Constituents of the Roots of *Cynanchum bungei* DECNE. Isolation and Structures of Four New Glucosides, Bungeiside-A, -B, -C, and -D

Jun LI,[a] Shigetoshi KADOTA,*[,b] Yukio KAWATA,[b] Masao HATTORI,[b] Guo-Jun XU,[a] and Tsuneo NAMBA[b]

China Pharmaceutical University,[a] 24 Tong Jia Xiang, Nanjing, China and Research Institute for Wakan-Yaku (Oriental Medicines), Toyama Medical and Pharmaceutical University,[b] 2630 Sugitani, Toyama 930-01, Japan. *Received April 13, 1992*

Four new glucosides, bungeiside-A, -B, -C, and -D, were isolated from the roots of *Cynanchum bungei* DECNE. The structures of the new compounds were determined by chemical and spectroscopic methods, including two-dimensional nuclear magnetic resonance (2D NMR) techniques, especially ^{1}H-detected heteronuclear multiple-bond multiple-quantum coherence.

Keywords *Cynanchum bungei*; Bai-Shu-Uh; Asclepiadaceae; bungeiside-A; bungeiside-B; bungeiside-C; bungeiside-D; acetophenone glucoside

Cynanchum bungei DECNE (Asclepiadaceae) is widely distributed in the north of China. The dried root (named Bai-Shu-Uh in Chinese; 白首烏) of this plant has been used traditionally as a tonic in the oriental system of medicine from early times. It was recorded by the ancient Ben Cao treatise that this crude drug has the same therapeutic action as "Ka-Shu-Uh" (in Chinese; 何首烏), which is the dried root of *Polygonum multiflorum* THUMB (Polygonaceae).[1] Several other kinds of plants have been used as "Bai-Shu-Uh" in China.[2] Chemical constituents of *Cynanchum* species have been studied by Mitsuhashi *et al.*[3] and the isolation and structure elucidation of many kinds of glycosides, which consist of C/D-*cis*-polyoxypregnane derivatives and 2,6-dideoxy-3-*O*-methyl sugars, were reported. However, until now no work has been done on the constituents of *C. bungei*. In the course of our chemical studies on biologically active constituents of Chinese medicines, we investigated the constituents of the roots of *C. bungei* and isolated four new acetophenone glucosides, named bungeiside-A (**7**), -B (**8**), -C (**9**), and -D (**10**), together with 2,4-dihydroxyacetophenone (**1**), 4-hydroxyacetophenone (**2**), blumenol A (**3**), β-sitosterol glucoside (**4**), (−)leucanthemitol (**5**), and 7-*O*-glucosyl liquiritigenin (**6**). This paper deals with the isolation and structure elucidation of the four new compounds and identification of the six known compounds.

The roots of *C. bungei* were pulverized and extracted with hot ethanol. The ethanol extract was separated into hexane-soluble, chloroform-soluble, ethyl acetate-soluble, and *n*-butanol-soluble fractions as shown in Chart 1. The chloroform-soluble fraction was subjected to column chromatography over silica gel eluted with methanol–chloroform to give compounds **1** to **4**. Compounds **1**, **2**, **3**, and **4** were identified as 2,4-dihydroxyacetophenone (**1**), 4-hydroxyacetophenone (**2**), blumenol A (**3**), and β-sitosterol glucoside (**4**), respectively.

