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N-CONTAINING COMPOUNDS FROM *Broussonetia papyrifera* SEEDS AND THEIR cAMP REGULATORY ACTIVITY IN N1E-115 CELLS

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From the n-*BuOH extract of* Broussonetia papyrifera *seeds, a novel chushizilactam A (**1**) and adenosine (**2**) were isolated. Compound **1** was identified as 5ζ-hydroxy-5-(hydroxymethyl)-4-methyl-3-vinyl-1*H*-pyrrol-2(5*H*)-one. Compounds **1** and **2** were evaluated for their cAMP-regulating activity by the AlphaScreen assay against N1E-115 cells.*

Keywords: *Broussonetia papyrifera*, Moraceae, lactam, cAMP regulatory activity.

The seeds of *Broussonetia papyrifera* (Moraceae) are known as a traditional Chinese medicine with tonic effects for the treatment of age-related disorders such as Alzheimer′s disease [1]. Previous reports indicated that its crude extract could improve learning and memory ability of mice [2]. This promoted us to conduct a chemical investigation. In this study, a novel lactam **1** and adenosine (**2**) were isolated and structurally identified. These compounds were evaluated for their cAMP-regulating activity by the AlphaScreen assay against N1E-115 cells.

Compound **1** was obtained as colorless crystals. Its molecular formula was established as $C_8H_{11}NO_3$ by positive HR-ESI-MS (*m/z* 192.0635 ($[M + Na]^+$; calcd 192.0636), indicating four degrees of unsaturation. The IR absorption of **1** at 1682 cm^{-1} was characteristic of a carbonyl group. The ^1H and ^{13}C NMR spectra disclosed a methyl, an oxymethylene, and a $CH{=}CH_2$ group. In addition, a carbonyl and three quaternary carbons, including two olefinic ones (Table 1), were also observed in the ^{13}C NMR and DEPT spectra. Except for a carbonyl and two double bonds, the molecular formula of **1** required a ring constructed by C-2 and C-4 via a *N*-atom. Due to unavailable COSY correlations, the assembly of **1** was mainly achieved by HMBC experiments, which was summarized as follows, i.e., H-7/C-4, C-3, and C-5, H-6/C-4 and C-5, and H-8/C-2 and C-4. These data suggested the planar structure of **1**. The relative configuration determination of **1** makes no sense because the lactam ring is a plane arising from the presence of an α,β-unsaturated ketone in the ring. Due to steric hindrance and the scarcity of the material, an effort to determine the absolute configuration at C-5 by the Mosher method was not successful. Thus, the structure of **1** was deduced as 5ζ-hydroxy-5-(hydroxymethyl)-4-methyl-3-vinyl-1*H*-pyrrol-2(5*H*)-one and given the name chushizilactam A.

In addition to compound **1**, a known *N*-containing compound, adenosine, was isolated and readily identified. Compound **1** represents novel five-membered lactams firstly characterized from plants of the genus *Broussonetia*.

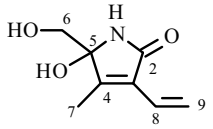

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TABLE 1. NMR Data of **1** (500 and 125 MHz, δ, ppm, J/Hz)

C atom	δ_C (DEPT)	δ_H	HMBC
2	174.1 (C=O)		
3	129.3 (C)		
4	155.6 (C)		
5	89.1 (C)		
6	64.9 (CH_2)	3.65 (d, J = 11.4)	C-4, C-5
		3.62 (d, J = 11.4)	
7	10.3 (CH_3)	2.00 (s)	C-3, C-4, C-5
8	127.0 (CH)	6.46 (dd, J = 17.7, 11.7)	C-2, C-3
9	120.2 (CH_2)	6.14 (dd, J = 17.7, 2.1)	C-3
		5.38 (dd, J = 11.7, 2.1)	

TABLE 2. The cAMP Regulatory Activity of Compounds **1** and **2**

	Counts per second	Standard error	*p* Value
DMSO	22131	2749	
1	20695	809	0.193
2	18684	517	0.008
10 μM Forskolin	11178	861	<0.001
1 μM cAMP	745	31	<0.001

Considering the medicinal applications of this plant, the cAMP regulation activity of the isolates was assessed by the AlphaScreen assay against N1E-115 neuroblastoma cells. The results showed that adenosine could significantly increase cAMP at a concentration of 50 μM, while compound **1** was inactive (Table 2).

