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6-NITRO-*ISO*-VANILLIC ACID, AN UNUSUAL CHROMOGEN FROM THE GENUS *CORTINARIUS**

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Key Word Index—*Cortinarius*; *Sericeocybe*; Agaricales; pigment; 6-nitro-*iso*-vanillic acid.

Abstract—6-Nitro-*iso*-vanillic acid, a new natural product, has been isolated from the fruit bodies of an Australian toadstool belonging to *Cortinarius* (*Sericeocybe*).

INTRODUCTION

Nitro compounds are found only occasionally as natural products, a small number having been isolated from higher plants, bacteria and fungi [1, 2]. Only three nitroaromatic metabolites have been reported hitherto from Basidiomycetes; *para*-nitroanisole (**1**) and *para*-nitrobenzaldehyde (**2**) occur in low concentrations in cultures of *Lepista diemii* [3], and 3,5,6-trichloro-1,4-dimethoxy-2-nitrobenzene (**3**) has been isolated from the fruit bodies of *Fomes robiniae* (Aphyllophorales) [4]. Of these, only the latter may be regarded as a contributor to the colour of the fungus in which it is produced.

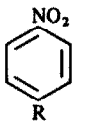
We report here the isolation and characterisation of the first nitroaromatic pigment, namely 6-nitro-*iso*-vanillic acid (**4**), from a toadstool belonging to the order Agaricales.

RESULTS AND DISCUSSION

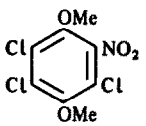
The fungus in question has been placed as a new taxon in the subgenus *Sericeocybe* of *Cortinarius*, close to *Cortinarius* (*Sericeocybe*) *anomalus* (Fr. ex Fr.) Fr. (Watling, R., personal communication).† It produces relatively large fruit bodies which, when fresh, have an exterior white to pale tan colour. In contrast, the interior flesh of the sporophores, particularly that of the tall stipe, is bright chrome yellow and the degree of pigmentation is intensified on administration of alkali.

The pigment **4** was extracted into ethanol from fresh fungi collected in *Eucalyptus* woodland near Marysville, Victoria. The yellow ethanolic extract, which produced an intensely yellow colour reaction with aqueous alkali, was concentrated, extracted with petrol to remove lipids and then continuously extracted with ethyl acetate to remove pigments from the (acidified) aqueous phase. Thin layer chromatography at this point revealed the presence of a single pale yellow substance [R_f 0.54, C_6H_6–EtOAc–HOAc–HCO_2H (12:6:1:1)] which gave a bright yellow colour reaction with base. This compound, for which the structure **4** was subsequently deduced, was isolated by chromatography on silica gel and crystallized as pale yellow needles.

The formula $C_8H_7NO_6$ followed from the presence in the mass spectrum of **4** of an abundant molecular ion at



1 R = OMe
2 R = CHO



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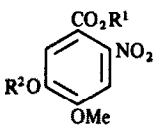
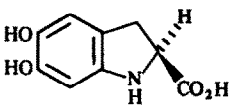


4 $R^1 = R^2 = H$
5 $R^1 = R^2 = Me$
6 $R^1 = H$, $R^2 = Ac$



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*Part 7 in the series 'Pigments of Fungi'.

†Voucher specimens of the fungus discussed here are lodged at the herbariums of the NSW Department of Agriculture, Biological and Chemical Research Institute, Rydalmere, NSW (accession number DAR 55995), and the Royal Botanic Garden, Edinburgh, U.K. (accession number WAT 19346).

m/z 213 and from appropriate combustion data. The number and nature of the substituents in the aromatic nucleus of the new pigment was deduced from the ^{1}H NMR and IR spectra. Thus, singlet resonances at δ4.00 (3H), 7.21 (1H) and 7.57 (1H) indicated a methoxy group and two *para*-disposed aromatic protons, while characteristic absorptions at 3540 cm^{-1} (free phenolic OH), 3000–2400 and 1695 cm^{-1} (CO_2H) and at 1535 and 1330 cm^{-1} (NO_2) identified the remaining three functional groups. **4** with diazomethane afforded the dimethyl derivative **5** and with acetic anhydride the monoacetate **6** was produced.