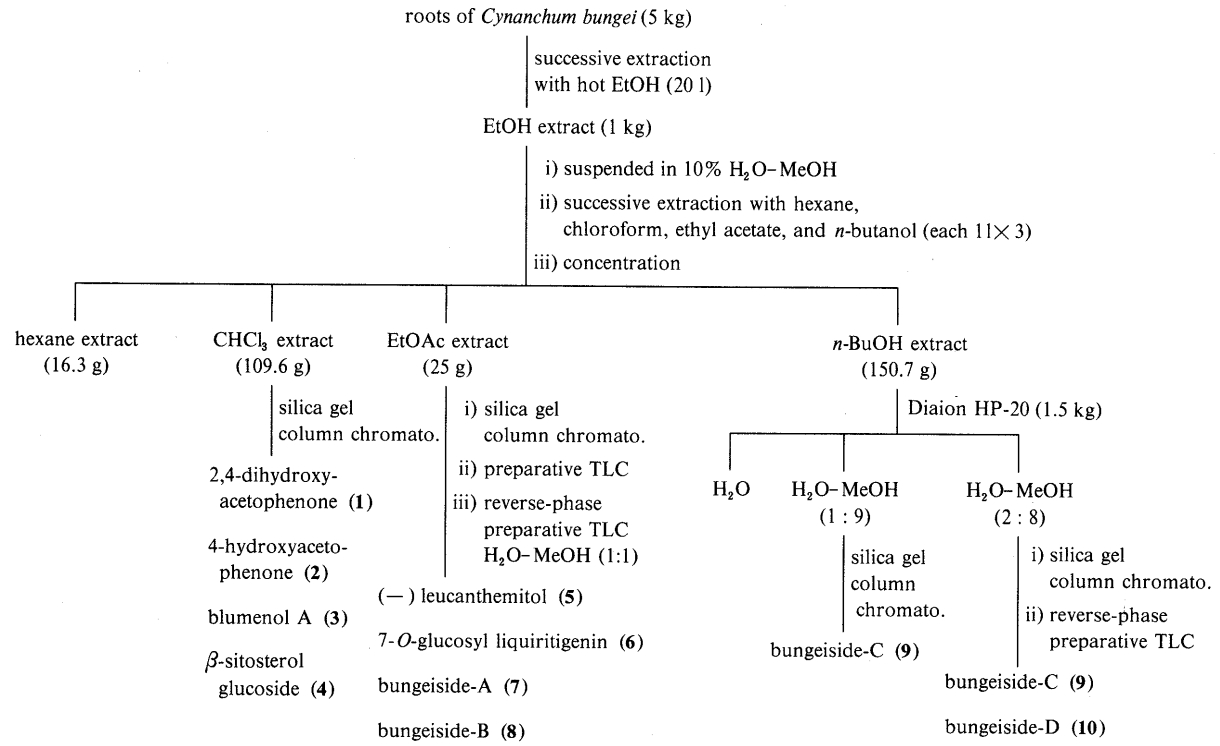

Chart 1. Isolation Scheme of the Constituents of *Cynanchum bungei*

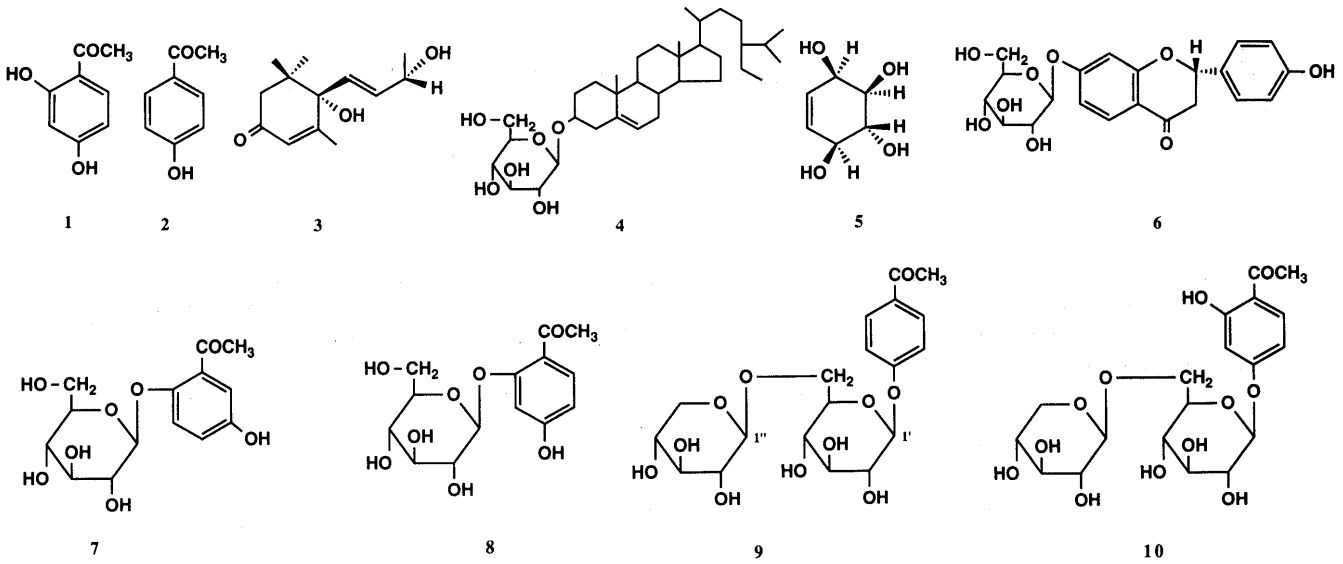

Chart 2

Compound **3** was obtained as colorless plates, mp 108—109°C, $C_{13}H_{20}O_3$, $[\alpha]_D$ +192.2° ($CHCl_3$). This compound was identified as blumenol A[4] by detailed analysis of its proton and carbon-13 nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectra with the aid of ^{1}H–^{1}H shift correlation spectroscopy (COSY) and ^{1}H–^{13}C COSY and ^{1}H–^{13}C long-range COSY spectroscopy.

The ethyl acetate-soluble fraction was separated by a combination of silica gel column chromatography and reversed-phase preparative TLC to give compounds **5** to **8**. Among these, compounds **5** and **6** were identified as (−)leucanthemitol (**5**)[5] and 7-*O*-glucosyl liquiritigenin (**6**),[6] respectively.

