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Alkaloids and saponins from blue cohosh

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Abstract

Blue cohosh, *Caulophyllum thalictroides* (L.) Michx. (Berberidaceae), is used primarily to cure menstrual disturbances and to ease childbirth. Alkaloids and saponins are considered to be responsible for its pharmacological activity. A detailed phytochemical investigation of blue cohosh resulted in the isolation of 15 compounds belonging to the alkaloids and the triterpene saponins. The structures of two alkaloids, caulophyllumines A (**1**) and B (**2**) and a saponin, cauloside H (**3**) both previously unknown were determined by spectroscopic techniques, including by 1- and 2-D NMR as well as by chemical analysis.

Keywords: Blue cohosh; *Caulophyllum thalictroides*; Berberidaceae; Caulophyllumine A; Caulophyllumine B; Cauloside H

1. Introduction

Blue cohosh, *Caulophyllum thalictroides* (L.) Michx. (Berberidaceae), is an indigenous perennial plant found in north–eastern North America. It is also known papoose root, squaw root, blue ginseng, leontice, or blueberry root (Jhoo et al., 2001). It is a well known dietary supplement used for regulation of the menstrual cycle and to ease childbirth and painful cramps. Native Americans used this plant for rheumatism, dropsy, colic, sore throat, cramp, epilepsy, hysterics, and inflammation of the uterus (Jhoo et al., 2001). Previously, a few triterpene saponins and alkaloids have been reported from blue cohosh (Jhoo et al., 2001; Kennelly et al., 1999; Flom et al., 1967). In a continuation to explore the chemical constituents of dietary supplements, the detailed phytochemical investigation of blue cohosh was carried out. This manuscript describes the isolation and characterization of two new alkaloids, caulophyllumines A (**1**) and B (**2**) and a new saponin, cauloside H (**3**) along with 12 known alkaloids and triterpene saponins. Their structures were determined by different spectroscopic techniques including 1- and 2-D NMR as well as by chemical analysis.

2. Results and discussion

Compound **1** gave a positive response to the Dragendorff reagent. An $[M+H]^+$ quasimolecular ion at *m/z* 280.1598 (calc 280.1549 for $C_{15}H_{22}NO_4$) in the positive HRESIMS indicated a molecular formula, $C_{15}H_{21}NO_4$. The ^{13}C NMR spectrum showed 15 resonances, which were resolved by a DEPT experiment into two methyl, five methylene, three methine, and five quaternary carbons. A pair of AB doublets at δ_H 6.45 (1H, *d*, $J = 9.0$ Hz) and 7.20 (1H, *d*, $J = 9.0$ Hz), characteristic of *ortho*-coupled protons in a tetra-substituted phenyl, and two singlets at δ_H 3.78 and 3.79 for two methoxyl groups were observed in the ^1H NMR spectrum of **1** (see Table 1). The ^{13}C NMR spectrum (see Table 1)

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Table 1
^1H and ^{13}C NMR spectroscopic data for caulophyllumines A (**1**) and B (**2**) in $CDCl_3$

