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Nine new compounds from the root bark of *Lycium chinense* and their α-glucosidase inhibitory activity†

Ya-Nan Yang,‡[a] Ya-Wen An,‡[a] Zhi-Lai Zhan,[b] Jing Xie,[a] Jian-Shuang Jiang,[a] Zi-Ming Feng,[a] Fei Ye[a] and Pei-Cheng Zhang*[a]

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Lycium chinense Mill. is a deciduous shrub in the Solanaceae family that is known for its fruits (Lycii fructus) and root bark (Lycii cortex). In our ongoing search for α-glucosidase inhibitors from the root bark of *L. chinense*, lyciumflavane A, one new flavane with an unusual benzofuran unit, one new amide possessing a naphthalene skeleton, one new sesquiterpene, three new lignan glucosides, and three new phenolic glucosides were isolated along with eight known compounds. Their structures were elucidated using NMR, HRESIMS, UV, ECD, and IR spectroscopic data. Their α-glucosidase inhibitory activity was screened using acarbose as a positive control (IC_{50} = 385 μM). Compound **1** showed strong inhibitory activity against α-glucosidase (IC_{50} = 20.89 μM).

Introduction

Lycium chinense Mill. is cultivated in the mainland of China and is an important source for health foods and traditional Chinese medicine.[1–3] The root bark of *L. chinense*, named Digupi in China, is conventionally used in traditional Chinese medicine prescriptions to treat diabetes, coughs, hypertension, and fever. Modern pharmacological studies revealed that extracts from the root bark of *L. chinense* can lower serum glucose levels and improve insulin resistance.[4,5] Previously, phytochemical investigations of this plant have shown the presence of alkaloids, lignanamides, cyclopeptides, lignans, and sterols.[6–12]

With the aim of discovering new bioactive natural products with hypoglycemic effects from the root bark of *L. chinense*, an oral sucrose tolerance test (OSTT) was performed. The results demonstrated that the water-soluble portion of the root bark of *L. chinense* obtained from an 80% EtOH extract could significantly decrease the postprandial blood glucose levels in normal ICR mice at a dose of 200 mg kg^{-1} (Fig. S1, ESI†), which is similar to the hypoglycemic effect of acarbose (20 mg kg^{-1}). Through bioactivity-guided isolation, one new flavane with an unusual benzofuran unit, one new amide possessing a naphthalene skeleton, one new sesquiterpene, three new lignan glucosides, and three new phenolic glucosides were obtained from the root bark of *L. chinense* along with eight known compounds (Fig. 1). In this paper, we reported the isolation and structure elucidation of compounds **1–9** and evaluated these compounds as α-glucosidase inhibitors.

Results and discussion

The molecular formula of **1** was determined to be $C_{22}H_{22}O_4$ by the positive ion peak at *m/z* 351.1586 $[M + H]^+$ in the HRESIMS, which indicated 12 degrees of unsaturation. The ^1H NMR spectrum of **1** (Table 1) revealed an ABX system aromatic ring at δ_H 6.88 (1H, d, J = 8.0 Hz), 6.29 (1H, dd, J = 2.0, 8.0 Hz), and 6.18 (1H, d, J = 2.0 Hz), a pentasubstituted aromatic ring at δ_H 7.17 (1H, s), three olefinic protons at δ_H 6.88 (1H, d, J = 2.0 Hz), 7.91 (1H, d, J = 2.0 Hz), and 5.07 (1H, m), an oxymethylene proton at δ_H 5.08 (1H, br d, J = 11.0 Hz), three methylene protons at δ_H 3.49 (1H, m), 3.42 (1H, m), 2.82 (1H, m), 2.66 (1H, m), 2.09 (1H, m), and 1.88 (1H, m), and two methyl groups at δ_H 1.67 (3H, s) and 1.61 (3H, s). The ^{13}C NMR and HSQC spectra of **1** displayed 22 carbon resonances including an isopentene group, two olefinic carbons and a C6–C3–C6 unit, which could be attributed to a flavone skeleton.

From the HMBC spectrum, the correlations of H-2 [δ_H 5.08 (1H, d, J = 10.0 Hz)] to C-1′ (δ_C 135.6) and C-6′ (δ_C 109.1) as well as the correlations of H-6′ [δ_H 7.17 (1H, s)] to C-2 (δ_C 74.6), C-1′ (δ_C 135.6), C-2′ (δ_C 122.3), C-4′ (δ_C 143.5), and C-5′ (δ_C 126.2) confirmed the basic skeleton of the flavane. The correlations of H-5 [δ_H 6.88 (1H, d, J = 8.0 Hz)] to C-9 (δ_C 156.4) and C-7 (δ_C 155.7) suggested the existence of 7-OH at ring A of the flavane. Moreover, the isopentene group was connected to the C-2′ (δ_C 122.3) of ring B because the H-1″ [δ_H 3.42 (1H, m)] was correlated with C-1′

[a]*State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing 100050, People's Republic of China. E-mail: pczhang@imm.ac.cn*

[b]*State Key Laboratory Breeding Base of Dao-di Herbs, National Resource Center for Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing, 100700, People's Republic of China*

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‡ These authors contributed equally.