Bungeiside-A (**7**) was obtained as a white amorphous powder and showed $[\alpha]_D$ −40.6° (MeOH). The infrared (IR) spectrum showed strong absorptions at 3400 (OH), 1670 (carbonyl), and 1620 cm^{-1} (phenyl). The fast atom bombardment mass spectrum (FAB-MS) and the negative ion FAB-MS exhibited the quasi-molecular ion peak at *m/z* 315 and 313, respectively, corresponding to the molecular formula $C_{14}H_{18}O_8$. The ^{1}H-NMR spectrum of **7**, analyzed with the aid of ^{1}H–^{1}H COSY, showed signals due to acetyl methyl protons (δ_H 2.59) and trisubstituted benzene protons [δ_H 6.91 (dd, J=9.0, 3.0 Hz), 6.97 (d, J=3.0 Hz), and 7.11 (d, J=9.0 Hz)] along with sugar protons (δ_H 3.17, 3.27, 3.29, 3.31, 3.47, 3.70, 4.82) (Table I). The ^{13}C-NMR of **7** showed a carbonyl carbon (δ_C 199.06), three aromatic methine carbons (δ_C 114.55, 117.64, 120.50), six sugar carbons (δ_C 60.75, 69.71, 73.41, 76.78, 77.11, 101.67), and an acetyl methyl carbon (δ_C 32.06) along with three quaternary carbons (δ_C 129.18, 149.67, 151.86) (Table I). In the nuclear Overhauser effect (NOE) experiments, irradiation of the anomeric proton at δ_H 4.82 caused an NOE increase of the signal at δ_H 7.11 (3-H), This observation led us to postulate the structure **7** or an alternative with the glucoside linkage and hydroxyl group at the 4 and 3 position respectively.

To establish the positions of the substituents, **7** was subjected to acid hydrolysis, which yielded 2,5-dihydroxyacetophenone as the aglycone and glucose as the sugar moiety. The aglycone, 2,5-dihydroxyacetophenone, was identified by comparing its ^{1}H-NMR and mass spectra (MS) with those of an authentic sample. This is the first reported isolation of this type of compound from a natural source. The structure was confirmed by measuring the ^{1}H-detected multiple-bond multiple-quantum coherence (HMBC)[7] spectrum. The carbon signal at δ_C 149.67 (C-2) is correlated with the protons at δ_H 4.82 (1′-H), 6.91 (4-H), and 6.97 (6-H), and the signal at δ_C 199.06 (C=O) is correlated with the protons at δ_H 2.59 ($COCH_3$) and 6.97 (6-H).

On the basis of the above results, the structure of bungeiside-A was determined to be 2-*O*-glucosyl-5-hydroxyacetophenone (**7**).

Bungeiside-B (**8**) was obtained as an amorphous white powder and showed $[\alpha]_D$ −17.3° (MeOH). The molecular formula for **8** was determined as $C_{14}H_{18}O_8$ on the basis of the negative ion FAB-MS, which exhibited the $[M-H]^-$ peak at *m/z* 313. Its ^{1}H-NMR spectrum showed a pattern very similar to that of **7**, except for a few signals due to trisubstituted benzene protons [δ_H 6.49 (dd, J=8.5, 2.0 Hz), 6.61 (d, J=2.0 Hz), and 7.58 (d, J=8.5 Hz)]. In the NOE experiments, irradiation at δ_H 4.93 (1′-H) enhanced the intensity of the signal at δ_H 6.61 (3-H). This observation led us to postulate the structure **8**, or the sugar moiety and hydroxyl group at the 3 and 4 positions, respectively, in the benzene ring. The acid hydrolysis of **8** gave 2,4-dihydroxyacetophenone (**1**) as the aglycone and glucose as the sugar. This result supports the structure **8**. The structure was further supported by an HMBC experiment. The carbon signal at δ_C 69.49 (C-4′) is correlated with the protons at δ_H 3.33 (3′-H) and 3.72 (6′-H). In turn, the carbon signals at δ_C 159.23 (C-2) and 196.42 (C=O) are correlated with the protons corresponding to the signals at δ_H 4.93 (1′-H). 6.61 (3-H), and 7.58 (6-H) and at δ_H 2.54 ($COCH_3$) and 7.58 (6-H), respectively. From these spectral data and the result of chemical analysis, bungeiside-B was determined to be 2-*O*-glucosyl-4-hydroxyacetophenone (**8**).

The butanol-soluble fraction was separated by a combination of column chromatography and reversed-phase preparative TLC to give bungeiside-C (**9**) and bungeiside-D (**10**).

TABLE I. ^{1}H(400 MHz) and ^{13}C(100 MHz) NMR Spectra of Bungeiside-A(**7**), -B(**8**), -C(**9**), and -D(**10**)

