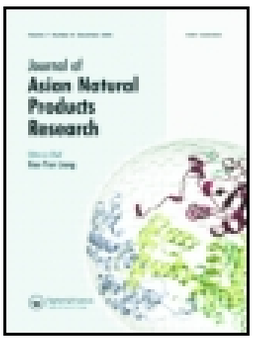

Journal of Asian Natural Products Research

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

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To cite this article: Wen-Xiang Wang, Shi-Ying Luo, Yi Wang, Li Xiang, Xiu-Hua Liu, Ce Tang & Yi Zhang (2020): Pterocephanoside A, a new iridoid from a traditional Tibetan medicine, *Pterocephalus hookeri*, Journal of Asian Natural Products Research, DOI: 10.1080/10286020.2020.1860951

To link to this article: https://doi.org/10.1080/10286020.2020.1860951

Published online: 17 Dec 2020.

JOURNAL OF ASIAN NATURAL PRODUCTS RESEARCH
https://doi.org/10.1080/10286020.2020.1860951

Pterocephanoside A, a new iridoid from a traditional Tibetan medicine, *Pterocephalus hookeri*

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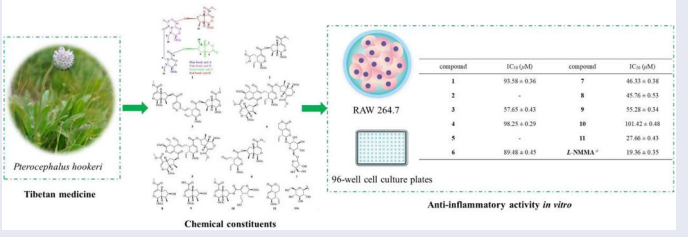
ABSTRACT

This work obtained and identified pterocephanoside A (**1**), one new iridoid glucoside derivative with rare structure of three iridoid glycosides linked to cyclopenta[c]pyran-3(1H)-one, and 10 known iridoids (**2–11**) from *Pterocephalus hookeri* through silica gel column chromatography and semi-preparative HPLC. The structure of the new compound was confirmed by 1D and 2D NMR and HRMS data analysis. Compounds **1** and **2** were isolated from this plant for the first time. The iridoids mostly possessed seco-iridoid subtype and iridoid subtype skeletons from *P. hookeri*. Compounds **1**, **3**, **4**, and **6–11** showed weak anti-inflammatory activity.



ARTICLE HISTORY
Received 10 August 2020
Accepted 3 December 2020

KEYWORDS
Dispsacaceae; pterocephanoside A; traditional Tibetan medicine; *Pterocephalus hookeri*; iridoid glycoside; anti-inflammatory activity

1. Introduction

Pterocephalus genus (Dipsacaceae) is distributed largely in Europe and Asia and comprises approximately 25 species. Among them, *Pterocephalus hookeri* and *P. bretschneideri* are traditional Tibetan medicine mainly distributed in Tibetan of China [1]. As the most commonly used Tibetan medicine, *P. hookeri* has been extensively prescribed for treating common diseases such as flu, cold, enteritis, and rheumatoid

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arthritis [2]. Several triterpenoid saponins [3,4], iridoids [5], monoterpenoids [4], pyridine alkaloids [6], tetrahydrofuranoid lignans [7], and bis-iridoids [6,7] have been identified in this plant by previous chemical investigations.

Total glycosides from *P. hookeri* display potent antiarthritic effect that can be attributed to its anti-inflammatory activity [8]. In this study, 11 iridoid glycosides including 1 novel tetramer containing seco-iridoid and iridoid subtype skeletons (**1**) were obtained from the water extract of *P. hookeri* whole grass. The content of iridoids (55 mg/g) was noticeably higher than that of triterpenoids (12 mg/g) in *P. hookeri* traditional Tibetan medicine [9]. The new compound **1** displayed secoiridoid/iridoid subtype skeletons consisting of secologanic acid condensed to the 7-OH of loganin iridoids. Nitric oxide production inhibition test was employed to gauge the anti-inflammatory activity of these compounds. Compounds **1**, **3**–**4**, and **6**–**11** showed weak anti-inflammatory activity.

