Characterization of the Triterpene Saponins of the Roots and Rhizomes of Blue Cohosh (*Caulophyllum Thalictroides*)

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Seven triterpene saponins were isolated from *n*-butanol fractions of blue cohosh (*Caulophyllum thalictroides*) roots and rhizomes. Their structures were established by spectral (^{1}H NMR, ^{13}C NMR, 2D-NMR, and APCI-MS) techniques and chemical reactions as hederagenin 3-*O*-α-L-arabinopyranoside (**1**); caulophyllogenin 3-*O*-α-L-arabinopyranoside (**2**); hederagenin 3-*O*-*β*-D-glucopyranosyl-(1→2)-α-L-arabinopyranoside (**3**); 3-*O*-α-L-arabinopyranosyl-hederagenin 28-*O*-α-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranosyl(1→6)-*β*-D-glucopyranoside (**4**); 3-*O*-α-L-arabinopyranosyl- caulophyllogenin 28-*O*-α-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranosyl(1→6)-*β*-D-glucopyranoside (**5**); 3-*O*-*β*-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl- echinocystic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranosyl(1→6)-*β*-D-glucopyranoside (**6**); 3-*O*-*β*-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl-hederagenin 28-*O*-α-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranosyl(1→6)-*β*-D-glucopyranoside (**7**). All seven compounds were identified in this species for the first time.

Keywords: *Blue cohosh; Caulophyllum thalictroides; NMR; Triterpene saponins*

INTRODUCTION

Blue cohosh (*Caulophyllum thalictroides* (L.) Michx., Berberidaceae), known as papoose root, squaw root, blue ginseng, leontice, or blueberry root, is an indigenous perennial plant found in north-eastern North America. The name "blue" comes from the bluish bloom and later blue berries following the flower. Native Americans used this plant for rheumatism, dropsy, colic, sore throat, cramp, epilepsy, hysterics, and inflammation of the uterus. It can also be used as an antispasmodic, an emmenagogue (menstrual flow stimulant), and a parturifacient (inducer of labor), including diuretic, diaphoretic, and expectorant effects (*1*, *2*). In the dietary supplement market, blue cohosh is recommended to use primarily for women, especially for amenorrhea (lack of menstruation) and dysmenorrhea (painful menstruation).

The genus *Caulophyllum* is known as having two species, one from eastern North America, the other from eastern Asia, which is considered as a variety of the North American one. The two species are morphological similar, however, *Caulophyllum robustum* differs from *Caulophyllum thalictroides* having one sessile leaf, flowering panicles usually shorter than the leaves, broad petals, and shorter filaments (*3*). Previous chemical studies on the components of roots and rhizome of *Caulophyllum robustum* have resulted in the identification of several triterpene saponins and cauloside A,B,C,D and G (*4*, *5*, *6*).

Earlier studies have revealed blue cohosh rhizomes and roots contain two kinds of saponins, caulosaponin, and caulophyllosaponin. The caulosaponin consists of caulosapogenin, $C_{42}H_{62}O_2(OH)_4$, and dextrose. However, the second saponin-like glycoside termed caulophyllosaponin consists of caulophyllosapogenin, $C_{56}H_{88}O_9$, and arabinose (*7*). Later, the formula for caulosapogenin was corrected as $C_{30}H_{48}O_4$, and it was identified as hederagenin. However, McShefferty et al. failed to identify the second sapogenin compound known as caulophyllosapogenin from blue cohosh (*8*).

Pharmacological studies reported glycosides in blue cohosh have significant oxytocic activity (hastening childbirth) by acting as smooth muscle stimulant. Furthermore, the aglycone obtained from acid hydrolysis of the glycosides matched with those obtained from caulosaponin (*9*). However, the glycosides also exert a toxic effect on cardiac muscle by constricting the coronary blood vessels (*9*). In addition, it is well-known that blue cohosh has a number of toxic alkaloids including quinolizidine alkaloids implicated as teratogens (*10*). Among them, *N*-methylcytisine showed teratogenic activity in the REC (rat embryo culture) (*11*).

