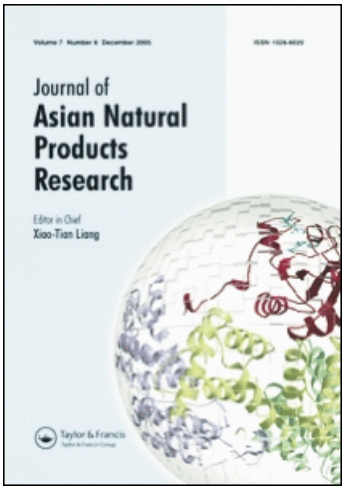

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Novel dimeric alkaloids from the roots of *Thalictrum atriplex*

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Two new bisbenzylisoquinoline alkaloids, neothalfine (**1**) and thaliatrine (**2**), together with three known dimeric alkaloids, thalifaberine, thalistine, and thalirecebine, have been isolated from the roots of *Thalictrum atriplex* Finet et Gagnep. Their structures have been established by spectroscopy. Compound **1** showed *in vitro* antiplatelet aggregation activities.

Keywords: *Thalictrum atriplex*; Bisbenzylisoquinoline alkaloids; Neothalfine; Thaliatrine

1. Introduction

Previous studies on the chemical constituents have indicated that plants of the genus *Thalictrum* (Ranunculaceae) are a rich resource of dimeric alkaloids, most of which possess many kinds of biological activities [1,2]. *Thalictrum atriplex* Finet et Gagnep. has been used as a Chinese and Tibetan folk medicine, distributed mainly in the southwest of China. Its roots have been used to treat infectious hepatitis, carbuncles, dysenteric diarrhea, and certain gastroenteric disorders [3]. No dimeric alkaloids from this species, or their biological activity, have been reported in the literature. In our research on this species, two new dimeric alkaloids, neothalfine (**1**) and thaliatrine (**2**) (figure 1), together with three known dimeric alkaloids have been isolated from the roots. Compound **1** contains two ether linkages connecting a benzylisoquinoline unit with a dehydroxyisoquinoline, a rare class of dimeric alkaloids. Compound **2** is a bisbenzylisoquinoline dimer, linked through one diphenyl ether. A biological assay indicates that **1** has potential antiplatelet aggregation activity.

2. Results and discussion

Neothalfine (**1**) was obtained as a white amorphous solid. The HR-FABMS of **1** shows the molecular ion peak at *m/z* 648, corresponding to the formula $C_{38}H_{36}O_8N_2$. The UV spectrum of **1** shows absorption maxima at 240, 256, 346 nm, indicating the presence

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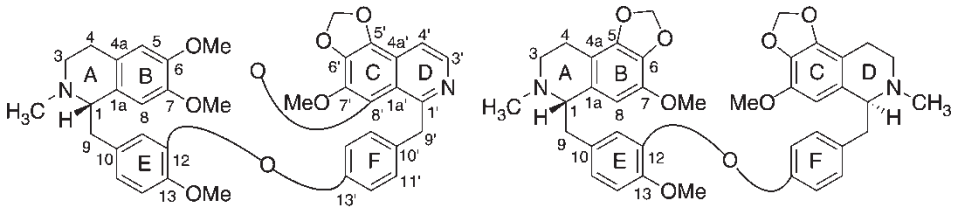

1 2

Figure 1. Structures of compounds **1** and **2**.

of a benzylisoquinoline moiety. From EIMS data, the base peak at *m*/*z* 648, corresponding to the molecular ion, indicates that **1** is a dimeric alkaloid with two diphenyl ether groups [4]. The ^{1}H NMR spectrum of **1** (table 1) reveals one *N*-methyl, four *O*-methyl, one methylenedioxyl, and ten aromatic protons. In the aliphatic region, from δ 2.38 to 3.12 (H-3, 4), only four protons are observed. The lack of four additional aliphatic protons of benzylisoquinoline reveals that **1** has a dehydro-benzylisoquinoline component. This hypothesis is supported by the two aromatic doublets of H-4′ (δ 7.45, $J = 5.8$ Hz) and H-3′ (δ 8.45, $J = 5.8$ Hz) and the low-field signals of H-9′ (δ 4.57, 4.79). The ^{13}C NMR spectrum exhibits five carbon signals in the aliphatic region, four *O*-methyls, one methylenedioxy, and one *N*-methyl, as well as 27 aromatic carbons, of which ten signals at δ 136.4–158.0 have been assigned to aromatic carbons linked oxygen. Signals at δ 158.0 and 140.6 are ascribed to C-1′ and C-3′ according to the HMQC correlation experiment. The ^{1}H NMR spectrum reveals an E ring that is a typical 1,2,4-substituted benzene ring, and signals at δ 5.99, 6.78, 6.76 are ascribed to H-11, H-14, and H-15, respectively, and also an F ring that is 1,4-substituted, with δ 6.67, 6.73 ascribed to H-12′,14′ and H-11′,15′. Therefore, **1** should possess a C-12-C-13′ linkage of a diphenyl ether; C-13 is substituted by an *O*-methyl (δ 3.87) since irradiation of δ 3.87 shows a NOE enhancement to H-14.

