



Biologically Active Anthraquinone Analogs from the Fungus *Eurotium* Sp.

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Four known anthraquinones: physcion, fluoroglauclin, catenarin and alaternin as well as a cyclic dipeptide with a triprenylated indole moiety, echinulin were purified from acetone extract of the mycelial mats of the fungus *Eurotium* sp. isolated from the leaves of the mangrove plant *Porteresia coarctata* (Roxb.) These compounds have previously been reported as fungal / angiosperm metabolites exhibiting anti-bacterial, anti-oxidant and cytotoxic properties. The structures of these compounds were finalized from their spectral data including 2D-NMR (COSY, HMQC & HMBC) studies, which also helped in the assignment of their proton and carbon signals.

Introduction

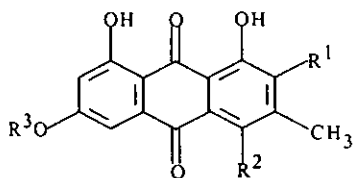
Microorganisms are a good source for novel bioactive compounds. The beta lactam antibiotics: penicillins, cephalosporins and the cyclic peptide, cyclosporin, widely used as immunosuppressant agent are some of the examples from fungal sources, while saframycins, the dimeric isoquinoline alkaloids, tetracyclins, etc., are potent antibiotics of bacterial origin. In addition, microorganisms also produce several useful compounds such as food additives, anti-oxidants, bio-pigments, etc.

As part of our ongoing programme on chemical examination of marine microorganisms, we have purified several compounds from a fungus, *Eurotium* sp., isolated from the leaves of the mangrove plant *Porteresia coarctata* (Roxb.) from Goa. Earlier we had reported isolation and structure determination of two antioxidants, tetrahydroauroglauclin and isodihydroauroglauclin from this source¹. Continuing our work, we herein report isolation and structure determination of 4 anthraquinone derivatives: physcion 1, fluoroglauclin 2, alaternin 3 and catenarin 4 as well as a cyclic peptide (diketopiperazine), echinulin 5, having a triprenylated indole moiety (Fig 1).

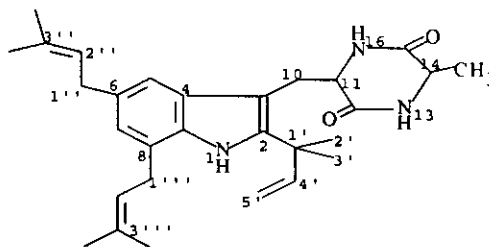
Materials and Methods

The fungus *Eurotium* sp. (NIO FMB #001) was obtained from the leaves of the mangrove plant *Porteresia coarctata* (Roxb.) from Goa coast. The fungal isolate was cultured on potato dextrose agar (PDA) slants for 10 days. After proper mycelial

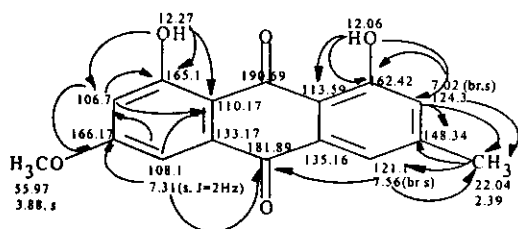
growth, the cultured mycelia were transferred into 500 ml flasks containing 100 ml of potato dextrose broth (PDB). PDB was prepared in 1:1 ratio of seawater and distilled water. The flasks were incubated in a rotating shaker (240 rpm) for about 4-5 days. The cultured mycelia were then transferred to 5 liter flasks, each containing 1 liter of the same liquid culture medium, PDB and incubated for 15 days at room temperature. At the end of 15 days, the fungal mat was filtered, washed with water and extracted with acetone (5 gm). The crude extract was purified over Sephadex LH-20 and silica gel columns repeatedly, yielding compounds 1-5



1. Physicon : R¹=R²=H, R³=CH₃
2. Fluoroglaucin : R¹=H, R²=OH, R³=CH₃
3. Alaternin : R¹=OH, R²=R³=H
4. Catenarin : R¹=R³=H, R²=OH



echinulin 5



HMBC of Physicon 1

Figure 1: Structures of compounds 1-5 alongwith their NMR data

Results and Discussions

The ¹H and ¹³C NMR spectrum of compound 1, [M+H]⁺=285, indicated presence of one O-Me (δ 3.88 s / 55.97 q), one vinyl Methyl (δ 2.39 s / 22.04 q), four aromatic protons in the region δ 6-6 to 7.6 and two phenolic hydroxyl groups (δ 12.06 s & 12.27 s). Its ¹³C NMR and DEPT 135 spectra indicated presence of 16

carbon atoms distributed as methyls-2, methines-4 and quarternary carbons-10 (Table 1). The carbon signals at δ 181.89 and 190.69 were indicative of the presence of quinone carbonyl groups. Comparison of these spectral values with those reported in literature indicated the compound to be physcion 1, the anthraquinone pigment isolated from several plants and fungi². The structure was further confirmed using 2D-NMR experiments such as COSY, HMQC and HMBC, which also led to the unambiguous assignment of all the NMR signals (Table 1, Structure 2).

