Note

Cyprotuoside C and Cyprotuoside D, Two New Cycloartane Glycosides from the Rhizomes of *Cyperus rotundus*

San-qing Lin, Zhong-liu Zhou,* and Chun-Yan Li

School of Chemistry and Chemical Engineering, Lingnan Normal University; 29 Cunjin Road, Zhanjiang 524048, China.

Received July 27, 2017; accepted November 1, 2017; advance publication released online November 9, 2017

Cyprotuoside C (1) and cyprotuoside D (2), two new cycloartane glycosides were isolated from the ethanol extract of the rhizomes of *Cyperus rotundus*. Their structures were identified as 24*R*-9,10-*seco*-cycloartan-1(10),9(11)-dien-3*β*,7*β*,24,25-tetraol 3-*O*-*β*-D-xylopyranosyl-(1→4)-[*α*-L-arabinopyranosyl-(1→6)]-*β*-D-glucopyranosyl-25-*O*-*β*-D-glucuronide (1) and 9,10-*seco*-cycloartan-1(10),9(11),23(24)-trien-3*β*,7*β*,25-triol 3-*O*-*β*-D-xylopyranosyl-(1→4)-{*α*-L-arabinopyranosyl-(1→6)-[*α*-L-rhamnopyranosyl-(1→2)]}-*β*-D-glucopyranosyl-25-*O*-*β*-D-glucuronide (2) by spectroscopic methods.

Key words *Cyperus rotundus*; cyprotuoside C; cyprotuoside D; cycloartane glycoside

Cyperus rotundus (*C. rotundus*), a perennial herb, is widespread in the tropical and subtropical regions all over the world. The rhizomes of *C. rotundus* is a kind of traditional Chinese medicine (TCM) named "Xiangfuzi," which is broadly used in folk medicine as an anti-inflammatory, antidepressant, analgesic, and antiemetic remedy for dysentery and women's diseases.[1–3] Previous investigations confirmed that the phytochemical constituents of *C. rotundus* contained terpenoids, steroids and flavonoids.[4–6] Among them, the terpenoids are the characteristic secondary metabolites.[1] In recent years, we have put our efforts to the discovery of secondary metabolites with various skeletons, in particular triterpene glycosides, from TCM.[7–10] In our ongoing research on the triterpene glycosides of this plant, we isolated two new cycloartane glycosides, cyprotuoside C (**1**) and cyprotuoside D (**2**) (Fig. 1). This paper reports the isolation and structure elucidation of the two new triterpene glycosides.