Because limited reports about glycosides in the blue cohosh were available in the literature, it was thought to be desirable to carry out systematic chemical investigations on the roots and rhizomes of blue cohosh. The isolation and structural elucidation of seven triterpene saponins from extract of blue cohosh roots and rhizomes are being reported in this paper.

MATERIALS AND METHODS

General Procedures. ^{1}H NMR and ^{13}C NMR and all 2D NMR spectra were obtained on a Varian 400 instrument and Varian 600 instrument (Varian Inc., Palo Alto, CA). Com-

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Table 1: ^{13}C NMR Chemical Shift Data of Compounds 1−7 (aglycone)[a]

	δ						
C	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1	39.2 t	39.7 t	38.3 t	39.5 t	39.6 t	39.9 t	39.5 t
2	25.9 t	26.5 t	27.5 t	27.0 t	27.0 t	27.1 t	26.4 t
3	83.9 d	83.6 d	88.8 d	83.3 d	83.3 d	91.3 d	83.7 d
4	43.6 s	44.0 s	41.4 s	43.9 s	43.9 s	40.8 s	44.1 s
5	48.7 d	48.3 d	47.2 d	48.0 d	48.2 d	57.1 d	48.2 d
6	18.8 t	18.5 t	18.5 t	18.9 t	18.8 t	19.4 t	18.9 t
7	33.2 t	33.9 t	33.5 t	33.3 t	33.6 t	34.2 t	33.3 t
8	40.3 s	40.7 s	40.8 s	40.7 s	40.8 s	40.4 s	40.7 s
9	48.1 d	48.2 d	47.2 d	48.1 d	48.0 d	50.0 d	48.0 d
10	37.5 s	37.8 s	36.0 s	37.7 s	37.7 s	37.9 s	37.7 s
11	23.9 t	24.6 t	22.9 t	23.3 t	24.5 t	24.5 t	24.0 t
12	123.3 d	123.5 d	121.0 d	123.8 d	123.5 d	123.6 d	123.7 d
13	145.0 s	145.2 s	144.6 s	144.3 s	144.7 s	144.7 s	144.9 s
14	42.7 s	42.8 s	42.4 s	42.4 s	42.7 s	42.7 s	43.0 s
15	28.6 t	36.7 t	29.2 t	28.9 t	36.5 t	36.5 t	28.9 t
16	24.4 t	75.4 d	23.5 t	24.6 t	75.0 d	75.3 d	24.6 t
17	47.0 s	49.6 s	46.1 s	46.3 s	49.7 s	47.8 s	47.2 s
18	42.4 d	42.2 d	42.4 d	42.5 d	42.0 d	42.0 d	42.5 d
19	47.0 t	47.9 t	45.5 t	47.2 t	47.8 t	48.2 t	47.2 t
20	31.5 s	31.5 s	30.5 s	31.6 s	31.3 s	31.3 s	31.6 s
21	34.8 t	36.3 t	34.0 t	34.9 t	36.2 t	36.3 t	34.7 t
22	33.2 t	32.8 t	33.0 t	33.3 t	32.0 t	32.0 t	33.3 t
23	65.2 t	65.1 t	70.0 t	64.8 t	64.8 t	27.3 q	65.7 t
24	13.3 q	12.6 q	12.6 q	13.4 q	13.5 q	16.4 q	13.4 q
25	16.5 q	16.7 q	15.5 q	16.6 q	16.7 q	16.3 q	16.5 q
26	17.7 q	18.0 q	17.1 q	17.8 q	17.8 q	17.9 q	17.8 q
27	26.5 q	27.5 q	25.6 q	27.0 q	28.0 q	28.6 q	26.4 q
28	180.0 s	181.3 s	179.4 s	178.1 s	177.3 s	177.3 s	178.1 s
29	33.6 q	33.6 q	33.0 q	33.5 q	33.4 q	33.3 q	33.5 q
30	24.1 q	25.1 q	23.5 q	24.1 q	25.1 q	25.1 q	24.1 q

[a] Samples were measured in CD_3OD, except for **1** which was measured in CD_3OD-$CDCl_3$ (5:1), and **3** which was measured in DMSO-d_6; q = CH_3; t = CH_2; d = CH; s = C.

