Food Chemistry 126 (2011) 1039–1043

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Phenylpropanoid glycosides from the seeds of *Michelia hedyosperma*

Xian-You Wang [a,c], Min Xu [a], Chong-Ren Yang [a,b,*], Ying-Jun Zhang [a,*]

[a] State Key Laboratory of Phytochemistry and Plant Resources of West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, PR China
[b] Weihe Biotech Research and Development Center, Yuxi 653101, PR China
[c] Pharmaceutical College of Henan University, Kaifeng 475001, PR China

ARTICLE INFO

Article history:
Received 30 June 2010
Received in revised form 21 October 2010
Accepted 18 November 2010
Available online 25 November 2010

Keywords:
Michelia hedyosperma
Magnoliaceae
Phenylpropanoid glycoside
Michehedyosides A–F

ABSTRACT

The seed of *Michelia hedyosperma* Law (Magnoliaceae) is a commonly used spice by the local people living in the south of Yunnan province, China. From which, six new phenylpropanoid glycosides, michehedyosides A–F (**1–6**) were obtained, in addition to six known compounds, eugenol, eugenol 4-*O*-*β*-D-glucopyranoside, martynoside, alaschanioside C, 3,4-methylenedioxycinnamyl alcohol, and (+)-pinoresinol 4-*O*-*β*-D-glucoside. Their structures were elucidated on the basis of detailed spectroscopic analyses and chemical methods.

1. Introduction

Phenylpropanoid glycosides (PPGs) are a group of water soluble natural products widely distributed in the plant kingdom, e.g., Smilacaceae (Zhang et al., 2008), Polygonaceae (Brown, Larson, & Sneden, 1998), and Magnoliaceae (Kelm & Nair, 2000). This class of compounds shows antibacterial, antiviral, analgesic, antispasmodic, neuroprotective, anti-feedant, cytotoxic, anti-inflammatory, antioxidant, enzyme inhibitory activities (Díaza et al., 2004; Xu, Su, & Zhou, 2007). Traditional study on identification of PPGs was carried out by a conventional high-sensitive polarimeter. Recently, rapid resolution liquid chromatography (RRLC) coupled with diode array detection (DAD) and electrospray ionisation time-of-flight mass spectrometry (ESI-TOF MS) method and HPLC–DAD–MS were developed to the exploration of the skeleton and the identity of PPGs in the molecule identification, which provide valuable structural information regarding the PPGs skeleton, fragmentation of the carbohydrate moiety, losses of the hydroxyl and glucose residue units (Qi et al., 2008; Wu et al., 2010). However, these methods appeared insufficient with regard to accurate peak identification in samples and were not sensitive for determination of low-content constituents (Wu et al., 2010).

Michelia hedyosperma Low (Magnoliaceae), an ornamental plant and important source of timber, is distributed in the southern China (Guan & Zhang, 2004). The seed is a commonly used spice by the local people of its growing area. Previous researches reported sesquiterpene lactones (Iida & Ito, 1982; Jacobsson, Kumar, & Saminathan, 1995; Ogura, Cordell, & Farnsworth, 1978) and alkaloids (Khan, Kihara, & Omoloso, 2002; Nijsiri, Kittisak, & Srirat, 1988; Talapatra, Patra, & Talapatra, 1975) from the genus *Michelia*. However, chemical constituents of *M. hedyosperma* have not been studied. In this paper, we report the isolation and structure elucidation of six new phenylpropanoid glycosides (**1–6**) from the seeds of *M. hedyosperma* by traditional methods (Fig. 1).

2. Materials and methods

2.1. General procedures

Optical rotations were measured with a HORIBA SEPA-300 high-sensitive polarimeter. IR spectra were recorded on a Bio-Rad FTS-135 spectrometer with KBr pellets. UV spectra were recorded on a UV 210A Shimadzu spectrometer. 1D- and 2D-NMR spectra were recorded in DMSO-d_6 or CD_3OD with a Bruker DRX-500 instrument operating at 500 MHz for ^1H, 125 MHz for ^{13}C, respectively. Coupling constants were expressed in Hertz and chemical shifts were given on a ppm scale with tetramethylsilane as internal standard. FABMS were recorded on a VG Auto Spec-300 spectrometer with glycerol as the matrix. HRESIMS was recorded on an API QSTAR Pular-1 mass spectrometer.

2.2. Chemicals and reagents

Column chromatography (CC) was performed on silica gel (200–300 mesh; Qingdao Marine Chemical Factory); Diaion HP20SS (Mitsubishi Chemical Industry, Ltd.); MCI-gel CHP20P (75–150 μm; Mitsubishi Chemical Industry, Ltd.); Chromatorex ODS

* Corresponding authors. Tel.: +86 871 522 3235; fax: +86 871 515 0124.
E-mail address: zhangyj@mail.kib.ac.cn (Y.-J. Zhang).

