DOI: 10.1002/ejoc.200900431

Novel Antioxidant *neo*-Clerodane Diterpenoids from *Scutellaria barbata*

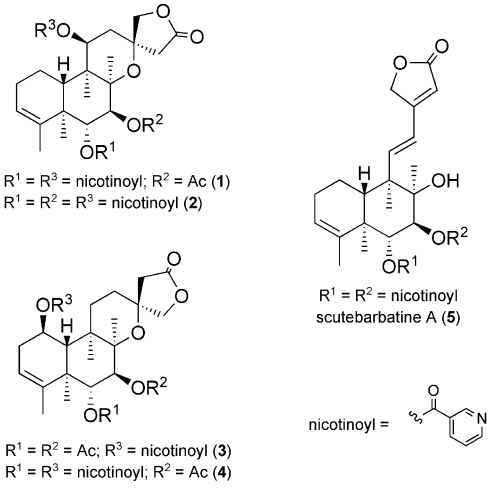
Van Hung Nguyen,*[a] Van Cuong Pham,*[a] Thi Thu Ha Nguyen,[a] Van Hieu Tran,[a] and Thi Mai Huong Doan[a]

Keywords: Terpenoids / Antioxidants / Natural products / Chirality / Structure elucidation

Four new *neo*-clerodane diterpenoids, barbatines A–D (**1**–**4**), have been isolated from the whole plant of *Scutellaria barbata* (Lamiaceae), along with the known scutebarbatine A (**5**), and their structures determined by spectral analysis, including mass spectrometry and 2D NMR spectroscopy. The absolute configurations of **2** and **5** were established from their CD spectra by using the exciton chirality rule. A biogenetic pathway for these compounds is proposed based on their structures. Barbatine A (**1**) and scutebarbatine A (**5**) showed a significant ability to protect cells against H_2O_2 with ED_{50} values of 16.8 and 5.0 μM, respectively.

Introduction

Scutellaria barbata D. Don (Lamiaceae) is widely used in traditional medicine in Vietnam, China, and Korea as an anti-inflammatory and antitumor agent.[1–3] This plant has attracted the attention of much research and *neo*-clerodane diterpenoids,[4–8] alkaloids,[8–11] and flavonoids[12–14] have been isolated from this plant. Apigenin and luteolin have also been isolated as antibiotics against methicillin-resistant *Staphylococcus aureus*.[15,16] Recently, the extracts of *S. barbata* were found to arrest cancer-cell growth in the G1 phase, to induce apoptosis in cancer cells, and to shrink solid cancers.[17,18]



In the course of our research program investigating biologically active compounds of Vietnamese medicinal plants, we isolated four new *neo*-clerodane diterpenoids (**1**–**4**) from the alkaloidal fraction of *S. barbata*, as well as the known compound scutebarbatine A (**5**). Compounds **1** and **5** were found to protect cells from oxidative damage induced by H_2O_2 and all the compounds showed weak cytotoxicity.

Results and Discussion

Compound **1** was obtained as a yellow solid and is optically active. Its IR spectrum indicates the presence of carboxylic functionalities. Its HRESIMS spectrum shows the protonated molecular ion $[M + H]^+$ at m/z = 619.2652. The ^1H and ^{13}C NMR spectra exhibit the signals of 34 carbon atoms, including four tertiary methyl groups resonating at δ_H = 1.21 (20-H), 1.37 (17-H), 1.43 (19-H), and 1.58 ppm (18-H), an acetyloxy group at δ_H = 1.79 ppm (2′′-H) and δ_C = 20.6 (C-2′′) and 170.7 ppm (C-1′′), and two nicotinoyloxy moieties (Table 1). The remaining signals arise from a carboxylic carbon atom, four sp^3 quaternary carbon atoms, four sp^3 methines, five methylenes, and a double bond assigned with the aid of DEPT and HSQC spectra. The signals at δ_C = 83.6 (C-8), 79.0 (C-16), 77.5 (C-13), 75.1 (C-6), 74.7 (C-11), and 74.1 ppm (C-7) are characteristic of carbon atoms bearing oxygen. The ^1H-^1H COSY spectrum reveals connectivities from10-H to 3-H via 1-H and 2-H, from 6-H to 7-H, and from 11-H to 12-H. The planar structure of **1** was deduced from an HMBC spectrum analysis (Figure 1, **A**). Cross-peaks of C-5 (δ_C = 43.2 ppm) with 3-H and 1-H were observed. The two carbon atoms C-18 and C-19 are correlated with 3-H and 6-H, respectively. HMBC correlations [17-H with C-7 (δ_C = 74.1 ppm) and C-9 (δ_C = 43.6 ppm); 20-H with C-8 (δ_C = 83.6 ppm) and C-10 (δ_C = 40.1 ppm)] were used to place the two other methyl groups

[a] Institute of Chemistry, VAST,
18 Hoang Quoc Viet road, Hanoi, Vietnam
Fax: +84-4-38361283
E-mail: phamvc@ich.vast.ac.vn
hungnv@ich.vast.ac.vn

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.200900431.

