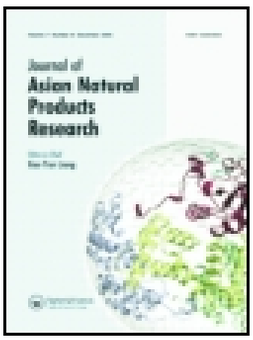


Journal of Asian Natural Products Research

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ganp20

Two new dammarane-type triterpenoids from the steamed roots of *Panax notoginseng*

Chong-Kang Yang , Jiao Xiong , Lian Zhang , Wen-Juan Liang & Yong Shen

To cite this article: Chong-Kang Yang , Jiao Xiong , Lian Zhang , Wen-Juan Liang & Yong Shen (2020): Two new dammarane-type triterpenoids from the steamed roots of *Panax notoginseng* , Journal of Asian Natural Products Research, DOI: 10.1080/10286020.2020.1825391

To link to this article: https://doi.org/10.1080/10286020.2020.1825391

JOURNAL OF ASIAN NATURAL PRODUCTS RESEARCH
https://doi.org/10.1080/10286020.2020.1825391

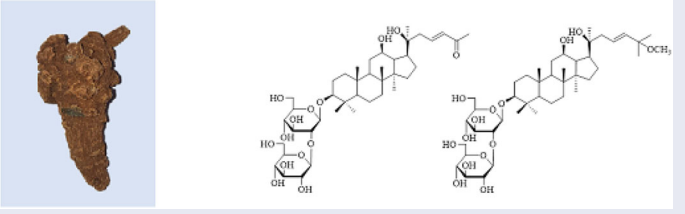
Two new dammarane-type triterpenoids from the steamed roots of *Panax notoginseng*

Chong-Kang Yang[a,b,c,d], Jiao Xiong[a,b,c], Lian Zhang[a,b,c], Wen-Juan Liang[e] and Yong Shen[a,b,c]

[a]Research & Development Department, Kunming Kangren Biotechnology CO., Ltd, Kunming 650033, China; [b]College of Agriculture and Biotechnology, Yunnan Agricultural University, Kunming 650201, China; [c]Research & Development Center for Functional Products, Yunnan Agricultural University, Kunming 650201, China; [d]Quality Management Department, Yunnan Baiyao Group Wuxi Pharmaceutical CO., Ltd, Wuxi 214028, China; [e]College of Food Science and Technology, Yunnan Agricultural University, Kunming 650201, China

ABSTRACT

Two new dammarane-type triterpenoids, notoginsenoside SY3 (**1**) and notoginsenoside SY4 (**2**), were isolated from the steamed roots of *Panax notoginseng*. Their structures were determined to be 3β, 12β, 20(*S*)-trihydroxy-27-anordammar-23(24)(*E*)-ene-3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside (**1**) and 3β, 12β, 20(*S*)-trihydroxy-25-methoxyldammar-23(24)(*E*)-ene-3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside (**2**) by IR, HRESIMS and NMR experiments.



ARTICLE HISTORY
Received 31 May 2020
Accepted 14 September 2020

KEYWORDS
Panax notoginseng; dammarane-type triterpenes; notoginsenoside SY3; notoginsenoside SY4

1. Introduction

Panax notoginseng (Burk.) F. H. Chen, a traditional Chinese medicine known as "Sanqi" in Chinese, is mainly distributed in Yunnan and Guangxi Provinces in China. Besides, it refers to a vital herbal material resource for the drug industry of Yunnan province [1]. The processed *notoginseng*, which is one critical form of this medicinal plant, has been employed as a tonic for its blood cell-increasing function [2]. Ginsenosides 20(*R*/*S*)-Rh_1, Rk_3, Rh_4, 20(*S*/*R*)-Rg_3, Rk_1 and Rg_5, the minor or even trace components in the raw roots, were reported as the main constituents of the processed roots [3, 4]. These mentioned saponins have gained prominence due to

CONTACT Wen-Juan Liang liangwenjuan2007@163.com; Yong Shen pharmsy@sina.com
Supplemental data for this article is available online at https://doi.org/10.1080/10286020.2020.1825391.

