Anthraquinones and Lignans from *Cassia occidentalis*

by **Shi-Fei Li**[a])[b]), **Ying-Tong Di**[a]), **Yue-Hu Wang**[a]), **Cheng-Jian Tan**[a]), **Xin Fang**[a]), **Yu Zhang**[a])[b]), **Yong-Tang Zheng**[c]), **Ling Li**[d]), **Hong-Ping He**[a]), **Shun-Lin Li***[a]), and **Xiao-Jiang Hao***[a])

[a]) State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, P. R. China (phone: +86-871-5223263; e-mail: lisl@mail.kib.ac.cn, haoxj@mail.kib.ac.cn)

[b]) Graduate School of Chinese Academy of Sciences, Beijing 100049, P. R. China

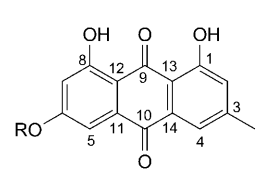
[c]) Laboratory of Molecular Immunopharmacology, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, P. R. China

[d]) Yunnan Pharmacological Laboratories of Natural Products, Kunming Medical College, Kunming 650031, P. R. China

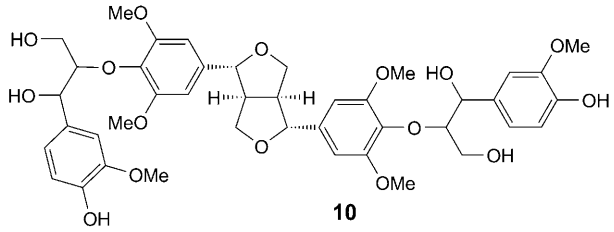
One new anthraquinone glycoside, 6-*O*-(α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyl)emodin (**1**), and two new sesquilignans, seslignanoccidentaliols A (**2**) and B (**3**), were isolated from the whole plant of *Cassia occidentalis*. The structures of the new compounds were established by means of spectroscopic methods, especially 2D-NMR data (^{1}H,^{1}H-COSY, HMQC, HMBC, and ROESY), as well as HR-ESI-MS analysis. One previously reported sesquilignan, *erythro*-guaiacylglycerol-β-*O*-4′-(5′)-methoxylariciresinol, was revised to be seslignanoccidentaliol A (**2**). The 6-*O*-(α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyl)emodin (**1**) exhibited moderate anti-HIV-1 activity with an EC_{50} value of 2.90 μg/ml and a therapeutic-index (*TI*) value of 260.

Introduction. – *Cassia occidentalis* L. (Leguminaceae) is used as traditional medicine in the tropical areas of the world. A wide range of the chemical constituents have been isolated from *C. occidentalis*, including sennoside, anthraquinone glycosides, fatty oils, flavonoid glycosides, gallactomannan, polysaccharides, and tannins [1]. In China, *C. occidentalis* L., named Wangjiangnan, was used for the treatment of some diseases such as cough, asthma, and headache [2]. As one part of our investigations on antiviral natural products from plants [3], the chemical constituents of *C. occidentalis* was studied, which led to the isolation of one new anthraquinone glycoside, 6-*O*-(α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyl)emodin[1]) (**1**), two new sesquilignans, seslignanoccidentaliols A[1]) (**2**) and B[1]) (**3**), together with two known anthraquinones, emodin (=1,3,8-trihydroxy-6-methylanthracene-9,10-dione; **4**) and physcion (**5**), and five known lignans, *threo*-buddlenol B (**6**), *erythro*-buddlenol B (**7**), *threo*-buddlenol C (**8**), *erythro*-buddlenol C (**9**), and hedyotisol A (**10**), from the AcOEt part of MeOH extracts of the plant. Among them, 6-*O*-(α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyl)emodin (**1**) showed moderate inhibitory activity against HIV-1. This article reports the structure elucidation of the new compounds as well as the bioassay results.

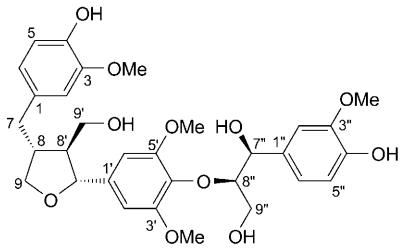
[1]) Arbitrary or trivial atom numbering, for systematic names, see *Exper. Part*.



