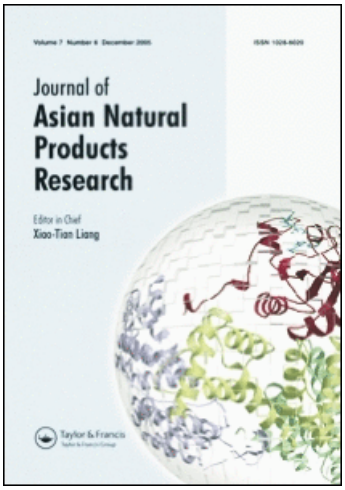

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Ferulates, amurenlactone A and amurenamide A from traditional Chinese medicine cortex Phellodendri Amurensis

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Two new ferulate compounds, amurenlactone A (**1**) and amurenamide A (**2**), along with 11 known compounds have been isolated from the cortex of *Phellodendron amurense*. The structures of the new compounds were established based on 1D, 2D NMR and mass spectral analyzes. The known compounds were identified by comparison with authentic samples.

Keywords: *Phellodendron amurense*; Rutaceae; cortex; amurenlactone A; amurenamide A

1. Introduction

The traditional Chinese medicine, cortex Phellodendri Amurensis, has been used for the treatment of bacillary dysentery, inflammation, tuberculosis and liver cirrhosis.[1,2] Several reports have been encountered on the isolation of isoquinoline alkaloids, phenolic compounds, flavonoids, limonoidal triterpenes and sterols from this plant.[2–6] However, most of the plant materials of these researches were obtained from the species growing in Japan, instead of China. Therefore, the cortex of *Phellodendron amurense* obtained from Liaoning province, a famous region yielding cortex Phellodendri Amurensis in China, was reinvestigated and resulted in the isolation of two new ferulate compounds amurenlactone A (**1**) and amurenamide A (**2**), along with 11 known compounds. We report herein the isolation and structural elucidation of the new compounds using chromatographic and spectral methods.

2. Results and discussion

Amurenlactone A (**1**) was obtained as white amorphous powder, $[\alpha]_D^{25}$ − 6.8. Both $FeCl_3$ and ferric hydroxamate tests showed positive results which indicated that compound **1** has a phenolic hydroxyl and a lactone group. The molecular formula was determined as $C_{17}H_{20}O_9$ by HR-ESI-MS at *m/z* 369.1184 $[M + H]^+$. The IR absorption bands at 3420 and 1786 cm^{-1} were consistent with the presence of a hydroxyl group and a γ-lactone ring. The ^1H NMR spectrum displayed one set of *trans*-double bond signals at δ 7.69 (1H, d, $J = 16.0$ Hz, H-7′) and 6.41 (1H, d, $J = 16.0$ Hz, H-8′). One set of ABX signals at δ 7.20 (1H, d, $J = 1.5$ Hz, H-2′), 7.11 (1H, dd, $J = 8.0$, 1.5 Hz, H-6′) and 6.81 (1H, d, $J = 8.0$ Hz, H-5′) were attributed to a 1,3,4-trisubstituted benzene ring. The HMQC correlations between H-7′ and C-7′ and the HMBC correlations from H-2′ to C-6′, C-7′ indicated that the *trans*-double bond was connected to the C-1′ of the benzene ring

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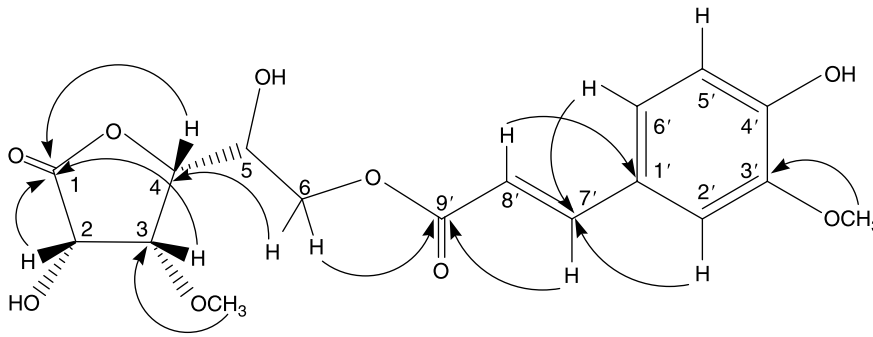

Figure 1. The selected HMBC correlations for compound **1**.

