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Bioactive phenolics and terpenoids from *Manglietia insignis*

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ABSTRACT

Four new compounds, maninsigins A–D (**1**–**4**), including two new neolignans (**1**–**2**) and two new sesquiterpenes (**3**–**4**), as well as ten known compounds (**5**–**14**), were isolated from the leaves and stems of *Manglietia insignis*. Their structures were established on the basis of extensive spectroscopic analyses. In addition, some compounds were tested for their cytotoxic and neurite outgrowth-promoting activities, as well as their antagonistic activity toward FXR ligand.

1. Introduction

Magnolia officinalis Rehd. et Wils is a very important traditional medicine and has been used in the treatments of asthma, abdominal distention and pain, dyspepsia, and asthmatic cough [1,2]. Phytochemical studies have revealed a variety of lignans and alkaloids as chemical constituents of the plant. The lignans showed cytotoxic, anti-inflammatory, antioxidative, antagonistic, and antitumor activities, while the alkaloids exhibited antiplasmodial and free radicals restraining activities [3–8]. *Manglietia insignis* (Wall.) Bl. is widely distributed in the west of China and has been partly used as a substitute of *M. officinalis* in Yunnan and Sichuan provinces of China. Previous researches have shown that *M. insignis* also contained representative bioactive components as that of *M. officinalis*, such as magnolol and magnocurarine [9]. However, phytochemical research on *M. insignis* is quite limited so far. Aiming at discovering chemical constituents with significant bioactivities, we conducted the phytochemical investigation of the leaves and stems of *M. insignis*, which led to the isolation of four new compounds, maninsigins A–D (**1**–**4**) including two neolignans (**1**–**2**) and two sesquitepenoids (**3**–**4**), and ten known lignans (**5**–**14**) (Fig. 1). Herein, the isolation, structural elucidation, and biological activities of these compounds are described.

2. Experimental

2.1. General experimental procedures

Optical rotations were measured with a JASCO DIP-370 digital polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. A BioRad FtS-135 spectrophotometer was used for scanning IR spectroscopy with KBr pellets.

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1D and 2D NMR spectra were recorded on Bruker AM-400, DRX-500 and Bruker Avance III-600 MHz spectrometers. Unless otherwise specified, chemical shifts (δ) were expressed in ppm with reference to the solvent signals. High-resolution electrospray-ionization (HRESIMS) was performed on a VG Autospec-3000 spectrometer under 70 eV. Column chromatography was performed using a silica gel (200–300 mesh, Qing-dao Marine Chemical, Inc., Qingdao, China). Semi-preparative HPLC was performed on an Agilent 1100 liquid chromatography with a Zorbax SB-C_{18}, 9.4 mm × 25 cm, column. Fractions were monitored by TLC and spots were visualized by heating the silica gel plates sprayed with 10% H_2SO_4 in EtOH.

2.2. Plant material

The leaves and stems of *M. insignis* (Wall.) Bl. were collected in Kunming Botanic Garden, Yunnan Province, People's Republic of China, in August 2007. The specimen was identified by Prof. Xun Gong and a voucher specimen (No. KIB 2007-08-11) has been deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