0308-8146/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2010.11.116

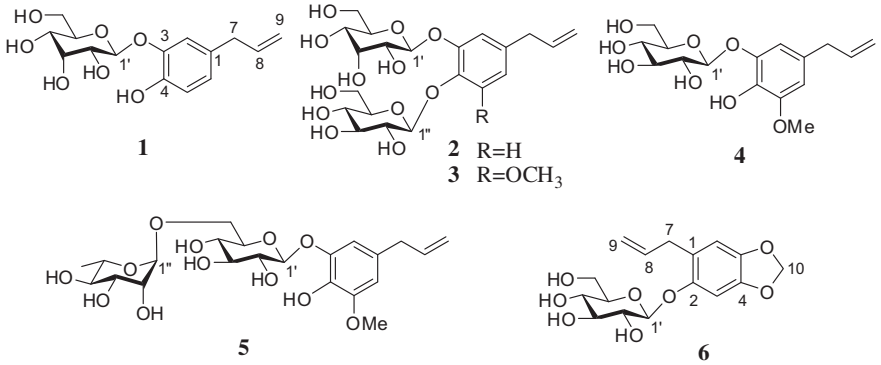

Fig. 1. Structures of new compounds **1–6** from the seeds of *Michelia hedyosperma*.

(100–200 mesh; Fuji Silysia Chemical Co. Ltd.). TLC was carried on silica gel H-precoated plates (0.2–0.25 mm thick, Qingdao Haiyang Chemical Co.,) with chloroform/methanol/water (7:3:0.51 or 8:2:0.2, v/v/v) and spots were detected by spraying with 10% sulphuric acid reagent followed by heating. GC analysis was run on Agilent Technologies HP5890 gas chromatograph equipped with an H_2 flame ionisation detector. The column was 30QC2/AC-5 quartz capillary column (30 m × 0.32 mm) with the following conditions: column temperature: 180/280 °C; programmed increase, 3 °C/min; carrier gas: N_2 (1 ml/min); injection and detector temperature: 250 °C; injection volume: 4 μl, split ratio: 1/50.

2.3. Plant materials

The seeds of *M. hedyosperma* were collected from Xishuangbanna, Yunnan, China, in December 2005, where it has been planted widely for seeds as spice.

2.4. Extraction and isolation

Grinded seeds (500 g) of *M. hedyosperma* were extracted with methanol under reflux condition for three times, and then defatted with petroleum ether. The defatted methanol extract (15 g) was subjected to Diaion column and eluted with H_2O/MeOH (1:0–0:1, v/v, each 800 ml) to give six fractions (I–VI). Fraction I (2.76 g) was chromatographed on a silica gel column, eluted with a gradient solvent system ($CHCl_3$/CH_3OH/H_2O, 8:2:0.2–6:4:1, v/v/v), and Rp-8 column (50–70% aq. MeOH) to afforded compounds **2** (591 mg) and **3** (35 mg). Fraction II (1.02 g) was subjected to chromatography of silica gel ($CHCl_3$/CH_3OH/H_2O, 9:1:0.1–7:3:0.5) and MCI-gel CHP20P (75–100% aq. MeOH) to produced **5** (218 mg). Similarly, compound **4** (45 mg) and alaschanioside C (9 mg) were isolated from fraction III (1.75 g). Compound **1** (223 mg) were obtained from fraction IV (1.3 g), while compound **6** (77 mg), martynoside (176 mg), eugenol 4-*O*-*β*-D-glucopyranoside (42 mg) and (+)-pinoresinol 4-*O*-*β*-D-glucoside (25 mg) were given from fraction V (2.81 g). Eugenol (8 mg) and 3,4-methylenedioxycinnamyl alcohol (167 mg) were produced from fraction VI (1.89 g) by repeated CC over silica gel ($CHCl_3$/CH_3OH/H_2O, 8:2:0.2–6:4:1, v/v/v), Rp-8 (50–70% aq. MeOH) and MCI-gel CHP20P (35–100% aq. MeOH).

Michehedyoside A (**1**): colourless wax; $[\alpha]_D^{25}$ – 46.3 (*c* 0.41, MeOH); IR (KBr) ν_{max}: 3357 (OH), 1078, 1602 cm^{-1}; UV (MeOH) λ_{max} nm (log ε): 284 (3.46), 204 (4.41); negative FABMS *m/z* 311 $[M–H]^-$, 149 $[M–H–162]^-$; HRESIMS *m/z* 311.1123 $[M–H]^-$ (calcd for $C_{15}H_{19}O_7$, 311.1130). ^1H and ^{13}C NMR see Tables 1 and 2.

Michehedyoside B (**2**): colourless wax; $[\alpha]_D^{25}$ – 62.7 (*c* 0.63, MeOH); IR (KBr) ν_{max}: 3406, 1072, 1596 cm^{-1}; UV (MeOH) λ_{max} nm (log ε): 273 (3.18), 205 (4.42); negative FABMS *m/z* 473 $[M–H]^-$, 311 $[M–H–162]^-$, 149 $[M–H–162–162]^-$; HRESIMS *m/z* 473.1672 $[M–H]^-$ (calcd for $C_{21}H_{29}O_{12}$, 473.1659); ^1H and ^{13}C NMR see Tables 1 and 2.