Table 2: ^{13}C NMR Chemical Shift Data of Compounds 1−7 (sugar moieties)[a]

	δ						
C	**1**	**2**	**3**	**4**	**5**	**6**	**7**
				Ara			
1	105.8 d	106.4 d	102.6 d	106.4 d	106.4 d	104.3 d	104.2 d
2	72.3 d	73.1 d	79.9 d	72.9 d	72.9 d	78.3 d	79.5 d
3	74.3 d	74.6 d	74.4 d	74.5 d	74.5 d	73.8 d	73.7 d
4	69.3 d	69.9 d	66.6 d	69.8 d	69.8 d	69.0 d	69.2 d
5	66.5 t	67.0 t	63.7 t	66.9 t	66.9 t	65.4 t	64.7 t
				Glc			
1			104.0 d			105.5 d	104.6 d
2			76.4 d			76.0 d	75.9 d
3			76.9 d			78.1 d	78.1 d
4			71.6 d			71.7 d	71.6 d
5			79.1 d			78.1 d	78.2 d
6			61.1 t			62.9 t	62.9 t
				C-28 Glc′			
1				95.7 d	95.7 d	96.2 d	95.7 d
2				73.8 d	73.9 d	73.9 d	73.8 d
3				78.1 d	78.1 d	78.2 d	78.1 d
4				70.9 d	70.8 d	70.9 d	70.9 d
5				76.8 d	78.1 d	76.8 d	76.8 d
6				69.3 t	69.3 t	69.4 t	69.3 t
				Glc″			
				104.2 d	104.2 d	104.7 d	104.6 d
2				75.3 d	75.2 d	75.0 d	75.3 d
3				76.7 d	76.7 d	76.7 d	76.7 d
4				79.5 d	79.4 d	78.3 d	78.3 d
5				78.1 d	76.8 d	77.9 d	77.8 d
6				61.8 t	61.8 t	61.9 t	61.8 t
				Rha			
1				102.3 d	102.9 d	102.9 d	102.9 d
2				72.4 d	72.4 d	72.5 d	72.4 d
3				72.2 d	72.1 d	72.2 d	72.2 d
4				73.7 d	73.7 d	73.9 d	74.0 d
5				70.6 d	70.6 d	70.7 d	70.6 d
6				17.9 q	17.8 q	17.9 q	17.9 q

[a] See Table 1 for the solvents used; q = CH_3; t = CH_2; d = CH.

pounds were analyzed in CD_3OD, CD_3OD-$CDCl_3$, and DMSO-d_6, with TMS as internal standard. APCI-Mass spectra were recorded on a Micromass Platform II system (Micromass Co., Beverly, MA) equipped with a Digital DECPC XL 560 computer for data analysis. Optical rotations were obtained on a JASCO DIP-1000 polarimeter. Thin-layer chromatography was performed on Sigma-Aldrich silica gel TLC plates (250 μm thickness, 2−25 μm particle size), with compounds visualized by spraying with 5% (v/v) H_2SO_4 in an ethanol solution. Silica gel (130−270 mesh), RP-18, and Diaion HP-20 for column chromatography were purchased from Aldrich Chemical Co. (Milwaukee, WI). All solvents used for chromatographic isolation were of analytical grade and purchased from Fisher Scientific (Springfield, NJ).

Plant Material. Blue cohosh roots and rhizomes were a gift from Natural Products, Inc. It was collected from North Carolina in 2000, and identified by Dr. H. H. Schmidt (Missouri Botanical Garden, MO). A voucher specimen has been deposited in the Department of Food Science, Cook College, Rutgers University.