Position	Bungeiside-A(**7**)		Bungeiside-B(**8**)		Bungeiside-C(**9**)		Bungeiside-D(**10**)		
	(DMSO-d_6)		(DMSO-d_6)		(DMSO-d_6 + D_2O)		(DMSO-d_6)	(DMSO-d_6 + D_2O)	(DMSO-d_6 + D_2O)
	$\delta H^{a,b)}$	$\delta C^{c,d)}$	$\delta H^{a,b)}$	$\delta C^{c,d)}$	$\delta H^{a,b)}$	$\delta C^{c,d)}$	$\delta H^{a,b)}$	$\delta H^{a,b)}$	$\delta C^{c,d)}$
1	—	129.18 s	—	119.49 s	—	131.21 s	—	—	114.91 s
2	—	149.67 s	—	159.23 s	7.96 d (8.5)	130.79 d	—	—	163.57 s
3	7.11 d (9.0)	117.64 d	6.61 d (2.0)	102.10 d	7.19 d (8.5)	116.37 d	6.58 d (2.0)	6.59 d (2.0)	103.59 d
4	6.91 dd (9.0, 3.0)	120.50 d	—	162.97 s	—	161.27 s	—	—	163.45 s
5	—	151.86 s	6.49 dd (8.5, 2.0)	109.32 d	7.19 d (8.5)	116.37 d	6.66 dd (9.0, 2.0)	6.67 dd (9.0, 2.0)	108.35 d
6	6.97 d (3.0)	114.55 d	7.58 d (8.5)	131.64 d	7.96 d (8.5)	130.79 d	7.86 d (9.0)	7.87 d (9.0)	133.52 d
1′	4.82 d (7.5)	101.67 d	4.93 d (7.5)	100.70 d	5.02 d (7.0)	100.03 d	4.99 d (7.5)	4.98 d (7.5)	99.64 d
2′	3.27 m	73.41 d	3.31 t (7.5)	73.32 d	3.34 t (7.0)	73.29 d	3.28 m	3.31 t (7.5)	73.14 d
3′	3.29 m	76.78 d	3.33 m	76.72 d	3.40 t (7.0)	76.39 d	3.32 t (7.5)	3.36 t (7.5)	76.39 d
4′	3.17 m	69.71 d	3.22 t (8.5)	69.49 d	3.30 dd (8.5, 7.0)	69.68 d	3.22 t (7.5)	3.27 t (7.5)	69.55 d
5′	3.31 m	77.11 d	3.35 m	77.14 d	3.66 dd (8.5, 6.0)	76.23 d	3.61 m	3.62 t (7.5)	75.93 d
6′	3.47 dd (12.0, 5.5)	60.75 t	3.53 dd (12.0, 5.5)	60.57 t	3.64 dd (10.0, 6.0)	68.34 t	3.62 m	3.65 dd (9.0, 7.5)	68.25 t
6′	3.70 d (12.0)	—	3.72 dd (12.0, 1.5)	—	3.98 d (10.0)	—	3.94 d (9.0)	3.94 d (9.0)	—
1″	—	—	—	—	4.25 d (8.0)	104.01 d	4.18 d (8.0)	4.20 d (8.0)	103.95 d
2″	—	—	—	—	3.05 t (8.0)	73.59 d	3.00 t (8.0)	3.03 t (8.0)	73.47 d
3″	—	—	—	—	3.15 t (8.0)	76.54 d	3.10 t (8.0)	3.14 t (8.0)	76.60 d
4″	—	—	—	—	3.37 m	69.74 d	3.30 m	3.33 dd (8.0, 5.5)	69.62 d
5″	—	—	—	—	2.98 t (11.0)	65.79 t	2.96 t (11.0)	2.98 t (11.0)	65.70 t
5″	—	—	—	—	3.72 dd (11.0, 5.0)	—	3.69 dd (11.0, 5.5)	3.72 dd (11.0, 5.5)	—
$COCH_3$	2.59 s	32.06 q	2.54 s	32.06 q	2.55 s	26.84 q	2.59 s	2.58 s	26.93 q
$COCH_3$	—	199.06 s	—	196.42 s	—	197.33 s	—	—	203.46 s
2-OH	—	—	—	—	—	—	12.5 s	—	—

δ value in ppm. *a)* Coupling constants in Hz. *b)* ^{1}H–^{1}H correlation spectra were measured. *c)* The multiplicities of carbon signals were determined by means of the distortionless enhancement by polarization transfer (DEPT) method, and are indicated as s, d, t, and q. *d)* ^{1}H–^{13}C COSY and HMBC spectra were measured. The assignment of each proton and carbon based on ^{1}H–^{13}C COSY including HMBC spectra.

Bungeiside-C (**9**) was obtained as colorless needles, mp 234—235°C, $[\alpha]_D$ −100.9° (MeOH). The negative ion FAB-MS exhibited the quasi-molecular ion peak at *m/z* 429, corresponding to the molecular formula $C_{19}H_{26}O_{11}$. It showed IR absorptions at 3440, 2940, 1660 (conjugated CO), 1600 (phenyl) cm^{-1}. The ^{1}H-NMR spectrum of **9**, analyzed with the aid of ^{1}H–^{1}H COSY, showed signals due to 1,4-disubstituted phenyl protons at δ_H 7.19 and 7.96 and acetyl methyl protons at δ_H 2.55, along with sugar protons (δ_H 2.98, 3.05, 3.15, 3.30, 3.34, 3.37, 3.40, 3.64, 3.66, 3.72, 3.98, 4.25, 5.02). The ^{13}C-NMR spectrum of **9** showed a carbonyl carbon (δ_C 197.33), two aromatic methine carbons (δ_C 116.37, 130.79), eleven sugar carbons (δ_C 65.79, 68.34, 69.68, 69.74, 73.29, 73.59, 76.23, 76.39, 76.54, 100.03, 104.01) and an acetyl methyl carbon (δ_C 26.84), along with two quaternary carbons (δ_C 131.21, 161.27) (Table I). Acid hydrolysis of **9** with 5% aqueous hydrochloric acid afforded 4-hydroxyacetophenone (**2**), along with glucose and xylose, which were identified by gas chromatographic (GC) comparison with authentic samples after trimethylsilylation. The above results suggested that **9** may be a glucoside having a 4-hydroxyacetophenone group, a glucose moiety and a xylose moiety.

