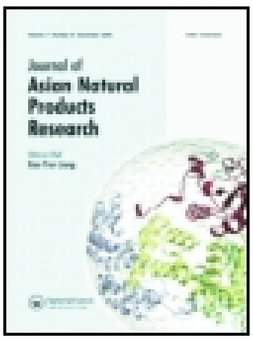


Journal of Asian Natural Products Research

ISSN: 1028-6020 (Print) 1477-2213 (Online) Journal homepage: http://www.tandfonline.com/loi/ganp20

Two new saponins from the leaves of Panax notoginseng

Ji-Wu Huang, Fang-You Chen, Chuang-Jun Li, Jing-Zhi Yang, Jie Ma, Xiao-Guang Chen & Dong-Ming Zhang

To cite this article: Ji-Wu Huang, Fang-You Chen, Chuang-Jun Li, Jing-Zhi Yang, Jie Ma, Xiao-Guang Chen & Dong-Ming Zhang (2017): Two new saponins from the leaves of Panax notoginseng, Journal of Asian Natural Products Research, DOI: 10.1080/10286020.2017.1403421

To link to this article: https://doi.org/10.1080/10286020.2017.1403421

JOURNAL OF ASIAN NATURAL PRODUCTS RESEARCH, 2017
https://doi.org/10.1080/10286020.2017.1403421

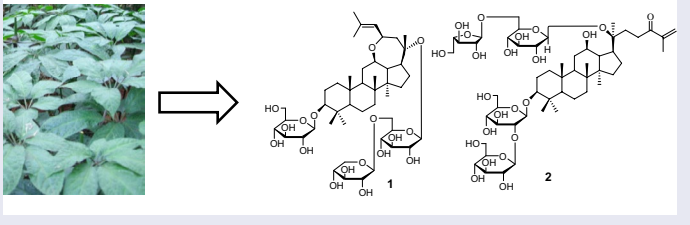
Two new saponins from the leaves of *Panax notoginseng*

Ji-Wu Huang, Fang-You Chen, Chuang-Jun Li, Jing-Zhi Yang, Jie Ma, Xiao-Guang Chen and Dong-Ming Zhang

State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China

ABSTRACT

Two new saponins, notoginsenosides Ng1 (**1**) and Ng2 (**2**), together with seven known compounds (**3–9**), were isolated from the leaves of *Panax notoginseng*. Their structures were elucidated by UV, IR, HRESIMS, and NMR experiments. Compounds **6** and **7** showed moderate cytotoxic activities against HCT-116, with IC_{50} values of 4.98 and 0.64 μmol/L, respectively.



ARTICLE HISTORY
Received 12 September 2017
Accepted 7 November 2017

KEYWORDS
Araliaceae; *Panax notoginseng*; saponins; anticancer

1. Introduction

Panax notoginseng (Burk.) F.H.Chen (Araliaceae), a traditional precious herb, is mainly cultivated in Yunnan Province and Guangxi Zhuang Autonomous Region [1]. Due to its excellent pharmacological effects on the immune system, cardiovascular system as well as anti-inflammatory and anti-tumor activities, chemical investigations have been made since decades ago and various bioactive components including saponins, flavonoids, and cyclopeptides have been isolated from this plant [2]. Modern pharmacological researches have revealed that the dammarane-type saponins are the main bioactive constituents in *P. notoginseng*. Over the past years, most studies were based on the root of this plant, and few phytochemical experiments have been performed on the leaves. For the further development and utilization of this valuable Chinese plant, we collected the air-dried leaves and conducted an extensive research on *P. notoginseng*. In this paper, we report the isolation and structure elucidation of two new saponins, notoginsenosides Ng1 (**1**) and Ng2 (**2**),

CONTACT Dong-Ming Zhang zhangdm@imm.ac.cn

along with seven known compounds notoginsenoside-LX (**3**), notoginsenoside FP2 (**4**), gypenoside-XVII (**5**), notoginsenoside Fe (**6**), ginsenoside Rd (**7**), ginsenoside Rb3 (**8**), and notoginsenoside E (**9**) (Figure 1). Furthermore, the anticancer assay was performed against five human cancer cell lines.

