The First Isolation of a Phlegmacin Type Pigment from the Ascomycete *Xylaria euglossa*

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A new pigment, phlegmacin A 8,8'-di-*O*-methyl ether (**1**), was isolated from fruiting bodies of *Xylaria euglossa* (Ascomycota) along with the known fungal pigments (*S*)-torosachrysone-8-*O*-methyl ether (**2**) and emodin-6,8-di-*O*-methyl ether (**3**). The structure of compound **1** was established on the basis of its spectroscopic data and the absolute configuration determined as *P* and 3'*S* by comparing the CD and ^{1}H NMR spectra of **1** with those of the phlegmacins **4** and **5**. Compound **1** represents the first phlegmacin type pigment isolated from the ascomycete.

Key words: Xylaria euglossa, Ascomycota, Dihydroanthracenone, Phlegmacin A 8,8'-Di-*O*-methyl ether

Introduction

The chemistry of pigments from higher fungi (macromycetes) has received a lot of attention and has been adequately reviewed [1, 2]. As part of our ongoing studies on active metabolites from higher fungi in Yunnan province of China [3 – 10], we investigated the chemical constituents of Chinese *Xylaria* species. In this paper, we report on a new pigment, phlegmacin A 8,8'-di-*O*-methyl ether (**1**), from fruit bodies of the ascomycete *Xylaria euglossa* Fr., where it occurs together with the known pigments (*S*)-torosachrysone-8-*O*-methyl ether (**2**) and emodin-6,8-di-*O*-methyl ether (**3**). *X. euglossa* grows on rotting wood and is found mainly on stumps and fallen branches in forests distributed in the southwest of China [11]. In previous studies, many unique secondary metabolites have been isolated from fruit bodies and cultures of *Xylaria* species, including cytochalasins [12, 13], xyloketals [14], lactones [15], sesquiterpenoids [16, 17], xylarin [18], xylaral [19], xylaramide [20], globoscin [21], malaysic acid [22], and maldoxin [23].

Results and Discussion

Pigment **1** was obtained from the methanol/chloroform extract of the fruit bodies as a green-yellow powder, $[\alpha]_D = +91.7$ ($c = 0.57$, $CHCl_3$). The high-resolution positive-ion ESI-MS indicated a molecular formula of $C_{34}H_{34}O_{10}$ ($[M+1]^+$ at *m/z* 603.2230, calcd. 603.2230), suggesting a pre-anthraquinone dimer [1, 24] containing four methoxy groups. This is supported by the presence of the corresponding monomer, (**2**) [1, 25 – 27], together with its oxidation product emodin-6,8-di-*O*-methyl ether (**3**) [28]. Both compounds have been isolated from *Cortinarius* and *Tricholoma* species before and were identified by comparison of their spectroscopic data with those of au-

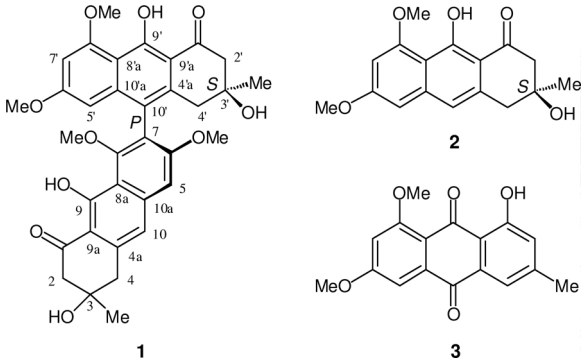

Fig. 1. Structures of phlegmacin A 8,8'-di-*O*-methyl ether (**1**), torosachrysone-8-*O*-methyl ether (**2**) and emodin-6,8-di-*O*-methyl ether (**3**).

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Table 1. ^{1}H and ^{13}C NMR data (400 MHz, $CDCl_3$) of **1** and **2**, δ in ppm, J in Hz.