Results and Discussion

Cyprotuoside C (**1**) was isolated as a white amorphous powder. Its molecular formula, $C_{52}H_{84}O_{23}$, was determined on the basis of its $[M+Na]^+$ peak (*m*/*z* 1099.5303) in the high resolution electrospray ionization (HR-ESI)-MS and ^{13}C-NMR data. IR spectrum showed the absorptions for the hydroxyl (3346 cm^{-1}) and olefin (1650 cm^{-1}) functionalities. A distortionless enhancement by polarization transfer (DEPT) experiment showed that the 52 carbons present in the ^{13}C-NMR spectrum consisted of 7 methyl, 11 methylene, 27 methine, and 7 quaternary carbons. The ^{1}H-NMR spectrum displayed six methyl singlets at δ 0.76, 0.93, 0.99, 1.02, 1.53, 1.58, one methyl doublet at δ 0.94 (3H, d, J=6.4 Hz), two olefinic protons at δ 5.31 (1H, dd, J=4.5, 2.4 Hz) and 5.41 (1H, br d, J=5.5 Hz), and a pair of diagnostic methylene protons at δ 2.74 (1H, d, J=14.4 Hz) and 3.03 (1H, d, J=14.4 Hz). Correspondingly, seven methyl carbons (δ 15.7, 16.1, 19.2, 24.2, 24.8, 21.7 and 19.1), four olefinic carbons (δ 120.2, 139.8, 119.3 and 136.9) and an isolated methylene carbon (δ 45.5) were exhibited in the ^{13}C-NMR spectrum (Table 1), respectively. The above information implied that compound **1** might possess a 9,10-*seco*-cycloartane triterpenoid skeleton.[11–13] Acid hydrolysis of **1** produced a triterpenoid aglycone (**1a**), and D-glucose, D-xylose, L-arabinose and D-glucuronic acid as sugar residues which were identified by comparison with the respective authentic samples by GC analysis. The ^{1}H- and ^{13}C-NMR spectra of **1a** confirmed that the structure of the aglycone moiety (**1a**) was the same as secomacrogenin B[13] (Table 1). Thus, the structure of the aglycone of **1** was determined as 24*R*-9,10-*seco*-cycloartan-1(10),9(11)-dien-3*β*,7*β*,24,25-tetraol. Anomeric region in the ^{1}H- and ^{13}C-NMR spectra of **1** revealed signals for four anomeric protons at δ 4.91 (1H, d, J=8.0 Hz), 5.03 (1H, d, J=7.6 Hz), 5.06 (1H, d, J=7.6 Hz) and 5.56 (1H, d, J=7.8 Hz) with their corresponding anomeric carbons at δ 102.6, 105.3, 105.5 and 105.0, respectively (Table 2). The relative stereochemistry of each monosaccharide was determined as *β*-D-glucose, *β*-D-xylose, *α*-L-arabinose and *β*-D-glucuronic acid based on the coupling constants of anomeric protons and ^{13}C-NMR data of the sugar moieties. The linkage of the sugar residues was deduced from the heteronuclear multiple bond correlation (HMBC) spectrum. HMBC spectrum showed cross peaks between the signals at δ_H 4.91 and δ_C 78.9 (C-3 of the aglycon), δ_H 5.56 and δ_C 79.8 (C-4 of the inner glucose), δ_H 5.06 and δ_C 68.1 (C-6 of the inner glucose) and δ_H 5.03 and δ_C 81.2 (C-25 of the aglycon) (Fig. 2). Therefore, the Xyl-(1→4)-[Ara-(1→6)]-Glc structure of the trisaccharide moiety was concluded to be linked to C-3 of the aglycone, and the glucuronic acid moiety to the C-25 of the aglycone, respectively. The combined application of ^{1}H–^{1}H correlation spectroscopy (^{1}H–^{1}H COSY), total correlation spectroscopy (TOCSY), heteronuclear single quantum correlation (HSQC) and HMBC experiments allowed the sequential assignments of all resonances for each monosaccharide. The rotating frame Overhauser enhancement spectroscopy (ROESY) correlations among H-7, H-5, H-29, H-28 and H-21, and H-3, H-30, H-8 and H-18 established the stereochemistry of H-7*α* and H-3*β* (Fig. 3). Based on all above, the structure of **1** was formulated as 24*R*-9,10-*seco*-cycloartan-1(10),9(11)-dien-3*β*,7*β*,24,25-tetraol 3-*O*-*β*-D-xylopyranosyl-(1→4)-[*α*-L-arabinopyranosyl-(1→6)]-*β*-D-glucopyranosyl-25-*O*-*β*-D-glucuronide, named cyprotuoside C.