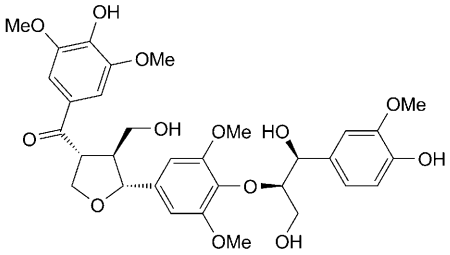
1[1]) R= Rha(1 → 6)Glc
4[1]) R= H
5[1]) R= Me



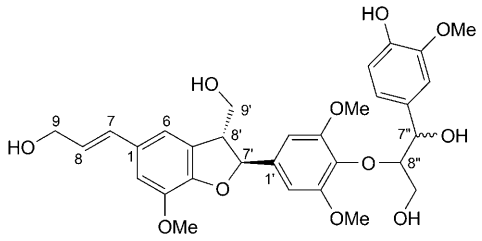
10



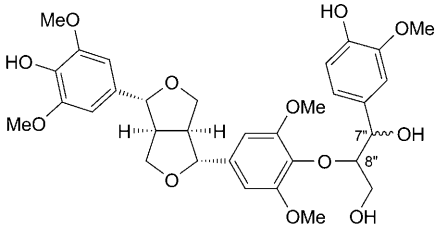
2[1])



3



6[1]) *threo*
7[1]) *erythro*



8[1]) *threo*
9[1]) *erythro*

Results and Discussion. – Compound **1** was isolated as an orange-brown amorphous solid. The molecular formula of **1** was determined to be $C_{27}H_{30}O_{14}$ on the basis of negative-ion-mode HR-ESI-MS (*m/z* 613.1326 ($[M+Cl]^-$)). The IR spectrum showed absorption bands of OH (3431 cm^{-1}) and chelated C=O (1628 cm^{-1}) groups. Compound **1** was established as 6-*O*-(α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranosyl)emodin (**1**) by an extensive 1D- and 2D-NMR study and by comparison with spectral data of compound **4**.

The ^{13}C-NMR (*Table 1*) spectra of **1** showed the prescence of 27 C-atoms comprising two Me, one CH_2, and fourteen CH groups and ten quaternary C-atoms. In the ^{13}C-NMR spectra of **1**, the signals due to the aglycone moiety were in good agreement with those of emodin (**4**), except for glycosylation shifts of the signals of C(5) ($\Delta\delta = +1.2$), C(6) ($\Delta\delta = -1.7$), and C(7) ($\Delta\delta = +0.4$), suggesting that the sugar moiety was linked to C(6) of the aglycone. Furthermore, in the NMR spectra of **1**, two anomeric C-atoms were identified at δ(C) 100.6 and 99.8, correlating with the anomeric H-atoms at δ(H) 5.11 and 4.49, respectively, which indicated that compound **1** was a emodin 6-glycosylglycoside. The sequential assignments of H- and C-atom resonances of each monosaccharide were carried out by analysis of the 2D-NMR data from COSY, HSQC, HMBC (*Fig. 1*), and ROESY experiments, as well as by their *m* pattern and

Table 1. *^1H- (400 MHz) and ^{13}C-NMR (100 MHz) Data of* **1**[1]) *and* **4**[1]) *in (D_6)DMSO.* δ in ppm, J in Hz.