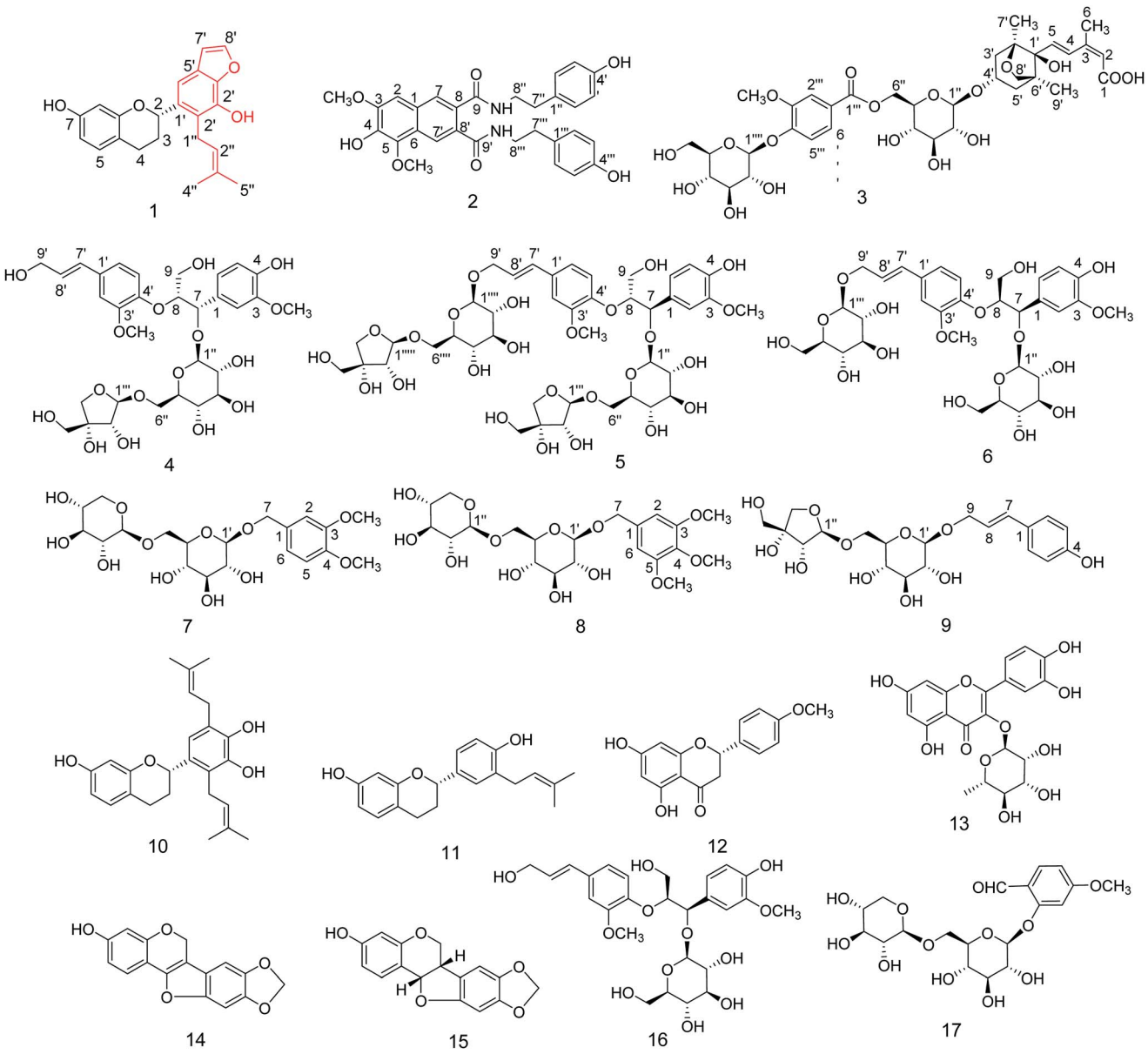

Fig. 1 Chemical structures of **1–17**.

(δ_C 135.6), C-2′ (δ_C 122.3), and C-3′ (δ_C 139.3). By considering the remaining two degrees of unsaturation and the molecular formula, the benzofuran unit was established, which was supported by the HMBC correlations (Fig. 2) from H-7′ [δ_H 6.88 (1H, d, J = 2.0 Hz)] to C-4′ (δ_C 143.5), C-5′ (δ_C 126.2), and C-8′ (δ_C 145.3) and from H-8′ [δ_H 7.91 (1H, d, J = 2.0 Hz)] to C-4′ (δ_C 143.5) and C-5′ (δ_C 126.2). In addition, the 2*S* configuration was confirmed by the negative Cotton effect at 280 nm in the ECD spectrum.[15] So, compound **1** was established as shown, and was accorded the trivial name lyciumflavane A.

Compound **2** was obtained as a yellow amorphous powder. The molecular formula, $C_{30}H_{30}N_2O_7$, was established by the protonated molecular ion peak at *m*/*z* 531.2131 $[M+H]^+$ (calcd for $C_{30}H_{31}N_2O_7$, 531.2131) in the HRESIMS, which corresponded to 17 degrees of unsaturation. In the ^{1}H NMR spectrum of **2** (Table 1), the presence of two *p*-tyramine moieties was deduced from two AA′BB′ spin system aromatic rings [δ_H 7.06 (2H, d, J = 8.5 Hz), 6.69 (2H, d, J = 8.5 Hz), 7.08 (2H, d, J = 8.5 Hz) and 6.71 (2H, d, J = 8.5 Hz)], two pairs of methylene proton signals [δ_H 2.72 (4H, m) and 3.36 (4H, m)], and two NH signals [δ_H 8.40 (1H, t, J = 5.5 Hz) and 8.34 (1H, t, J = 5.5 Hz)]. Simultaneously, another three aromatic protons at δ_H 7.16 (1H, s), 7.75 (1H, s), and 7.83 (1H, s), and two methoxy groups at δ_H 3.93 (3H, s) and 3.87 (3H, s) were also observed. The ^{13}C NMR spectrum (Table 2) of **2** revealed 30 carbon signals, 20 of which were assigned to two *p*-tyramine moieties, two carbonyl groups and two methoxy groups, and the remaining 10 carbons were assigned to a naphthalene unit combined with the degrees of unsaturation. The HMBC correlations (Fig. 2) of H-8″/C-9, H-8‴/C-9′, H-7/C-9, and H-7′/C-9′ suggested that **2** was an amide possessing a naphthalene skeleton.

