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The metabolites of the mangrove fungus *Verruculina enalia* No. 2606 from a salt lake in the Bahamas

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Abstract

Two metabolites enalin **A** (**1**) and **B** (**2**), together with hydroxymethyl furfural (**3**) and three cyclodipeptides (**4, 5** and **6**), were isolated from the mangrove fungus *Verruculina enaria* from a salt lake in the Bahamas. Their structures were determined by spectroscopic methods, mainly by 2D NMR spectroscopic analyses. A possible biosynthetic scheme to **1** and **2** is presented.

Keywords: *Verruculina enalia*; Marine fungi; Metabolites; Enalins; Coumaranone

1. Introduction

A strain (No. 2606) of the fungus *Verruculina enalia*, an ascomycete, was isolated from the decayed wood of a Cassurina tree from a salt lake in the Bahamas. Although *V. enalia* is a very common tropical species found on mangrove wood worldwide, this is its first collection from a salt lake. As a part of our study on the metabolites of mangrove fungi (Lin et al., 2000, 2001), we examined the chemical constituents of *V. enalia* of which none have previously been reported. Two new phenolic compounds, enalin **A** (**1**) and **B** (**2**), were isolated together with hydroxymethyl furfural (**3**) and three cyclodipeptides (**4, 5** and **6**) from its fermentation broth. Enalin **A** (**1**) is a coumaranone, a type of compound distributed widely from microorganisms to higher plants and having antimicrobial, antifungal, phytotoxic (Furumoto et al., 1997) and antidiabetic (Manickam et al., 1997) activities.

2. Results and discussion

A 120 l fermentation broth was concentrated and extracted with ethyl acetate, and the extract was repeatedly subjected to silica gel column chromatography. Enalin **A** (**1**) was subsequently obtained as colorless needles, mp 190–192 °C, $[\alpha]_D = -17.7°$, and having the molecular formula of $C_{10}H_{10}O_4$ as determined by FABMS and elemental analysis. The numbers of hydrogen and carbon atoms in the ^1H and ^{13}C NMR spectra were in agreement with the molecular formula.

The IR absorptions of compound **1** at 3435 and 1679 cm^{-1} showed the presence of hydroxyl and carbonyl group(s). In the ^1H NMR spectrum, there was a phenolic hydroxyl signal at δ 8.32. The ^{13}C NMR spectrum disclosed not only the presence of a ketone carbonyl group (δ 200.4) but signals for all 10 carbons; those included two methyl groups (δ 22.3 and 16.8) and a carbonyl functionality (δ 200.4), with the six remaining signals being aromatic carbons at δ 118.6, 123.6, 124.4, 130.2, 142.1 and 159.7, and a quaternary carbon at δ 104.4. The data suggested that **1** had a benzene ring system, a ketone, and possibly a hemiacetal functionality (δ 104.4). The ^1H NMR signals of two coupling protons at δ 6.67 (*d*, $J = 8.5$ Hz) and 7.05 (*d*, 8.5 Hz) indicated a pair of adjacent protons on the benzene ring, and the six unsaturation equivalents, required by the molecular formula, suggested that the compound had an additional ring.

The HMBC data established the overall structure of compound **1** (Scheme 1), especially the multiple correlations from the OH-7 (δ 8.32) to C-6, C-7 and C-8, and

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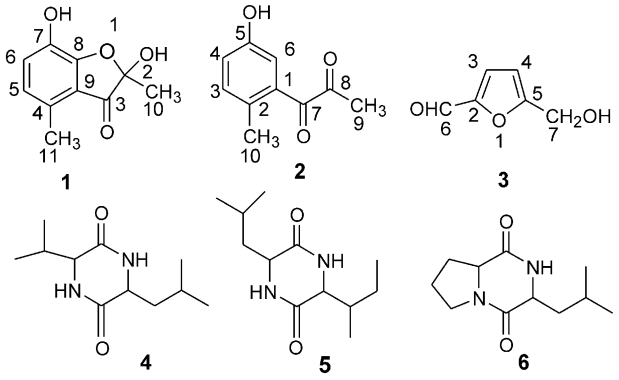


from the hydroxy (δ 6.40) to C-2, C-3 and C-10 which unambiguously assigned the positions of the hydroxyl groups. Additionally, the correlations from CH_3-11 to C-9,C-4 and C-5 indicated the methyl group was attached to C-4.