Me-17 and Me-20. These data indicate the presence of the two rings **a** and **b**. The **a/b** ring fusion was determined from cross-peaks of C-4 with 6-H and of C-9 with 1-H. Moreover, the linkage between C-9 and C-11 was established on the basis of cross-peaks of C-11 with 20-H. ^3J-HMBC correlations [C-13 with 11-H (δ_H = 5.69 ppm), C-16 with 14-H (δ_H = 2.89 and 2.84 ppm) and 12-H (δ_H = 2.23 and 2.13 ppm), and C-15 (δ_C = 173.9 ppm) with 16-H (δ_H = 4.48 and 4.29 ppm)] suggest a spiro system (**c/d**). The correlation of C-1′′ (δ_C = 170.7 ppm) with 7-H reveals the bonding of the acetyloxy group at C-7. Similarly, the two nicotinoyloxy fragments were determined to be linked to C-6 and C-11 by ^3J-HMBC correlations of C-1′ (δ_C = 164.4 ppm) with 6-H and of C-1′′′ (δ_C = 165.1 ppm) with 11-H. The relative configuration of **1** was assigned on the basis of ^{1}H-^{1}H vicinal coupling constant analysis and NOESY experiments. In the ^{1}H NMR spectrum, 10-H appears as a broad doublet (J = 11.0 Hz), which indicates it adopts an axial position on the **a** ring. In the same way, 6-H and 7-H exhibit a strong coupling constant of 10.0 Hz (*anti*) and had a *trans* diaxial relationship. 11-H exhibits coupling constants of 12.5 (*anti*) and 4.0 Hz (*gauche*), and adopts an axial position on the **c** ring. In the NOESY spectrum, spatial interactions of 19-H with 1_{ax}-H and 20-H can be noted. This suggests that C-19 is axial on both the **a** and **b** rings with C-20 being axial on the **b** ring. 11-H shows spatial cross-peaks with 17-H and one of the protons of methylene-14, which indicates that 11-H, C-17, and C-14 are in axial positions on the **c** ring. These analyses permitted the assignment of a *trans*-fused junction for the **a/b** rings and a *cis* **b/c** ring fusion (Figure 1, **B**). This new *neo*-clerodane diterpenoid was named barbatine A and its structure is close to that of 6-*O*-nicotinoyl-7-*O*-acetylscutebarbatine G, except for the inversion of the C-13 chiral center.[10]

Table 1. NMR spectroscopic data for compounds **1** and **2** (^{1}H: 500 MHz, ^{13}C: 125 MHz, $CDCl_3$).

	1		**2**	
Position	δ_C [ppm]	δ_H [ppm], mult. (J [Hz])	δ_C [ppm]	δ_H [ppm], mult. (J [Hz])
1	18.1	2.55, m	18.2	2.59, dd (6.5, 12.0)
		1.82, m		1.84, m
2	26.0	2.17, m	26.1	2.21, m
3	123.4	5.32, br. s	123.5	5.34, br. s
4	140.8		140.7	
5	43.2		43.3	
6	75.1	5.59, d (10.0)	75.2	5.76, d (10.5)
7	74.1	5.42, d (10.0)	75.5	5.65, d (10.5)
8	83.6		83.6	
9	43.6		43.7	
10	40.1	2.62, br. d (11.0)	40.2	2.62, br. d (12.0)
11	74.7	5.69, dd (4.0, 12.5)	74.7	5.72, dd (4.0, 13.0)
12	35.4	2.23, dd (13.0, 13.0)	35.1	2.27, dd (13.0, 13.0)
		2.13, dd (4.0, 13.0)		2.15, dd (4.0, 13.0)
13	77.5		77.7	
14	42.8	2.89, d (17.0)	42.8	2.84, br. s
		2.84, d (17.0)		
15	173.9		173.7	
16	79.0	4.48, d (9.5)	79.0	4.49, d (9.5)
		4.29, d (9.5)		4.33, d (9.5)
17	19.5	1.37, s	19.8	1.43, s
18	20.5	1.58, s	20.5	1.59, s
19	17.3	1.43, s	17.3	1.48, s
20	16.8	1.21, s	16.9	1.27, s
1′	164.4		164.4	
2′	126.0		125.7	
3′	150.7	9.20, d (2.0)	150.7	8.95, d (1.5)
5′	153.8	8.80, dd (2.0, 5.0)	153.5	8.63, dd (1.5, 5.0)
6′	123.5	7.41, dd (5.0, 8.0)	123.1	7.20, dd (5.0, 8.0)
7′	136.9	8.25, dt (2.0, 8.0)	136.6	7.97, dt (1.5, 8.0)
1′′	170.7		165.1	
2′′	20.6	1.79, s	125.5	
3′′			150.6	9.20, d (1.5)
5′′			154.0	8.84, dd (1.5, 4.5)
6′′			123.7	7.46, dd (4.5, 8.0)
7′′			137.3	8.28, dt (1.5, 8.0)
1′′′	165.1		165.0	
2′′′	125.5		124.7	
3′′′	151.0	9.19, d (1.5)	151.1	8.96, d (1.5)
5′′′	154.0	8.84, dd (1.5, 5.0)	153.8	8.67, dd (1.5, 5.0)
6′′′	123.7	7.47, dd (5.0, 8.0)	123.2	7.27, dd (5.0, 8.0)
7′′′	137.3	8.28, dt (1.5, 8.0)	137.1	8.07, dt (1.5, 8.0)

