Three New Glycosides from the Stems of *Miliusa balansae*

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Three new glycosides with the same saccharides, namely miliusoside A (**1**), miliusoside B (**2**), and miliusoside C (**3**), together with five known compounds were isolated from the stems of *Miliusa balansae*. Their structures were elucidated on the basis of detailed spectroscopic analysis and by comparison with the spectra of related model compounds. There was a rarely encountered α-D-apiose moiety occurring in all new compounds.

Introduction. – *Miliusa balansae* FINET et GAGNEP is an evergreen shrub, belonging to the family Annonaceae, which is used to treat gastropathy and glomerulonephropathy [1]. Previous investigations led to the isolation of flavonoids, dihydrochalcones, styryl derivatives, homogentistic acid derivatives, norditerpenes, and alkaloids [2–6]. Herein, we report the isolation and characterization of three new glycosides, *i.e.*, 2-hydroxy-5-(2-hydroxyethyl)phenyl *O*-α-D-apiofuranosyl-(1 → 6)-*O*-β-D-glucopyranoside (**1**), 2-(4-hydroxylphenyl)ethyl *O*-α-D-apiofuranosyl-(1 → 6)-*O*-β-D-glucopyranoside (**2**), and megastigm-7-ene-3,6,9-triol-9-*O*-α-D-apiofuranosyl-(1 → 6)-*O*-β-D-glucopyranoside (**3**). In addition, five known compounds were isolated and identified by comparison with literature values, *i.e.*, osmanthuside H [7], cuchiloside [8], 1-(α-L-rhamnosyl-(1 → 6)-β-D-glucopyranosyloxy)-3,4,5-trimethoxybenzene [9], 3,4,5-trimethoxyphenol-β-D-glucopyranoside [10], and alangionoside B (**4**) [11] (*Fig. 1*).

Results and Discussions. – Compound **1** was obtained as an amorphous powder, with the molecular formula $C_{19}H_{28}O_{12}$, based on the $[M + Na]^+$ peak at *m/z* 471.1475 in the HR-ESI-MS, and confirmed by ^{1}H- and ^{13}C-NMR experiments (*Table 1*). The ^{1}H-NMR spectrum of **1** in CD_3OD (*Table 1*) revealed three aromatic H-atom signals at δ(H) 6.74–6.77 (*m*, 1 H), 6.78–6.81 (*m*, 1 H), and 7.03 (*d*, J = 1.2 Hz, 1 H), and signals of two CH_2 groups at 3.67–3.74 (overlapped, 2 H), 2.87 (*t*, J = 7.2 Hz, 2 H). In an attempt to obtain a better resolution for aromatic H-atom signals, the NMR data of **1** were then acquired in C_5D_5N. The ^{1}H-NMR spectrum of **1** in C_5D_5N further supported the presence of a 1,2,4-trisubstituted benzene ring (δ(H) 7.65 (*d*, J = 2.0 Hz, 1 H), 7.18 (*d*, J = 8.5 Hz, 1 H), and 7.03 (*dd*, J = 8.5, 2.0 Hz, 1 H)). Acid hydrolysis of **1** yielded D-glucose and D-apiose, which were identified by TLC and GC analyses. The orientation of anomeric H-atoms was deduced from ^{1}H-NMR data as β (δ(H) 4.35 (*d*, J = 7.5 Hz)) for glucose and α (δ(H) 5.00 (*d*, J = 4.5 Hz)) for apiose. This was