An extensive NOE experiment indicated that C-6 and C-7 are substituted by *O*-methyl at δ 3.49, and 3.74, and the oxygen-bridge is at C-5, since irradiation of 7-OMe (δ 3.74) shows NOE enhancements to 6-OMe (δ 3.49), and H-8 (δ 6.45), while irradiation of H-1 (δ 3.57) shows NOE enhancements to H-8, not to 6,7-OMe. Irradiation of H-4′ (δ 7.45) did not enhance the *O*-methyl (δ 3.56), revealing that *O*-methyl (δ 3.56) is at either C-7′ or C-8′, while the methylenedioxy is at C-5′,C-6′. Irradiation of H-9′ (δ 4.57, 4.79) gave no NOE enhancement to OCH_3 (δ 3.56), which enables the assignment of 7′-OCH_3 and the 8′-linkage of the oxygen-bridge (figure 2). All these assignments are supported by comparison with those of thalfine [5]. The HMBC experiment (table 1) allowed assignments of all the carbons.

The absolute stereochemistry of **1** was established as *S* for C-1 by comparison of its CD spectrum with that of thalfine [5], which has an *S* configuration of C-1.

Thaliatrine (**2**) was obtained as a white amorphous solid. The HR-FABMS of **2** showed the molecular ion at *m*/*z* 666 corresponding to the formula $C_{39}H_{42}O_8N_2$. The very weak molecular ion peak on the EIMS revealed **2** was a dimeric alkaloid with one diphenyl ether group [4]. The absence of any other strong peaks around the base peak *m*/*z* 220 suggests that the two tetrahydroisoquinoline moieties have the same substitutions [4]. Thus, rings B and C are both substituted by a methoxy and a methylenedioxy. The many overlapped and duplicate signals on the ^{1}H and ^{13}C NMR spectrum also indicate that the two tetrahydroisoquinoline moieties possess same structure. By comparison of ^{1}H NMR spectrum of **1**, compound **2** has

Table 1. ^{1}H and ^{13}C NMR data for compounds **1** and **2**[a].

	1			***2***		
Position	δ_C	δ_H	*HMBC*	δ_C	δ_H	*HMBC*
1	63.4	3.57 m	C-4a,C-8,C-10	65.0	4.12 m	C-4a,C-8,C-10
1a	134.8			132.0		
3	51.3	2.38–3.12 m		45.0	2.95–3.20 m	
4	23.0	2.50 m		18.0	2.80 m	
4a	119.8			126.0		
5	148.7			146.0		
6	136.4			134.8		
7	151.6			142.0		
8	105.4	6.46 s	C-1,C-4a,C-6,C-7	107.0	5.70 s	C-1,C-4a,C-6,C-7
9	36.4	3.18–3.29 m	C-1a,C-1,C-11,C-15	40.0	3.05 m	C-1a,C-1,C-11,C-15
10	132.6			132.5		
11	118.9	5.99 d (2.5)	C-9,C-13,C-15	112.8	6.63 d (2.5)	C-9,C-13,C-15
12	148.7			145.0		
13	148.8			150.0		
14	110.8	6.78 d (8.2)	C-10,C-12,C-13	123.0	6.90 d (8.0)	C-10,C-12,C-13
15	123.5	6.76 dd (8.2, 2.5)	C-9,C-11,C-13	126.0	6.84 dd (8.0, 2.5)	C-9,C-11,C-13
1′	158.0			65.0	4.12 m	C-4a′,C-8′,C-10′
1a′	118.4			134.0		
3′	140.6	8.45 d (5.8)	C-4′,C-4a′	45.0	2.95–3.20 m	
4′	111.8	7.45 d (5.8)	C-3′,C-1a′,C-5′	18.0	2.80 m	
4a′	118.3			123.0		
5′	137.9			146.0		
6′	142.3			134.8		
7′	139.7			142.0		
8′	137.9			106.9	5.64 s	C-1′,C-4a′,C-6′,C-7′
9′	46.7	4.57 d (14.3) 4.79 d (14.3)	C-1a′,C-1′,C-11′	40.0	2.72 m	C-1a′,C-1′,C-11′
10′	137.1			134.5		
11′	128.9	6.73 dd (8.7, 2.5)	C-9′,C-12′,C-13′	130.9	6.98 d (8.0)	C-9′,C-12′,C-13′
12′	120.8	6.67 dd (8.7, 2.5)	C-11′,C-13′,C-10′	116.9	6.76 d (8.0)	C-11′,C-13′,C-10′
13′	154.6			157.0		
14′	120.8	6.67 dd (8.7, 2.5)		116.9	6.76 d (8.0)	
15′	128.9	6.73 dd (8.7, 2.5)		130.9	6.98 d (8.0)	
MeN-1	43.8	2.28 s	C-1,C-3	42.0	2.60 s	C-1,C-3
MeN-1′				42.0	2.60 s	
MeO-6	59.9	3.49 s	C-6			
MeO-7	56.2	3.74 s	C-7	56.2	3.61 s	C-7
MeO-7′	60.4	3.57s	C-7′	56.2	3.58 s	C-7′
MeO-13	56.2	3.87 s	C-13	57.1	3.78 s	C-13
—OCH_2O -5, 6				101.6	5.93 s	C-5,C-6
—OCH_2O -5′, 6′	102.6	6.15 s	C-5′,C-6′	101.6	5.93 s	C-5′,C-6′

