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ISOQUINOLINE ALKALOIDS FROM *Broussonetia papyrifera* FRUITS

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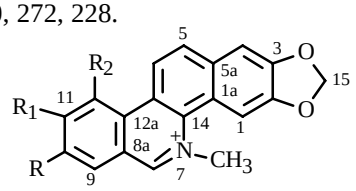
*A new isoquinoline alkaloid, named broussonpapyrine (**1**), along with three other known isoquinoline alkaloids, namely nitidine (**2**), oxyavicine (**3**), and liriodenine (**4**), were isolated from* Broussonetia papyrifera *fruits. The structures of these compounds were determined by 1D and 2D NMR, MS techniques, and chemical methods. This is the first report of the isoquinoline alkaloids isolated from this plant.*

Key words: *Broussonetia papyrifera,* isoquinoline alkaloid, broussonpapyrine.

Broussonetia papyrifera (L.) Vent. (Moraceae) is a deciduous plant widely distributed in China. In traditional Chinese medicine, the fruits of this plant have been used for impotency and to treat ophthalmic disorders [1] for more than one thousand years, but until now the related active components remain unknown. Extracts of whole plants from *B. papyrifera* have shown antifungal, antithepatotoxic, antioxidant, and lens aldose reductase inhibitory activities [2]. Our investigation on *n*-butanol extract resulted in the isolation of a new benzophenanthridine alkaloid, named broussonpapyrine (**1**), along with three known alkaloids, nitidine (**2**) [3], oxyavicine (**3**) [4, 5], and liriodenine (**4**) [6] from *Broussonetia papyrifera* fruits. All four alkaloids were isolated from *Broussonetia* (L.) Vent. for the first time. This present paper describes the structures of these compounds.

Broussonpapyrine (**1**) was isolated as yellow needles and gave a positive result in the Dragendorff test, mp 201–203°C, UV (MeOH, λ_{max}, nm): 342, 330, 303 sh, 280, 272, 228.



1, 2

1: R = H, $R_1 = R_2 = OCH_3$

2: $R = R_1 = OCH_3$, R_2 = H

The SEIMS afforded the positive ion at *m/z* 348 $[M]^+$; its molecular formula was determined as $C_{21}H_{18}NO_4^+$ by HRSEIMS and NMR data. The ^{1}H NMR spectrum (Table 1) showed two pairs of doublets for two pairs of ortho coupled protons at δ 8.20, 8.64 (J = 9 Hz) and 8.21, 8.68 (J = 9 Hz), two methoxys at δ 4.15 (s) and 4.25 (s), one N-methyl at δ 5.00 (s) and one methylenedioxy at δ 6.28 (s), and three singlets for aromatic protons at δ 7.58, 8.16, and 9.98. Its ^{13}C NMR and DEPT spectrum indicated the presence of 21 carbons: three methyl carbons, one methylene carbon, seven methine carbons, and ten quaternary carbons.

The downfield signals were similar to benzophenanthridine alkaloids [7]. The ^{13}C NMR data of the methyl at δ 52.94 suggested it maybe linked to nitrogen atom. The structure was further established by long-range correlation in the HMBC spectrum (Table 1) and NOESY. Its HMBC spectrum shows the correlations of δ 9.98 (H-6) with δ 133.5 (C-14), δ 52.94 (N-CH_3), δ 119.9 (C-9), and δ 130.1 (C-12a). On the basis of the above spectroscopic studies, the structure of compound **1** was elucidated; it was named broussonpapyrine.

