Pergamon

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Isolation of a Fluorone Pigment from the Indian Paint Fungus *Echinodontium tinctorium* and *Pyrofomes albomarginatus*

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Abstract: An orange pigment named echinotinctone was isolated from the wood-rotting polypores *Echinodontium tinctorium* and *Pyrofomes albomarginatus*. Its structure was elucidated as 2,6-dihydroxy-1,8-dimethyl-3*H*-xanthen-3-one (**1**) on the basis of spectroscopic and chemical studies. **1** represents the first naturally occurring pigment with a simple fluorone chromophore.

INTRODUCTION

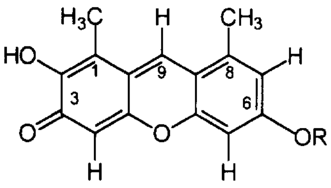
Echinodontium tinctorium (Ell. & Ev.) Ell. & Ev. (Echinodontiaceae) is a wood-rotting fungus from the western United States and Canada which causes damage to living trees of *Abies grandis* and other conifers. It produces hoof-shaped conks attached to the trees which can easily be identified by the greyish teeth extending from the lower surface of the fruit body. J. B. Swan, the original collector reported that the Indians from the Admirality Islands (Alaska) obtained a rust-red powder from the ground basidiocarps which was used for the preparation of dyes and paints.[1] Interestingly, the Wasco Indians of Oregon used the fungus as a bactericidal agent and the crude material has been found to exhibit antitumor properties.[2]

In an earlier investigation a lanostane derivative, echinodol, was isolated from this fungus,[2] however, the nature of its pigments remained unknown. In this publication we describe the structural elucidation of an orange pigment for which the name echinotinctone is proposed. The pigment was also found in the orange-brown, woody fruiting bodies of *Pyrofomes albomarginatus* (Lév.) Ryv. from Malaysia.

RESULTS AND DISCUSSION

Extraction of the powdered fruit bodies of *E. tinctorium* with methanol yielded an orange-brown solution. On TLC the orange-yellow zones showed strong tailing which prohibited the identification of individual components. Exposure to ammonia resulted in a colour change to purple-violet. Several attempts to separate the pigments by chromatography yielded only ill-defined fractions with very broad NMR signals. Despite of these difficulties we were able to isolate a minor pigment by repeated gel chromatography.

The compound, named echinotinctone, has the molecular formula $C_{15}H_{12}O_4$ and displays UV/Vis absorption maxima at 242, 442 and 468 nm. Its rust-red methanolic solution is decolourized by the addition of $NaBH_4$ and re-oxidized by shaking in air. The ^{1}H NMR spectrum of **1** (Table 1) indicated the presence of two methyl groups at δ 2.29 and 2.56, four aromatic protons and two exchangeable hydroxy groups. Treatment with diazomethane led to the formation of a lemon-yellow mono methyl ether **2**.



1: R = H
2: R = CH_3

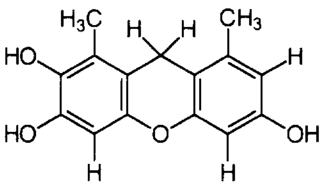


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Table 1. ^{1}H NMR data for compounds **1** and **2** (600 MHz, δ-values)

H	**1**[a]	**2**[b]	Long range correlations[c]
4	6.25, d, J=1.1 Hz	6.43, d, J=1.1 Hz	H-9, 1-CH_3
5	6.73, s (br)	6.82, s (br)	H-9, OCH_3
7	6.75, s (br)	6.79, s (br)	8-CH_3
9	8.07, s (br)	7.97, s (br)	H-4, H-5
1-CH_3	2.29, s	2.39, s	H-4
8-CH_3	2.56, s	2.59, s	H-7
OH	8.87, 10.77		
OMe	--	3.89, s	H-5

[a] In DMSO-d_6. [b] In $CDCl_3$. [c] Observed in the ^{1}H COSY long range spectrum of **2**.

The UV/Vis spectra and ^{1}H NMR data of **1** and **2** suggested the presence of a 3*H*-xanthen-3-one (fluorone) chromophore.[3] The substitution pattern at the xanthen-3-one system was deduced from the ^{1}H COSY (Table 1) and NOESY spectra of methyl ether **2**. As indicated in Figure 1, five successive NOE correlations connect 5-H with the methyl group at C-1 which proves structure **2** for the methyl derivative. Echinotinctone is therefore 2,6-dihydroxy-1,8-dimethyl-3*H*-xanthen-3-one (**1**).