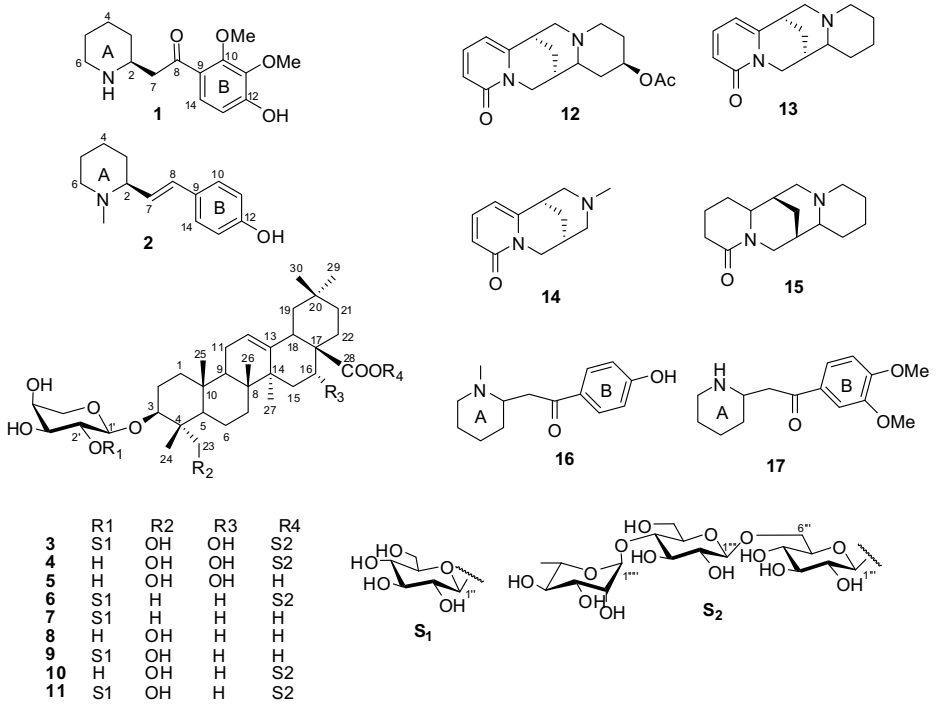
Position	**1**		**2**	
	δ_C	δ_H	δ_C	δ_H
2	53.0 *d*	3.16 *m*	68.7 *d*	2.59 *t* (8.4)
3	31.0 *t*	1.44 *m*, 1.63 *m*,	32.9 *t*	1.34 *m*, 1.58 *m*
4	24.3 *t*	1.71 *m*, 1.84 *m*	23.9 *t*	1.68[a], 1.77 *m*
5	24.8 *t*	1.57 *m*, 1.66 *m*	25.4 *t*	1.68[a]
6	46.0 *t*	2.78 *ddd*, (3.2, 11.6, 14.8) 3.18 *m*	56.6 *t*	2.15 *ddd* (3.2, 10.0, 13.2) 3.04 *br d* (11.6)
7	47.5 *t*	2.95 *d* (8.8)	128.8[a] *d*	5.94 *dd* (8.4, 15.6)
8	198.1 *s*	–	131.9 *d*	6.42 *d* (15.6)
9	121.7 *s*	–	128.8[a] *s*	–
10	155.1 *s*	–	128.0 *d*	7.17 *d* (8.2)
11	141.8 *s*	–	116.5 *d*	6.78 *d* (8.2)
12	159.9 *s*	–	157.0 *s*	–
13	113.9 *d*	6.45 *d* (9.0)	116.5 *d*	6.78 *d* (8.2)
14	126.6 *d*	7.20 *d* (9.0)	128.0 *d*	7.17 *d* (8.2)
10O*Me*	60.5 *q*	3.78 *s*		
11O*Me*	60.9 *q*	3.79 *s*		
N*H*		2.13 *s*		
N*Me*			44.3 *q*	2.31 *s*

[a] Overlapped, δ in ppm, J in Hz.

