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A novel *N*-cinnamoylcyclopeptide containing an allenic ether from the fungus *Xylaria* sp. (strain # 2508) from the South China Sea

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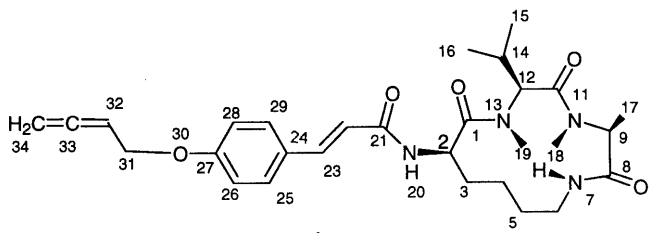
Abstract—A novel cyclic peptide containing an allenic ether of a *N*-(*p*-hydroxycinnamoyl)amide, and two aromatic allenic ethers were isolated from the endothytic fungus *Xylaria* sp. from the South China Sea. Their structures were determined by analysis of spectroscopic data, mainly 2D NMR experiments.

1. Introduction

Fungi of the genus *Xylaria* are a rich source of bioactive metabolites.[1,2] Recently, we have studied a strain (# 2508) of *Xylaria* sp. from the seed of an angiosperm tree from the South China Sea coast. Strain # 2508 grew rapidly in GYT (glucose, yeast extract, peptone) and produced a number of metabolites that included three aromatic allenic ethers **1**, **2** and **3** that were isolated from the medium. In this paper, we describe the structural elucidation of xyloallenolide A (**1**), the but-2,3-dienyl ether of *p*-hydroxycinnamic acid **2** and the corresponding methyl ester **3**. The structure of the cyclic peptide **1** is unique. This is also the first report of the natural occurrence of the acid **2** but the methyl ester **3** was previously described as eucalyptene A, which was isolated from *Clitocybe eucalyptorum* by Arnone et al.[3]

Xyloallenoide A (**1**) was isolated as a solid and its molecular formula, $C_{29}H_{40}N_4O_5$, was established by FABMS of the peak at m/z 525 [M+H] and elemental analysis. Bands at 1960 (allene), 1660, 1625, 1605 cm^{-1} in the IR spectrum suggested the presence of allenic and amide groups. The ^1H NMR spectrum of **1** (Table 1) showed an A_2MX_2 spin system due to the 31, 32, and 34 protons, and signals at δ 6.28 (d, 1 H, $J = 15.5$ Hz) and 7.56 (d, 1 H, $J = 15.5$ Hz), 6.90 (d, 2 H, $J = 9$ Hz)



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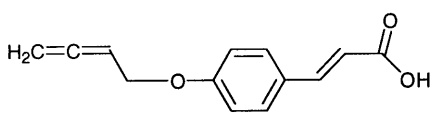

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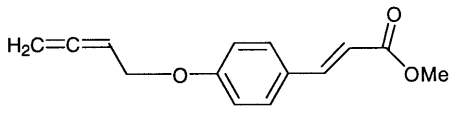

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Table 1. The NMR data of **1**

	^{13}C	^{1}H	^{1}H–^{1}H COSY	HMBC	ROESY
1	173.0			H-2, 3, 12, 19, 20	
2	49.1	5.11 (dt, 2.0, 7.0 Hz)	H-3, 4, 20,	H-3, 20	
3	27.9	1.91 (m,)	H-2, 4, 5	H-2, 4, 5	
4	17.9	1.60 (m)	H-2, 3, 6	H-5, 2,	
5	24.7	1.59 (dd, 7.0, 10.5 Hz)	H-6, 3	H-6	
6	36.2	3.59 (m)	H-4, 5, 7	H-4	
8	170.2			H-6, 7, 9, 17	
9	55.2	4.73 (q, 7.0 Hz)	H-17	H-17, 18	
11	169.4			H-12, 14, 18	
12	57.9	5.18 (d, 10.5 Hz)	H-14, 19	H-14, 16, 19	
14	27.2	2.43 (m)	H-15, 16	H-12, 15, 16	
15	19.6	0.90 (d, 6.5 Hz)	H-14,	H-12, 14, 16	
16	18.0	0.80 (d, 6.5 Hz)	H-14	H-12, 14, 15	
17	15.9	1.48 (d, 7.0 Hz)	H-9	H-9	H-18, 9
18	29.4	2.93 (s)		H-9	H-17, 19
19	30.5	3.09 (s)	H-12	H-12	NH-7, 18
21	165.2			H-2, 20, 22, 23	
22	117.7	6.28 (d, 15.5 Hz)	H-33	H-23	
23	141.2	7.56 (d, 15.5 Hz)	H-33	H-25	
24	127.5			H-22, 13, 26	
25, 29	129.4	7.42 (d, 9.0 Hz)	H-26	H-26	
26, 28	115.2	6.90 (d, 9.0 Hz)	H-25	H-25	
27	159.8			H-25, 26, 31	
31	65.9	4.58 (dt, 2.5, 7.0 Hz)	H-32, 34	H-32, 34	H-32, 34
32	86.8	5.38 (tt, 7.0 Hz)	H-31, 34	H-31, 34	H-31, 34
33	209.5			H-31, 32, 34	
34	76.7	4.87 (dt, 2.5, 7.0 Hz)	H-31, 32	H-32	H-31, 32
	NH-20	6.36 (S)	H-2		
	NH-7	5.80 (S)	H-6		H-19