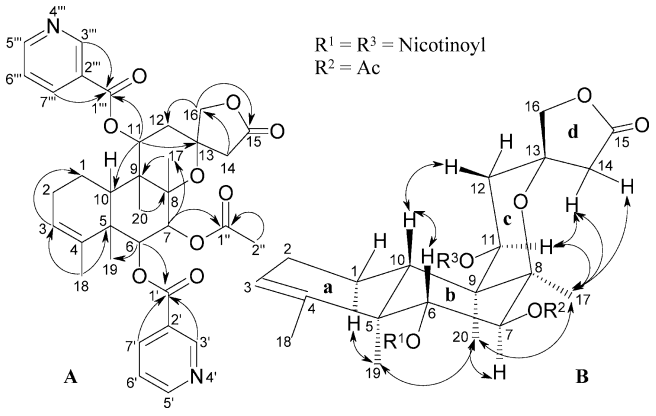


Figure 1. Selected HMBC (A) and NOE (B) correlations of **1**.

Compound **2** is optically active and has a molecular formula of $C_{38}H_{39}N_3O_9$, as determined by HRESIMS analysis. The IR spectrum suggests the presence of a γ-lactone and ester groups. The 1D NMR spectra of **2** are similar to those of **1** except for the replacement of the acetyl group by a nicotinoyl group. Complete interpretation of the ^{1}H and ^{13}C NMR data for **2** with the aid of ^{1}H-^{1}H COSY, HSQC, and HMBC spectra indicate the presence of the *neo*-clerodane skeleton as in compound **1** as well as three nicotinoyloxy moieties (Table 1). The linkage of these nicotinoyloxy fragments to C-6, C-7, and C-11 of the *neo*-clerodane moiety was deduced from the HMBC cross-peaks [correlations of C-1′ (δ_C = 164.4 ppm) with 6-H (δ_H = 5.76 ppm), C-1′′ (δ_C = 165.1 ppm) with 7-H (δ_H = 5.65 ppm), and C-1′′′ (δ_C = 165.0 ppm) with 11-H (δ_H = 5.72 ppm)]. The relative configuration of **2** was determined to be similar to that of **1**. In the ^{1}H NMR spectrum, 10-H appears as a broad doublet with coupling constants of 11.0 (*anti*) and <1.0 Hz (*gauche*), and adopts an axial disposition on the **a** ring. Similarly, 6-H, 7-H, and 11-H adopt axial positions, as deduced from their coupling constants (Table 1). Strong spatial interactions of 14-H with 11-H and 17-H were observed. Hence, C-14 and C-17 are axial on the **c** ring. NOE

interactions of 20-H with 7-H and 19-H were also observed. Thus, C-20 and C-19 are in axial positions on the **b** ring.

The CD spectrum of **2** shows a negative first Cotton effect at 236 nm ($\Delta\varepsilon$ = –28.6 mdeg) and a positive second Cotton effect at 218 nm ($\Delta\varepsilon$ = +4.8 mdeg; Figure 2). By applying the exciton chirality rule[19] to these data, *R* and *S* configurations were assigned to C-6 and C-7, respectively. Based on the relative configuration established above, the absolute configuration 5*R*,6*R*,7*S*,8*R*,9*R*,10*R*,11*S*,13*R* was determined for **2**. This compound was named barbatine B.

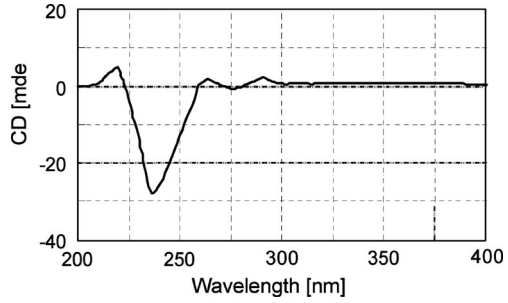


Figure 2. CD spectrum of **2**.

The difference between the ^{1}H chemical shifts of methylene-14 in **1** (δ_H = 2.89 and 2.84 ppm) and **2** (δ_H = 2.84 ppm) can be explained by an aromatic shielding effect of the nicotinoyl ring at C-7 in the structure of **2**, whereas for **1** with an acetyl group at C-7, such an effect is not present. This is in agreement with the lowest-energy conformation of **2** (Figure 3) obtained by the AM1 method with the HyperChem program (v. 8.0.3)[20] in which one of the C-14 protons is oriented towards the plane of the nicotinoyl ring at C-7 and is thus shielded.

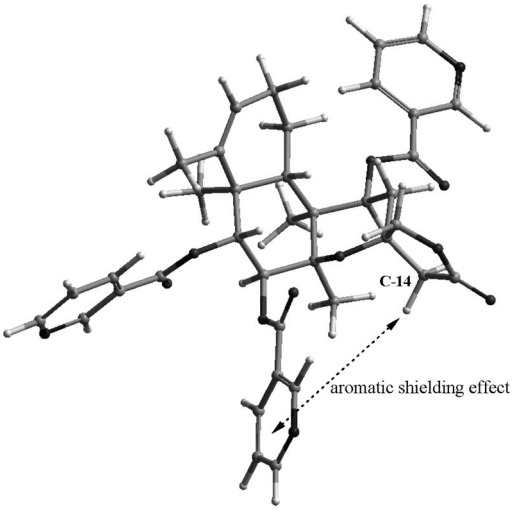


Figure 3. Prefered conformation of **2** according AM1.