2. Result and discussion

The new compound **1** was observed as pale yellow powder with $[\alpha]_D^{25}$ −86.5 (c 0.022, MeOH). Its molecular formula was $C_{58}H_{78}O_{30}$ as indicated by the HR-ESI-MS (Supplementary Figure S7) at *m/z* 1253.4513 $[M-H]^-$. The maximum absorption at 238 nm was displayed in UV spectrum (Supplementary Figure S8). The IR spectrum (Supplementary Figure S13) of **1** showed absorption bands of the hydroxy group at 3412 cm^{-1} and the carbonyl groups at 1694 and 1634 cm^{-1}, respectively. Acid hydrolysis of **1** generated D-glucose identified by GC analysis. The relevant signals of **1** were in accordance with three β-glucopyranose moieties (Table 1) as suggested by the three typical protons acting as doublets at δ_H 4.68 (1H, d, $J = 7.9$ Hz, H-1'A), 4.67 (1H, d, $J = 7.9$ Hz, H-1'B), and 4.66 (1H, d, $J = 7.9$ Hz, H-1'D), respectively [10]. The ^{13}C NMR spectrum (Supplementary Figure S2) showed three glucopyranoside units (δ_C 100.1, 99.5, 100.4, 74.4, 74.7, 74.8, 77.7, 78.0, 78.2, 71.5, 71.5, 71.6, 78.3, 78.3, 78.4, 62.6, 62.8, and 62.9) and 58 carbon resonance signals (Table 2) ascribed to 3 methyls, 10 methylenes, 37 methines, and 8 quaternary carbons.

In the 1D NMR spectra, three singlets at δ_H 7.54 (1H, s, H-3A), 7.48 (1H, s, H-3B), and 7.45 (1H, s, H-3D) together with δ_C 154.1, 151.9, 153.2, 111.0, 109.8, and 112.6 and three carbonyl signals at δ_C 168.2, 168.3, and 169.4 indicated the presence of three olefinic protons of the enols [7,11]. Moreover, protons signals at δ_H 5.57 (1H, d, $J = 4.6$ Hz, H-1A), 5.48 (1H, d, $J = 4.6$ Hz, H-1B), and 5.19 (1H, d, $J = 5.8$ Hz, H-1C) and the carbons at δ_C 97.8, 97.2, and 98.3 based on 1D NMR data (Supplementary Figure S9) established the existence of three protons on hemiacetal [5]. In addition, signals at δ_H 9.31 (1H, s, H-7B) and δ_C 197.1 were ascribed to an aldehyde, and signals at δ_H 6.76 (1H, t, $J = 7.0$ Hz, H-7A) and δ_C 135.3 and 156.1 revealed a trisubstituted olefin. These findings indicated the rationality of α, β-unsaturated aldehyde based on NMR spectra. Its ^1H NMR spectrum (Supplementary Figure S1) uncovered two sets of terminal olefin proton signals at δ_H 5.30 (1H, d, $J = 10.5$ Hz, H-10aA), 5.38 (1H, d, $J = 17.2$ Hz, H-10bA), 5.75–5.77 (1H, m, H-8A), 5.02 (1H, d, $J = 10.5$ Hz, H-10aB), 5.06 (1H, d, $J = 17.2$ Hz, H-10bB), and 5.63–5.65 (1H, m, H-8B) and three sets of β-glucopyranosyl units signals δ_H 3.21–3.92,

Table 1. ^{1}H NMR (700 MHz) spectral data of **1** in CD_3OD.