and 7.42 (d, 2 H, $J = 9$ Hz) assigned to the cinnamoyl unit. In the HMBC spectrum, correlations between H-31 and C-27, C-32 and C-33, and from H-22 and H-23 to C-21 defined the (*p*-hydroxycinnamoyl)but-2,3-dienyl ether moiety, which accounted for $C_{13}H_{11}O_2$. This left a $C_{16}H_{29}N_4O_3$ entity to be assigned. The ^{13}C NMR spectrum contained three unassigned carbonyl groups at δ 173.0, 170.2 and 169.4, which accounts for the three oxygen atoms. There are a total of six carbon atoms attached to the four nitrogens, three methines at δ 57.2, 55.2 and 49.1, a methylene at 36.2, and two methyls at 30.5 (δ_H 3.09) and 39.2 (δ_H 2.93). Interpretation of the COSY and HMBC experiments defined the amino acids in the cyclic peptide as *N*-methyl alanine, *N*-methyl valine and lysine. The linkages between the amino acid units were assembled using the HMBC data (Fig. 1).

In the ROESY spectrum of **1**, correlations between the Me-17 and Me-18, Me-18 and Me-19, Me-19 and both H-14 and NH-7, together with an absence of correlations from Me-19 to H-12 and H-2, Me-18 to H-9 supposed that the amino acids had the configurations 2*R**, 9*S** and 12*S**. While a cyclic tripeptide is not uncommon in nature, the derivatization with a but-2,3-dienyl ether of *p*-hydroxycinnamic acid is unprecedented. Biological evaluation of xyloallenolide (**1**) is in progress.

Comparison of the spectral data of acid **2** and methyl ester **3** with corresponding data for the aromatic allenic ether portion of **1** showed a very good correlation and allowed the structures of **2** and **3** to be elucidated from spectroscopic data. This analysis revealed that **3** was identical in all respects to eucalyptene A.[3]

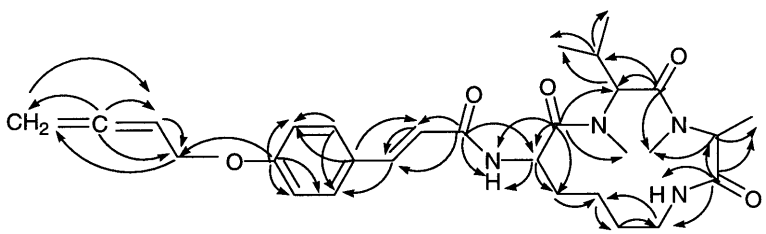

Figure 1. The correlations of **1**.

2. Experimental

2.1. Instruments

The following instruments were used: an Inova-500 NMR spectrometer, a VGZAB mass spectrometer, a Nicolet 5DX-FTIR spectrophotometer, a Shimadzu UV-240 spectrophotometer, a Perkin–Elmer 241 polarimeter, and a Perkin–Elmer 240C elemental analyzer.

2.2. Fungal strain

A strain of the fungus *Xylaria* sp. (# 2508) was isolated from a seed of an angiosperm tree in Mai Po, Hong Kong, and was stored in Department of Applied Chemistry, Zhongshan University, Guangzhou, PR China.