displayed resonances for a phenyl ring (δ_C 121.7, 155.1, 141.8, 159.9, 113.9, and 126.6), two methoxyl groups (δ_C 60.5 and 60.9) and an oxo group (δ_C 198.1). Furthermore, the resonances for a methine ($\delta_{C/H}$ 53.0/3.16), five methylenes ($\delta_{C/H}$ 31.0/1.44, 1.63; 24.3/1.71, 1.84; 24.8/1.57, 1.66; 46.0/2.78, 3.18 and 47.5/2.95) and an N*H* (δ_H 2.13) were observed in the ^{13}C and ^1H NMR spectra. An up-field resonance of a ketone suggested its conjugation with a phenyl ring. The HMBC correlations of OMe-10 (δ_H 3.78) with C-10 (δ_C 155.1), OMe-11 (δ_H 3.79) with C-11 (δ_C 141.8), H-13 (δ_H 6.45) with C-9 (δ_C 121.7) and C-11 (δ_C 141.8), and H-14 (δ_H 7.20) with C-10 (δ_C 155.1), C-12 (δ_C 159.9) and C-8 (δ_C 198.1) suggested the assignment of phenyl ring and positions of carbonyl and methoxyl groups. In turn cross-peaks in the ^1H–^1H COSY spectrum [$\delta_{H/H}$ 2.95 (H_2-7)/3.16 (H-2)/1.44, 1.63 (H_2-3)/1.71, 1.84 (H_2-4)/1.57, 1.66 (H_2-5)/2.78, 3.18 (H_2-6)] indicated a spin system, sequencing as CH_2-7–CH-2–CH_2-3–CH_2-4–CH_2-5–CH_2-6. The facts of odd molecular mass (*m*/*z* 279), N*H* resonance (δ_H 2.13), and characteristic chemical shifts of methine ($\delta_{C/H}$ 53.0/3.16, C-2) and methylene ($\delta_{C/H}$ 46.0/2.78, 3.18, C-6) (Kennelly et al., 1999; Hootele et al., 1985) helped to recognize the C-2–NH–C-6 unit. Ultimately, a 2-substituted piperidine ring was determined. The following HMBC associations [H_2-7 (δ_H 2.95) with C-8 (δ_C 198.1) and C-3 (δ_C 31.0), and H-2 (δ_H 3.16) with C-7 (δ_C 47.5)] established the connectivity between a piperidine ring and an acetophenone moiety. A literature survey showed that piperidine derivatives possessing C-2 β-substituted chiral center had a negative optical rotation sign, whereas the α-substituted ones gave a positive sign (see Table 2). As compound **1** had a negative optical rotation sign, the substitution at C-2 was believed to be β (see Table 2) and its absolute configuration was determined to be *S* by Chan-Ingold-Prelog sequencing. In brief, the ^1H and ^{13}C NMR spectroscopic data of **1** (see Table 1) were assigned by HMQC, HMBC, and ^1H–^1H COSY experiments (see Fig. 1) and/or by comparison with the published data for identical compounds (Kennelly et al., 1999; Hart et al., 1968; Hootele et al., 1985). Finally, caulophyllumine A (**1**) was characterized as piperidine 2*S*-(1-(4-hydroxy-2,3-dimethoxyphenyl)ethanonyl).