Michehedyoside C (**3**): white amorphous powder; $[\alpha]_D^{25}$ – 52.8 (*c* 0.12, MeOH). IR (KBr) ν_{max}: 3441, 1070, 1599 cm^{-1}; UV (MeOH) λ_{max} nm (log ε): 268 (3.09), 207 (4.67); negative FABMS *m/z* 503 $[M–H]^-$, 341 $[M–H–162]^-$, 179 $[M–H–162–162]^-$; HRESIMS *m/z* 503.1773 [M–H] – (calcd for $C_{22}H_{31}O_{13}$, 503.1764); ^1H and ^{13}C NMR see Tables 1 and 2.

Michehedyoside D (**4**): white amorphous powder; $[\alpha]_D^{25}$ – 53.6 (*c* 0.43, MeOH); IR (KBr) ν_{max}: 3406, 1070, 1612 cm^{-1}; UV (MeOH) λ_{max} nm (log ε): 277 (3.36), 206 (4.57); negative FABMS *m/z* 341 $[M–H]^-$, 179 $[M–H–162]^-$; HRESIMS *m/z* 341.1243 $[M–H]^-$(calcd for $C_{16}H_{21}O_8$, 341.1236). ^1H and ^{13}C NMR see Tables 1 and 2.

Michehedyoside E (**5**): white amorphous powder; $[\alpha]_D^{25}$ – 67.0 (*c* 0.17, MeOH); IR (KBr) ν_{max}: 3419, 1069, 1612 cm^{-1}; UV (MeOH) λ_{max} nm (log ε): 279 (3.38), 206 (4.53); negative FABMS *m/z* 487 $[M–H]^-$, 341 $[M–H–146]^-$, 179 $[M–H–146–162]^-$; HRESIMS *m/z* 487.1801 $[M–H]^-$ (calcd for $C_{22}H_{31}O_{12}$, 487.1815). ^1H and ^{13}C NMR see Tables 1 and 2.

Michehedyoside F (**6**): white amorphous powder; $[\alpha]_D^{25}$ – 51.2 (*c* 0.24, MeOH); IR (KBr) ν_{max}: 3395, 1075, 1505 cm^{-1}; UV (MeOH) λ_{max} nm (log ε): 296 (3.68), 235 (3.73), 204 (4.39); negative FABMS *m/z* 339 $[M–H]^-$, 177 $[M–H–162]^-$; HRESIMS *m/z* 339.1084 $[M–H]^-$ (calcd for $C_{16}H_{19}O_8$, 339.1079); ^1H and ^{13}C NMR see Tables 1 and 2.

Table 1
^{13}C NMR (125 MHz) spectroscopic data for compounds **1–6** (in DMSO-d_6, δ in ppm).

No.	1	2	3	4	5	6
1	130.8 (s)	134.6 (s)	135.9 (s)	134.5 (s)	134.5 (s)	121.8 (s)
2	116.9 (d)	118.3 (d)	110.1 (d)	107.3 (d)	107.5 (d)	149.5 (d)
3	145.5 (s)	147.7 (s)	150.9 (s)	148.0 (s)	148.0 (s)	99.4 (s)
4	145.0 (s)	145.7 (s)	133.8 (s)	129.8 (s)	129.9 (s)	145.7 (s)
5	115.8 (d)	118.6 (d)	152.7 (s)	145.7 (s)	145.7 (s)	141.9 (d)
6	122.6 (d)	122.5 (d)	107.9 (d)	109.9 (d)	109.7 (d)	108.5 (s)
7	39.3 (t)	39.3 (t)	39.8 (t)	39.5 (t)	39.5 (t)	33.5 (t)
8	138.1 (d)	137.8 (d)	137.3 (d)	137.9 (d)	138.0 (d)	137.4 (d)
9	115.5 (t)	115.8 (t)	115.9 (t)	115.6 (t)	115.7 (t)	115.1 (t)
OCH_3			55.3 (q)	55.9 (q)	56.0 (q)	
OCH_2O						100.7 (t)
1′	100.4 (d)	100.0 (d)	100.3 (d)	102.8 (d)	102.7 (d)	102.6 (d)
2′	71.2 (d)	71.3 (d)	70.8 (d)	73.4 (d)	70.9 (d)	73.3 (d)
3′	70.5 (d)	70.6 (d)	70.7 (d)	76.0 (d)	75.8 (d)	76.9 (d)
4′	67.1 (d)	67.1 (d)	67.0 (d)	69.8 (d)	70.0 (d)	69.8 (d)
5′	75.0 (d)	74.8 (d)	75.0 (d)	77.2 (d)	75.7 (d)	76.7 (d)
6′	61.0 (t)	61.0 (t)	60.9 (t)	60.7 (t)	66.5 (t)	60.9 (t)
1″		102.1 (d)	103.6 (d)		100.6 (d)	
2″		73.5 (d)	74.1 (d)		70.9 (d)	
3″		77.1 (d)	77.1 (d)		72.2 (d)	
4″		69.8 (d)	70.0 (d)		73.4 (d)	
5″		76.5 (d)	76.4 (d)		68.5 (d)	
6″		60.8 (t)	61.0 (t)		18.0 (t)	

Table 2
^{1}H NMR (500 MHz) spectroscopic data for compounds **1–6** (in DMSO-d_6, δ in ppm, J in Hz).

