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Fungal depside, guisinol, from a marine derived strain of *Emericella unguis*

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Abstract

A marine isolate of the fungus *Emericella unguis* gave a new antibacterial depside, guisinol (**1**). The structure determination was based on mass spectrometry and NMR spectroscopical studies. Structurally **1** resembles the depsidones emeguisin A → C previously isolated from another strain of the same species. Five other isolates of *E. unguis* also produced the same qualitative profile of secondary metabolites including guisinol and dechloronidulin. Other species of *Emericella* did not produce any of these compounds and they are thus of chemotaxonomic significance. © 1998 Elsevier Science Ltd. All rights reserved.

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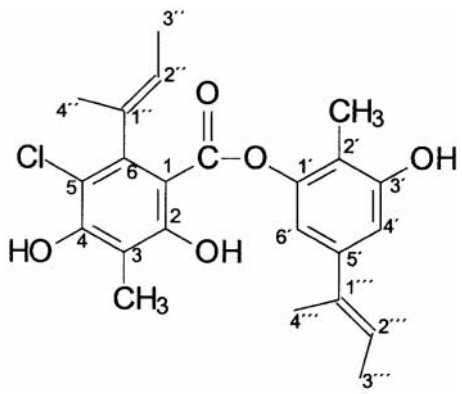
1. Introduction

Two isolates of *Emericella unguis* (anamorph: *Aspergillus unguis*) were obtained from a mollusc and a medusa collected in Venezuelan waters in 1997. From *E. unguis* several secondary metabolites of the depsidone type, 2-chlorounguinol, emeguisins A → C, and 3-ethyl-5,7-dihydroxy-3,6-dimethylphthalide were previously described (Kawahara, Nakajima, Satoh, Yamazaka, & Kawai, 1988; Kawahara, Nozawa, Nakajima, Kawai, & Yamazaki, 1988). The latter compound is believed to be involved in the biogenesis of the depsidones (Kawahara, Nakajima, Satoh, Yamazaka, & Kawai, 1988). In addition, nornidulin, nidulin and unguinol have also been reported from *Emericella nidulans* (anamorph *A. nidulans*) and *A. mellinus* (Turner & Aldridge, 1983), but the latter two organisms have been shown to be *E. unguis* (Samson, 1979). Furthermore haiderin, rubinin, shirin and nasrin has been isolated from *E. unguis* (Kamal, Haider, Qureshi, & Khan, 1970).

In a preliminary screening extracts of the two isolates of *E. unguis* exhibited strong antibiotic activity against *Staphylococcus aureus*. Based on the HPLC profile of secondary metabolites one of the isolates was selected for further investigation. The residue from the ethylacetate:chloroform:methanol (3:2:1) extract was partitioned between heptane and methanol containing 20% H_2O. Column chromatography of the polar fraction led to the isolation of an antibacterial depside, guisinol (**1**).

2. Results and discussion

The molecular ions at *m*/*z* 416 and 418 in a ratio of 3:1 suggested the presence of one chlorine atom and hence the molecular formula $C_{23}H_{25}O_5Cl$. The ^{13}C



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NMR spectrum confirmed the presence of 23 carbon atoms and revealed the presence of one ester carbonyl carbon at δ 169.6. The ^{1}H NMR spectrum (Table 1) of **1** displayed signals from two *meta* coupled (COSY) protons at δ 6.72 (1H, d, $J = 2$ Hz) and 6.57 (1H, d, $J = 2$ Hz) assigned to H-6′ and H-4′. A set of signals at δ 5.84 (1H, dq, $J = 1$, 6 Hz), 1.76 (3H, dd, $J = 1$, 6 Hz), and 1.96 (3H, t, $J = 1$ Hz) were shown by a single bond HMQC experiment to correlate with carbon signals at δ 122.8, 14.2, 15.2, respectively, indicating the presence of a 1-methylprop-1-enyl group. Multiple bond HMQC revealed these proton signals to be correlated to the olefinic carbon at 143.2, confirming the presence of a 1-methylprop-1-enyl group in the compound. The same pattern was observed for the proton signals at δ 5.31 (1H, dq, $J = 5$, 1 Hz), 1.72 (3H, dd, $J = 6$, 1 Hz), and 1.95 (3H, t, $J = 1$ Hz) which correlated with the carbon signals at δ 122.9, 13.5, and 17.0, respectively, and were assigned to another 1-methylprop-1-enyl group.