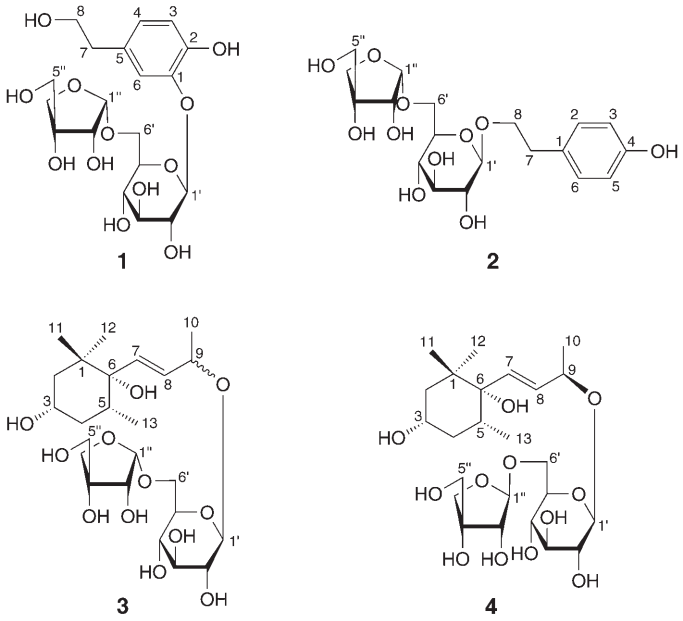


Fig. 1. *Structures of compounds* **1**, **2**, **3**, *and* **4**

confirmed by comparison of the ^{13}C-NMR data of the apiose in **1** with those of methyl α-D- and β-D-apiofuranosides [12]. The position of attachment of the disaccharide moiety and the interglycosidic linkage were established from the HMBC correlation between H–C(1′) (δ(H) 4.75 (*d*, J = 7.5 Hz)) and C(1) (δ(C) 146.9), and H–C(1″) (δ(H) 5.00 (*d*, J = 4.5 Hz)) and C(6′) (δ(C) 68.0). Thus, compound **1** was characterized as *2-hydroxy-5-(2-hydroxyethyl)phenyl* O-α-D-*apiofuranosyl-(1 → 6)*-O-β-D-*glucopyranoside*, and named as *miliusoside A*.

Compound **2** was obtained as an amorphous powder, with the molecular formula $C_{19}H_{28}O_{11}$, based on the $[M + Na]^+$ peak at *m*/*z* 455.1526 in the HR-ESI-MS, and confirmed by ^{1}H- and ^{13}C-NMR experiments (*Table 1*). The ^{1}H-NMR spectrum of **2** showed signals for two CH_2 groups at δ(H) 2.83 (*t*, J = 6.6 Hz, 2 H), and δ(H) 3.94–4.00 (*m*, 1 H) and δ(H) 3.70–3.74 (*m*, 1 H). Signals for aromatic H-atoms at δ(H) 6.64 (*d*, J = 8.4 Hz, 2 H) and 7.02 (*d*, J = 8.4 Hz, 2 H) suggested a 2-(4-hydroxyphenyl)-ethanol moiety in **2**. Acid hydrolysis of **2** yielded D-glucose and D-apiose, which were identified by TLC and GC analyses. Comparison of the ^{1}H- and ^{13}C-NMR data of **2** with those of **1** suggested that **2** contained the same disaccharide moiety. The position of attachment of the glycosidic chain and the interglycosidic linkage in **2** were determined from the HMBC correlations between H–C(1′) (δ(H) 4.75 (*d*, J = 7.5 Hz)) and C(8) (δ(C) 72.3), and H–C(1″) (δ(H) 5.00 (*d*, J = 4.5 Hz)) and C(6′) (δ(C) 68.1). Therefore, compound **2** is identified as *2-(4-hydroxylphenyl)ethyl* O-α-D-*apiofuranosyl-(1 → 6)*-O-β-D-*glucopyranoside*, and named as *miliusoside B*.

Compound **3** was obtained as an amorphous powder, with the molecular formula $C_{24}H_{42}O_{12}$, based on the $[M + Na]^+$ peak at *m*/*z* 545.2555 in the HR-ESI-MS, and

Table 1. *^{1}H- and ^{13}C-NMR Data of* **1** *and* **2**[a]). δ in ppm, J in Hz.