The disposition of the four substituents about the aromatic ring in **4** was tentatively formulated as that shown by consideration of its UV spectrum in which an absorption maximum at 343 nm in ethanol is shifted bathochromically (to λ_{max}429 nm) and intensified on addition of base. This suggested that the phenolic hydroxyl is *para* to the nitro group and, since the hydroxy group does not chelate intramolecularly with the carboxylic acid moiety, the pigment must have the 1,2,4,5-tetrasubstituted benzene nucleus shown in **4**. ^{13}C NMR spectra of **4** and of its derivative **5** and **6** may be assigned by reference to correlation tables [5] (Table 1) and are in full accord with the structures shown.

Final proof of the identity of the *Cortinarius* pigment with 6-nitro-*iso*-vanillic acid (**4**) was obtained by comparison of the natural product with synthetic material obtained by nitration of veratric acid followed by cleavage with alkali of the labile 3-OMe ether [6]. Material obtained in this way proved to be chromatographically and spectroscopically indistinguishable from the natural product.

Although no studies have been performed on the biosynthesis of **4** it seems likely that the nitro group results from oxidation of an amino precursor [1]. If this is the case then **4** could be a derivative of anthranilic acid or a degradation product of *cyclo*-DOPA (**7**). Thus, the probable biogenesis of **4** in the shikimate–chorismate pathway is in marked contrast with the polyketide mode of pigment production which prevails in many other *Cortinarius* toadstools [7].

Table 1. ^{13}C NMR spectroscopic data of nitroaromatics **4–6**

Carbon	Multiplicity†	Chemical shift, (δ*) 4	5‡	6
C-1	*s*	122.8	121.7	117.3
C-2	*d*	116.1	110.8	125.8
C-3	*s*	149.9	152.5	143.0
C-4	*s*	151.3	150.4	155.5
C-5	*d*	108.5	107.0	109.4
C-6	*s*	142.0	141.3	149.2
OMe	*q*	57.0	56.6	57.6
COOR	*s*	166.4	166.3	164.6
COOMe	*q*	—	53.3	—
MeCO	*q*	—	—	20.3
MeCO	*s*	—	—	168.4

*δ values are reported in ppm downfield from TMS as internal standard.

†This refers to the multiplicity of each signal in the proton off-resonance decoupled spectrum.

‡The spectrum of **5** was recorded in $CDCl_3$.

Somewhat surprisingly, 6-nitro-*iso*-vanillic acid (**4**) proved totally inactive at concentrations of 10 μg/ml in the plate diffusion assay against *Penicillium notatum*, *Nematospora coryli*, *Acinetobacter calcoaceticus*, *Bacillus brevis*, *B. subtilis*, and *Mucor miehei* (Anke, T., personal communication). This inactivity may be due in part to the dissociation of **4** in aqueous solution [8].

EXPERIMENTAL

^{1}H NMR: 100 MHz, ^{13}C NMR: 25 MHz, acetone-d_6 (unless stated otherwise) with TMS as int. standard; UV: EtOH; mps: uncorr. CC: Merck Kieselgel 60 (0.015–0.04 mm). Petrol: bp 60–80°. Combustion analyses were performed by the Australian Microanalytical Service, Melbourne.