(Figure 1). The HMBC correlations between the methoxy proton at δ 3.89 (3H, *s*) and C-3′, and H-7′ and the carbonyl carbon C-9′ at δ 169.0, suggested that **1** has a feruloyl moiety. There were four oxymethine proton signals at δ 4.65 (1H, d, $J = 4.4$ Hz, H-2), 4.11 (1H, dd, $J = 4.4$, 3.3 Hz, H-3), 4.47 (1H, dd, $J = 8.0$, 3.3 Hz, H-4) and 4.19 (1H, m, H-5), and one oxymethylene proton signal at δ 4.30 (2H, m, H-6) in the ^{1}H NMR spectrum. The ^{1}H–^{1}H COSY spectrum showed that these protons correlated in turn, and their corresponding carbons were confirmed by HMQC spectrum (Table 1). The carbonyl carbon signal at δ 177.4 (C-1) in ^{13}C NMR spectrum and the HMBC correlations between C-1 and H-2, H-3, and H-4 (Figure 1), combined with the IR absorption band at 1786 cm^{-1}, indicated that compound **1** should include a hexano-γ-lactone moiety. The methoxy at δ 3.56 (3H, s) was connected to C-3 according to the HMBC correlation between the methoxy and C-3. The feruloyl moiety was linked to C-6 since the long-range correlation of H-6 with C-9′ was displayed in the HMBC spectrum (Figure 1). Therefore, **1** was considered to have the structure of 6-*O*-feruloyl-hexano-γ-lactone.

Pei-Lin Wu *et al.* isolated a compound vittarilide-A from *Vittaria anguste-elongata* with similar structure as **1** except that the substituting groups of C-3 and 3′ in vittarilide-A are hydroxyls,[7] and its ^{13}C NMR spectral data were similar to those of **1** except for C-3 (Table 1). In vittarilide-A, the H-3 *trans* to H-2 and *cis* to H-4 were proven by the larger coupling constant of $J_{\text{H-2/H-3}} = 9.3$ Hz and the smaller coupling constant of $J_{\text{H-3/H-4}} = 5.0$ Hz. Comparing with vittarilide-A, the smaller coupling constant of $J_{\text{H-2/H-3}} = 4.4$ Hz and $J_{\text{H-3/H-4}} = 3.3$ Hz were observed in **1**, which suggested that the relative configuration of H-3 to H-2 and H-4 is all *cis*. Correlations between H-2 and H-3, H-3 and H-4 in NOESY experiment also proved that these three protons projected in the same direction. The similar coupling constants of $J_{\text{H-2/H-3}}$, $J_{\text{H-3/H-4}}$ and $J_{\text{H-4/H-5}}$ compared with mannono-γ-lactone, as well as the negative optical rotation of **1**, indicated that it is a derivative of L-mannono-γ-lactone.[8,9] Therefore, **1** was determined as 6-*O*-feruloyl-3-*O*-methyl-L-mannono-γ-lactone, named as amurenlactone A (**1**).

Amurenamide A (**2**) was obtained as yellow oil, $[\alpha]_D^{25} - 4.4$. $FeCl_3$ test showed positive results which indicated that compound **2** has phenolic hydroxyl group. The molecular formula was determined as $C_{17}H_{23}O_9N$ by HR-ESI-MS at *m/z* 386.1446 $[M + H]^+$. The IR absorption band at 1674 ($\nu_{C=O}$), 1631 (β_{NH}) and 1429 (ν_{C-N}) cm^{-1} suggested the presence of primary amide (because of the existence of hydroxyl group in **2**, the absorption band of ν_{NH} was not shown in the IR spectrum). The low field of the ^{1}H NMR spectrum was similar to compound **1**, which displayed that **2** has the same feruloyl fragment as **1**. The ^{1}H–^{1}H COSY spectrum showed correlations of four oxymethine protons at δ 4.44 (1H, d,

Table 1. ^{1}H and ^{13}C NMR spectral data (δ) of compounds **1**–**2** (CD_3OD), and vittarilide-A (acetone-d_6).

