Constituents of *Agaricus xanthodermus* Genevier: The First Naturally Endogenous Azo Compound and Toxic Phenolic Metabolites

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Extraction of fresh sporophores of the fungus *Agaricus xanthodermus* yields 4,4′-dihydroxyazobenzene, phenol, *p*-quinol, and 4,4′-dihydroxybiphenyl. This is the first report of an azo compound arising endogenously in nature, while phenol, *p*-quinol and 4,4′-dihydroxybiphenyl have not previously been isolated from higher fungi. Phenol is present in fruitbodies in sufficiently high concentration to account for the toxicity of *A. xanthodermus*.

The mushroom *Agaricus xanthodermus* is one of only a few members of the *Agaricus* genus which cause discomfort when eaten. The white fruitbodies of this fungus exude a characteristic inky [1] or "carbolic" [2] aroma which, together with a distinctive chrome yellow stain produced when the flesh is damaged, distinguishes *A. xanthodermus* from closely related edible species such as the "field mushroom" *A. campestris*. Nevertheless, *A. xanthodermus* is often mistaken for its innocuous relatives whereupon ingestion can cause alarming symptoms such as coma, vomiting and diarrhoea in susceptible individuals [1, 3].

We report herein, firstly, the presence in fruitbodies of *A. xanthodermus* of phenolic metabolites which account for the toxicity of the fungus and, secondly, the identification of a yellow compound produced when fruitbodies are damaged.

Materials and Methods

Sporophores of *A. xanthodermus* were collected in Kew, Victoria during April, 1983 (voucher specimens are held in the herbariums of the New South Wales Department of Agriculture Biological and Chemical Research Institute, Rydalmere, N.S.W., Australia, and the Royal Botanic Garden, Edinburgh, U.K., under collection numbers DAR-49095 and WAT 16914, respectively). The fungus was finely chopped and immersed in ethanol for 3 h. Evaporation of the bright yellow solution and dilution of the residual aqueous suspension with water followed by exhaustive extraction with diethyl ether gave a yellow oil after removal of the organic solvent. The mixture of metabolites thus obtained could be fractionated using column chromatography (polyamide-6; solvent: gradient from dichloromethane to dichloromethane/ethanol 1:1) whereupon four colourless fractions and one zone containing a mobile yellow metabolite were collected. Further chromatography of the yellow material on a column of silica gel (solvent: dichloromethane/ethyl acetate 7:3) was necessary to obtain the pure pigment. A methyl ether derivative was prepared from a small portion of this compound using etherial diazomethane and the product was purified using a column of silica gel (solvent: dichloromethane/ethyl acetate 9:1).

An authentic sample of 4,4′-dihydroxyazobenzene was obtained upon coupling phenol with diazotized *p*-aminophenol and its dimethyl ether was produced using diazomethane. TLC comparison of pure metabolites with authentic substances was performed on silica gel plates using dichloromethane/ethyl acetate 3:1 and 7:3.

Results

Extraction of fresh fruitbodies of *A. xanthodermus* followed by column chromatography using polyamide-6 yields three pure colourless metabolites. Phenol (**1**) (0.08% fresh weight of fungus), a semicrystalline mass, was identified by its N.M.R., I.R., and mass spectra and by the formation of a crystalline 3,5-dinitrobenzoate, m.p. 144–145° (lit. [4] m.p. 145–146°). *p*-Quinol (**2**) (4×10^{-3}%) and 4,4′-dihydroxybiphenyl (**3**) (1×10^{-3}%) could be readily

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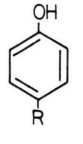
identified by direct chromatographic and spectroscopic comparison with commercial specimens. The only yellow constituent of *A. xanthodermus* which proves mobile on polyamide-6 and subsequently on silica gel crystallizes from aqueous ethanol as yellow needles (0.9×10^{-3}%), m.p. 219–221°; ^{1}H NMR (100 MHz, CD_3OD): δ6.8 and 7.74 (both 4H, d, $J = 8.5$ Hz); ^{13}C NMR (25 MHz, CD_3OD): δ115.7 (d), 124.4 (d), 146.5 (s), and 160.4 (s); MS: *m/e* (rel. int.) 214 (41, M^+), 121 (31), 93 (100), 65 (47) and 39 (28), and is identical with synthetic 4,4′-dihydroxyazobenzene (**4**), m.p. 216° (lit. [5], m.p. 216–218°). The identity was confirmed on methylation of the naturally derived azobenzene **4** which afforded 4,4′-dimethoxyazobenzene (**5**), m.p. 165° (lit. [5], m.p. 160°), indistinguishable from authentic material.