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
1		146.9		130.7
2		146.5	7.02 (d, J = 8.4)	130.9
3	6.74–6.77 (m)	117.1	6.64 (d, J = 8.4)	116.1
4	6.78–6.81 (m)	125.5		156.8
5		131.0	6.64 (d, J = 8.4)	116.1
6	7.03 (d, J = 1.2)	119.9	7.02 (d, J = 8.4)	130.9
7	2.87 (t, J = 6.6)	39.2	2.83 (t, J = 6.6)	36.4
8	3.67–3.74 (m)	64.5	3.94–4.00 (m), 3.70–3.74 (m)	72.3
Glc				
1′	4.75 (d, J = 7.5)	104.5	4.30 (d, J = 7.8)	104.5
2′	3.48 (dd, J = 7.4, 9.4)	75.3	3.47 (dd, J = 7.4, 9.4)	75.0
3′	3.46 (t, J = 9.4)	77.9	3.46 (t, J = 9.4)	77.9
4′	3.32 (t, J = 9.4)	71.6	3.34 (t, J = 9.4)	71.8
5′	3.58–3.68 (m)	77.0	3.38–3.44 (m)	76.6
6′	3.96 (dd, J = 12.0, 6.2), 3.66 (dd, J = 12.0, 2.0)	68.0	3.96 (dd, J = 12.0, 6.2), 3.66 (dd, J = 12.0, 2.0)	68.1
Api				
1″	5.00 (d, J = 4.5)	104.0	5.00 (d, J = 4.5)	104.2
2″	3.90 (d, J = 5.0)	73.6	3.89 (d, J = 4.5)	73.6
3″		78.0		78.0
4″	4.01 (d, J = 9.9), 3.82 (d, J = 9.9)	75.3	4.02 (d, J = 10.0), 3.83 (d, J = 10.0)	75.3
5″	3.46 (d, J = 11.0), 3.50 (d, J = 11.0)	65.2	3.47 (d, J = 11.0), 3.49 (d, J = 11.0)	65.1

[a]) Recorded in CD_3OD, at 300 and 75 MHz, resp.

confirmed by ^{1}H- and ^{13}C-NMR experiments (*Table 2*). The ^{1}H-NMR spectrum of **3** showed signals for two alkene H-atoms at δ(H) 5.77 (dd, J = 16.0, 7.0 Hz) and 5.61 (d, J = 16.0 Hz), and those for four Me groups at δ(H) 1.30 (d, J = 6.0 Hz), 0.97 (s), 0.89 (s), and 0.80 (d, J = 6.0 Hz). By comparison of the ^{1}H- and ^{13}C-NMR data of **3** with those of **4** (alangionoside B) [11] indicated that **3** was similar to **4** except for the orientation of the apiose (*Table 2*). Acid hydrolysis of **3** yielded D-glucose and D-apiose, which were identified by TLC and GC analysis. HMBC Correlation (*Fig. 2*) between H–C(1′) (δ(H) 4.35 (d, J = 7.5)) and C(9) (δ(C) 78.0) indicated that the glucose was linked to C(9) of aglycone. Moreover, another HMBC (*Fig. 2*) correlation between H–C(1″) (δ(H) 5.00, (d, J = 4.5)) and C(6′) (δ(C) 67.8) suggested that glucose and apiose were connected by a (1 → 6) linkage. NOE Correlations (*Fig. 3*) were observed between H–C(5) (δ(H) 1.88–1.95 (m)) and H–C(3) (δ(H) 3.78–3.80 (m)), between H–C(5) (δ(H) 1.88–1.95 (m)) and H–C(7) (δ(H) 5.61 (d, J = 16.0)), and between H–C(3) (δ(H) 3.78–3.80 (m)) and H–C(11) (δ(H) 0.97 (s)), indicating that H–C(3) and H–C(5) were coplanar and β-oriented, and OH–C(6) was α-configured. Therefore, compound **3** was identified as *megastigma-7-ene-3,6,9-triol-9*-O-α-D-*apiofuranosyl-(1 → 6)*-O-β-D-*glucopyranoside*, and named as *miliusoside C*.

The absolute configuration of compound **3** may be proposed as (3*S*,5*R*,6*R*) in view of the reported absolute configuration of alangionoside B (**4**).

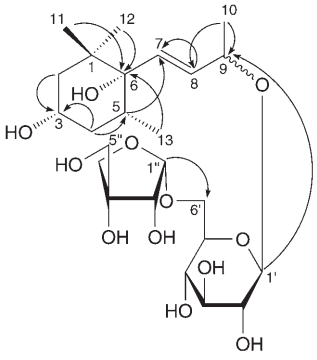
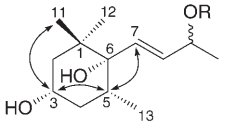


Fig. 2. Selected HMBC correlations (H → C) *of* **3**



R = α-D-Api(1→6)β-D-Glc

Fig. 3. *Important NOE correlations of* **3**

α-D-Apiose is rarely encountered in natural products, examples including 4-[*O*-α-D-apiofuranosyl-(1″ → 2′)-β-D-glucopyranosyloxy]benzaldehyde and 2-{4-[*O*-α-apiofuranosyl-(1″ → 6′)-β-D-glucopyranosyloxy]phenyl}ethanol [13–15]. On the other hand, it is the first report of α-D-apiofuranosides from the genus *Miliusa*. Of particular interest is the fact that both α-D-apiose and β-D-apiose coexist in the same plant.