*Isolation of 6-nitro-*iso*-vanillic acid* (**4**). The fresh fungus (460 g), collected in the Olinda State Forest, Victoria, during June 1985, was macerated and extracted with EtOH (3 × 1l) at room temp. and the combined extracts were concentrated under red. pres. The resulting aq. suspension was diluted with H_2O, washed with petrol, adjusted to pH 1 with dil. aq. HCl and continuously extracted overnight with EtOAc. Evaporation of dried ($MgSO_4$) organic phase left a pale yellow residue (1.47 g) which was triturated with portions of hot $CHCl_3$ until the washings no longer exhibited a bright yellow colour reaction with base. Evaporation of the combined $CHCl_3$ washings afforded a brown oil which was chromatographed on a column of silica gel using CH_2Cl_2–EtOAc–HOAc (10:5:1) as eluant. A single yellow zone which eluted from the column gave (**4**), pale yellow needles (from EtOAc–petrol), R_f 0.54 [C_6H_6–EtOAc–HOAc–HCO_2H (12:6:1:1)], mp 179–183° (lit [6] 181–182°) (196 mg, 4.3 × 10^{-2}%) (Found: C, 45.25; H, 3.7; N, 6.75. Calc. for $C_8H_7NO_6$: C, 45.1; H, 3.3; N, 6.55%); IR ν_{max}^{KBr} cm^{-1}: 3540, 3200–2400, 1695, 1535, 1435, 1333, 1280, 1220, 1050, 993, 880, 795, 655; UV λ_{max}^{EtOH} nm (log ε): 270.5 (3.94), 300 (3.50), 343 (3.56); UV λ_{max}^{EtOH} $^{+OH^-}$ nm (log ε): 270.5 (4.53), 429 (4.76); ^{1}H NMR: δ4.00 (3H, *s*, OMe), 7.21 (1H, *s*, H-2), 7.57 (1H, *s* H-5); EIMS (probe) 70 eV, *m/z* (rel. int.): 213 $[M]^+$ (100), 167 $[M-NO_2]^+$ (15), 124 (10), 111 (21), 107 (13), 96 (20), 85 (20), 83 (31), 79 (15), 55 (14), 53 (14), 51 (22), 50 (15), 45 (12), 30 (15); dimethyl derivative (CH_2N_2) pale yellow needles (from EtOH–H_2O), mp 143–144° (lit [9] 143°) (Found: C, 49.8; H, 4.7; N, 5.7. Calc. for $C_{10}H_{11}NO_6$: C, 49.8; H, 4.6; N, 5.8%); IR ν_{max}^{KBr} cm^{-1}: 1730, 1580, 1522, 1462, 1437, 1345, 1290, 1270, 1260, 1220, 1130, 1050, 985, 878, 802, 788, 767; UV λ_{max}^{EtOH} nm (log ε): 245 (4.16), 293.5 (3.68), 338.5 (3.64); ^{1}H NMR ($CDCl_3$): δ3.91 (3H, *s*, CO_2Me), 3.98 (6H, *s*, OMe), 7.08 (1H, *s*, H-2), 7.46 (1H, *s* H-5); EIMS (probe) 70 eV, *m/z* (rel. int.): 242 $[M+1]^+$ (11), 241 $[M]^+$ (100), 211 (9), 210 (31), 195 $[M-NO_2]^+$ (11), 137 (12), 136 (18), 109 (9), 93 (11), 82 (8), 77 (8), 59 (12), 50 (12); acetate (Ac_2O–H_2SO_4) colourless needles (from HOAc–H_2O), mp 218–221° (lit [9] 214°) (Found: C, 47.25; H, 3.45; N, 5.5. Calc. for $C_{10}H_9NO_7$: C, 47.05; H, 3.55; N, 5.5%; IR ν_{max}^{KBr} cm^{-1}: 3438–3068, 2850–2622, 1776, 1704, 1615, 1575, 1538, 1426, 1394, 1371, 1331, 1296, 1284, 1249, 1201, 1175, 1140, 1050, 1010, 937, 849, 801; UV λ_{max}^{EtOH} nm (log ε): 204 (4.27), 210 (4.25), 229 (4.18), 260 (2.79), 320 (2.79); ^{1}H NMR: δ2.32 (3H, *s*, OAc), 4.02 (3H, *s*, OMe), 7.62 and 7.68 (each 1H, *s*, ArH); EIMS (probe) 15 eV, *m/z* (rel. int.): 255 $[M]^+$ (11), 214 (8), 213 (100), 195 (27).

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