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# Fungal Metabolites: Tetrahydroauroglaucin And Isodihydroauroglaucin From The Marine Fungus, *Eurotium* Sp.

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**Abstract:** Two poly substituted aromatic compounds: tetrahydroauroglaucin **1** and isodihydroauroglaucin **2** were identified from a marine fungus, *Eurotium* sp. isolated from leaves of the mangrove, *Porteresia coarctata* (Roxb). These compounds were reported earlier from terrestrial fungal source of the same genus. The structures of these compounds were finalized from their spectral data and comparison with literature values. Complete  $^1\text{H}$  and  $^{13}\text{C}$  NMR signal assignments are reported here for the first time based on extensive 2D NMR studies including COSY, HMQC, HMBC.

**Keywords:** Cephalosporin, mangrove, metabolites, penicillin, antibiotics.

## Introduction

Marine organisms and fungi are well-known source for novel biologically active secondary metabolites. However, only recently have marine microorganisms such as fungi, bacteria, etc., received broader attention of biologists and chemists<sup>1</sup>. To date more than 100 metabolites from different marine fungi have been described<sup>2</sup>. This includes the antibiotics: penicillin, cephalosporin, the immunosuppressant agent: cyclosporin A, etc.

As part of our ongoing program on chemical examination of marine micro organisms, we examined the marine fungus *Eurotium* sp. isolated from the leaves of the mangrove plant *Porteresia coarctata* (Roxb) from Goa. Herein we report isolation, purification and structure determination of two known metabolites: tetrahydroauroglaucin [2-(1E-heptenyl)-3,6-dihydroxy-5-(3-methyl-2-butenyl)- benzaldehyde **1**] and isodihydro auroglaucin **2** 2-(3E,5E-heptadienyl)-3,6-dihydroxy-5-(3-methyl-2-butenyl)-benzaldehyde **2**]. These compounds were earlier reported from terrestrial fungi *Eurotium* sp<sup>3</sup> and *Aspergillus ruber*<sup>4</sup>. The complete NMR assignments for both the compounds based on extensive 2D NMR studies are discussed here for the first time

## Materials and Methods

The fungus *Eurotium* sp. (NIO FMB #001) was obtained from the leaves of the mangrove plant *Porteresia coarctata* from Goa coast. The fungal isolate was cultivated on potato dextrose agar (PDA) slants for 10 days. After proper mycelial growth, the cultured mycelia were transferred into 500ml flasks containing 100ml potato dextrose broth (PDB). PDB was prepared in 1:1, seawater: distilled water. The flasks were incubated in a rotating shaker (240 rpm) for about 4-5 days. The cultured mycelia were then transferred to 5 lit. flasks, each containing 1 lit. of the same liquid culture medium PDB and incubated for 15

days at room temperature. At the end of 15 days, fungal mat was filtered, washed with water and extracted with acetone (5 gms). The crude extract was initially separated into four sub fractions by Gel Permeation Chromatography (Sephadex LH-20,  $\text{CHCl}_3$ -MeOH, 1:1). Fraction 3, rich in aromatic aldehydes as indicated by  $^1\text{H}$  NMR spectrum, was further purified on silica gel columns (Petroleum ether-EtOAc) to yield compounds 1 and 2 in the pure form.

## Results and Discussion

Compound 1 exhibited strong  $[\text{M}+\text{H}]^+$  and  $[\text{M}+\text{Na}]^+$  peaks at 303 and 325 respectively, indicating its molecular weight to be 302. Its  $^{13}\text{C}$  NMR and DEPT spectra indicated 19 carbon signals: three methyls, five methylene, five methines and six quaternary carbons. The signals at  $\delta$  11.72(1H,s), and 5.23 (1H, brs) were assigned to one bonded and another non-bonded hydroxyl groups. The signal at  $\delta$  10.09(1H, s) and 196.3(d) indicated presence of an aldehyde group. The downfield signal of one of the phenolic protons ( $\delta$  11.72) as well as the shift in aldehyde absorption frequency in IR spectrum ( $1622\text{ cm}^{-1}$ ) indicated ortho hydroxy benzaldehyde moiety. The molecular formula of the compound was determined to be  $\text{C}_{19}\text{H}_{26}\text{O}_3$  from the above results, indicating an index of hydrogen deficiency of seven. These could be accounted for by the benzene ring, aldehyde and two unsaturations. The  $^1\text{H}$  NMR and COSY spectrum also indicated presence of 3-methyl-2-butenyl and 1-heptenyl moieties. The coupling constant ( $J=16.15\text{Hz}$ ) of vinyl protons of the latter alkyl chain indicated its stereochemistry as E. The structure of this molecule was finalized as tetrahydroauroglaucin [2-(1E-heptenyl)-3,6-dihydroxy-5-(3-methyl-2-butenyl)-benzaldehyde] 1 from the above data<sup>4</sup>. This was further confirmed by extensive 2D NMR studies including COSY, HMQC and HMBC, which also helped in assignment of all proton and carbon signals (Table 1).

