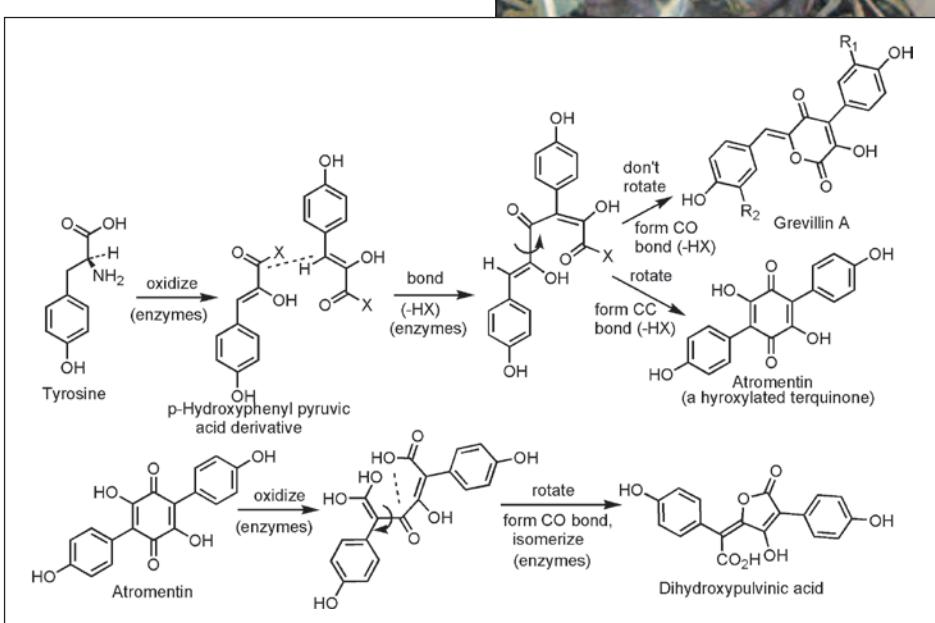


# Bluing Components and Other Pigments of Boletes

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Boletes, as well as many other fungi and lichens, contain compounds arising from enzyme controlled dimerization of p-hydroxyphenylpyruvic acid, which is derived by oxidation of the amino acid tyrosine, followed by either CO bond formation to produce grevillins such as grevillin A, or CC bond formation to produce hydroxylated terphenylquinone derivatives such as atromentin, as well as products of further reactions, including oxidative bond cleavage and rearrangement of atromentin with dehydration to dihydroxypulvinic acid (see Scheme 1), or further oxidation reactions. I discuss some pigments of boletes here. The material below on bolete pigments comes from the unrivaled review of mushroom pigments by Gill and Steglich (1987) which occupies an entire volume of the review series.



Scheme 1. Outline of biosynthetic pathways to produce the bolete pigments atromentin, grevillin A, and pulvinic acid derivatives (Gill and Steglich, 1987).

The compounds of boletes that turn blue upon bruising or cutting were suggested to be quinones by Bertrand

(1901; 1902) and more specifically, a hydroxylated anthraquinone carboxylic acid by Kögl and a coworker in 1935.

Figure 1. Bluing reaction of *Boletus luridus*, which contains both variegatic and xerocomic acids (Scheme 2). (Newport State Park, Door Co., WI)

The true structures of the compounds causing the bluing reaction of many members of *Boletus* and *Suillus* were not known until Edwards et al. (1968) showed the bluing to be caused by enzymatic oxidation of a more highly hydroxylated pulvinic acid derivative than the dihydroxy compound of Scheme 1, variegatic acid (in 1968 for *B. erythropus*, *B. appendiculatus*, *S. varietatus* and *S. bovinus*, later including *B. pulverulentus*, one of the fastest and most intensely bluing species, and this compound has been found in over 100 other species of the Boletales). The structure of the closely related xerocomic acid was reported by Steglich et al. (1968). It also contributes to bluing

reactions, and was first isolated from *Boletus (Xerocomus) chrysenteron* and also has been found in over 95 other species in the Boletales, mostly the same as the ones containing variegatic acid. Both are oxidized to quinone methide derivatives by oxidase enzymes present

*B. chrysenteron*, and *B. rubellus*. Many related pigments are less widespread. For example, the greenish-yellow color of *Pulveroboletus ravenelii* (present as 8% of its dried weight) and of *B. subglobosus* is caused by vulpinic acid, and *Pulv. auriflammeus* contains five related, more

highly oxygenated and often chlorinated pigments.