Extraction and Isolation. The grounded roots and rhizomes (1 kg) of blue cohosh were extracted with 95% ethanol at room temperature for two weeks. The extract was concentrated to dryness under reduced pressure, and the residue (160 g) was dissolved in water and partitioned with hexane. Then the water layer was extracted successively with ethyl acetate and *n*-butanol. The *n*-butanol extract was evaporated in vacuo to give residue (25 g). The residue was subjected to Diaion HP-20 using an H_2O/EtOH gradient system (100/0, 70/30, 30/70, and 0/100). Fraction I (8 g) eluted by water 100% (3 L) was subjected to silica gel column chromatography with chlorform/MeOH solvent system (8:1−2:1) to give 20 fractions. Fractions 3−5 were combined together and subjected to RP-18 silica gel column chromatography with H_2O/MeOH (2:3) solvent system to afford compound **1** (90 mg). Fractions 6−9 were combined and rechromatographed with silica gel with chlorform/MeOH solvent system (7:1) to yield compound **2** (80 mg). Fractions 11−13 were combined and subjected to RP-18 silica gel column chromatography with H_2O/EtOH (1:4) to give compound **3** (80 mg).

Fraction II (7 g) eluted by 30% EtOH (3 L) was subjected to silica gel column chromatography with ethyl acetate/MeOH/H_2O solvent system (4:1:0.8−1:1:0.8) to give 15 fractions. Fractions 3−6 were combined and rechromatographed on a silica gel column and eluted with chloroform/MeOH/H_2O (2.5/1/0.1−2.0/1/0.1) giving two subfractions (1 and 2). Subfraction 1 was then purified on a RP-18 silica gel column eluted with H_2O/MeOH (1.5:3.5) to afford 80 mg of compound **4**. Subfraction 2 was rechromatographed on a silica gel column eluted with chloroform/MeOH/H_2O (2.0/1/0.1) to give compound **5** (100 mg). Fractions 7−11 were combined and subjected to RP-18 silica gel column chromatography with H_2O/MeOH (1.5:3.5) solvent system to give compounds **6** (90 mg) and **7** (80 mg).

Acid Hydrolysis and Alkaline Hydrolysis. Compounds **1**−**7** were hydrolyzed on TLC plate with concentrated HCl vapor at 80 °C for 30 min. Then, the TLC plate developed with CH_3Cl/MeOH/H_2O (7:3:0.5) solvent system in comparison with standard sugars. Spots were detected with aniline phthalate sugar reagent and 5% (v/v) H_2SO_4 in an ethanol solution.

Compounds **4**−**7** were hydrolyzed with 0.5M KOH at 80 °C for 2 h. After neutralization by the addition of 2N HCl, the solution was concentrated to dryness under vacuum. The residues were then fractionated with *n*-butanol and water. The aqueous fraction was concentrated to dryness and the residues were hydrolyzed on TLC plate with concentrated HCl vapor. Then, the alkaline hydrolyzed sugars were compared to the standard sugars with the same methods of acid analysis.

Table 3: ^{1}H NMR Chemical Shift Data of Compounds 1−7 (sugar moieties)[a]