Enalin **B** (**2**) was obtained as an oil and its molecular formula of $C_{10}H_{10}O_3$ was determined by FABMS and elemental analysis and by overall NMR spectroscopic interpretation. The ^{13}C NMR spectrum of compound **2** contained signals for 10 carbons, these being two ketone carbonyl groups (δ 201.6 and 194.3), two methyl groups (δ 20.5 and 26.1) and six olefinic carbons (δ 118.3, 120.9, 131.4, 132.4, 133.4 and 153.6), respectively. The ^1H NMR spectrum of **2** showed an AMX spin system with characteristic couplings [7.13 (*d*, 8 Hz), 6.97 (*dd*, 8, 2.5 Hz) and 7.10 (*d*, 2.5 Hz)], suggesting that it contained a trisubstituted benzene ring. In the IR spectrum, a strong absorption at 3402 cm^{-1} showed hydroxyl group(s), and two intense bands at 1707 and 1672 cm^{-1} indicated two conjugated ketones. The structure of **2** was thus defined on the basis of a standard series of 1D and 2D NMR spectroscopic experiments, including COSY, HMQC and HMBC analyses.

Enalin **A** and **B** are both polyketides, and enalin **A** is an analogue of the known cycloarthropsone previous isolated from the fungus *Arthropssis truncata*. Ayer and Craw (1992) have examined the biosyntheses of these metabolites, and a possible biosynthetic pathway and interrelationship of enalin **A** and **B** are shown in Scheme 1 based on their work.

Lastly, the structures of compounds **3, 4, 5** and **6** were elucidated from spectroscopic data and from comparison with literature values (Trigos and Reyna, 1995; Nitecki and Halpern, 1968; Czeski et al., 1995).

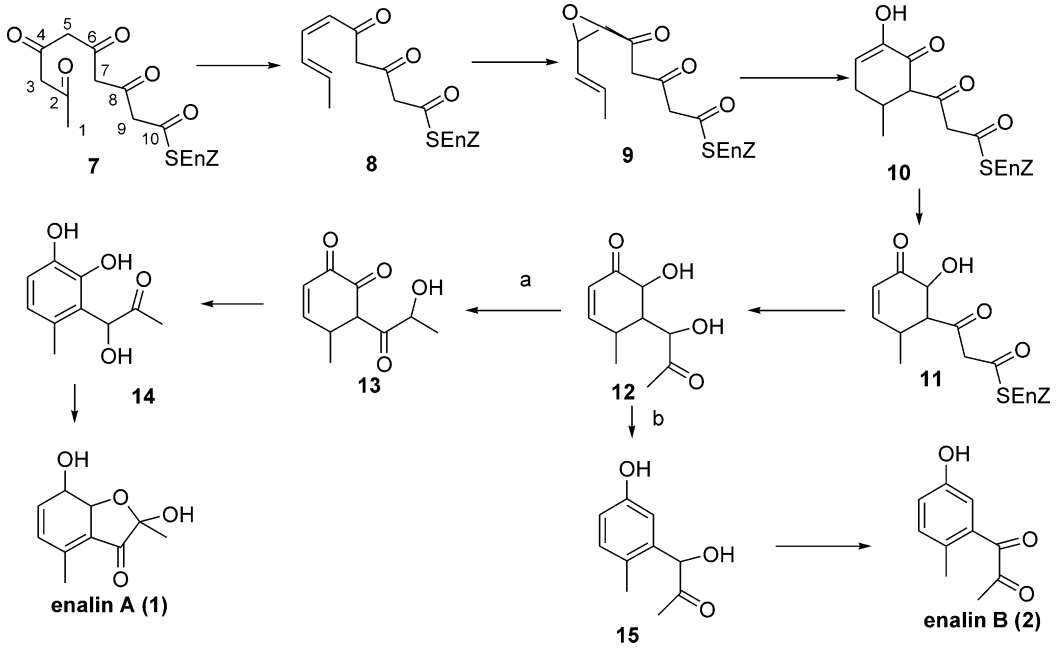
3. Experimental

3.1. General

NMR spectroscopic data were recorded on a Varian Inova 500NB NMR spectrometer, mass spectra on a VG–ZAB–HS mass spectrometer, IR spectra on Bruker EQUINOX 55 spectrophotometer, UV spectra on a Shimadzu UV-2501PC spectrophotometer, optical rotations on a Horiba High Sensitivity Polarimeter SEPA-300, and elemental analyses on a Elementar Vario EL CHNS-O elemental analyzer.