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Experimental Part

General. Column chromatography (CC): silica gel *H* (200–300 mesh; *Qingdao Marine Chemical Industry*), *Sephadex LH-20* gel (*Pharmacia*), Semi-prep. HPLC: *ODS* column (250 × 10 mm, 5 μm; *Alltech*), with *Waters 2996* photodiode-array detector (280 nm) and *Waters*™ *600* pump; flow rate, 2.5 ml/min. GC: *Agilent 6890 N* gas chromatograph, with a *HP-5* cap. column (28 m × 0.32 mm) and a FID detector operated at 260° (column temp. 180°), 1.0 ml/min N_2 as carrier gas. M.p.: *X-4* micro-melting-point apparatus; uncorrected. Optical rotations: *Perkin-Elmer 243 B* digital polarimeter. UV Spectra: *UV-240* (*Shimadzu*) spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *NEXUS-470 FTIR* (*Nicolet*). NMR Spectra: *Varian INOVA-500*, *INOVA-300*, and *UNITY-500* spectrometers, TMS as an internal standard. HR-ESI-MS: *APEX II FT-ICRMS* (*Bruker Daltonics*) spectrometer.

Plant Material. The stems of *Miliusa balansae* FINET et GAGNEP were collected in August 2004 from Guangxi Zhuang Autonomous Region, P. R. China. The identification of the plant was performed by *P.-F. T.* A voucher specimen (CM200408) was deposited with the Herbarium of Peking University Modern Research Center for Traditional Chinese Medicine.

Extraction and Isolation. The dried stems (20 kg) of *M. balansae* were extracted three times with hot 80% EtOH (20 l) for 2 h each time. After removal of the solvent under reduced pressure at 60°, the residue (1 kg) was suspended in H_2O (1 l) and defatted with petroleum ether (3 × 1 l). The aq. layer was further extracted with AcOEt (3 × 1 l) and BuOH (3 × 1 l) successively. The BuOH extract (240 g) was

Table 2. *^{1}H- and ^{13}C-NMR Data of* **3** *and* **4**[a]). δ in ppm, J in Hz.

Position	**3**		**4**	
	δ(H)	δ(C)	δ(H)	δ(C)
1		40.5		40.6
2	1.62–1.67 (*m*), 1.34–1.41 (*m*)	45.9	1.66 (*t*, *J* = 12), 1.32–1.43 (*m*)	45.9
3	3.78–3.80 (*m*)	67.4	3.80 (*tt*, *J* = 4.0, 12.0)	67.5
4	1.65–1.68 (*m*), 1.38 (*d*, *J* = 12.5)	39.9	1.61–1.69 (*m*), 1.39 (*d*, *J* = 12.0)	39.9
5	1.88–1.95 (*m*)	35.5	1.89–1.97 (*m*)	35.4
6		78.2		78.3
7	5.61 (*d*, *J* = 16.0)	136.1	5.63 (*d*, *J* = 16.0)	136.1
8	5.77 (*dd*, *J* = 7.0, 16.0)	133.4	5.78 (*d*, *J* = 6.0, 16.0)	133.7
9	4.37–4.40 (*m*)	78.0	4.38 (*q*, *J* = 6.0)	78.0
10	1.30 (*d*, *J* = 6.0)	21.5	1.30 (*d*, *J* = 6.0)	21.5
11	0.97 (*s*)	25.3	0.98 (*s*)	25.4
12	0.89 (*s*)	26.1	0.90 (*s*)	26.3
13	0.80 (*d*, *J* = 6.0)	16.5	0.82 (*d*, *J* = 7.0)	16.6
Glc				
1′	4.35 (*d*, *J* = 7.5)	104.1	4.32 (*d*, *J* = 8.0)	102.5
2′	3.47 (*dd*, *J* = 7.4, 9.4)	75.3	3.16 (*t*, *J* = 8.0)	75.4
3′	3.30 (*t*, *J* = 9.4)	77.9	3.30 (*t*, *J* = 9.6)	78.1
4′	3.40 (*t*, *J* = 9.4)	71.6	3.39 (*t*, *J* = 9.6)	71.6
5′	3.32–3.34 (*m*)	76.5	3.31–3.34 (*m*)	77.9
6′	3.96 (*dd*, *J* = 11.0, 2.0), 3.66 (*dd*, *J* = 11.0, 4.0)	67.8	3.90–3.95 (*m*), 3.55–3.58 (*m*)	68.5
Api				
1″	5.00 (*d*, *J* = 4.5)	102.7	4.98 (*d*, *J* = 3.0)	111.0
2″	3.90 (*d*, *J* = 5.0)	73.6	3.92 (*d*, *J* = 3.0)	76.9
3″		78.0		80.6
4″	4.02 (*d*, *J* = 9.5), 3.83 (*d*, *J* = 9.5)	75.3	3.76 (*d*, *J* = 10.0), 3.97 (*d*, *J* = 10.0)	75.0
5″	3.46 (*d*, *J* = 11.0), 3.50 (*d*, *J* = 11.0)	65.1	3.58 (*s*)	65.7

