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RESEARCH ARTICLE

A new bisbenzylisoquinoline alkaloid from the roots of *Stephania tetrandra*

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Abstract

A new bisbenzylisoquinoline alkaloid, (1*S*,1′*S*)-13-hydroxy-6,6′,7-trimethoxy-2,2′-dimethylberbaman (**1**) along with four known alkaloids, tetrandrine (**2**), fangchinoline (**3**), tetrahydropalmatine (**4**) and dehydrocrebanine (**5**) were isolated from the roots of *Stephania tetrandra*. Their chemical structure was determined by HR-ESI-MS and 1D-, 2D NMR spectra and comparison with published results. The absolute configuration of compound **1** was determined by the electronic circular dichroism (ECD) spectroscopy technique.

KEYWORDS

(1S,1′S)-13-hydroxy-6, 7-trimethoxy-2, 2-dimethylberbaman, alkaloid, Menispermaceae, *Stephania tetrandra*

1 | INTRODUCTION

Stephania tetrandra S. Moore (Menispermaceae) is a medical plant, distributed widely in tropical and subtropical regions of Asia, Africa and also found in Northern Vietnam. The roots of this species has been used in traditional medicine for the treatment of stomachache, arthritis, fever, oedema, asthma, cancer, dysentery, hyperglycaemia, hypertension, malaria and tuberculosis.[1,2] Previous investigations on this plant revealed that alkaloids are the main constituents, in which bisbenzylisoquinolines are the most common.[3–11] Of these compounds, tetrandrine is an active bisbenzylisoquinoline possessing a broad spectrum of pharmacological properties as hypotensive effects, anticancer, antidiabetic, anti-inflammatory, anti-microbial, antinociceptive, anti-fibrotic, anti-depressant, antirheumatoid arthritis, anti-adipogenic, neuroprotective and memory-improving activities.[12,13] This paper reported the isolation and structural elucidation of a new bisbenzylisoquinoline alkaloid, (1*S*,1′*S*)-13-hydroxy-6,6′,7-trimethoxy-2,2′-dimethylberbaman (**1**) along with four known alkaloids, tetrandrine (**2**), fangchinoline (**3**), tetrahydropalmatine (**4**) and dehydrocrebanine (**5**) (Figure 1) from the roots of *Stephania tetrandra* collected in Vietnam.

2 | MATERIALS AND METHODS

2.1 | Plant materials

The roots of *S. tetrandra* was collected in Da Bac district, Hoa Binh province, Vietnam in July 2023 and identified by Dr. Do Van Hai, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology (VAST). A voucher specimen has been preserved at the Laboratory of Drug Research and Discovery, Institute of Chemistry, VAST, Hanoi, Vietnam.

2.2 | Experimental procedures

Optical rotation was measured with a Jasco P2000 polarimeter. HR-ESI-MS was acquired on a Sciex X500R QTOF mass spectrometer. 1D and 2D NMR spectra were

