A novel bisbenzylisoquinoline alkaloid from *Lindera aggregata*

Lishe Gan[a], Xia Zhao[a], Wei Yao[b], Limao Wu[a], Lianda Li[a] and Changxin Zhou[a]*

[a]*Institute of Modern Chinese Medicine (IMCM), College of Pharmaceutical Sciences, Zhejiang University, Hangzhou 310058, P. R. China*

[b]*Zhejiang Chem-Tech Group Co., Ltd., 387 Tianmushan Rd., Hangzhou, 310023 P. R. China*

A novel bisbenzylisoquinoline alkaloid, linderegatine (**1**), as well as two known isoquinoline alkaloids (+)-reticuline (**2**) and pallidine (**3**) were isolated from the roots of the Chinese medicinal plant *Lindera aggregata*. Their structures were determined on the basis of extensive spectroscopic studies.

Keywords: bisbenzylisoquinoline alkaloid, *Lindera aggregata*

Lindera aggregata (Sims.) Kosterm. [*L. strychnifolia* (Sieb. et Zucc.) F. Vill.] (Lauraceae) is an evergreen shrub which is widely distributed in Southern China, Japan and Southeast Asia.[1] The dry roots of *L. aggregata* are a well-known traditional herbal medicine in China and Japan, and they have been used for the treatment of cardiac, renal, cystic and rheumatic diseases for hundreds of years.[2] Previous phytochemical investigations have demonstrated that isoquinoline alkaloids,[3-5] sesquiterpene lactones,[6] flavonoids,[7] and tannins[8] are the four main active components of *L. aggregata*. The alkaloids have shown potential therapeutic effects on rheumatoid arthritis.[9] In the present study, a novel bisbenzylisoquinoline alkaloid, linderegatine (**1**), together with the two known isoquinoline alkaloids, (+)-reticuline (**2**) and pallidine (**3**), were isolated from the ethanolic extract of the roots of *L. aggregata*.

Linderegatine (**1**) was obtained as a yellow amorphous solid. Its molecular formula, $C_{35}H_{34}N_2O_7$, was established on the basis of a positive HRESIMS pseudo-molecular ion peak at *m/z* 595.2431 $[M+H]^+$ (calcd for $C_{35}H_{35}N_2O_7^+$, 595.2439). This suggested a bisbenzylisoquinoline alkaloid with 20 degrees of unsaturation. The UV spectrum of **1** showed absorption bands for a large conjugated systems (λ_{max} 288, log ε = 4.87) typical of benzylisoquinoline alkaloids.[10] The IR spectrum showed vibrations for benzene ring (1588, 1510, 1450 cm^{-1}) and conjugated carbonyl (1664 cm^{-1}) groups. The ^1H NMR spectrum of **1** displayed, in addition to three aromatic methoxyl groups at δ 3.90 (3H, s), 3.78 (3H, s), 3.74 (3H, s), 11 phenyl protons which resonated at δ 6.48–7.91 and one CH–N proton at δ 4.45 (1H, t, *J* = 6.1, 8.0). This confirmed a bisbenzylisoquinoline skeleton. The above deduction was firmly reinforced by the ^{13}C NMR and DEPT spectra, in which a total of 35 carbon signals could be discerned, including 15 quarternary carbons, 12 methines, five methylenes and three methyls. Moreover, signals at δ 194.4 confirmed the existence of a conjugated carbonyl group and two higher abundance signals at δ 133.6, 117.0 indicated a 1,4-substituted benzene ring. The structure of **1** was fully established by analysis of its 2D NMR data.

