kept in the herbarium of the Pacific Institute of Bioorganic Chemistry of the Far-Eastern Branch of the RAS.

Isolation of compounds 1a, 2—4. Crushed air-dried roots (weight 525 g) were treated with $CHCl_3$ (1 L × 3) and exhaustively extracted with 80% EtOH at ~20 °C. The combined ethanol extract was concentrated *in vacuo* to remove ethanol, 1 L of water was added, and the mixture was repeatedly extracted with *n*-butanol. The combined butanol extract was concentrated *in vacuo* at a temperature not higher than 50 °C, and the residue (76 g) was dissolved in 100 mL of warm MeOH and poured in 1 L of acetone. The resulting precipitate was washed on a filter with cold acetone and dried at ~20 °C to give 47 g of a mixture of triterpenoid compounds. A part of the mixture (35 g) was passed through a column with silica gel with the $CHCl_3$—EtOH solvent system as the eluent (7 : 1→2 : 2). With increasing polarity of the eluent, two fractions were isolated, I (10.4 g) and II (6.0 g). Fraction I (5.0 g) was passed additionally through a similar column with the $CHCl_3$—MeOH solvent system (5 : 1→3 : 1) as the eluent to give the glycoside fraction A consisting of compounds **1a** and **3** (2.87 g, TLC, BuOH—EtOH—25% NH_4OH (5 : 2 : 3), one spot with R_f 0.35). Fraction II (2.5 g) was passed through a column with silica gel using the $CHCl_3$—MeOH solvent system (4 : 1→2 : 1) as the eluent to give glycoside fraction B consisting of compounds **2** and **4** (0.93 g, TLC, BuOH—EtOH—25% NH_4OH (5 : 2 :3), one spot with R_f 0.20). The overall fractions A (210 mg) and B (190 mg) were separated and purified by HPLC on an RP-18 column using $MeCN$—H_2O solvent systems (27.5 : 72.5 and 30 : 70) to give glycosides **1a** (57 mg), **2** (19 mg), **3** (75 mg), and **4** (45 mg).

Chinensioside A (1a), amorphous; $C_{59}H_{96}O_{26}$; $[\alpha]_D$ −17.0 (*c* 0.44, MeOH); IR (KBr), ν/cm[−1]: 3419 and 1056 (OH), 2938 (CH), 1732 (C(28)=O), 1643 and 881 (C(20)=C(29)H_2), 1452, 1386. MALDI-TOF(+) MS, *m/z* (I_{rel} (%)): 1243.6 [M + Na][+] (100), 1097 [M + Na − Rha][+] (10), 965 [M + Na − Rha − Ara][+] (11), 773 [M + Na − Rha − 2 Glc][+] (7), 493 [23-hydroxybetulininc acid + Na − 2 H][+] (14). ^{1}H NMR spectrum of an aliquot portion: 0.89, 0.96, 1.06, 1.16, and 1.69 (all s, each 3 H, C(24)H_3, C(25)H_3, C(26)H_3, C(27)H_3, C(30)H_3); 3.39 (m, 1 H, C(19)H); 4.67 and 4.68 (both br.s, each 1 H, C(29)H_B, C(29)H_A); the spectrum of the carbohydrate part is given in Table 1. ^{13}C NMR spectrum of the aglycon part: 39.06 (C(1)), 25.77 (C(2)), 80.86 C((3)), 42.50 (C(4)), 47.60 (C(5)), 17.85 (C(6)), 34.01 (C(7)), 40.88 (C(8)), 50.70 (C(9)), 36.59 (C(10)), 20.90 (C(11)), 26.01 (C(12)), 38.09 (C(13)), 43.28 (C(14)),

(C(12)H=C(13)). MALDI-TOF(+) MS, *m/z* (I_{rel} (%)): 1243.6 [M + Na]$^+$ (100), 1097 [M + Na − Rha]$^+$ (10), 965 [M + Na − Rha − Ara]$^+$ (6), 773 [M + Na − Rha − 2 Glc]$^+$ (10), 493 [hederagenin + Na − 2 H]$^+$ (47).