No.	1	2	3	4	5	6
2	6.90 (d, 1.6)	6.95 (d, 1.8)	6.69 (d, 1.4)	6.61 (d, 1.8)	6.46 (br s)	
3	–					6.78 (s)
5	6.70 (d, 8.1)	7.04 (d, 8.3)				
6	6.64 (dd, 8.2, 1.7)	6.74 (dd, 8.4, 1.6)	6.55 (d, 1.4)	6.47 (dd, 1.9)	6.50 (br s)	6.58 (br s)
7	3.18 (d, 6.5)	3.25 (d, 6.4)	3.25 (d, 6.9)	3.21 (d,.5.0)	3.18 (d, 6.5)	3.32 (d, 6.9)
						3.27 (d, 6.7)
8	5.88 (tdd, 6.5, 17.2, 10.2)	5.90 (tdd, 6.4, 16.8, 9.9)	5.90 (tdd, 6.9, 16.5, 10.1)	5.90 (tdd, 8.3, 17.8, 12.6)	5.90 (tdd, 6.5, 17.2, 10.2)	5.88 (m)
9	5.02 (dd, 17.2, 1.5)	5.18 (dd, 16.8, 2.8)	5.11 (dd, 16.5, 3.0)	5.23 (dd, 17.8, 2.8)	5.02 (dd, 17.2, 1.5)	5.02 (dd, 15.2, 4.0)
	5.00 (dd, 10.2, 1.5)	5.00 (dd, 9.9, 2.8)	5.02 (dd, 10.1, 3.0)	5.00 (dd, 12.6, 2.8)	5.00 (dd, 10.2, 1.5)	5.00 (dd, 11.0, 4.0)
OCH_3			3.70 (s)	3.70 (s)	3.68 (s)	
OCH_2O						5.88 (s)
1′	4.93 (d, 8.0)	5.02 (d, 7.7)	4.93 (d, 7.9)	4.58 (d, 6.9)	4.57 (d, 8.3)	4.56 (d, 6.4)
2′	3.44 (dd, 8.0, 2.7)	3.27 (m)	3.47 (m)	3.25 (m)	3.21 (m)	3.34 (m)
3′	3.94 (t, 2.7)	3.92 (t, 2.7)	3.92 (t, 2.7)	3.26 (m)	3.42 (m)	3.36 (m)
4′	3.39 (dd, 9.8, 2.5)	3.26 (m)	3.43 (m)	3.14 (m)	3.40 (m)	3.15 (m)
5′	3.64 (m)[a]	3.63 (m)	3.61 (m)	3.27 (m)	3.27 (m)	3.28 (m)
6′	3.41 (dd, 5.6, 12.0)	3.43[b]	3.47 (dd, 5.8, 12.0)	3.46 (dd, 5.8, 11.9)	3.43 (dd, 5.8, 12.0)	3.68 (dd, 1.3, 11.8)
	3.64[a]	3.64 (dd, 2.0,11.8)	3.61 (dd, 1.8,12.0)	3.69 (m)	3.89 (dd,1.0, 12.0)	3.46 (dd, 6.0, 11.8)
1″		4.71 (d, 7.3)	4.77 (d, 7.2)		4.53 (br s)	
2″		3.28 (m)	3.25 (m)		3.29 (m)	
3″		3.26 (m)	3.02 (m)		3.45 (m)	
4″		3.43 (m)	3.10 (m)		3.43 (m)	
5″		3.27(m)	3.18 (m)		3.26 (m)	
6″		3.64 (dd, 5.2,11.8)	3.61 (dd, 1.8,12.0)		1.07 (d, 6.1)	
		3.43[b]	3.47 (dd, 5.8, 12.0)			

[a] Overlapped with each other at the same column.
[b] Overlapped with water peak.

2.5. Acid hydrolysis of compounds *1–6*

A solution of **1–6** (about 5.0 mg) in 2% HCl (dioxane-H_2O, 1:1, 1 ml) was heated at 95 °C for 2 h based on a previous reference (Zhang, Zhang, Jacob, Li, & Yang, 2008). The reaction solution was extracted with chloroform 3 times. The aqueous layer was passed through an Amberlite IRA-401 (OH^- form) column and the eluate was condensed to dryness to yield the monosaccharide mixture.

2.6. Sugar analysis of compounds *1–6*

The solutions of those sugar parts obtained as described above in pyridine (2 ml) were added to L-cysteine methyl ester hydrochloride (1.5 mg) and kept at 60 °C for 1 h each. Then trimethylsilyl imidazole (1.5 ml) was added to the reaction mixture and kept at 60 °C for 30 min. Supernatant was subjected to GC analysis under the following conditions: Column temperature: 180/280 °C, programmed increase: 3 °C/min, carrier gas: N_2 (1 ml/min), injector and detector temperature: 250 °C, injection volume: 4 μl, split ratio: 1/50. Configuration identification of D-glucose, D-allose, L-rhamnose was carried out by comparison with its derivative's retention time (Hara, Okabe, & Mihashi, 1987). Retention time in GC of standard D/L-glucose, D/L-allose and L/D-rhamnose derivatives were 19.450/19.856, 19.964/20.053 and 15.849/16.312 min, respectively. By comparing with the retention time of the authentic sugars in the form of derivatives under the same condition, the sugar moieties of compounds **1–6** were determined to be D-allose for **1–3**, D-glucose for **2–6**, and L-rhamnose for **5**. All chemical reagents and standard sugars were purchased from Sigma–Aldrich Corporation.