The HSQC spectrum firstly revealed the attachment and assignment of all the protons to their associated carbons. The connectivity of each proton/carbon unit was then ascertained by the HMBC experiment. In the HMBC spectrum (Fig. 2), correlations between H_2-10,14/C-α, H_2-10,14/C-12, H_2-10,14/C-14,10, H_2-11,13/C-9, H_2-11,13/C-12 showed the existence of the 4-hydroxybenzoyl unit in monomer A; correlations between H-3a,3b/C-1, H-3a,3b/C-4a, H_2-4/C-5, H_2-4/C-8a, H-5/C-4, H-5/C-8a, H-5/C-7, H-8/C-1, H-8/C-6 and H-8/C-4a revealed the dihydro-isoquinoline backbone of monomer A. The backbone of the tetrahydroisoquinoline unit in monomer B were elucidated by HMBC correlations between H-1'/C-8', H-3'a, 3'b/C-1', H-3'a, 3'b/C-4a', H_2-4'/C-8a', H-5'/C-4', H-5'/C-7', H-5'/C-8a', H-8'/C-6', H-8'/C-1', H-8'/C-4a'. The presence and the linkage of the 3-hydroxy-4-methoxybenzyl unit in monomer B were deduced from correlations of H-10'/C-α', H-10'/C-12', H-10'/C-14', H-14'/C-α', H-14'/C-10', H-13'/C-9', H-1'/C-9'. The three OMe groups were unambiguously assigned to C-6, C-6' and C-12' by the corresponding HMBC correlations and confirmed by NOESY correlations of 6-OCH3/H-5, 6'-OCH3/H-5', and 12'-OCH3/H-13'. Analysis of the ^{13}C NMR chemical shifts of similar structures[11,12] allowed the assignment of the linkage of the two monomer parts by an ether bond C-12–O–C-11', which was further confirmed by recording the ^{13}C NMR spectrum of **1** in CD_3OD/CH_3OH (1 : 1). The resulted spectrum showed splitted peaks for carbon signals of C-7 and C-7', which indicated they were hydroxylated. Finally, the negative specific optical rotation for **1** (–16°) indicated an *R* configuration on C-1'.[13] On the basis of the above deduction, the structure of linderegatine (**1**) was finally elucidated as shown in Fig. 1.

Linderegatine (**1**) is the first bisbenzylisoquinoline alkaloid to be isolated from *L. aggregata* and the first 1, α-didehydro-bisbenzylisoquinoline alkaloides from the genus *Lindera*. The structure of the alkaloids **2** and **3** were identified as (+)-reticuline[13] and pallidine,[14] respectively, by comparison of their NMR and specific rotation data with those reported in the literature.

Experimental

Optical rotations were measured on a JASCO p-1030 polarimeter. IR spectra were recorded on a Bruker VECTOR 22 FT/IR-4100 spectrometer. UV spectra were recorded on a Hitachi U-4100

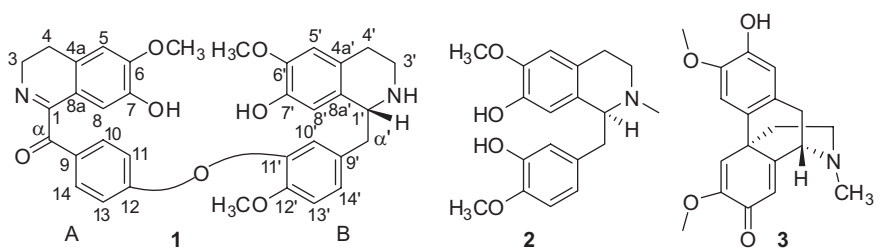

Fig. 1 Structure of alkaloids **1**–**3**.

* Correspondent. E-mail: zhoucx10@zju.edu.cn

Table 1 ^{1}H (400 MHz) and ^{13}C(100 MHz) NMR data of **1** in CD_3OD

No.	δ_H (multi, *J* in Hz)	δ_C	No.	δ_H (multi, *J* in Hz)	δ_C
1		167.8	1'	4.45 (t, 6.1, 8.0)	57.3
3	3.79 (2H, m)	47.8	3'	a 3.37 (1H, m)	
				b 3.15 (1H, m)	40.6
4	2.83 (2H, m)	26.0	4'	2.89 (2H, m)	26.9
4a		131.4	4'a		124.6
5	6.90 (1H, s)	111.9	5'	6.68 (1H, s)	112.6
6		152.6	6'		148.8
7		146.4	7'		146.2
8	6.67 (1H, s)	114.4	8'	6.48 (1H, s)	114.4
8a		120.3	8a'		126.4
α		194.4	α'	a 3.25 (1H, dd, 14.0, 6.1)	
				b 3.03 (1H, dd, 14.0, 8.0)	40.7
9		130.3	9'		130.8
10, 14	7.91 (2H, d, 8.8)	133.6	10'	6.93 (1H, s)	124.8
11, 13	6.91 (2H, d, overlap)	117.0	11'		144.0
12		165.0	12'		152.3
6-OCH_3	3.90 (3H, s)	56.5	13'	7.13 (1H, d, 8.4)	114.6
			14'	7.20 (1H, d, 8.3)	128.7
			6'-OCH_3	3.78 (3H, s)	56.3
			12'-OCH_3	3.74 (3H, s)	56.3