Compound **3** was isolated as an optically active yellow solid. Its IR spectrum also suggests the presence of a γ-lactone and ester groups. The HRESIMS spectrum indicates the molecular formula $C_{30}H_{37}NO_9$. Signals of a nicotinoyloxy moiety and two acetyloxy groups were observed in the ^{1}H and ^{13}C NMR spectra. In addition, signals of four tertiary methyl groups, characteristic of the *neo*-clerodane diterpenoid skeleton as in compounds **1** and **2**, were observed at δ_H = 1.08 (20-H), 1.11 (17-H), 1.34 (19-H), and 1.66 ppm (18-H). However, in the ^{1}H NMR spectrum, the signals arising from methylene-14 (δ_H = 3.10 and 2.54 ppm), methylene-16 (δ_H = 4.31 and 4.11 ppm), and methine-1 (δ_H = 5.77 ppm) are significantly different to those observed in the ^{1}H NMR spectra of **1** and **2**. Analyses of the COSY, HSQC, and HMBC spectra revealed the planar structure of **3** in which the nicotinoyloxy is linked to C-1 and the two acetyloxy groups are bonded to C-6 and C-7 of the *neo*-clerodane moiety. 10-H appears as a doublet with a coupling constant of 9.5 Hz (*anti*), which indicates a *trans* diaxial relationship with 1-H. 6-H and 7-H were determined to be in an axial position on the **b** ring on the basis of their large coupling constants (*J* = 10.0 Hz). In the NOESY spectrum, 19-H shows cross-correlation peaks with 1-H and 20-H. This indicates their axial dispositions. 17-H presents spatial interactions with 16-H. Thus, the configuration of the C-13 chiral center of **3** is inverted in comparison with that of compounds **1** and **2**. Complete analysis of the NOESY spectrum revealed *trans*- and *cis*-fused ring junctions of the **a/b** and **b/c** rings, respectively (Figure 4). This *neo*-clerodane was named barbatine C.

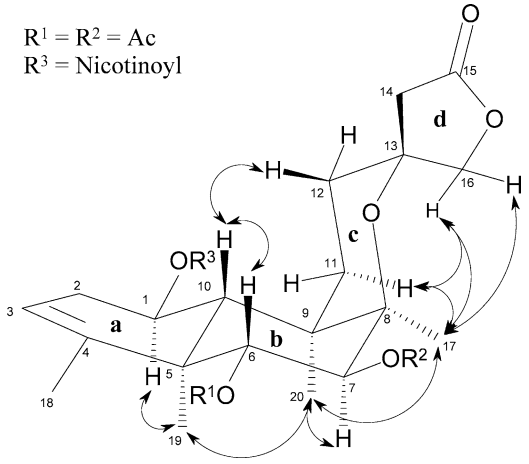


Figure 4. Key NOE correlations of **3**.

Compound **4** is optically active, its molecular formula of $C_{34}H_{38}N_2O_9$ was deduced from the HRESIMS spectrum. The 1D NMR spectra of **4** are similar to those of **3**, except for the presence of a nicotinoyloxy group in place of an acetyloxy group. This suggests that **4** has a *neo*-clerodane skeleton like compound **3**. Analysis of the HMBC spectrum indicated that the two nicotinoyloxy groups are linked to C-1 and C-6 by correlations of C-1′ (δ_C = 164.2 ppm) with 6-H (δ_H = 5.71 ppm) and of C-1′′′ (δ_C = 164.5 ppm) with 1-H (δ_H = 5.84 ppm). The remaining acetyloxy group is bonded to C-7, as indicated by the HMBC cross-peak of C-1′′ (δ_C = 170.8 ppm) with 7-H (δ_H = 5.43 ppm). The relative configuration of **4** is identical to that of **3**, as deduced from an analysis of the $^3J_{H–H}$ coupling constants (Table 2) and NOESY data. 11_{ax}-H shows spatial interactions with 17-H

and one of the C-16 protons revealed a 1,3-diaxial relationship for C-16 and C-17. This compound is reported here for the first time and was named barbatine D.

Table 2. NMR spectroscopic data for compounds **3** and **4** (^{1}H: 500 MHz, ^{13}C: 125 MHz, $CDCl_3$).