	1		**4**	
	δ(H)	δ(C)	δ(H)	δ(C)
C(1)		161.5		161.3
H–C(2)	7.20 (br. *s*)	124.3	7.16 (br. *s*)	123.9
C(3)		148.7		148.0
H–C(4)	7.55 (br. *s*)	120.8	7.49 (br. *s*)	120.3
H–C(5)	7.23 (*d*, J = 2.3)	109.0	7.12 (*d*, J = 2.4)	107.8
C(6)		163.8		165.5
H–C(7)	6.92 (*d*, J = 2.3)	109.1	6.59 (*d*, J = 2.4)	108.7
C(8)		164.1		164.3
C(9)		190.2		189.5
C(10)		181.2		181.1
C(11)		134.9		134.9
C(12)		110.9		[a])
C(13)		113.6		113.2
C(14)		133.0		132.6
Me–C(3)	2.42 (*s*)	21.6		21.4
Glc:				
H–C(1′)	5.11 (*d*, J = 7.0)	99.8		
H–C(2′)	3.59–3.62 (*m*)	73.1		
H–C(3′)	3.29–3.32 (*m*)	76.2		
H–C(4′)	3.14–3.15 (*m*)	69.8		
H–C(5′)	3.13 (overlapped)	75.8		
CH_2(6′)	3.84 (*d*, J = 9.7), 3.39 (overlapped)	66.1		
Rha:				
H–C(1″)	4.49 (br. *d*, J = 7.3)	100.6		
H–C(2″)	3.61 (overlapped)	70.3		
H–C(3″)	3.45–3.47 (*m*)	70.7		
H–C(4″)	3.08–3.12 (*m*)	72.1		
H–C(5″)	3.37 (overlapped)	68.3		
Me(6″)	1.06 (*d*, J = 6.2)	17.9		

[a]) Undiscovered.

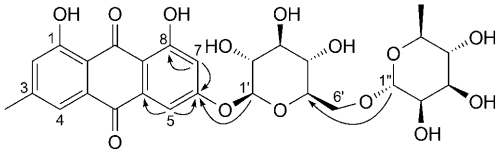


Fig. 1. *Key HMBC* (H → C) *data of* **1**[1])

coupling constants in the ^{1}H-NMR spectrum. The sugar units were identified as an α-L-rhamnopyranosyl (Rha) and a β-D-glucopyranosyl (Glc) unit [4]. In addition, acid hydrolysis of **1** produced L-rhamnose and D-glucose as sugar residues, as determined by GC analysis. A HMBC between C(6) on the aglycone and the H–C(1) of Glc confirmed that the sugar chain was linked at C(6). The linkage of Rha to C(6′) of Glc was supported by a HMBC between H–C(1″) at δ(H) 4.49 (br. *d*, J = 7.3 Hz) and C(6′)

at δ(C) 66.1. Thus, compound **1** was identified to be 6-*O*-(α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranosyl)emodin.

Compound **2** was obtained as a white powder. It had the molecular formula $C_{31}H_{38}O_{11}$ as deduced from the positive-ion-mode HR-ESI-MS (*m/z* 609.2311 ($[M+Na]^+$, $C_{31}H_{38}O_{11}Na^+$). Its IR spectrum showed absorptions of OH (3432 cm^{-1}) and aromatic moieties (1596, 1516, and 1462 cm^{-1}). The ^{1}H-NMR data of **2** (*Table 2*) indicated the presence of one 1,3,4,5-tetrasubstituted benzene ring (δ(H) 6.74 (br. s, 2 H, H−C(2′,6′))) [5], two 1,3,4-trisubstituted benzene moieties (δ(H) 6.67 (*dd*, *J* = 7.8, 1.8 Hz, 1 H), 6.74 (br. *s*, 1 H), and 6.84 (*d*, *J* = 1.8 Hz, 1 H); δ(H) 6.83 (*d*, *J* = 1.7 Hz, 1 H), 6.79 (br. *s*, 1 H), and 7.05 (br. *s*, 1 H)), and four MeO and three CH_2O groups. From the ^{1}H,^{1}H-COSY data (*Fig. 2*), four partial structures **a** (C(7)−C(8)(C(9))−C(8′)(C(9′))−C(7′)), **b** (C(7″)−C(8″)−C(9″)), **c** (C(5)−C(6)), and **d** (C(5″)−C(6″)) were deduced. Among them, the partial structure consisting of a tetrahydrofuran ring was supported by the HMBC (*Fig. 2*) of H−C(9) to C(7′). The correlations of H−C(7) to C(1), C(2), and C(6), and of H−C(7′) to C(1′) and C(2′,6′) indicated that partial structure **a** was connected to C(1) and C(1′). In addition, partial structure **b** ('guaiacylglyceryl' (=2-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-1-(hydroxymethyl)ethyl) moiety) was conncected to C(4′) *via* an ether bridge as shown by the correlation of H−C(8″) to C(4′). The relative configuration of H−C(8)/H−C(8′) and H−C(7′)/H−C(8′) were confirmed to be *trans* by the obvious correlations H−C(8′)/H_a−C(7), and H−C(7′)/H−C(8) in the ROESY plot (*Fig. 3*), and an *erythro*-configuration of H−C(7″)/H−C(8″) was determined by the *J* value of H−C(7″) (*d*, *J* = 4.4 Hz) [6]. Thus, the structure of seslignanoccidentaliol A (**2**) was established as shown.