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

No.	1	2	3	4	5	6
2	5.08, br d (11.0)	7.16, s	5.65, s	7.07, d (2.0)	7.07, d (2.0)	6.97, d (2.0)
3	2.09, m					
	1.88, m					
4	2.82, m		8.05, d (16.5)			
	2.66, m					
5	6.88, d (8.0)		6.02, d (16.5)	6.67, d (8.0)	6.67, d (8.0)	6.77, d (8.0)
6	6.29, dd (2.0, 8.0)		1.81, s	6.80, dd (2.0, 8.0)	6.80, dd (2.0, 8.0)	7.00, d (8.0)
7		7.75, s		4.96, d (4.5)	4.95, d (4.5)	4.94, d (4.0)
8	6.18, d (2.0)			4.38, m	4.39, m	4.50, m
9				3.65, m	3.62, m	3.66, m
				3.21, m	3.23, m	3.21, m
2′				7.01, d (2.0)	7.03, d (2.0)	7.05, d (1.5)
3′			1.96, m			
			1.73, m			
4′			3.95, m			
5′			1.63, m	6.96, d (8.0)	6.97, d (8.0)	6.67, d (8.0)
			1.57, m			
6′	7.17, s			6.87, dd (2.0, 8.0)	6.89, dd (2.0, 8.0)	6.87, dd (8.0, 2.0)
7′	6.88, d (2.0)	7.83, s	0.99, s	6.43, d (16.0)	6.55, d (16.0)	6.55, d (16.5)
8′	7.91, d (2.0)		3.48, m	6.23, dt (6.0, 16.0)	6.20, m	6.23, dt (16.0)
9′			0.67, s	4.08, m	4.15, m	4.39, d (6.0)
					4.37, m	4.16, m
1″	3.42, m		4.25, d (8.0)	3.98, d (7.5)	3.97, d (7.0)	4.41, d (7.5)
	3.49, m					
2″	5.07, m	7.06, d (8.5)	2.94, m	3.61, m	3.00, m	2.99, m
3″		6.69, d (8.5)	3.28, m	3.12, m	3.01, m	3.13, m
4″	1.67, s		3.11, m	3.11, m	2.99, m	3.03, m
5″	1.61, s	6.69, d (8.5)	3.17, m	3.13, m	3.27, m	3.06, m
6″		7.06, d (8.5)	4.49, d (10.5)	3.83, d (10.0)	3.48, m	3.68, m,
			4.29, m	3.49, m	3.85, m	3.57, d (1.0)
7″		2.72, m				
8″		3.36, m				
1‴				4.89, d (3.0)	4.88, d (3.0)	4.20, d (8.0)
2‴		7.08, d (8.5)	7.47, d (1.5)	3.83, m	3.78, m	3.00, m
3‴		6.71, d (8.5)				3.14, m
4‴				3.87, d (9.5)	3.85, m	3.04, m
				3.60, d (9.5)	3.59, m	
5‴		6.71, d (8.5)	7.15, d (8.5)	3.36, m	3.35, m	3.08, m
6‴		7.08, d (8.5)	7.54, dd (8.5, 1.5)			3.68, m
						3.45, m
1⁗			4.99, d (6.5)		4.20, d (8.0)	
2⁗			3.18, m		2.99, m	
3⁗			3.28, m		3.13, m	
4⁗			3.17, m		3.06, m	
5⁗			3.28, m		3.13, m	
6⁗			3.64, d (10.5)		3.42, m	
			3.47, m		3.84, m	
1⁗′					4.89, d (3.0)	
2⁗′					3.71, m	
4⁗′					3.59, m	
					3.87, m	
5⁗′					3.36, m	
3-OCH_3		3.93, s		3.71, s	3.71, s	3.78, s
5-OCH_3		3.87, s				
3′-OCH_3				3.77, s	3.77, s	3.71, s
3‴-OCH_3			3.80, s			

The linkage points of the two methoxy groups were confirmed to be at C-3 and C-5 based on the correlations between the methoxy protons (δ_H 3.93) with C-3 and the methoxy protons (δ_H 3.87) with C-5 in the HMBC spectrum. Thus, compound **2** was elucidated as shown and was named lyciumamide A.

Compound **3** was isolated as a yellow powder. The molecular formula was established to be $C_{35}H_{48}O_{18}$ by the HRESIMS ion peak at m/z $[M + Na]^+$ 779.2723 ($C_{35}H_{48}O_{18}Na$, calcd for 779.2733). The UV, IR, and NMR spectra of **3** were similar to dihydrophaseic acid 4′-*O*-(6″-*O*-galloyl)-β-D-glucopyranoside;[16] the differences

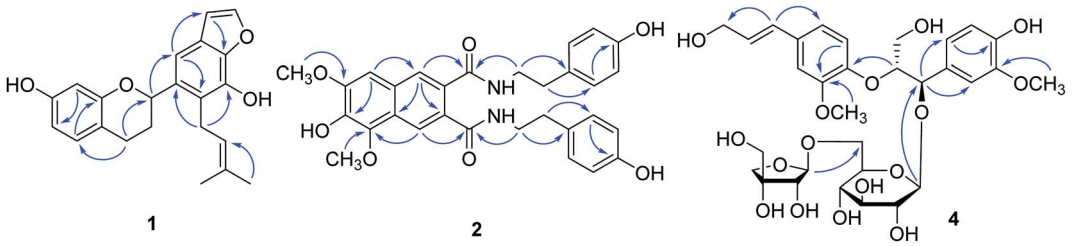

Fig. 2 Key HMBC correlations of **1**, **2**, and **4**.

between the compounds were that **3** contained an additional glucose unit and a vanilloyl group in place of the galloyl in dihydrophaseic acid 4′-*O*-(6″-*O*-galloyl)-β-D-glucopyranoside. In the HMBC spectrum of **3**, the correlations from H-1‴′ (δ_H 4.99) to C-4‴ (δ_C 150.7) established the linkage position of the additional glucose. Compound **3** was assigned as lyciumoside A.

