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Two novel neo-clerodane diterpenoids from *Scutellaria barbata*

Hanna Lee [a], YuJin Kim [a], Inho Choi [a], Byung Sun Min [b], Sang Hee Shim [a,*]

[a] School of Biotechnology, Yeungnam University, 214-1 Dae-dong, Gyeongsan, Gyeongbuk 712-749, South Korea
[b] College of Pharmacy, Catholic University of Daegu, Gyeongsan, Gyeongbuk 712-702, South Korea

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ABSTRACT

Two novel neo-clerodane diterpenoids, barbatellarines A (**1**) and B (**2**), were isolated from the whole plants of *Scutellaria barbata*, along with the known compound scutebarbatine F (**3**). The chemical structures and relative stereochemistry of the isolated compounds were established by NMR (1D and 2D) and mass spectroscopic analyses. Compounds **2** and **3** were evaluated for in vitro cytotoxic activity against the HL-60 (human leukemia), MCF7 (human breast cancer), and LLC (Lewis lung carcinoma) cancer cell lines. Compound **2** exhibited weak cytotoxic activity against HL-60 cells, with an IC_{50} value of 41.4 μM.

The traditional Chinese medicinal herb 'Banzhilian', derived from the dry whole plant of *Scutellaria barbata* D. Don, is commonly used for the treatment of tumors, hepatitis, cirrhosis and other diseases.[1] Previous investigations of this plant have revealed the presence of over 30 flavonoids, more than 10 neo-clerodane-type diterpenoids, triterpenoids, and sterol glucosides, some of which exhibit interesting biological activities.[2–10] In the course of our search for anti-tumor compounds, chemical investigation of *S. barbata* led to the isolation of two novel neo-clerodane-type diterpenoids, barbatellarines A (**1**) and B (**2**), along with the known compound scutebarbatine F (**3**).[5] Details of the isolation, structural elucidation, and cytotoxic activities are described herein.

Dried whole plants of *S. barbata* (1 kg) were extracted with MeOH (3 h × 5), and the methanolic extract was suspended in water and then partitioned successively with *n*-hexane, chloroform, ethyl acetate, and *n*-butanol. Fractionation of the chloroform extract of the plant by silica gel column chromatography followed by repeated reverse-phase HPLC led to the isolation of barbatellarines A (**1**) and B (**2**).[11]

Barbatellarine A (**1**)[12] was obtained as a white amorphous powder and gave a quasimolecular ion $[M+Na]^+$ at *m/z* 593.2358 (calcd 593.2363) in the positive HRFABMS, consistent with the molecular formula $C_{31}H_{38}O_{10}$ (13 unsaturations). The NMR data (Table 1) revealed the presence of four singlet methyl groups, two acetyl groups, one benzoyl group, one lactone group, and one olefin group. Accordingly, a tetracyclic structure was required to fulfill the unsaturation requirement in compound **1**. neo-Clerodane diterpenoids have been isolated previously from *Scutellaria* sp., and compound **1** exhibited signals consistent with this class of compounds: four tertiary methyl singlets at δ_H 1.30, 1.20, 1.16, and 1.10, the 13-spiro-15,16-γ-lactone moiety at δ_H 4.13 (for an oxymethylene), and an isolated methylene group at δ_H 3.05 and 2.62. Three isolated spin systems were identified through detailed examination of ^1H–^1H COSY data. The first spin system included the signals of a disubstituted double bond (δ_H 5.64, δ_C 128.0; δ_H 5.50, δ_C 134.9) coupled with an oxymethine signal (δ_H 5.86, δ_C 71.2), which was in turn vicinally coupled with a non-oxygenated sp^3 methine (δ_H 3.32, δ_C 36.9). This CH–CH–CH–CH spin system corresponded to the C10/C1–C3 unit in the neo-clerodane skeleton, which was verified by HMBC correlations from H-3 to C-1, C-2, and C-5 and from H-10 to C-1, C-4, C-5, and C-19. The weak correlation signal between H-1 and H-2 in the ^1H–^1H COSY spectrum and its small coupling constant (1.8 Hz) indicated that the dihedral angle was almost 90° according to the Karplus equation. In addition, HMBC correlations from H-1 to a benzoyl carbonyl carbon at δ_C 165.7 indicated attachment of the benzoyl group at C-1.

The second isolated spin system comprised two oxygenated methines at δ_H 5.90 and 5.26, corresponding to C6–C7 in the neo-clerodane diterpenoid, which was verified by HMBC correlations from H-6 to C-5, C-7, and C-19. In addition, HMBC correlations from H-6 to one ester carbonyl carbon (δ_C 171.6) and from H-7 to the other ester carbonyl carbon (δ_C 170.7) indicated that two acetyl groups were attached to C-6 and C-7, respectively.