3. Results and discussion

The MeOH extract of the seeds of *M. hedyosperma* was defatted with petroleum ether and then applied to repeated column chromatography (CC) on Diaion HP20SS, silica gel, Rp-18 and MCI-gel CHP20P column to afford six new compounds, michehedyosides A–F (**1–6**). In addition, six known compounds were identified as eugenol (Yamaguchi, Numata, Uemura, Kaneto, & Yokoyama, 1975), eugenol 4-*O*-β-D-glucopyranoside (Fujita & Nakayama, 1992), martynoside (Jia, Gao, & Liu, 1994), alaschanioside C (Gao & Jia, 1995), *trans*-3,4-methylenedioxycinnamyl alcohol (Kashima, Tanoguchi, Arimoto, & Yamaguchi, 1991) and (+)-pinoresinol 4-*O*-β-D-glucoside (Chiba, Hisada, Nishibe, & Thieme, 1980; Chiba, Okabe, Hisada, & Shima, 1979), respectively, by direct comparison of their spectral and physical data with those of authentic samples and literatures. All compounds were isolated from *M. hedyosperma* for the first time.

Michehedyoside A (**1**), obtained as a colourless wax, had a molecular formula $C_{15}H_{20}O_7$, as derived from the negative HRFABMS (*m/z* 311.1130 $[M-H]^-$) and ^{13}C NMR (DEPT) spectrum (Table 1). Negative ion FABMS showed fragment ion peak at *m/z* 149 $[M-H-162]^-$, indicating the existence of one hexosyl unit. The IR spectrum of **1** indicated the existence of hydroxyl group (3357, 1078 cm^{-1}) and aromatic ring (1602 cm^{-1}). The ^{13}C NMR and DEPT spectra (Table 1) displayed the presence of one methylene at δ 39.3, eight olefinic carbons arising from a phenyl and a terminal double bonds (δ 138.1, 115.5), together with a set of signals due to a hexosyl unit (δ 100.4, 71.2, 70.5, 67.1, 75.0, 61.0). The ^{1}H NMR spectrum (Table 2) showed three aromatic protons at δ 6.90 (1H, *d*, *J* = 1.7 Hz), 6.64 (1H, *dd*, *J* = 8.1, 1.7 Hz), and 6.70 (1H, *d*, *J* = 8.1 Hz) arising from a 1,3,4-trisubstituted benzene ring, three olefinic protons at δ 5.88 (1H, tdd, *J* = 6.5, 17.2, 10.2 Hz), 5.02 (1H, *J* = dd, 17.2, 1.5 Hz), and 5.00 (1H, *J* = dd, 10.2, 1.5 Hz) referring to a terminal double bonds, a doublet methylene at δ 3.18 (2H, *d*, *J* = 6.5 Hz), as well as an anomeric proton at δ 4.93 (1H, *d*, *J* = 8.0 Hz). These NMR features suggested compound **1** was dihydroxyallylbenzene glycoside. Acidic hydrolysis of **1** with 2% HCl afforded D-allose, which was identified by GC analysis of its trimethylsilyl imidazole derivatives (Hara et al., 1987). In the HMBC spectrum of **1**, correlations of the methylene (δ 3.18) with the aromatic carbons [δ 116.9 (C-2), 115.8 (C-5)] and the terminal double bond carbons [δ 115.5 (C-9), 138.1 (C-8)] confirmed the connection of the allyl with C-1. Moreover, the HMBC correlation from the anomeric proton (δ 4.93) to C-3 (δ 145.5) and the ROESY

correlation of the same anomeric proton with H-2 (δ 6.90) determined the connection of allosyl group at C-3. Therefore, the structure of michehedyoside A (**1**) was elucidated as 3,4-dihydroxy-allylbenzene 3-*O*-β-D-allopyranoside.

Michehedyoside B (**2**) was obtained as a colourless wax and possessed a molecular formula $C_{21}H_{30}O_{12}$, as determined by the negative HRFABMS (*m/z* 473.1659 $[M-H]^-$) and ^{13}C NMR (DEPT) spectrum (Table 1). The negative ion FABMS showed the fragment ion peaks at *m/z* 473 $[M-H]^-$, 311 $[M-H-162]^-$ and 149 $[M-H-162-162]^-$, indicating the existence of two hexosyl units in **2**. The ^{13}C (Table 1) and ^1H (Table 2) NMR spectra of **2** were closely related to those of **1**, except that **2** had one more β-D-glucopyranosyl unit. Acidic hydrolysis of **2** gave D-glucose and D-allose, as determined by GC analysis as **1**. In the HMBC spectrum of **2**, correlations from the allosyl anomeric proton at δ 5.02 (1H, d, J = 7.7 Hz) to C-3 (δ 147.7) and the glucosyl anomeric proton at δ 4.71 (1H, d, J = 7.3 Hz) to C-4 (δ 145.7) revealed their connections at C-3 and C-4 of aglycone, respectively. Moreover, the ROESY correlations of the allosyl anomeric proton (δ 5.02) with H-2 (δ 6.95) and the glucosyl anomeric proton (δ 4.71) with H-5 (δ 7.04) confirmed the linkage positions of β-D-allopyranosyl to C-3 (δ 147.7) and β-D-glucopyranosyl to C-4 (δ 145.7). In conclusion, michehedyoside B (**2**) was identified as 3,4-dihydroxy-allylbenzene 3-*O*-β-D-allo-pyranosyl-4-*O*-β-D-glucopyranoside.