2. Results and discussion

Notoginsenoside Ng1 (**1**) was isolated as white powder. Its molecular formula was established as $C_{47}H_{78}O_{17}$ by the HRESIMS ion peak at *m/z* 937.5150 $[M + Na]^+$. ^1H NMR spectrum of **1** (Table 1) exhibited eight methyl signals (δ_H 0.82, 0.94, 1.01, 1.10, 1.31, 1.51, 1.68, 1.83) and an olefinic proton signal at δ_H 5.54 (1H, d, J = 7.2 Hz). The ^{13}C NMR spectrum of **1** presented 47 carbon signals, of which the anomeric carbons of three sugar moieties (δ_C 99.3, 106.4, and 106.9) were observed. The signals at δ_C 129.2 (C-24) and 131.2 (C-25) showed the existence of two olefinic carbon signals. The ^{13}C NMR data (Table 1) were similar to those of notoginsenoside-LX (**3**) [3], except for the appearance of a xylopyranosyl unit.

Acid hydrolysis of compound **1** provided D-glucose (Glc) and D-xylose (Xyl) [t_R (min): 20.33 and 14.76] by GC. The standard monosaccharide derivatives were made and analyzed by GC under the same condition. Retention times were consistent. According to HSQC, HMBC, and ROESY experiments, two β-D-glucopyranoses (δ_H 4.99 and 5.11) and one β-D-xylopyranose (δ_H 4.99) were identified. In the HMBC spectrum of **1** (Figure 2), the location and sequence of the sugar moieties were observed, with key long-range correlations from δ_H 4.99 (H-1′ of Glc I) to δ_C 88.7 (C-3), from 5.11 (H-1″ of Glc II) to 82.0 (C-20), and from δ_H 4.99 (H-1‴ of Xyl) to 70.8 (C-6″ of Glc II). The correlations between H-23 (δ_H 4.85, 1H, t, J = 8.4 Hz) and C-12 (δ_C 79.6) in HMBC spectrum indicated the presence of the ether linkage between C-23 and C-12. The α-orientation of H-23 and H-12 was demonstrated by the ROESY correlations between H-12 (δ_H 3.68, 1H, overlapped) and H-9 (δ_H 1.48, 1H, overlapped)/H-30 (δ_H 1.10, 3H, s)/H-17 (δ_H, 3.21, 1H, td, J = 10.8, 4.2 Hz), as well as correlations between H-23 (δ_H 4.85, 1H, t, J = 8.4 Hz) and H-12 (δ_H 3.68, 1H, overlapped)/H-17 (δ_H, 3.21, 1H, td, J = 10.8, 4.2 Hz). The ROESY correlations between H-21 (δ_H 1.51, 3H, s) and H-13 (δ_H 1.59, 1H, t, J = 10.2 Hz), as well as between H-13 and H-19 (δ_H 0.82, 3H, s), indicated the configuration of C-21 was β (Figure 2).