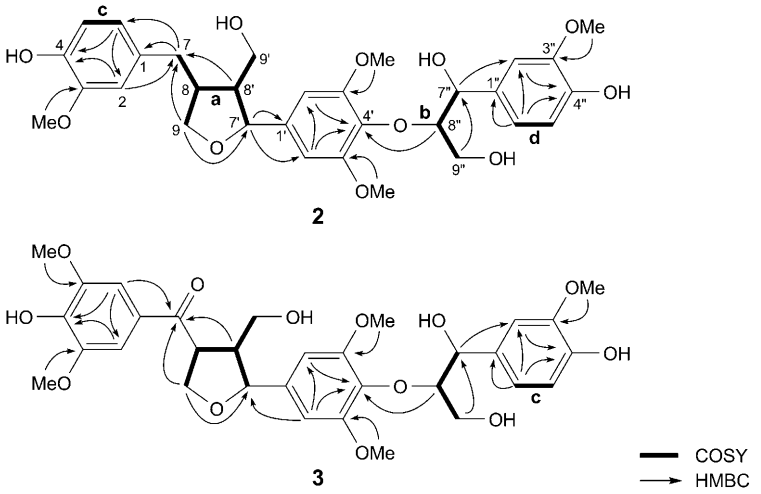


Fig. 2. *Selected 2D-NMR correlations of* **2** *and* **3**[1])

In the literature [7], however, a natural product, named *erythro*-guaiacylglycerol-β-*O*-4′-(5′)-methoxylariciresinol, with the same planar structure as **2** was found. Almost the same ^{1}H- and ^{13}C-NMR data suggested that both compounds should have the same

Table 2. *^{1}H- and ^{13}C-NMR Data of Compounds* **2** *and* **3**. δ in ppm, J in Hz.