$^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of compound 2 indicated its structure to be similar to that of compound 1. The strong pseudomolecular ion  $m/z$  323 ( $\text{M}+\text{Na}$ ) indicated its molecular weight to be 300, two units lower than that of compound 1. The difference was in the appearance of terminal methyl group of C7 chain as a doublet (1.78 ppm, 7Hz), and presence of two conjugated double bonds in that chain. The stereochemistry of these double bonds were assigned as E,E in agreement with those of isodihydroauroglaucin and  $^{13}\text{C}$  NMR chemical shifts. The structure of this compound was finalized as 2-(3E,5E-heptadienyl)-3,6-dihydroxy-5-(3-methyl-2-butenyl)-benzaldehyde 2 from the above results. Detailed  $^1\text{H}$  and  $^{13}\text{C}$  NMR assignments based on extensive COSY, HMQC, HMBC correlations are given in table 2.

## Conclusion

Compounds 1 & 2 are typical fungal metabolites known to possess anti-oxidant properties and exhibit synergism with tocopherol.<sup>3</sup> They were initially isolated from terrestrial fungi belonging to the genus *Aspergillus* and *Eurotium*. This is the first report of the isolation of these compounds from a marine fungus, also belonging to the genus *Eurotium*. Earlier the structures of these compounds were finalized mainly from 1D NMR studies. The present

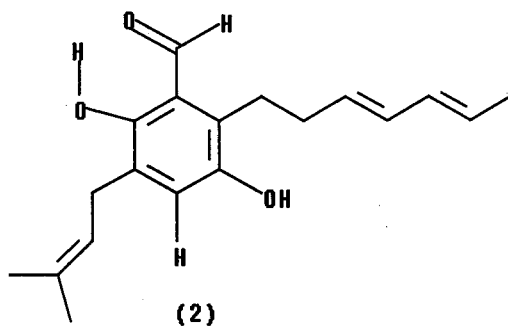
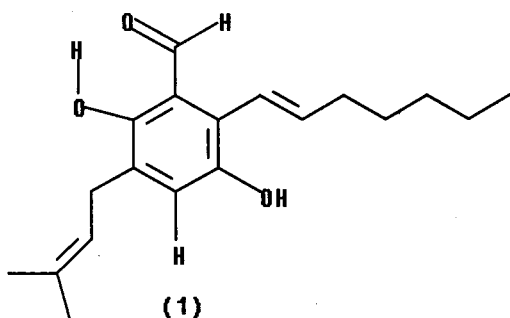
study has confirmed the structures of these two compounds and also helped in assigning all the proton and carbon signals unambiguously based on extensive 2D NMR experiments mentioned above.

**Table 1:**  $^1\text{H}$  and  $^{13}\text{C}$  NMR assignments and HMBC correlations of 2-(1E- heptenyl)-3,6-dihydroxy-5-(3-methyl 2-butenyl)-benzaldehyde 1.

Carbon no.	$^1\text{H}$ NMR	$^{13}\text{C}$ NMR	HMBC correlations
CHO	10.09(1H,s)	196.3	117.1, 130.2 155.1
1		117.1	
2		124.6	
3		144.6	
4	7.02 (1H,s)	125.1	27.2,124.6, 144.6,155.1, 130.2, 118.5
5		130.2	
6		155.1	
1'	3.31 (2H,d, J= 7.44Hz)	27.2	121.0, 125.1, 130.2, 133.8, 155.1
2'	5.29(1H,t, J=7.73Hz)	121.0	17.7, 27.2
3'		133.8	
4'	1.66 (3H,s)	17.7	25.7, 121.0, 133.8
5'	1.76 (3H,s)	25.7	17.7, 121.0, 133.8
1''	6.48(1H,d, J=16.15Hz)	120.1	33.4, 117.1, 124.6, 144.6
2''	5.97 (1H,dt, J=16.12,6.8Hz)	142.6	33.4,124.6
3''	2.32 (2H,q, J=7.02,6.79Hz)	33.4	28.7, 31.4, 120.1, 142.6
4''	1.52 (2H,m)	28.7	31.4, 33.4
5''	1.36(2H,m)	31.4	13.9, 22.4, 28.7
6''	1.34 (2H,m)	22.4	13.9, 28.7, 31.4
7''	0.92 (3H,t, J=6.76Hz)	13.9	22.4, 31.4
	11.72 (1H,s)		117.1, 130.2 155.1
	5.23(1H,s, exchanged with D <sub>2</sub> O)		

