Two New Lignans from *Phryma leptostachya* L.

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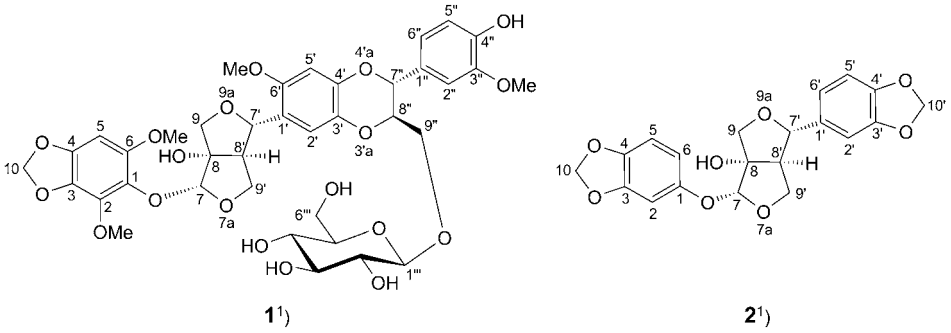
Haedoxancoside A (**1**), a new sesquilignan glycoside, and phrymarolin V (**2**), a new lignan, together with two known compounds, were isolated from *Phryma leptostachya* L. Their structures were determined on the basis of comprehensive spectroscopic analysis, mainly 1D- and 2D-NMR spectroscopy as well as mass spectrometry. This is the first report of a sesquilignan glycoside isolated from a species of the Phrymaceae family.

Introduction. – The medicinal herb plant *Phryma leptostachya* L., the sole species of the family Phrymaceae [1], known as 'Washixi' in the traditional Chinese medicine (TCM) of the Yi nationality, has long been used as a secret remedy for human's scabies [2]. People in Southwestern of China also use this herb to drive or kill mosquitos and flys [3]. The roots of this plant have been used as natural insecticide in East Asia for a long time [4–6].

Many lignans that have a unique oxygenated 3,7-dioxabicyclo[3.3.0]octane skeleton have been isolated from *P. leptostachya* [7–9]. Our previous investigations on the title plant have led to the identification of 11 lignans [10], most of them featuring the oxygenated 3,7-dioxabicyclo[3.3.0]octane skeleton. Our follow-up work on this plant has resulted in the isolation from the EtOH extract of a new sesquilignan glycoside, haedoxancoside A[1]) (**1**), and a new lignan, phrymarolin V[1]) (**2**; *Fig. 1*), along with two known constituents. This is the first report of a sesquilignan glycoside isolated from this plant. Herein, the isolation and structure elucidation of the new compounds are described.

Results and Discussion. – Haedoxancoside A (**1**) was obtained as white powder. Its HR-ESI-MS indicated a quasi-molecular ion $C_{38}H_{43}O_{19}^{-}$ ($[M-H]^-$ at m/z 803.2402), which was supported by the ^{1}H- and ^{13}C-NMR, DEPT, and HSQC spectra of **1**. In the ^{1}H- and ^{13}C-NMR spectra (*Table 1*), the signals at δ(H) 4.94 (*d*, $J = 7.5$ Hz, 1 H) and δ(C) 102.5 suggested the presence of a sugar unit in **1**, which was confirmed by the coexistence of the signals at δ(H) 3.44, 3.52, 3.43, 4.00, 3.70, and 3.47 and δ(C) 77.8, 74.9, 71.3, 78.2, and 62.1. The characteristic *d* of the anomeric H-atom at δ(H) 4.94 with the large coupling constant ($J = 7.5$ Hz) and the remaining signals assigned to the sugar

[1]) Trivial atom numbering; for systematic names, see *Exper. Part.*



1[1]) **2**[1])

Fig. 1. *Compounds* **1** *and* **2**, *isolated from* Phryma leptostachya L.

Table 1. *^{1}H- and ^{13}C-NMR Data* (CD_3OD; 400 and 100 MHz, resp.) *of Compound* **1**[1]). δ in ppm, J in Hz.