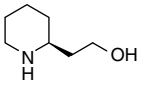
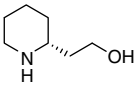
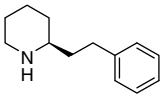
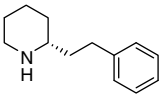
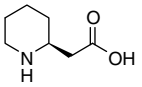
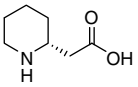
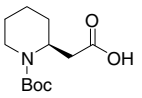
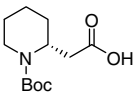
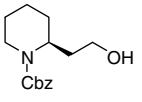
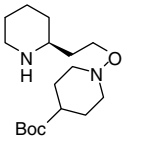
Compound **2** also gave positive response to the Dragendorff test. The positive HRESIMS displayed an $[M+H]^+$ quasimolecular ion at *m*/*z* 218.1527 (calc 218.1545 for $C_{14}H_{20}NO$), which in conjunction with ^{13}C NMR spectroscopic data, established a molecular formula, $C_{14}H_{19}NO$. The 14 resonances in the ^{13}C NMR spectrum of **2** were differentiated by a DEPT experiment as a methyl, four methylene, seven methine, and two quaternary carbons. The ^1H NMR spectrum of **2** displayed a pair of AB doublets at δ_H 6.78 (2H, *d*, $J = 8.2$ Hz) and 7.17 (2H, *d*, $J = 8.2$ Hz), characteristic of a *para*-substituted phenyl ring. Another doublet at δ_H 6.42 (1H, *d*, $J = 15.6$ Hz, H-8), showed a correlation in the ^1H–^1H COSY spectrum (see Fig. 1) with a resonance at δ_H 5.94 (1H, *dd*, $J = 8.4$, 15.6 Hz, H-7) which in turn correlated with a methine at δ_H 2.59 (1H, *t*, $J = 8.4$ Hz, H-2). The coupling constants, 15.6 Hz indicated a *trans*-olefinic moiety in the molecule. When its ^1H and ^{13}C NMR spectroscopic data (see Table 1) were compared with those of thalictroidine (**16**) (Kennelly et al., 1999), the resonances of a ketone and a methylene were absent in **2**, showing instead a *trans*-olefinic moiety in between rings A and B. The HMBC correlations (see Fig. 1) of N*Me* (δ_H 2.31) with C-2 (δ_C 68.7) and C-6 (δ_C 56.6), H-7 (δ_H 5.94) with C-3 (δ_C 32.9) and C-9 (δ_C 128.8), H-8 (δ_H 6.42) with C-2 (δ_C 68.7) and C-10/14 (δ_C 128.0) and H-10/14 (δ_H 7.17) and H-11/13 (δ_H 6.78) with C-12 (δ_C 157.0) confirmed the positions of N*Me*, olefinic, and hydroxyl moieties. The absolute configuration at C-2 was determined to be *S* in a similar manner to that of compound **1**. Ultimately, caulophyllumine B (**2**) was characterized as piperidine 1-methyl-2*S*-(1-(4-hydroxyphenyl)ethenyl).

Compounds **1** and **2**, piperidine-acetophenone conjugates, are rare in the plant kingdom. Thalictroidine (**16**) isolated from blue cohosh (Kennelly et al., 1999) and piperidylacetophenone (**17**) from *Boehmeria* genus (Hart et al., 1968) are other examples of such a type of compounds.



	R1	R2	R3	R4
3	S1	OH	OH	S2
4	H	OH	OH	S2
5	H	OH	OH	H
6	S1	H	H	S2
7	S1	H	H	H
8	H	OH	H	H
9	S1	OH	H	H
10	H	OH	H	S2
11	S1	OH	H	S2

Table 2
Piperidine derivatives having C-2 chiral center and their specific rotations

Compound	$[\alpha]_D$	Config.	Reference	Compound	$[\alpha]_D$	Config.	Reference
	–[a]	*R*	Beyerman et al. (1971)		+35	*S*	Aldrich-522902[b]
	−16	*R*	Beyerman et al. (1971)		+17	*S*	Beyerman et al. (1971)
	−1	*S*	Aldrich-670170[b]		+1	*R*	Aldrich-670057[b]
	−7	*S*	Kiguchi et al. (1990)		+7	*R*	Kiguchi et al. (1990)
	−26	*S*	Fluka-80615[b]		+27	*R*	Fluka-80617[b]
	−46	*S*	Aldrich-15558[b]		+68	*R*	Aldrich-516341[b]
	−24	*S*	Danilewicz et al. (2002)				
	−12	*S*	Danilewicz et al. (2002)				

[a] Value could not find.
[b] Aldrich and Fluka catalog numbers.