[a]) Recorded in CD_3OD, at 500 and 125 MHz, resp.

subjected to CC (SiO_2; $CHCl_3$/MeOH 100:1 → 3:1) to give *Fractions A–R*. *Fr. O* (10 g) was subjected to CC (*Sephadex LH-20*; MeOH/H_2O 10:90) to afford *Fr. 1–4*. *Fr. 2* was subjected to CC (SiO_2; $CHCl_3$/MeOH 100:15) to give *Fr. 2.1–2.3*. *Fr. 2.3* was subjected to CC (ODS; MeOH/H_2O 10:90) to yield *Fr. 2.3.1–2.3.5*. *Fr. 2.3.2* was subjected to semiprep. HPLC (MeOH/H_2O 15:85) to provide compounds **1** (6.3 mg, t_R 17.4 min), osmanthuside H (7.8 mg, t_R 20.84 min), and cuchiloside (9.2 mg, t_R 24.9 min). *Fr. 2.3.3* was subjected to semiprep. HPLC (MeOH/H_2O 15:85) to yield compounds **2** (6.7 mg, t_R 21.7 min) and 1-(α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranosyl)-3,4,5-trimethoxybenzene (11.2 mg, t_R 20.3 min). *Fr. 2.3.4* was subjected to semiprep. HPLC (MeOH/H_2O 18:82) to yield compounds **3** (3.8 mg, t_R 12.9 min) and alangionoside B (**4**; 2.7 mg, t_R 11.4 min). *Fr. M* (25.35 g) was subjected to CC (SiO_2; $CHCl_3$/MeOH/H_2O 10:1:0.1) to afford *Fr. 1–5*. *Fr. 4* was subjected to CC (*Sephadex LH-20*; H_2O) and semiprep. HPLC (MeOH/H_2O 38:62) to yield 3,4,5-trimethoxyphenol-β-D-glucopyranoside (7.4 mg, t_R 9.4 min).

Miliusoside A (=*2-Hydroxy-5-(2-hydroxyethyl)phenyl* O-α-D-*Apiofuranosyl-(1→6)*-O-β-D-*glucopyranoside*; **1**). Amorphous powder. $[\alpha]_D^{20} = -75.0$ ($c = 0.056$, MeOH). UV (MeOH): 278 (3.22), 220 (3.75). IR (KBr): 3419, 2926, 1760, 1605, 1514, 1434, 1279, 1233, 1039, 799, 604. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-ESI-MS: 471.1475 ($[M + Na]^+$, $C_{19}H_{28}NaO_{12}^+$; calc. 471.1473).

Miliusoside B (=*2-(4-Hydroxylphenyl)ethyl* O-α-D-*Apiofuranosyl-(1→6)*-O-β-D-*glucopyranoside*; **2**). Amorphous powder. $[\alpha]_D^{20} = -70.0$ ($c = 0.050$, MeOH). UV (MeOH): 278 (3.13), 223 (138.2). IR (KBr): 3419, 2927, 2881, 1614, 1516, 1449, 1370, 1236, 1080, 1032, 829, 551, 518, ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-ESI-MS: 455.1526 ($[M + Na]^+$, $C_{19}H_{28}NaO_{11}^+$; calc. 455.1524).