2.3. Extraction and isolation

The plant material of *M. insignis* (8.5 kg) was ground and exhaustively extracted with Me_2CO–H_2O (V/V = 7:3, 3 × 25 L) at room temperature. The solvent was evaporated in vacuo, and the crude extract was dissolved in H_2O and partitioned with EtOAc. The EtOAc portion (110 g) was chromatographed on a silica gel column (80–100 mesh, 15 × 120 cm, 0.6 kg) being eluted with $CHCl_3$–Me_2CO (1:0, 20:1, 10:1, 5:1, 2:1, 1:1, and 0:1, each 12 L) to afford fractions I–VII. Fraction III (9.2 g) was applied to RP-18 (3 × 40 cm), eluted with a MeOH–H_2O (40%–100%, each 8 L) gradient system, to afford five fractions. Fraction III-2 (1.3 g) was repeatedly chromatographed on a silica gel (a, 200–300 mesh, 3 × 35 cm, petroleum ether–Me_2CO, 12:1, 9:1, 6:1, and 2:1, each 0.9 L; b, 200–300 mesh, 1.5 × 35 cm, $CHCl_3$–Me_2CO, 30:1, 20:1, 15:1, 10:1, each 0.6 L) and Sephadex LH-20 (1.5 × 120 cm, MeOH) to yield **1** (9.0 mg), **4** (3.5 mg) and **6** (10.0 mg). Fraction III-3 (1.7 g) was chromatographed on a silica gel (a, 200–300 mesh, 3 × 35 cm, petroleum ether–Me_2CO, 18:1, 13:1, 8:1, 4:1 and 2:1, each 1.2 L; b, 200–300 mesh, 1.5 × 35 cm, $CHCl_3$–Me_2CO, 30:1, 20:1, 12:1, 6:1, each 0.8 L), further over an RP-18 column [1.5 × 35 cm, MeOH–H_2O, 56%, (4 L)], followed by Sephadex LH-20 (1.5 × 120 cm, MeOH) to yield **3** (3.5 mg), **5** (2.9 mg),

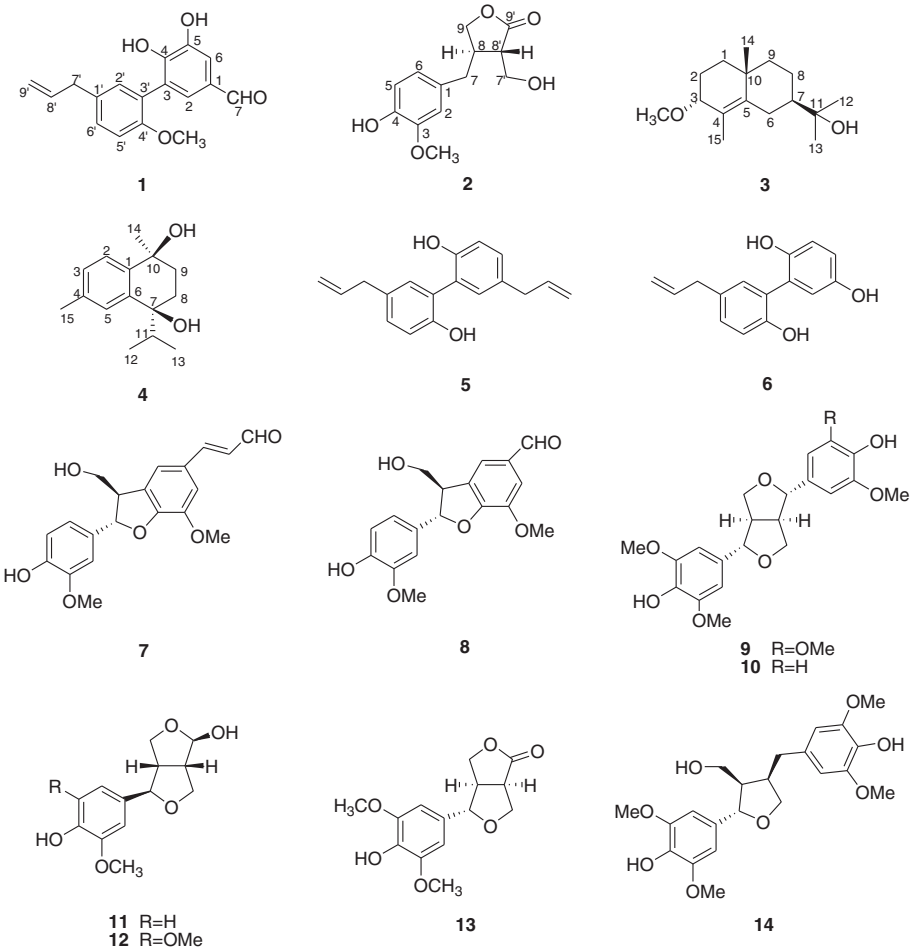


Fig. 1. The structures of compounds **1**–**14**.