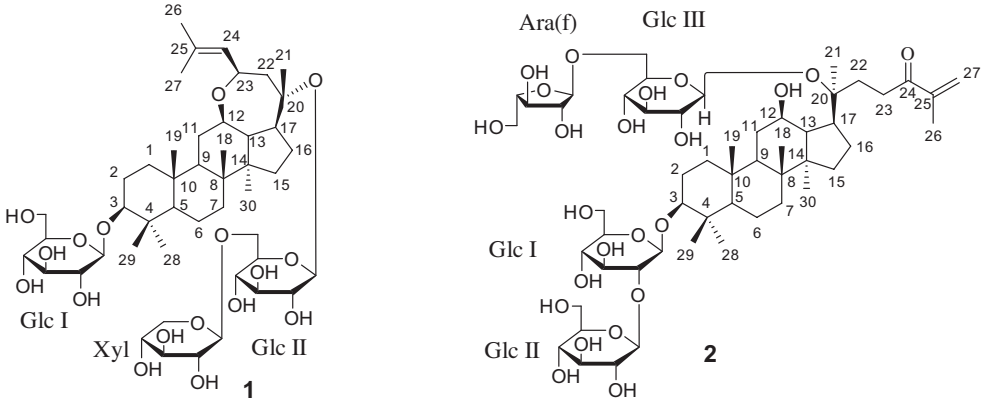


Figure 1. Structures of compounds **1** and **2**.

Table 1. ^{1}H and ^{13}C NMR (600 MHz, 150 MHz in pyridine-d_5) spectral data for compounds **1** and **2.**

Position	**1** δ_C	**1** δ_H (*J* in Hz)	**2** δ_C	**2** δ_H (*J* in Hz)
1	39.4	0.85 *, 1.52 *	39.2	0.73 *t* (13.2), 1.55 *
2	26.8	1.83 *, 2.27 *	26.8	1.82 *, 2.20–2.22 m
3	88.7	3.37 dd (11.4, 2.4)	89.0	3.28 dd (11.4, 4.2)
4	39.7		39.7	
5	56.4	0.73 d (11.4)	56.4	0.65–0.68 m
6	18.5	1.37 *, 1.48 *	18.4	1.36 *, 1.47 *
7	35.2	1.19–1.21 m, 1.37 *	35.1	1.16–1.19 m, 1.47 *
8	39.8		40.0	
9	50.6	1.48 *	50.2	1.36 *
10	37.1		36.9	
11	30.1	1.37 *, 1.92 *	30.9	1.47 *, 1.97 dd (10.8, 4.8)
12	79.6	3.68 *	70.1	4.16 *
13	49.8	1.59 *t* (10.2)	49.5	2.03 *t* (10.8)
14	51.3		51.5	
15	32.6	1.07 dd (11.4, 7.8), 1.48 *	30.7	1.01 *, 1.55 *
16	25.6	2.10–2.14 m, 2.27 *	26.7	1.36 *, 1.82 *
17	46.6	3.21 *t*d (10.8, 4.2)	51.9	2.61 q (10.2)
18	15.5	0.94 s	16.0	0.93 s
19	16.5	0.82 s	16.3	0.82 s
20	82.0		83.2	
21	24.6	1.51 s	21.9	1.59 s
22	52.1	2.27 *, 2.84 *	32.7	3.17 ddd (16.8, 10.2, 6.0),
3.43 ddd (14.4, 9.6, 4.2)				
23	72.6	4.85 *t* (8.4)	29.8	2.10 ddd (15.0, 9.6, 6.0), 2.73–2.77 m
24	129.2	5.54 d (7.2)	202.5	
25	131.2		144.4	
26	26.7	1.68 s	17.8	1.86 s
27	18.9	1.83 s	125.4	5.78 s, 6.37 s
28	28.1	1.31 s	28.1	1.29 s
29	16.8	1.01 s	16.6	1.12 s
30	17.0	1.10 s	17.4	0.97 s
	3-*O*-sugar Glc I	3-*O*-sugar Glc I		
1′	106.9	4.99 *	105.1	4.94 d (7.8)
2′	75.8	4.06 *	83.5	4.26 *
3′	78.9	4.25 *	78.1	4.16 *
4′	71.9	4.25 *	72.0	3.96 *
5′	78.4	4.06 *	78.0	4.34 *
6′	63.1	4.42–4.43 m, 4.62 d (11.4)	62.9	4.34 *, 4.58 d (10.8)
	20-*O*-sugar Glc II	Glc II		
1″	99.3	5.11 d (7.8)	106.1	5.39 d (7.2)
2″	75.3	3.93 td (8.4, 3.0)	77.2	4.16 *
3″	78.8	4.25 *	79.4	4.16 *
4″	71.8	4.16 *	71.7	4.34 *
5″	76.9	4.16 *	78.3	3.96 *
6″	70.8	4.38 *, 4.76 d (10.2)	62.7	4.26 *, 4.50 s
	Xyl	20-*O*-sugar Glc III		
1‴	106.4	4.99 *	98.0	5.13 d (7.8)
2‴	74.9	4.06 *	74.9	3.96 *
3‴	78.3	4.16 *	78.4	4.16 *
4‴	71.1	4.25 *	71.7	4.16 *
5‴	67.2	3.68 *, 4.38 *	76.7	4.09 *
6‴			68.8	4.16 *, 4.72 q (9.6)
			Ara(f)	
1⁗			110.3	5.71 d (1.2)
2⁗			83.5	4.89 br s
3⁗			78.8	4.83 q (6.0)
4⁗			85.9	4.76 d*t* (7.2, 4.2)
5⁗			62.7	4.26 *, 4.34 *