	3		**4**	
Position	δ_C [ppm]	δ_H [ppm], mult. (*J* [Hz])	δ_C [ppm]	δ_H [ppm], mult. (*J* [Hz])
1	71.7	5.77, ddd (6.0, 6.0, 9.5)	71.6	5.84, ddd, (6.0, 6.0, 9.5)
2	33.0	2.70, m 2.16, m	33.1	2.76, m 2.22, m
3	119.9	5.29, br. s	120.3	5.33, m
4	143.3		143.2	
5	44.3		44.7	
6	73.1	5.39, d (10.0)	74.3	5.71, d (10.5)
7	74.0	5.23, d (10.0)	73.9	5.43, d (10.5)
8	80.8		80.9	
9	38.6		38.8	
10	43.1	2.71, d (9.5)	43.4	2.81, d (9.5)
11	28.6	2.01, m 1.59, m	28.6	2.06, m 1.64, ddd (3.0, 14.5, 14.5)
12	29.2	2.04, m 1.69, m	29.3	2.09, m 1.75, ddd (4.0, 4.0, 14.5)
13	76.4		76.6	
14	44.2	3.10, d (17.5) 2.54, d (17.5)	44.3	3.15, d (17.5) 2.60, d (17.5)
15	173.5		173.4	
16	76.3	4.31, d (9.0) 4.11, d (9.0)	76.4	4.19, d (8.5) 4.15, d (8.5)
17	19.6	1.11, s	19.6	1.16, s
18	20.0	1.66, s	20.2	1.66, s
19	16.6	1.34, s	16.8	1.52, s
20	21.1	1.08, s	21.1	1.15, s
1′	169.7		164.2	
2′	20.7	1.99, s	126.0	
3′			151.0	9.20, dd (1.0, 2.0)
5′			153.9	8.81, dd (2.0, 5.0)
6′			123.6	7.43, ddd (1.0, 5.0, 8.0)
7′			136.9	8.25, dt (2.0, 8.0)
1″	170.8		170.8	
2″	21.4	2.06, s	20.6	1.79, s
1‴	164.4		164.5	
2‴	126.0		125.9	
3‴	150.7	9.12, d (1.5)	150.8	9.16, dd (0.5, 2.0)
5‴	153.7	8.78, dd (1.5, 4.5)	153.8	8.80, dd (2.0, 4.5)
6‴	123.5	7.41, dd (4.5, 7.5)	123.5	7.41, ddd (0.5, 4.5, 8.0)
7‴	136.8	8.19, dt (1.5, 7.5)	136.8	8.22, dt (2.0, 8.0)

The configurations of the C-13 chiral center in the structures of **1**–**4** should be indicated by the chemical shifts of the methylene-14 and -16 groups. In the cases of **1** and **2**, in which C-14 is in an axial disposition, the chemical shifts of the two protons of C-14 are not significantly different (they even overlap in the case of **2**), whereas for **3** and **4** (the configuration of C-13 is inverted and C-14 is equatorial) the chemical shifts of these protons are remarkably different (Table 2). Moreover, owing to 1,3-diaxial interactions with 11_{ax}-H and methyl-17, the ^{13}C chemical shifts of C-14 for **1** and **2** and of C-16 for **3** and **4** are displaced upfield.

Compound **5** was obtained as an optically active yellow solid. Its HRESIMS spectrum indicates a molecular formula of $C_{32}H_{34}N_2O_7$. The ^{1}H and ^{13}C NMR spectra reveal two nicotinoyloxy moieties and four tertiary methyl groups at δ_H = 1.08 (17-H), 1.29 (20-H), 1.46 (19-H), and 1.58 ppm (18-H). Furthermore, three olefinic protons were observed at δ_H = 5.94 (14-H), 6.40 (11-H), and 6.46 ppm (12-H). Full interpretation of the 1D NMR spectra (Table 3) with the aid of COSY, HSQC, and HMBC spectra revealed that **5** is identical to scutebarbatine A, which has already been isolated from this plant.[8] The *trans* configuration of the double bond C11=C12 was assigned from the large coupling constant (*J* = 17.0 Hz) observed between 11-H and 12-H.

R and *S* configurations were assigned to C-6 and C-7, respectively, by applying the exciton chirality rule[19] to the CD spectrum data of **5** [negative first Cotton effect at 237 nm ($\Delta\varepsilon$ = –24.9 mdeg) and a positive second Cotton effect at 217 nm ($\Delta\varepsilon$ = +5.6 mdeg)]. Taking into account the relative configurations established on the basis of the ^{1}H-^{1}H vicinal coupling constants and NOE interactions, the absolute configuration 5*R*,6*R*,7*S*,8*R*,9*R*,10*R* was defined for **5**.

As **1**, **3**, and **4** were isolated from the same plant as **2** and **5**, the absolute stereochemistry of the chiral centers of the *neo*-clerodane moiety was suggested to be identical for these compounds. As a consequence, based on the relative configurations determined above, compound **1** has the same absolute configuration as **2**, whereas the absolute stereochemistry 1*R*,5*R*,6*R*,7*S*,8*R*,9*R*,10*R*,13*S* was suggested for compounds **3** and **4**.