	δ_H (*J*, Hz)						
C	**1**	**2**	**3**	**4**	**5**	**6**	**7**
				Ara			
1	4.34 d (6.2)	4.35 d (6.3)	4.25 d (6.5)	4.35 d (6.1)	4.32 d (6.1)	4.38 d (6.1)	4.38 d (6.3)
2	3.52 m	3.53 m	3.42 m	3.52 m	3.50 m	3.50 m	3.50 m
3	3.28 m	3.32 m	3.08 m	3.20 m	3.20 m	3.18 m	3.20 m
4	3.90 br s	3.90 br s	4.02 br s	3.96 br s	3.95 br s	3.84 br s	3.92 br s
5	3.55 m	3.60 m	3.42 m	3.54 m	3.52 m	3.50 m	3.52 m
	3.83 m	3.82 m	3.90 m	3.78 m	3.80 m	3.84 m	3.80 m
				Glc			
1			4.93 d (7.5)			4.51 d (7.8)	4.45 d (7.5)
2			3.45 m			3.28 m	3.20 m
3			3.58 m			3.25 m	3.23 m
4			3.52 m			3.25 m	3.20 m
5			3.64 m			3.35 m	3.40 m
6			4.01 m			3.85 m	3.82 m
				C-28 Glc′			
1				5.38 d (7.8)	5.35 d (7.8)	5.30 d (7.9)	5.30 d (7.8)
2				3.34 m	3.40 m	3.32 m	3.35 m
3				3.40 m	3.45 m	3.40 m	3.40 m
4				3.40 m	3.40 m	3.41 m	3.40 m
5				3.31 m	3.34 m	3.35 m	3.34 m
6				3.62 m	3.70 m	3.80 m	3.85 m
				4.06 br d	4.08 br d	4.08 br d	4.08 br d
				Glc″			
1				4.45 d (7.8)	4.45 d (7.7)	4.55 d (7.8)	4.55 d (7.8)
2				3.25 m	3.20 m	3.21 m	3.21 m
3				3.56 m	3.45 m	3.40 m	3.45 m
4				3.50 m	3.50 m	3.50 m	3.50 m
5				3.54 m	3.50 m	3.52 m	3.51 m
6				3.56 m	3.65 m	3.64 m	3.65 m
				3.85 m	3.83 m	3.85 m	3.86 m
				Rha			
1				4.95 br s	4.90 br s	4.86 br s	4.89 br s
2				3.85 m	3.82 m	3.85 m	3.80 m
3				3.62 m	3.68 m	3.65 m	3.65 m
4				3.41 m	3.37 m	3.40 m	3.40 m
5				3.40 m	3.40 m	3.42 m	3.40 m
6				1.38 d (6.2)	1.32 d (6.4)	1.30 d (6.4)	1.38 d (6.2)

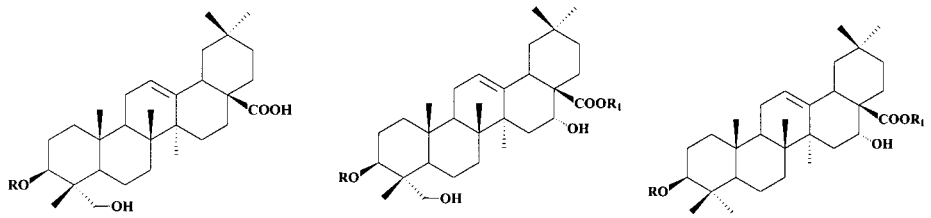
[a] See Table 1 for the solvents used.

RESULT AND DISCUSSION

The *n*-butanol fraction of blue cohosh extracts was fractionated by a combination of HP-20, silica gel, and RP-18 columns chromatography to afford seven compounds. Their structures were elucidated by interpretation of 1D and 2D NMR spectra and compared with literature data.

Compound **1** was isolated as an amorphous solid, $[\alpha]_D^{25}$ + 66° (MeOH, c = 0.82), and had a molecular formula of $C_{35}H_{56}O_8$, which was determined by positive APCI MS (*m/z* 605 $[M+H]^+$, 473 $[M+H\text{-pentose}]^+$), as well as ^{13}C NMR data. The acid hydrolysis of compound **1** gave arabinose as the sugar component. Its spectral features and physicochemical properties suggested compound **1** to be a triterpene saponin. Of the 35 carbons, 30 were assigned to the aglycone part, 5 to a sugar moiety (Tables 1 and 2). The ^{13}C NMR spectrum of compound **1** has six sp^3 carbons at δ 13.3, 16.5, 17.7, 24.1, 26.5, and 33.6 ppm, and two olefinic carbons at δ 123.3 (d) and 145.0 (s). Furthermore, the ^{1}H NMR spectral information showed six methyl proton singlets (δ 0.73, 0.83, 0.93, 0.96, 1.00, 1.19 ppm) and a broad single peak at δ 5.30. These spectral evidences indicated the aglycone possessed an olean-12-ene skeleton. The ^{13}C NMR data for the aglycone part was assigned to hederagenin, known as one of the common aglycones from triterpene glycosides, by comparison with reported data (*12*). Anomeric protons [δ 4.34 (*d*, J = 6.3 Hz)] for sugar moiety can be easily distinguished from other protons with the ^{1}H NMR spectra. Moreover, the chemical shift of C-3 (δ 83.9) was shifted to downfield by about 10 ppm from that in the parent sapogenin−hederagenin (*12*), suggesting sugar moiety is attached at C-3 of the aglycone unit. When compared with literature data, the spectral characteristics of compound **1** were found to be identical with those of hederagenin 3-*O*-α-L-arabinopyranoside (*13*). The compound was identical with cauloside A, previously isolated from *C. robustum* (*5*).