*Overlapped.

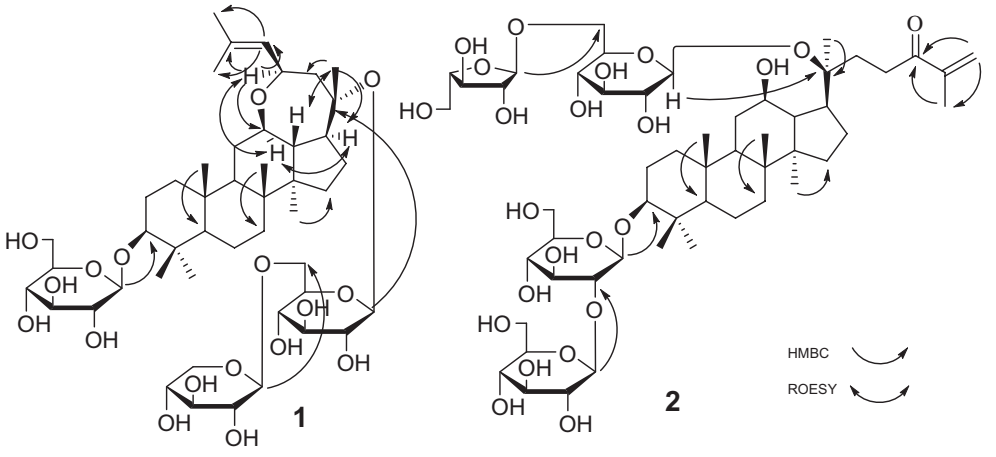


Figure 2. Key HMBC and ROESY correlations of compounds **1** and **2**.

From the above evidence, the structure of compound **1** was elucidated as (20*S*)-3-*O*-*β*-D-glucopyranosyl-20-*O*-*β*-D-xylopyranosyl(1→6)-*β*-D-glucopyranosyl-12*β*,23*R*-epoxydammar-24-ene (Figure 1), named notoginsenoside Ng1.

Notoginsenoside Ng2 (**2**) was also obtained as white powder. Its molecular formula was established as $C_{53}H_{88}O_{23}$ by the HRESIMS ion peak at *m/z* 1115.5613 $[M + Na]^+$. The enone absorption was showed at 219 nm in its UV spectrum. ^{1}H NMR spectrum of **2** (Table 1) showed seven methyl signals (δ_H 0.82, 0.93, 0.97, 1.12, 1.29, 1.59, 1.86), two olefinic proton signals at δ_H 5.78 (1H, s) and 6.37 (1H, s), and four anomeric proton signals at δ_H 4.94 (1H, d, *J* = 7.8 Hz), 5.13 (1H, d, *J* = 7.8 Hz), 5.39 (1H, d, *J* = 7.2 Hz), and 5.71 (1H, d, *J* = 1.2 Hz). The ^{13}C NMR spectrum of **2** showed four anomeric carbon signals (δ_C 98.0, 105.1, 106.1, and 110.3) and two olefinic carbon signals at δ_C 144.4 (C-25) and 125.4 (C-27). The signal at δ_C 202.5 (C-24) showed the existence of a carbonyl group. The carbon signals in the ^{13}C NMR spectrum (Table 1) of **2** were similar to those of notoginsenoside-B [4], except the five signals in **2** due to an arabinofuranosyl unit.