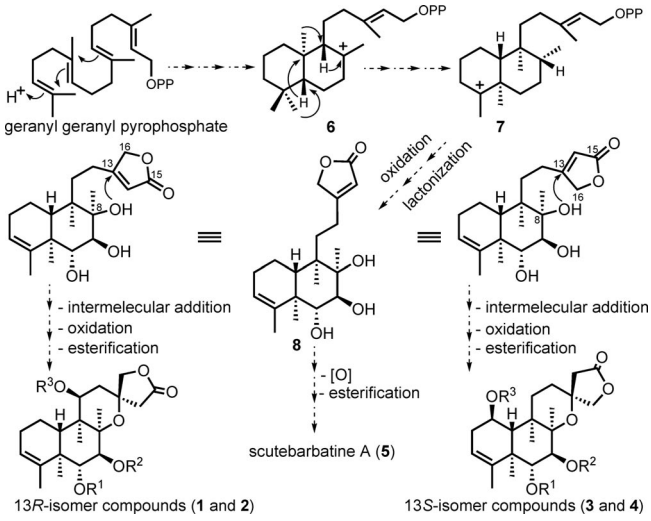
The antioxidant activity of the isolates was examined. Barbatine A (**1**) and scutebarbatine A (**5**) showed an interesting capacity to prevent cell damage induced by H_2O_2 with ED_{50} values of 16.8 and 5.0 μM, respectively. The cytotoxicity of these compounds was also evaluated against different cancer cell lines (KB, MCF-7, and Hep-G2), however, only barbatine D (**4**) slightly inhibited KB cell growth with an IC_{50} value of 32.0 μM.

The *neo*-clerodane diterpenoids **1**–**4** contain a (15→16) lactone moiety. However, compounds **1** and **2** differ from **3** and **4** by an inversion of the chiral center C-13. Based on the structures of the isolates from the *Scutellaria* genus, we can propose a biosynthetic pathway. Clerodanes are believed to be biogenetically closely related to the labdanes. The precursor **6** is biosynthesized by proton-initiated cyclization of geranylgeranyl pyrophosphate. Clerodane **7** is produced from **6** through a series of successive hydride and methyl shifts (Scheme 1).[21] Oxidation of **7** followed by lactonization yield the intermediate **8**, which is oxidized and then esterified with nicotinic acid or nicotinamide adenosine dinucleotide[22,23] to afford scutebarbatine A (**5**). Due to rotation around the C12–C13 bond of intermediate **8**, additive cyclizations of the OH group at C-8 to the double bond lead to the formation of two diastereoisomers with an inverted configuration at C-13 (13*R* for **1** and **2**, and 13*S* in the case of **3** and **4**). The presence of scutebarbatine A (**5**) in *S. barbata* and of several constituents having the same

Table 3. NMR spectroscopic data for compound **5** (^{1}H: 500 MHz, ^{13}C: 125 MHz, $CDCl_3$).

Position	δ_C [ppm]	δ_H [ppm], mult. (*J* [Hz])	Position	δ_C [ppm]	δ_H [ppm], mult. (*J* [Hz])
1	19.3	1.69, m 1.38, m	17	22.5	1.08, s
2	26.2	2.05, m	18	20.1	1.58, s
3	123.4	5.26, br. s	19	17.4	1.46, s
4	140.6		20	15.5	1.29, s
5	43.4		1′	164.8	
6	76.2	5.94, d (10.0)	2′	125.8	
7	76.6	5.74, d (10.0)	3′	150.7	9.00, d (1.5)
8	76.9		5′	153.6	8.65, dd (1.5, 4.5)
9	48.4		6′	123.3	7.23, dd (4.5, 8.0)
10	42.8	2.40, br. d (11.5)	7′	136.7	8.04, dt (1.5, 8.0)
11	146.6	6.40, d (17.0)	1′′	164.7	
12	122.1	6.46, d (17.0)	2′′	124.8	
13	162.0		3′′	151.0	9.04, d (1.5)
14	115.1	5.94, s	5′′	153.8	8.68, dd (1.5, 4.5)
15	174.0		6′′	123.3	7.27, dd (4.5, 8.0)
16	70.7	5.01, s	7′′	137.1	8.10, dt (1.5, 8.0)

skeleton as **8** previously isolated from *S. barbata*,[24] *S. baicalensis*,[25] and *S. Drummondii*[26] supports this hypothesis.



Scheme 1. Proposed biogenetic pathway for *neo*-clerodane diterpenoids of *Scutellaria* genus.

Experimental Section

General: Infrared spectra were recorded as thin films on NaCl plates with a Fourier transform (FTIR) Nicolet Impact 410 spectrometer. Melting points are uncorrected. Optical rotations were measured with a Polartronic D Schmidt + Haensch polarimeter. Mass spectra were recorded with a HB 5989 B series II spectrometer by using the electrospray technique. ^{13}C NMR spectra were recorded with a Bruker AC 500 spectrometer operating at 125.76 MHz. ^{1}H and 2D NMR spectra were recorded with a Bruker Avance 500 spectrometer operating at 500.13 MHz. ^{1}H chemical shifts are referenced to $CHCl_3$ at δ = 7.24 ppm and ^{13}C chemical shifts to the central peak of $CDCl_3$ at δ = 77.0 ppm. For HMBC experiments the delay (1/2*J*) was 70 ms and for the NOESY experiments the mixing time was 150 ms.