7 (8.0 mg), **8** (2.0 mg) and **14** (2.0 mg). Fraction IV (14 g) was subjected to RP-18 (3×40 cm), developed with MeOH–H_2O (30%–100%, each 8 L) gradient system, to afford five fractions. Fraction IV-3 (2.3 g) was subjected to semi-preparative HPLC (46% MeOH–H_2O) to yield **2** (2.1 mg), **11** (2.5 mg), **12** (2.0 mg). Fraction IV-4 (2.3 g) was chromatographed on a silica gel column (200–300 mesh, 3×40 cm), eluted with $CHCl_3$–Me_2CO (20:1, 15:1, 11:1, 7:1 and 2:1, each 1.2 L), further over a Sephadex LH-20 column (1.5×120 cm, MeOH), followed by a semi-preparative HPLC (40% MeOH–H_2O) to yield **9** (11.6 mg), **10** (12.0 mg), **13** (1.8 mg).

Maninsigin A (**1**): yellow oil; $[\alpha]_D^{25}$+0.89 (*c* 1.50, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 288 (3.77), 219 (4.15), 203 (4.12) nm; IR (KBr) ν_{max} 3424, 3075, 2926, 2835, 2730, 1678, 1605, 1589, 1501, 1496, 1439, 1248, 1248, 915, 808 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; positive EIMS *m/z* 285 $[M+H]^+$; positive HREIMS *m/z* 285.1125 $[M+H]^+$ (calcd for $C_{17}H_{17}O_4$, 285.1126).

Maninsigin B (**2**): yellow oil; $[\alpha]_D^{25}$−0.80 (*c* 1.42, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 281 (3.24), 226 (3.58), 204 (4.4) nm; IR (KBr) ν_{max} 3425, 2928, 2853, 1764, 1628, 151 4, 1463, 1382, 1031 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; positive ESIMS *m/z* 275 $[M+Na]^+$; positive HRESIMS *m/z* 275.0905 $[M+Na]^+$ (calcd for $C_{13}H_{16}O_5Na$, 275.0895).

Maninsigin C (**3**): white powder; $[\alpha]_D^{25}$+84.2 (*c* 3.59, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 204 (4.0) nm; IR (KBr) ν_{max} 3441, 2932, 2855, 1629, 1462, 1377, 1083 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; positive ESIMS *m/z* 275 $[M+Na]^+$; HRESIMS *m/z* 275.1987 $[M+Na]^+$ (calcd for $C_{16}H_{28}O_2Na$, 275.1987).

Maninsigin D (**4**): white powder; $[\alpha]_D^{25}$−9.4 (*c* 0.70, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 214 (3.92), 202 (4.20) nm; IR (KBr) ν_{max} 3425, 3022, 2961, 2874, 1629, 1499, 1461, 1383, 1005 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; positive ESIMS *m/z* 257 $[M+Na]^+$; positive HRESIMS *m/z* 257.1524 $[M+Na]^+$ (calcd for $C_{15}H_{22}O_2Na$, 257.1517).

Table 1
^{13}C NMR and ^1H NMR spectroscopic assignments of compounds **1** and **2**.[a]

Position	**1**		**2**	
	δ_C	δ_H	δ_C	δ_H
1	126.9 s	–	131.9 s	–
2	113.9 d	7.49 (s)	113.2 d	6.76 (d, 1.8)
3	125.7 s	–	149.1 s	–
4	146.6 s	–	146.1 s	–
5	146.5 s	–	116.2 d	6.67 (d,8.0)
6	125.8 s	7.44 (s)	122.2 d	6.61 (dd,1.8,8.0)
7α	191.2 d	9.89 (s)	33.5 t	2.50 (dd, 12.0, 16.0)
7β	–	–		2.86 (overlap)
8	–	–	40.7 d	2.95 (overlap)
9α	–	–	73.2 t	4.02 (dd, 5.9, 8.7)
9β	–	–		4.09 (dd, 7.0, 8.7)
MeO-3	–	–	56.3 q	3.78 (^{3}H, s)
1′	134.7 s	–	–	–
2′	132.4 d	7.25 (s)	–	–
3′	130.8 s	–	–	–
4′	153.3 s	–	–	–
5′	112.3 d	7.05 (d, 8.4)	–	–
6′	130.0 d	7.28 (d, 8.4)	–	–
7′α	39.2 t	3.43 (d, 6.5)	59.6 t	3.84 (dd, 7.7, 13.9)
7′β	–	–	–	3.91 (dd, 4.9, 13.9)
8′	137.1 d	5.98 (m)	46.9 d	2.80 (m)
9′	116.2 t	5.12 (^{2}H, m)	180.7 s	–
MeO-4′	56.9 q	3.96 (^{3}H, s)	–	–