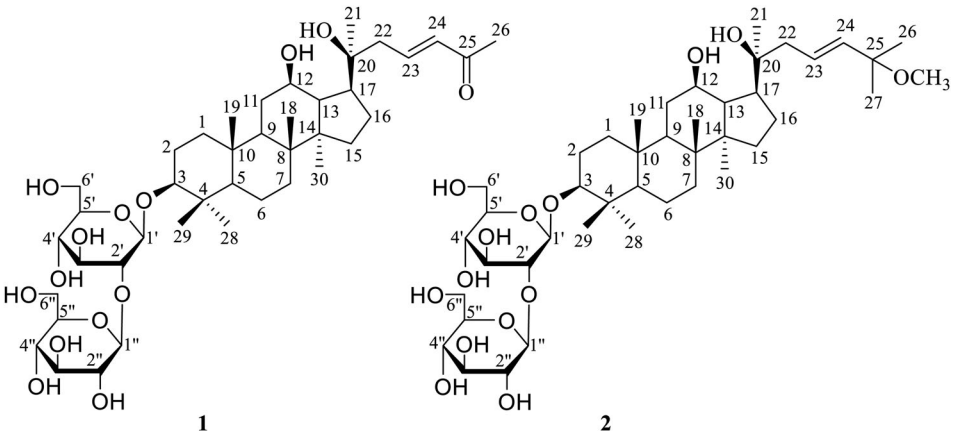


Figure 1. Structures of compounds **1** and **2**.

their potential pharmaceutical values of anticancer and enhancement on immune [5–9]. To be specific, ginsenoside Rg_3 is the main component of 'Shenyi capsule' which has been used for the treatment of cancer in clinic [10]. In the present study, two new saponins, notoginsenoside SY3 (**1**) and notoginsenoside SY4 (**2**) (Figure 1) were isolated from the steamed roots of *P. notoginseng* and the isolation and structural elucidation were also described.

2. Results and discussion

Compound **1** was obtained as white amorphous powder, and its molecular formula was assigned as $C_{41}H_{68}O_{14}$ based on the HRESIMS ion peak at *m/z* 807.4508 $[M+Na]^+$. The IR spectrum exhibited absorption bands for hydroxyl (3408 cm^{-1}), carbonyl (1658 cm^{-1}) and olefinic carbons (1453 cm^{-1}) groups in **1**. Compound **1** displayed 41 resonances in the ^{13}C NMR (DEPT) spectrum (Table 1) attributing to seven methyls, ten methylenes, eighteen methines and six quaternary C-atoms, which covered two anomeric carbons (δ_C 105.6, 106.6) of sugar moieties, a pair of olefinic carbons (δ_C 134.1, 147.4) and one carbonyl carbon (δ_C 198.3). In the ^1H NMR spectrum (Table 1) of **1**, seven singlet methyls [δ_H 0.85, 0.97, 1.06, 1.13, 1.30, 1.39 and 2.26 (each 3H, s)], two olefinic protons [δ_H 6.37 (1H, d, $J=16.0$ Hz) and δ_H 7.40-7.43 (1H, m)], and two anomeric protons at δ_H 4.96 (1H, d, $J=7.5$ Hz, H-1′), 5.41 (d, $J=7.2$ Hz, H-1″) were observed. The large coupling constants of the anomeric protons were compatible with the β-configuration for all sugar moieties [11], which were determined to be D-glucosyl moieties based on acidic hydrolysis experiment. The ^1H and ^{13}C NMR spectral data (Table 1) of compound **1** were identical to those of 3β, 12β, 20(*S*), 25- tetrahydroxydammar-23(24)(*E*)-ene-3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside [12], except a ketone signal (δ_C 198.3) appeared on the side chain of **1** and one methyl signal was lost. Detailed analysis of the HMBC correlations (Figure 2) from the olefinic protons at δ_H 7.40-7.43 (1H, m) and 6.37 (1H, d, $J=16.0$ Hz), and H_3-26 at δ_H 2.26 to the ketone carbonyl carbon at δ_C 198.3 indicated the ketone carbonyl group bound to C-25. Moreover, the long-range correlations between δ_H 4.96 (H-1′) and δ_C 89.4 (C-3), δ_H 5.41 (H-1″) and δ_C 83.9 (C-2′) in

Table 1. ^{1}H and ^{13}C NMR spectral data for compounds **1** and **2**.