Cyprotuoside D (**2**) was obtained as a white amorphous

* To whom correspondence should be addressed. e-mail: zlzhou@hotmail.com

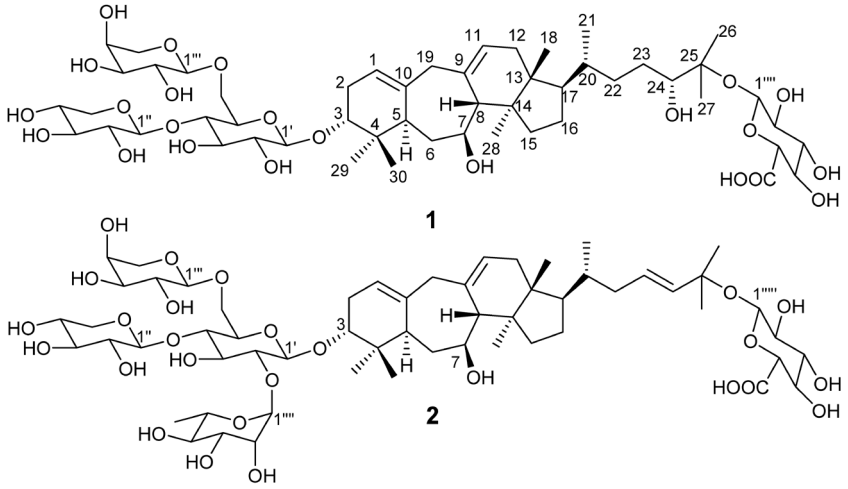

Fig. 1. Chemical Structures of **1** and **2** Isolated from the Rhizomes of *Cyperus rotundus*

Table 1. ^{1}H-NMR (400 MHz) and ^{13}C-NMR Data of the Aglycone Moieties of **1**, **1a** and **2** (δ, ppm: J, Hz)

No.	**1**[a]		**1a**[b]		**2**[a]	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	5.31 (dd, 4.5, 2.4)	120.2	5.31 (dd, 4.8, 2.4)	117.3	5.33 (dd, 4.4, 2.4)	120.4
2	1.87 (m), 2.15 (m)	30.8	1.91 (m), 2.33 (m)	32.4	1.85 (m), 2.19 (m)	31.1
3	3.97 (dd, 9.4, 6.0)	78.9	3.55 (dd, 9.6, 5.8)	75.0	3.99 (dd, 9.6, 6.2)	79.2
4	—	37.1	—	38.21	—	37.6
5	1.72 (dd, 12.4, 5.2)	55.5	1.71 (dd, 12.4, 5.0)	45.3	1.71 (dd, 12.4, 5.0)	55.3
6	1.86 (m), 1.98 (m)	35.2	1.82 (m), 1.91 (m)	36.1	1.84 (m), 1.98 (m)	35.2
7	3.93 (ddd, 12.0, 7.2, 4.6)	74.4	3.91 (ddd, 11.8, 7.2, 4.4)	74.6	3.92 (ddd, 12.0, 7.2, 4.4)	74.4
8	2.18 (d, 12.0)	55.6	2.06 (d, 12.0)	55.7	2.18 (d, 12.0)	55.4
9	—	136.9	—	135.6	—	136.7
10	—	139.8	—	139.4	—	139.8
11	5.41 (br d, 5.5)	119.3	5.41 (d, 6.0)	126.2	5.39 (br d, 5.4)	119.0
12	2.12 (m), 1.95 (m)	34.3	1.97 (m), 2.08 (m)	37.7	2.09 (m), 1.91 (m)	34.1
13	—	45.8	—	46.0	—	45.6
14	—	49.5	—	48.3	—	49.0
15	1.51 (m), 1.59 (m)	33.6	1.50 (m), 1.66 (m)	35.6	1.49 (m), 1.57 (m)	33.8
16	1.17 (m), 1.88 (m)	29.4	1.41 (m), 1.98 (m)	28.8	1.08 (m), 1.89 (m)	28.4
17	1.60 (m)	52.2	1.61 (m)	51.0	1.66 (m)	53.7
18	0.93 (s)	16.1	0.72 (s)	15.3	0.94 (s)	16.3
19a	3.03 (d, 14.4)	45.5	3.03 (d, 14.4)	45.3	3.01 (d, 14.4)	45.2
19b	2.74 (d, 14.4)		2.77 (d, 14.4)		2.72 (d, 14.4)	
20	1.43 (m)	37.5	1.41 (m)	36.6	1.41 (m)	37.3
21	0.94 (d, 6.4)	19.1	0.90 (d, 6.0)	18.8	0.96 (d, 6.0)	19.8
22	2.11 (m), 1.13 (m)	34.7	1.51 (m), 1.26 (m)	33.6	2.27 (m), 1.89 (m)	39.6
23	1.95 (m), 1.32 (m)	28.6	1.53 (m), 1.37 (m)	28.8	5.64 (m)	129.8
24	3.81 (dd, 6.6, 5.4)	78.8	3.35 (dd, 6.6, 5.7)	79.1	5.49 (d, 16.0)	138.5
25	—	81.2	—	73.5	—	78.4
26	1.53 (s)	24.8	1.18 (s)	23.9	1.43 (s)	27.6
27	1.58 (s)	21.7	1.23 (s)	26.9	1.43 (s)	27.6
28	0.99 (s)	19.2	0.94 (s)	19.3	1.01 (s)	18.9
29	1.02 (s)	24.2	1.04 (s)	24.7	1.06	24.7
30	0.76 (s)	15.7	0.73 (s)	13.9	0.79	16.1