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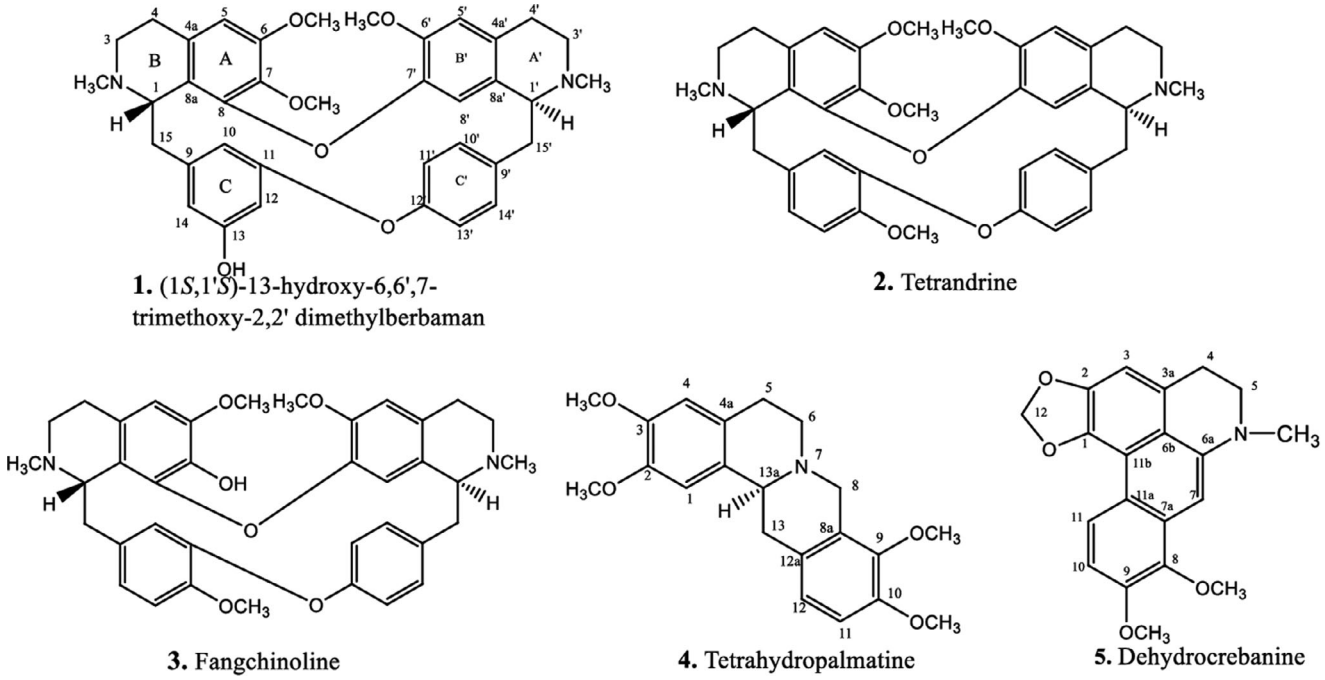

FIGURE 1 Chemical structures of **1–5**.

recorded on a Bruker Avance III HD 600 MHz spectrometer. The electronic circular dichroism spectrum was recorded on a Chirascan Circular Dichroism Spectrometer. Column chromatography was performed with silica gel 60 and Sephadex LH-20. Thin-layer chromatography was carried out on pre-coated silica gel 60 F_{254} plates. The spots were detected under UV light (254 and 365 nm) and by spraying with a solution of vanillin/sulfuric acid and heating with a heat gun.

2.3 | Extraction and isolation

The dried and powdered roots of *S. tetrandra* (1.2 kg) were extracted with 95% methanol three times at room temperature. The extract was concentrated under reduced pressure and then added into a 1% H_2SO_4 solution. The acidic solution was filtered, basified with 5% NaOH solution to pH = 9–10 and extracted with CH_2Cl_2 to give 25 g crude alkaloid mixture. The mixture was chromatographed on a silica gel column and eluted with CH_2Cl_2-MeOH (4:0.1 to 3:1, v/v) to yield six fractions (1-6). Fraction 1 was re-chromatographed on silica gel column (CH_2Cl_2-MeOH = 4:0.1 to 4:0.3, v/v) to give compounds **4** (7 mg) and **5** (5 mg). Fraction 2 was purified on silica gel column (CH_2Cl_2-MeOH = 4:0.4, v/v) to afford compound **2** (5.72 g). Fraction 4 was separated on silica gel column (*n*-hexane–acetone = 3:2, v/v) to give four sub-fractions (4.1–4.4). Sub-fraction 4.2 was purified on a Sephadex LH-20 column (MeOH) to yield compound **1** (5 mg). Sub-fraction 4.3 was purified on a silica gel column (*n*-hexane–acetone = 3:2, v/v) and then Sephadex LH-20 column (MeOH) to yield compound **3** (10 mg).