Compound **4** was obtained as a white powder. The molecular formula, $C_{31}H_{42}O_{16}$, was established by a sodiated molecular ion peak observed at *m*/*z* 693.2377 $[M + Na]^+$ (calcd for $C_{31}H_{42}O_{16}Na$, 693.2365) in the HRESIMS. The ^{1}H NMR spectrum of **4** (Table 1) exhibited two ABX spin system aromatic protons at δ_H 7.07 (1H, d, J = 2.0 Hz), 6.67 (1H, d, J = 8.0 Hz), 6.80 (1H, dd, J = 2.0, 8.0 Hz), 7.01 (1H, d, J = 2.0 Hz), 6.96 (1H, d, J = 8.0 Hz), and 6.87 (1H, dd, J = 2.0, 8.0 Hz), and two olefinic protons at δ_H 6.43 (1H, d, J = 16.0 Hz) and 6.23 (1H, dt, J = 6.0, 16.0 Hz). Two oxygenated methine protons at δ_H 4.96 (1H, d, J = 4.5 Hz) and 4.38 (1H, m), four oxygenated methylene protons at δ_H 3.65 (1H, m), 3.21 (1H, m), and 4.08 (2H, m), two methoxy groups at δ_H 3.71 (3H, s) and 3.77 (3H, s), and two anomeric protons at δ_H 3.98 (1H, d, J = 7.5 Hz) and 4.89 (1H, d, J = 3.0 Hz) were observed in the upfield region. The ^{13}C NMR and HSQC spectra displayed a total of 31 carbon signals. Apart from one glucopyranose moiety, one apiofuranose moiety, and two methoxy groups, the remaining 18 carbon signals were assigned to two C6–C3 units. The key HMBC correlations (Fig. 2) of H-7 at δ_H 4.96 with C-1, 2, 6, 8, and 9, of H-7′ at δ_H 6.43 with C-1′, 2′, 6′, 8′, and 9′, and of H-8 at δ_H 4.38 with C-4′ at δ_C 147.7 suggested that **4** was an 8-*O*-4′ system neolignan.[17] The methoxy groups were confirmed to be at C-3 and C-3′ based on the HMBC correlations of the methoxy groups at δ_H 3.71 and 3.77 with C-3 and C-3′, respectively. The HMBC correlation of H-1″ (δ_H 3.98) with C-7 (δ_C 76.1) revealed that the glucopyranose unit was attached to C-7, while the correlation of H-1‴′ (δ_H 4.89) with C-6″ (δ_C 67.3) suggested that the apiofuranose unit was attached to C-6″. On the basis of the above analysis, the planner structure of **4** was similar to that of ligusinenoside D,[18] except for the position of sugar moiety. Acid hydrolysis of **4** yielded **4a**, D-glucopyranose and D-apiofuranose, which were identified by GC analysis of their trimethylsilyl L-cysteine derivatives. Furthermore, the β-linkage of D-glucopyranose and D-apiofuranose was determined by their anomeric protons at δ_H 3.98 (1H, d, J = 7.5 Hz) and δ_H 4.89 (1H, d, J = 3.0 Hz). The ^{1}H NMR experiment of **4a** was performed in $CDCl_3$. A small coupling constant ($J_{7,8}$ = 4.5 Hz) between H-7 and H-8 indicated the *erythro* configuration of C-7 and C-8 unambiguously.[19] This result combined with the negative Cotton effect at 231 nm in the CD spectrum of **4** established the 7*S*,8*R* configurations for **4**.[19] From the above data, the structure of **4** was assigned as shown (Fig. 1), and this compound was named lyciumlignan A.

The molecular formula of **5** was determined to be $C_{42}H_{60}O_{25}$ on the basis of the sodiated molecular ion peak observed at *m*/*z* 987.3316 $[M + Na]^+$ in the HRESIMS. A comparison of the UV and NMR data of **5** with those of **4** revealed that the aglycone of **5** was also an 8-*O*-4′ system neolignan. Its ^{1}H NMR spectrum exhibited four anomeric protons at δ_H 3.97 (1H, d, J = 7.0 Hz), 4.88 (1H, d, J = 3.0 Hz), 4.20 (1H, d, J = 8.0 Hz), and 4.89 (1H, d, J = 3.0 Hz), which indicated the existence of two glucopyranose units and two apiofuranose units with the β-linkage. The key HMBC correlations of H-1″ (δ_H 3.97) to C-7 (δ_C 76.3), H-1⁗ (δ_H 4.20) to C-9′ (δ_C 68.6), H-1‴ (δ_H 4.88) to C-6″ (δ_C 67.4), and H-1⁗ (δ_H 4.89) to C-6⁗ (δ_C 67.7) clearly presented the planner structure for compound **5** as 4,9-dihydroxy-3,3′-dimethoxy-7-en-8,4′-oxyneolignan-7-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyl-9′-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside. Acid hydrolysis of **5** resulted in **5a**, D-glucopyranose, and D-apiofuranose. The D-configurations of these two sugars were determined by GC analysis. In the ^{1}H NMR spectrum of **5a** ($CDCl_3$), the characteristic coupling constant of H-7 ($J_{7,8}$ = 7.5 Hz) allowed for the determination of the *thero* configuration of C-7 and C-8.[18] By considering the negative Cotton effects at 231 nm ($\Delta\varepsilon$ −1.82) in **5** that were exhibited in the CD spectrum, the 7*R*,8*R* configurations were confirmed.[18] Thus, **5** was characterized as shown and named lyciumlignan B.

Detailed analysis of the 1D NMR spectra (Tables 1 and 2) suggested that the structure of **6** was similar to that of **5**. The difference between these two compounds was the absence of two apiofuranose units at C-6″ and C-6‴ in **6**. Using the same method that was described for **5**, the absolute configurations of C-7 and C-8 in **6** were established as 7*R*,8*S*. Therefore, the structure of **6** (lyciumlignan C) was determined to be (7*R*,8*S*)-4,9-dihydroxy-3,3′-dimethoxy-7-en-8,4′-oxyneolignan-7-*O*-β-D-glucopyranosyl-9′-*O*-β-D-glucopyranoside.