Many species of *Suillus* contain grevillins, which are orange to dark



Figure 7. *Pulveroboletus* (*Boletus* in Smith's nomenclature) *auriflammeus*, which contains five more oxidized or chlorinated derivatives of vulpinic acid (Scheme 2). (Elkmont, Great Smoky Mountain National Park, NC)

Scheme 2. Outline of biosynthetic pathways to produce the bolete pigments atromentin, grevillin A, and pulvinic acid derivatives (Gill and Steglich, 1987).

in their fruitbodies. See Scheme 2 for the structures. However, many of the species containing variegatic and xerocomic acids do not turn blue at all, presumably because they lack the oxidase enzymes that are necessary to produce the blue quinone methides. It is stated that variegatic and xerocomic acids are only present in the order Boletales, so that their isolation from *Gomphidius*, *Omphalotus*, *Hygrophoropsis*, *Coniophora*, *Leucogyrophana*, *Rhizopogon*, and *Serpula* species is strong evidence for their inclusion in the Boletales. Air oxidation of variegatic acid, especially if traces of acid are present, produces variegatorubin, which is the common red pigment present in Boletes. It is present in all species reported to contain variegatic acid, even those that are not red, and is possibly formed during work-up of the samples. Especially high concentrations are reported for *B. erythropus*, *B. (Chalciporus) piperatus*, *B. frostii* (ssp. *floridanus*; northern material, ssp. *frostii*, appears not to have been studied by 1987), *B. rhodoxanthus*, *B. splendens*,

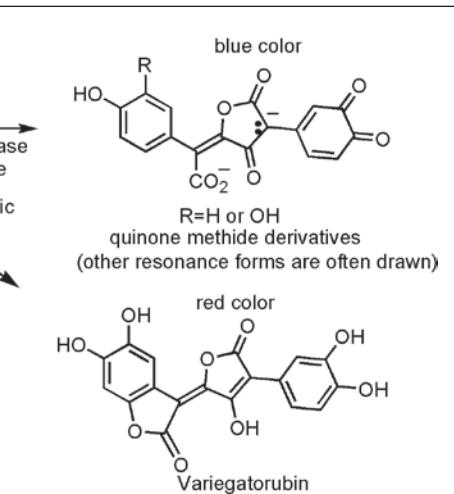


Figure 2. Bluing of *Boletus pulverulentus*, reported to contain only variegatic acid (Scheme 2). (Governor Dodge State Park, Iowa Co., WI)

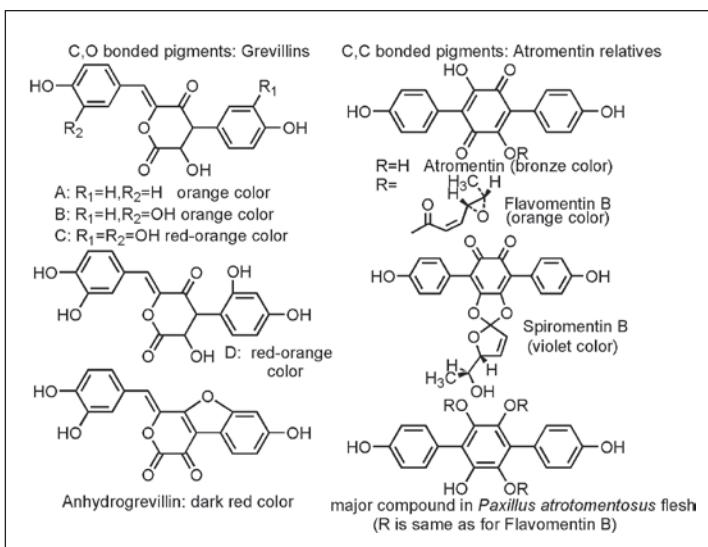


Figure 3. An especially red *Boletus frostii*, presumably containing large amounts of variegatorubin (Scheme 2). It has a rather weak bluing reaction. (Walking Iron Dane Co. Park, WI)



Figure 6. *Pulveroboletus ravenelii* which contains vulpinic acid (Scheme 2). (Porcupine Mountain State Park, Upper Peninsula of Michigan)

red pigments containing pyrandione rings that are also derived from the biosynthetic pathways shown in Scheme 1 (see Scheme 3 for structures). Grevillin A has been isolated from *S. grevillei*, *S. bresadolae*, *S. americanus*, *S. luteus*, *S. placidus*, and *S. punctatipes*, B and C from the same species as A plus *S. granulatus*, *S. tridentinus*, *S. pictus*, *S. lakei*, *S. aeruginascens*, *S. collinitus*, and *S. sibiricus*, D from many of the same species and *S. albidiipes*, *S. leptopus*, *S. pseudobrevipes*, *S. riparius*, and *S. umbonatus*. Grevillin B has also been isolated from *Gasteroboletus laricinus*.