Glycoside 4, amorphous; $C_{65}H_{106}O_{31}$; [α]$_D$ −12.9 (*c* 0.41, MeOH). IR (KBr), ν/cm^{-1}: 3418 (OH), 1745 (C(28)=O), 1642 (C(12)H=C(13)). MALDI-TOF(+) MS, *m/z* (I_{rel} (%)): 1405.6 [M + Na]$^+$ (100), 1259 [M + Na − Rha]$^+$ (17), 1244 [M + Na − Glc]$^+$ (47), 935 [M + Na − Rha − 2 Glc]$^+$ (30), 493 [hederagenin + Na − 2 H]$^+$ (60). ^{1}H NMR, aglycon part: 0.86, 0.89, 0.99, 1.11, 1.13, 1.18 (all s, each 3 H, C(25)H$_3$, C(29)H$_3$, C(24)H$_3$, C(26)H$_3$, C(27)H$_3$, C(30)H$_3$); 3.17 (m, 1 H, C(18)H); 5.41 (br.s, 1 H, C(12)H=C(13)); the spectrum of the carbohydrate fragment at the aglycon C(3) atom: Ara: 4.99 (d, 1 H, C(1)H, *J* = 6.5 Hz); Rha: 6.28 (s, 1 H, C(1)H); 1.67 (d, 3 H, C(6)H$_3$, *J* = 6.1 Hz); Glc: 5.13 (d, 1 H, C(1)H, *J* = 7.9 Hz); the spectrum of the carbohydrate fragment at the aglycon C(28) atom: Rha: 5.87 (d, 1 H, C(1)H, *J* = 1.1 Hz); 1.71 (d, 3 H, C(6)H$_3$, *J* = 6.2 Hz); Glc: 5.00 (d, 1 H, C(1)H, *J* = 7.7 Hz), Glc: 6.25 (d, 1 H, C(1)H, *J* = 8.1 Hz). ^{13}C NMR, aglycon part: 38.14 (C(1)), 25.96 (C(2)), 80.81 (C(3)), 43.23 (C(4)), 47.57 (C(5)), 17.92 (C(6)), 32.58 (C(7)), 39.68 (C(8)), 47.94 (C(9)), 36.64 (C(10)), 23.58 (C(11)), 122.63 (C(12)), 143.80 (C((13)), 41.92 (C(14)), 28.06 (C(15)), 23.14 (C(16)), 46.80 (C(17)), 41.43 (C(18)), 45.98 (C(19)), 30.45 (C(20)), 33.77 (C(21)), 32.33 (C(22)), 64.80 (C(23)), 13.64 (C(24)), 15.91 (C(25)), 17.32 (C(26)), 25.76 (C(27)), 176.20 (C(28)), 32.79 (C(29)), 23.44 (C(30)); the spectrum of the carbohydrate fragment at the aglycon C(3) atom: Ara: 103.94 (C(1)), 76.12 (C(2)), 74.31 (C(3)), 79.73 (C(4)), 63.76 (C(5)); Rha: 101.38 (C(1)), 71.91 (C(2)), 72.23 (C(3)),73.86 (C(4)), 69.38 (C(5)), 18.27 (C(6)); Glc: 106.28 (C(1)), 75.17 (C(2)), 78.24 (C(3)), 71.09 (C(4)), 78.41 (C(5)), 62.32 (C(6)); the spectrum of the carbohydrate fragment at the aglycon C(28) atom: Glc: 95.37 (C(1)), 73.64 (C(2)), 78.41 (C(3)), 70.75 (C(4)), 77.74 (C(5)), 69.07 (C(6)); Glc: 105.54 (C(1)), 75.02 (C(2)), 76.28 (C(3)), 78.24 (C(4)), 76.84 (C(5)), 61.15 (C(6)); Rha: 102.48 (C(1)), 72.18 (C(2)), 72.46 (C(3)), 73.70 (C(4)), 70.04 (C(5)), 18.16 (C(6)).

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