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Xylactam, a New Nitrogen-containing Compound from the Fruiting Bodies of Ascomycete *Xylaria euglossa*

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Abstract A novel nitrogen-containing compound, named xylactam (**1**), was isolated from the fruiting bodies of ascomycete *Xylaria euglossa* together with two known compounds penochalasin B2 and neoechinulin A. Their structures were elucidated on the basis of spectral data.

Keywords *Xylaria euglossa*, xylactam, ascomycete

Xylaria euglossa Fr. is a rot-wood-inhabiting ascomycete, mainly occurring on stumps and fallen branches of forested areas in the Southwest of China [1]. Many unique secondary metabolites have been found in the fungi of this genus. During the study of Xylaria sp., various new metabolites had been discovered, including cytochalasins [2, 3], globoscin [4], lactones [5], maldoxin [6], sesquiterpenoids [7, 8], xylaramide [9], xylarin [10], and xyloketals [11]. As a part of our search for naturally occurring bioactive metabolites of the higher fungi in Yunnan province [12], we have carried out a detailed chemical investigation on the fungus Xylaria euglossa Fr. and isolated a new nitrogen-containing compound, xylactam (1), along with two known alkaloids, penochalasin B2 and neoechinulin A from extracts of the fruiting bodies.

Fruiting bodies of *Xylaria euglossa* were collected at Ailao mountain of Yunnan province, P. R. China, in July, 2002 and identified by Prof. Mu Zang, Kunming Institute of Botany, the Chinese Academy of Sciences. The voucher specimen was deposited in the Herbarium of the Kunming Institute of Botany, the Chinese Academy of Sciences. The air-dried fruiting bodies of *X. euglossa* (0.5 kg) were crushed and extracted with chloroform/methanol (1/1, v/v) four times at room temperature. The combined extracts were concentrated *in vacuo* to give a syrup (25 g), which was partitioned between chloroform and water. The chloroform soluble part (17 g) was subjected to silica gel column chromatography employing a gradient elution with chloroform/methanol (from 100 : 0 to 85 : 15, v/v) to give six fractions. Fraction II (100 : 1, v/v) was chromatographed on silica gel (petroleum ether/acetone 85 : 15), yielding penochalasin B2 (30 mg). Fraction III (98 : 2, v/v) was rechromatographed through silica gel, eluting with petroleum ether/acetone 80 : 20 (v/v) to give neoechinulin A (5 mg). Fraction VI (85 : 15, v/v) was passed through Sephadex LH-20, eluting with chloroform/methanol (1 : 1, v/v). A fraction exhibited strong yellow and white-blue fluorescence at 254 nm and was further purified on silica gel in petroleum ether/acetone (7 : 3, v/v) to afford compound **1** (8 mg).

Xylactam (**1**) was obtained as a white powder. Its molecular formula was determined as $C_{23}H_{31}NO_6$ from the quasi-molecular ion peak at *m/z* 418.2211 ($[M+H]^+$, calcd. 418.2229) in the positive-ion HR-ESI-MS. Twenty-three signals in the ^{13}C-NMR (DEPT) spectra of **1** were recognized (9×C, 2×CH, 11×CH_2, 1×CH_3), including three carbonyl C-atoms (δ 172.4, 173.5 and 204.0) and six aromatic quartery C-atoms (δ 102.5, 120.8, 125.9, 128.2, 155.0, 164.8). To fulfill the molecular formula of

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compound **1**, the presence of one benzene ring, three carbonyl carbons (–COOH, –CO–, –CONH–) and two phenolic hydroxyl groups (the exchangeable proton signals at δ_H 10.02 and 13.78) was suggested. In the mass spectrum of **1** a significant fragment ion at m/z=181 ($C_{12}H_{21}O$) was recognized. The signals in the ^{1}H-NMR spectrum for one A_2B_2-system at δ 2.97 and 3.12 were assigned to one isolated –CH_2CH_2– group, and signals at δ 6.13 (H-12, d, J=16) and 7.02 (H-13) were consistent with the presence of one *trans* double bond in the structure. The correlations from H-9 (δ_H 2.97) and H-12 to C-11 (δ_C 204.0) were observed in the HMBC spectrum of **1**. All these data suggested the existence of a long chain $CH_3(CH_2)_8CH$=$CHCO$–CH_2CH_2– in the structure of **1**. The ^{1}H-NMR spectrum also presented a characteristic amide NH at δ 6.81 (1H, exchangeable with D_2O) and ^{13}C-NMR (DEPT) spectrum of **1** showed the presence of an amide functionality at δ 173.5 (C-1) and δ 44.6 (C-3). In addition, the correlations between NH (δ_H 6.81) and C-3a (δ_C 125.9), C-7a (δ_C 128.2) was observed in the HMBC spectrum of **1**. Combined all these evidence and comparision with the data of xylaral (**2**) from *Xylaria polymorpha* previously reported [13], compound **1** can be considered as one substructure A with four substituent groups.