	Unit A	Unit B	Unit C	Unit D
1	5.57 d (4.6)	5.48 d (4.6)	4.20/4.40 dd (4.1, 11.6)	5.19 d (5.8)
2				
3	7.54, s	7.48, s		7.45, s
4			2.74–2.76, m	
5	3.01–3.03, m	4.09 d (4.6)	2.88–2.90, m	3.01–3.03, m
6	2.36–2.38/3.16–3.18, m		1.49–1.51/2.15–2.17, m	1.61–1.63/2.15–2.17, m
7	6.76 t (7.0)	9.31, s	5.24–5.26, m	5.15 t (5.2)
8	5.75–5.77, m	5.63–5.65, m	2.06–2.08, m	2.13–2.15, m
9	2.79–2.81, m	2.87–2.59, m	2.22–2.24, m	1.97–1.99, m
10	5.30/5.38 d (10.5, 17.2)	5.02/5.06 d (10.5, 17.2)	1.06 d (6.8)	1.01 d (6.8)
11				
11-OMe				3.71, s
1′	4.68 d (7.9)	4.67 d (7.9)		4.66 d (7.9)
2′	3.22 brt (8.6)	3.22 brt (8.6)		3.22 brt (8.6)
3′	3.21–3.41, m	3.21–3.41, m		3.21–3.41, m
4′	3.21–3.41, m	3.21–3.41, m		3.21–3.41, m
5′	3.21–3.41, m	3.21–3.41, m		3.21–3.41, m
6′	3.64–3.70, m	3.64–3.70, m		3.64–3.70, m
6′	3.88–3.92, m	3.88–3.92, m		3.88–3.92, m

Table 2. ^{13}C NMR (150 MHz) spectral data of **1** in CD_3OD.

	unit A	unit B	unit C	unit D
1	97.8	97.2	69.8	98.3
2				
3	154.1	151.9	176.5	153.2
4	111.0	109.8	35.1	112.6
5	33.5	30.7	34.1	33.5
6	29.6	143.6	40.0	40.2
7	156.1	197.1	80.2	77.8
8	135.3	135.6	40.4	41.1
9	45.3	46.6	46.9	46.9
10	120.6	119.4	13.6	14.3
11	168.2	168.3		169.4
11-OMe				51.8
1′	100.1	99.5		100.4
2′	74.4	74.7		74.8
3′	77.7	78.0		78.2
4′	71.5	71.5		71.6
5′	78.3	78.3		78.4
6′	62.6	62.8		62.9

illustrating the existence of three iridoid glucoside units in compound **1**. One methyl group in ^{1}H-NMR spectrum at δ_H 1.01 (3H, d, $J = 6.8$ Hz, H-10D) and two olefinic signals at δ_H 7.45 (1H, s, H-3D) were identified as an iridoid of loganic-type. NMR spectroscopic data for compound **1** revealed four distinct parts (Figure S10) displayed as units A, B, C, and D. Among them, units A, B, and D in **1** were extremely consistent with those in strychoside B in literature [12]. Except for the relative signal of units A, B, and D, one methoxy, three methylene, and four methine protons signals were found in the remaining signals at δ_H 4.20/4.40 (2H, dd, $J = 4.1$, 11.6 Hz, H-1C), 2.74–2.76 (1H, m, H-4C), 2.88–2.90 (1H, m, H-5C), 1.49-1.51/2.15–2.17 (2H, m, H_2-6C), 5.24–5.26 (1H, m, H-7C), 2.06–2.08 (1H, m, H-8C), 2.22–2.24 (1H, m, H-9C), and 1.06 (3H, d, $J = 6.8$ Hz, H_3-10C). In addition, one ester carbonyl group was found