EXPERIMENTAL

Melting point was determined on a XRC-1 micro-melting point apparatus and uncorrected. Optical rotation was recorded on a Horiba SEPA-300 polarimeter. UV spectrum was obtained on a Shimadzu double-beam 210A spectrometer, λ_{max} in nm. IR spectrum was determined by a Bruker Tensor 27 spectrometer, with KBr pellets, in cm^{-1}. NMR spectra were measured on a DRX-500 spectrometer, with TMS as an internal standard. FAB-MS was determined on a VG Autospec-3000 spectrometer. HR-ESI-MS was collected by an API QSTAR Pulsar 1 spectrometer. Column chromatography (CC) was carried out on silica gel (200–300 mesh, 10–40 μM; Qingdao Marine Chemical Inc., Qingdao, China) and Sephadex LH-20 (Amersham Pharmacia, Uppsala, Sweden). Semipreparative HPLC was done on an Agilent 1100 liquid chromatography with a Zorbax SB-C_{18} column (9.4 × 250 mm, i.d.).

Plant Material. The seeds of *B. papyrifera* were purchased from Yunnan Corporation of Materia Medica (YCMM), Yunnan Province, China, and identified by Mr. H. Y. Sun at YCMM. A voucher specimen (CHYX0043) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming, China.

Extraction and Isolation. The dried powders of *B. papyrifera* seeds (30 kg) were extracted three times with 95% EtOH under reflux. The extracts were concentrated and suspended in water followed by successive partition with petroleum ether, EtOAc, and *n*-BuOH. The *n*-BuOH extract (50 g) was separated by silica gel CC using $CHCl_3$–MeOH gradient to afford Fr. 1 and 2. Fraction 2 (15 g) was submitted to silica gel CC eluted with $CHCl_3$–MeOH (85:15) followed by gel filtration on Sephadex LH-20 (MeOH–H_2O, 90:10) to produce Fr. 2.1–Fr. 2.4. Fraction 2.1 (5 g) was subjected to silica gel CC eluted with gradient $CHCl_3$–MeOH to yield Fr. 2.1.1–Fr. 2.1.5. Fraction 2.1.3 (700 mg) was passed through Sephadex LH-20 (MeOH–H_2O, 90:10) followed by semipreparative HPLC (MeOH–H_2O, 35:65) to afford **1** (4 mg) and **2** (20 mg).

5ζ-Hydroxy-5-(hydroxymethyl)-4-methyl-3-vinyl-1*H*-pyrrol-2(5*H*)-one (1). Colorless crystals, mp 144–145°C; $[\alpha]_D^{27}$ +50.0° (*c* 0.10, MeOH). UV (MeOH, λ_{max}, nm): 217 (log ε 4.20). IR (KBr, ν_{max}, cm^{-1}): 3265, 1682, 1416, 1042, 937.

^{1}H and ^{13}C NMR, see Table 1. FAB-MS *m/z* 170 $[M + H]^+$; HR-ESI-MS *m/z* $[M + Na]^+$ 192.0635 (calcd for $C_8H_{11}NO_3Na$, 192.0636).

AlphaScreen cAMP Assay. The activity of the compounds against G_s-coupled receptor was tested by the AlphaScreen cAMP assay as described previously [3]. The concentration of the test compounds was 50 μM, and 10 μM for forskolin, a generic activator of cAMP synthesis directly stimulating adenylate cyclase. A mixture containing 5 μL anti-cAMP acceptor beads, 5 μL of 5 μM cAMP solution, and 15 μL biotinylated-cAMP/streptavidin donor beads was used as a positive control. Plates were read on a 2104 EnVision® Multilabel Plate Reader (Perkin–Elmer) with an excitation wavelength of 680 nm and an emission wavelength of 570 nm. The cAMP level change was calculated. The statistical tests were performed using one-way ANOVA analysis in software SPSS11.5 (SPSS, Chicago, IL).

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