Extraction and Isolation: The plant *S. barbata* was collected in Lang Son, Vietnam, in June 2008 and identified by Prof. Nguyen Xuan Phuong. A specimen (PHUONG 16128) was deposited at the Institute of Ecology and Natural Resources, VAST. The dried and ground whole plant of *S. barbata* (2.0 kg) was extracted with CH_2Cl_2 (3 × 4 L) at room temperature. The solvents were removed under reduced pressure. The CH_2Cl_2 solubles (129 g) were suspended in 5% aqueous HCl (250 mL) and washed with EtOAc (3 × 200 mL). The aqueous solution was neutralized with 25% NH_4OH until pH ≈ 8 and then extracted with EtOAc (3 × 100 mL). The EtOAc solubles (2.9 g) were purified by open column chromatography over silica gel and eluted with *n*-hexane/EtOAc (100:0 to 0:100) to afford 18 fractions (1–18). Fraction 13 was subjected to preparative TLC (20% of EtOAc in *n*-hexane) to yield compound **3** (8 mg). Fraction 17 was purified on a Sephadex LH-20 column and eluted with CH_2Cl_2/MeOH (1:9) to provide **1** (15 mg) and **4** (10 mg). Similarly, **2** (11 mg) and **5** (3 mg) were obtained from fraction 18 by purification on a Sephadex LH-20 column (10% MeOH in CH_2Cl_2).

Barbatine A (1): Yellow solid; m.p. 125–127 °C. $[\alpha]_D^{25}$ = –27.2 (*c* = 0.81, $CHCl_3$). IR (thin film, NaCl): $\tilde{\nu}_{max}$ = 2929, 1784, 1724, 1530, 1640, 1435, 1280, 1033 cm^{-1}. UV (MeOH): λ_{max} [log(ε/M^{-1}cm^{-1})] = 219 [4.20], 262 [3.79] nm. HRMS (ESI, +ve): calcd. for $C_{34}H_{39}N_2O_9$ 619.2656 [M + H]$^+$; found 619.2652. For the NMR data, see Table 1.

Barbatine B (2): Yellow solid; m.p. 154–156 °C. $[\alpha]_D^{20}$ = –79.0 (*c* = 2.9, $CHCl_3$). IR (thin film, NaCl): $\tilde{\nu}_{max}$ = 2929, 1785, 1730, 1588, 1422, 1286, 1221, 1104 cm^{-1}. UV (MeOH): λ_{max} [log(ε/M^{-1}cm^{-1})] = 219 [4.48], 263 [4.03] nm. HRMS (ESI, +ve): calcd. for $C_{38}H_{40}N_3O_9$ 682.2765 [M + H]$^+$; found 682.2771. For the NMR data, see Table 1.

Barbatine C (3): Yellow solid; m.p. 130–132 °C. $[\alpha]_D^{20}$ = –40.9 (*c* = 4.2, $CHCl_3$). IR (thin film, NaCl): $\tilde{\nu}_{max}$ = 2982, 1785, 1747, 1722, 1593, 1375, 1252, 1116, 1028, 773 cm^{-1}. UV (MeOH): λ_{max} [log(ε/M^{-1}cm^{-1})] = 218 [4.07], 262 [3.57] nm. HRMS (ESI, +ve): calcd. for $C_{30}H_{38}NO_9$ 556.2547 [M + H]$^+$; found 556.2542. For the NMR data, see Table 2.

Barbatine D (4): Yellow solid; m.p. 119–121 °C. $[\alpha]_D^{25}$ = –43.9 (*c* = 0.66, $CHCl_3$). IR (thin film, NaCl): $\tilde{\nu}_{max}$ = 2923, 1785, 1728, 1591, 1551, 1423, 1278, 1118 cm^{-1}. UV (MeOH): λ_{max} [log(ε/M^{-1}cm^{-1})] = 220 [4.27], 262 [3.76] nm. HRMS (ESI, +ve): calcd. for

$C_{34}H_{39}N_2O_9$, 619.2656 $[M + H]^+$; found 619.2661. For the NMR data, see Table 2.

Scutebarbatine A (5): Yellow solid; m.p. 154–156 °C (ref.[8] 148–150 °C). $[\alpha]_D^{25} = -60.0$ ($c = 0.2$, $CHCl_3$). IR (thin film, NaCl): $\tilde{\nu}_{max}$ = 2966, 1783, 1728, 1626, 1548, 1435, 1289, 1117 cm^{-1}. UV (MeOH): λ_{max} [log (ε/M^{-1} cm^{-1})] = 218 [4.20], 260 [4.24] nm. HRMS (ESI, +ve): calcd. for $C_{32}H_{35}N_2O_7$ 559.2444 $[M + H]^+$; found 559.2446. For the NMR data, see Table 3.

Cell Viability Assay: Rat liver cells (1×10^4) were seeded in each well of a microtiter plate and allowed to attach overnight at 37 °C in air/CO_2 (95:5). Cells were treated with various doses of test samples and plates were maintained for 2 h at 37 °C and then H_2O_2 (100 μM) was added for another 2 h. MTT [3-(4,5-dimethylthiazol-yl-2)-2,5-diphenyltetrazolium bromide] in PBS (phosphate buffered saline) (50 μL) was then added to each well followed by incubation for 4 h at 37 °C. Formazan crystals forming from MTT were dissolved in DMSO. The optical density was determined with a microculture plate reader at 492 nm.[27,28] The ED_{50} values were defined as the dose of a sample that is effective for 50% of the population exposed to the sample.

Cytotoxicity Assays: Cytotoxicity evaluations were performed by following the previously described protocols.[29]

Supporting Information (see footnote on the first page of this article): 1D and 2D NMR spectra of the isolated compounds **1**–**5**.