	1			**2**			**vittarilide-A**
No.	δ_C	δ_H	HMBC	δ_C	δ_H	HMBC	δ_C
1	177.4			175.8			175.3
2	72.9	4.65 (1H, d, $J = 4.4$ Hz)	C-1	72.9	4.44 (1H, d, $J = 2.5$ Hz)	C-1	74.5
3	80.0	4.11 (1H, dd, $J = 3.3$, 4.4 Hz)	C-1, 3-O*C*H_3	83.4	3.74 (1H, t, $J = 3.1$ Hz)	C-1, 3-O*C*H_3	74.5
4	81.5	4.47 (1H, dd, $J = 3.3$, 8.0 Hz)	C-1, C-3, C-5	72.3	3.90 (1H, t, $J = 3.1$ Hz)		80.1
5	69.4	4.19 (1H, m)		70.7	4.03 (1H, m)	C-3	69.0
6	65.4	4.30 (2H, m)	C-4, C-9′	66.3	4.19 (1H, dd, $J = 6.6$, 11.1 Hz) 4.28 (1H, dd, $J = 4.3$, 11.1 Hz)	C-4, C-9′	66.4
1′	127.7			127.5			127.5
2′	111.9	7.20 (1H, d, $J = 1.5$ Hz)	C-3′, C-4′, C-6′, C-7′	111.4	7.34 (1H, d, $J = 1.5$ Hz)	C-3′, C-4′, C-6′, C-7′	115.1
3′	149.4			148.7			146.2
4′	150.7			150.0			148.7
5′	116.5	6.81 (1H, d, $J = 8.0$ Hz)	C-1′, C-3′, C-4′, C-6′	116.0	6.87 (1H, d, $J = 8.0$ Hz)	C-1′, C-3′, C-4′, C-6′	116.3
6′	124.2	7.11 (1H, dd, $J = 1.5$, 8.0 Hz)	C-2′, C-4′, C-7′	123.9	7.14 (1H, dd, $J = 1.5$, 8.0 Hz)	C-2′, C-4′, C-7′	122.5
7′	147.4	7.69 (1H, d, $J = 16.0$ Hz)	C-1′, C-9′	145.8	7.61 (1H, d, $J = 16.0$ Hz)	C-1′, C-9′	146.0
8′	115.1	6.41 (1H, d, $J = 16.0$ Hz)	C-1′, C-9′	115.8	6.38 (1H, d, $J = 16.0$ Hz)	C-1′, C-9′	115.3
9′	169.0			167.5			167.5
3-OCH_3	60.8	3.56 (3H, s)	C-3	59.0	3.48 (3H, s)	C-3	
3′-OCH_3	56.5	3.89 (3H, s)	C-3′	56.3	3.90 (3H, s)	C-3′	

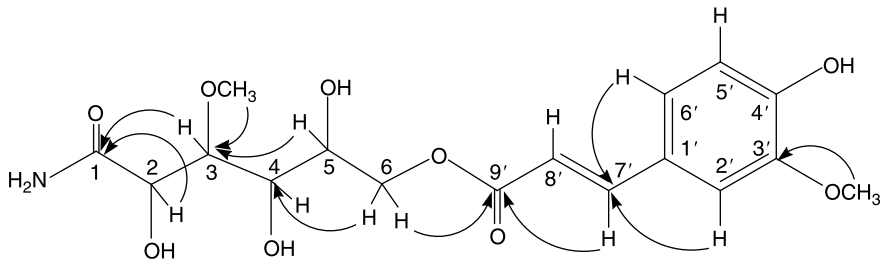

Figure 2. The selected HMBC correlations for compound **2**.

$J = 2.5$ Hz, H-2), 3.74 (1H, t, $J = 3.1$ Hz, H-3), 3.90 (1H, t, $J = 3.1$ Hz, H-4) and 4.03 (1H, m, H-5) successively, and the correlations of the oxymethylene protons at δ 4.19 (1H, dd, $J = 6.6$, 11.1 Hz, H-6α) and 4.28 (1H, dd, $J = 4.3$, 11.1 Hz, H-6β) with H-5 were also observed. Only H-2 and H-3 correlated with the carbonyl carbon at δ 175.8 (C-1) in the HMBC spectrum indicating that this structural fragment in **2** should be a straight chain, which was also supported by the unsaturation degree of **2**. The methoxy protons at δ 3.48 (3H, s) correlated with C-3 in HMBC spectrum, so it should be connected to C-3. Since the correlation of H-6 with C-9′ was observed in the HMBC spectrum, the feruloyl should be connected to C-6 (Figure 2). According to the molecular formula, it is concluded that there is an $-NH_2$ group in **2**, and IR spectrum showed the absorption for a primary amide group; therefore, **2** was determined as 6-*O*-feruloyl-3-methoxy-2,4,5-trihydroxycaproylamine, named as amurenamide A. The relative configurations of C-2, C-3, C-4 and C-5 were not determined.