	2[a])		**3**[b])	
	δ(H)	δ(C)	δ(H)	δ(C)
C(1)		133.2		128.4
H–C(2)	6.84 (*d*, *J* = 1.8)	112.9	7.41 (br. *s*)	107.3
C(3)		147.9		148.4
C(4)		145.6		142.0
H–C(5)	6.74 (br. *s*)	115.7		148.4
H–C(6)	6.67 (*dd*, *J* = 7.8, 1.8)	121.7	7.41 (br. *s*)	107.3
H_a–C(7)	2.93 (*dd*, *J* = 16.8, 6.3)	33.4		198.1
H_b–C(7)	2.54 (*dd*, *J* = 16.8, 13.8)			
H–C(8)	2.66–2.75 (*m*)	43.4	4.27–4.31 (*m*)	49.7
H_a–C(9)	4.00 (*dd*, *J* = 10.5, 8.5)	73.3	4.22 (br. *s*)	71.1
H_b–C(9)	3.70–3.75 (*m*)		4.21 (*d*, *J* = 2.8)	
C(1′)		141.5		139.3
H–C(2′)	6.74 (br. *s*)	103.6	6.79 (br. *s*)	104.5
C(3′)		153.9		154.0
C(4′)		135.1		135.6
C(5′)		153.9		154.0
H–C(6′)	6.74 (br. *s*)	103.6	6.79 (br. *s*)	104.5
H–C(7′)	4.90 (*d*, *J* = 5.6)	83.5	4.71 (*d*, *J* = 7.6)	84.1
H–C(8′)	2.31–2.38 (*m*)	53.8	2.63–2.68 (*m*)	54.7
H_a–C(9′)	3.87–3.93 (*m*)	60.4	3.73 (*d*, *J* = 5.2)	61.2
H_b–C(9′)	3.72 (overlapped)			
C(1″)		133.7		133.7
H–C(2″)	7.05 (br. *s*)	110.8	7.03 (*d*, *J* = 1.6)	110.7
C(3″)		148.2		147.9
C(4″)		146.4		146.3
H–C(5″)	6.79 (br. *s*)	115.2	6.77 (br. *s*)	115.1
H–C(6″)	6.83 (*d*, *J* = 1.7)	119.9	6.83 (overlapped)	120.0
H–C(7″)	4.98 (br. *d J* = 4.4)	73.3	4.97 (*d*, *J* = 4.4)	73.1
H–C(8″)	4.14–4.18 (*m*)	87.8	4.13–4.16 (*m*)	87.8
H_a–C(9″)	3.87 (overlapped)	60.8	3.82 (overlapped)	60.8
H_b–C(9″)	3.42–3.51 (*m*)		3.41 (*dd*, *J* = 12.0, 2.8)	
*Me*O–C(3)	3.84 (*s*)	56.1	3.89 (*s*)	56.6
*Me*O–C(5)	–	–	3.89 (*s*)	56.6
*Me*O–C(3′,5′)	3.85 (*s*)	56.4	3.85 (*s*)	56.5
*Me*O–C(3″)	3.82 (*s*)	56.1	3.82 (*s*)	56.1

[a]) In CD_3OD. [b]) In (D_6)Acetone.

relative configuration. However, the relative configuration of H–C(8)/H–C(8′) in [7] was determined to be *cis* only by a H–C(8)/H–C(8′) correlation in the NOESY plot, which is opposite to our experiment. Since H–C(8) and H–C(8′) are vicinal H-atoms and their NOE correlation for conformational analysis is unreliable, we consider that the relative *cis*-configuration of H–C(8)/H–C(8′) in [7] is wrong, and should be *trans.* Moreover, the 1D-NMR spectroscopic data of the tetrahydrofuran moiety of **2** (*Table 2*) were almost identical to those of lignans with the same moiety, which also supported our results [8–10].

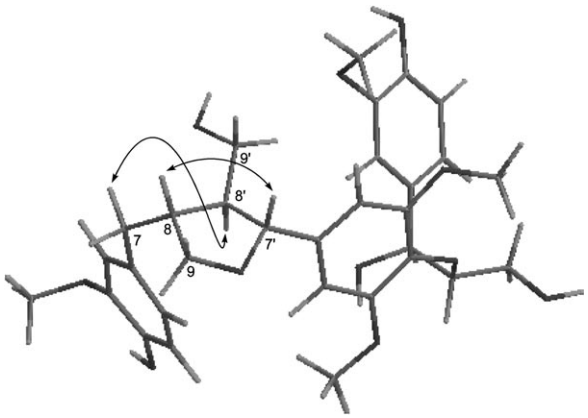


Fig. 3. *Key ROESY* (H ↔ H) *correlations of* **2**[1])