Table 3
^{1}H and ^{13}C NMR spectroscopic data for cauloside H (**3**) in C_5D_5N

Aglycone			Sugars		
Position	δ_C	δ_H[a] (HMQC)	Position	δ_C	δ_H[a] (HMQC)
1	38.6 *t*	0.82, 1.40	3-Ara-1	103.9 *d*	4.77 *d* (6.2)
2	25.8 *t*	1.89 *m*	2	79.4 *d*	4.12 *t* (6.2)
3	82.4 *d*	3.76 *dd* (8.8, 4.0)	3	73.3[b] *d*	3.94 *br d* (6.2)
4	43.3 *s*	–	4	68.3 *d*	4.00 *br* s
5	47.4 *d*	1.29 *br d* (12.0)	5	65.1 *t*	3.51, 3.96
6	18.0 *t*	1.14, 1.40	2′-Glc-1	104.5 *d*	4.81 *d* (7.6)
7	32.8 *t*	1.14, 1.53 *m*	2	75.5 *d*	3.59
8	39.8 *s*	–	3	77.4 *d*	3.45
9	47.0[b] *d*	1.64	4	71.0 *d*	3.64 *dd* (8.4, 8.8)
10	36.7 *s*	–	5	77.9 *d*	3.68–3.79
11	23.7 *t*	0.76, 1.79	6	62.2 *t*	3.86
12	122.5 *d*	5.33 *br s*			4.28 *br d* (10.8)
13	144.4 *s*	–	28-Glc-1	95.4 *d*	5.71 *d* (8.0)
14	41.9 *s*	–	2	73.3[b] *d*	3.61
15	35.6[b] *t*	1.40, 2.04	3	77.5 *d*	3.68–3.79
16	73.9 *d*	4.82 *br s*	4	70.0 *d*	3.76 *t* (8.4)
17	49.0 *s*	–	5	75.9 *d*	3.68–3.79
18	41.1 *d*	3.12 *br d* (12.0)	6	68.6 *t*	3.94
19	47.0[b] *t*	1.05			4.28 *br d* (11.6)
		2.41 *t* (13.2)	6‴-glc-1	104.1 *d*	4.59 *d* (7.6)
20	30.5 *s*	–	2	74.7 *d*	3.49
21	35.6[b] *t*	1.40, 1.40	3	76.5 *d*	3.68–3.79
22	31.6 *t*	1.79, 2.04	4	78.2 *d*	3.84
23	63.9 *t*	3.36 *d* (12.8)	5	77.7 *d*	3.68–3.79
		3.77 *d* (12.8)	6	60.8 *t*	3.74, 3.89
24	13.2 *q*	0.72 *s*	4⁗-Rha-1	102.3 *d*	5.21 *br s*
25	16.1 *q*	0.82[b] *s*	2	71.9 *d*	4.16 *br s*
26	17.4 *q*	0.82[b] *s*	3	72.0 *d*	4.01 *dd* (3.6, 9.2)
27	27.0 *q*	1.44 *s*	4	73.3[b] *d*	3.79 *dd* (9.2, 10.4)
28	176.3 *s*	–	5	70.1 *d*	4.35 *m*
29	33.0 *q*	0.78 *s*	6	18.2 *q*	1.37 *d* (6.2)
30	24.5 *q*	0.85 *s*			

[a] Multiplicity not clear for some signals due to overlapping.
[b] Overlapped, δ in ppm, J in Hz.

Compound **3** showed an $[M+K]^+$ pseudomolecular ion in the positive HRESIMS at m/z 1291.5793 (calc 1291.5725 for $C_{59}H_{96}KO_{28}$), which in association with its ^{13}C NMR spectroscopic data indicated a molecular formula, $C_{59}H_{96}O_{28}$. The ^{13}C NMR spectrum had 59 resonances, of which 30 were attributed to a triterpene skeleton and 29 to five sugar moieties. A DEPT NMR experiment permitted differentiation of the 59 ^{13}C NMR resonances into seven methyl, 14 methylene, 30 methine, and eight quaternary carbons. The ^{1}H and ^{13}C NMR spectroscopic assignment of **3** (see Table 3) were based on HMQC, HMBC (see Fig. 1), and ^{1}H–^{1}H COSY spectral analyses, and by comparison with the published data (Jhoo et al., 2001). The aglycone of **3** was found to be caulophyllogenin and its sugar moieties are the same as those of for cauloside G (Jhoo et al., 2001). A broad singlet appeared in the ^{1}H NMR spectrum for H-16 established its equatorial (β) orientation, similar to that of other 16-hydroxyl saponins isolated from blue cohosh (Jhoo et al., 2001). The sugars obtained after acid hydrolysis were identified as glucopyranose, arabinopyranose, and rhamnopyranose by comparing their R_f to those of the standard sugar samples on co-TLC. The absolute configurations of glucose, arabinose, and rhamnose were determined as D, L, and L, respectively, by GC–MS analysis of their acetylated thiazolidine derivatives (Hara et al., 1987). Accordingly, cauloside H (**3**) was identified as 3-*O*-β-D-glucopyranosyl-(1 → 2)-α-L-arabinopyranosylcaulophyllogenin 28-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl(1 → 6)-β-D-gluco- pyranoside.