Position	δ(H)	δ(C)	Position	δ(H)	δ(C)
C(1)		132.3	C(1′)		124.4
C(2)		139.7	H–C(2′)	7.19 (*s*)	115.5
C(3)		132.8	C(3′)		138.4
C(4)		146.3	C(4′)		144.5
H–C(5)	6.45 (*s*)	91.2	H–C(5′)	6.57 (*s*)	101.1
C(6)		149.6	C(6′)		152.0
H–C(7)	5.24 (*s*)	105.8	H–C(7′)	4.85 (*d*, J = 6.2)	85.7
C(8)		93.6	H–C(8′)	2.55–2.52 (*m*)	59.4
CH_2(9)	4.25 (*d*, J = 9.7),	78.8	CH_2(9′)	4.64 (*dd*, J = 9.7, 5.1),	72.1
	3.64 (*dd*, J = 9.7, 5.1)			3.97 (*dd*, J = 9.7, 2.2)	
CH_2(10)	5.87 (*s*)	102.5			
C(1″)		132.9	H–C(1‴)	4.94 (*d*, J = 7.5)	102.5
H–C(2″)	7.09 (*d*, J = 1.7)	112.9	H–C(2‴)	3.44[a])	77.8
C(3″)		150.9	H–C(3‴)	3.52 (*d*, J = 8.9)	74.9
C(4″)		148.4	H–C(4‴)	3.43[a])	71.3
H–C(5″)	7.22 (*d*, J = 8.4)	117.6	H–C(5‴)	4.00[a])	78.2
H–C(6″)	7.01 (*dd*, J = 8.4,1.7)	121.5	CH_2(6‴)	3.70, 3.47[a])	62.1
H–C(7″)	4.96 (*d*, J = 7.5)	77.7	MeO–C(3″)	3.22 (*s*)	56.8
H–C(8″)	4.01–4.00 (*m*)	79.6	MeO–C(2)	3.89 (*s*)	60.6
CH_2(9″)	3.90–3.87,	62.4	MeO–C(6)	3.70 (*s*)	57.5
	3.71–3.68 (2*m*)		MeO–C(6′)	3.72 (*s*)	56.3

[a]) Signal pattern unclear due to overlapping.

unit indicated the presence of a β-glucose moiety. The conclusion was corroborated by the hydrolysis of **1** with 6% HCl solution, which gave only one sugar, identified as glucose by the GC assay of its 1-(trimethysilyl)-1*H*-imidazole derivative and comparison with an authentic sample. The optical rotation of the glucose obtained from acid hydrolysis suggested that the glucosse unit in **1** had D-configuration. Except for the signals ascribed to the sugar moiety, the NMR data of **1** were similar to those of haedoxan J [10], suggesting the presence of the 3,7-dioxabicyclo[3.3.0]octane skeleton in the molecule. In the ^{1}H-NMR spectrum the signals of three benzene rings were