Compound **3** was obtained as a white, optically active powder. It exhibited an $[M+Na]^+$ ion peak at *m/z* 653.2210 in the positive-ion mode HR-ESI-MS, corresponding to the molecular formula $C_{32}H_{38}O_{13}$. The IR spectrum displayed absorption bands of OH (3440 cm^{-1}) and aromatic moieties (1639, 1512, and 1461 cm^{-1}). Its ^1H- and ^{13}C-NMR spectroscopic data (*Table 2*) were similar to those of compound **2** indicating that **3** was a sesquilignan. Comparison of the ^{13}C-NMR spectrum of compound **3** with that of **2** showed that **3** had an additional C=O group at δ(C) 198.1, an sp^2 quatenary C-atom (δ(C) 148.4) and one more MeO group, in place of an sp^3 CH_2 (δ(C) 33.4) and an sp^2 CH group (δ(C) 115.7) in **2**. Comprehensive analysis of the ^1H- and ^{13}C-NMR data and 2D-NMR spectra of **3**, especially ^1H,^1H-COSY, HMQC, HMBC, and ROESY, elucidated the structure of **3**. Thus HMBCs (*Fig. 2*) from H−C(2), H−C(6), and H−C(8′) to C(7) (δ(C) 198.1) indicated that the C=O group was located at C(7), while the additional MeO was at C(5) due to the HMBC of MeO−C(5) to C(5). The remaining structure and relative configuration of **3** were identical to those of **2** as determined by HMBC (*Fig. 2*) and ROESY NMR experiments. In 1982, *Nakatsubo* and co-workers [11] reported the acetylation product of **3** in a study on the degradation process of trimeric lignin model compounds on a fungus. They speculated the presence of compound **3** as an intermediate in the reaction system but did not obtained it. The isolation and structure elucidation of compound **3** is reported for the first time.

The seven known compounds were identified as emodin (**4**) [12], physicon (**5**) [12], *threo*-buddlenol B (**6**) [6][13], *erythro*-buddlenol B (**7**) [6][13], *threo*-buddlenol C (**8**) [6][14], *erythro*-buddlenol C (**9**) [6][14], and hedyotisol A (**10**) [13] by comparison of their spectroscopic data with literature data.

Compound **1** was tested for cytotoxicity against C8166 cells (CC_{50}) and showed moderate cytotoxic activity with a CC_{50} value of 756 μg/ml. Its anti-HIV-1 activity was evaluated by an inhibition assay for the cytopathic effects of HIV-1 (EC_{50}), with AZT as a positive control. Compound **1** showed anti-HIV-1 activity with an EC_{50} value of 2.90 μg/ml and a therapeutic-index (*TI*) value of 260.

This work was supported by the *National Natural Science Foundation of China* (30760306) and the *Yunnan Provincial Science Foundation* (2006C009Z).

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh, 10–40 μm; *Qingdao Haiyang Chemical Factory*, China); *RP-C_{18}* reversed-phase SiO_2 (40–63 μm; *Daiso Co.*, Japan), and *Sephadex LH-20* (*Amersham Pharmacia Biotech*, Sweden). HPLC: *Agilent-1100* workstation *Zorbax-SB-C_{18}* column (250 × 9.4 mm); DAD detector). GC: *HP5890* (*Agilent*, America) with a quartz cap. column (30 mm × 0.32 mm × 0.25 μm); FID detection. Optical rotations: *Horiba-SEPA-300* polarimeter or *Jasco-DIP-370* digital polarimeter. UV Spectra: *Shimadzu-UV-2401PC* spectrometer; λ_{max} in nm. IR Spectra: *Bio-Rad-FTS-135* spectrometer; KBr pellets; $\tilde{\nu}$ in cm^{-1}. 1D- and 2D-NMR Spectra: *DRX-500* or *Bruker-AM-400* spectrometer; δ in ppm, J in Hz; Me_4Si as internal standard. ESI- and HR-ESI-MS: *VG-Auto-Spec-3000* spectrometer; in m/z (rel. %).

Plant Material. The whole plant of *C. occidentalis*, collected in Xishuangbanna of Yunnan Province, P. R. China, in October 2007, was identified by Prof. *Jing-Yun Cui*, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences.

Extraction and Isolation. The whole air-dried plant of *C. occidentalis* (12.0 kg) was extracted with MeOH under refluxing to give a crude extract. After removal of the solvent by evaporation, the residue was suspended in H_2O and then extracted successively with petroleum ether, AcOEt, and BuOH. The AcOEt extract was concentrated to yield a residue (300 g). Then, this residue was subjected to CC (SiO_2, $CHCl_3$/MeOH 10:1, 5:1, 1:1, and 0:1): *Fractions 1–4*. *Fr. 1* was subjected repeatedly to CC (*RP-C_{18}* and SiO_2): **4** (15.0 mg) and **5** (5.0 mg). *Fr. 2* was subjected repeatedly to CC (*RP-C_{18}* and SiO_2): *Frs. 2A–2E*. *Fr. 2C* was subjected to repeated CC (SiO_2, $CHCl_3$/MeOH 7:1 and 3:1): *Frs. 2C1–2C5*. Further purification of *Frs. 2C2* and *2C3* by CC (*Sephadex LH-20*) and semi-prep. HPLC yielded **2** (8.0 mg), **3** (6.0 mg), **6** (10.0 mg), and **7** (10.0 mg). Compounds **8**–**10** were obtained by the same procedures from *Fr. 2C4*. *Fr. 2C5* was purified by recrystallization and repeated CC (SiO_2): **1** (15.0 mg).