at δ_C 176.5. The above deduction indicated the existence of a 7-methylcyclopenta[c]-pyran-3(1H)-one moiety. Complete structure assignments and further information were obtained from the exhaustive analysis of HSQC, ^{1}H-^{1}HCOSY, and HMBC data (Supplementary Figure S3–S5). δ_H 2.36-2.38/3.16-3.18 (2H, m, H_2-6A) was correlated with δ_H 6.76 (1H, t, $J = 7.0$ Hz, H-7A) and δ_H 3.01-3.03 (1H, m, H-5A) in ^{1}H-^{1}H COSY spectrum (Figure S11). The signals at δ_H 6.76 (1H, t, $J = 7.0$ Hz, H-7A) displayed long-range correlations with the carbons at δ_C 30.7 (C-5B) and 197.1 (C-7B) in the HMBC spectrum (Supplementary Figure S11), indicating the linkage location of units A and B. In addition, long-range correlations were observed between the signals at δ_H 5.15 (1H, t, $J = 5.2$ Hz, H-7D) and the carbon at δ_C 168.3 (C-11B) and between the signal of proton at δ_H 5.24-5.26 and the carbon at δ_C 168.2 (C-11A) in HMBC spectrum. These findings indicated the linkage location of units A and C, units B and D, respectively. The three anomeric protons signals as doublets at δ 4.68, 4.67, and 4.66 exhibited long range correlations with δ_C 97.8 (C-1A), 97.2 (C-1B), and 98.3 (C-1D) could help to correctly determine the linkage location of three glucoses. This location was also supported by long-range heteronuclear correlations of H-1A/C-1'A, H-1B/C-1'B, and H-1D/C-1'D in the HMBC spectrum.

The relative configuration of **1** was ultimately defined by coupling constants and NOESY experiments (Supplementary Figure S6). A J value of 4.0 Hz between H-1b and H-9b implied their *trans*-relationship [13,14]. With the assumption that B-1 had β-configuration, stereochemistries for all positions in unit B were assigned.

The published compounds possess β-configuration at C-1 of the loganin [15,16]. Units A, B, and D were determined as loganin moieties according to NMR data [6,17]. NOESY correlations (Supplementary Figure S12) clearly observed between H-5a and H-9a uncovered their β-orientation in the iridoid skeleton. This orientation was also observed for H-5B and H-9B. Thus, the structure of **1** was determined as shown in Figure 1 and named as pterocephanoside A.

The known compounds **2**–**11** were identified as triplostoside A (**2**) [18], dipasperoside A (**3**) [19], dipsanoside A (**4**) [5], dipsanoside B (**5**) [5], cantleyoside (**6**) [20], 6′-*O*-β-apiofuranosylsweroside (**7**) [21], loganin (**8**) [22], loganic acid (**9**) [23], sylvestroside I (**10**) [15], and sweroside (**11**) [24] according to their NMR data. Previous studies on *P. hookeri* promoted the investigation of its chemical constituents and anti-inflammatory activity. Eleven compounds (**1**–**11**) have been extracted from *P. hookeri*, and their anti-inflammatory activity was evaluated. Compounds **1**, **3**, **4**, and **6**–**11** displayed weak anti-inflammatory activity (Table 3). In particular, the new compound (**1**) had the weakest inhibitory effect with IC_{50} value of 93.58 μM. This work mainly focused on chemical constituents and their anti-inflammatory activity. Iridoids with anti-inflammatory activity are considered as chemotaxonomic markers of *P. hookeri* and thus require further investigation.

3. Experiment

3.1. General experimental procedures

Shimadzu 210 A spectrophotometer was employed to detect UV spectrum (Shimadzu Co., LTD., Shanghai, China). Horiba SEPA-300 digital polarimeter was used to