[a] Spectra of **1** were recorded in $CDCl_3$, spectra of **2** were recorded in CD_3OD, and all chemical shifts (δ) were in ppm.

2.4. Cytotoxicity assay

The following human tumor cell lines were used: HL-60, MMC-7721, A549, MCF-7, and SW480. All cells were cultured in RPMI-1640 or DMEM medium (Hyclone, Logan, UT), supplemented with 10% fetal bovine serum (Hyclone) at 37 °C in a humidified atmosphere with 5% CO_2. Cell viability was assessed by conducting colorimetric measurements of the amount of insoluble formazan formed in living cells based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma, St. Louis, MO) [10]. Briefly, 100 μL of adherent cells was seeded into each well of a 96-well cell culture plate and allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before drug addition, both with an initial density of 1×10^5 cells/mL in 100 μL of medium. Each cell line was exposed to the test compound at various concentrations in triplicate for 48 h, with cisplatin and paclitaxel (Sigma) as positive controls. After the incubation, MTT (100 μg) was added to each well, and the incubation continued for 4 h at 37 °C. The cells were lysed with 100 μL of 20% SDS-50% DMF after removal of 100 μL of medium. The optical density of the lysate was measured at 595 nm in a 96-well microtiter plate reader (Bio-Rad 680). The IC_{50} value of each compound was calculated by Reed and Muench's method [11].

2.5. Neurite outgrowth-promoting activity

The neurotrophic activities of the test compounds were examined according to an assay using PC12 cells as reported [12,13]. Briefly, PC12 cells were maintained in F12 medium supplemented with 12.5% horse serum (HS), and 2.5% fetal bovine serum (FBS), and incubated at 5% CO_2 and 37 °C. Test

Table 2
^1H NMR and ^{13}C NMR Assignments of Compounds **3** and **4**.[a]

Position	**3**		**4**	
	δ_C	δ_H	δ_C	δ_H
1α	35.4 t	1.50 (m)	141.4 s	–
1β	–	1.32 (m)	–	–
2α	23.0 t	1.87 (m)	125.1 d	7.43 (d, 8.0)
2β	–	1.60 (overlap)	–	–
3	80.7 d	3.41 (s)	127.7 d	7.04 (d, 8.0)
4	125.2 s	–	136.2 s	–
5	142.4 s	–	125.9 d	7.24 (s)
6α	27.5 t	2.67 (dd, 2.1, 13.8)	139.5 s	–
6β	–	1.63 (overlap)	–	–
7	51.4 d	1.27 (m)	73.5 s	–
8α	24.2 t	1.65 (m)	26.9 t	1.89 (m)
8β	–	1.43 (m)	–	1.80 (m)
9α	43.5 t	1.60 (overlap)	34.2 t	2.06 (m)
9β	–	1.20 (m)	–	1.78 (m)
10	35.9 s	–	70.1 s	–
11	73.2 s	–	36.9 d	2.37 (m)
12	26.4 q	1.15 (s)	15.0 q	0.57 (d, 6.9)
13	27.2 q	1.16 (s)	17.5 q	1.04 (d, 6.9)
14	23.3 q	1.00 (s)	28.8 q	1.36 (s)
15	17.4 q	1.70 (s)	19.9 q	2.28 (s)
MeO-3	57.2 q	3.36 (s)	–	–

[a] Spectra of **3** and **4** were recorded in CD_3OD.