Acid hydrolysis of compound **2** provided D-glucose and L-arabinose (Ara) [t_R (min): 20.33 and 14.79] by GC. The standard monosaccharide derivatives were made and analyzed by GC under the same condition. Retention times were consistent. According to HSQC, HMBC, and ROESY experiments, three *β*-D-glucopyranoses and one *α*-L-arabinofuranose were identified. In the HMBC experiment of **2** (Figure 2), long-range correlations were observed between δ_H 4.94 (H-1′ of Glc I) and δ_C 89.0 (C-3), 5.39 (H-1″ of Glc II) and 83.5 (C-2′ of Glc I), 5.13 (H-1‴ of Glc III) and 83.2 (C-20), 5.71 (H-1⁗ of Ara) and 68.8 (C-6‴ of Glc III).

From the above evidence, the structure of compound **2** was characterized as (20*S*)-3-*O*-[*β*-D-glucopyranosyl(1→2)*β*-D-glucopyranosyl]-20-*O*-[*α*-L-arabinofuranosyl(1→6)*β*-D-glucopyranosyl]-12*β*-hydroxydammar-25-en-24-one, named notoginsenoside-Ng2.

By comparison with literatural data, compounds **3–9** were identified as notoginsenoside-LX (**3**) [3], notoginsenoside FP2 (**4**) [5], gypenoside-XVII (**5**) [6], notoginsenoside Fe (**6**) [7], ginsenoside Rd (**7**) [8], ginsenoside Rb3 (**8**) [8], and notoginsenoside E (**9**) [9], respectively.

Cytotoxic assay of saponins **1**–**9** was carried out by MTT (3-(4,5-dimethylthiazolyl)-2,5-diphenyltetrazolium bromide) method as represented in the reference [10].

3. Experimental

3.1. General experimental procedures

Optical rotations were performed on an Autopol V automatic polarimeter (RUDOLPH, Hackettstown, NJ, U.S.A.). UV spectra were taken with a JASCO V-650 spectrophotometer (JASCO, Tokyo, Japan). IR spectra were recorded on a Nicolet 5700 spectrometer using an FT-IR microscope transmission method (Thermo Electron, Madison, WI, U.S.A.). NMR spectra were acquired with VNS-600 (VARIAN, Palo Alto, CA, U.S.A.). HRESIMS were measured using an Agilent 1100 series LC/MS ion trap mass spectrometer (Agilent Technologies, Santa Clara, CA, U.S.A.). GC was performed on the Agilent technologies 7890A apparatus (Agilent Technologies, Inc, Germany) with flame ionization detector and a HP-5 column (60 m × 0.25 mm). The column temperature was programmed from 200 to 280 °C at a rate of 10 °C/min. Nitrogen was used as the carrier gas at 1.5 ml/min. The injector and detector temperature was at 300 °C and the injection volume was 1.0 µl. MPLC system was composed of two C-605 pumps, a C-635 UV detector (Büchi, Flawil, Switzerland), and an ODS column (YMC, 600 × 60 mm, 50 µm, Kyoto, Japan). Column chromatography was performed with MCI gel (CHP20/P120, Mitsubishi Chemical, Tokyo, Japan), Celite (Sinopharm Chemical Reagent Co., Ltd, Shanghai, China), macroporous resin (HPD-100, Hebei Cangzhou Baoen Chemical Co. Ltd, Cangzhou, China). Preparative HPLC was performed on a Shimadzu LC-6AD instrument with a SPD-20A detector (Shimadzu, Kyoto, Japan) and a reversed-phase C18 column (YMC-Pack ODS-A, 250 × 20 mm, 5 µm, Kyoto, Japan) at 25 °C at a flow rate of 10.0 ml/min using the following eluent MeOH/H_2O 72:28 (HPLC system I); CH_3CN/H_2O 24:76 (HPLC system II), 25:75 (HPLC system III), 35:65 (HPLC system IV), detection at 203 nm.