present: those at δ(H) 7.22 (*d*, *J* = 8.4 Hz, H–C(5″)), 7.09 (*d*, *J* = 1.7 Hz, H–C(2″)), and 7.01 (*dd*, *J* = 8.4, 1.7, H–C(6″)) were typical of a 3,4-substituted phenyl group, and those at δ(H) 6.57 (*s*, H–C(5′)), 7.19 (*s*, H–C(2′)), and 6.45 (*s*, H–C(5)) suggested the existence of a 3,4,6- and a 2,3,4,6-substituted phenyl group, respectively. In the HMBC spectrum of **1**, the correlations δ(C) 93.6 (C(8))/δ(H) 5.24 (*s*, H–C(7)), 4.25 (1 H–C(9)), and 3.64 (1 H–C(9)) implied the existence of the fragment CH(7)–C(8)–CH_2(9), which was determined to be linked to C(1) *via* an O-atom as shown by the HMBC cross-peak δ(H) 5.24 (H–C(7))/δ(C) 132.3 (C(1)) (*Fig. 2*). Another fragment CH(7′)–CH(8′)–CH_2(9′) was identified on the basis of the HMBC cross-peaks δ(C) 59.4 (C(8′))/δ(H) 4.85 (H–C(7′)), 4.64 (1 H–C(9′)), and 3.97 (1 H–C(9′)), and the fragment was located at C(1′) as established by the HMBC cross-peak δ(H) 4.85 (H–C(7′))/δ(C) 124.4 (C(1′)) (*Fig. 2*). Furthermore, from the ^{1}H,^{1}H-COSY cross-peaks δ(H) 4.96 (H–C(7″))/δ(H) 4.01 – 4.00 (H–C(8″)) and δ(H) 4.01 – 4.00 (H–C(8″))/δ(H) 3.90 – 3.87 and 3.71 – 3.68 (CH_2(9″)), the fragment CH(7″)–CH(8″)–CH_2(9″) was inferred and was located at C(1″), as suggested by the HMBC δ(H) 4.96 (H–C(7″))/δ(C) 132.9 (C(1″)). Therefore, the molecule of **1** contained a phenoxypropyl and two phenylpropyl groups, two furan rings, and a glucose unit, hence it is a sesquilignan glycoside, and the glucose unit was located at C(9″) as revealed by the HMBC δ(H) 4.94 (H–C(1‴) (Glu))/δ(C) 62.4 (C(9″)). The signals at δ(H) 3.89 (*s*, 3 H), 3.72 (*s*, 3 H), 3.70 (*s*, 3 H), 3.22 (*s*, 3 H), and 5.87 (*s*, 2 H) implied the presence of four aromatic MeO groups and one O–CH_2–O moiety. In the HMBC, the correlations δ(H) 3.89/δ(C) 139.7, δ(H) 3.22/δ(C) 150.9, δ(H) 3.70/δ(C) 149.6, and δ(H) 3.72/δ(C) 152.0 demonstrated that these MeO groups were located at C(2), C(3″), C(6), and C(6′), respectively, and the correlations δ(H) 5.87/δ(C) 132.8 (C(3)) and 146.3 (C(4)) indicated that O–CH_2–O was attached at C(3) and C(4) (*Fig. 2*). The NOESY correlation pattern belonging to the aglycon moiety of **1** was similar to that of haedoxan J [10], signifying that the relative configurations of the asymmetric C-atoms of **1** were the same as those of haedoxan J (*Fig. 2*). Thus, the structure of **1** was elucidated as *rel*-(1*R*,3a*R*,4*S*,6a*S*)-1-{(2*S*,3*S*)-2,3-dihydro-2-(4-hydroxy-3-methoxyphenyl)-3-[(β-D-glucopyranosyloxy)methyl]-7-methoxy-1,4-benzo-

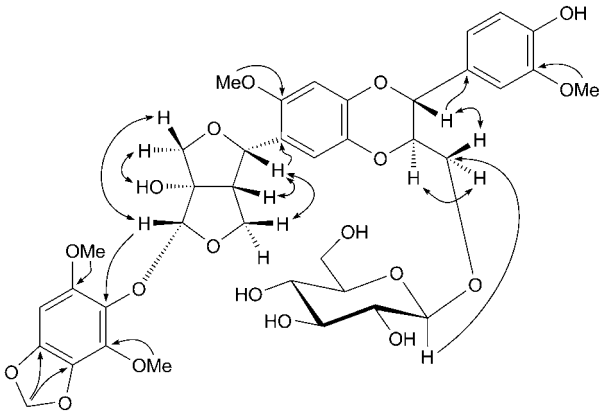


Fig. 2. *Key HMBC* (H → C) *and NOESY* (H ↔ H) *features of compound* **1**

dioxin-6-yl}-4-[(4,6-dimethoxy-1,3-benzodioxol-5-yl)oxy}dihydro-1*H*,3*H*-furo[3,4-*c*]-furan-3a(4*H*)-ol[1]), and it was named haedoxancoside A[1]).