The remaining proton signals corresponded to two methyl groups (δ 1.99 and 2.20) and three hydroxylic protons (δ 3.49, 6.25 and 11.30) exchangeable with D_2O. The remaining 14 signals in the ^{13}C NMR spectrum corresponded to 12 aromatic carbon atoms and two methyl groups. The remaining DBE of 8 and comparison of the ^{13}C NMR values (Table 1) with emeguisin A of mass 414, suggested **1** to be a hydrogenated depsidone. The appearance of a new aromatic proton (δ 6.72) and an additional hydroxylic proton signal indicated an opening of the ether bond. This is supported by the low field carbon signal (δ 143.5, C-5a) in emeguisin A being replaced by a high field signal (δ 110.8) in **1**.

Table 1
NMR data for guisinol **1**[a]

Position	δ_H	δ_C	HMQC	COSY
1		104.9		
2		154.4		
3		112.1		
4		160.8		
5		111.3		
6		135.0		
1′		154.3		
2′		115.1		
3′		149.5		
4′	6.57 d (2)	110.2	C2′,C3′,C5′,C6′	H6′
5′		134.0		
6′	6.72 d (2)	110.8	C1′,C2′,C4′,C5′	H4′
CO		169.6		
1″		143.8		
2″	5.31 dq (1,5)	122.9	C1″, C3″, C4″	H3″,H4″
3″	1.72 dd (1,6)	13.5	C6, C2″	H2″,H4″
4″	1.95 t (1)	17.0	C1, C1′, C2′	H3′,H1′
1‴		143.2		
2‴	5.84 dq (1,6)	122.8	C1‴, C3‴, C4‴	H3‴,H4‴
3‴	1.76 dd (1,6)	14.2	C5′, C2‴	H2‴,H4‴
4‴	1.96 t (1)	15.2	C5′, C1‴, C2‴	H3‴,H1‴
3-Me	2.20 s	8.7	C2, C4, C5[b]	
2′-Me	1.99 s	9.0	C1′, C2′, C3′	
4-OH	3.49			
2-OH	11.30		C1, C2, C4[b]	
3′-OH	6.25		C1′[b], C4′	

[a] J values are given in parentheses (Hz).
[b] Denotes a four-bond coupling. Has been observed previously in a similar compound (Poch & Gloer, 1991)

The substituent patterns of the aromatic rings were inferred from a multiple bond HMQC experiment. Two proton signals at δ 6.72 and 6.57 were assigned to H-6′ and H-4′ based on long range correlations (Table 1). *A priori*, the position of the chlorine atom could be on either C4 or C5. Calculated ^{13}C δ values only agreed with C5-Cl and C4-OH. Moreover the location of the chlorine atom on the A-ring was supported by the EIMS fragmentation pattern. Depsides exhibit prominent molecular ions in the mass spectrum with concomitant cleavage of the depside bond forming two fragments (Hunneck et al., 1968). Thus the mass spectrum of guisinol exhibited significant peaks at m/z 239/241 (ratio 3:1), 211/213 (ratio 3:1) confirming the presence of a chlorine atom on the carbonyl fragment. A prominent peak at m/z 178 corresponded to the remaining fragment. Furthermore, the choice of C5-Cl is in accordance with a previous X-ray structure of 2-chlorounguinol (Kawahara, Nakajima, Satoh, Yamazaka & Kawai, 1988) isolated from the same species. The position of the remaining substituent was determined from the HMQC correlations shown in Table 1.

The stereochemistry of the 1-methylprop-1-enyl groups are *E*, based on the upfield shift of the *cis* methyl groups. Calculated ^{13}C δ values shows a shift from low field (*Z*) to upfield (*E*). Furthermore, this is supported by an observed NOE effect on C4″ and C4‴, when C3″ and C3‴ has been irradiated respectively. This is in agreement with findings for other depsidones with 1-methylprop-1-enyl substituents (Kawahara, Nozawa, Nakajima, Kawai & Yamazaki, 1988; Poch & Gloer, 1991; Hamano et al., 1992).