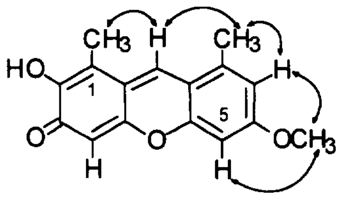
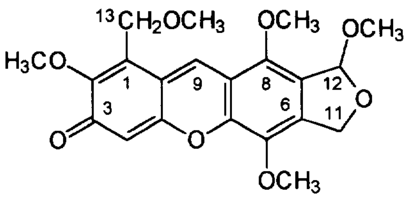


Figure 1. NOESY correlations of **2**



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Structure **1** is supported by the ^{13}C NMR data of echinotinctone including a detailed analysis of the HMQC and HMBC[4] spectra (Table 2). The chemical shifts of the carbons in rings A and B are in good agreement with those of trametin pentamethyl ether (**4**).[5]

Table 2. ^{13}C NMR data of echinotinctone (**1**) and trametin pentamethyl ether (**4**) (150.9 MHz, in DMSO-d$_6$ and CDCl$_3$, respectively; δ-values in ppm with solvent as internal standard)

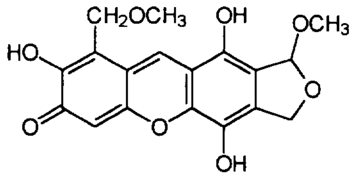
	1		**4**[a]	
C-atom	δ	HMBC correlations to	δ	HMBC correlations to
1	112.0	1-CH$_3$	121.2	H-4, H-13
2	148.0	H-4, 1-CH$_3$	153.7	H-4, H-13, 2-OCH$_3$
3	177.0	H-4	180.4	
4	101.0		106.4	
4a	157.5	H-4, H-9	156.8	H-4, H-9
5	115.4	H-7	135.8	H-11, 5-OCH$_3$
6	161.6	H-5	138.3	H-11, H-12
7	99.7	H-5, 8-CH$_3$	120.7	H-11
8	139.3	H-9, 8-CH$_3$	147.5	H-9, H-12, 8-OCH$_3$
8a	111.6	H-7, H-9, 8-CH$_3$	113.9	H-9
9	130.3		130.0	
9a	119.7	H-4, 1-CH$_3$	124.1	H-9, H-13
10a	153.5	H-5, H-9	144.9	H-9
1-CH$_3$	10.1			
8-CH$_3$	18.3	H-7		

[a] Further signals at δ 70.1 (C-11), 106.2 (C-12), 64.5 (C-13), 60.9 (2-OCH$_3$), 61.1 (5-OCH$_3$), 60.2 (8-OCH$_3$), 54.6 (12-OCH$_3$), 57.8 (13-OCH$_3$).

Reduction of methyl ether **2** with $NaBH_4$ in CD_3OD solution afforded dihydro derivative **3**. The ^1H NMR spectrum taken directly from the reaction mixture indicated the disappearance of the aromatic proton at δ 7.97 and the formation of a methylene group at δ 3.71. Long range ^1H-^1H correlations connected the latter signal to protons H-4 and H-5 as well as to the protons of both methyl groups.

Echinotinctone (**1**) was also isolated from the orange-brown methanolic extract of the polypore *Pyrofomes albomarginatus*. Like in *E. tinctorium* **1** is accompanied by a major fraction of closely related pigments which exhibit similar UV/Vis spectra and change their colour to purple-violet on exposure to ammonia.

Echinotinctone represents the first natural product with a simple fluorone chromophore.[4] Familiar compounds of this type are the xanthene dyes fluorescein and eosin. More complex examples lacking the hydroxy group in 6-position are the santal wood pigments, e.g. santalin A (**6**)[6], and trametin dimethyl ether (**5**),[5] which has been obtained by extraction of *Gloeophyllum odoratum* and *G. sepiarium* (Aphyllophorales) with methanol.[3]



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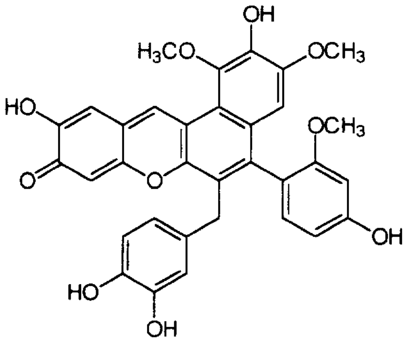

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Experimental

^1H NMR and ^{13}C NMR spectra were recorded on a Bruker AMX-600 spectrometer operating at 600.13 and 150.9 MHz, respectively. Chemical shifts are given in ppm relative to residual protio solvent signals. EI and HRMS were recorded at 70 eV on a Finnigan MAT 95Q instrument.