a) Compound **1a** was tested in C_5D_5N. *b*) Compounds **1** and **2** was tested in $CDCl_3$.

powder. Its HR-ESI-MS showed a quasi-molecular ion peak at *m*/*z* 1227.5779 (calculated value 1227.5775) $[M+Na]^+$, which indicated a molecular formula of $C_{58}H_{92}O_{26}$. The ^{1}H- and ^{13}C-NMR spectroscopic data of **2** were similar to those of **1**, with the exception of a double bond [δ_H 5.49 (1H, d, J=16 Hz) and 5.64 (1H, m); δ_C 138.5, 129.8] located at C-23 and C-24 in **2**, instead of a hydroxyl group linked to C-24 in **1**. This was revealed by the HMBC correlations from H-23 to C-20, C-22, C-24 and C-25, H-24 to C-22, C-23, C-25, C-26 and C-27 as well as the ^{1}H–^{1}H correlations of the spin

Table 2. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) Data of the Sugar Moieties of **1** and **2** (δ, ppm: J, Hz)

Position	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1′	4.91 (d, 8.0)	102.6	4.87 (d, 8.0)	101.1
2′	3.87 (m)	75.4	4.53 (m)	79.4
3′	4.20 (m)	76.7	4.10 (m)	75.5
4′	4.55 (m)	79.8	4.56 (m)	79.9
5′	4.02 (m)	74.6	4.11 (m)	75.8
6′	4.73 (dd, 2.5, 11.8)	68.1	4.76 (dd, 2.0, 11)	68.2
	4.81 (dd, 5.4, 11.8)		4.82 (dd, 5.2, 11)	
1″	5.56 (d, 7.8)	105.0	5.54 (d, 7.8)	105.3
2″	4.01 (m)	74.9	4.03 (m)	74.8
3″	4.32 (m)	78.6	4.29 (m)	78.4
4″	4.24 (m)	71.1	4.23 (m)	70.9
5″	3.94 (m), 4.25 (m)	67.2	3.96 (m), 4.27 (m)	67.5
1‴	5.06 (d, 7.6)	105.5	5.07 (d, 7.6)	105.7
2‴	4.49 (m)	72.6	4.48 (m)	72.6
3‴	4.01 (m)	74.5	4.02 (m)	74.4
4‴	4.26 (m)	70.2	4.24 (m)	69.9
5‴	3.69 (m), 4.27 (m)	67.7	3.70 (m), 4.28 (m)	67.6
1⁗	5.03 (d, 7.6)	105.3	6.13 (brs)	101.4
2⁗	4.13 (m)	75.1	4.68 (m)	72.2
3⁗	4.35 (m)	78.1	4.36 (m)	72.4
4⁗	4.61 (m)	73.6	4.38 (m)	74.1
5⁗	4.67 (d, 10)	78.1	4.80 (m)	69.5
6⁗		173.8	1.81 (d, 6.8)	18.7
1⁗′			5.05 (d, 7.6)	105.4
2⁗′			4.11 (m)	75.0
3⁗′			4.34 (m)	78.1
4⁗′			4.61 (m)	73.8
5⁗′			4.66 (d, 10)	78.0
6⁗′				173.8