Miliusoside C (=*Megastigma-7-ene-3,6,9-triol-9*-O-α-D-*apiofuranosyl-(1→6)*-O-β-D-*glucopyranoside*; **3**). Amorphous powder. $[\alpha]_D^{20} = -86.7$ ($c = 0.015$, MeOH). IR (KBr): 3421, 2930, 1631, 1463, 1370, 1316, 1030, 750, 606. ^{1}H- and ^{13}C-NMR: see *Table 2*. HR-ESI-MS: 545.2555 ($[M + Na]^+$, $C_{24}H_{42}NaO_{12}^+$; calc. 545.2569).

Acid Hydrolysis and Analysis of Sugars. Compounds **1**–**3** (2 mg) was hydrolyzed with 2N aq. CF_3COOH (10 ml) at 110° for 8 h in a sealed tube. The mixture was diluted with H_2O (20 ml) and extracted with AcOEt (3 × 10 ml).

The aq. layer was evaporated under reduced pressure, and the residue was analyzed using TLC by comparison with the standard sugars. The solvent system was $CHCl_3$/MeOH/H_2O 8:5:1. Spots were visualized by spraying with 95% EtOH/H_2SO_4/anisaldehyde 9:0.5:0.5 (*v*/*v*), then heated at 120° for 10 min. The R_f of glucose and apiose were 0.23 and 0.45, resp. For GC analysis, the aq. layer was evaporated, and the residue was dissolved in anh. pyridine (100 µl). 0.1M L-Cysteine methyl ester hydrochloride (200 µl) was added, and the mixture was warmed at 60° for 1 h. HMDS/TMCS (hexamethyldisilazane/Me_3SiCl/pyridine 2:1:10) (*Acros Organics*, Belgium) was added, and the mixture was warmed at 60° for 30 min. The thiazolidine derivatives were analyzed by GC; D-glucose (t_R 11.63 min) and D-apiose (t_R 5.12 min) were detected from compounds **1**, **2**, and **3**.

REFERENCES

[1] D. L. Wu, 'Contributions of Plant Investigation in the Upper Reaches of Hongshui River', Science Press, Beijing, 1996, p. 120.
[2] R. Wu, Q. Ye, N. Y. Chen, G. L. Zhang, *Chin. Chem. Lett.* **2001**, *12*, 247.
[3] C. Kamperdick, N. H. Van, S. T. Van, *Phytochemistry* **2002**, *61*, 991.
[4] D. T. Huong, C. Kamperdick, S. T. Van, *J. Nat. Prod.* **2004**, *67*, 445.
[5] B. Chen, Q. Ye, B. G. Li, G. L. Zhang, *Indian J. Heterocycl. Chem.* **2002**, *12*, 81.
[6] G. G. Harrigan, A. A. L. Gunatilaka, D. G. I. Kingston, *J. Nat. Prod.* **1994**, *57*, 68.
[7] M. Sugiyama, M. Kikuchi, *Phytochemistry* **1993**, *32*, 1553.
[8] N. G. Bisset, A. K. Choudhury, P. J. Houghton, *Phytochemistry* **1989**, *28*, 1553.
[9] J. O. Andrianaivoravelona, C. Terreaux, S. Sahpaz, J. Rasolondramanitra, K. Hostettmann, *Phytochemistry* **1999**, *52*, 1145.
[10] L. Verotta, M. DellAgli, A. Giolito, M. Guerrini, P. Cabalion, E. Bosisio, *J. Nat. Prod.* **2001**, *64*, 603.
[11] H. Otsuka, K. Kamada, C. Ogimi, E. Hirata, A. Takushi, Y. Takeda, *Phytochemistry* **1994**, *35*, 1331.
[12] T. Ishii, M. Yanagisawa, *Carbohydr. Res.* **1998**, *313*, 189.
[13] I. Kitagawa, K. Hori, M. Sakagami, F. Hashiuchi, M. Yoshikawa, J. Ren, *Chem. Pharm. Bull.* **1993**, *41*, 1350.
[14] I. Dupin, Z. Gunata, J. C. Sapis, C. Bayonove, *J. Agric. Food Chem.* **1992**, *40*, 1886.
[15] B. I. Kamara, E. V. Brandt, D. Ferreira, E. Joubert, *J. Agric. Food Chem.* **2003**, *51*, 3874.

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