2.3.1 | (1S,1′S)-13-hydroxy-6,6′,7-trimethoxy-2,2′-dimethylberbaman (1)

A white solid. $[\alpha]_D^{25}$ + 245° (*c* 0.1, $CHCl_3$). HR-ESI-MS *m/z* 609.2936 $[M+H]^+$ ($C_{37}H_{41}N_2O_6^+$ calcd for 609.2959). ^1H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) are shown in Table 1.

2.3.2 | Tetrandrine (2)

A white solid. HR-ESI-MS *m/z* 623.3090 $[M+H]^+$ ($C_{38}H_{43}N_2O_6^+$ calcd for 623.3116). ^1H NMR (600 MHz, $CDCl_3$): 7.34 (1H, dd, *J* = 8.4, 2.4 Hz, H-10′), 7.14 (1H, dd, *J* = 8.4, 2.4 Hz, H-11′), 6.88 (1H, dd, *J* = 8.4, 1.2 Hz, H-14), 6.85 (1H, dd, *J* = 8.4 Hz, H-13), 6.80 (1H, dd, *J* = 8.4, 2.4 Hz, H-13′), 6.55 (1H, d, *J* = 1.2 Hz, H-10), 6.51 (1H, s, H-5′), 6.30 (1H, s, H-5), 6.29 (1H, dd, *J* = 8.4, 2.4 Hz, H-14′), 6.00 (1H, s, H-8′), 3.93 (3H, s, 12-OCH_3), 3.86 (1H, dd, *J* = 11.4, 6.0 Hz, H-1′), 3.75 (3H, s, 6-OCH_3), 3.51 (1H, m, H-3a), 3.42 (1H, m, H-3′a), 3.37 (3H, s, 6′-OCH_3), 3.24 (1H, dd, *J* = 12.0, 5.4 Hz, H-15′a), 3.19 (3H, s, 7-OCH_3), 2.97-2.88 (4H, m, H-4′a, H-4a, H-3b, H-3′b), 2.79 (1H, dd, *J* = 12.0, 11.4 Hz, H-15′b), 2.73 (1H, m, H-4′b), 2.69 (1H, dd, *J* = 13.8, 10.2 Hz, H-15a), 2.62 (3H, s, 2′-NCH_3), 2.51 (1H, d, *J* = 13.8 Hz, H-15b), 2.40 (1H, m, H-4b), 2.33 (3H, s, 2-NCH_3). ^{13}C NMR (150 MHz, $CDCl_3$): 153.7 (C-12′), 151.4 (C-6), 149.4 (C-11), 148.6 (C-6′), 148.4 (C-8), 147.1 (C-12), 143.8 (C-7′), 137.9 (C-7), 135.2 (C-9′), 135.0 (C-9), 132.6 (C-14′), 130.1 (C-10′), 128.2 (C-4′a), 128.1 (C-8′a), 128.0 (C-4a), 123.0 (C-8a), 122.8 (C-14), 122.0 (C-13′), 121.9 (C-11′), 120.2 (C-8′), 116.2 (C-10), 112.8 (C-5′), 111.6 (C-13), 105.9 (C-5), 63.9 (C-1′), 61.4 (C-1), 60.3 (7-OCH_3), 56.2

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TABLE 1 NMR data of compounds **1** and tetrandrine (**2**).[14]