compounds were dissolved in DMSO. For the neurite outgrowth-promoting activity bioassay, PC12 cells were seeded at a density of 2×10^4 cells/mL in 48-well plate coated with poly-L-lysine. After 24 h, the medium was changed to that containing 10 μM of each test compound plus 5 ng/mL NGF, or various concentrations of NGF (50 ng/mL for the positive control, 5 ng/mL for the negative control). The final concentration of DMSO was 0.05%, and the same concentration of DMSO was added into the negative control. After 72 h incubation, the neurite outgrowth was assessed under a phase contrast microscope. Neurite processes with a length equal to or greater than the diameter of the neuron cell body were scored as neurite bearing cells. The ratio of the neurite-bearing cells to total cells (with at least 100 cells examined/view area; 5 viewing area/well) was determined and expressed as a percentage.

2.6. Yeast two-hybrid system-based assay

The restriction and modification enzymes in this work were obtained from NEB. *P*-nitrophenyl α-D-galactopyranoside, guggulsterone (GS), yeast nitrogen base without amino acids, agar, lithium acetate, dimethyl sulfoxide (DMSO) and glucose were all purchased from Sigma. The yeast expression plasmids pGADT7 and pGBKT7 were from Clontech (Palo Alto, CA). Chenodeoxycholic acid (CDCA) was from Merck. The dropout supplement free from leucine and tryptophan (-Leu/-Trp DO supplement) was bought from Takara. The yeast strain AH109 was purchased from Clontech (Palo Alto, CA). The agonistic or antagonistic activities of the compounds were tested by a yeast two-hybrid system for FXR constructed by yeast co-transformation with pGBKT7-FXR LBD and pGADT7-SRC1 according to the lithium acetate method [14]. Human FXRα-LBD (200–473 aa) was sub-cloned into vector pGBKT-7 using NdeI and BamHI restrict enzyme sites. The primers used for PCR amplification were listed as follows: FXRα-LBD (sense) 5′-ATCATATGGAAATTCAGTGTAAATCTAAG-CG-3′, (anti-sense) 5′-ATGGATCCTCACTGCACGTCCCA-3′. The combination plasmid pGADT7-SRC1 was prepared as described previously [15]. After co-transforming the two constructs into yeast strain AH109, we successfully evaluated FXR/SRC1 interactions by conducting a convenient α-galactosidase assay. Yeast transformations were incubated with either a control vehicle (DMSO) or the indicated compounds for 24 h in an hFXR agonist testing, and in antagonist assays treated with tested compounds plus 10 μM CDCA. The α-galactosidase activity was then measured using *p*-nitrophenyl α-D-galactopyranoside as the substrate [16]. The α-galactosidase activity was calculated according to the following formula:

$$\alpha-\text{galactosidase activity}[\text{milliunits}/(\text{mL}\times\text{cell})] = \frac{OD_{410}\times V_f\times 1000}{(\varepsilon\times b)\times t\times V_i\times OD_{600}}$$

where t is the elapsed time of incubation, V_f is the final volume of assay (200 μL), V_i is the volume of culture medium supernatant added (16 μL), OD_{600} is the optical density of overnight culture, and $\varepsilon\times b$ is the *p*-nitrophenol molar absorptivity at 410 nm × the light path (cm) = 10.5 mL/μmol.

3. Results and discussion

Powdered leaves and stems of *M. insignis* were extracted with 70% aqueous acetone. The filtrate was concentrated and partitioned between H_2O and EtOAc. The EtOAc fraction was dried under reduced pressure, and then submitted to silica gel, MCI CHP-20 gel, RP-18 gel column chromatography (CC), Sephadex LH-20, and semi-preparative HPLC to yield four new compounds (**1**–**4**) and ten known ones. The ^1H and ^{13}C NMR spectroscopic data of **1**–**4** are listed (Tables 1, 2).