Michehedyoside C (**3**) was isolated as a white amorphous powder and had a molecular formula $C_{22}H_{32}O_{13}$, as deduced by the HRFABMS (*m/z* 503.1773 $[M-H]^-$) and ^{13}C NMR (DEPT) spectrum (Table 1). The negative FABMS displayed the fragment ion peaks at *m/z* 503 $[M-H]^-$, 341 $[M-H-162]^-$ and 179 $[M-H-162-162)]^-$, indicating the existence of two hexosyl units. Acidic hydrolysis of **3** afforded D-glucose and D-allose as sugar moieties. The NMR data of **3** were similar to **2**, except for the appearance of an additional methoxyl group in **3**, which led the ABX coupling system in **2** changed as a *meta*-coupled system in **3** [δ 6.69 (d, J = 1.4 Hz) and 6.55 (d, J = 1.4 Hz)]. The linkage position of the additional methoxyl group was determined at C-5 by the HMBC correlation of the methoxyl proton (δ 3.70) with C-5 (δ 152.7) and the ROESY correlation of the methoxyl proton (δ 3.70) with H-6 (δ 6.55) (Fig. 2.). Accordingly, the structure of michehedyoside C (**3**) was elucidated as 3,4-dihydroxy-5-methoxy-allylbenzene 3-*O*-β-D-allopyranosyl-4-*O*-β-D-glucopyranoside.

Michehedyoside D (**4**) was obtained as a white amorphous powder. The HRESIMS displayed a quasi-molecular ion peak at *m/z* 341.1243 $[M-H]^-$, corresponding to a molecular formula $C_{16}H_{22}O_8$ for **4**. In the negative FABMS, the fragment ion peaks at *m/z* 341 $[M-H]^-$ and 179 $[M-H-162]^-$ indicated the existence of one hexosyl unit in **4**. Acidic hydrolysis of **4** gave D-glucose as a sugar moiety. The ^{13}C (Table 1) and ^1H (Table 2) NMR spectra of **4** showed the presence of one 1,3,4,5-tetrasubstitued benzene ring [δ 6.61 and 6.47 (each 1H, d, J = 1.8 Hz)], one allyl group with terminal double bonds [δ 3.21 (d, J = 5.0 Hz, H-7), 5.90 (tdd, J = 8.3, 17.8, 12.6 Hz, H-8), 5.23 (dd, J = 17.8, 2.8 Hz, H-9a), and 5.00 (dd, J = 12.6, 2.8 Hz, H-9b); δ 39.5 (C-7), 137.9 (C-8) and 115.6 (C-9)], one methoxyl group (δ 55.3), and one hexosyl unit [anomeric proton at δ 4.58 (d, J = 6.9 Hz, H-1′)]. These NMR features were very similar to those of **3**. The only difference was that **4** had only one glucosyl unit in molecule. In the HMBC spectrum of **4**, correlation of the glucosyl H-1 (δ 4.58) with aromatic carbon (δ 145.7, C-5) was observed. Moreover, the ROESY correlations of the glucosyl H-1 with the aromatic H-2 (δ 6.61), and the methoxyl proton (δ 3.70) with the aromatic H-6 (δ 6.47) confirmed the linkage positions of glucosyl and methoxyl groups at C-3 and C-5, respectively. Based on the above evidences, michehedyoside D (**4**) was determined as 3,4-dihydroxy-5-methoxy-allylbenzene 3-*O*-β-D-glucopyranoside.

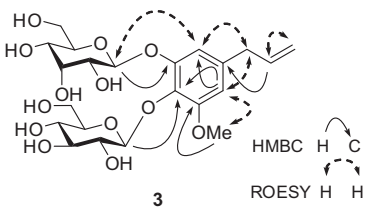

Fig. 2. Key HMBC and ROESY correlations of compound **3**.