**Table 2 :**  $^1\text{H}$  and  $^{13}\text{C}$  NMR and HMBC values of 2-(3,5-heptadienyl)-3,6-dihydroxy-5-(3-methyl-2-butenyl)-benzaldehyde 2.

Carbon no.	$^1\text{H}$ NMR	$^{13}\text{C}$ NMR	HMBC correlations
CHO	10.23 (1H,s)	195.3	117.2,127.4,155.7
1		117.2	
2		127.4	
3		145.1	
4	6.92 (1H,s)	125.8	127.4,145.1,155.7
5		133.1	
6		155.7	
1'	3.34 (2H,d, J=7.18Hz)	26.9	121.1,133.7,155.7
2'	5.27 (1H,t, J=6.09Hz)	121.1	-----
3'		133.7	
4'	1.73 (3H,s)	17.6	121.1, 133.7, 25.6
5'	1.83 (3H,s)	25.6	121.1, 133.7, 17.6
1''	2.81(2H,t, J=7.30Hz)	23.9	145.1, 117.2, 127.4, 34.2
2''	2.37(2H,m)	34.1	131.0,23.9
3''	5.62(1H,m)	131.8	-----
4''	5.99(1H,m)	128.8	-----
5''	5.99(1H,m)	129.3	-----
6''	5.62(1H,m)	131.1	-----
7''	1.78(3H,d, J=7.0H)	17.9	129.3
	11.93(1H,s)	-----	117.2,127.4, 155.7, 128.7
	4.70(1H,s,exchanged with D <sub>2</sub> O)		



## Experimental Section

FTIR spectra were recorded on a Shimatzu spectrophotometer (8201PC). NMR ( $^1\text{H}$ ,  $^{13}\text{C}$ , COSY, HMQC & HMBC) spectra were recorded on a Bruker (Avance 300) spectrometer. Mass spectra were obtained on PE Sciex-QSTAR. All solvents were distilled prior to use.

**2-(1-Heptenyl)-3,6 dihydroxy-5 (3-methyl 2-butenyl)-benzaldehyde :** Yellow crystals, MP 70°C; IR (KBr)  $\nu_{\text{max}}$ : 3265, 2924, 2856, 1622, 1606, 1581, 1488, 1261, 983, 958, 889, 704  $\text{cm}^{-1}$ ; For  $^1\text{H}$ ,  $^{13}\text{C}$  NMR, and HMBC values, see Table 1; MS m/z: 325(M+Na) $^+$ , 303(M+H) $^+$ , 282, 247, 245, 229, 215, 149 and 114.

**2-(3,5-Heptadienyl)-3,6-dihydroxy-5- (3-methyl-2-butenyl)-benzaldehyde:** Orange crystal, IR( Neat)  $\nu_{\text{max}}$ : 3417, 2925, 2854, 1643, 1434, 1296, 1128, 993 and 761 $\text{cm}^{-1}$ ; For  $^1\text{H}$  &  $^{13}\text{C}$  NMR values and HMBC correlations, see Table 2; MS m/z: 323(M+H) $^+$ , 301 (M+H) $^+$ , 299, 231, 215, 149 and 114

## Acknowledgment

We express our deep gratitude to Dr.E.Desai, Director, NIO, Goa, for constant encouragement and facilities. Thanks are also due to Shri. Umesh Pawa (Labindia, New Delhi) for recording MS data and Shri. Siddharth Bhonsle, NCL, Pune & Dr. Alka Pandey, Agharkar Research Institute, Pune, for taxonomical studies of the fungus. Financial support in the form of Research Associateship from CSIR for one of the coauthors (PD) and Project Assistantship from DOD for two coauthors (ST and DG) are also sincerely acknowledged

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