Depsides are best known as lichen metabolites. Guisinol is the first depside to be isolated from *E. unguis*. The structure of **1** is related to the depsidones emeguisins A → C (Kawahara, Nozawa, Nakajima, Kawai & Yamazaki, 1988), previously known from another strain of *E. unguis*. Depsidones are generally accepted to be biosynthesized from depsides with direct formation of the seven-membered ring by oxidative coupling (Turner & Aldridge, 1983). Guisinol is thus a possible precursor for depsidones of the emeguisin A → C types.

Guisinol showed antibacterial activity against *S. aureus* (5 mg/ml DMSO, 15 μl added to a 4 mm well).

Inhibition was not observed when guisinol was tested against the Gram negative *Vibrio parahaemolyticus*.

Four other isolates of *E. unguis* were screened for production of guisinol and in all cases this depside was produced in addition to dechloronidulin. A series of isolates of other *Emericella* species were screened for production of guisinol and dechloronidulin (*E. falconensis*, *E. lata*, *E. navahoensis*, *E. nidulans*, *E. quadrilineata*, *E. rugulosa*, *E. similis*, *E. sublata*, *E. variecolor*, *E. violacea*), they were not detected in any instance. Thus guisinol and dechloronidulin appears to be specific indicators of the species *E. unguis*.

3. Experimental

3.1. General procedures

NMR spectra were recorded in $CDCl_3$ on a Varian 400 FT-NMR spectrometer at 400.0 and 100.6 MHz for ^1H and ^{13}C NMR spectra, respectively. The HPLC data were obtained on a HPLC system combined with a Millenium 996 photodiode array detector from Waters. The UV spectrum was recorded on Hewlett Packard 8452A diode array spectrophotometer.

3.2. Collection, isolation and fermentation

Two strains of *E. unguis* (isolate 1 (M87-2) and 2 (M90B-10)) were collected in the Paria Bay, Venezuela, in January 1997. Isolate 1 originated from a *Stomolopus meliagris*, isolate 2 from the soft part of an unidentified mollusc. Further isolates of *E. unguis* (IBT 16746 from Galapagos Islands, IBT K546 from Bahamas, IBT M101-1B, the latter also from Venezuela) and 42 isolates of other species of *Emericella* were screened for production of guisinol and dechloronidulin using authentic standards and HPLC with diode array detection (Frisvad & Thrane, 1987). A comparison of the metabolite profiles obtained from HPLC analysis, showed that two isolates of *E. unguis* (M87-2 and M90B-10) express the same metabolites, although in variable amounts. Isolate 1 was chosen for further metabolite studies, due to the high amount of metabolites. The fungus was grown on solid YES medium, (yeast extract, 20 g/l; sucrose, 150 g/l) for a period of 14 days at 25°C.

3.3. Extraction and separation

Mycelium and agar of isolate 1 were harvested and extracted with a mixture of ethylacetate:chloroform: methanol (3:2:1) containing 1% formic acid. The dried extract was defatted by partition between methanol:water (80:20) and hexane. The polar fraction (7 g) was separated by VLC. The column was packed with RP-18 (40–63 μm) and the fractionation performed with an increasing gradient of methanol:water (60:40 → 100:0) containing 1% formic acid. The methanol:water (90:10) fraction was further purified on a Waters RCM Prep Nova-pak HR C18 6 μm column coupled with a Waters HPLC system with photo diode array detection. The four fractions include nornidulin (120 mg) and compound **1** (40 mg).

Guisinol (**1**) was obtained as yellowish oil; λ_{max}^{MeOH} nm (log ϵ): 318 (3.81), 254 (4.09), 216sh(4.53) nm; NMR data, see Table 1; HREIMS m/z 416.1396 ($\Delta = +1.3$ ppm)(11)/ 418(4), 239(100)/ 241(38), 211(14)/213(5), 178(89), 163(21).

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