	1		2	
Position	δ_C	δ_H	δ_C	δ_H
1	39.6	0.76-0.78 m, 1.51 *	40.6	1.03 *, 1.71 *
2	27.2	1.84-1.86 m, 2.18 *	27.2	1.72 *, 1.85-1.87m
3	89.4	3.32 dt (10.2, 5.0)	91.3	3.19 *
4	40.2		40.9	
5	56.8	0.70 dd (11.8, 2.2)	57.5	0.78 d (10.1)
6	18.9	1.41 *, 1.56 *	19.2	1.46 *, 1.56 *
7	35.6	1.25 *, 1.52-1.53 m	35.9	1.29 *, 1.54 *
8	40.6		41.0	
9	50.9	1.41 *	51.3	1.45 *
10	37.4		37.9	
11	31.7	1.03-1.06 m, 1.56-1.57 m	31.9	1.23-1,25 m, 1.82 *
12	71.4	3.96 *	72.2	3.56 *
13	49.5	2.01 *	51.3	1.75-1.76 m
14	52.3		52.6	
15	32.7	1.03 *, 2.05-2.06 m	32.1	1.03-1.04 m, 1.54 *
16	27.2	1.37 *, 1.90-1.91 m	27.2	1.32 *, 1.97 *
17	54.7	2.38 td, (10.7, 7.1)	54.7	2.02-2.05 m
18	16.3	1.06 s	16.2	1.03 s
19	16.9	0.85 s	16.7	0.85 s
20	73.7		74.4	
21	28.4	1.39 s	27.2	1.11 s
22	40.5	2.51 dd (14.0, 8.9), 2.89 d (14.3)	40.2	2.19-2.21 m, 2.35-2.37 m
23	147.4	7.40-7.43 m	128.2	5.73-5.77 m
24	134.1	6.37 d, (16.0)	138.4	5.47 d (16.0)
25	198.3		76.6	
26	27.1	2.26 s	26.2	1.26 s
27			26.3	1.26 s
28	28.6	1.30 s	28.4	1.06 s
29	17.1	1.13 s	16.8	0.91 s
30	17.5	0.97 s	17.1	0.92 s
OCH_3			50.6	3.15 s
3-O-Glc				
1′	105.6	4.96 d, (7.5)	105.4	4.45 d (7.4)
2′	83.9	4.28 t, (8.7)	81.0	3.55 *
3′	78.8	4.35 *	77.9	3.26 *
4′	72.1	4.17 *	71.9	3.21 *
5′	78.8	3.95 *	78.4	3.38 *
6′	63.3	4.38 *, 4.59 d (11.7)	63.1	3.64 *, 3.81 *
Glc				
1"	106.6	5.41 d (7.2)	104.5	4.68 d (7.5)
2"	77.7	4.17 *	76.3	3.23 *
3"	78.5	4.28 *	78.5	3.62 *
4"	72.1	4.35 *	71.6	3.36 *
5"	78.6	3.95 *	78.4	3.38 *
6"	63.2	4.51 *, 4.52 *	62.8	3.66 *, 3.86 *

*Overlapped.

HMBC spectrum revealed the connectivity between the aglycone and the glycosyl moieties (Figure 2). The geometry of the $\Delta^{23,24}$ double bond was assigned as *E* owing to the large coupling constant between H-23 and H-24 ($J = 16.0$ Hz) [13]. The 20*R* configuration of **1** was confirmed by comparing the chemical shifts of C-17 and C-21 with literature data [11, 12, 14]. Accordingly, the structure of compound **1** was characterized as 3*β*, 12*β*, 20(*S*)-trihydroxy-27-anordammar-23(24)(*E*)-ene-3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranoside, named as notoginsenoside SY3.