Maninsigin A (**1**) was assigned the molecular formula, $C_{17}H_{16}O_4$, by HREIMS experiment (*m/z* 285.1125 $[M+H]^+$), requiring 10° of unsaturation. The ^1H NMR spectrum exhibited three proton signals for one allyl group at δ_H 5.98 (1H, m), 5.12 (2H, m), and 3.43 (2H, d, J = 6.5 Hz), three signals for one ABX-aromatic system at δ_H 7.25 (1H, s), 7.05 (1H, d, J = 8.4 Hz) and 7.28 (1H, d, J = 8.4 Hz), two singlets for one AB-aromatic system at δ_H 7.49 (1H, s) and 7.44 (1H, s), and an aldehyde signal at δ_H 9.89 (1H, s) (Table 1). The ^{13}C NMR and DEPT spectra showed 15 carbon signals (Table 1). Comparison of the NMR data of **1** with those of magnaldehyde D [17] suggested that the differences of the two compounds can be rationalized to be an additional methoxyl and one more hydroxyl group located at the aromatic ring in **1**. The methoxyl group located at C-4′ was deduced by the HMBC correlation from the methoxyl proton with C-4′ (δ_C 153.3), which was further confirmed by the ROESY correlation of this methoxyl proton with H-5′. The additional hydroxyl group located at C-5 was deduced from the spin–spin coupling of H-2 and H-6, and both of them were singlets, together with the HMBC correlations of both H-2 and H-6 with C-1 and C-7, and H-7 with C-1, C-2, and C-6 (Fig. 2). Thus, the structure of **1** was determined as shown and given the name as maninsigin A.

Maninsigin B (**2**) was assigned the molecular formula of $C_{13}H_{16}O_5$ from the molecular ion peak at *m/z* 275.0905 $[M+Na]^+$ in HRESIMS. The ^1H NMR spectrum showed the presence of one set of ABX-type aromatic signals at δ_H 6.67 (^1H, d, J = 8.0 Hz), 6.76 (^1H, d, J = 1.8 Hz), 6.61 (^1H, dd, J = 8.0, 1.8 Hz), and a methoxyl group at δ_H 3.79 (^3H, s) and other signals (δ_H 2.0–4.1, ^8H) (Table 1). The ^{13}C NMR and DEPT spectra exhibited 13 carbon signals, including seven sp^2 carbons (six aromatic carbons and a carbonyl one), three methylenes (two oxygenated ones), two methines and a methoxyl group (Table 1). The $^1H-^1H$ COSY correlations of H_2-9/H-8/H-8′/H_2-7′ which showed the connection pattern of C-9–C-8–C-8′–C-7′, together with the HMBC correlations of H-8, H-9, H-7′, and H-8′ with the lactone carbonyl (C-9′, δ_C 180.7), determined the presence of a γ-butyrolactone ring by the linkage between C-9 and C-9′ as shown (Fig. 2). The HMBC correlations of H-7 with C-1 (δ_C 131.9), C-2 (δ_C 113.2),

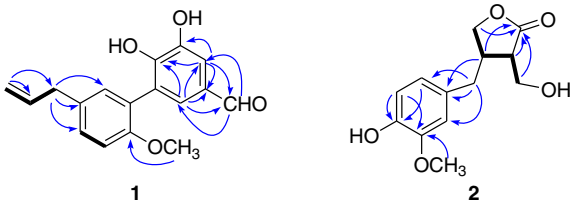


Fig. 2. Key HMBC (H→C) and $^1H-^1H$ COSY (—) correlations of **1** and **2**.

and C-6 (δ_C 122.2); of H-8 with C-1 suggested that C-8 was connected with the aromatic ring through C-7 (δ_C 33.5). The methoxyl group was deduced to be located at C-3 by the HMBC correlation of its proton signal with C-3, which was further confirmed by the ROESY correlation of this methoxyl proton with H-2. A hydroxyl group was located at C-4 deduced by its downfield chemical shift at δ_C 146.1, and the HMBC correlations of H-5, H-6 and H-2 with C-4. The relative configurations of **2** were established on the basis of the ROESY spectrum. The key correlation between H-8′ and H-9β (δ_H 4.09) revealed that H-8′ and H-9β were cofacial and the correlation between H-8 and H-7′ (δ_H 3.84) indicated that they were on the other side. Thus, the relative configuration of **2** was determined. H-8′ was arbitrarily defined as a β-orientation, and accordingly, H-8 was assigned the α-orientation (Fig. 3). Therefore, the structure of **2** was established.