3.2. Plant material

Panax notoginseng leaves were collected from Wen-Shan, Yunnan, China, in June 2015, and identified by associate Professor Ma Lin of Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College (IMM CAMS&PUMC). A voucher specimen has been deposited at the Herbarium of IMM CAMS&PUMC (ID-22816).

3.3. Extraction and isolation

The air-dried *P. notoginseng* leaves (25 kg) were extracted with 95% ethanol (150 L × 2) and then concentrated under vacuum at 50 °C to give a residue (5.1 kg). The ethanol extract (3.6 kg) was subjected to Celite column (petroleum ether; chloroform; acetone; acetone/EtOH 4:1; acetone/EtOH 1:1; 95% EtOH; MeOH) to afford seven fractions: Fr.A-Fr.G. Fr.E (379 g) was applied to macroporous resin column (HPD-100, 4.0 kg) using EtOH/H_2O (0, 30%, 60%, 95%) to yield 4 fractions, E0-E3. E2 (113 g) was subjected to a MCI gel column (H_2O/EtOH 9:1-95:5) to obtain five fractions, E2A-E2E. E2B (30 g) was applied to a Sephadex LH-20 gel column to afford ten subfractions, E2Ba-E2Bj. E2Bc (10 g) was

loaded onto a MPLC system and eluted with 45–95% MeOH/H_2O (9 h) to yield 25 fractions (B1-B25). The following four compounds were isolated by preparative HPLC: compounds **3** (19.1 mg; t_R 46.0 min), **4** (7.6 mg; t_R 95.0 min), and **2** (8.7 mg; t_R 113.0 min) were purified from fraction B16 by HPLC system II; compound **1** (10.0 mg; t_R 45.0 min) was purified from fraction B18 by HPLC system III. E2D (15 g) was applied to MPLC system and eluted with 60–90% MeOH/H_2O (9 h) to yield 25 fractions (D1-D25). The following five compounds were isolated by preparative HPLC: compounds **9** (4.7 mg; t_R 13.0 min), **8** (19.8 mg; t_R 43.0 min), and **7** (33.3 mg; t_R 57.0 min) were purified from fraction D18 by HPLC system I; compounds **5** (3.0 mg; t_R 35.0 min) and **6** (28.0 mg; t_R 57.0 min) were purified from fraction D20 by HPLC system IV.

3.3.1. Notoginsenoside Ng1 (1)

White powder; $[\alpha]_D^{20}$ −13.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε): 203 (2.58), 254 (2.01) nm; IR (microscope) ν_{max}: 3404, 2942, 1639, 1451, 1380, 1079 cm^{-1}; ^1H NMR (600 MHz, pyridine-d_5) and ^{13}C NMR (150 MHz, pyridine-d_5) spectral data, see Table 1; HRESIMS: *m/z* 937.5150 $[M + Na]^+$ (calcd for $C_{47}H_{78}O_{17}Na$, 937.5131).

3.3.2. Notoginsenoside Ng2 (2)

White powder; $[\alpha]_D^{20}$ +3.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε): 203 (2.84), 219 (2.84), 254 (2.69) nm; IR (microscope) ν_{max}: 3361, 2932, 1732, 1662, 1388, 1074 cm^{-1}; ^1H NMR (600 MHz, pyridine-d_5) and ^{13}C NMR (150 MHz, pyridine-d_5) spectral data, see Table 1; HRESIMS: *m/z* 1115.5613 $[M + Na]^+$ (calcd for $C_{53}H_{88}O_{23}Na$, 1115.5609).