	1	**2**	**1**	**2**
1			202.4 (*s*)	202.6 (*s*)
2	2.87 (m)	2.59 (*d*, $J = 17.3$), 2.63 (*d*, $J = 17.3$)	52.0 (*t*)	48.9 (*t*)
3	–	–	70.9 (*s*)	69.7 (*s*)
4	3.12 (*d*, $J = 17.0$), 3.17 (*d*, $J = 17.0$)	2.82 (*d*, $J = 15.8$), 2.86 (*d*, $J = 15.8$)	43.7 (*t*)	43.1 (*t*)
5	6.95 (*s*)	6.39 (*d*, $J = 2.0$)	102.6 (*d*)	98.8 (*d*)
6	–	–	160.0 (*s*)	161.9 (*s*)
7	–	6.24 (*d*, $J = 2.0$)	121.8 (*s*)	97.7 (*d*)
8	–	–	158.7 (*s*)	160.8 (*s*)
9	–	–	164.8 (*s*)	164.7 (*s*)
10	7.06 (*s*)	6.70 (*s*)	117.3 (*d*)	117.0 (*d*)
4a	–	–	136.6 (*s*)	136.9 (*s*)
8a	–	–	113.9 (*s*)	109.4 (*s*)
9a	–	–	109.6 (*s*)	109.8 (*s*)
10a	–	–	141.3 (*s*)	141.8 (*s*)
3-Me	1.50 (*s*)	1.17 (*s*)	29.0 (*q*)	27.6 (*q*)
6-OMe	3.45 (*s*)	3.71 (*s*)	61.7 (*q*)	55.0 (*q*)
8-OMe	3.77 (*s*)	3.76 (*s*)	56.0 (*q*)	55.6 (*q*)
9-OH	14.93 (*s*)	–	–	–
1’	–	–	202.4 (*s*)	–
2’	2.82 (*m*)	–	51.4 (*t*)	–
3’	–	–	70.5 (*s*)	–
4’	2.58 (*d*, $J = 16.7$), 2.80 (*d*, $J = 16.7$)	–	41.8 (*t*)	–
5’	6.16 (*s*)	–	98.3 (*d*)	–
6’	–	–	164.7 (*s*)	–
7’	6.46 (*s*)	–	97.5 (*d*)	–
8’	–	–	162.0 (*s*)	–
9’	–	–	165.9 (*s*)	–
10’	–	–	119.8 (*s*)	–
4’a	–	–	135.4 (*s*)	–
8’a	–	–	110.6 (*s*)	–
9’a	–	–	110.0 (*s*)	–
10’a	–	–	141.8 (*s*)	–
3’-Me	1.28 (*s*)	–	28.9 (*q*)	–
6’-OMe	3.61 (*s*)	–	55.1 (*q*)	–
8’-OMe	4.01 (*s*)	–	56.2 (*q*)	–
9’-OH	15.47 (*s*)	–	–	–

thentic samples. The negative optical rotation of **2**, $[\alpha]_D^{20} = -12.0$ ($CHCl_3$), agrees with that reported for (*S*)-torosachrysone-8-*O*-methyl ether in the literature [26].

Comparison of the ^{1}H NMR spectrum of the new pigment **1** with that of **2** (Table 1) classifies **1** as a phlegmacin type dimer [29–31], in which the two halves of the molecule are connected *via* a biaryl bond between C-7 and C-10’. The ^{1}H spectrum of **1** contains signals for two aliphatic C-methyl groups, four methylene groups, four methoxy groups, four isolated aromatic protons, and two strongly chelated phenolic hydroxy groups. The proton at C-5 appears as singlet ($\delta = 6.95$) rather than a doublet ($\delta = 6.39$, $J = 2.0$)

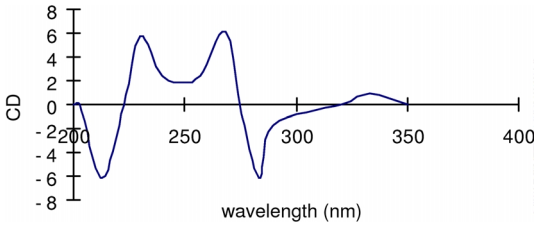

Fig. 2. CD ($\Delta\varepsilon$) spectrum of **1** in $CHCl_3$.

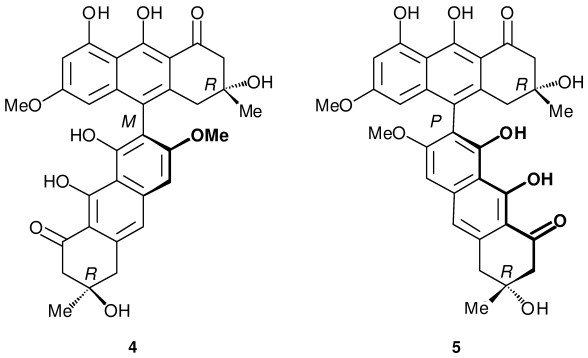

Fig. 3. Structures of $(M, 3R, 3''''R)$-phlegmacin (phlegmacin A_1) (**4**) and $(P, 3R, 3''''R)$-phlegmacin (phlegmacin B_1) (**5**).

as observed for monomer **2** (Table 1). In the HMBC spectrum of **1** the protons at C-4 correlate with three aromatic quaternary carbons (C-10’, C-4’a, C-9’a), whereas in the spectrum of **2** the corresponding protons show cross peaks to two aromatic quaternary carbons (C-4a, C-9a) and an aromatic methine carbon (C-10). From this evidence the 7-10’ connectivity is firmly established.