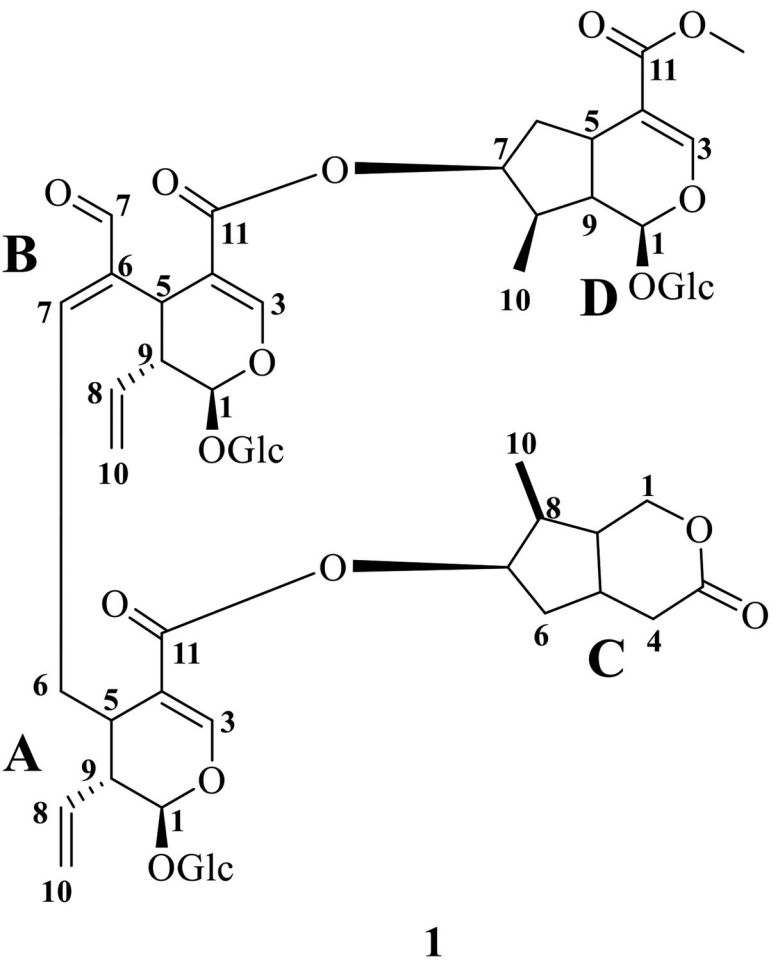

Figure 1. The structure of compound **1** isolated from *Pterocephalus hookeri*.

Table 3. Inhibitory effect of compounds **1 − 11** on LPS induced NO production in RAW 264.7 cells.

Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
1	93.58 ± 0.36	**7**	46.33 ± 0.38
2	—	**8**	45.76 ± 0.53
3	57.65 ± 0.43	**9**	55.28 ± 0.34
4	98.25 ± 0.29	**10**	101.42 ± 0.48
5	—	**11**	27.66 ± 0.43
6	89.48 ± 0.45	***L*-NMMA**[a]	19.36 ± 0.35

[a]Positive control. "—" is stand for the inhibitory rate <50% at a dose of 100 μM.

measure specific rotations (Horiba Co., LTD., Shanghai, China). Bruker DRX-700 spectrometer was applied to survey NMR data with TMS as the solvent peak (Bruker Technologies Co., LTD., Beijing, China). APIQSTAR time-of-flight mass spectrometer was utilized to acquire mass spectra (Agilent Technologies Inc, Beijing, China). Silica gel column chromatography (200–300 mesh) and GF_{254}TLC silica gel plates were employed for isolation as supported by Qingdao Haiyang Chemical Co., Ltd, China.

3.2. Plant material

The whole grass of *Pterocephalus hookeri* was gathered in September 2018 in Tibetan areas, and voucher samples (No. PH20181108–201A) were deposited in Ethnic Medicine Academic Heritage Innovation Research Center of Chengdu University of Traditional Chinese Medicine.