The ^{1}H-NMR signals due to the aglycone moiety of **9** are similar to those of **2**, indicating that the sugar moiety is linked to the C-4 hydroxyl group of **2**. The chemical shift of the C-6′ carbon of the glucose moiety (δ_C 68.34) revealed the presence of a linkage involving C-6′ and C-1″ between glucose and xylose. Next, we measured the HMBC spectrum in order to determine the positions of the substituents. The carbonyl carbon signals at δ_C 197.33 ($COCH_3$) and 161.27 (C-4) showed long-range correlations with the proton signals at δ_H 7.96 (2- and 6-H), 2.55 ($COCH_3$) and at δ_H 7.96 (2- and 6-H), 7.19 (3- and 5-H), and 5.02 (1′-H), respectively. On the other hand, the carbon signal at δ_C 104.01 (C-1″) is correlated with the proton signals at δ_H 3.98 (6′-H), 3.72 (5″-H), 3.64 (6′-H), 3.05 (2″-H), and 2.98 (5″-H).

On the basis of the above findings, the structure of bungeiside-C was determined to be 4-*O*-priverosyl acetophenone as represented by the formula **9**.

Bungeiside-D (**10**) was obtained as colorless needles, mp 238—240°C, $[\alpha]_D$ −68.3° (MeOH), and IR ν_{max} cm^{-1}: 3350, 2900, 1630, and 1590. The negative ion FAB-MS exhibited the quasi-molecular ion peak at *m/z* 445, corresponding to the molecular formula $C_{19}H_{26}O_{12}$. The ^{1}H- and ^{13}C-NMR pattern of **10** resembled that of **8**, except for signals of the sugar moiety. The proton signal pattern of the aglycone moiety of **10** and the presence of a hydroxyl signal at δ_H 12.5 due to hydrogen bonding indicated that the sugar moiety is linked to the C-4 hydroxyl group of **1** (Table I and Experimental). Treatment with 5% aqueous hydrochloric acid gave crystalline 2,4-dihydroxyacetophenone (**1**) along with glucose and xylose, which were identified by GC comparison with authentic samples after trimethylsilylation. Finally, the sequence of glucose and xylose was found to be the same as that of **9** from the HMBC spectrum. On the basis of the above spectral and chemical evidence, the structure of bungeiside-D was concluded to be 4-*O*-priverosyl-2-hydroxyacetophenone (**10**).

As is shown in Table I, the chemical shift of the acetyl methyl carbon is about δ_C 27 for bungeiside-C (**9**) and -D (**10**), while it is about δ_C 32 for bungeiside-A (**7**) and bungeiside-B (**8**). This shift is due to steric compression by the bulky group on the *ortho*-hydroxyl with respect to the acetyl group; in order that the carbonyl oxygen can remain as far as possible from the oxygen at the *ortho*-position, the methyl group comes relatively closer to the bulkyl group, causing the acetyl methyl signal to appear at low field relative to that of the *p*-substituted compounds. If the hydroxyl group is at the *ortho*-position with respect to the acetyl

group, a six-membered chelate is formed due to hydrogen bonding, so that the carbonyl carbon is observed at δ_C 203 for **1** and **10**, while in other cases where there is no possibility of chelate formation, *e.g.* **2**, **7**, **8**, and **9**, the carbonyl carbon is observed at δ_C 196—199.

We have isolated four acetophenone glucosides from the roots of *C. bungei*. In 1966, Mitsuhashi *et al.*,[8] reported paeonol as the first example of an acetophenone-related compound occurring in the Asclepiadaceae family. Our present result provides the first example of acetophenone glucosides. The distribution of acetophenone glucosides in other Asclepiadaceae plants and their biological activities require further study.

Experimental

Melting points were determined on a Kofler-type apparatus and are uncorrected. IR spectra were taken on a Hitachi 260-01 IR spectrometer in KBr discs. Optical rotations were measured on a JASCO DIP-4 automatic polarimeter at 25 °C. ^{1}H- and ^{13}C-NMR spectra were taken on a JEOL GX-400 spectrometer in dimethylsulfoxide (DMSO)-d_6 or in pyridine-d_5 solutions with tetramethylsilane as an internal standard, and chemical shifts are recorded in δ values. ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY, and HMBC were obtained with the usual pulse sequence, and data processing was performed with the standard JEOL software. MS and high-resolution (HR) MS were obtained with a JEOL JMS DX-300 spectrometer using a direct inlet system and glycerol was used as a matrix in FAB-MS measurements. Column chromatography was done with Wakogel C-200 (Wako Pure Chemical Co., Osaka, Japan). TLC and preparative TLC were carried out on precoated Merck Kieselgel F_{254} plates (0.25 or 0.5 mm) or precoated Merck RP-18 F_{254} reversed-phase plates (0.25 mm) with MeOH–$CHCl_3$ (2:8), MeOH–H_2O (2:3), and MeOH–H_2O (1:1) as the developing solvents, and spots were detected under UV light, or by using $Ce(SO_4)_2$–10% H_2SO_4 (1:99) reagent.