Acid Hydrolysis of **1**. Compound **1** (5.0 mg) was refluxed with 1M HCl/dioxane 1:1 (*v*/*v*, 2 ml) at 80° for 3 h. The mixture was neutralized with 1M NaOH and filtered. The filtrate was extracted with $CHCl_3$ and H_2O, and then the H_2O-souble fraction was concentrated. The dried sugar residue was diluted in abs. pyridine (1 ml), L-cysteine methyl ester hydrochloride (1.5 mg) was added, and the mixture was heated at 60° for 1 h. Then 1-(trimethylsilyl)-1*H*-imidazole (1.5 ml) was added, and the mixture was heated at 60° for another 30 min. An aliquot (4 μl) of the supernatant was removed and directly subjected to GC analysis (column temp. 180–280° at 3°/min, carrier gas N_2 (1 ml/min), injector and detector temp. 250°, split ratio 1:50). The configurations of L-rhamnose and D-glucose for **1** were determined by comparison of the retentions times of the corresponding derivatives with standard L-rhamnose and D-glucose, giving single peaks at t_R 15.826 and 19.208 min, resp.

6-O-(α-L-Rhamnopyranosyl-(1→6)-β-D-glucopyranosyl)emodin (= *3-{[6-O-(6-Deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxyl}-1,8-dihydroxyanthracene-9,10-dione*; **1**): Orange-brown amorphous solid. $[\alpha]_D^{19.2} = -124.3$ ($c = 0.18$, MeOH). UV (MeOH): 505, 304, 258, 237, 226, 202. IR (KBr): 3431, 2922, 1628, 1479, 1387, 1263, 1217, 1067. ^{1}H- and ^{13}C-NMR: *Table 1*. HR-ESI-MS: 613.1326 ($[M + Cl]^-$, $C_{27}H_{30}O_{14}Cl^-$; calc. 613.1324).

Seslignanoccidentaliol A (= rel-*(1R,2S)-2-{2,6-Dimethoxy-4-{(2S,3R,4S)-tetrahydro-4-[(4-hydroxy-3-methoxyphenyl)methyl]-3-(hydroxymethyl)furan-2-yl}phenoxy}-1-(4-hydroxy-3-methoxyphenyl)propane-1,3-diol*; **2**): White powder. $[\alpha]_D^{27.2} = 0.0$ ($c = 0.15$, MeOH). UV (MeOH): 280, 204. IR (KBr): 3432, 2937, 1596, 1516, 1462, 1425, 1273, 1235, 1123, 1034. ^{1}H- and ^{13}C-NMR: *Table 2*. HR-ESI-MS: 609.2312 ($[M + Na]^+$, $C_{31}H_{38}O_{11}Na^+$; calc. 609.2311).

Seslignanoccidentaliol B (= rel-*(4-Hydroxy-3,5-dimethoxyphenyl){(3R,4S,5R)-tetrahydro-5-{4-[(1R,2S)-2-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-1-(hydroxymethyl)ethoxy]-3,5-dimethoxyphenyl}-4-(hydroxymethyl)furan-3-yl)methanone*; **3**): White powder. $[\alpha]_D^{24.1} = +33.3$ ($c = 0.09$, MeOH). UV (MeOH): 301, 283, 205. IR (KBr): 3440, 2926, 1711, 1639, 1512, 1462, 1424, 1378, 1352, 1280, 1123, 1034. ^{1}H- and ^{13}C-NMR: *Table 2*. HR-ESI-MS: 653.2190 ($[M + Na]^+$, $C_{32}H_{38}O_{13}Na^+$; calc. 653.2210).