Known compounds were characterized as leonticin D (**4**), caulosides A (**8**), B (**5**), C (**9**), D (**10**), and G (**11**) (Jhoo et al., 2001), ciwujianoside A (**6**) (Gao and Wang, 2006), saponin PE (**7**) (Zhong et al., 2001), *O*-acetylbaptifolin (**12**) (Saito et al., 1989), anagyrine (**13**), *N*-methylcytisine (**14**), and lupanine (**15**) (Sagen et al., 2002).

3. Concluding remarks

This is the first detailed report on blue cohosh with respect to its two classes of natural products. We found two saponins containing oleanolic acid aglycone (**6** and **7**), though common in other plants, have not been reported

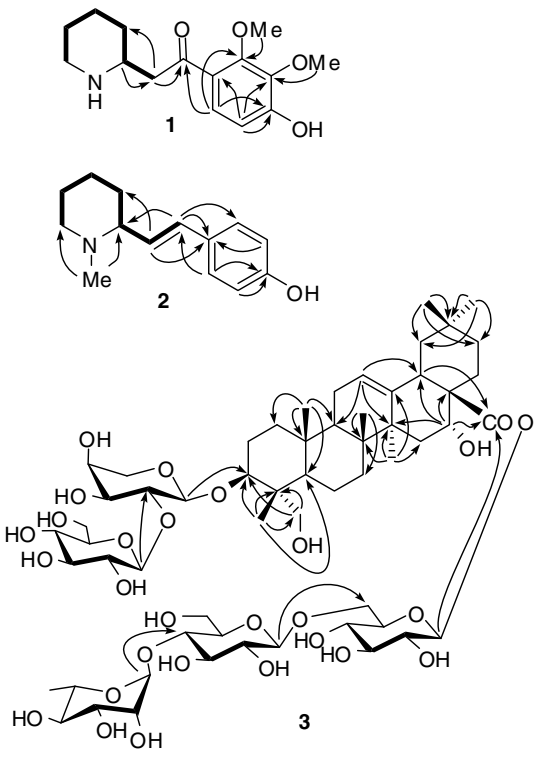


Fig. 1. Selected COSY (—) for compounds **1** and **2** and HMBC (→) correlations for compounds **1**–**3**.

so far from *Caulophyllum* species. The new compounds (**1**–**3**), new from blue cohosh (**6**, **7** and **12**), and other known ones might be useful in chemical finger printing of *Caulophyllum*.

4. Experimental

4.1. General experimental procedures

Optical rotations were measured on a Rudolph Research Auto Pol IV polarimeter. NMR spectra were recorded on a Varian AS 400 NMR spectrometer. ESIMS and HRESIMS data were obtained on an Agilent Series 1100 SL mass spectrometer. Gravity CC was performed using silica gel (J. T. Baker, 40 μm for flash chromatography) and reversed-phase RP-18 silica (Polarbond, JT Baker). Thin layer chromatography (TLC) and preparative thin layer chromatography (PTLC) were carried out on silica gel 60 F_{254} plates (Merck, Germany). Sugar samples were purchased from Sigma–Aldrich (St. Lousis, MO).