Extraction and Separation of the Constituents of Roots of *Cynanchum bungei* Decne Dried roots (5 kg) of *C. bungei*, collected on Tai mountain (Tai an city, China) in November, 1990, were pulverized and extracted three times (3 h) with hot ethanol (20 l). The EtOH extract was evaporated to dryness and the residue (800 g) was suspended in 10% MeOH aqueous solution (500 ml) and fractionated by successive extractions with hexane (1 l × 3), $CHCl_3$ (1 l × 3), EtOAc (1 l × 3), and BuOH (1 l × 3) to give the hexane-soluble fraction (16.3 g), $CHCl_3$-soluble fraction (109.6 g), EtOAc-soluble fraction (25 g), and BuOH-soluble fraction (150.7 g).

The $CHCl_3$-soluble fraction (100 g) was subjected to column chromatography on silica gel (1.5 kg). The column was successively eluted with $CHCl_3$, 2% MeOH–$CHCl_3$, 5% MeOH–$CHCl_3$, 10% MeOH–$CHCl_3$, 20% MeOH–$CHCl_3$, and 30% MeOH–$CHCl_3$ and the eluates were separated into 54 fractions.

Fraction 19 (2% MeOH–$CHCl_3$ eluate) was again separated by chromatography on silica gel with MeOH–$CHCl_3$ (2:98, 5:95, and 1:9) as the eluents. The eluate frs. 18—22 were combined and subjected to chromatography over silica gel with 20% acetonehexane to give 2,4-dihydroxyacetophenone (**1**) (50 mg). Fraction 22 (2% MeOH–$CHCl_3$ eluate) was further separated by chromatography on silica gel with acetone–hexane (2:8, 3:7, 4:6, and 1:1) as the eluents. The eluate frs. 6—8 were combined and purified by preparative TLC with acetone–hexane (4:6) to give 2,4-dihydroxyacetophenone (**1**) (20 mg). Rechromatography of frs. 9—10 on a silica gel column with 2% acetone–$CHCl_3$ gave 4-hydroxyacetophenone (**2**) (70 mg). Fraction 51 (20% MeOH–$CHCl_3$ eluate) was again separated by chromatography over silica gel and the eluates were separated into seven fractions. Fraction 51-6 (20% MeOH–$CHCl_3$ eluate) was purified by reversed-phase preparative TLC (Merck RP-18) with MeOH–H_2O (3:2) to afford blumenol A (**3**) (19 mg). Fraction 53 (20% MeOH–$CHCl_3$ eluate) was rechromatographed on silica gel with (20% MeOH–$CHCl_3$), giving twelve fractions. Fr. 53-11 was purified by preparative TLC with 20% MeOH–$CHCl_3$ to give β-sitosterol glucoside (**4**) (22 mg).

2,4-Dihydroxyacetophenone (**1**): Yellow needles, mp 142—143 °C. IR ν_{max} cm^{-1}: 3300, 1630, 1610. ^{1}H-NMR (DMSO-d_6) δ: 2.52 (3H, s, $COCH_3$), 6.25 (1H, d, J=2.5 Hz, 3-H), 6.37 (1H, dd, J=8.5, 2.5 Hz, 5-H), 7.74 (1H, d, J=8.5 Hz, 6-H), 10.61 (1H, s, 4-OH), 12.65 (1H, s, 2-OH). ^{13}C-NMR (DMSO-d_6) δ: 26.23 (q, $COCH_3$), 102.28 (d, C-3), 108.08 (d, C-5), 112.82 (s, C-1), 133.58 (d, C-6), 164.24 (s, C-4), 164.88 (s, C-2), 202.55 (s, $COCH_3$). MS *m/z*: 152 (M^+), 137 (base peak), 109, 81.

4-Hydroxyacetophenone (**2**): Colorless needles, mp 109—110 °C, IR ν_{max} cm^{-1}: 3300, 1660, 1600, 1575. ^{1}H-NMR (DMSO-d_6) δ: 2.47 (3H, s, $COCH_3$), 6.84 (2H, d, J=8.5 Hz, H-3, H-5), 7.83 (2H, d, J=8.5 Hz, 2-H, 6-H). ^{13}C-NMR δ: 26.20 (q, $COCH_3$), 115.09 (d, C-3, C-5), 128.57 (s, C-1), 130.64 (d, C-2, C-6), 161.96 (s, C-4), 195.87 (s, $COCH_3$). MS *m/z*: 136 (M^+), 121 (base peak), 93, 65.