Phrymarolin V (**2**) was obtained as a white powder. The molecular formula was deduced as $C_{20}H_{18}O_8$, on the basis of the HR-ESI-MS ($[M-H]^-$ at *m*/*z* 385.0928). Detailed comparison of the ^{1}H- and ^{13}C-NMR spectra of **2** (*Table 2*) with those of phrymarolin I [6] indicated that compound **2** was an analogue of phrymarolin I, except for the absence of MeO groups in **2**, which suggested that the structure of **2** contained two 3,4-disubstituted phenyl groups. The linkage of C(7) to C(1) *via* an O-atom was deduced from the HMBC δ(H) 5.32 (*s*, H–C(7))/δ(C) 151.1 (C(1)). The HMBCs δ(H) 5.96 (*s*, OCH_2O)/δ(C) 143.5 (C(3)) and 148.1 (C(4)) and δ(H) 5.97 (*s*, OCH_2O)/δ(C) 147.4 (C(3′)) and 148.2 (C(4′)) indicated that both phenyl groups were substituted by an OCH_2O group at C(3) and C(4), and C(3′) and C(4′), respectively. The correlation pattern in the NOESY plot of **2** was also similar to that of phrymarolin I, indicating that the relative configurations of the asymmetric C-atoms in **2** were the same as those of phrymarolin I [6]. Thus, the structure of **2** was established as *rel*-(1*R*,3a*R*,4*S*,6a*S*)-1-(1,3-benzodioxol-5-yl)-4-(1,3-benzodioxol-5-yloxy)dihydro-1*H*,3*H*-furo[3,4-*c*]furan-3a(4*H*)-ol, and it was named phrymarolin V[1]).

The two known compounds were identified as apigenin and oleanic acid by comparing their physical and spectroscopic data with literature values [11][12].

Table 2. *^{1}H- and ^{13}C-NMR Data* ($CDCl_3$; 400 and 100 MHz, resp.) *of Compound* **2**[1]). δ in ppm, *J* in Hz

Position	δ(H)	δ(C)	Position	δ(H)	δ(C)
C(1)		151.1	C(1′)		134.3
H–C(2)	6.68 (*d*, *J* = 2.4)	100.7	H–C(2′)	7.00 (*d*, *J* = 1.5)	119.9
C(3)		143.5	C(3′)		147.4
C(4)		148.1	C(4′)		148.2
H–C(5)	6.74 (*d*, *J* = 8.4)	109.9	H–C(5′)	6.79 (*d*, *J* = 7.9)	108.1
H–C(6)	6.57 (*dd*, *J* = 8.4, 2.4)	108.1	H–C(6′)	6.88 (*dd*, *J* = 7.9, 1.5)	106.9
H–C(7)	5.32 (*s*)	103.4	H–C(7′)	4.56 (*d*, *J* = 6.5)	89.4
C(8)		92.3	H–C(8′)	2.70–2.69 (*m*)	59.2
CH_2(9)	4.34, 3.79 (2*d*, *J* = 9.6)	77.7	CH_2(9′)	4.32 (*dd*, *J* = 9.4, 6.8), 3.87 (*dd*, *J* = 9.4, 2.1)	69.9
CH_2(10)	5.96 (*s*)	101.1	CH_2(10′)	5.97 (*s*)	101.5

Experimental Part

General. Reagents were of anal. grade. Anal. TLC: silica gel plates (*GF_{254}*; *Yantai Institute of Chemical Technology*, Yantai, P. R. China). Column chromatography (CC): silica gel (SiO_2; 200–300 and 300–400 mesh; *Qingdao Marine Chemical Factory*, Qingdao, P. R. China), and *Lichroprep-RP_{18}* gel (40–60 μm; *Merck*, Darmstadt, Germany). GC: *Agilent 6980N*; in hexane. UV Spectra: *Shimadzu-UV-260* spectrophotometer; in MeOH; λ_{max} (log ε) in nm. IR Spectra: *Avatar-360-ESP* spectrophotometer (*Thermo Nicolet*); as KBr tablets; in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Bruker-DRX-400* spectrometer; in CD_3OD or $CDCl_3$; δ in ppm with $SiMe_4$ as internal standard and *J* in Hz. HR-ESI-MS: *Bruker-Apex-7.0-Tesla* FT-MS apparatus (*Bruker*, Germany), in *m*/*z*.

Plant Material. The whole plants of *P. leptostachya* were collected in Hanyuan, Sichuan Province, P. R. China, in May 2009, and identified by Dr. *Hong-Ping Deng*. A voucher specimen (PL20090523) has been deposited in the Herbarium of Materia Medica, College of Pharmaceutical Sciences, Southwest University, R. P. China.