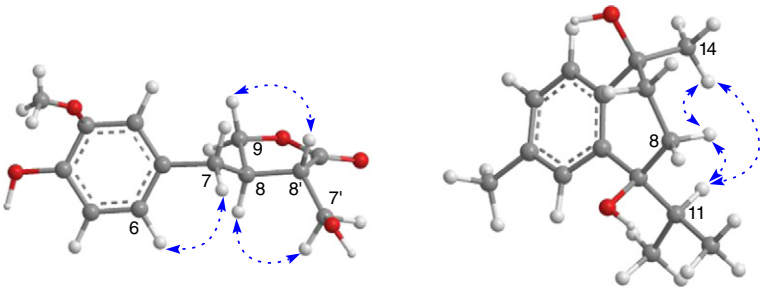
Maninsigin C (**3**) gave an $[M+Na]^+$ peak at *m/z* 275.1987 in the HRESIMS, consistent with a molecular formula of $C_{16}H_{28}O_2$. The ^1H NMR spectrum showed three methyl groups at δ_H 1.01, 1.16, and 1.17, and one methoxyl group at δ_H 3.36. The ^{13}C NMR and DEPT spectra of **3** exhibited 16 carbons signals attributed to three methyls, one methoxyl group, five methylenes, two methines (an oxygenated one), two quaternary sp^2 carbons, and two quaternary sp^3 carbons (an oxygenated one) (Table 2). The above evidence hinted that compound **3** was a sesquiterpenoid. Analyses of the $^1H-{}^1H$ COSY, HSQC, HMBC, and ROESY spectra suggested that **3** had a skeleton of eudesmane and was a derivative of machikusanol [4]. The only difference was the appearance of an additional methoxyl group in **3**. This methoxyl group was located at C-3 by the HMBC correlation of its proton signals with C-3 (δ_C 80.7). The stereochemistry of **3** was established by a ROESY NMR experiment, in which the major interactions were the same as those of machikusanol [4]. Thus, compound **3** was elucidated as 3α-methoxy-γ-eudesmol, and given the trivial name as maninsigin C.

Maninsigin D (**4**) was assigned a molecular formula of $C_{15}H_{22}O_2$ as supported by the HRESIMS (*m/z* 257.1517 $[M+Na]^+$), corresponding to 5° of unsaturation. The ^1H NMR spectrum of **4** showed three aromatic signals at δ_H 7.43, 7.04, and 7.24, one isopropyl group with signals at δ_H 2.37, 0.57 and 1.04, and two methyl groups at δ_H 1.36 and 2.28 (Table 2). The ^{13}C NMR and DEPT spectra exhibited the coexistence of six sp^2 carbons (three methines and three quaternary carbons) indicative of the presence of one benzene ring, four methyl carbons, two methylenes and two oxygenated quaternary sp^3 carbons (Table 2). Analyses of $^1H-{}^1H$ COSY, HSQC, HMBC, and ROESY spectra suggested that it was a cadinane-type sesquiterpenoid. Two hydroxyl groups located at C-7 and C-10 were deduced from the downfield chemical shift of C-7 (δ_C 73.5) and C-10 (δ_C 70.1). The relative stereochemistry of **4** was deduced by its ROESY spectrum (Fig. 3). The ROESY correlations of H-11 with H-14 showed that H-12 and H-14 were on the same side and assigned as the α-orientation. Thus, the structure of **4** was determined as shown.

The known compounds were identified as mognolol (**5**) [18], randaiol (**6**) [17], (+)-balanophonin (**7**) [19], ficusal (**8**) [20], syringaresinol (**9**) [21], isopterocarpolone (**10**) [22], (1*R**,2*R**,5*R**,6*S**)-6-(4-hydroxy-3-methoxyphenyl)-3,7-dioxabicyclo[3,3,0]octan-2-ol (**11**) [23], (1*R**,2*R**,5*R**,6*S**)-6-(4-hydroxy-3,5-dimethoxyphenyl)-3,7-dioxabicyclo[3,3,0]octan-2-ol (**12**) [24], 2-(3′,5′-dimethoxy-4′-hydroxyphenyl)-3,7-dioxabicyclo[3.3.0]octan-6-one (**13**) [25] and (+)-5,5′-dimethoxyl ariciresinol (**14**) [26].