Anti-HIV-1 Assay. The cytotoxicity assay against C8166 cells (CC_{50}) was assessed by using the MTT (= 2-(4,5-dimethylthiazol-2-yl)-3,5-diphenyl-2*H*-tetrazolium bromide) method, and the anti-HIV-1 activity was evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC_{50}) [15].

REFERENCES

[1] J. C. Chukwujekwu, P. H. Coombes, D. A. Mulholland, J. van Staden, *S. Afr. J. Bot.* **2006**, *72*, 295.
[2] Editorial Committee, 'Chinese Bencao', Shanghai Science and Technology Press, 1999, Vol. 4, p. 410.
[3] Y. Li, L. Wang, S. Li, X. Chen, Y. Shen, Z. Zhang, H. He, W. Xu, Y. Shu, G. Liang, R. Fang, X. Hao, *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 8083; Y.-H. Wang, Z.-K. Zhang, F.-M. Yang, Q.-Y. Sun, H.-P. He, Y.-T. Di, S.-Z. Mu, Y.-Lu, Y. Chang, Q.-T. Zheng, M. Ding, J.-H. Dong, X.-J Hao, *J. Nat. Prod.* **2007**, *70*, 1458.
[4] H. Yoshimitsu, M. Nishida, F. Hashimoto, M. Tanaka, Y. Sakata, M. Okawa, T. Nohara, *J. Nat. Med.* **2007**, *61*, 334.
[5] X.-W. Yang, P.-J. Zhao, Y.-L. Ma, H.-T. Xiao, Y.-Q. Zuo, H.-P. He, L. Li, X.-J. Hao, *J. Nat. Prod.* **2007**, *70*, 521.
[6] T. Miyase, A. Ueno, N. Takizawa, H. Kobayashi, H. Oguchi, *Chem. Pharm. Bull.* **1987**, *35*, 3713; C. H. Ludwig, B. J. Nist, J. L. McCarthy, *J. Am. Chem. Soc.* **1964**, *96*, 1186; G. D. Manners, E. P. Swan, *Can. J. Chem.* **1971**, *49*, 3607; K. Kijima, H. Otsuka, T. Ide, C. Ogimi, E. Hirata, A. Takushi, Y. Takeda, *Phytochemistry* **1998**, *48*, 669.
[7] H.-Y. Han, H.-W. Liu, N.-L. Wang, X.-S. Yao, *Nat. Prod. Res.* **2008**, *22*, 984.
[8] J. Lee, D. Lee, D. S. Jang, J.-W. Nam, J.-P. Kim, K. H. Park, M. S. Yang, E.-K. Seo, *Chem. Pharm. Bull.* **2007**, *55*, 137.
[9] J. Lee, E.-K. Seo, D. S. Jang, T. J. Ha, J.-P. Kim, J.-W. Nam, G. Bae, Y. M. Lee, M. S. Yang, J. S. Kim, *Chem. Pharm. Bull.* **2009**, *57*, 298.
[10] R.-Q. Mei, Y.-H. Wang, G.-H. Du, G.-M. Liu, L. Zhang, Y.-X. Cheng, *J. Nat. Prod.* **2009**, *72*, 621.
[11] Y. Kamaya, F. Nakatsubo, T. Higuchi, *Agric. Biol. Chem.* **1983**, *47*, 299.
[12] S. N. Ram, S. P. D. Dwivedi, V. B. Pandey, A. H. Shah, *Fitoterapia* **1988**, *59*, 78.
[13] P. J. Houghton, *Phytochemistry* **1985**, *24*, 819.
[14] S. Matsuda, S. Kadota, T. Tai, T. Kikuchi, *Chem. Pharm. Bull.* **1984**, *32*, 5066.
[15] J. H. Wang, S. C. Tam, H. Huang, D.-Y. Ouyang, Y.-Y. Wang, Y.-T. Zheng, *Biochem. Biophys. Res. Commun.* **2004**, *317*, 965.

Received December 18, 2009