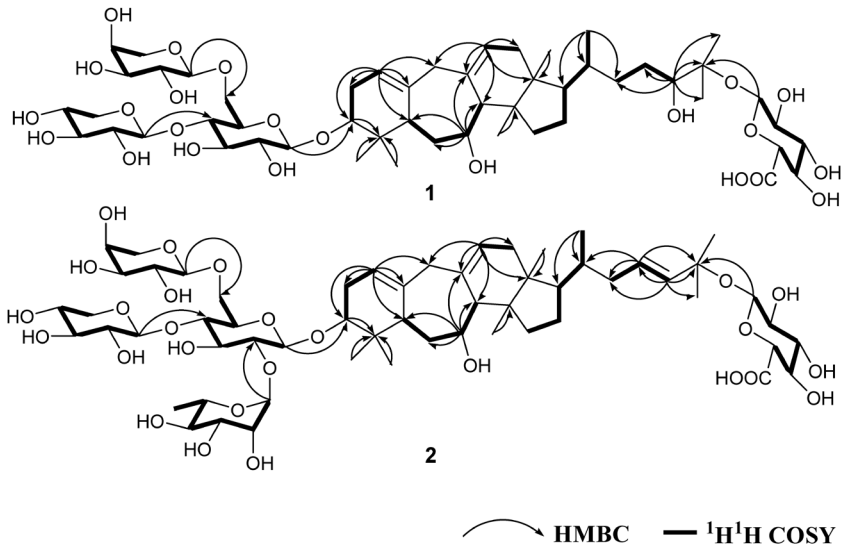

Fig. 2. Key HMBC and ^{1}H–^{1}H COSY Correlations of **1** and **2**

system H-22/H-23/H-24 (Fig. 2). Furthermore, comparison of ^{1}H- and ^{13}C-NMR spectra of **2** with those of **1**, indicated that **2** had one more rhamnosyl group [δ_H 6.13 (1H, brs) and 1.81 (3H, d, J=6.8 Hz); δ_C 101.4, 72.2, 72.4, 74.1, 69.5, 18.7] attached at C-2′ of the inner glucose. The suggestion was in accord with the observation of the downfield shift of C-2′ signal from δ_C 75.4 in **1** to δ_C 79.4 in **2**. This was further established by the HMBC correlation from H-1⁗′ [δ_H 6.13 (1H, brs)] to C-2′ (δ_C 79.4) (Fig. 2). Acid hydrolysis of **2** was performed in the same manner as that of **1** and D-glucose, D-xylose, L-arabinose, L-rhamnose and D-glucuronic acid as sugar residues were identified by GC analysis from **2**. The

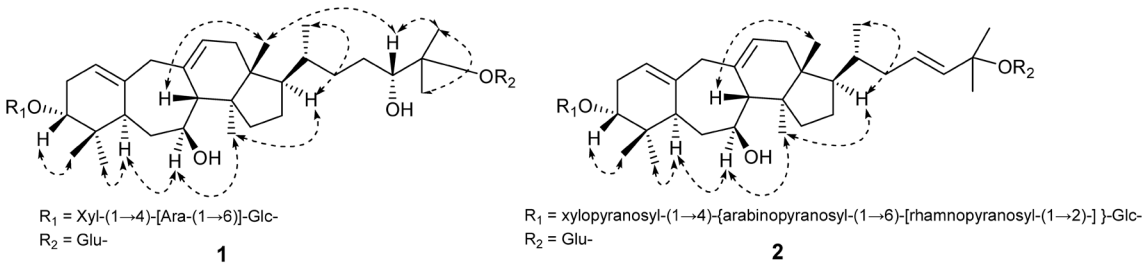

Fig. 3. Key ROESY Correlations of **1** and **2**

coupling constants of anomeric protons and ^{13}C-NMR data of the sugar moieties demonstrated that the glucose, xylose and glucuronic acid have a *β*-configuration, while the arabinose and rhamnose have an *α*-configuration. Consequently, the structure of **2** was established as 9,10-*seco*-cycloartan-1(10),9(11),23(24)-trien-3*β*,7*β*,25-triol 3-*O*-*β*-D-xylopyranosyl-(1→4)-{*α*-L-arabinopyranosyl-(1→6)-[*α*-L-rhamnopyranosyl-(1→2)-]}-*β*-D-glucopyranosyl-25-*O*-*β*-D-glucuronide, named cyprotuoside D.