C	1		2	C	1		2
	δ_H (mult., *J* in Hz)	δ_C	δ_C		δ_H (mult., *J* in Hz)	δ_C	δ_C
1	3.86 (1H, d, 10.2)	61.4	61.6	1′	3.90 (1H, dd, 10.8, 6.0)	63.8	64.1
3	3.53 (1H, m) 2.91 (1H, m)	43.9	44.3	3′	3.46 (1H, m) 2.88 (1H, m)	45.3	45.4
4	2.91 (1H, m) 2.43 (1H, m)	22.0	22.2	4′	2.98 (1H, m) 2.76 (1H, m)	25.3	25.4
4a		127.9	128.3	4a′		127.9	128.1
5	6.30 (1H, s)	105.9	105.9	5′	6.49 (1H, s)	112.7	112.9
6		151.5	151.5	6′		148.7	148.7
7		138.0	138.0	7′		144.0	143.9
8		148.4	148.6	8′	6.01 (1H, s)	120.3	120.3
8a		123.1	123.1	8a′		127.9	128.2
9		134.0	135.1	9′		135.5	135.4
10	6.47 (1H, s)	115.4	116.4	10′	7.33 (1H, dd, 8.4, 2.4)	130.2	132.8
11		147.4	149.5	11′	7.08 (1H, dd, 8.4, 2.4)	121.7	122.0
12	6.80 (1H, s)	115.4	147.2	12′		153.3	153.9
13		143.7	111.7	13′	6.68 (1H, dd, 8.4, 2.4)	122.0	122.1
14	6.81 (1H, s)	123.4	122.9	14′	6.19 (1H, dd, 8.4, 2.4)	132.8	130.3
15	2.72 (1H, dd, 13.8,10.2) 2.51 (1H, d, 13.8)	41.9	42.1	15′	3.28 (1H, dd, 12.0, 5.4) 2.80 (1H, dd, 12.0, 11.4)	38.3	38.4
6-OCH_3	3.74 (3H, s)	55.9	56.0	6′-OCH_3	3.27 (3H, s)	55.7	56.0
7-OCH_3	3.19 (3H, s)	60.2	60.4				
12OCH_3	–	–	56.3				
2-NCH_3	2.33 (3H, s)	42.1	42.5	2′-NCH_3	2.62 (3H, s)	42.6	42.8

(12-OCH_3), 55.9 (6′-OCH_3), 55.8 (6-OCH_3), 45.2 (C-3′), 44.1 (C-3), 42.6 (2′-NCH_3), 42.3 (2-NCH_3), 41.9 (C-15), 38.1 (C-15′), 25.3 (C-4′), 22.1 (C-4).

2.3.3 | Fangchinoline (3)

A white solid. ^{1}H NMR (600 MHz, $CDCl_3$): 7.33 (1H, dd, *J* = 8.4, 2.4 Hz, H-10′), 7.13 (1H, dd, *J* = 8.4, 2.4 Hz, H-11′), 6.87 (1H, dd, *J* = 8.4, 1.8 Hz, H-14), 6.84 (1H, d, *J* = 8.4 Hz, H-13), 6.80 (1H, dd, *J* = 8.4, 2.4 Hz, H-13′), 6.57 (1H, d, *J* = 1.8 Hz, H-10), 6.52 (1H, s, H-5′), 6.31 (1H, dd, *J* = 8.4, 2.4 Hz, H-14′), 6.28 (1H, s, H-5), 6.05 (1H, s, H-8′), 3.92 (12-OCH_3), 3.88 (1H, dd, *J* = 10.8, 6.0 Hz, H-1′), 3.76 (3H, s, 6-OCH_3), 3.49 (1H, m, H-3a), 3.38 (1H, m, H-3′a), 3.35 (3H, s, 6′-OCH_3), 3.24 (1H, dd, *J* = 12.6, 6.0 Hz, H-15′a), 2.97-2.84 (4H, m, H-4′a, H-4a, H-3b, H-3′b), 2.77-2.70 (3H, m, H-15′b, H-4′b, H-15a), 2.62 (3H, s, 2′-NCH_3), 2.55 (1H, d, *J* = 13.8 Hz, H-15b), 2.41 (1H, m, H-4b), 2.33 (3H, s, 2-NCH_3). ^{13}C NMR (150 MHz, $CDCl_3$): 153.8 (C-12′), 149.4 (C-11), 148.8 (C-6′), 147.1 (C-12), 145.8 (C-6), 143.5 (C-7′), 141.8 (C-8), 135.1 (C-9), 135.0 (C-9′), 134.6 (C-7), 132.6 (C-14′), 130.1 (C-10′), 128.8 (C-8′a), 128.2 (C-4′a), 123.5 (C-4a), 123.4 (C-8a), 122.8 (C-14), 121.9 (C-11′, C-13′), 120.6 (C-8′), 116.3 (C-10), 113.2 (C-5′), 111.6 (C-13), 104.9 (C-5).