The CD spectrum of phlegmacin pigment **1** (Fig. 2) exhibits a negative Cotton effect at longer wavelength and a positive one at shorter wavelength (a so-called A-type curve). The same type of spectrum is shown by $(M, 3R, 3''''R)$-phlegmacin (phlegmacin A_1), for which structure **4** has recently been established by a combination of biosynthetic studies, quantum mechanical CD calculations, and NOE measurements [31, 32].

Since the sign of the Cotton effect of dimeric pre-anthraquinones is only determined by the chiral axis, the absolute axial configuration given in formula **1** can be safely assigned to the *Xylaria* pigment. According to the rules of Prelog and Helmchen [34], **1** possesses *P*-configuration [35].

In the ^{1}H NMR spectra of dimeric pre-anthraquinones, the biaryl axis has a distinctive influence on the difference in chemical shifts of the methylene protons at C-4’, which can be used to determine the relative configuration at C’-3 [36]. In dimers of the phleg-

macin type, the occurrence of the 4'-methylene protons as well-separated AB-quartet ($\Delta\delta = 0.15 - 0.35$ ppm) indicates an *anti*-relationship between the OH-group at C-3' and the bulk of the second torosachrysone (anthraquinone) moiety, whereas more narrow signals ($\Delta\delta \leq 0.08$ ppm) are typical for a *syn*-relationship of the two residues [31].

In the ^{1}H NMR spectrum of phlegmacin dimethyl ether **1**, the 4'-protons appear as well separated AB-quartet, which suggests an *anti*-relationship of the two residues and, accordingly, the 3'*S*-configuration. This rule does not apply for C-3, since the 4-protons are too remote from the chiral axis [31]. Pigment **1** is thus defined as a phlegmacin A 8,8-*O*-dimethyl ether with *P*-axial configuration, 3'*S*-configuration, and undetermined stereochemistry at C-3. Its NMR data are in agreement with those of phlegmacin B_1 (**5**), which has the same relative configuration [31].

Octaketides from the acetate-malonate biosynthetic pathway form an important group of fungal pigments. Dimeric pre-anthraquinones (dihydroanthracen-1(2*H*)-ones) occur in several *Cortinarius*, *Dermocybe*, and *Tricholoma* species and are responsible for the spectacular colors of these toadstools [1, 24, 25]. It has been established that these dimers are formed biosynthetically by phenolic coupling of monomeric precursors such as atrochrysone and/or torosachrysone [31, 33]. The co-occurrence of phlegmacin A 8,8-*O*-dimethyl ether **1** with its monomer, (*S*)-torosachrysone-8-*O*-methyl ether (**2**), in *Xylaria euglossa* suggests a similar biosynthetic relationship [37]. Interestingly, this is the first report of a phlegmacin type pigment from an ascomycete.

Experimental Section

General

Column chromatography (CC): silica gel (200–300 mesh) and Sephadex LH-20. Optical rotation: Horiba SEPA-300 digital polarimeter. CD spectrum: CDJ-801 instrument; $\lambda(\Delta\varepsilon)$ in nm. UV spectrum: Shimadzu UV-2401PC spectrometer; λ_{max} in nm. IR spectrum: Bio-Rad FTS-135 spectrometer, KBr pellets; ν in cm^{-1}. ^{1}H and ^{13}C NMR spectra: Bruker AM-400 spectrometer; δ in ppm, J in Hz. MS: VG Autospec-3000 spectrometer and API QSTAR Pulsar 1 spectrometer.