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TABLE 1. ^{1}H NMR (500 MHz), ^{13}C NMR (DEPT) (125 MHz), and HMBC Spectral Data of **1** (CD_3OD)

Atom	^{1}H (δ, ppm)	^{13}C	DEPT	HMBC (C/H)
1	7.58, s	107.1	CH	C-2, C-3, C-4a, C-12
2		151.0	C	
3		150.8	C	
4	8.16, s	105.1	CH	C-2, C-3, C-12a, C-4b
4a		121.8	C	
4b		133.5	C	
6	9.98, s	151.8	CH	C-4b, (N)-C(H_3), C-7, C-10a
6a		120.9	C	
7	8.21, d	119.9	CH	C-6, C-9, C-10a
8	8.68, d	127.5	CH	C-6a, C-10
9		152.1	C	
10		147.5	CH	
10a		130.1	C	
10b		134.3	C	
11	8.64, d	119.5	CH	C-10, C-10a, C-12a, C-4b
12	8.20, d	132.6	CH	C-10b, C-4a, C-1
12a		127.1	C	
N-CH_3	5.00, s	52.94	CH_3	C-6, C-4b
9-OCH_3	4.15, s	57.6	CH_3	C-9
10-OCH_3	4.25, s	62.8	CH_3	C-10
-O-CH_2-O-	6.28, s	104.3	CH_2	C-2, C-3

EXPERIMENTAL

General Experimental Procedures. Melting points (mp) were determined using an X-4 micromelting-point apparatus (Beijing, China) and were uncorrected. UV spectra were measured on a Shimadzu UV-2501 spectrometer (Kyoto, Japan). IR spectra were obtained on KBr pellets using a Nicolet impact 410 spectrometer (Madison, USA). The ^{1}H and ^{13}C NMR spectra were obtained on an INOVA-500 and 125 MHz (Viarian, San Francisco, USA) with TMS as an internal standard. SEIMS measurements were undertaken on an HP5989A spectrometer (Palo Alto, USA). TLC and column chromatography were performed on plates precoated with silica gel F254 and silica gel (200–300 mesh; Qingdao Marine Chemical Ltd., Qingdao, China), respectively. Solvents were distilled prior to use.

Plant Materials. *Broussonetia papyrifera* fruits were collected from Bozhou, Anhui Province, China in August 2003 and was identified by Pr. H.C. Zheng, in the Department of Pharmacognosy, Second Military Medical University. A voucher specimen was deposited in the Department of Pharmacognosy, Second Military Medical University.

Extraction and Isolation. The air-dried and roughly powdered *B. papyrifera* fruit (10 kg) was extracted three times with 80% ethanol under reflux. After removal of the solvent by evaporation, the extracts were partitioned between H_2O and peltroleum ether, $CHCl_3$, EtOAc, and *n*-BuOH, successively. The *n*-BuOH extract was chromatographed on a silica gel (300–400 mesh; 1500 g) column, eluting with $CHCl_3$–MeOH mixture to afford 25 fractions. Fraction 5 was separated by column chromatography (CC) over silica gel (300–400 mesh; 600 g) eluted with a $CHCl_3$–MeOH gradient (6:1) to obtain compound **3**. Fraction 10 (1.4 g) was further separated by CC (3×80 cm) over silica gel eluted with $CHCl_3$–MeOH (30:1–1:1) to yield compound **1** (26 mg) and **2** (15 mg). Fraction 11 (1.2 g) was isolated by CC to yield compound **4** (16 mg).

Compound 1: yellow needles, mp 201–203°C, ($CHCl_3$–MeOH 15:1), IR (KBr, cm^{-1}): 1607, 1514, 1440, 1169, 950, 860. HRSEI-MS ($[M]^+$ 348.1238, calcd. 348.1236). ^{1}H NMR, ^{13}C NMR spectral data are given in Table 1.