Compound **2** was an amorphous solid, $[\alpha]_D^{25}$ +1.7° (MeOH, c = 0.65). The molecular formula was estimated as $C_{35}H_{56}O_9$ with negative APCI MS (*m/z* 619 $[M-H]^-$, 487 $[M-H\text{-pentose}]^-$), and ^{13}C NMR spectra. Compound **2** afforded arabinose as the sugar component on acid hydrolysis. Of the 35 carbons, 30 were assigned to the aglycone part and 5 to sugar moiety (Tables 1, 2 and 3). The ^{1}H and ^{13}C NMR spectra of compound **2** have similar spectral features with compound **1**, having an olean-12-ene skeleton and one sugar moiety. Noticeable differences are found at ^{13}C NMR signals of C-15, C-16, and C-17 which were shifted to downfield by δ 8.1, 51.0, 2.6 ppm, respectively, as compared to compound **1**. This evidence proved that the hydroxyl group is attached to the C-16. The ROESY experiment showed cross-peak between H-16 and H-15β. However, cross-peak between C-27 methyl proton and H-16 was not detected. It was supported the hydroxyl group of C-16 having an α-con-



I II III

Comp.	Aglycone	R	R_1
1	I	α-L-arabinopyranosyl	H
2	II	α-L-arabinopyranosyl	H
3	I	β-D-glucopyranosyl-(1→2)- α-L-arabinopyranosyl	H
4	I	α-L-arabinopyranosyl	α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside
5	II	α-L-arabinopyranosyl	α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside
6	III	β-D-glucopyranosyl-(1→2)- α-L-arabinopyranosyl	α-L-rhamnopyranosyl-(1→4)- β -D-glucopyranosyl(1→6)-β-D-glucopyranoside
7	I	β-D-glucopyranosyl-(1→2)- α-L-arabinopyranosyl	α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside

Figure 1. Compounds **1** − **7**.

figuration. Moreover, according to ^{13}C NMR published data, 16α-OH was found at a lower field than that of the epimer having 16*β*-OH ascribe to the 1,3-diaxial Me....OH interaction (*14*), and it was confirmed by comparison with literature data (*14*, *15*). The aglycone part was assigned to caulophyllogenin. These spectra signals were in agreement with those of caulophyllogenin 3-*O*-α-L-arabinopyranoside (*15*). The compound was the same with cauloside B, which was isolated from *C. robustum* (*4*).

Compound **3** was isolated as an amorphous solid, $[\alpha]_D^{25}$ + 44° (pyridine, $c = 0.43$). The molecular formula was estimated as $C_{41}H_{66}O_{13}$ with negative APCI (*m/z* 765 [M−H]$^-$, 471 [M−H-hexose-pentose]$^-$) and ^{13}C NMR spectra. The acid hydrolysis of **3** afforded arabinose and glucose as sugar components. ^{13}C NMR spectra of **3** showed signals of aglycone parts were in agreement with those of hederagenin by comparison with compound **1**. ^1H and ^{13}C NMR Spectral data indicated compound **3** has two sugar moieties, which are present *β*-D-glucopyranosyl unit at a terminal and α-L-arabinopyranosyl unit at an inner [anomeric protons: δ 4.25 (*d*, $J = 6.5$ Hz), 4.93 (*d*, $J = 7.5$ Hz), anomeric carbons: δ 102.6, 104.0]. Spectral characteristics of compound **3** were in agreement with those of hederagenin 3-*O*-*β*-D-glucopyranosyl-(1→2)-α-L-arabinopyranoside, which is known as cauloside C (*6*), when compared with literature data (*16*).