The third isolated spin system CH_2–CH_2 was connected to quaternary carbons C-9 and C-13, respectively, based on HMBC correlations from H_3-20 to C-11 and from H_2-14 and H_2-16 of the γ-lactone ring to C-12, respectively. This result indicated that this isolated CH_2–CH_2 system connected the decalin moiety of the neo-clerodane skeleton with the γ-lactone moiety. Furthermore, the decalin moiety was

* Corresponding author. Tel.: +82 53 810 3028; fax: +82 53 810 4769.
E-mail address: shshim29@ynu.ac.kr (S.H. Shim).

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Table 1
NMR data for barbatellarine A (**1**) and barbatellarine B (**2**)[a]

Position	Barbatellarine A		Barbatellarine B	
	δ_H[a] (mult., *J* in hertz)	δ_C[a]	δ_H[a] (mult., *J* in hertz)	δ_C[a]
1α	5.86 (br d, 10.2)	71.2	2.00 (br d, 13.8)	19.4
1β			1.84 (m)	
2	5.64 (dd, 10.2, 1.8)	128.0	2.16 (m)	25.8
3	5.50 (d, 10.2)	134.9	5.32 (s)	123.1
4		72.8		141.2
5		47.3		43.0
6	5.90 (d, 10.8)	71.1	5.78 (d, 10.2)	75.7
7	5.26 (d, 10.8)	73.3	5.69 (d, 10.2)	75.9
8		80.8		78.8
9		38.4		47.4
10	3.32 (d, 10.2)	36.9	2.43 (br d, 12)	40.6
11α	2.00 (dt, 3.0, 14.0)	29.13	5.65 (br d, 10.8)	74.3
11β	1.70 (dt, 3.0, 14.0)			
12α (12A)	1.53 (td, 3.6, 14.0)	29.07	3.57 (d, 15.6)	32.9
12β (12B)	2.27 (td, 3.0, 14.0)		2.68 (dd, 10.8, 15.0)	
13		76.8		167.7
14α	2.62 (d, 18.0)	44.0	5.88 (s)	116.8
14β	3.05 (d, 18.0)			
15		173.9		173.7
16A	4.13 (s)	76.5	4.88 (d, 17.4)	73.0
16B	4.13 (s)		4.68 (d, 17.4)	
17	1.16 (s)	19.7	1.02 (s)	16.1
18	1.20 (s)	25.5	1.59 (s)	20.3
19	1.30 (s)	13.5	1.46 (s)	17.3
20	1.10 (s)	21.4	1.31 (s)	22.4
1′		165.7		164.7
2′		129.9		125.9
3′	7.93 (d, 7.8)	129.5	8.96 (s)	150.6
4′	7.44 (t, 7.8)	128.7		
5′	7.58 (t, 7.8)	133.6	8.62 (s)	153.3
6′	7.44 (t, 7.8)	128.7	7.20 (s)	123.3
7′	7.93 (d, 7.8)	129.5	8.00 (d, 7.8)	136.7
1″				165.6
2″				128.4
3″, 7″			7.81 (7.8)	129.6
4″, 6″			7.34 (br t, 7.8)	128.4
5″			7.49 (t, 7.2)	133.5
6-OCOCH$_3$		171.6		
6-OCOCH$_3$	2.02 (s)	21.5		
7-OCOCH$_3$		170.7		
7-OCOCH$_3$	2.08 (s)	20.8		
11-OCOCH$_3$				170.8
11-OCOCH$_3$			2.05 (s)	20.8

[a] Data were recorded in $CDCl_3$ at 600 MHz (^{1}H, COSY, HMQC, HMBC) and 100 MHz (^{13}C).

connected to the γ-lactone ring through an ether linkage to form a tetrahydropyran ring, as indicated by the highly downfield-shifted ^{13}C NMR resonances for C-8 and C-13 at δ_C 80.8 and 76.8, respectively, as well as HMBC correlations of H_2-14 and H_2-16 with C-13 and of H_3-20 with the oxygenated quaternary carbon C-8. At this point, all of the oxygenated carbon signals were accounted for, with the exception of a quaternary carbon at δ_C 72.8. HMBC correlations of H-2, H_3-18, and H_3-19 with this quaternary carbon indicated that a hydroxyl group was attached to C-4, even though the hydroxyl proton signal did not appear in the ^{1}H NMR spectrum in $CDCl_3$.