Michehedyoside E (**5**), white amorphous powder, displayed a quasi-molecular ion peak at *m/z* 487.1801 $[M-H]^-$ in the HRESIMS, corresponding to a molecular formula $C_{22}H_{32}O_{12}$. The negative FABMS data at *m/z* 487 $[M-H]^-$, 341 $[M-H-146]^-$ and 179 $[M-H-146-162]^-$, combining with the ^{13}C NMR data (Table 1), indicated that **5** had one hexosyl and one 6-deoxyhexosyl units. Acidic hydrolysis of **5** yielded L-rhamnose and D-glucose as sugar moieties. The ^{13}C (Table 1) and ^1H (Table 2) NMR spectra of **5** were closely related to those of **4**, except for the appearance an additional rhamnosyl unit in **5**. The HMBC correlations of the glucosyl H-1 (δ 4.57) with C-3 (δ 145.7) and the rhamnosyl H-1 (δ 4.53) with the glucosyl C-6′ (δ 66.5), indicated the linkage positions of the glucosyl unit at C-3 and the rhamnosyl unit at glucosyl C-6′, respectively. In addition, the ROESY correlations between the glucosyl anomeric proton (δ 4.57) with H-2 (δ 6.46) and the methoxyl proton with H-6 (δ 6.50) further confirmed the glucosyl unit linked at C-3 and the methoxyl group at C-5. Accordingly, michehedyoside E (**5**) was established as 3,4-dihydroxy-5-methoxyl-allylbenzene 3-*O*-α-L-rhamnopyranosyl-(1–6)-β-D-glucopyranoside.

Michehedyoside F (**6**) was isolated as a white amorphous powder. The HRESIMS exhibited a quasi-molecular ion peak at *m/z* 339.1084 $[M-H]^-$, establishing a molecular formula $C_{16}H_{20}O_8$ for **6**. The negative FABMS displayed fragment ion peaks at *m/z* 339 $[M-H]^-$ and 177 $[M-H-162]^-$, suggesting the existence of one hexosyl unit. The ^{13}C (DEPT) (Table 1) and ^1H (Table 2) NMR spectra of **6** showed the existence of one 1,2,4,5-tetrasubstituted benzene ring [δ 121.8 (s), 149.5 (d), 99.4 (s), 145.7 (s), 141.9 (d), and 108.5 (s); δ 6.78 and 6.58 (each 1H, s)], one terminal double bonds (δ 137.4 and 115.1), one aliphatic methylene (δ 33.5), and one hexosyl [anomeric proton at δ 4.56 (d, J = 6.4 Hz)] units. Acid hydrolysis of **6** produced D-glucose as the sugar moiety. These NMR data suggested that **6** was an allylbenzene glucoside. However, compared with **1**–**5**, compound **6** showed the presence of a typical methylenedioxyl group (δ 5.88 (2H, s), δ 100.7). In the HMBC spectrum of **6**, the correlations of the methylenedioxyl protons (δ 5.88) with C-4 (δ 145.7) and C-5 (δ 141.9), the allyl methylene protons (δ 3.32 and 3.27) with C-6 (δ 108.5), the aromatic proton (δ 6.58, H-2) with C-1, and the glucosyl anomeric proton (δ 4.56) with C-2 (δ 149.5) were observed. In addition, the ROESY correlations of the allyl methylene protons (δ 3.32 and 3.27) with H-6 (δ 6.58) and the anomeric proton (δ 4.56) with H-3 (δ 6.78) further confirmed the glucosyl unit located on C-2, while the methylenedioxyl group was linked between C-4 and C-5 positions. Thus, michehedyoside F (**6**) was deduced as 2-hydroxy-4,5-methylenedioxyl-allylbenzene 2-*O*-β-D-glucopyranoside.

Based on the structures, the isolated compounds from the seeds of *M. hedyosperma* could be classified into phenylpropanoid glycosides (**1**–**6**, eugenol, eugenol 4-*O*-β-D-glucopyranoside, martynoside, and 3,4-methylenedioxycinnamyl alcohol) and lignin glycosides [alaschanioside C and (+)-pinoresinol 4-*O*-β-D-glucoside]. Among them, phenylpropanoid glycosides are the main chemical composition in the seeds of *M. hedyosperma*. Phenylpropanoid glycosides (PPGs) are known to possess various interesting pharmacological properties, including antioxidant, antiviral, and inhibition of blood platelet aggregation and LTB4 synthesis, antibacterial and antifungal activities (Ismailoglu, Saracoglu, Harput,

& Sahin-Erdemli, 2002). Our research result supported that the seeds of *M. hedyosperma* could be a healthy plant resource for spice in the view of phytochemistry and the previous pharmacological publications.

Acknowledgements

The authors are grateful to the analytical group at State Key Laboratory of Phytochemistry & Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, for measuring all the spectral data. This work was supported by the NSFC U0632010, 2008ZX09401-004, 2011CB915503 and The State Key Laboratory of Phytochemistry and Plant Resources in West China, Chinese Academy of Sciences (P2008-ZZ08).

References

Brown, L. L., Larson, S. R., & Sneden, A. T. (1998). Vanicosides C–F, new phenylpropanoid glycosides from *Polygonum pensylvanicum*. *Journal of Natural Products, 61*, 762–766.

Chiba, M., Hisada, S., Nishibe, S., & Thieme, H. (1980). ^{13}C NMR analysis of symplocosin and (+)-epinoresinol glucoside. *Phytochemistry, 19*, 335–336.

Chiba, M., Okabe, K., Hisada, S., & Shima, K. (1979). Elucidation of the structure of a new lignan glucoside from *Olea europaea* by Carbon-13 nuclear magnetic resonance spectroscopy. *Chemical and Pharmaceutical Bulletin, 27*, 2868–2873.