2.3.4 | Tetrahydropalmatine (4)

A white solid. ^{1}H NMR (600 MHz, $CDCl_3$): 6.88 (1H, dd, *J* = 8.4 Hz, H-12), 6.79 (1H, dd, *J* = 8.4 Hz, H-11), 6.74 (1H, s, H-1), 6.62 (1H, s, H-4), 3.89 (3H, s, 2-OCH_3), 3.87 (3H, s, 9-OCH_3), 3.86 (3H, s, 3-OCH_3), 3.85 (3H, s, 10-OCH_3). ^{13}C NMR (150 MHz, $CDCl_3$): 150.2 (C-10), 147.5 (C-2), 147.4 (C-3), 145.0 (C-9), 129.5 (C-13b), 128.4 (C-12a), 127.7 (C-8a), 126.6 (C-4a), 123.8 (C-12), 111.4 (C-4), 111.1 (C-11), 108.7 (C-1), 60.2 (9-OCH_3), 59.2 (C-13a), 56.2 (2-OCH_3), 55.9 (3-OCH_3), 55.8 (10-OCH_3), 53.9 (C-8), 51.5 (C-6), 36.2 (C-13), 29.0 (C-5).

2.3.5 | Dehydrocrebanine (5)

A yellow solid. ^{1}H NMR (600 MHz, $CDCl_3$): 8.64 (1H, d, *J* = 9.0 Hz, H-11), 7,01 (1H, d, *J* = 9.0 Hz, H-10), 6.88 (1H, s, H-3), 6.85 (1H, s, H-7), 6.18 (2H, s, H-12), 3.99 (3H, s, 9-OCH_3), 3.97 (3H, s, 8-OCH_3), 3.37 (2H, t, *J* = 6.0 Hz, H-5), 3.21 (2H, t, *J* = 6.0 Hz, H-4), 3.13 (3H, s, NCH_3). ^{13}C NMR (150 MHz, $CDCl_3$): 150.3 (C-9), 145.2 (C-2), 144.1 (C-6a), 141.7 (C-1), 141.6 (C-8), 129.6 (C-7a), 127.5 (C-3a), 123.5 (C-11), 118.7 (C-11a), 118.4 (C-6b), 117.5 (C-11b), 108.7 (C-10), 106.8 (C-3), 100.8 (C-12), 94.0 (C-7), 60.6 (8-OMe), 56.3 (9-OMe), 50.6 (C-5), 40.6 (NCH_3), 30.8 (C-4).

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3 | RESULTS AND DISCUSSION

The crude alkaloids in the dichloromethane extract from the roots of *S.terandra* were repeatedly chromatographed on silica gel and Sephadex LH-20 columns to give a new bisbenzylisoquinoline alkaloid, (1*S*,1′*S*)-13-hydroxy-6,6′,7-trimethoxy-2,2′-dimethylberbaman (**1**) and four known alkaloids **2**–**5**.