4.2. Plant material

Roots of *C. thalictroides* (blue cohosh) (4.4 kg) were purchased from Mountain Rose Herbs™ (www.mountainroseherbs.com) on June 26, 2006 and authenticated by Dr. V. Joshi at the National Center for Natural Products Research, University of Mississippi, where a voucher specimen (No. 2973) has been deposited.

4.3. Extraction and isolation

Roots powder (4.0 kg) was extracted with MeOH (4.0 L × 24 h × 4) at room temperature. The combined extracts were evaporated under reduced pressure to afford a brown powder (592 g). A portion (292.0 g) was dissolved in 5% HCl in H_2O (2.0 L) and extracted with EtOAc (2.0 L × 4). The aqueous layer was then removed, NH_4OH was added to make it basic (pH 9) and the whole was extracted with EtOAc (2.0 L × 4). The EtOAc soluble part was evaporated to obtain the alkaloidal fraction (1.2 g) (A). The H_2O layer was neutralized with 5% HCl and extracted with *n*-butanol (1.5 L × 4). The combined organic layers were evaporated to obtain a dried brown material (234 g) (B).

The alkaloidal fraction (A, 1.1 g) was subjected to CC [flash silica gel (40 μm, 150 g), column (42 × 1 in), $CHCl_3$–MeOH, 19:1 (1.6 L), 9:1 (2 L) and 0:1 (1 L)] to afford six fractions A1–A6. Anagyrine (4.4 mg), *N*-methylcytisine (48.0 mg), and *O*-acetylbaptifolin (14.0 mg) were purified from fraction B1 (163 mg) by repeated PTLC using silica gel plates (EtOAc–$CHCl_3$–MeOH–H_2O, 12:8:8:2 and $CHCl_3$–MeOH–NH_4OH, 180:20:1). To fraction B4 (235 mg), $CHCl_3$ was added, with the soluble part subjected to repeated PTLC (silica gel plates, EtOAc–$CHCl_3$–MeOH–H_2O, 12:8:8:2 and EtOH–MeCOMe–MeOH–NH_4OH, 40:40:6:1) to give lupanine (15.1 mg), caulophyllumines A (2.2 mg), and B (2.7 mg).

A portion of the *n*-butanol solubles (100 g) was resolved into 11 fractions (B1–B11) by CC [flash silica gel (1.2 kg), column (30 × 4 in), $CHCl_3$–MeOH, 9:1 (6 L), 5.7:1 (6 L), 4:1 (12 L), 3:1 (6 L), 2.3:1 (4 L), 1.9:1 (4 L) and 0:1 (2 L)]. Caulosides A (96.0 mg) and C (1.93 g) were purified from fractions B3 (2.26 g) and B6 (2.48 g), respectively, as insoluble material when EtOAc was added to these fractions. Fraction B4 (1.3 g) afforded caulosides A (81.1 mg) and B (298.2 mg) by CC [flash silica gel (500 g), column (51 × 1.7 in), EtOAc–$CHCl_3$–MeOH–H_2O, 15:8:4:1 (2 L)]. Cauloside B (76.9 mg) and saponin PE (24.7 mg) were obtained from fraction B5 (0.7 g) by CC over reversed-phase silica gel [RP-18 (150 g), column (20 × 1 in), MeOH–H_2O, 8:2 (3 L)]. Cauloside D (4.8 g) was purified from fraction B9 (12.2 g) by CC [flash silica gel (1.0 kg), column (40 × 3 in), EtOAc–$CHCl_3$–MeOH–H_2O, 12:8:8:2 (14 L)]. Fraction B11 (34.6 g) was fractionated in to six sub-fractions (B11A–B11F) by CC [flash silica gel (1.6 kg), column (52 × 3 in), $CHCl_3$–MeOH–H_2O, 65:35:10 (lower layer, 15 L). Sub-fraction B11C contained pure cauloside G (3.1 g). Leonticin D (809 mg) and ciwujianoside A (24.2 mg) were recovered from sub-fraction B11B by CC [flash silica gel (600 g), column (51 × 1.7 in), $CHCl_3$–MeOH–H_2O, 65:35:10 (lower layer, 3 L). Cauloside H (50.1 mg) was purified from sub-fraction B11E via CC on reversed-phase silica gel [RP-18 (150 g), column (20 × 1 in), MeOH–H_2O, 15:5 (1 L)].