Compound **2** was isolated as a white amorphous powder. Its molecular formula was determined to be $C_{43}H_{74}O_{14}$ based on the pseudo-molecular ion at *m/z* 837.4971

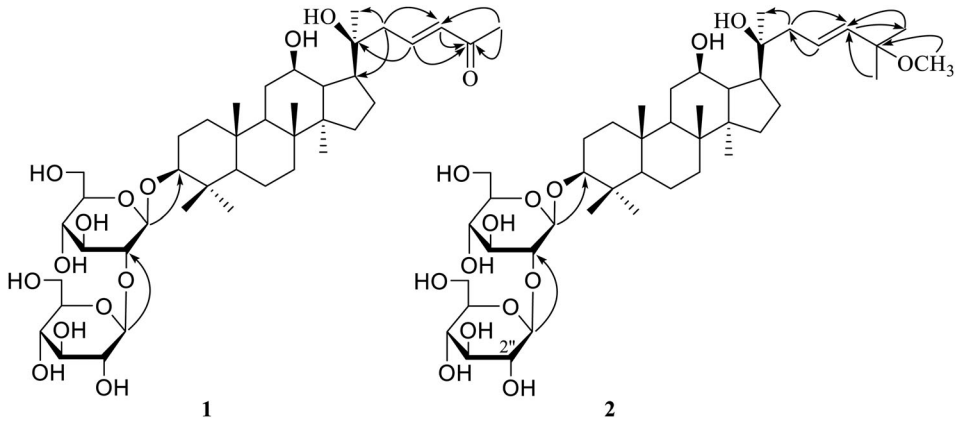


Figure 2. Key HMBC correlations of compounds **1** and **2**.

$[M+Na]^+$. The IR spectrum of **1** displayed strong absorption bands at 3385, 2878 and 1637 cm^{-1} due to hydroxyl, methoxy and olefinic carbonyl functional groups. Compound **2** exhibited 43 resonances in the ^{13}C NMR (DEPT) spectrum, including 8 methyls, a methoxyl (δ_C 50.6), 10 methylenes, 18 methines, as well as 6 quaternary C-atoms. The signals at δ_C 138.4 and 128.2 in the ^{13}C NMR spectrum indicated that two olefinic carbons were present. In the ^1H NMR spectrum (Table 1), seven singlet methyls [δ_H 0.85, 0.91, 0.92, 1.03, 1.06, 1.11, 1.26 × 2 (each 3H, s)], a methoxyl proton [δ_H 3.15 (3H, s)], a pair of olefinic protons [δ_H 5.47 (1H, d, J = 16.0 Hz), 5.73-5.77 (1H, m)] and two anomeric protons signals [δ_H 4.45 (d, J = 7.4 Hz), 4.68 (d, J = 7.5 Hz)] were observed. As revealed from acid hydrolysis of **2**, D-glucose was the only sugar component. According to the coupling constant of the anomeric protons, the β configuration for both of the glucose residues was verified [11]. The ^1H and ^{13}C NMR spectra (Table 1) of compound **2** were similar to those of 3β, 12β, 20(*S*), 25-tetrahydroxydammar-23(24)(*E*)-ene-3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside [12], expect for the appearance of an additional methoxy signal (δ_H 3.15 and δ_C 50.6) in **2**. In detailed inspection of the HMBC spectrum (Figure 2), a cross-peak between δ_H 3.15 (OCH_3) and δ_C 76.6 (C-25) indicated the methoxyl group was at C-25 position. The long-range correlations between the aglycone and the glycosyl moiety of **2** were the same as those of **1**. The chemical shifts of C-17 and C-21 observed at δ_C 54.7 and 27.2, respectively, suggested that the configuration of C-20 was *S* [14]. The *E* configuration for the double bond between C-23 and C-24 was determined as described previously. From the above data, the structure of compound **2** was established to be 3β, 12β, 20(*S*)-trihydroxy-25-methoxyldammar-23(24)(*E*)-ene-3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside, named as notoginsenoside SY4.