Díaza, A. M., Abadb, M. J., Fernándeza, L., Silvánb, A. M., Santosa, J. D., & Bermejob, P. (2004). Phenylpropanoid glycosides from *Scrophularia scorodonia*: In vitro anti-inflammatory activity. *Life Sciences, 74*, 2515–2526.

Fujita, T., & Nakayama, M. (1992). Perilloside A, a monoterpene glucoside from *Perilla Frutescens*. *Phytochemistry, 31*, 3265–3267.

Gao, J. J., & Jia, Z. J. (1995). Lignan, iridoid and phenylpropanoid glycosides from *Pedicularis alaschanica* Maxim (Scrophulariaceae). *Indian Journal of Chemistry, 34B*, 466–468.

Guan, Z. B., & Zhang, L. X. (2004). The study on conservation and utilization of endangered plant *Michelia hedyosperma*. *Chinese Wild Plant Resources, 23*, 11–14.

Hara, S., Okabe, H., & Mihashi, K. (1987). Gas–liquid chromatographic separation of aldose enantiomers as trimethylsilyl ethers of methyl 2-(polyhydroxyalkyl)–thiazolidine-4(R)-carboxylates. *Chemical and Pharmaceutical Bulletin, 35*, 501–596.

Iida, T., & Ito, K. (1982). Sesquiterpene lactones from *Michelia fuscata*. *Phytochemistry, 21*, 701–703.

Ismailoglu, U. B., Saracoglu, I., Harput, U. S., & Sahin-Erdemli, I. (2002). Effects of phenylpropanoid and iridoid glycosides on free radical-induced impairment of endothelium-dependent relaxation in rat aortic rings. *Journal of Ethnopharmacology, 79*, 193–197.

Jacobsson, U., Kumar, V., & Saminathan, S. (1995). Sesquiterpene lactones from *Michelia champaca*. *Phytochemistry, 39*, 839–843.

Jia, Z. J., Gao, J. J., & Liu, Z. M. (1994). Iridoid and phenylpropanoid glycosides from *Pedicularis plicata* Maxim. *Indian Journal of Chemistry, 33B*, 460–464.

Kashima, T., Tanoguchi, M., Arimoto, M., & Yamaguchi, H. (1991). Studies on the constituents of the seeds of *Hernandia ovigera* L. VIII. Syntheses of (±)-desoxy-podophyllotoxin and (±)-β-peltatin-A methyl ether. *Chemical and Pharmaceutical Bulletin, 39*, 192–194.

Kelm, M. A., & Nair, M. G. (2000). A brief summary of biologically active compounds from *Magnolia* spp. *Studies in Natural Products Chemistry, 24*, 845–873.

Khan, M. R., Kihara, M., & Omoloso, A. D. (2002). Antimicrobial activity of *Michelia champaca*. *Fitoterapia, 73*, 744–748.

Nijsiri, R., Kittisak, L., & Srirat, K. (1988). Constituents of *Michelia rajaniana*, two new germacranolide amides. *Journal of Natural Products, 51*, 1220–1225.

Ogura, M., Cordell, G. A., & Farnsworth, N. R. (1978). Anticancer Sesquiterpene lactones of *Michelia compressa* (Magnoliaceae). *Phytochemistry, 17*, 957–961.

Qi, L. W., Wen, X. D., Cao, J., Li, C. Y., Li, P., Yi, L., et al. (2008). Rapid and sensitive screening and characterization of phenolic acids, phthalides, saponins and isoflavonoids in Danggui Buxue Tang by rapid resolution liquid chromatography/diode-array detection coupled with time-of-flight mass spectrometry. *Rapid Communications in Mass Spectrometry, 22*, 2493–2509.

Talapatra, S. K., Patra, A., & Talapatra, B. (1975). Alkaloids of *Michelia lanuginosa* Wall. *Tetrahedron, 31*, 1105–1107.

Wu, Q., Yuan, Q., Liu, E. H., Qi, L. W., Bi, Z. M., & Li, P. (2010). Fragmentation study of iridoid glycosides and phenylpropanoid glycosides in *Radix scrophulariae* by rapid resolution liquid chromatography with diode-array detection and electrospray ionization time-of-flight mass spectrometry. *Biomedical Chromatography, 24*, 808–819.

Xu, J. Y., Su, K., & Zhou, J. (2007). Study Progress of Phenylpropanoid Glycosides (II). *Lishizhen Medicine and Material Medica Research, 18*, 1770–1772.

Yamaguchi, H., Numata, A., Uemura, E., Kaneto, H., & Yokoyama, A. (1975). The effect of the alkaline earth salts on the NMR spectrum of phenols. *Chemical and Pharmaceutical Bulletin, 23*, 1169–1170.

Zhang, L. J., Liao, C. C., Huang, H. C., Shen, Y. C., Yang, L. M., & Kuo, Y. H. (2008). Antioxidant phenylpropanoid glycosides from *Smilax bracteata*. *Phytochemistry, 69*, 1398–1404.

Zhang, Y., Zhang, Y. J., Jacob, M. R., Li, X. C., & Yang, C. R. (2008). Steroidal saponins from the stem of *Yucca elephantipes*. *Phytochemistry, 69*, 264–270.