3.4. Acidic hydrolysis

Solutions of compounds **1** and **2** (2.0 mg each) in 2.5 N HCl (4.0 ml) were stirred at 90 °C for 12 h. After cooling, each reaction mixture was diluted to 4.0 ml with water and then extracted by EtOAC (8.0 ml × 3). The aqueous layer was repeatedly evaporated to dryness with water until neutral. The aqueous layer residues were dissolved in anhydrous pyridine (2.0 ml) and stirred with L-cysteine methyl (1.0 mg) for 2 h at 60 °C and then dried by a stream of N_2. Furthermore, 0.5 ml of N-trimethylsilylimidazole was added and the mixture was stirred at 60 °C for another 2 h. After cooling, each reaction mixture was diluted to 2.0 ml with water and then extracted by hexane (4.0 ml × 3). The hexane layer was dried under N_2 steam at room temperature. The residue was dissolved in hexane (1.0 ml), and the solution was analyzed by GC.

3.5. Cytotoxic activities

As the data shown in Table 2, compounds **6** and **7** exhibited moderate cytotoxic activities against HCT-116, with IC_{50} values 4.98 and 0.64 μmol/L, respectively.

Table 2. Cytotoxicities of compounds **6** and **7.**

	IC_{50} (μmol/L)				
Compound	HCT-116	HepG2	BGC-823	A549	A2780
6	4.98	>10	>10	>10	>10
7	0.64	>10	>10	>10	>10
Taxol	<0.01	<0.01	<0.01	<0.01	0.735

Acknowledgments

We are grateful to the Department of Instrumental Analysis of IMM CAMS&PUMC for the measurement of the UV, IR, HRESIMS, and NMR.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This research program was financially supported by the National Natural Science Foundation of China [grant number 81630094] and CAMS Innovation Fund for Medical Sciences (CIFMS) [grant number 2016-I2M-1-010] and [grant number 2016-I2M-2-003].

References

[1] H.Z. Fu, R.J. Zhong, D.M. Zhang, and D. Wang, *J. Asian. Nat. Prod. Res*. **15**, 1139 (2013).
[2] T. Wang, R.X. Guo, G.H. Zhou, X.D. Zhou, Z.Z. Kou, F. Sui, C. Li, L.Y. Tang, and Z.J. Wang, *J. Ethnopharmacol*. **188**, 234 (2016).
[3] D.W. Li, J.Q. Cao, X.L. Bi, X.C. Xia, W. Li, and Y.Q. Zhao, *J. Gins. Res*. **38**, 28 (2014).
[4] M. Yoshikawa, T. Murakami, T. Ueno, K. Yashiro, N. Hirokawa, N. Murakami, J. Yamahara, H. Matsuda, R. Saijoh, and O. Tanaka, *Chem. Pharm. Bull*. **45**, 1093 (1997).
[5] X.Y. Wang, D. Wang, X.X. Ma, Y.J. Zhang, and C.R. Yang, *Helv. Chim. Acta*. **91**, 60 (2008).
[6] R.W. Teng, H.Z. Li, D.Z. Wang, Y.N. He, and C.R. Yang, *Chin. J. Magn. Reson*. **19**, 25 (2002).
[7] T.R. Yang, R. Kasai, J. Zhou, and O. Tanaka, *Phytochemistry*. **22**, 1473 (1983).
[8] H.Z. Li, Y.J. Zhang, and C.R. Yang, *Nat. Prod. Res. Dev*. **18**, 549 (2006).
[9] M. Yoshikawa, T. Murakami, T. Ueno, N. Hirokawa, K. Yashiro, N. Murakami, J. Yamahara, H. Matsuda, R. Saijoh, and O. Tanaka, *Chem. Pharm. Bull*. **45**, 1056 (1997).
[10] N. Ganot, S. Meker, L. Reytman, A. Tzubery, and E.Y. Tshuva, *Jove-J. Vis. Exp*. **81**, e50767 (2013).