Discussion

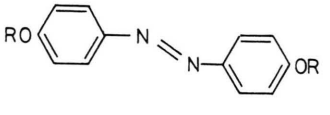
Agaricus xanthodermus is known colloquially as "yellow stainer" due to the chrome yellow colour which develops rapidly in the flesh when sporophores are damaged. The compounds responsible for most of this vivid stain are unstable to the conditions used to isolate and purify metabolites **1**–**3**, however, we have isolated and characterized one stable yellow constituent of *A. xanthodermus* which we identify as the azobenzene **4**. This metab-



1 R = H

2 R = OH

3 R = p-HO-C_6H_4-



4 R = H

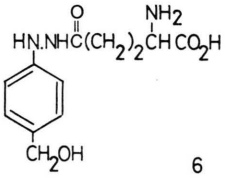
5 R = Me

olite **4** represents the first azo compound to be isolated as an endogenous natural product. Azoxy compounds such as calvatic acid (*p*-carboxyphenylazoxycyanide) [6], azoxybenzene-4,4′-dicarboxylic acid [7] and lyophyllin [8] as well as hydrazine derivatives such as gyromitrin [9] are well known fungal metabolites as are several other molecules containing nitrogen-nitrogen bonds [10]. However, compounds containing the azo linkage have been isolated previously only from soil microorganisms in which they arise as metabolic transformation products of herbicidal aniline derivatives [11].

4,4′-Dihydroxyazobenzene (**4**) is visible chromatographically immediately the fruitbodies of *A. xanthodermus* are macerated in solvent. We have been unable to obtain extracts without producing the vivid yellow stain and consequently we cannot preclude the possibility that **4** arises by rapid enzymic or aerial oxidation of a colourless precursor on its exposure to atmospheric oxygen. However, at the low levels detected, azobenzene **4** could be present as such in undamaged sporophores without imparting observable colour.

This is also the first report of phenol, *p*-quinol or 4,4′-dihydroxybiphenyl as constituents of higher fungi. Phenol, a surprisingly rare natural product, is present in fruitbodies of *A. xanthodermus* in concentrations (ca. 0.1% fresh weight) sufficient to account for the immediate toxicity of the fungus. The reported [1, 3] symptoms of *A. xanthodermus* poisoning are entirely consistent with those associated with phenol ingestion [12] and, furthermore, the characteristic odour of the sporophores is in accord with the presence and volatility of **1**. *p*-Quinol (**2**), also markedly toxic [12], is widely distributed in higher plants as the O-glucoside arbutin but its occurrence in a basidiomycete has not been described previously. 4,4′-Dihydroxybiphenyl has been found once before in nature as a constituent of the phycomycete *Cunninghamella elegans* [13].

The isolation of azobenzene **4**, phenol, *p*-quinol and the biphenyl **3** from *A. xanthodermus* is consistent with earlier reports of constituents of *Agaricus* species. Thus, agaritine [β-N(γ-L-(+)-glutamyl)-4-(hydroxymethyl)phenylhydrazine] (**6**) and related



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compounds [14] are found in young sporophores of several *Agaricus* fungi including *A. xanthodermus* [15], and an enzyme capable of cleaving acylhydrazines such as **6** to the component arylhydrazine and glutamate has been detected in *A. bisporus* [16].

Furthermore, *A. bisporus* also contains a second enzyme which oxidizes arylhydrazines to the corresponding aryldiazonium cation [17]. Interestingly, the 4-(hydroxymethyl)benzenediazonium cation itself is claimed to be a constituent of *A. bisporus* [18]. Thus, it is conceivable that each of the metabolites of *A. xanthodermus* reported here could share a common biogenetic precursor in the form of an equivalent of the *p*-hydroxyphenyldiazonium ion (**1**, $R = N_2^+$). Alternatively, the azo compound **4** could originate from a C_6N precursor such as *p*-aminophenol which is a known *Agaricus* metabolite [16]. *p*-Aminophenol was detected by TLC in extracts of *A. xanthodermus* but was not isolated.

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