Compound **7** exhibited a molecular formula of $C_{20}H_{30}O_{12}$ according to the positive HRESIMS ion observed at *m*/*z* 485.1633 $[M + Na]^+$. The ^{1}H NMR spectrum (Table 3) of **7** revealed a set of ABX system aromatic protons at δ_H 7.00 (1H, d, J = 2.0 Hz), 6.88 (1H, overlap), and 6.88 (1H, overlap), two oxymethylene protons at δ_H 4.70 (1H, d, J = 12.0 Hz) and 4.50 (1H, d, J = 12.0 Hz), and two methoxyl protons at δ_H 3.73 (3H, s) and 3.74 (3H, s). Moreover, two anomeric protons at δ_H 4.16 (1H, d, J = 8.0 Hz) and 4.24 (1H, d, J = 7.5 Hz) observed in the upfield region

Table 2 ^{13}C NMR data of **1–6** in DMSO-d_6 (125 MHz, δ in ppm)

No.	1	2	3	4	5	6
1		123.2	177.5	128.6	128.4	129.7
2	74.6	102.4	126.8	111.6	111.7	111.8
3	29.6	151.0	141.4	147.1	147.2	149.6
4	24.6	139.8	131.0	145.8	145.8	145.7
5	129.9	140.5	129.9	114.7	114.7	115.1
6	108.0	126.7	20.5	120.1	120.1	119.7
7	155.7	126.3		76.1	76.3	77.6
8	102.8	126.8		82.9	83.0	81.8
9	156.4	168.4		60.5	60.6	59.8
10	112.1					
1′	135.6		81.0	130.2	129.7	129.7
2′	122.3		85.3	110.0	110.1	109.7
3′	139.3		42.0	149.6	149.6	147.6
4′	143.5		72.8	147.7	148.1	147.6
5′	126.2		41.8	115.7	115.7	114.6
6′	109.1		47.5	119.1	119.4	119.4
7′	107.7	120.0	19.5	128.5	131.7	131.4
8′	145.3	131.7	75.0	128.4	123.8	124.1
9′		168.6	16.0	61.6	68.6	68.7
1″	23.9	129.6	102.2	100.3	100.2	102.1
2″	123.8	129.5	73.3	73.3	73.4	74.2
3″	129.8	115.0	77.1	76.6	76.6	77.1
4″	25.4	155.6	70.6	70.0	70.0	69.9
5″	17.7	115.0	73.6	75.3	75.3	76.8
6″		129.5	64.3	67.3	67.4	61.0
7″		34.1				
8″		41.1				
1‴		129.6	123.0	109.3	109.2	102.2
2‴		129.7	112.7	75.9	75.9	73.5
3‴		115.1	148.6	78.8	78.8	76.9
4‴		155.6	150.7	73.4	73.3	70.1
5‴		115.1	114.5	63.3	63.1	76.5
6‴		129.7	122.8			61.1
7‴		34.3	165.1			
8‴		41.1				
1⁗			99.6		101.8	
2⁗			73.1		73.4	
3⁗			76.8		76.6	
4⁗			69.4		70.3	
5⁗			76.5		75.6	
6⁗			60.4		67.7	
1⁗					109.3	
2⁗					75.9	
3⁗					78.8	
4⁗					73.2	
5⁗					63.3	
3-OCH_3		55.8		55.4	55.7	55.5
5-OCH_3		60.4				
3′-OCH_3				55.7	55.4	55.4
3‴-OCH_3			55.7			

confirmed the presence of two sugar moieties with a β-linkage. The ^{13}C NMR spectrum showed 20 carbon signals that could be assigned to a glucopyranose unit, a xylopyranose unit, two methoxy groups, and a benzylalcohol moiety. The HMBC correlation peaks of the two methoxy groups at δ_H 3.73 and 3.74 with C-3 and C-4 confirmed the linkage positions of these methoxy groups. The sequence of two sugar units was determined to be β-xylopyranosyl-(1→6)-*O*-β-glucopyranosyl based on correlations from H-1″ to C-6′ in the HMBC spectrum. The unusual downfield shift of the H-7 (δ_H 4.70, 4.50) and C-7 (δ_C 69.3) was an important indicator that the sugar moiety occurred at C-7. This was supported by the HMBC correlation that was observed from H-1′ (δ_H 4.16) to C-7. Finally, the structure of **7** was elucidated as 3′,4′-dimethoxy-benzylalcohol-1-*O*-β-D-xylopyranosyl-(1→6)-*O*-β-D-glucopyranoside.

A comparison of the IR, UV, and NMR data (Table 3) of **8** with those of **7** revealed that the difference between these two compounds was the presence of an additional methoxy group at C-5 in **8**. This result was supported by the HMBC correlation from OCH_3-3, 5 to C-3, 5, and the singlet proton signal at δ_H 6.71 (2H, s) in the ^{1}H NMR spectrum. So, compound **8** was elucidated as 3′,4′,5′-trimethoxy-benzylalcohol-1-*O*-β-D-xylopyranosyl-(1→6)-*O*-β-D-glucopyranoside.