Experimental

General Optical rotations were measured using a JASCO P-1010 digital polarimeter (Horiba, Tokyo, Japan) and IR spectra were obtained from a PerkinElmer, Inc. Spectrum One FT-IR spectrometer (PerkinElmer, Inc., Waltham, U.S.A.). MS data were detected on a Finnigan LCQ Advantage Spectrometer (Thermo Scientific, Massachusetts, U.S.A.) and a Shimadzu GC-MS model QP2010 Plus spectrophotometer (Shimadzu, Kyoto, Japan), respectively. NMR spectra were recorded on 400 MHz FT-NMR spectrometer (Varian Inova AS 400, Varian, U.S.A.). GC analysis was performed on an Agilent Technologies HP6890 gas chromatograph equipped with an H_2 flame ionization detector (Agilent Technologies Inc., California, U.S.A.). The column was HP-5 quartz capillary column (30 m×0.32 mm×0.25 *μ*m) with the following conditions: column temperature 180–260°C; carrier gas, N_2 (2 mL/min); injector and detector temperature, 270°C. Column chromatography separations were carried out on silica gel (200–300 mesh, Qingdao Haiyang Chemical Co., Ltd., China), octadecyl silane (ODS) (50 mesh, AA12S50, YMC), and Diaion HP-20 (Pharmacia, U.S.A.). All other chemicals and reagents of analytical grade were obtained from Sigma-Aldrich, unless indicated otherwise.

Plant Materials The rhizomes of *Cyperus rotundus* were collected in Zhanjiang, Guangdong Province of China in September 2009, and were identified by Professor Wenqing Yin (School of Chemistry & Chemical Engineering of Guangxi Normal University, Ministry of Education Key Laboratory of Chemistry and Molecular Engineering of Medicinal Resource, Guilin). A voucher specimen (No. 20090903) has been deposited in the authors' laboratory.

Extraction and Isolation The dry rhizomes of *C. rotundus* (10 kg) were extracted three times under reflux with 95% aqueous ethanol (150 L×2 h). After removing the solvent under reduced pressure, the residue was suspended in water and then sequentially extracted with petroleum ether, CH_2Cl_2, EtOAc and *n*-BuOH. The *n*-BuOH extract (152 g) was submitted through a column chromatography (9×90 cm) of Diaion HP-20, eluting with H_2O and CH_3OH. The methanol fraction (98 g) was repeatedly chromatographed over normal phase silica gel and the eluting solutions were monitored by TLC to produce four fractions (Frs.1–4). Fraction 4 (11.3 g) was subjected to ODS column chromatography (8×30 cm) eluting with CH_3OH–H_2O (0 : 1–1 : 0) to obtain 5 fractions (Frs. A–E). Fraction D (6.6 g) was subjected to repeated silica gel column chromatography with $CHCl_3$–MeOH–H_2O (7 : 3 : 0.3) and then subjected to repeated ODS column chromatography with a MeOH–H_2O (40 : 60–90 : 10) to yield **1** (21 mg) and **2** (18 mg).

Cyprotuoside C (**1**): white amorphous powder; $[\alpha]_D^{25}$+71.2 (*c* 1.0, MeOH); IR ν_{max} (KBr): 3346, 2929, 2354, 1721, 1650, 1451, 1384, and 1036 cm^{-1}; HR-ESI-MS *m/z*: 1099.5303 $[M+Na]^+$ (Calcd for $C_{52}H_{84}O_{23}Na$, 1099.5301). ^{1}H-NMR (400 MHz, C_5D_5N) and ^{13}C-NMR (100 MHz, C_5D_5N) spectral data see Tables 1 and 2.

Cyprotuoside D (**2**): white amorphous powder; $[\alpha]_D^{25}$+57.3 (*c* 1.0, MeOH); IR ν_{max} (KBr): 3349, 2931, 2351, 1722, 1649, 1453, 1381, 1033 cm^{-1}; HR-ESI-MS *m/z*: 1227.5779 $[M+Na]^+$ (Calcd. for $C_{58}H_{92}O_{26}Na$, 1227.5775). ^{1}H-NMR (400 MHz, C_5D_5N) and ^{13}C-NMR (100 MHz, C_5D_5N) spectral data see Tables 1 and 2.