On the basis of the above spectral data, the planar structure of **1** was obtained as shown. The relative stereochemistry of **1** was

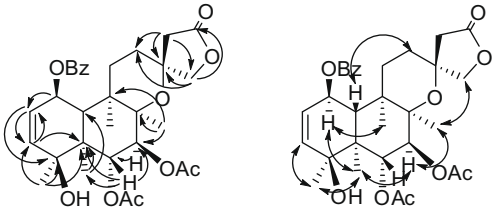

Figure 2. Key HMBC and NOESY correlations observed for barbatellarine A (**1**).

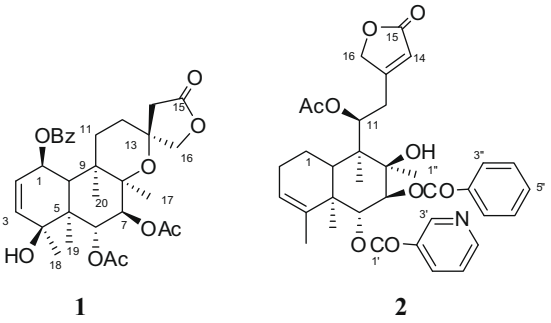

Figure 1. Chemical structures of barbatellarine A (**1**) and B (**2**).

established based on the NOESY spectrum, as well as on coupling constants. Furthermore, NMR assignments for two geminal methylene groups (H_2-11 and H_2-12) were made by analysis of NOESY data as shown in Table 1. Strong NOESY correlations between H-1 and H_3-19 and H_3-20 indicated that the benzoyl group at C-1 was on the face opposite to the methyl groups, as shown in Figure 2. The coupling constant (10.8 Hz) between H-6 and H-7 indicated that both of the protons were in axial positions. Strong NOESY correlations between H-7 and H_3-17 and H_3-19 suggested that the acetyl group at C-7 was in β-configuration. Even though a NOESY correlation between H-6 and H-10 was not observed, the acetyl group at C-6 was deduced to be in α-configuration since the coupling constant indicates that H-6 is in the axial position; this arrangement is analogous to other neo-clerodane diterpenoids with substituents at both C-6 and C-7.[3,4,9] Thus the overall chemical structure of **1** was determined as shown in Figure 1. Individual assignments for all methylene proton signals were made on the basis of NOESY correlations as shown in Table 1.

Barbatellarine B (**2**)[13] was obtained as a white amorphous powder and gave a quasimolecular ion $[M+H]^+$ at *m/z* 618.2702 (calcd 618.2703) in the positive HRFABMS, consistent with the molecular formula $C_{35}H_{39}NO_9$ (17 unsaturations). The NMR data (Table 1) revealed the presence of four singlet methyl groups, one acetyl group, one benzoyl group, one nicotinic ester group, one α,β-unsaturated lactone group, and one olefin group. Among them, four tertiary methyl groups, a nicotinyl ester group, and an α,β-unsaturated lactone were characteristic signals for neo-clerodane diterpenoids from *Scutellaria* sp. The singlet signal at δ_H 5.88 and the isolated oxygenated methylene signals at δ_H 4.68 and 4.88 indicated that **2** had an α,β-unsaturated lactone ring instead of the 13-spiro-15,16-γ-lactone moiety found in **1**.

The ^{1}H–^{1}H COSY spectrum revealed the presence of an isolated spin system CH–CH_2–CH_2–CH corresponding to C10/C1–C3. ^{1}H–^{1}H COSY spectrum revealed another isolated spin system CH–CH, whose position was determined by HMBC correlations as shown in Figure 3. HMBC correlations of H-6 (δ_H 5.78) to the nicotinyl carbonyl carbon (δ_C 164.7) and of H-7 (δ_H 5.69) to the benzoyl carbonyl carbon (δ_C 165.6) indicated that the nicotinyl group and the benzoyl group were attached to C-6 and C-7, respectively. In

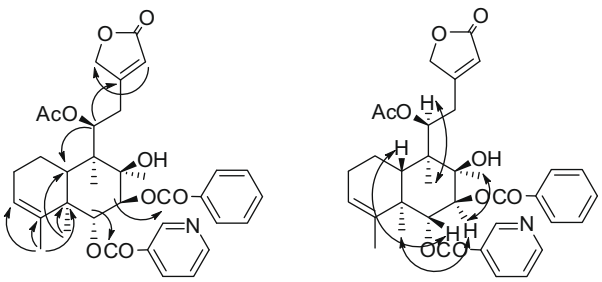

Figure 3. Key HMBC and NOESY correlations observed for barbatellarine B (**2**).

addition, the attachment of a hydroxyl group to C-8 was evident from HMBC correlations of C-8 with H_3-17 and H_3-20, as well as the downfield shift of its ^{13}C NMR resonance (δ_C 78.8).

