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ANTHRAQUINONES FROM *TRICHODERMA POLYSPORUM*

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Key Word Index—*Fomes annosus*; *Trichoderma polysporum*; anthraquinones; formajorin D.

Abstract—The antagonistic compounds produced by *Trichoderma polysporum* when in contact with the basidiomycete fungus *Fomes annosus* were identified as the known anthraquinones pachybasin (**2**), chrysophanol (**3**) and emodin (**4**). Bioassays showed a marked inhibition of *F. annosus* by the *O*-acetyl derivatives of **2**, **3** and **4**.

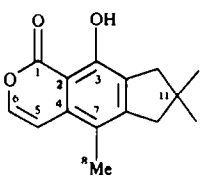
INTRODUCTION

In a continued search to find a successful method for the biological control of the pathogenic basidiomycete fungus *Fomes annosus* (syn. *Heterobasidium annosum* (Fr) Bref), a series of fungi were grown in paired plate culture with *F. annosus* (Fr) Cooke (Strain 608). Of the test fungi *Trichoderma polysporum*, isolated from the roots of *Picea sitchensis*, exhibited an antagonism which took the form of a distinct demarcation line which was associated with increased production of aerial hyphae and deposition of crystalline material by both fungi at the line of mycelial contact. This antagonism, coupled with a report [1] that growth of *F. annosus* on the roots of pine was inhibited by *T. viride* Pers ex Fries, suggested the possible importance of such antagonistic compounds and prompted their analysis and biological testing.

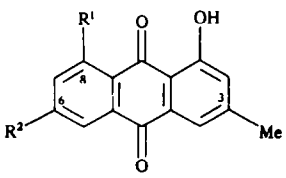
The crystals produced by *F. annosus* in the presence of *T. polysporum* proved to be 3-hydroxy-7,11,11-trimethylcyclopenta-[g]-benzopyran-1-one (fomajorin D, **1**), a sesquiterpene isocoumarin which has previously been isolated from cultures and fructifications of *F. annosus* [2, 3].

RESULTS AND DISCUSSION

Separation of the pigment produced by *T. polysporum* in paired cultures with *F. annosus* was achieved by CC. The components isolated were 1-hydroxy-3-methylanthraquinone (pachybasin, **2**), 1,8-dihydroxy-3-methylanthraquinone (chrysophanol, **3**) and 1,6,8-trihydroxy-3-methylanthraquinone (emodin, **4**). The structures of the anthraquinones were assigned on the basis of their mp, elemental analyses and spectroscopic data and formation of their *O*-acetyl and *O*-methyl derivatives. The anthraquinones isolated in this antagonism have previously been isolated from *T. viride* and from *Phomea foveata* Foister. This is the first reported presence in *T. polysporum*.



1



2 $R^1 = R^2 = H$
3 $R^1 = OH$, $R^2 = H$
4 $R^1 = R^2 = OH$

Biological testing of the original pigment mixture, the isolated anthraquinones and their *O*-acetyl and *O*-methyl derivatives was carried out against two growing strains of *F. annosus*. There was a decrease in linear growth rate of the fungal strains when treated with the *O*-acetyl derivatives of the anthraquinones; however no marked inhibition of growth was observed when grown with the other test compounds.

EXPERIMENTAL

Mps are uncorr., MS were recorded at 70 eV, direct inlet and ^{1}H NMR at 60 MHz ($CDCl_3$, TMS, δ values). *Trichoderma polysporum* was isolated from the roots of *Picea sitchensis* at Glenealy, Co. Wicklow (1982) and *Fomes annosus* (Fr) Cooke (strain 608); both fungi are maintained at the Botany Department, University College, Dublin.

Extraction and isolation. Ten ½ PDA agar plates were inoculated with *T. polysporum* and *F. annosus* and were incubated for 10 days at 25°. Crystalline deposits appeared along the line of contact between the two fungal mycelia. The crystalline material deposited by *F. annosus* was extracted using cotton wool soaked in $CHCl_3$. Evaporation of the solvent yielded a pale yellow solid which was purified by prep. TLC (developer: *n*-hexane–EtOAc, 7:3) and gave 3-hydroxy-7,11,11-trimethylcyclopenta-[g]-benzopyran-1-one (**1**, 7 mg), identical (mp, TLC, ^{1}H NMR, MS, IR) with an authentic sample [2].

The crystalline material deposited by *T. polysporum* was extracted on to $CHCl_3$-soaked cotton wool and evaporation of the solvent afforded a yellow solid. This pigment mixture (250 mg) was separated by CC using $CaCO_3$ and Kiselguhr gel (1:1). Gradient elution (petrol–C_6H_6, C_6H_6–$CHCl_3$, $CHCl_3$–MeOH) yielded in order of increasing polarity 1-hydroxy-3-methylanthraquinone (**2**, 35 mg), mp 117° (needles, EtOH) (lit. [4] mp 177–178°); (Found: C, 75.9; H, 4.5. Calc. for $C_{15}H_{10}O_4$: C, 75.6; H, 4.2%); 1,8-dihydroxy-3-methylanthraquinone (**3**, 20 mg), mp 195–196° (bronze plates EtOH) (lit. [4] mp 195–196°); (Found: C, 71.1; H, 4.2. Calc. for $C_{15}H_{10}O_4$ C, 70.9; H, 3.9%); 1,6,8-trihydroxy-3-methylanthra-

quinone (4, 8 mg), mp 258° (red needles, $CHCl_3$); (Found: C, 66.8; H, 3.8. Calc. for $C_{15}H_{10}O_5$ C, 66.7; H, 3.7%). Spectroscopic properties (^1H NMR, IR, UV) of 2, 3 and 4 were identical to those reported in the lit. [4].

Acetylation of 2, 3 *and* 4. The compounds when acetylated using Ac_2O–pyridine (10:1) yielded the mono-, di- and tri-*O*-acetyl derivatives of 2, 3 and 4 respectively. Identical (mp, IR, ^1H NMR, MS) to those reported in ref. [4].

Methylation of 2, 3 *and* 4. The components, when methylated using CH_2N_2–Et_2O, yielded the *O*-dimethyl ether of 2, 1,8-*O*-dimethyl ethers of 3 and 4. Physical and chemical data were in agreement with those published [4].

Biological testing. The $CHCl_3$ solns (50 μg/ml and 100 μg/ml) of the original anthraquinone mixture, and of the isolated anthraquinones 2, 3 and 4, their *O*-acetyl and *O*-methyl ether derivatives were prepared and filter paper discs were impregnated with the samples (1 μl). Controls were impregnated with $CHCl_3$ (1 μl). Papers were air-dried under sterile conditions and were placed just beyond the edge of actively growing cultures of *Fomes annosus* (strain 608). The cultures of *Fomes annosus* were incubated at 24° and linear growth rate was recorded over 14 days.

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