[a] δ (ppm); ^{1}H (500 MHz) and ^{13}C (125 MHz); $CDCl_3$; *J* (Hz) in parentheses.

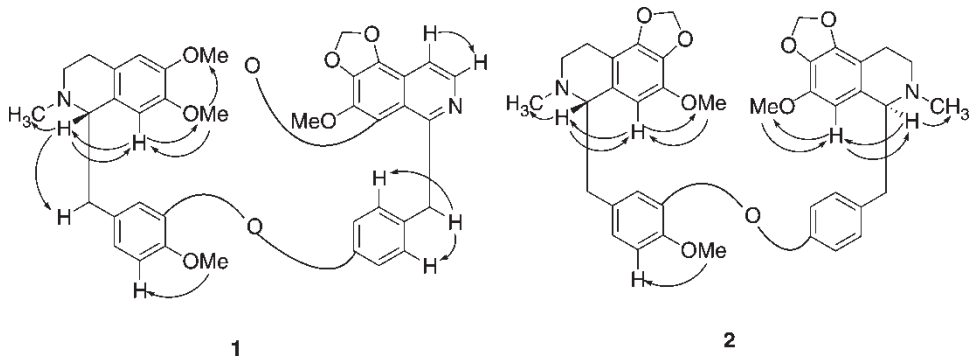

Figure 2. Key NOE correlations for **1** and **2**.

the same substituent status as **1** in rings E and F. Furthermore, the ^{1}H and ^{13}C NMR spectra of **2** (table 1) exhibit two *N*-methyl groups, three methoxy groups, and two methylenedioxyls. In the NOE experiment, irradiation of δ 5.70 created NOE enhancements to an aliphatic proton (H-1) and a methoxy group (δ 3.58), indicating that δ 5.70 is to be assigned to H-8 and *O*-methyl (δ 3.58) is at C-7 while the methylenedioxy is at C-5, C-6. The placement of *O*-methyl (δ 3.61) and another methylenedioxy on ring C were also confirmed through an NOE experiment. Irradiation of OCH_3 (δ 3.78) resulted in NOE enhancement to H-14 (δ 6.90, $J = 8.0$ Hz), revealing that OCH_3 (δ 3.78) is on C-13. All of the carbon signals were assigned according to 2D CH-correlation NMR and HMBC experiments.

The absolute configuration of **2** was established from its CD spectrum by comparison with the CD spectrum of thalastine; the two compounds had similar CD spectra, thus **2** should have the *S* configuration at C-1, as with thalistine [6].

All compounds were tested for activity against platelet aggregation induced by adenosine diphosphate (ADP) and collagen (Coll) *in vitro*. Compound **1** exhibited potential inhibition of platelet aggregation.