2.3. Culture conditions

Starter cultures (from Professor E. B. Gareth Jones and Dr. L. L. P. Vrijmoed) were maintained on cornmeal seawater agar. Plugs of agar supporting mycelial growth were cut and transferred aseptically to a 250 mL Erlenmeyer flask containing 100 mL of liquid medium GYT (glucose 10 g/L, peptone 2 g/L, yeast extract 1 g/L, NaCl 30 g/L). The flask was incubated at 30°C on a rotary shaker for 5–7 days. The mycelium was aseptically transferred to a 300 litre fermenter containing 170 L of GYT medium and was incubated at 30°C for 80 h.

2.4. Extraction and separation of metabolites

The cultures (170 L) were filtered through cheesecloth. The filtrate was concentrated to 3.5 L below 50°C, and extracted five times by shaking with an equal volume of ethyl acetate. The combined extracts were chromatographed on silica gel using a gradient elution from petroleum to ethyl acetate, to obtain **3** (150 mg) from the 5% ethyl acetate/petroleum fraction, **2** (100 mg) from the 50% EtOAc/petroleum fraction and **1** (120 mg) from the 100% EtOAc fraction.

2.4.1. Xyloallenolide (1). Colorless crystals, mp 82–85°C; $[\alpha]_D^{25} = -34.6$ (c 0.058, $CHCl_3$). Anal. found: C, 66.16; H, 7.83; N, 10.37. Calcd for $C_{29}H_{40}N_4O_5$: C, 66.41; H, 7.63; N, 10.69; MS (FAB): 525, 467, 412, 382, 199, 147, 86, 58; IR (KBr): 3415, 3065, 2960, 2935, 2880, 1960, 1660, 1625, 1605, 1510, 1460, 1410, 1250, 1225, 1175, 1110, 1000, 830 cm^{-1}; ^1H NMR ($CDCl_3$, TMS), ^{13}C NMR ($CDCl_3$) and 2D NMR (see Table 1); UV λ_{max} nm ($CHCl_3$): 216.8 (ε 3.8×10^4), 291.0 (ε 2.9×10^4).

2.4.2. But-2,3-dienyl ether of *p*-hydroxycinnamic acid (2). Colorless crystals, mp 151–152°C; MS (FAB): 217, 209, 164, 148; ^1H NMR ($CDCl_3$) δ: 7.74 (d, 1 H, $J = 15.5$ Hz), 7.50 (d, 2 H, $J = 9$ Hz), 6.93 (d, 2 H, $J = 9$ Hz), 6.32 (d, 1 H, $J = 15.5$ Hz), 5.34 (tt, 1 H, $J = 6.5$ Hz), 4.89 (dt, 2 H, $J = 2.5$, 6.5 Hz), 4.61 (dt, 2 H, $J = 2.5$, 6.5 Hz). ^{13}C NMR ($CDCl_3$) δ: 209.6 (C), 171.8 (C), 160.5 (C), 146.7 (CH), 130.1 (CH), 127.0 (C), 115.3 (CH), 114.7 (CH), 86.9 (CH), 77.1 (CH_2), 66.2 (CH_2); UV λ_{max} nm ($CHCl_3$): 220.5 (ε 1.5×10^4), 294.5 (ε 2.1×10^4).

2.4.3. Eucalyptene (3). Colorless crystals, mp 68–70°C; MS (FAB): 231, 199, 178, 167, 118, 107; ^1H NMR ($CDCl_3$) δ: 7.60 (d, 1 H, $J = 16$ Hz), 7.50 (d, 2 H, $J = 9$ Hz), 6.9 (d, 2 H, $J = 9$ Hz), 6.3 (d, 1 H, $J = 16$ Hz), 5.39 (tt, 1 H, $J = 7$ Hz), 4.88 (dt, 2 H, $J = 2.5$, 7 Hz), 4.60 (dt, 2 H, $J = 2.5$, 7 Hz), 3.80 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ: 209.6 (C), 167.7 (C), 160.2 (C), 144.5 (CH), 129.7 (CH), 127.4 (C), 115.4 (CH), 115.2 (CH), 86.7 (CH), 77.4 (CH_2), 65.9 (CH), 51.6 (CH_3); IR (KBr): 3420, 2935, 2875, 1960, 1720, 1635, 1605, 1510, 1285, 1250, 1170, 1010, 985, 860, 820 cm^{-1}. Anal. found: C, 72.90; H, 5.93. Calcd for $C_{14}H_{14}O_3$: C, 73.04; H, 6.13.

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