4.3.1. Caulophyllumine A (**1**)

Brown powder; $[\alpha]_D^{28}$ − 11.6 (*c* 0.27, MeOH); UV (MeOH) λ_{max} (log ε) 280 (3.04); IR (NaCl) υ_{max} 3346, 1668 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic analyses, see Table 1; ESIMS, *m/z* 280 $[M + H]^+$; HRESIMS, *m/z* 280.1598 $[M + H]^+$, $C_{15}H_{21}NO_4 + H$, requires 280.1549.

4.3.2. Caulophyllumine B (**2**)

Brown powder; $[\alpha]_D^{28}$ − 13.2 (*c* 0.22, MeOH); UV (MeOH) λ_{max} (log ε) 265 (3.41); for ^1H and ^{13}C NMR spectroscopic analyses, see Table 1; ESIMS, *m/z* 218 $[M + H]^+$; HRESIMS, *m/z* 218.1527 $[M + H]^+$, $C_{14}H_{19}NO + H$, requires 218.1545.

4.3.3. Cauloside H (**3**)

White powder; $[\alpha]_D^{28}$ − 9.5 (*c* 0.21, MeOH); IR (NaCl) υ_{max} 3376, 1684 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic analyses, see Table 3; ESIMS, *m/z* 1253 $[M + H]^+$, 1275 $[M + Na]^+$, 1291 $[M + K]^+$; HRESIMS, *m/z* 1291.5793 $[M + K]^+$, $C_{59}H_{96}O_{28} + K$, requires 1291.5725.

4.4. Sugar analysis of compound **3**

Compound **3** (5 mg) was hydrolyzed with 1 N HCl (2 ml) for 3 h at 95 °C. The reaction mixture was cooled, neutralized and partitioned between EtOAc (2 ml) and H_2O (2 ml). The aqueous layer was analyzed using TLC ($CHCl_3$–MeOH–H_2O, 8:5:1) with comparison to authentic standards of D-glucose, D-galactose, L-arabinose, D-xylose, and L-rhamnose. The spots were visualized by spraying with anisaldehyde-H_2SO_4 followed by heating. The sugars obtained on hydrolysis showed comparable R_f values to those of D-glucose (R_f 0.28), L-arabinose (R_f 0.36), and L-rhamnose (R_f 0.45).

The absolute configurations of glucose, arabinose and rhamnose, were determined as described by Hara et al. (1987). The aqueous layer obtained on acid hydrolysis gave the sugar residue after drying. The residue was dissolved in pyridine (1 ml) and 0.1 M L-cysteine methyl ester hydrochloride in pyridine (2 ml) was added. The mixture was heated at 60 °C for 1 h. An equal volume of Ac_2O was added with heating continued for another 1 h. Acetylated thiazolidine derivatives were subjected to GC–MS analysis (Conditions: Column, JW DB-5, 30 m × 0.25 mm, 0.25 μm; carrier gas He; injection temperature 280 °C, detection temperature 280 °C, column temperature; 150 °C (1 min), 10 °C/min to 250 °C (30 min). The configurations were determined by comparing their retention times (t_R L-arabinose 13.53 min, t_R L-rhamnose 17.17 min t_R D-glucose 22.09 min) with acetylated thiazolidine derivatives prepared in a similar way from standard sugars (Sigma–Aldrich).

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