3. Experimental

3.1 General experimental procedures

Melting points were detected on a Fisher-Johns hot-stage apparatus and are uncorrected. Optical rotation was obtained in $CHCl_3$ at 20°C, using a Perkin–Elmer 341 polarimeter. CDs were taken on a JASCO polarimeter. UV spectra were recorded on a Philips Pye Unicam PU8800 spectrophotometer, while IR spectra were measured on a Perkin–Elmer 983G spectrophotometer. ^{1}H and ^{13}C NMR, ^{1}H—^{1}H COSY, HMQC, HMBC, and NOESY spectra were recorded on a Bruker AM-500 spectrometer, using $CDCl_3$ as solvent and TMS as internal standard. MS measurements were carried out on a ZABSPEC spectrometer. Column chromatography was carried out on silica gel (120–230 mesh, Merck) Preparative TLC was accomplished on silica gel GF_{254} (Merck). For column chromatography, Sephadex LH-20 (Pharmacia) was also employed. Eluates were detected on TLC using Dragendorff's reagent.

3.2 Plant material

The roots of *Thalictrum atriplex* Finet et Gagnep. were collected in August 1996 at Ma-Er-Kang County, Si-Chuan Province, People's Republic of China, and identified by Professor Wen-Cai Wang, Institute of Botany, Chinese Academy of Sciences. A voucher specimen (no. 960814) has been deposited in the herbarium of the Institute of Medicinal Plant Development, Peking Union Medical College & Chinese Academy of Medical Sciences.

3.3 Extraction and isolation

The air-dried roots of *T. atriplex* (4.0 kg) were exhaustively extracted with 95% EtOH. The EtOH was then removed under reduced pressure, and the resultant residue (900 g) was dissolved in water. After filtration, the solution was then extracted several times with light petroleum. The aqueous layer was then extracted with $CHCl_3$, EtOAc and n-BuOH,

successively. The $CHCl_3$ residue (53 g) was fractionated over a silica-gel column, gradiently eluting with $CHCl_3$–MeOH, to give 67 fractions (each 500 ml). Fractions 28–36, 46–57, 58–67 were then combined to give fractions A, B, C, respectively, after monitoring by TLC. Fractions A, B and C were subsequently further separated by column chromatography over silica gel, eluting EtOAc-MeOH (9:1). Fractions 9–13 of A then gave compound **1** (530 mg), fractions 36–41 of B were purified with Sepadex-LH_{-20} to yield thalifaberine [7] (50 mg). Fractions 21–32 of B and 14–20 and 23–26 of C were subjected to preparative TLC, using the solvent system light petroleum (60–90°C)-CH_3COCH_3–MeOH–NH_4OH (6:4:0.1:1), to yield pure compound **2** (170 mg), thalistine [6,8] (150 mg), and thaliracebine [5] (100 mg), respectively.

Neothalfine (1). A white amorphous powder (CH_3OCH_3), mp 132–135°C; $[\alpha]_D$ + 36.4 (*c* 0.5, $CHCl_3$); CD (*c*8.25×10^{-4} M, MeOH): $\Delta\varepsilon$ (λ nm) −5.1346 (289), + 9.2962 (264), + 10.3812 (235), + 2.1634 (221), + 20.5107 (210); UV λ_{max} (MeOH) (nm): 210, 240 (sh), 256, 340; IR (KBr) ν_{max} (cm^{-1}): 2960, 1610, 1520, 1460, 1420, 1350, 1280, 1240, 1180, 1140, 1120, 1090, 1065, 1045, 980; ^1H and ^{13}C NMR, see table 1; EIMS *m/z* $[M]^+$ 648 (100), 633 (97), 422 (10), 324 (99), 220 (60), 205 (80), 190 (70), 174 (35); HR-FABMS *m/z* 648.2479 (calcd. for $C_{38}H_{36}O_8N_2$ 648.2471).

Thaliatrine (2). A white amorphous powder (CH_3OCH_3), mp 89–90°C; $[\alpha]_D$ + 42.2 (*c* 0.9, $CHCl_3$); CD (*c*8.25×10^{-4} M, MeOH): $\Delta\varepsilon$ (λ nm) −0.6783 (299), + 0.5723 (286), + 14.14 (227); UV λ_{max} (MeOH) (nm): 210, 274; IR (KBr) ν_{max} (cm^{-1}): 2960, 1650, 1610, 1516, 1460, 1380, 1280, 1230, 1140, 1065, 960; ^1H and ^{13}C NMR, see table 1; EIMS *m/z* $[M]^+$ 666 (5), 445 (75), 430 (95), 220 (100), 205 (10), 190 (15), 174 (8), 147 (12); HR-FABMS *m/z* 666.3048 (calcd. for $C_{39}H_{42}O_8N_2$ 666.3041).

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