Extraction and Isolation. The whole plants were air-dried and powdered, and the material (9 kg) was extracted with 95% EtOH by percolation. The percolate was concentrated and the crude extract (1.1 kg) then suspended in H_2O (2500 ml) and partitioned in turn by extraction with petroleum ether ($3 \times$ 2000 ml), AcOEt (3×2000 ml), and BuOH (3×2000 ml). The AcOEt-soluble part was subjected to CC (SiO_2 (200–300 mesh; 2.8 kg), 12×100 cm, petroleum ether/AcOEt $100:0 \rightarrow 0:100$): *Frs. 1–11*. *Fr. 9* (76.3 g) was subjected to repeated CC (SiO_2 (300–400 mesh), petroleum ether/acetone 9:1): **2** (21 mg), apigenin (8 mg), and oleanic acid (50 mg). *Fr. 11* (18.7 g) was subjected to repeated CC (SiO_2 (300–400 mesh), $CHCl_3$/MeOH 9:1): *Frs. 11.1–11.3*. *Fr. 11.2* was purified by semi-prep. HPLC (MeOH/H_2O 2:3): **1** (38 mg).

rel-*{(2*R,*3*R*)-7-{(1*S,*3a*S,*4*R,*6a*R*)-4-[(4,6-Dimethoxy-1,3-benzodioxol-5-yl)oxy]tetrahydro-3a-hydroxy-1*H,*3*H*-furo[3,4-*c*]furan-1-yl}-2,3-dihydro-3-(4-hydroxy-3-methoxyphenyl)-6-methoxy-1,4-benzodioxin-2-yl}methyl β-*D*-Glucopyranoside* (**1**): White powder. M.p. 181–183°. $[\alpha]_D^{20} = +1.4$ ($c = 1.6$, MeOH). UV (MeOH): 292 (4.06), 234 (4.07). IR (KBr): 3480, 3005, 2940, 2890, 1600, 1500, 1445, 1434, 1330, 980, 930. ^{1}H- and ^{13}C-NMR: *Table 1*. HR-ESI-MS: 803.2402 ($C_{38}H_{43}O_{19}^-$, $[M-H]^-$; calc. 803.2477).

rel-*(1*R,*3a*R,*4*S,*6a*S*)-1-(1,3-Benzodioxol-5-yl)-4-(1,3-benzodioxol-5-yloxy)dihydro-1 H,3*H-*furo[3,4-c]furan-3a(4*H*)-ol* (**2**): White powder. M.p. 159–160°. $[\alpha]_D^{20} = +155$ ($c = 3.6$, dioxane). UV (MeOH): 297 (4.06), 227 (3.73). IR (KBr): 3320, 2998, 2767, 1743, 930, 1630–1650. ^{1}H- and ^{13}C-NMR: *Table 2*. HR-ESI-MS: 358.0928 ($C_{20}H_{17}O_8^-$, $[M-H]^-$; calc. 385.1002).

Acid Hydrolysis of Compound **1** *and GC Assay of the Sugar.* Compound **1** (20 mg) was refluxed in 6% HCl soln. at 80° for 2 h. The mixture was extracted with AcOEt (4×10 ml), the H_2O phase lyophilized, and the residue subjected to CC (SiO_2, $CHCl_3$/MeOH/H_2O 7:3:1): 3.2 mg of monosaccharide. The monosaccharide was dissolved in pyridine (0.5 ml) containing L-cysteine methyl ester hydrochloride (4 mg), and incubated at 60° for 2 h. The mixture was dried under reduced pressure, 1-(trimethylsilyl)-1*H*-imidazole (0.5 ml) was added, and the mixture heated at 60° for 2 h [13]. The mixture was partitioned between hexane (0.5 ml) and H_2O (0.5 ml), and the hexane extract was assayed by GC (capillary column *HP-5MS* ($30 \text{ m} \times 0.25 \text{ mm} \times 0.25$ m; *Agilent*), oven temp. from 200° (1 min) to 250° (3 min) at 1.5°/min; detection temp. 250°; flow rate 1 ml/min) [14][15]. In the acid hydrolyzate of **1**, glucose was identified according to the retention time of the derivative, t_R 17.93 min, compared with that of the derivative of authentic D-glucose prepared under the same conditions. The optical rotation of the glucose obtained from acid hydrolysis of **1** was $[\alpha]_D^{20} = +71.6$ ($c = 0.17$, H_2O).

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