3.3. Extraction and isolation

The whole grass of *P. hookeri* (5 kg) was extracted three times using purified water at metal container. The aqueous extract was evaporated and then extracted sequentially with petroleum ester, ethyl acetate, and *n*-butanol. The *n*-butanol extract (513 g) was eluted with a gradient of ethanol/water (from 0:1 to 1:0, v/v) and chromatographed over D101 macroporous resin to gain five fractions (Frs. A–E). Subsequently, seven sub-fractions (Frs. B1–B7) were obtained from fraction B (60 g) through column chromatography (CH_2Cl_2-CH_3OH, 1:0-0:1, v/v), and compounds **1** (21 mg) and **3** (4 mg) were isolated from fraction B3 through pre-HPLC (CH_3OH/H_2O, 39:61, 3 ml·min^{-1}, 237 nm). Fraction B4 was isolated by preparative TLC with the developing solvent of dichloromethane-methanol-water (8:1:0.2, v/v) and preparative HPLC (CH_3OH–H_2O, 36:64, 3 ml·min^{-1}, 237 nm) to gain **2** (2.9 mg), **5** (3.8 mg), and **6** (5.2 mg). Compounds **4** (3 mg) and **7** (1.8 mg) were separated from Fr. B5a and Fr. B5b of Fr. B5 through preparative HPLC (CH_3OH–H_2O, 36:64, 3 ml·min^{-1}, 237 nm).

3.3.1. Compound 1

Yellowish powder (CH_3OH), $[\alpha]_D^{25}$ −86.5 (c 0.022, MeOH); UV (MeOH) λ_{max} (log ε): 238 (4.25) nm; IR (KBr) ν_{max} 3412, 2923, 2358, 1694, 1291, 1076 cm^{-1}; ^{1}H and ^{13}C NMR spectral data (CD_3OD, 700 MHz for ^{1}H and 175 MHz for ^{13}C) see Tables 1 and 2. HR-ESI-MS: *m/z* 1253.4513 $[M–H]^-$ (calcd for $C_{58}H_{77}O_{30}$, 1253.4500).

3.5. Measurement of anti-inflammatory activity in vitro

Nitric oxide production inhibition test was employed to gauge the anti-inflammatory activity of these *P. hookeri* compounds on macrophages. The Cell Bank of the Chinese Academy of Sciences (Shanghai, China) provided the RAW 264.7 cell line for the cellular experiment. The inhibition activity of NO production was evaluated based on a previous method. The RAW264.7 cells were cultured using Dulbecco's modification of Eagle's medium (DMEM) containing 10% FBS, 100 units/ml penicillin, and 100 μg/ml streptomycin and seeded in 96-well cell culture plates (1.5×10^5 cells/well). The cells were treated with serial dilutions of the compounds with a maximum concentration of 100 μM, followed by stimulation with LPS (1 μg/ml) for 18 h. Griess reagent (reagent A and reagent B, Sigma) was employed to evaluate the NO production of the supernatant. Microplate reader (Thermo, Waltham, MA) was used to gauge the absorbance at 570 nm. Nitric oxide synthase (NOS) inhibitor NG-Methyl-L-arginine acetate salt was applied as the positive control (L-NMMA, Sigma) [25].

3.6. Acid hydrolysis

Pterocephanoside A (2 mg) was processed in 4 ml of 1,4-dioxane/10% HCl (1:1) at 80 °C for 4 h. The mixture was neutralized with 1 M NaOH in MeOH after cooling, the solution was extracted with EtOAc (5 ml × 3) after adding 5 ml of water, and the residue of water layer after evaporating was silylated with pyridine: HMDS: TMCS (9:3:1). Finally, GC was used to determine sugar content by comparing it with the retention time of the standards at 13.15 min.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the National Natural Science Foundation of China [grant number 81903922]; National Key Research and Development Program of China [grant number 2017YFC1703900]; Xinglin Scholars Research Promotion Project of Chengdu University of Traditional Chinese Medicine (BSH2019002).