Compound 2: yellow needles, mp 281–283°C, ($CHCl_3$–MeOH 15:1), ESI-MS $[M]^+$ *m/z* 348; UV (MeOH, λ_{max}, nm): 329, 303 sh, 281, 272 and 231. IR (KBr, cm^{-1}): 3414, 2935, 1710, 1604, 1514, 1441, 1168, 980, 830. ^{1}H NMR (DMSO-d_6, δ, ppm, J/Hz): 9.82 (1H, s, H-8), 8.83 (1H, d, J = 9, H-6), 8.38 (1H, s, H-12), 8.30 (1H, s, H-1), 8.28 (1H, d, J = 9, H-5), 7.89 (1H, s, H-9), 7.76 (1H, s, H-4), 6.29 (2H, s, -OCH_2O-), 4.90 (3H, s, N-CH_3), 4.25 (3H, s, 11-OCH_3), 4.05 (3H, s, 10-OCH_3). ^{13}C NMR (DMSO-d_6): 158.3 (C-8), 151.5 (C-10), 151.2 (C-11), 148.8 (C-2), 148.3 (C-3), 132.4 (C-14), 132.0 (C-5a), 129.9

(C-5), 124.0 (C-12a), 124.3 (C-13), 119.8 (C-8a), 119.3 (C-1a), 119.1 (C-6), 108.7 (C-12), 105.6 (C-9), 104.4 (C-1), 103.2 (C-4), 102.6 (-O-CH_2-O-), 57.2 (10-OCH_3), 56.2 (11-OCH_3), 51.3 (N-CH_3) [3].

Compound 3: powder, mp 259–260°C, ($CHCl_3$–MeOH 6:1). The SIMS afforded the positive ion at *m/z* 351 $[M+Na]^+$; its molecular formula was determined as $C_{20}H_{13}NO_5$ by MS and NMR data and confirmed by HRSEIMS ($[M+H]^+$ 348.3262, calcd. 348.3264). UV (MeOH, λ_{max}, nm): 247, 278, 288, 321, 333, 368. IR (KBr, cm^{-1}): 1650 (C=O), 1610, 1480 (Ar-H), 940. ^1H NMR ($CDCl_3$, δ, ppm, J/Hz): 7.17 (1H, s, H-1), 7.62 (1H, s, H-4), 7.89 (1H, s, H-9), 7.60 (1H, s, H-12), 7.54 (1H, d, J = 9, H-5), 7.91 (1H, d, J = 9, H-6), 3.96 (3H, s, N-CH_3), 6.12 (2H, s, -O-CH_2-O-). ^{13}C NMR ($CDCl_3$): 164.1 (C-8), 152.4 (C-11), 148.2 (C-10), 147.6 (C-3), 147.1 (C-2), 135.9 (C-14), 132.0 (C-5a), 131.1 (C-12a), 123.3 (C-5), 120.9 (C-1a), 120.8 (C-8a), 118.6 (C-6), 116.8 (C-13), 106.6 (C-9), 104.8 (C-4), 102.7 (C-1), 101.9 (10,11-O-CH_2-O-), 101.6 (15-O-CH_2-O-), 100.7 (C-12), 51.3 (N-CH_3) [4, 5].

Compound 4: yellow needles, mp 281–282°C, ($CHCl_3$–MeOH 15:1), UV (MeOH, λ_{max}, nm): 248, 268, 309, 413. IR (KBr, ν, cm^{-1}): 1670, 1600, 1580. ESI-MS: *m/z* 288, $[M+H]^+$, 310 $[M+Na]^+$. ^1H NMR (CD_3OD, δ, ppm, J/Hz): 6.36 (2H, s, OCH_2O), 7.16 (1H, s, H-3), 7.57 (1H, dt, J = 8.0, 1.1, H-9), 7.72 (1H, dt, J = 8.0, 1.1, H-10), 7.74 (1H, d, J = 5.2, H-4), 8.57 (1H, dd, J = 8.0, H-8), 8.65 (1H, d, J = 8.0, H-11), 8.90 (1H, d, J = 5.2, H-5); ^{13}C NMR (CD_3OD): 147.9 (C-1), 151.7 (C-2), 103.3 (C-3), 124.2 (C-4), 145.0 (C-5), 145.5 (C-11b), 182.4 (C-7), 132.8 (C-7a), 128.6 (C-8), 128.9 (C-9), l33.9 (C-10), 127.4 (C-11), 108.9 (C-11a), 123.4 (C-6a), 102.5 (-OCH_2O-) [8].

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