Compound **1** was obtained as a white solid. Its molecular formula was deduced

as $C_{37}H_{40}N_2O_6$ by positive HR-ESI-MS molecular ion peak at *m/z* 609.2936 $[M+H]^+$ (calcd. for $C_{37}H_{41}N_2O_6^+$, 609.2959) and ^{13}C NMR data. The ^1H NMR spectrum of **1** displayed the presence of a para-disubstituted benzene ring at δ_H 7.33 (1H, dd, $J = 8.4$, 2.4 Hz, H-10′), 7.08 (1H, dd, $J = 8.4$, 2.4 Hz, H-11′), 6.68 (1H, dd, $J = 8.4$, 2.4 Hz, H-13′), 6.19 (1H, dd, $J = 8.4$, 2.4, H-14′); a 1,3,5-trisubstituted benzene ring [δ_H 6.81 (1H, s, H-14), 6.80 (1H, s, H-12), 6.47 (1H, s, H-10)], and three aromatic singlet protons at δ_H 6.49 (1H, s, H-5′), 6.30 (1H, s, H-5), 6.01 (1H, s, H-8′). In addition, three methoxy groups at δ_H 3.74 (3H, s, 6-OCH_3), 3.27 (3H, s, 6′-OCH_3), 3.19 (3H, s, 7-OCH_3), two N-methyl groups at δ_H 2.62 (3H, s, 2′-NCH_3), 2.33 (3H, s, 2-NCH_3) and aliphatic protons in the range δ_H 3.90–2.43 were observed. The ^{13}C NMR and HSQC spectra of **1** revealed 37 carbons, including 14 aromatic quaternary carbons [δ_C 153.3 (C-12′), 151.5 (C-6), 148.7 (C-6′), 148.4 (C-8), 147.4 (C-11), 144.0 (C-7′), 143.7 (C-13), 138.0 (C-7), 135.5 (C-9′), 134.0 (C-9), 127.9 (C-4a, C-4a′, C-8a′), 123.1 (C-8a)], 10 aromatic methine carbons [δ_C 132.8 (C-14′, 130.2 (C-10′), 123.4 (C-14), 122.0 (C-13′), 121.7 (C-11′), 120.3 (C-8′), 115.4 (C-10, C-12), 112.7 (C-5′), 105.9 (C-5)], two sp^3 methine carbons [δ_C 63.8 (C-1′), 61.4 (C-1)], six sp^3 methylene carbons [δ_C 45.3 (C-3′), 43.9 (C-3), 41.9 (C-15), 38.3 (C-15′), 25.3 (C-4′), 22.0 (C-4)], and five methyl carbons [δ_C 60.2 (7-OCH_3), 55.9 (6-OCH_3), 55.7 (6′-OCH_3), 42.6 (2′-NCH_3), 42.1 (2-NCH_3)]. These data suggested that compound **1** to be a bisbenzylisoquinoline alkaloid. The ^1H and ^{13}C NMR data of **1** showed a similarity to those of tetrandrine (**2**),[14] except for the signals of C-ring. Three singlets at δ_H 6.81 (1H, s), 6.80 (1H, s) and 6.47 (1H, s) determined ring C of **1** as a 1,3,5-trisubstituted aromatic ring. These was also proved through the HMBC correlations from H-15a (δ_H 2.72) and H-15b (δ_H 2.51) to C-9 (δ_C 134.0), C-10 (δ_C 115.4) and C-14 (δ_C 123.4); from H-10 (δ_H 6.47) and H-14 (δ_H 6.81) to C-9 (δ_C 134.0) and C-15 (δ_C 41.9); from H-10 (δ_H 6.47) to C-11 (δ_C 147.4) and C-14 (δ_C 123.4); from H-14 (δ_H 6.81) to C-10 (δ_C 115.4), C-12 (δ_C 115.4); from H-12 (δ_H 6.80) and H-14 (δ_H 6.81) to C-10 (δ_C 115.4) and C-13 (δ_C 143.7). Three methoxy groups was located at C-6, C-7 and C-6′ based on the HMBC correlations from H-5 (δ_H 6.30) to C-6 (δ_C 151.5), C-7 (δ_C 138.0), from H-8′ (δ_H 6.01) to C-6′ (δ_C 148.7), and protons of three methoxy groups (δ_H 3.74, 3.19, 3.27) to C-6 (δ_C 151.5), C-7 (δ_C 138.0) and C-6′ (δ_C 148.7). The ECD spectrum of **1** showed a positive cotton effect at 225 nm which indicated the absolute configuration of **1** was 1*S*, 1′*S*.[11] Therefore, the structure of **1** was determined to be (1*S*,1′*S*)-13-hydroxy-6,6′,7-trimethoxy-2,2′-dimethylberbaman.

The four known compounds were determined as tetrandrine (**2**),[14] fangchinoline (**3**),[15] tetrahydropalmatine (**4**),[16] and dehydrocrebanine (**5**)[17] by the analysis of their spectral data and comparison with the literature.

SUPPORTING INFORMATION

Supporting information can be found online in the Supporting Information section at the end of this article

ACKNOWLEDGMENTS

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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