Compound **4**, an amorphous solid, $[\alpha]_D^{25}$ + 17.5° (pyridine, $c = 0.44$), has an estimated molecular formula of $C_{53}H_{86}O_{22}$ with negative APCI MS (*m/z* 1073 [M−H]$^-$, 941 [M−H-pentose]$^-$, 749 [M−H-2 hexose]$^-$, 603 [M−H-2 hexose-deoxyhexose]$^-$, 471 [M−H-2hexose-deoxyhexose-pentose]$^-$), as well as ^{13}C NMR and DEPT spectra. Upon acid hydrolysis, compound **4** afforded arabinose, glucose and rhamnose as its sugar components. The alkaline hydrolysis of **4** gave glucose and rhamnose as sugar components of C-28. The spectral characteristics of compound **4** indicated that structure of the aglycone part was the same with hederagenin. ^1H and ^{13}C NMR Spectral data showed compound **4** had four sugar moieties. [anomeric protons: δ 4.35 (*d*, $J =$ 6.1 Hz), 5.38 (*d*, $J = 7.8$ Hz), 4.45 (*d*, $J = 7.8$ Hz), and 4.95 (*br s*), anomeric carbons: δ 95.7, 102.3, 104.2, and 106.4]. The glycosidic connectivity was confirmed by an HMBC spectrum which showed cross-peaks between H_{Ara}-1 (δ 4.35) and C-3 (δ 83.3), $H_{Glc'}$-1(δ 5.38) and C-28-(δ 178.1), $H_{Glc''}$-1(δ 4.45) and $C_{Glc'}$-6(δ 69.3), H_{rha}-1(δ 4.95)and $C_{Glc''}$-4(δ 79.5). When compared with literature data, ^1H and ^{13}C NMR spectral signals of **4** were in agreement with those of 3-*O*-α-L-arabinopyranosyl-hederagenin 28-*O*-α-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranosyl(1→6)-*β*-D-glucopyranoside (*16*) which is known as cauloside D (*5*).

Compound **5** was isolated as an amorphous solid, $[\alpha]_D^{25}$−28° (MeOH, $c = 0.64$). The molecular formula was estimated as $C_{53}H_{86}O_{23}$ with negative APCI MS (*m/z* 1089 [M−H]$^-$, 619 [M−H-2 hexose-deoxyhexose]$^-$, 487 [M−H-2 hexose-deoxyhexose-pentose]$^-$), together with ^{13}C NMR and DEPT spectra. Acid hydrolysis of **5** gave arabinose, glucose and rhamnose as the sugar components. Moreover, alkaline hydrolysis of **5** afforded glucose and rhamnose for its sugar components of C-28. Comparison of spectral properties with that of compound **2** revealed that compound **5** had the same aglycone, caulophyllogenin, as compound **2**. ^1H and ^{13}C NMR spectra showed compound **5** had four sugar moieties. According to 2D NMR spectra, including, COSY, TOCSY, HMBC, HMQC spectra, the sugar moieties of **5** are the same as those of compound **4**. Consequently, the structure of **5** was characterized as 3-*O*-α-L-arabinopyranosyl-caulophyllogenin 28-*O*-α-L-

rhamnopyranosyl-(1→4)-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside, and it was confirmed by literature data (*15*). Compound **5** was the same compound as leonticin D, which was isolated from *Leontice kiangnanensis* (*15*).