3. Experimental

3.1. General experimental procedures

Optical rotations were determined on a Jasco model 1020 polarimeter (Horiba, Tokyo, Japan). IR (KBr discs, cm^{-1}) spectra were recorded on a Bio-Rad FTS-135 spectrometer (Bio-Rad, Hercules, CA). 1D and 2D NMR were recorded on Bruker

DRX-500 or AVANCE 800 spectrometers (Bruker, Bremerhaven, Germany). High resolution electrospray ionization mass spectrum (HRESIMS) were run on a VG Spec3000 spectrometer (VG, Manchester, UK) and Waters AutoSpec Premier P776 (Waters, Milford, MA). D101 macroreticular resin (Tianjin Haiguang Chemical Co., Ltd, Haiguang, Tianjing, China), silica gel (200–300 mesh, Qingdao Makall Chemical Company, Makall, Qingdao, China) and RP C18 silica gel (300–400 mesh, Merck KGaA, Merck, Darmstadt, Germany) were used in the open column chromatography. Gas chromatography (GC) was performed on the Agilent technologies 6890 N apparatus (Agilent, Santa Clara, USA). TLC was carried on the GF254 plates (Makall, Qingdao, China). Analytical-grade reagents of chloroform ($CHCl_3$), ethanol (EtOH), and methanol (MeOH) were purchased from Kemiou Pure Chemical Co. Ltd. (Kemiou Tianjin, China). Fractions were visualized by silica gel plates sprayed with 10% H_2SO_4 ethanol solution followed by heating.

3.2. Plant material

The air-dried roots of *Panax notoginseng* (Burk.) F. H. Chen were collected from Wenshan county, Yunnan province, China, in December 2017, and authenticated by Prof. Qing-Er Yang from South China Botanical Garden, Chinese Academy of Sciences. The voucher specimen (No. YAU 2017-12-1) has been deposited in the College of Agriculture and Biotechnology, Yunnan Agricultural University. The raw plant sample was crushed into small pieces and then steamed at high temperature (120 °C) and pressure (0.12 MPa) for 12 h, yielding the steamed roots of *P. notoginseng* preparation.

2.3. Extraction and isolation

The steamed roots of *P. notoginseng* (15 kg) were extracted with 70% EtOH for three times (3 h × 3) under reflux. After removal of the ethanol under reduced pressure, the ethanolic extract was subjected to D101 resin column, eluting with H_2O and 90% EtOH, successively. The EtOH eluent was combined and concentrated under reduced pressure to give the total saponin fraction (2.1 kg), which was subjected to column chromatography (CC) on silica gel (10 kg, 200–300 mesh) eluted with a gradient $CHCl_3$-MeOH-H_2O (9:1:0.1→7:3:0.5) to provide eight fractions (Frs. A–G). Fr.D (418 g) was carried out on silica gel CC ($CHCl_3$-MeOH-H_2O, 8.5:1.5:0.15) to give six fractions (Frs.D1 − D6). Fr.D3 (8.2 g) was repeatedly chromatographed over silica gel ($CHCl_3$-MeOH-H_2O, 8.5:1.5:0.15) and Rp-18 columns (MeOH-H_2O, 8:2) to obtain **1** (5 mg) and **2** (6 mg).

2.3.1. Notoginsenoside SY3 (1)

White amorphous powder; Libermann-Burchard and Molish reactions were positive; $[\alpha]_D^{22}$ +9.78 (*c* 0.12, MeOH); IRν_{max} 3408, 2968, 2945, 2878, 1658, 1453, 1388, 1367, 1197, 1077, 1034 and 895 cm^{-1}; ^1H NMR (800 MHz, Pyr) and ^{13}C NMR (200 MHz, Pyr) spectral data are shown in Table 1; HRESIMS: *m/z* 807.4508 $[M + Na]^+$ (calcd for $C_{41}H_{68}O_{14}Na$, 807.4501).

2.3.2. Notoginsenoside SY4 (2)

White amorphous powder; Libermann-Burchard and Molish reactions were positive; $[\alpha]_D^{22}$ +8.65 (*c* 0.19, MeOH); IRν_{max} 3385, 2968, 2944, 2878, 1719, 1637, 1453, 1386, 1199, 1076, 1035 and 893 cm^{-1}; ^1H NMR (500 MHz, MeOD) and ^{13}C NMR (125 MHz, MeOD) spectral data are shown in Table 1; HRESIMS: *m/z* 837.4971 $[M + Na]^+$ (calcd for $C_{43}H_{74}O_{14}Na$, 837.4971).