The analysis of ^1H–^1H COSY data led to the identification of another isolated spin system, CH–CH_2; this system was connected to C-9 and C-13, respectively, based on HMBC correlations of the oxymethine proton at δ_H 5.65 with C-10, C-13, and the acetyl carbonyl carbon, suggesting that the acetyl group was attached to C-11. The planar structure of **2** was established in this way. Barbatellarine B is most closely related to scutebarbatine B,[3] differing only in the reduction at C11–C12 and the addition of an acetyl group at C-11 in place of the C11–C12 double bond found in scutebarbatine B. However, barbatellarine B is structurally similar to scuterivulactone A in terms of the side chain (C11–C16).[1]

The relative stereochemistry of **2** was established by the NOESY spectrum as shown in Figure 3. Strong NOESY correlations between H-10 and H-6 and between H-7 and H_3-17 and H_3-19 indicated that the nicotinyl ester group was in an α configuration while the benzoyl group was in a β configuration. The relative stereochemistry of the decalin moiety in **2** was analogous to that of scutebarbatine B.[3] Furthermore, the stereochemistry of the acetyl group was assigned as a β-configuration based on the strong NOESY correlation between H-11 and H_3-20, which was analogous to that found in scuterivulactone A.[1] As reported for scuterivulactone A,[1] the side chain at C-9 did not move freely but existed in a relatively fixed conformation with respect to the decalin ring.

Even though barbatellarine A (**1**) is a member of the neo-clerodane-type diterpenoids, which have been commonly reported in *Scutellaria* sp., to the best of our knowledge, a neo-clerodane with a double bond between C-2 and C-3 has not been identified yet. Barbatellarine B (**2**) is structurally analogous to scutebarbatine B in the decalin ring moiety, while the side chain at C-9 of **2** is identical to that of scuterivulactone A. It has been reported that the C11–C12 double bond found in scuterivulactone A is formed by the loss of a molecule of acetic acid when the precursor compound is left in a solution of pyridine for several days (or when a solution in $CHCl_3$ is shaken with 28% ammonia water).[1] However, **2** did not form the double bond at C11–C12 when left for days in solution in $CHCl_3$, methanol, and CH_3CN.

Compounds **2** and **3** were evaluated for their in vitro cytotoxic activity against HL-60 (human leukemia), MCF-7 (human breast cancer), and LLC (Lewis lung carcinoma) cancer cell lines using the MTT assay method.[14] Compound **2** exhibited weak cytotoxic activity against HL-60 cells with an IC_{50} value of 41.4 μM using adriamycin as a positive control (IC_{50} = 0.18 μM). Compound **3** was inactive against all cell lines tested, and compound **1** was not evaluated in the cytotoxic activity assay due to the limited amount of sample.