CC: Sephadex LH-60 and LH-20 (Pharmacia). TLC: Merck silica gel 60 F_{254} on aluminium sheets (C_6H_6 : HCO_2Et : HCO_2H = 10:5:3).

Echinodontium tinctorium was collected in September 1990 near Clark Fork Field Campus, Idaho, USA, *Pyrofomes albomarginatus* in April 1994 near Sungai Halong, Malaysia (leg. N. Arnold, det. H. Besl, confirm. L. Ryvarden). Voucher specimen are kept in the Bayerische Botanische Staatssammlung, München.

Trametin pentamethyl ether (**4**) was obtained from trametin dimethyl ether (**5**) by treatment with dimethyl sulfate/K_2CO_3 in anhydrous DMF.[5]

Isolation of echinotinctone (**1**): Air-dried, ground fruit bodies of *E. tinctorium* (600 g) were defatted by successive extraction with several 1.5 l-portions of petrol ether (40-60°C), $CHCl_3$, and EtOAc. Further extraction with acetone (1.5 l) yielded a dark brown residue (2 g) which was put on a Sephadex LH-60 column. Elution with methanol afforded a yellow-brown forerun followed by a chestnut-red zone which contained echinotinctone (**1**) (TLC: R_f= 0.25 - 0.30). Rechromatography of **1** on a Sephadex LH-20 column with methanol yielded the pure compound (17 mg, 2.9 x 10^{-3}%). Further elution of the Sephadex LH-60 column with methanol (2 l) yielded a mixture of rust-red pigments (0.5 g) which showed strong tailing on TLC and turned purple-violet on exposure to ammonia.

Echinotinctone (**1**): rust-red amorphous solid, m.p. 272°C (decomp.); UV [MeOH, λ_{max} (log ε)] 242 (3.54), 260 sh (3.32), 420 sh (3.19), 442 (3.30), 468 nm (3.22); IR (KBr) ν_{max} 3950 (st), 3400 (st, br), 1715 (m), 1645 (m), 1615 (st), 1555 (st), 1515 (m), 1210 cm^{-1} (sst); HRMS *m/z* (%) 256.0730 (100, M^+, $C_{15}H_{12}O_4$ requires 256.0736), 227 (76), 211 (19); ^1H and ^{13}C NMR spectra see Tables 1 and 2, respectively.

Echinotinctone-6-O-methyl ether (**2**): An aliquote of the echinotinctone solution obtained from the Sephadex LH-20 column was evaporated to a small volume and dissolved again in methanol containing 10% H_2O (35 ml). The same volume of ethereal diazomethane was added in portions at 0 °C and the flask shaken gently. After 1 h the solvent was removed and the residue chromatographed on Sephadex LH-20. Elution with acetone afforded **2** as a lemon-yellow semisolid, R_f = 0.42; UV (MeOH, λ_{max}) 240 (sh), 260 (sh), 276 (sh), 416 (sh), 440, 462 nm (sh); HRMS *m/z* (%) 270.0864 (100, M^+, $C_{16}H_{14}O_4$ requires 270.0892), 241 (34), 225 (8).

Reduction of **2** *with sodium borohydride:* 0.5 mg of **2** was dissolved in CD_3OD (0.5 ml) in a NMR tube. Argon was bubbled through the solution and a crystal of $NaBH_4$ was added. The ^1H NMR and long range COSY spectra of **3** were obtained directly from this solution.

Isolation of **1** *from Pyrofomes albomarginatus:* Air-dried, defatted fruit bodies of *P. albomarginatus* (200 g) were ground and extracted with methanol (3 x 600 ml). The solvent was evaporated *in vacuo* and the residue (3.92 g) chromatographed on a Sephadex LH-20 column as described before. The chromatographic procedures were controlled by TLC and afforded echinotinctone (**1**) (55 mg, 2.75 x 10^{-2} %) which still contained a minor impurity.

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REFERENCES AND NOTES

Dedicated to Professor Rudolf Gompper on the occasion of his 70th birthday.

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