In addition, 11 compounds obakunone (**3**), obaculactone (**4**), kihadanin B (**5**), berberine (**6**), β-sitosterol (**7**), daucosterol (**8**), palmatine (**9**), syringin (**10**), methyl 3-*O*-feruloyl quinate (**11**), jatrorrhizine (**12**) and sanleng acid (**13**) were also isolated from the cortex of *P. amurense*.

3. Experimental

3.1 General experimental procedures

UV spectra were measured on a Hitachi UV-3210 spectrophotometer; IR spectra were measured on a Brucker Is-55 spectrophotometer as KBr disks. Optical activities were measured on a Perkin–Elmer 241MC polarimeter. NMR spectra were recorded on Bruker ARX-300 spectrometers, using tetramethylsilane as internal standard; all chemical shifts are reported in parts per million (ppm, δ). ESI mass spectra were recorded on a LCQ LC-MS spectrometer and HR-ESI-MS spectra were recorded on a ZAB-HS GC-MS spectrometer. Column chromatography was carried out on silica gel (100–140 and 200–300 mesh; Qingdao Haiyang Chemical Co., Shandong, China) and Sephadex LH-20 (Sigma, USA). Pre-coated TLC plates were used without activation (silica gel 60 F254, 0.25 mm, Merck, Germany).

3.2 Plant material

The cortex of *P. amurense* were collected in September 1999 from Tieling, Liaoning province, China, and authenticated by Professor Zheng Cui of Shenyang Pharmaceutical University, China. A voucher specimen (No. SPU-PA-99-9) has been deposited in the School of Traditional China Materia Medica, Shenyang Pharmaceutical University, Shenyang, China.

3.3 Extraction and isolation

The air-dried and powdered cortex of *P. amurense* (7.2 kg) was extracted three times with 70% ethanol under reflux. The extract was concentrated under reduced pressure to give dark brown syrup. The syrup was successively partitioned with petroleum

ether, chloroform, and *n*-butanol to obtain chloroform extract 120 g and *n*-butanol extract 276 g. Compounds **3**–**7** and **13** were isolated from chloroform extract by repeated chromatography on silica gel column.

The *n*-butanol extract was chromatographed over silica gel column using a gradient of chloroform–methanol (100:1 to 100% methanol) to afford six fractions (PBFr I–VI). PBFr II was subjected to column chromatography over silica gel with chloroform–methanol (10:1) followed by Sephadex LH-20 chromatography with methanol, and further purified by preparative TLC with chloroform–methanol–ammonia (10:1:0.5) to yield **2** (10 mg). PBFr III was chromatographed over silica gel using a gradient of chloroform–methanol to afford three fractions (PBFr III-1, -2 and -3). PBFr III-1 was subjected to column chromatography over silica gel with cyclohexane and ethyl acetate (1:1) and rechromatographed over silica gel column with chloroform–methanol (20:1), yielding **1** (7.7 mg). Compounds **8**–**11** and **12** were also isolated from *n*-butanol extract by column chromatography on silica gel or Sephadex LH-20 and preparative TLC successively.

3.3.1 Amurenlactone A (*1*)

$C_{17}H_{20}O_9$, white amorphous powder. $[\alpha]_D^{25}$ − 6.8 (*c* 0.0051, MeOH). UV (MeOH) λ_{max} nm (log ε): 204 (3.97), 233 (3.79), 326 (3.94). IR ν_{max} cm^{-1} 3420 (OH), 1786 (C═O). ^1H NMR and ^{13}C NMR spectral data (Table 1). Negative ESI-MS (probe) 12 eV, *m/z* (rel. int.): 367 $[M - H]^-$ (76), 335 $[M - H_2O\text{-}Me]^-$ (100), 233 (14). HR-ESI-MS *m/z* 369.1184 $[M + H]^+$ (calcd for $C_{17}H_{21}O_9$, 369.1180).

3.3.2 Amurenamide A (*2*)

$C_{17}H_{23}O_9N$, yellow oil, $[\alpha]_D^{25}$ − 4.4 (*c* 0.0037, MeOH). UV (MeOH) λ_{max} nm (log ε): 202 (3.70), 218 (3.63), 324 (3.62). IR ν_{max} cm^{-1} 3422 (OH), 1674 (NH_2—C═O). ^1H NMR and ^{13}C NMR spectral data (Table 1). Positive ESI-MS (probe) 12 eV, *m/z* (rel. int.): 386 $[M + H]^+$(50), 368 $[M - NH_3]^+$(100). HR-ESI-MS *m/z* 386.1446 $[M + H]^+$ (calcd for $C_{17}H_{24}O_9N$, 386.1446).

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