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11. The air-dried whole plant (1 kg) of *Scutellaria barbata* D. Don was finely cut and extracted three times (3 h × 3) with refluxing methanol. Evaporation of the solvent under reduced pressure provided the methanolic extracts, which were partitioned successively between H_2O and *n*-hexane, $CHCl_3$, EtOAc, and *n*-BuOH. The $CHCl_3$-soluble extract was separated into six fractions (fractions I–VI) by chromatography on a silica gel column with a gradient of methanol in methylene chloride. Fraction IV (5.7 g) was further separated by chromatography on a silica gel column with a gradient of cyclohexane–acetone (14.5:0.5→10:5) to afford 26 subfractions (subfractions IV-1–IV-26). Among them, subfraction IV-25 (36.2 mg) was subjected to semi-preparative reverse-phase HPLC (Luna 5u C18 100A column; 250 × 10 mm; flow rate, 2 mL/min; 50–80% CH_3CN in H_2O for 10 min, followed by 80–90% CH_3CN in H_2O for 30 min; UV detection at 254 nm) to afford compound **1** (2.7 mg, t_R = 15.8 min). Fraction V (626.6 mg) was also chromatographed over a silica gel using cyclohexane–acetone (80:20→50:50) as an eluent, which afforded thirteen subfractions (subfractions V-1–V-13). Subfraction V-9 (26.4 mg) was further subjected to semi-preparative reverse-phase HPLC (Luna 5u C18 100A column; 250 × 10 mm; flow rate, 2 mL/min; 20–60% CH_3CN in H_2O for 10 min, followed by 60–80% CH_3CN in H_2O for 30 min; UV detection at 210 nm) to provide compound **2** (6.9 mg, t_R = 21.6 min). Subfraction V-12 (15.8 mg) was further subjected to semi-preparative reverse-phase HPLC (Luna 5u C18 100A column; 250 × 10 mm; flow rate, 2 mL/min; 60–80% MeOH in H_2O for 30 min; UV detection at 210 nm) to provide compound **3** (2.7 mg, t_R = 22.3 min).
12. Barbatellarine A (**1**): white amorphous powder; $[\alpha]_D$ −25.1 (c 1.0 × 10^{-3} g/mL, MeOH); ^1H NMR and ^{13}C NMR, see Table 1; HMBC correlations ($CDCl_3$, H-# → C-#) H-1→C-2 and C-1′; H-2→C-3 and C-4; H-3→C-1, C-2, and C-5; H-6→C-5, C-7, C-19, and 6-O*C*OCH$_3$; H-7→C-6 and 7-O*C*OCH$_3$; H-10→C-1, C-4, C-5, C-11, and C-19; H-12α→C-14 and C-16; H-12β→C-14 and C-16; H-14α→C-12, C-13, and C-15; H-14β→C-12, C-13, and C-15; H_2-16→C-12, C-13, C-14, and C-15; H_3-17→C-7, C-8, C-9, and C-11; H_3-18→C-3, C-4, and C-5; H_3-19→C-5 and C-10; H_3-20→C-9, C-10, and C-11; H-3′, 7′→C-1′, C-2′, C-4′, C-5′, and C-6′; H-4′, 6′→C-2′, C-3′, C-5′, and C-7′; H-5′→C-3′, C-4′, C-6′, and C-7′; 6-OCOC*H*$_3$→6-O*C*OCH$_3$; 7-OCOC*H*$_3$→7-O*C*OCH$_3$; NOESY correlations ($CDCl_3$, H-#→H-#) H-1↔H_3-19 and H_3-20; H-2↔H-3; H-3↔H_3-18; H-7↔H_3-17, H_3-19, and H_3-20; H-10↔H-12β and H-14β; H-11α↔H-11β and H-12α; H-11β↔H-12α and H-12β; H-12α↔H-12β and H-14α; H-14α↔H-14β; H_2-16↔H_3-17; HRESIMS obsd *m/z* 593.2358, calcd for $C_{31}H_{38}O_{10}$+Na, 593.2363.
13. Barbatellarine B (**2**): white amorphous powder; $[\alpha]_D$ −25.0 (c 1.0 × 10^{-3} g/mL, MeOH); ^1H NMR and ^{13}C NMR, see Table 1; HMBC correlations ($CDCl_3$, H-#→C-#) H-1α→C-9; H-1β→C-9; H-2→C-1; H-6→C-7 and C-1′; H-7→C-6 and C-1″; H-10→C-5, C-9, and C-19; H-11→C-10, C-13, and 11-O*C*OCH$_3$; H-12α→C-13, C-14, and C-16; H-12β→C-11 and C-13; H-14→C-15 and C-16; H-16A→C-13 and C-15; H-16B→C-13 and C-15; H_3-17→C-7, C-8, and C-9; H_3-18→C-3, C-4, and C-5; H_3-19→C-4, C-5, C-6, and C-10; H_3-20→C-8, C-9, C-10, and C-11; H-3′→C-2′, C-5′, and C-7′; H-5′→C-3′; H-6′→C-2′, C-5′, and C-7′; H-7′→C-1′ and C-5′; H-3″ and H-7″→C-1″, C-4″, and C-5″; H-4″ and H-6″→C-7″; H-5″→C-3″ and C-4″; 11-OCOC*H*$_3$→11-O*C*OCH$_3$; NOESY correlations ($CDCl_3$, H-#→H-#) H-1α↔H-1β and H-2; H-1β↔H-2 and H-10; H-2↔H-3; H-3↔H_3-18; H-6↔H-10; H-7↔H_3-17 and H_3-19; H-10↔H-12A; H-11↔H_3-20; H-12A↔H-12B; H-16A↔H-16B; H_3-17↔H_3-20; H_3-18↔H_3-19; H_3-19↔H_3-20; H-3′↔H-7′; H-5′↔H-6′; H-6′↔H-7′; H-3″ and H-7″↔H-4″ and H-6″; H-4″ and H-6″↔H-5″; HRFABMS obsd *m/z* 618.2702, calcd for $C_{35}H_{39}NO_9$+H, 618.2703.
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