Scheme 3. Pigments related to grevillin A and atromentin (Gill and Steglich, 1987).

Anhydrogrevillin has been found in most of the species that produce grevillin D, including *S. americanus* and *S. granulatus*, and may be an artifact of the isolation methods. The poisonous *Paxillus atrotomentosus* contains several relatives of atromentin, including flavomentin B, and spiromentin B (Scheme 3, right column). As better separation and analytical methods have been developed, it has been found that



Figure 4. *Suillus grevillii*, which contains grevillins A, B, and C (Scheme 3). (Fern Ridge, Vilas Co., WI)

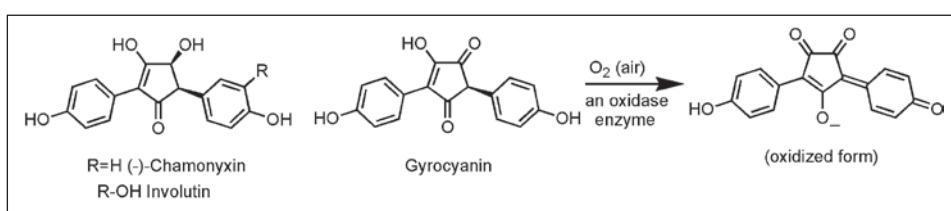
the principal component of its colorless flesh is the reduced tri-R-substituted compound shown at the bottom right

of Scheme 3, along with smaller amounts of the tetra-R substituted compound. Hydrolysis of these esters is very facile, and it is not obvious to what extent the pigments that have been isolated are hydrolysis and air oxidation artifacts of the isolation procedures used in the earlier work.

Other boletes have developed an alternate cleavage of atromentin, which can be accomplished without enzymes by

oxidizing it with potassium ferricyanide ( $K_3Fe(CN)_6$ ) in the presence of calcium cations, ultimately producing bis-phenolated cyclopentenones such as those shown in Scheme 4. Chamonixin is found as the (+) enantiomer in *Chamonia caespitosa* and as the (-) enantiomer in *Paxillus involvtus*, which also contains the more highly hydroxylated involutin, which causes the brown discoloration observed upon bruising its flesh. The bluing reaction of *Gyroporus cyanescens* is not caused by oxidation of variegatic acid, as stated on a popular Internet website (Tom Volk's Fungi <<http://tomvolkfungi.net/>>; Mushroom of the Month for July 2003) but by oxidation of gyrocyanin, a more highly oxidized bis-phenol-substituted cyclopentenone. (There are also mistakes in the structure of variegatic acid and its oxidation product at the same website.)

Finally, and on an unrelated topic except for its blue color, the blue of



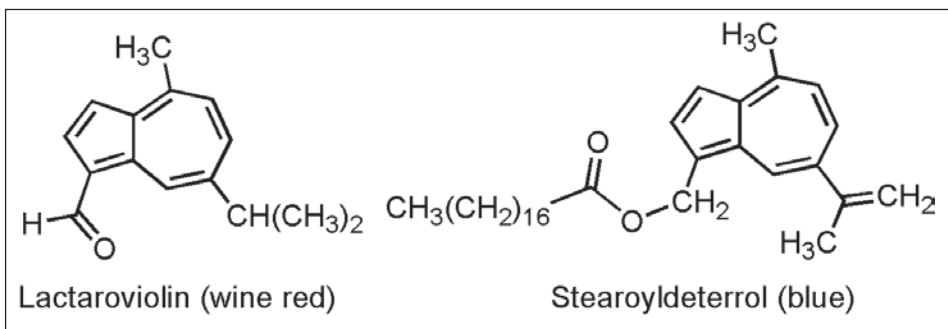
Scheme 4. Some diphenol derivatives of cyclopentenone from *Paxillus* and *Gyroporus* (Gill and Steglich, 1987).



Figure 5. Bluing of *Gyroporus cyanescens*, which contains gyrocyanin (Scheme 4). (Rowan Creek, Columbia Co., WI)



Figure 8. *Paxillus involutus*, which contains involutin (Scheme 4).  
(Whitefish Dune State Park, Door Co.)



Scheme 5. Pigments isolated from *Lactarius indigo* (Daniewski and Vidari, 1999).

*Lactarius indigo* has nothing to do chemically with the blue colors produced in boletes. The pigments involved are the azulene derivatives shown in Scheme 5. A dozen other related pigments produce the colors of the other *Lactarius* species having colored milk (Daniewski and Vidari, 1999).

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Indigo lactarius and chants  
- photo courtesy of G. Sayers



For this issue, even Professor Tom Volk got into the spirit, sporting his blue look. - photo courtesy of B. Bunyard

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