Compound **9** was obtained as a white powder. The molecular formula, $C_{20}H_{28}O_{11}$, was established by the positive molecular ion peak at *m*/*z* 467.1531 $[M + Na]^+$ (calcd for $C_{20}H_{28}O_{11}Na$, 467.1524) in the HRESIMS. The ^{1}H NMR spectrum of **9** revealed an AA′BB′ spin system [δ_H 7.25 (2H, d, J = 9.0 Hz) and 6.71 (2H, d, J = 9.0 Hz)], two *trans* olefince proton signals [δ_H 6.53 (1H, d, J = 16.0 Hz) and 6.10 (1H, d, 16.0 Hz)], two oxymethylene proton signals [δ_H 4.44 (1H, m) and 4.13 (1H, m)], and two anomeric proton signals [δ_H 4.19 (1H, d, J = 7.5 Hz) and 4.88 (1H, d, J = 3.0 Hz)]. In the ^{13}C NMR spectrum (Table 3), aside from the 11 carbon signals of a glucopyranose unit and an apiofuranose unit, the remaining nine carbon signals could be attributed to a *p*-coumaryl alcohol moiety. Subsequently, the two sugar units were determined to be β-apiofuranosyl-(1→6)-*O*-β-glucopyranosyl by the characteristic correlation of H-1″ with C-6′ in the HMBC spectrum. In combination with the HMBC correlation of H-1′ with C-9, compound **9** was defined as 4′-hydroxystyrone-1-*O*-β-D-apiofuranosyl-(1→6)-*O*-β-D-glucopyranoside.

Based on the spectroscopic data and a comparison with those found in the literature, the eight known compounds (Fig. 1) were established as kazinol A (**10**),[20,21] 7,4′-dihydroxy-isopentene flavane (**11**),[22] acacetin (**12**),[23] quercitrin (**13**),[24] maackianin (**14**),[25] maackiain (**15**),[26] (7*R*,8*S*)-4,9,9′-trihydroxy-3,3′-dimethoxy-7′-en-8,4′-oxyneolignan-7-*O*-β-D-glucopyranoside (**16**),[27] and 2-hydroxy-4-methoxybenzaldehyde-2-*O*-β-D-glucopyraneosyl-1-6-*O*-β-D-xylopyranoside (**17**).[28]

Experimental

General experimental procedures

The optical rotations, UV spectra, and ECD spectra were measured on JASCO P-2000 polarimeter, JASCO V650 spectrometer, and JASCO J-815 spectrometer (JASCO, Easton, MD, USA), respectively. Infrared (IR) spectra were recorded on a Nicolet 5700 spectrometer (Thermo Scientific, Waltham, MA, USA). 1D and 2D NMR spectra were recorded with a Bruker 500 MHz spectrometer (Bruker-Biospin, Billerica, MA, USA). High-resolution electrospray ionization mass spectrometry (HRESIMS) was performed on an Agilent 6520 HPLC-Q-TOF (Agilent Technologies, Waldbronn, Germany). Flash chromatography was performed using a Combiflash RF200 apparatus (Teledyne Isco Corp., Lincoln, NE, USA). Preparative and semi-preparative HPLC (pHPLC and semi-pHPLC) were performed using a Shimadzu preparative chromatography system (Shimadzu Corp.,

Table 3 ^{1}H and ^{13}C NMR data of **7–9** in DMSO-d_6 (500 MHz for ^{1}H NMR, 125 MHz for ^{13}C NMR)

	7			**8**			**9**	
No.	δ_H (J in Hz)	δ_C	No.	δ_H (J in Hz)	δ_C	No.	δ_H (J in Hz)	δ_C
1		130.3	1		133.6	1		132.0
2	7.00, br s	111.4	2	6.71, s	104.8	2	7.25, d (9.0)	127.6
3		148.1	3		152.7	3	6.71, d (9.0)	115.4
4		148.5	4		136.5	4		157.2
5	6.88, overlap	111.9	5		152.7	5	6.71, d (9.0)	115.4
6	6.88, overlap	120.2	6	6.71, s	104.8	6	7.25, d (9.0)	127.6
7	4.70, d (12.0)	69.3	7	4.70, d (12.5)	69.3	7	6.53, d (16.0)	132.0
	4.50, d (12.0)			4.54, d (12.5)				
8			8			8	6.10, dt (16.0)	122.4
9			9			9	4.44, m	68.8
							4.13, m	
1′	4.16, d (8.0)	101.5	1′	4.17, d (7.5)	101.7	1′	4.19, d (7.5)	101.7
2′	3.01, m	73.3	2′	3.00, m	73.3	2′	2.98, m	73.4
3′	3.08, m	76.6	3′	3.08, m	76.5	3′	3.13, m	76.6
4′	3.55, m	70.0	4′	3.55, m	70.0	4′	2.99, m	70.3
5′	3.27, m	75.8	5′	3.24, m	75.8	5′	3.25, m	75.6
6′	3.95, m	68.4	6′	3.95, d (10.5)	68.4	6′	3.41, d (3.0)	67.7
	3.54, m			3.55, m			3.85, brd	
1″	4.24, d (7.5)	104.0	1″	4.24, d (8.0)	104.1	1″	4.88, d (3.0)	109.2
2″	2.97, m	73.3	2″	2.98, m	73.4	2″	3.75, m	75.9
3″	3.05, m	76.6	3″	3.14, m	76.6	3″		78.8
4″	3.27, m	69.6	4″	3.27, m	69.6	4″	3.82, d (9.5)	73.2
							3.59, d (9.5)	
5″	3.02, m	65.7	5″	3.68, m	65.7	5″	3.34, m	63.1
	3.68, m			3.02, m				
3-OCH_3	3.73, s	55.4	3,5-OCH_3	3.73, s	59.9			
4-OCH_3	3.74, s	55.5	4-OCH_3	3.63, s	55.8			

Tokyo, Japan) with YMC-Pack ODS-A columns (250 mm × 20 mm, 5 μm; 250 mm × 10 mm, 5 μm; YMC Corp., Kyoto, Japan). GC analysis was performed using on an Agilent 7890A instrument. HPLC-DAD analysis was conducted on an Agilent 1200 instrument with a YMC C_{18} column (250 mm × 4.6 mm, 5 μm).

Plant material

The root bark of the *L. chinense* was collected from Ningan Town, Zhongning County, Ningxia Hui autonomous region, People's Republic of China, in March 2012. A voucher specimen (ID-S-2592) was deposited in the Herbarium of the Institute of Materia Medica, Chinese Academy of Medical Science, Beijing.