2.4. Acid hydrolysis

The acid hydrolysis of new compounds and sugar determination were based on Gu's method by GC analysis [15]. Compounds **1** and **2** (each 5 mg) were hydrolyzed in 2 M HCl (5 ml) at 65 °C for 6 h, respectively. The reaction mixture was extracted with $CHCl_3$ for three times (3 × 5 ml). The aqueous layer was neutralized with 2 M NaOH and dried to produce a monosaccharide mixture. Then, a solution of the sugar mixture in pyridine (2 ml) was added to L-cysteine methyl ester hydrochloride (1.5 mg) and kept at 60 °C for 1 h. After this, trimethylsilylimidazole (1.5 ml) was added to the reaction mixture in an ice − water bath and kept at 60 °C for 30 min. The mixture was subjected to GC analysis, performed on an Agilent 6890 N gas chromatograph, equipped with a 30 m × 0.32 mm HP-5 column and a H_2 flame ionization detector with the following conditions: column temperature, 200–260 °C; programmed increase, 10 °C/min; carrier gas, N_2 (1 ml/min); injector and detector temperature, 260 °C; injection volume, 1 μl; and split ratio 1/50. The retention times for the authentic samples (Sigma), after being treated in the similar manner, were 19.81 min (D-glucose) and 19.93 min (L-glucose), respectively. The peaks of the hydrolysate of two compounds were detected at t_R 19.82 for D-glucose. Co-injection of each hydrolysate with standard D-glucose gave single peaks.

Acknowledgments

The authors are grateful to the staff of analytical group at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences for the spectral data.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

[1] 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).
[2] C.Z. Gu, J.J. Lv, X.X. Zhang, H. Yan, H.T. Zhu, H.R. Luo, D. Wang, C.R. Yang, M. Xu, and Y.J. Zhang, *Fitoterapia* **103**, 97 (2015).
[3] P.Y. Liao, D. Wang, Y.J. Zhang, and C.R. Yang, *J. Agric. Food Chem.* **56**, 1751 (2008).
[4] D. Wang, P.Y. Liao, H.T. Zhu, K.K. Chen, M. Xu, Y.J. Zhang, and C.R. Yang, *Food Chem.* **132**, 1808 (2012).
[5] B. Chen, and X.B. Jia, *Molecules* **18**, 8109 (2013).

[6] B. Chen, Y.P. Shen, D.F. Zhang, J. Cheng, and X.B. Jia, *Nat. Prod. Res.* **27**, 2351 (2013).
[7] S. Sun, L.W. Qi, G.J. Du, S.R. Mehendale, C.Z. Wang, and C.S. Yuan, *Food Chem.* **125**, 1299 (2011).
[8] S. Sun, C.Z. Wang, R. Tong, X.L. Li, A. Fishbein, Q. Wang, T.C. He, W. Du, and C.S. Yuan, *Food Chem* **118**, 307 (2010).
[9] D.F. Toh, D.N. Patel, E.C.Y. Chan, A. Teo, S.Y. Neo, and H.L. Koh, *Chin. Med.* **6**, 4 (2011).
[10] P. Lu, W. Su, Z-h Miao, H-r Niu, J. Liu, and Q-l Hua, *Chin. J. Integr. Med.* **14**, 33 (2008).
[11] R.G. Teng, H.Z. Li, J.T. Chen, D.Z. Wang, Y.N. He, and C.R. Yang, *Magn. Reson. Chem.* **40**, 483 (2002).
[12] J.R. Wang, L.F. Yau, R. Zhang, Y. Xia, J. Ma, H.M. Ho, P. Hu, M. Hu, L. Liu, and Z.H. Jiang, *J. Agric. Food Chem.* **62**, 2558 (2014).
[13] T.L. Tran, Y.R. Kim, J.L. Yang, D.R. Oh, T.T. Dao, and W.K. Oh, *Bioorg. Med. Chem.* **22**, 499 (2014).
[14] H. Yang, J.Y. Kim, S.O. Kim, Y.H. Yoo, and S.H. Sung, *J. Ginseng Res.* **38**, 194 (2014).
[15] C.Z. Gu, J.J. Lv, X.X. Zhang, Y.J. Qiao, H. Yan, Y. Li, D. Wang, H.T. Zhu, H.R. Luo, C.R. Yang, M. Xu, and Y.J. Zhang, *J. Nat. Prod.* **78**, 1829 (2015).