Blumenol A (**3**): Colorless plates, mp 108—109 °C, $[\alpha]_D$ +192.2° (c=0.4, $CHCl_3$). MS *m/z*: 224 (M^+), 206, 168, 150, 135, 124 (base peak). High-resolution MS *m/z*: Found 224.1430, Calcd for $C_{13}H_{20}O_3$ (M^+) 224.1428. IR ν_{max} cm^{-1}: 3360, 2960, 1660. ^{1}H-NMR (pyridine-d_5) δ: 1.13 (3H, s, 11-H_3), 1.28 (3H, s, 12-H_3), 2.02 (3H, d, J=1.0 Hz, 13-H_3), 2.41, 2.69 (each 1H, ABq, J=16.5 Hz, 2-H), 4.70 (1H, q, J=6.0 Hz, 9-H), 6.11 (1H, br s, 4-H), 6.25 (1H, d, J=15.5 Hz, 7-H), 6.32 (1H, dd, J=15.5, 5.5 Hz, 8-H). ^{13}C-NMR (pyridine-d_5) δ: 19.41 (q, C-13), 23.51 (q, C-12), 24.45 (q, C-11), 24.51 (q, C-10), 41.63 (s, C-1), 50.29 (t, C-2), 67.32 (d, C-9), 78.97 (s, C-6), 126.67 (d, C-4), 129.13 (d, C-7), 137.23 (d, C-8), 164.55 (s, C-5), 197.80 (s, C-3).

β-Sitosterol Glucoside (**4**): Amorphous powder. ^{1}H-NMR (pyridine-d_5) δ: 0.67 (3H, s, 18-H_3) 0.86, 0.89 (each 3H, d, J=7.0 Hz, 26- or 27-H_3), 0.90 (3H, t, J=7 Hz, 29-H_3), 0.94 (3H, s, 19-H_3), 1.00 (3H, d, J=6.5 Hz, 21-H_3), 3.98 (1H, m, 5′-H), 3.99 (1H, m, 3-H), 4.05 (1H, t, J=8.0 Hz, 2′-H), 4.24 (1H, t, J=8.0 Hz, 4′-H), 4.31 (1H, t, J=8.0 Hz, 3′-H), 4.38 (1H, dd, J=11.5, 5.5 Hz, 6′-H), 4.55 (1H, dd, J=11.5, 2.5 Hz, 6′-H), 5.03 (1H, d, J=8.0 Hz, 1′-H), 5.38 (1H, m, 6-H).

The EtOAc-soluble fraction (24 g) was subjected to column chromatography on silica gel (700 g). The column was successively eluted with $CHCl_3$, 5% MeOH–$CHCl_3$, 10% MeOH–$CHCl_3$, 20% MeOH–$CHCl_3$, and 30% MeOH–$CHCl_3$ and the eluates were separated into five fractions.

Fraction 5 (20% MeOH–$CHCl_3$) (1.0 g) was separated by preparative TLC with 20% MeOH–$CHCl_3$ to give an amorphous powder, which was again purified by reversed-phase preparative TLC with MeOH–H_2O (1:1) to afford four compounds: (—)leucanthemitol (**5**) (10 mg), 7-*O*-glucosyl liquiritigenin (**6**) (10 mg), bungeiside-A (**7**) (20 mg), and bungeiside-B (**8**) (20 mg), in order of increasing polarity.

(—)Leucanthemitol (**5**): Amorphous powder, $[\alpha]_D$ −161° (c=0.3, MeOH). Negative ion FAB-MS: 145 $[M-H]^-$. ^{1}H-NMR (DMSO-d_6) δ: 3.73 (1H, dt, J=7.0, 2.0 Hz, 1-H), 3.43 (1H, dd, J=10.0, 7.0 Hz, 2-H), 3.21 (1H, dd, J=10.0, 4.5 Hz, 3-H), 3.98 (1H, t, J=4.5 Hz, 4-H), 5.63 (1H, ddd, J=10.0, 4.5, 2.0 Hz, 5-H), 5.55 (1H, dd, J=10.0, 2.0 Hz, 6-H). ^{13}C-NMR (DMSO-d_6) δ: 66.22 (d), 71.16 (d), 72.07 (d), 72.35 (d), 127.11 (d), 132.82 (d).

7-*O*-Glucosyl Liquiritigenin (**6**): Amorphous powder, $[\alpha]_D$ −26.7° (c=0.5, MeOH). ^{1}H-NMR (DMSO-d_6) δ: 2.67 (1H, dd, J=16.5, 2.7 Hz, 3-H), 3.17 (1H, m, 3″-H), 3.17 (1H, m, 4″-H), 3.25 (1H, t, J=7.0 Hz, 2″-H), 3.32 (1H, m, 5″-H), 3.45 (1H, dd, J=11.5, 5.5 Hz, 6″-H), 3.69 (1H, dd, J=11.5, 3.0 Hz, 6″-H), 4.89 (1H, d, J=7.0 Hz, 1″-H), 5.53 (1H, dd, J=13.0, 2.7 Hz, 2-H), 6.35 (1H, d, J=2.0 Hz, 8-H), 6.51 (1H, dd, J=9.0, 2.0 Hz, 6-H), 7.06 (2H, d, J=9.0 Hz, 3′- and 5′-H), 7.44 (2H, d, J=9.0 Hz, 2′- and 6′-H), 7.65 (1H, d, J=9.0 Hz, 5-H). ^{13}C-NMR (DMSO-d_6) δ: 43.20 (t, C-3), 60.69 (t, C-6″), 69.71 (d, C-4″), 73.23 (d, C-2″), 76.63 (d, C-5″), 77.05 (d, C-3″), 78.63 (d, C-2), 100.28 (d, C-1″), 102.58 (s, C-10), 110.60 (d, C-6), 113.51 (d, C-8), 116.18 (d, C-3′, C-5′), 127.99 (d, C-2′, C-6′), 128.42 (s, C-1′), 132.37 (d, C-5), 157.47 (s, C-4′), 163.06 (s, C-9), 164.73 (s, C-7), 189.92 (s, C-4).