Extraction and isolation

The powdered plant material (100 kg) was extracted three times with 80% EtOH (600 L) under reflux. The solvent was evaporated and the crude residue (8.0 kg) was partitioned with EtOAc (3 × 45 L). The EtOAc and H_2O solvent were removed under reduced pressure, which yielded Fr. 1 and Fr. 2, respectively. Fr. 2 (1.375 kg) was chromatographed with a macroporous resin column (HP-20, 200 × 15 cm) and eluted successively with H_2O, 15%, 30%, 50%, 70%, and 95% EtOH. The 30% EtOH fraction was concentrated and was further chromatographed over a macroporous resin (SP-700, 200 × 15 cm) and eluted successively with 15%, 20%, 25%, 30%, 45%, 50%, and 95% EtOH, which yielded fractions A–G.

Fr. C (72 g) was subjected to Combiflash RF200 apparatus with a C_{18} column (55 × 8 cm, 50 μm) and eluted with MeOH–H_2O (from 5 : 95 to 100 : 0) to provide Fr. C1–Fr. C25. Fr. C5 (3 g) was chromatographed over Sephadex LH-20 and eluted with gradient mixtures of MeOH–H_2O (from 10 : 90 to 95 : 5) to yield Fr. C5-1–C5-15. Fr. C5-4 was purified with pHPLC (MeOH–H_2O, 45 : 55) to yield **5** (20 mg). Fraction C5-11 was chromatographed over Sephadex LH-20 using MeOH–H_2O (from 0 : 100 to 60 : 40) as the gradient mobile phase system and was further purified with pHPLC to yield **3** (26 mg), **6** (16 mg), and **17** (35 mg). Fraction C7 (5 g) was subjected to the Rp-C_{18} (50 μm) and eluted with MeOH–H_2O (from 10 : 90 to 100 : 0) to yield Fr. C7-1–C7-8. **4** (17 mg) was obtained from Fr. C7-3 by pHPLC (MeOH–H_2O, 30 : 70). Fr. C7-5 was purified using pHPLC with MeOH–H_2O (40 : 60) as the mobile phase to yield **16** (12 mg) and **9** (24 mg). Fr. C10 (1.8 g) was subjected to the Rp-C_{18} (50 μm) and eluted with MeOH–H_2O (15 : 85) to give six fractions (Fr. C10-1–C10-6). Purification of Fr. C10-2 (1.8 g) with MeOH–H_2O (20 : 80) on pHPLC yielded **7** (32 mg) and **8** (19 mg).

Fraction G (4 g) was chromatographed over Sephadex LH-20 with a gradient of H_2O–MeOH (from 60 : 40 to 0 : 100) to yield Fr. G1–G11. Fr. G2 was purified by pHPLC (MeOH–H_2O, 50 : 50) to yield **13** (25 mg) and **2** (20 mg). **11** (33 mg) and **12** (27 mg) were obtained from Fr. G5 by pHPLC (MeOH–H_2O, 65 : 35) as the mobile phase. Fr. G7 was purified by pHPLC (MeOH–H_2O, 70 : 30) to yield **14** (29 mg) and **15** (22 mg). Fr. G9 and Fr. G10

were purified by pHPLC with MeOH–H_2O (70 : 30) to yield **1** (28 mg) and **10** (15 mg), respectively.

Structure characterization

Lyciumflavane A, 1. $[\alpha]_D^{20}$ −8.7 (*c* 0.10, MeOH); CD (MeOH) λ_{max} ($\Delta\varepsilon$): 219 (−1.03) and 276 (−0.08) nm; UV (MeOH) λ_{max} (log ε): 207 (4.24), 255 (2.95), and 285 (2.53) nm; IR (KBr) ν_{max}: 3364, 2926, 1598, 1509, 1460, 1378, 1261, 1155, 1114, 1041, 956, and 801 cm^{-1}; and (+)-HRESIMS: *m*/*z* 351.1586 $[M + H]^+$ (calcd for $C_{22}H_{23}O_4$, 351.1596). For the NMR data, see Tables 1 and 2.

Lyciumamide A, 2. UV (MeOH) λ_{max} (log ε): 202 (4.15), 237 (3.79), and 258 (4.05) nm; IR (KBr) ν_{max}: 3325, 2940, 1635, 1515, 1452 1241, 1122, 1087, 910, 827, and 560 cm^{-1}; and (+)-HRESIMS: *m*/*z* 531.2131 $[M + H]^+$ (calcd for $C_{30}H_{31}N_2O_7$, 531.2131). For the NMR data, see Tables 1 and 2.

Lyciumoside A, 3. $[\alpha]_D^{20}$ −5.31 (*c* 0.10, MeOH); CD (MeOH) λ_{max} ($\Delta\varepsilon$): 217 (−2.67), 251 (−0.77), and 276 (−0.31) nm; UV (MeOH) λ_{max} (log ε): 207 (4.25), 215 (4.28), 256 (4.26), and 297 (3.49) nR (KBr) ν_{max}: 3379, 2930, 1712, 1601, 1417, 1274, 1074, 1026, 883, and 764 cm^{-1}; and (+)-HRESIMS: *m*/*z* 779.2723 $[M + Na]^+$ (calcd for $C_{35}H_{48}O_{18}Na$, 779.2738). For the NMR data, see Tables 1 and 2.

Lyciumlignan A, 4. $[\alpha]_D^{20}$ −45.5 (*c* 0.10 MeOH); CD (MeOH) λ_{max} ($\Delta\varepsilon$): 231 (−1.10), 255 (+0.84), and 284 (+1.99); UV (MeOH) λ_{max} (log ε): 206 (4.57), 268 (3.89), and 301 (3.49); IR (KBr) ν_{max}: 3371, 2936, 2881, 1698, 1604, 1513, 1268, 1034, 823, and 621 cm^{-1}; and (+)-HRESIMS: *m*/*z* 693.2377 $[M + Na]^+$ (calcd for $C_{31}H_{42}O_{16}Na$, 693.2371). For the NMR data, see Tables 1 and 2.