Table 1: ¹H, ¹³C NMR and HMBC correlations of Physcion 1 and Alaternin 3

Carbon No. of 1	Carbon Chemical Shift (δ)	Proton chemical shift	HMBC Correlations	Carbon chemical shift (ppm) of 3	Proton Chemical Shift (ppm)	HMBC correlations
1	162.41(s)	12.06(OH)	162.41, 124.3, 113.59	167.88 (s)?		
2	124.3 (d)	7.02(brs)	162.4, 113.59, 22.04, 121.1	158.33 (s)		
3	148.34(s)			144.14 (s)		
4	121.1(d)	7.56(brs)	22.04, 181.89, 124.3, 113.59	120.48 (d)	7.47 (brs)	113.58, 129.68, 180.78, 18.80
4a	135.16(s)			129.68 (s)		
5	108.1(d)	7.31(d,2Hz)	181.89, 110.17, 166.47, 106.7	108.75 (d)	7.06 (2Hz)	180.78, 108.75
6	166.17(s)			165.78 (s)		
7	106.7(d)	6.63(d,2Hz)	165.1, 110.17, 108.1, 166.47	107.64 (d)	6.48 (2Hz)	165.78, 108.75
8	165.1(s)	12.27(OH)	165.1, 110.17, 106.7	165.04 (s)		
8a	110.17(s)			111.58 (s)		
9	190.69(s)			189.73 (s)		
9a	113.59(s)			113.58 (s)		
10	181.89(s)			180.78 (s)		
10a	133.17			134.72 (s)		
Vinyl Me	22.04(q)	2.39(s)	148.34, 121.1, 124.3	18.80 (q)		120.48, 129.68, 144.14
Ome	55.97(q)	3.88(s)	166.47			

The IR spectrum of compound 2, $[M+H]^+=301$, had peaks at 3400, 1645, 1434, 1301, 1272 and 772cm^{-1} . Its ¹H and ¹³C NMR spectra were similar to that of compound 1, but for the absence of the proton signal at δ 7.56 (4-H in physcion) and the appearance of an additional hydroxyl proton signal at δ 13.32 (Table 2). The structure of this compound was finalized as 4-hydroxy physcion (fluoroglauicin) 2 from these results (Table 2).

The more polar fraction was insoluble in chloroform, but freely miscible with methanol. Its ¹H NMR spectrum indicated it to be a 2:1 mixture of two anthraquinone derivatives. Careful purification of this mixture over silica gel column

yielded the more abundant compound **3** in pure form $[M+H]^+=287$. The 1H NMR spectrum of compound **3** recorded in MeOH-*d*₄ had three aromatic proton signals at δ 7.37 (1H, brs), 6.95 (1H, d, $J=2$ Hz), and 6.40 (1H, d, $J=2$ Hz) and one vinyl methyl peak at δ 2.38 (3H, s). Its ^{13}C NMR spectrum was similar to that of Physcion **1**, but for the absence of the carbon signal at δ 124.3(d) and the appearance of a new signal at δ 158.33(s), indicating hydroxylation of that carbon atom (Tables 1 & 2). The structure of this compound was finalized as 2-hydroxy emodin (alaternin) **3** from these results. Compound **4**, $[M+H]^+=287$, was evidently isomeric with compound **3**. The only difference in its 1H NMR spectrum was the disappearance of the proton signal at δ 7.37 and the presence of a new signal at δ 6.82, indicating the shift of the hydroxyl group from C-2 to C-4 position. The vinyl methyl signal now appeared slightly up field, at δ 2.15 (Table 2). The structure of this compound was finalized as 4-hydroxy emodin (catenarin) from these results.

The molecular formula of compound **5**, $[M+H]^+=462$, $[M+Na]^+=484$ and $[M-H]^-=460$ was calculated to be $C_{29}H_{38}N_3O_2$. The strong peak at 1650 cm^{-1} in its IR spectrum indicated presence of an amide group. The 1H NMR spectrum of this compound had one methyl doublet and six methyl singlets in the region δ 1.4-2.0. Its ^{13}C NMR spectrum indicated the presence of 29 carbon signals as expected (Table 3). The coupling of the doublet signal at δ 1.48 ($J=7$ Hz) to the signal at δ 4.04 (1H, brq, 7Hz) indicated presence of the amino acid alanine. Detailed analysis of the HMQC and HMBC spectra of this molecule indicated presence of three prenyl groups, thus accounting for all the remaining methyl signals. The rest of the carbon, hydrogen and nitrogen atoms neatly fitted with that of the amino acid, tryptophan. Apparently, the three prenyl groups are attached to this amino acid. The structure of this molecule was thus confirmed to be echinulin **6**, a metabolite produced by several fungi and plants. The stereochemistry of this compound is assumed to be SS (as in natural L-amino acids), as reported for similar compounds earlier³.