Bungeiside-A (**7**): Amorphous white powder, $[\alpha]_D$ −40.6° (c=0.5, MeOH). Positive ion FAB-MS: 315 $[M+H]^+$. Negative ion FAB-MS: 313 $[M-H]^-$. IR ν_{max} cm^{-1}: 3400, 2920, 1670, 1620. ^{1}H- and ^{13}C-NMR: Table I.

Bungeiside-B (**8**): Amorphous white powder, $[\alpha]_D$ −17.3° (c=0.7, MeOH). Negative ion FAB-MS: 313 $[M-H]^-$. ^{1}H- and ^{13}C-NMR: Table I.

The BuOH-soluble fraction (150 g) was chromatographed on a Diaion HP-20 column. The column was eluted successively with H_2O, MeOH–H_2O (1:9, 2:8, 3:7, 4:6, 1:1, 7:3), and MeOH and eluates were separated into eight fractions. Fraction 2 (10% MeOH–H_2O eluate) (10 g) was again separated by chromatography over silica gel with 40% MeOH–$CHCl_3$ to give bungeiside-C (**9**) (25 mg). Fraction 3 (20% MeOH–H_2O eluate) was separated by chromatography over silica gel with 20% MeOH–$CHCl_3$ to give an amorphous powder (600 mg). A portion (70 mg) of this amorphous powder was further purified by reversed-phase preparative TLC with MeOH–H_2O (1:1), giving two bands. The less polar band afforded bungeiside-C (**9**) (30 mg), while the more polar band gave bungeiside-D (**10**) (30 mg).

Bungeiside-C (**9**): Colorless needles, mp 234—235 °C, $[\alpha]_D$ −100.9°

($c=0.4$ MeOH). Negative ion FAB-MS: 429 $[M-H]^-$. IR ν_{max} cm^{-1}: 3440, 2940, 1660, 1600. ^1H- and ^{13}C-NMR: Table I.

Bungeiside-D (**10**): Colorless needles, mp 238—240°C, $[\alpha]_D$ −68.3° ($c=0.5$, MeOH). Negative ion FAB-MS: 445 $[M-H]^-$. IR ν_{max} cm^{-1}: 3350, 2900, 1630, 1590. ^1H- and ^{13}C-NMR: Table I.

Acid Hydrolysis of 7 Bungeiside-A (**7**) (4.2 mg) was refluxed with 5% aqueous HCl solution (0.5 ml) for 2 h. The reaction mixture was extracted with $CHCl_3$, and the extract was dried over anhydrous $MgSO_4$ and evaporated under reduced pressure to give a syrup. The residue was separated by preparative TLC with MeOH–$CHCl_3$ (3:7) to give 2,5-dihydroxyacetophenone (1.5 mg), which was identified by ^1H-NMR and MS comparisons with an authentic sample. The aqueous layer afforded glucose and xylose, which were identified by GC comparison with authentic samples after trimethylsilylation.

Acid Hydrolysis of 8 By the same procedure as described above, hydrolysis of bungeiside-B (**8**) (5.5 mg) gave 2,4-dihydroxyacetophenone (**1**) (1.7 mg) and glucose.

Acid Hydrolysis of 9 Bungeiside-C (**9**) (3.5 mg) was refluxed with 5% aqueous HCl solution (0.5 ml) for 2 h. The reaction mixture was extracted with $CHCl_3$, and the extract was dried over anhydrous $MgSO_4$ and evaporated under reduced pressure to give a syrup. The residue was separated by preparative TLC with MeOH–$CHCl_3$ (2:8) to give 4-hydroxyacetophenone (**2**) (1.4 mg). The aqueous layer afforded glucose and xylose, which was identified by GC comparison with authentic samples after trimethylsilylation.

Acid Hydrolysis of 10 Bungeiside-D (**10**) (4.0 mg) was refluxed with 5% aqueous HCl solution (0.5 ml) for 2 h. The reaction mixture was extracted with $CHCl_3$, and the extract was dried over anhydrous $MgSO_4$ and evaporated under reduced pressure to give a syrup. The residue was separated by preparative TLC with MeOH–$CHCl_3$ (2:8) to give 2,4-dihydroxyacetophenone (**1**) (1.0 mg). The aqueous layer afforded glucose and xylose, which were identified by GC comparison with authentic samples after trimethylsilylation.

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