3.2. Fungal strain

A strain (No. 2606) of the fungus *V. enalia*, an ascomycete, was isolated from decayed wood of a *Cassurina*



Scheme 1. Possible biogenesis of enalin **A** and **B**.

tree from a salt lake in the Bahamas; voucher specimens are stored in the Department of Biology and Chemistry, City University of Hong Kong, Hong Kong, and the Department of Applied Chemistry, Zhongshan University, Guangzhou, PR China.

3.3. Culture conditions

Starter cultures (from Professor E.B.G. Jones and Dr. L.L.P. Vrijmoed) were maintained on cornmeal seawater agar. Plugs of agar supporting mycelial growth were excised and transferred aseptically to a 250 ml Erlenmeyer flask containing 100 ml liquid medium (glucose 5 g/l, peptone 1 g/l, yeast extract 0.5 g/l, beef extract 0.5 g/l, and natural sea water 50 ml/l). The flask was incubated at 30 °C on a rotary shaker for 5–7 days, and the mycelium was aseptically transferred to 500 ml Erlenmeyer flasks containing culture liquid (200 ml). The flasks were then incubated at 30 °C for 45 days.

3.4. Extraction and separation of metabolites

The cultures (120 l) were filtered through cheesecloth. The filtrate was concentrated to 5 l in vacuo below 50 °C and extracted three times by shaking with an equal volume of ethyl acetate. The combined organic extracts were applied to a silica gel column, then being eluted with a gradient of petroleum ether to ethyl acetate to offered compounds **3** (55 mg), **1** (510 mg), **2** (20 mg), **4** (50 mg), **5** (120 mg) and **6** (30 mg).

3.4.1. Compound *1*

Yellow color needles: mp 190–192 °C, $[\alpha]_D = -17.7°$; IR (KBr) 3435, 3211, 2996, 1679, 1630, 1630, 1596, 1413, 1382, 1285, 1214, 1170, 1093, 999 cm^{-1}; UV: λ^{ϵ}_{max} (MeOH) 234 nm (3164), 275 (3227), 358 nm (1044); for ^1H NMR ($CDCl_3$,TMS), ^{13}C NMR ($CDCl_3$) and 2D NMR, see Table 1; FABMS *m*/*z* 195 (M + 1), 177, 154, 107, 89, 77, 51. Anal. found: C 61.55, H 5.24, calc. (for $C_{10}H_{10}O_4$): C 61.86, H 5.16.

3.5. Compound *2*

Oily, FBMS *m*/*z* 179 (M + 1), 163, 146, 135, 107; for ^1H NMR ($CDCl_3$, TMS), ^{13}C NMR ($CDCl_3$) and 2D NMR, see Table 1; IR (KBr) 3402, 3060, 2966, 2928, 1707, 1673, 1610, 1575, 1499, 1300, 1224, 1158, 857, 586; UV: λ^{ϵ}_{max} ($CHCl_3$) 227 nm (6374), 230 (6525), 330 nm (1864). Anal. found: C 67.33, H 5.80, calc. (for $C_{10}H_{10}O_4$): C 67.42, H 5.618.

Table 1
NMR spectroscopic data for compounds **1** and **2**

		^{13}C	^1H	HMBC	COSY
1	1				
	2	104.4 (C)		H-10, OH-2	
	3	200.4 (C)		H-10, OH-2	
	4	130.2 (C)		H-6, 11	
	5	123.6 (CH)	6.67 (*d*, 8.5 Hz)	H-11	H-6, 11
	6	124.4 (CH)	7.05 (*d*, 8.5 Hz)	H-5, OH-7	H-5
	7	142.1 (C)		H-5, 6, OH-7	
	8	159.7 (C)		H-6, OH-7	
	9	118.6 (C)		H-5, 11	
	10	22.3 (C)	1.53 (*s*)	OH-2	
	11	16.8 (CH_3)	2.41 (*s*)	H-5	H-5
		OH-2	6.40 (*s*)		
		OH-7	8.32 (*s*)		
2	1	131.4 (C)		H-3	
	2	132.4 (C)		H-4, 6, 10	
	3	133.4 (CH)	7.13 (*d*, 8 Hz)	H-10	H-4, 10
	4	120.9 (CH)	6.97 (*dd*, 8, 2.5 Hz)	H-3. 6	H-3, 6
	5	153.6 (C)		H-3, 4, 6	
	6	118.3 (CH)	7.10 (*d*, 2.5 Hz)	H-4	
	7	194.3 (C)		H-6	
	8	201.6 (C)		H-9	
	9	26.1 (CH_3)	2.51 (*s*)		
	10	20.5 (CH_3)	2.43 (*s*)	H-3	H-3
	11	OH	6.48		

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