Material

X. euglossa was collected at the 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.

Extraction and isolation

The air-dried fruiting bodies of *X. euglossa* (0.5 kg) were crushed and extracted four times with chloroform/methanol (1/1, v/v) 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 CC. Gradual elution with chloroform/methanol from 100:0 to 90:10 (v/v) yielded four fractions. Fraction II (chloroform/methanol, 100:1, v/v) was separated again on silica gel with petroleum ether/acetone (98:2, 95:5, 90:10). From the 95:5-fraction, compound **3** (7 mg) was obtained by preparative silica gel TLC (petroleum ether/acetone, 90:10, v/v) and detected by its yellow-red fluorescence under UV light at 365 nm. Fraction III (chloroform/methanol, 95:5, v/v) was chromatographed again on silica gel with petroleum ether/acetone (85:15, v/v) to yield compound **2** (32 mg). Fraction VI (chloroform/methanol, 90:10, v/v) was passed through a Sephadex LH-20 column with chloroform/methanol (1:1, v/v) as eluent. The fractions, exhibiting a strong yellow and white-blue fluorescence under UV light at 254 nm, were purified further by silica gel CC with petroleum ether/acetone (85:15, v/v) to afford compound **1** (15 mg).

*Phlegmacin A 8,8-O-dimethyl ether (**1**):* Green-yellow powder, $[\alpha]_D^{25} = +91.7$ ($c = 0.57$, $CHCl_3$). – CD ($CDCl_3$): $\lambda(\Delta\varepsilon) = 200$ (0.03), 203 (0), 213 (−6.07), 223 (0), 230 (5.73), 240 (2.36), 250 (1.82), 257 (2.36), 268 (6.07), 275 (0), 283 (−6.07), 290 (−1.68), 320 (0), 333 (0.88), 350 (0). – UV ($CHCl_3$): $\lambda_{max} = 278$, 206, 388, 317. – IR (KBr): $\nu = 3442$, 2962, 2930, 2851, 1612, 1458, 1402, 1379, 1323, 1287, 1209, 1163, 1105, 1062. – ^{1}H and ^{13}C NMR: see Table 1. – EI-MS: *m/z* (%) = 602 (100) [M^+], 584 (45) [M^+-H_2O], 566 (10) [M^+-$2H_2O$], 302 (3), 284 (13), 269 (10). – FAB-MS (neg.): 601 [M^--1]. – HR-ESI-MS (pos.): $m/z = 603.2230$ [M^++1] (calcd. for $C_{34}H_{34}O_{10}$, 603.2230).

*(S)-Torosachrysone-8-O-methyl ether (**2**):* Yellow needles, m. p. 207 − 210° ($CHCl_3$), $[\alpha]_D^{20} = -12$ ($c = 0.3$, $CHCl_3$), $[\alpha]_{546}^{22} = -15.5$ ($CHCl_3$) [26]. – ^{1}H and ^{13}C NMR: see Table 1. – EI-MS: *m/z* (%) = 302 (100) [M^+], 284 (15) [M^+-H_2O], 269 (30) [M^+-H_2O-Me], 244 (55), 149 (23), 113 (25).

*Emodin-6,8-di-O-methyl ether (**3**):* Yellow powder, m. p. 210–213°C (C_6H_6-Petroleum ether). – EI-MS: *m/z* (%) = 298 (100) [M^+], 280 (45), [M^+-H_2O], 269 (40), 252 (55), 149 (40). – ^{1}H NMR ($CDCl_3$, 500 MHz): $\delta = 7.05$ (*s*, 2-H)); 7.54 (*s*, 4-H); 7.44 (*d*, $J = 2.4$, 5-H); 6.77 (*d*, $J = 2.4$, 7-H); 2.41 (*s*, C-Me); 4.01 (*s*, 6-OMe); 3.97 (*s*, 8-OMe). –

^{13}C NMR ($CDCl_3$, 500 Hz): 21.9 (*q*, C-Me), 56.0 (*q*, 8-OMe), 56.6 (*q*, 6-OMe), 103.9 (*d*, C-5), 104.7 (*d*, C-7), 114.7 (*s*, C-9a), 115.2 (*s*, C-8a), 120.0 (*d*, C-4), 124.8 (*d*, C-2), 137.7 (*s*, C-10a), 146.9 (*s*, C-3), 146.9 (*s*, C-4a), 162.6 (*s*, C-6), 162.9 (*s*, C-1), 165.2 (*s*, C-8), 183.0 (*s*, C-10), 187.4 (*s*, C-9).

Acknowledgement

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[37] The co-occurrence of monomer **2** and dimer **1** suggests 3*S*-configuration for both halves of the latter molecule. This plausible hypothesis, however, lacks experimental verification.