Anthraquinones as well as auroglaucin derivatives, found in several plants and fungi, are well known anti-oxidants. Choi *et. al.*⁴, had evaluated several anthraquinone analogs for inhibition of peroxidation of linoleic acid by thiocyanate method⁴. They observed that alaternin is very efficient as it inhibits both oxygen and nitrogen mediated peroxidation in a dose dependent manner. It is also non-hepatotoxic and anti-mutagenic. Wakulinski *et. al.*⁴, had studied 88 fungal strains for anthraquinone production and found that almost 29 % of them produce catenarin. They also observed that a high concentration of this pigment is present in those organisms, which lack ability to biosynthesize melanins. Catenarin also inhibited the growth of other fungi, the most sensitive species being *Epicoccum nigrum*, whose

growth is inhibited upto 90%⁴. Besides anthraquinones are also known to inhibit sulfide production by sulfate-reducing bacteria *Desulfovibrio desulfuricans* in sewage, oil wells, process tanks or biomass fermentation⁵.

At the same time, it did not inhibit the growth of other microorganisms such as *Thiobacillus denitrificans* or *Escherichia coli*. Echinulin, the diketopiperazine isolated from several angiosperms and fungi has been reported to be active against *Mycobacterium tuberculosis*³.

While going through the literature on anthraquinone pigments, it was noticed that a vast majority of them have one methyl group and several hydroxyl or methoxyl groups in the aromatic rings. Depending upon the substitution pattern, three ways of systematic nomenclature are in vogue for these compounds, which mention the same methyl substituent in position 2, 3 or 6. Thus, the systematic name for Physcion and alaternine are 1,8-dihydroxy - 6 - methoxy - 3 - methyl-9,10-anthraquinone and 1,2,6,8 - tetra-hydroxy-3-methyl - 9,10 -anthraquinone, while fluoroglaucin is known as 1,4,5-trihydroxy - 7 -methoxy-2-methyl-9,10-anthraquinone and emodin is 1,3,8-trihydroxy - 6 - methyl - 9,10 - anthra- quinone. The problem becomes more complicated while describing 2-hydroxy emodin and its 7-hydroxy isomer. If the new hydroxyl group is between the methyl and the hydroxyl groups (alaternin), it is known as 1,2,6,8-tetrahydroxy - 3 - methyl - 9,10-anthra- quinone⁶. But, when the new hydroxyl group is between the two hydroxyl groups of emodin (7-hydroxy analog of alaternin), it is known as 1,2,3,8-tetra hydroxy-6-methyl anthraquinone⁷. Due to this, some workers name both the isomers as 2-hydroxy emodin. This has led to some confusion, leading, in certain cases, to erroneous identification of the latter isomer of alaternin as alaternin [641-90-7]⁷ itself.

Table 2: ¹H NMR values of compounds 1-4.

Proton No.	Physcion 1	Erythroglaucin 2	Alaternine 3	Catenarin 4
1-OH	12.06 (s)	12.31 (s)	---	---
2-H / OH	7.02(brs)	7.08(brs)	---	6.82 (brs)
3-Me	2.39 (s)	2.31 (s)	2.38 (s)	2.15 (s)
4-H / OH	7.56 (brs)	13.32 (s)	7.37 (brs)	---
5-H	7.31 (2Hz)	7.36 (2Hz)	6.95 (2Hz)	6.92 (2Hz)
6-OH/ O Me	3.88(s)	3.90 (s)	---	---
7-H	6.63 (2Hz)	6.65 (2Hz)	6.40 (2Hz)	6.36 (2Hz)
8-OH	12.27	12.40 (s)	---	---

Table 3: ¹H, ¹³C NMR and HMBC correlations of echinulin 5

Carbon No	¹³ C NMR	¹ H NMR	HMBC	Carbon No	¹³ C NMR	¹ H NMR	HMBC
1-NH	---	8.00	141.3, 104.02, 132.16	16-NH	---	5.94	
2	141.3			1'	38.90		
3	128.94			2'	27.74	1.46	27.74, 38.90
4	104.02			3'	27.74	1.46	27.74, 38.90
5	114.97	7.08	104.02, 131.5, 123.4, 34.50	4'	145.70	6.04(dd, 10.5, 17.6)	38.90,
6	123.34			5'	112.24	5.10(d,11) 5.11(d,17)	38.90
7	122.80	6.76	132.16, 31.25, 123.4, 114.97	1''	34.50	3.35(d7.1)	123.4, 122.8, 114.97, 124.4
8	132.16			2''	124.40	5.30	
9	131.5			3''	133.81		
10	29.41	3.13(dd, 11.7,14.7) 3.63 (dd, 14.7,3.6)	54.6, 128.94, 104.02 ---	4''	17.80	1.69(s)	25.61, 133.8, 124.4
11	54.60	4.34 (dd, 2.4, 11.6)		5''	25.61	1.69(s)	133.8, 17.7, 124.4
12	168.34			1'''	31.25	3.48(d, 7.3)	122.8, 131.5, 132.1
13,NH	---	5.62	50.73, 168.34	2'''	122.8	5.37(brt, 7.4)	
14-H	50.73	4.04	19.80	3'''	132.9		
14-Me	19.80	1.48(d,7)	167.73, 50.7	4'''	17.8	1.82(s)	25.6, 122.8, 132.9
15	167.73			5'''	25.61	1.76(s)	17.8, 122.8, 132.9

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