Acid Hydrolysis of Compounds 1 and 2 A solution (18 mg) of **1** in 1 M HCl (8 mL) was heated at 100°C for 3 h under an N_2 atmosphere. After cooling, the reaction mixture was neutralized with Na_2CO_3, and then extracted with $CHCl_3$ (3 ×10 mL). The $CHCl_3$ phase was chromatographed on silica gel column eluting with $CHCl_3$–CH_3OH (9 : 1) to give compound **1**a (9 mg). A solution of **2** (2 mg) in 1 M HCl (2 mL) was heated at 100°C for 3 h under an N_2 atmosphere. The reaction mixture was diluted with H_2O and extracted with $CHCl_3$ (3×10 mL). The aqueous layer was concentrated to dryness to give a residue and dissolved in pyridine (1 mL), and then L-cysteine methyl ester hydrochloride (5 mg) was added to the solution. The mixture was heated at 60°C for 3 h, and trimethylchlorosilane (0.5 mL) was added, followed by heating at 60°C for 3 h. Finally, the solution was concentrated to dryness and taken up in water, followed by extraction with *n*-hexane (3×5 mL). The *n*-hexane fraction was analyzed by GC, and the retention times of the D-glucose, L-arabinose, D-xylose, D-glucuronic acid, and L-rhamnose standards were 8.96, 4.84, 6.08, 9.05, 7.31 min, respectively.

Acknowledgments This study was supported by the National Natural Science Foundation of China (31400295), the China Spark Program (YQ2015109), Science and Technology Planning Project of Guangdong Province (2014A020221057), Distinguished Young Teachers in Higher Education of Guangdong (YQ2015109), Foundation of Education Bureau of Guangdong Province (2015KTSCX087, 2016KQNCX099), and Foundation for Youths of Lingnan Normal University (QL1401).

Conflict of Interest The authors declare no conflict of interest.

References

1) Yang J. L., Shi Y. P., *Planta Med.*, **78**, 59–64 (2012).
2) Xu Y., Zhang H. W., Wan X. C., Zou Z. M., *Magn. Reson. Chem.*, **47**, 527–531 (2009).
3) Zhou Z. L., Yin W. Q., Yang Y. M., He C. H., Li X. N., Zhou C. P., Guo H., *Chem. Pharm. Bull.*, **64**, 73–77 (2016).
4) Huang K. L., Xiao G., Huang J. H., Wang H., Pan X., *Youjiang Minzu Yixueyuan Xuebao*, **36**, 491–492 (2014).
5) Kum C. J., Kim E. Y., Kim J. H., Lee B., Min J. H., Heo J., Kim J. H., Yeom M., Sohn Y., Jung H. S., *J. Ethnopharmacol.*, **205**, 186–194 (2017).
6) Hu Q. P., Cao X. M., Hao D. L., Zhang L. L., *Scientific Reports*, **7**, 45231 (2017).
7) Zhou Z. L., Feng Z. C., Yin W. Q., Zhang H. L., *Chem. Nat. Compd.*, **49**, 682–684 (2013).
8) Zhou Z. L., Feng Z. C., Fu C. Y., Zeng L., *Nat. Prod. Res.*, **27**, 1343–1347 (2013).
9) Zhou Z. L., Feng Z. C., Xia J. M., *Asian Journal of Chemistry*, **25**, 9457–9459 (2013).
10) Zhou Z. L., Lin S. Q., Yin W. Q., *J. Asian Nat. Prod. Res.*, **18**, 662–668 (2016).
11) Fu X., Li X. C., Smillie T. J., Carvalho P., Mabusela W., Syce J., Johnson Q., Folk W., Avery M. A., Khan I. A., *J. Nat. Prod.*, **71**, 1749–1753 (2008).
12) Ali Z., Khan S. I., Fronczek F. R., Khan I. A., *Phytochemistry*, **68**, 373–382 (2007).
13) Isaev I. M., Iskenderov D. A., Isaev M. I., *Chem. Nat. Compd.*, **46**, 36–38 (2010).