Acknowledgments

This work was supported by a grant from the Vietnam Academy of Sciences and Technology. We thank Dr. Do Thi Thao (Institute of Biotechnology – VAST) for biological assays and Prof. Vu Xuan Phuong (Institute of Ecology and Natural Resources – VAST) for plant collection and identification.

[1] T. T. Do, T. T. V. Trinh, Q. C. Nguyen, V. H. Nguyen, *J. Med. (Vietnamese)* **2005**, *11*, 10–13.
[2] B. K. H. Tan, J. Vanitha, *Curr. Med. Chem.* **2004**, *11*, 1423–1430.
[3] T. K. Lee, D. K. Lee, D. I. Kim, Y. C. Lee, Y. C. Chang, C. H. Kim, *Int. Immunopharmacol.* **2004**, *4*, 447–454.
[4] S. J. Dai, L. Shen, Y. Ren, *J. Integr. Plant Biol.* **2008**, *50*, 699–702.
[5] T. T. Do, Q. H. Le, K. H. Do, Q. C. Nguyen, V. H. Nguyen, *Asian J. Sci. Technology Development* **2008**, *25*, 478–481.
[6] S. J. Dai, J. Y. Tao, K. Liua, Y. T. Jiang, L. Shen, *Phytochemistry* **2006**, *67*, 1326–1330.
[7] M. Bruno, F. Piozzi, S. Rosselli, *Nat. Prod. Rep.* **2002**, *19*, 357–378.
[8] Z. Q. Wang, F. M. Xu, Y. Zhu, *Chin. Chem. Lett.* **1996**, *7*, 333–334.
[9] S. J. Dai, D. D. Liang, Y. Ren, K. Lui, L. Shen, *Chem. Pharm. Bull.* **2008**, *56*, 207–209.
[10] S. J. Dai, G. F. Wang, M. Chen, K. Liu, L. Shen, *Chem. Pharm. Bull.* **2007**, *55*, 1218–1221.
[11] S. J. Dai, M. Chen, K. Liu, Y. T. Jiang, L. Shen, *Chem. Pharm. Bull.* **2006**, *54*, 869–872.
[12] X. Hu, J. You, C. Bao, H. Zhang, X. Meng, T. Xiao, K. Zhang, Y. Wang, H. Wang, H. Zhang, A. Yu, *Anal. Chim. Acta* **2008**, *610*, 217–223.
[13] M. Sonoda, T. Nishiyama, Y. Matsukawa, M. Moriyasu, *J. Ethnopharmacol.* **2004**, *91*, 65–68.
[14] M. Li-Weber, *Cancer Treat. Res.* **2009**, *35*, 57–68.
[15] Y. Sato, S. Suzaki, T. Nishikawa, M. Kihara, H. Shibata, T. Higuti, *J. Ethnopharmacol.* **2000**, *72*, 483–488.
[16] D. I. Kim, T. K. Lee, I. S. Lim, H. Kim, Y. C. Lee, C. H. Kim, *Toxicol. Appl. Pharmacol.* **2005**, *205*, 213–224.
[17] I. Cohen, USP 200710110832 A1, **2007**; CAPLUS, AN 2007:536005.
[18] X. Yin, J. Zhou, C. Jie, D. Xing, Y. Zhang, *Life Sci.* **2004**, *75*, 2233–2244.
[19] N. Harada, K. Nakanishi. *Circular Dichroic Spectroscopy − an Application for Organic Stereochemistry*, Tokyo Kagaku Dojin, Tokyo, **1982**.
[20] *Hyperchem*, Hypercube, Inc., Gainesville, FL, **2002**.
[21] S. R. Wilson, L. A. Neubert, J. C. Huffman, *J. Am. Chem. Soc.* **1976**, *98*, 3669–3674.
[22] R. M. Smith, *The Alkaloids* (Ed.: R. H. F. Manske), Academic Press, New York, **1977**, vol. XVI, pp. 215–248.
[23] H. J. Lees, G. R. Waller, *Phytochemistry* **1972**, *11*, 2233–2240.
[24] H. Kizu, Y. Imoto, T. Tomimori, T. Kikuchi, S. Kadota, K. Tsubono, *Chem. Pharm. Bull.* **1997**, *45*, 152–160.
[25] B. Esquivel, E. Flores, S. Hernandez-Ortega, R. A. Toscano, *Phytochemistry* **1995**, *38*, 175–179.
[26] A. A. Hussein, M. C. de la Torre, M. L. Jimeno, B. Rodriguez, M. Bruno, F. Piozzi, O. Servettaz, *Phytochemistry* **1996**, *43*, 835–837.
[27] A. C. Mello-Filho, R. Meneghini, *Biochim. Biophys. Acta* **1985**, *847*, 82–89.
[28] R. P. Huang, A. Peng, M. Z. Hossain, Y. Fan, A. Jagdale, A. L. Boynton, *Carcinogenesis* **1999**, *20*, 485–492.
[29] V. C. Pham, A. Jossang, P. Grellier, T. Sévenet, V. H. Nguyen, B. Bodo, *J. Org. Chem.* **2008**, *73*, 7565–7573.

Received: April 18, 2009
Published Online: October 5, 2009

As a result of a technical error, the arrows in Scheme 1 of the Early View version were not converted properly; it has since been corrected.