Lyciumlignan B, 5. $[\alpha]_D^{20}$ −66.7 (*c* 0.10 MeOH); CD (MeOH) λ_{max} ($\Delta\varepsilon$): 231 (−1.82), 253 (+0.79), and 285 (+2.02); UV (MeOH) λ_{max} (log ε): 205 (4.54), 268 (3.95), and 304 (3.32); IR (KBr) ν_{max}: 3368, 2974, 2931, 2885, 1513, 1419, 1268, 1049, 825, and 617 cm^{-1}; and (+)-HRESIMS: *m*/*z* 987.3316 $[M + Na]^+$ (calcd for $C_{42}H_{60}O_{25}Na$, 987.3321). For the NMR data, see Tables 1 and 2.

Lyciumlignan C, 6. $[\alpha]_D^{20}$ +16.93 (*c* 0.07 MeOH); CD (MeOH) λ_{max} ($\Delta\varepsilon$): 230 (+0.80); UV (MeOH) λ_{max} (log ε): 205 (4.35), 271 (3.71), and 301 (3.61); IR (KBr) ν_{max}: 3384, 2930, 1565, 1513, 1420, 1267, 1078, 1031, 670, and 618 cm^{-1}; and (+)-HRESIMS: *m*/*z* 723.2485 $[M + Na]^+$ (calcd for $C_{32}H_{44}O_{17}Na$, 723.2476). For the NMR data, see Tables 1 and 2.

3′,4′-Dimethoxy-benzylalcohol-1-*O*-β-D-xylopyranosyl-(1→6)-*O*-β-D-glucopyranoside, 7. $[\alpha]_D^{20}$ −35.9 (*c* 0.10 MeOH); UV (MeOH) λ_{max} (log ε): 203 (4.09), 221 (3.61), and 279 (2.93) nm; IR (KBr) ν_{max}: 3612, 3429, 2932, 2835, 1514, 1449, 1368, 1258, 1050, and 668 cm^{-1}; and (+)-HRESIMS: *m*/*z* 485.1633 $[M + Na]^+$ (calcd for $C_{20}H_{30}O_{12}Na$, 485.1635). For the NMR data, see Table 3.

3′,4′,5′-Trimethoxy-benzylalcohol-1-*O*-β-D-xylopyranosyl-(1→6)-*O*-β-D-glucopyranoside, 8. $[\alpha]_D^{20}$ −15.9 (*c* 0.10 MeOH); UV (MeOH) λ_{max} (log ε): 207 (4.19), 233 (3.55), and 269 (3.17) nm; IR (KBr) ν_{max}: 3368, 2935, 1594, 1461, 1423, 1334, 1239, 1126, 1044, and 832 cm^{-1}; and (+)-HRESIMS: *m*/*z* 515.1741 $[M + Na]^+$ (calcd for $C_{21}H_{32}O_{13}Na$, 515.1741). For the NMR data, see Table 3.

4′-Hydroxystyrone-1-*O*-β-D-apiofuranosyl-(1→6)-*O*-β-D-glucopyranoside, 9. $[\alpha]_D^{20}$ −10.4 (*c* 0.10 MeOH); UV λ_{max} (MeOH) nm: 206 (4.25), 263 (4.05), and 297 (3.46); (+)-HRESIMS: *m*/*z* 467.1531 $[M + Na]^+$ (calcd for $C_{20}H_{28}O_{11}Na$, 467.1529); and IR (KBr) ν_{max}: 3360, 2928, 1609, 1514, 1446, 1269, 1050, 853, 767, and 621 cm^{-1}. For the NMR data, see Table 3.

Determination of the absolute configuration of sugar

Compound **4** (8 mg) was dissolved in 0.5 M HCl (8 mL) and refluxed for 4 h. The reaction solution was then extracted with EtOAc (3 × 10 mL). The EtOAc layer was concentrated to create a residue that was further purified using pHPLC with MeOH–H_2O (45 : 55) to yield **4a** (3.5 mg). The aqueous layer was evaporated under a vacuum to create a monosaccharide residue. The residue was mixed with L-cysteine methyl ester hydrochloride (2 mg), and was dissolved in fresh anhydrous pyridine (2.0 mL). The reaction mixture was maintained at 60 °C for 2 h. Then, the dried *N*-trimethylsilylimidazole (0.2 mL) was added to the mixture. The solution was incubated at 60 °C for 2 h and partitioned between *n*-hexane and H_2O. The *n*-hexane layer was subjected to GC analysis under the following conditions: capillary column, HP-5 (30 m × 0.32 mm, with a 0.25 μm film, Dikma); detector temperature, 300 °C; injection temperature, 300 °C; initial temperature, 200 °C that was raised to 280 °C at a rate of 10 °C min^{-1}; the final temperature was maintained for 30 min; detection, FID; and carrier gas, N_2 gas. D-glucose and D-apiose were confirmed by comparing the retention time of their derivatives to the standard sugar derivatized in a similar manner, which exhibited retention times of 20.5 and 14.5 min, respectively. The hydrolysis procedures for **5–9** were similar to that of **4**. The D-xylose was confirmed by the retention time (14.9 min) of its derivative.

Inhibitory activity of α-glucosidase

The inhibitory activity of compounds **1–17** on α-glucosidase was determined by the same method as described in the literature.[13,14]

Conclusions

In our ongoing effort to discover new bioactive natural products with hypoglycemic effects from the root bark of *L. chinense*, 17 compounds were isolated, including one new flavane with an unusual benzofuran unit, one new amide possessing a naphthalene skeleton, one new sesquiterpene, three new lignan glucosides, three new phenolic glucosides, and eight known compounds. A literature survey revealed that this is the first report of a flavane with an unusual benzofuran unit that showed strong inhibitory activity against α-glucosidase (IC_{50} = 20.89 μM).

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