

ANTIBIOTICS FROM BASIDIOMYCETES

EVIDENCE FOR THE OCCURRENCE OF THE 4-HYDROXYBENZENEDIAZONIUM ION IN THE  
EXTRACTS OF AGARICUS XANTHODERMUS GENEVIER (AGARICALES)

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**Abstract:** Extraction of fruitbodies of Agaricus xanthodermus with sodium sulfite solution yielded the antibiotically active 4-hydroxybenzenediazonium ion in form of its stable sulfonate

In the course of our screening for antibiotically active metabolites from basidiomycetes, we found that extracts of fruitbodies of A. xanthodermus contained an antimicrobial, cytotoxic, antineoplastic, and prophage-inducing metabolite.<sup>1</sup> The specimen, A. xanthodermus, belongs to the widely distributed poisonous mushrooms.

Now we wish to report on the occurrence of the 4-hydroxybenzenediazonium ion (1) in the extracts of A. xanthodermus. This diazonium ion is responsible for the antibiotical activity and could be isolated in form of its stable sulfonate which we named agaridin (2).

Chopped fruitbodies of A. xanthodermus were immersed in 0.05 % sodium sulfite solution for 2 hours. After centrifugation, inactive proteins were precipitated by addition of acetone. The supernatante was concentrated and lyophilized. The solid was then dissolved in methanol and passed through a column of silica gel using chloroform-methanol (2:1) as the eluent. The crude metabolites-containing product thus obtained was subjected to fractionation by means of gel-chromatography (Sephadex LH 20/methanol). Fractionation and isolation were monitored by prophage-induction test.<sup>1</sup>

Agaridin crystallizes from methanol-ether as yellow needles (0.025 %), m.p. > 250 °C (dec.), Anal. Calcd. for C<sub>6</sub>H<sub>5</sub>N<sub>2</sub>O<sub>4</sub>Na·CH<sub>3</sub>OH (256): C 32.81 %, H 3.51, N 10.93, S 12.50, Na 8.98, O (diff.) 31.27, Found: C 32.69, H 3.46, N 11.06, S 12.01, Na 10.12, O (diff.) 30.66, EI-MS, m/z 48 (SO), 64 (SO<sub>2</sub>), 77 (C<sub>6</sub>H<sub>5</sub>), 94.0423 (C<sub>6</sub>H<sub>6</sub>O, 100 %), 186.0687 (C<sub>12</sub>H<sub>10</sub>O<sub>2</sub>, 20 %), UV (MeOH), 237 nm (ε 10,200), 329 (ε 19,300), IR (KBr), 3200 cm<sup>-1</sup> (OH), 1610, 1593, 1478, 840 (benzene), 1200 - 1250, 1050, 630 (SO<sub>3</sub>), Raman (solid state, He/Ne-laser), 1442 cm<sup>-1</sup> (azo), <sup>1</sup>H NMR (DMSO-d<sub>6</sub>), δ 7.29 ppm (m, 4 H), <sup>13</sup>C NMR (DMSO-d<sub>6</sub>), δ 116.21 ppm (d, C3/C5), 125.55 (d, C2/C6), 143.34 (s, C1), 162.16 (s, C4).

All the physico-chemical properties are compatible with the 4-hydroxyben-

zenediazonium sulfite structure and its more stable form, 4-hydroxybenzenediazosulfonate.<sup>2,3</sup>

The proposed structure was confirmed by the conversion of agaridin into p-quinol (4) upon heating 1 in 0.25 n HCl. p-Quinol was readily identified by direct chromatographic comparison with an authentic sample.

In a recent publication, Gill and Strauch reported on the isolation of phenol (3), p-quinol (4), 4,4'-dihydroxydiphenyl (5), and 4,4'-dihydroxyazobenzene (6) from *A. xanthodermus*.<sup>4</sup>

In accordance with these results, it seems reasonable to assume that the 4-hydroxybenzenediazonium ion (1) reported here could be the common biogenic precursor of these metabolites (fig.)

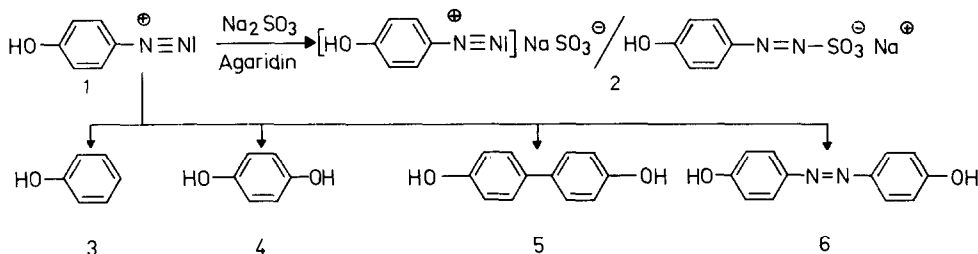


Fig. Metabolism of the 4-hydroxybenzenediazonium ion

The 4-hydroxybenzenediazonium ion itself is presumable generated from an unknown metabolite of *A. xanthodermus* by an enzyme system present in this basidiomycete.

A related example amongst natural products is provided by the 4-hydroxymethylbenzenediazonium ion which is claimed to be a constituent of *A. bisporus*.<sup>5</sup> This basidiomycete contains an enzyme system which oxidizes arylhydrazines to the corresponding aryl diazonium cations.<sup>5,6</sup>

The biological properties of 1 will be described in a forthcoming paper.<sup>7</sup>

#### References and Notes

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2. The first description of 4-hydroxybenzenediazosulfonate was given by R. Schmitt and L. Glutz, *Ber. Dtsch. Chem. Ges.*, **1869**, *2*, 51.
3. After completion of our investigation, we became aware of the work of Hilbig et al. which confirms the identity of agaridin with 4-hydroxybenzenediazosulfonate. They also report on the constituents of *A. xanthodermus*: S. Hilbig, T. Andries, W. Steglich, and T. Anke, *Angew. Chem.*, in press (W. Steglich, personal communication).
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