Considering the various bioactivities of the chemical constituents from the Magnoliaceae family reported previously [3–8,27], some related bioassays were also carried out in our present study. Firstly, all compounds were assayed for their cytotoxicity against the HL-60, SMMC-7721, A-549, MCF-7, and SW-480 human tumor cell lines by the MTT method with *cis*-platin as positive control [10,11]. Compound **7** displayed weak cytotoxic activity against HL-60, SMMC-7721, MCF-7, and SW-480 human tumor cell lines with the IC_{50} values of 13.4, 39.4, 19.2, and 19.4 μM, respectively (Table 3). Besides, compound **2** also showed some signs of cytotoxicity against the HL-60 and MCF-7 cell lines with the IC_{50} values of 24.3 and 25.4 μM, respectively (Table 3). In addition, the effects of compounds **5**, **9** and **10** on neurite outgrowth from PC12 cells were also evaluated according to previously reported procedures [12,13]. Compounds **9** and **10** had no neurite outgrowth effect on PC12 cells, while compound **5** showed some evidence of neurite outgrowth activity on PC12 cells in the presence of NGF (5 ng/mL) at a concentration of 10 μM (Table 4).

FXR, which is highly expressed in the liver, intestine, kidney, adrenal glands, and adipose tissue, is a master regulator of the synthesis and pleiotropic actions of endogenous Bas [28]. Since FXR ligands may be used as chemical tools in studies aiming at further defining the physiological role of FXR and as the potential therapeutic agents for the treatment of diseases linked to cholesterol, glucose and bile acid metabolism and homeostasis; therefore, the discovery of novel FXR ligands is quite desirable and significantly important. On account of the



2 **4**

Fig. 3. Selected ROESY (HH) correlations of **2** and **4**.

Table 3
IC_{50} values of active compounds against human tumor cell lines.

Compounds[a]	HL-60 IC_{50} (μM)	SMMC-7721 IC_{50} (μM)	A-549 IC_{50} (μM)	MCF-7 IC_{50} (μM)	SW480 IC_{50} (μM)
2	24.3	>40	>40	25.4	>40
7	13.4	39.4	>40	19.2	19.4
Cis-platin[b]	1.5	14.5	14.1	15.0	16.9

[a] Other compounds than selected ones were inactive for all of cell lines (IC_{50} > 40 μM).
[b] *Cis*-platin was used as positive control.

Table 4
Neurite outgrowth-promoting activity of compounds **5**, **9**, and **10.**

Differential ratio of PC12 (%) 72 h	Negative control (5 ng/ml NGF)	Positive control (50 ng/ml NGF)	**5**	**9**	**10**
10 μM	5.0%	53.5%	–	16.2%	–

Table 5
Anti-FXR activity of compounds **1**, **7**, and **8**.[a]

Compounds	Activation (25 μM)	Inhibition rate % (25 μM)
1	0.875	44.29 (IC_{50} = 55.6 μM)
7	0.890	24.75
8	0.891	13.38
DMSO	1.00	0
CDCA (10 μM)	2.70	ND
GS (25 μM)	ND	60.72 (IC_{50} = 6.47 μM)

[a] Each experiment was repeated at least three times determined by Y2H assays. This system employed the interaction between hFXR-LBD and the coactivator SRC 1 (see Experimental section). ND: not determined. GS: positive control.

effective influence of magnolol on diabetic nephropathy in type 2 diabetic Goto-Kakizaki rats [29], the antagonistic activity toward FXR ligand using yeast two-hybrid system of compounds **1**, **7** and **8** were also tested in vitro [14–16], and compound **1** could availably suppress CDCA-induced FXR activation with an IC_{50} value of 55.6 μM (Table 5). Therefore, the studies of the chemical constituents of *M. insignis* and their bioactivities will give valuable information for the further studies on the plants of the genus *Manglietia* or the family of Magnoliaceae.

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