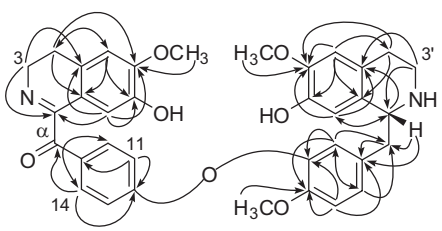

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Fig. 1 Structure of alkaloids **1**–**3**.

spectrometer. NMR spectra were measured on Varian Inova-400 spectrometer with TMS as internal standard. ESIMS were carried out on a Finnigan LC Q^{DECA} instrument, respectively. All solvents were of analytical grade (Shanghai Chemical Plant, Shanghai, People's Republic of China). Silica gel (200–300 mesh) was used for column chromatography and precoated silica gel GF254 plates (Qingdao Haiyang Chemical Co. Ltd., Qingdao, P.R. China) were used for TLC. C_{18} reverse-phased silica gel (150–200 mesh, Merck), MCI gel (CHP20P, 75–150 μm, Mitsubishi Chemical Industries Ltd.) and Sephadex LH-20 gel (Amersham Biosciences) were used for column chromatography.

Plant material

The dried root material was obtained from the Factory of Traditional Chinese Medicine Sliced Tablets, Hangzhou, Zhejiang province, P. R. China. A sample has been deposited in Institute of Modern Chinese Medicine, Zhejiang University. (accession number LA-2007-I).

Extraction and purification

The air-dried and ground root material (5.0 kg) of *L. aggregata* was percolated with 95% EtOH at room temperature. Removal of the solvent under reduced pressure gave 370 g of crude extract, which was dissolved in 1l of H_2O to form a suspension and the pH was adjusted to 3 with 2 M HCl. The acidic suspension was first partitioned with EtOAc (1l × 3) to remove the neutral components. The aqueous phase was then basified with 5% Na_2CO_3 to pH = 10 and extracted with EtOAc (1l × 3) to obtain 32 g of crude alkaloids. The crude alkaloids were subjected to a MCI gel column (H_2O–EtOH, 1 : 0-0 : 1) to give six major fractions 1–6. Fraction 1 was separated by a silica gel column ($CHCl_3$–CH_3OH, 20:1), followed by a RP-18 Column (30% aqueous EtOH) and a Sephadex LH-20 gel column to give compound **3** (38 mg). Fraction 5 was further purified by silica gel column ($CHCl_3$–CH_3OH, 25 : 1) and a subsequent silica gel CC (EtOAc–CH_3OH–Et_2NH, 20 : 1 : 0.1) to give compound **2** (68 mg). Fraction 6 was further chromatographed by repeated silica gel column eluted with $CHCl_3$–CH_3OH, 25 : 1 and EtOAc–CH_3OH–Et_2NH, 15 : 1 : 0.1 to give linderegatine **1** (18 mg)

Linderegatine (**1**): Yellow amorphous solid. $[\alpha]_D^{20}$ –16°(*c* 1.0, MeOH); UV (MeOH) λ_{max} nm (log ε): 207 (5.30), 288 (4.87); IR (film) ν_{max} cm^{-1} 3405, 2932, 1712, 1593, 1510, 1276, 1020, 780; ^{1}H NMR, ^{13}C NMR, Table 1; ESIMS (positive) *m/z* 595 $[M+H]^+$ (100); (negative) *m/z* 593 (100); HRESIMS (positive) *m/z* 595.2431 (calcd for $C_{35}H_{35}N_2O_7^+$, 595.2439).

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