References

[1] R. Wang, Z.Y. Dong, X.L. Zhang, J.X. Mao, F.C. Meng, X.Z. Lan, Z.H. Liao, and M. Chen, *Toxins* **11**, 142 (2019).
[2] D. Gülcemal, M. Masullo, O. Alankuş-Calişkan, T. Karayildirim, S.G. Senol, S. Piacente, and E. Bedir, *Magn. Reson. Chem.* **48**, 239 (2010).
[3] J. Tian, F.E. Wu, M.H. Qiu, and R.L. Nie, *Phytochemistry* **32**, 1535 (1993).
[4] Y.C. Wu, J. Lu, X.Q.Y. Lu, R. Li, J. Guo, F.J. Guo, and Y.M. Li, *Phytochem. Lett.* **13**, 30 (2015).
[5] X.Y. Tian, Y.H. Wang, S.S. Yu, and W.S. Fang, *Org. Lett.* **8**, 2179 (2006).
[6] Y.C. Wu, C.X. Guo, Y.Z. Zhu, Y.M. Li, F.J. Guo, and G.F. Zhu, *Fitoterapia* **98**, 104 (2014).
[7] Y.C. Wu, Y.J. Ying, C.X. Guo, and G.F. Zhu, *Biochem. Syst. Ecol.* **56**, 209 (2014).
[8] X.F. Shen, Y. Zeng, J.C. Li, C. Tang, Y. Zhang, and X.L. Meng, *Pharm. Biol.* **55**, 560 (2017).
[9] C. Tang, J. Wen, J. Wang, K.H. Zhao, G. Fan, X.L. Meng, Z.M. Zou, and Y. Zhang, *Chin. J. Chin. Mater. Med.* **42**, 1234 (2017).
[10] F.O. Abdullah, F.H. Hussain, M. Clericuzio, A. Porta, and G. Vidari, *Chem. Biodivers.* **14**, e1600281 (2017).
[11] S. Huang, J.F. Zhang, L.H. Shan, Y. Zhang, and X.L. Zhou, *Heterocycles* **94**, 485 (2017).
[12] A. Itoh, N. Oya, E. Kawaguchi, S. Nishio, Y. Tanaka, E. Kawachi, T. Akita, T. Nishi, and T. Tanahashi, *J. Nat. Prod.* **68**, 1434 (2005).
[13] A.J. Chulia, J. Vercauteren, and M. Kaouadji, *Phytochemistry* **36**, 377 (1994).
[14] A.J. Chulia, J. Vercauteren, and A.M. Mariotte, *Phytochemistry* **42**, 139 (1996).
[15] L. Tomassini, S. Foddai, M. Serafini, and M.F. Cometa, *J. Nat. Prod.* **63**, 998 (2000).
[16] H. Tsunao, T. Hideki, and I. Tetsuo, *Phytochemistry* **22**, 1977 (1983).
[17] J.F. Zhang, S. Huang, L.H. Shan, L. Chen, Y. Zhang, and X.L. Zhou, *Chin. J. Org. Chem.* **35**, 2441 (2015).
[18] W.G. Ma, D.Z. Wang, Y.L. Zeng, and C.Y. Yang, *Acta Botanica Yunnanica* **14**, 92 (1992).
[19] F. Li, K. Tanaka, S. Watanabe, Y. Tezuka, and I. Saiki, *Chem. Pharm. Bull. (Tokyo)* **61**, 1318 (2013).

[20] S.R. Jensen, S.E. Lyse-Petersen, and B.J. Nielsen, *Phytochemistry* **18**, 273 (1979).
[21] D. Prasad, V. Juyal, R. Singh, V. Singh, G. Pant, and M.S.M. Rawat, *Fitoterapia* **71**, 420 (2000).
[22] K. Machida, J. Asano, and M. Kikuchi, *Phytochemistry* **39**, 111 (1995).
[23] M. Wu, P. Wu, M.F. Liu, H.H. Xie, Y.M. Jiang, and X.Y. Wei, *Phytochemistry* **70**, 746 (2009).
[24] Q.L. Chen, Z.Y. Shi, G.Z. Tu, and W.J. Sun, *Chin. J. Chin. Mater. Med.* **19**, 43 (2005).
[25] D.W. Reif, and S.A. McCreedy, *Arch. Biochem. Biophys.* **320**, 170 (1995).