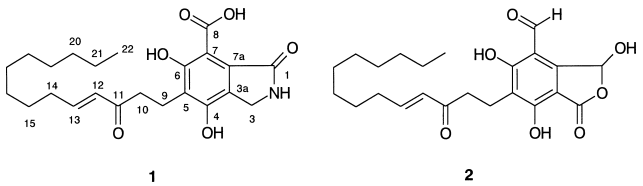
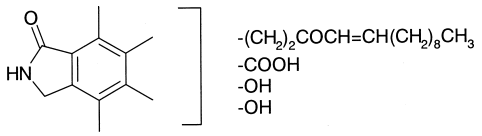


Fig. 1 Structure of xylactam (**1**) and xylaral (**2**).

The HMBC spectrum revealed that the exchangeable proton at δ_H 13.78 belonged to a phenolic OH group coupled to carbons at δ_C 102.5 (C-7), 120.8 (C-5) and 164.8 (C-6). The latter signal was attributed to a carbon bearing a phenolic hydroxyl, and the strong coupling of the OH proton to the carbon at δ_C 102.5 and 120.8 suggested the direct neighborhood of the OH group to the aromatic



A

Table 1 ^{1}H- and ^{13}C-NMR data and HMBC correlations of **1**

Position			HMBC	^{1}H, ^{1}H-COSY
1		173.5 (C)		
2	6.81 (s)		C-3a, C-7a	
3	4.48 (s)	44.6 (CH_2)	C-1, C-4, C-7a	
3a		125.9 (C)		
4		155.0 (C)		
5		120.8 (C)		
6		164.8 (C)		
7		102.5 (C)		
7a		128.2 (C)		
8		172.4 (C)		
9	2.97 (t, J=5.2)	16.8 (CH_2)	C-4, C-6, C-11	H-10
10	3.12 (t, J=5.2)	39.3 (CH_2)	C-5, C-11	H-9
11		204.0 (C)		
12	6.13 (d, J=16)	129.0 (CH)	C-11, C-14	H-13
13	7.02 (m)	151.4 (CH)	C-11, C-14, C-15	H-12, H-14
14	2.20 (m)	32.6 (CH_2)	C-12, C-13	H-13, H-15
15	1.42 (m)	27.8 (CH_2)	C-13	H-14
16～19	1.24 (m)	29.1～29.4 (CH_2)		
20	1.24 (m)	31.8 (CH_2)		
21	1.24 (m)	22.6 (CH_2)		
22	0.84 (t, J=6)	14.1 (CH_3)	C-20	H-21
4-OH	10.02 (s)		C-5, C-3a, C-4	
6-OH	13.78 (s)		C-5, C-7, C-6	

carbons. The exchangeable proton at δ_H 10.02 exhibited strong HMBC correlations to carbons at δ_C 120.8 (C-5) and 125.9 (C-3a) as well as 155.0 (C-4). The HMBC spectrum also displayed correlations between H-3 (δ_H 4.48) and C-1 (δ_C 173.5), C-4 (δ_C 155.0), C-7a (δ_C 128.2), and also between H-9 (δ_H 2.97) and C-4 (δ_C 155.0), C-6 (δ_C 164.8). A combination of the substructure A and substituents leads to structure **1** for xylactam.

The spectral data and physical properties of penochalasin B2 and neoechinulin A were identical with the data previously reported [14, 15]. Penochalasin B was reported to exhibite potent cytotoxicity against cultured P 388 cells [14].

Xylactam (**1**). White powder. EI-MS: 417 (9, $[M]^+$), 399 (5, $[M-H_2O]^+$), 246 (19), 236 (34), 218 (100), 204 (20), 181 (45). FAB-MS (posit.): 418 (100, $[M+1]^+$), 400 (40, $[M+1-H_2O]^+$). HR-ESI-MS (posit.): 418.2211 ($[M+1]^+$, calc. 418.2229). UV ($CHCl_3$): 242, 277, 325. IR (KBr): 3432, 3190, 2953, 2926, 2854, 1655, 1601, 1490, 1450, 1391, 1338, 1295, 1227, 1105, 766. ^{1}H-, ^{13}C-NMR, HMBC, and ^{1}H-^{1}H COSY: Table 1.

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