Compound **6**, an amorphous solid, $[\alpha]_D^{25}$ −15° (MeOH, $c = 0.41$), possessed the molecular formula $C_{59}H_{96}O_{27}$, as determined by negative APCI MS (*m/z* 1235 $[M-H]^-$, 765 [M−H-2 hexose-deoxyhexose]$^-$, 603 [M−H-3 hexose-deoxyhexose]$^-$, 471 [M−H-3 hexose-deoxyhexose-pentose]$^-$), and confirmed with ^{13}C NMR and DEPT data. Compound **6** gave arabinose, glucose and rhamnose as its sugar components on acid hydrolysis. The alkaline hydrolysis afforded glucose and rhamnose for its C-28 sugar components. According to ^1H and ^{13}C NMR spectral data, the aglycone part of compound **6** is different from those of compounds **1** and **2**, which are hederagenin and caulophyllogenin, respectively. According to ^{13}C NMR, signals of C-15, C-16, and C-17 were shifted to downfield by δ 7.9, 50.9, 0.8 ppm, respectively, when compared to compound **1**. These characteristics were the same as those of compound **2**, which had a hydroxyl group at C-16 having an α-configuration. Furthermore, noticeable differences between compounds **2** and **6** were found that chemical shifts at C-4 and C-23 for compound 6 were shifted to upfield by 3.2 and 37.8 ppm, respectively. In addition, compound **6** has seven sp^3 carbons, which was confirmed by ^1H (δ 0.81, 0.87, 0.91, 0.99, 0.99, 1.30, 1.39 ppm) and ^{13}C NMR (δ 16.3, 16.4, 17.9, 25.1, 27.3, 28.6, 33.3 ppm) spectra. Consequently, the aglycone part was assigned to echinocystic acid, which has seven methyl groups. ^1H and^{13}C NMR spectra showed compound **6** had five sugar moieties. Their glycosidic connectivity was identified by HMBC, HMQC spectrum. From these results, including literature data, compound **6** was elucidated as 3-*O*-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl-echinocystic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside, which was reported from *Pithecellobium dulce* (*17*).

Compound **7** was isolated as an amorphous solid, $[\alpha]_D^{25}$ + 3.6° (pyridine, $c = 0.58$). The molecular formula was estimated as $C_{59}H_{96}O_{27}$ with negative APCI MS (*m/z* 1235 $[M-H]^-$, 765 [M−H-2 hexose-deoxyhexose]$^-$, 603 [M−H-3 hexose-deoxyhexose]$^-$, 471 [M−H-3 hexose-deoxyhexose-pentose]$^-$), and in accordance with ^{13}C NMR and DEPT spectra data. Acid hydrolysis of compound **7** afforded arabinose, glucose, and rhamnose as its sugar components. Alkaline hydrolysis of **7** gave glucose and rhamnose for its C-28 sugar components. The aglycone moiety of compound **7** showed agreement with those of hederagenin by comparison with the spectral data of compound **1**. 2D NMR spectra, including ^1H-^1H COSY, TOCSY, HMBC and HMQC, showed compound **7** had the same sugar moieties as that of compound **6**. As a consequence, compound **7** was identified as 3-*O*-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl-hederagenin 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside, and it was confirmed by literature data (*16*). Compound **7** was the same compound as cauloside G. (*6*).

Column chromatographic fractionations afforded 7 triterpene saponins. Previous research identified those compounds from several plants, including *Caulophyllum robustum*, *Leontice Kiangnanensis*, and *Pithecellobium dulce*; however, the seven triterpene saponins were identified for the first time in this species. Compounds **1**, **2**, **3**, **4**, and **7** were identical compounds as cauloside A, B, C, D, and G, respectively, which were isolated from *Caulophyllum robustum*. Our study showed a significant similarity for the saponin constituents between those two species.

Earlier biological studies showed compounds **1**, **2**, **3**, **4**, and **7** have antimicrobial activity (*18*). In addition, other biological studies reported compounds **2** and **3** have cytotoxicity to developing sea urchin embryos by changing cell permeability. It is well-known that cytotoxic glycoside causes a disturbance of cell membrane permeability that can cause leakage of important cellular components (*19*, *20*). Pharmacological studies of triterpene saponin components of blue cohosh have not yet been performed extensively. However, taking into consideration the previous pharmacological research results for the glycosides in this plant, biological effects of triterpene saponins in blue cohosh still remain of interest.

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