

***Merulius incarnatus* Schwein., a Rare Mushroom with Highly Selective Antimicrobial Activity**

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BACKGROUND

In the course of our search for new drug candidates from polypores, we studied a rare North American mushroom, *Merulius incarnatus* (Corticiaceae). The polypores and corticioid fungi are members of Aphyllophorales, a group of morphologically complex, terrestrial Basidiomycetes. Many of these fungi are saprobic wood decayers and most often are found on logs, stumps, or other dead wood. Polypores are considered by many as a major source of pharmacologically active natural products. Their secondary metabolites exhibit a wide range of biological activities such as antimicrobial, antiviral, antifungal, anticancer, cardiovascular, anti-inflammatory, antioxidant, immunostimulating, nematocidal, and other activities (Zjawiony, 2004).

Merulius incarnatus Schwein. (1822), also known under the later name *Phlebia incarnata* Nakesone et Bursdall (1994), can be found on dead logs and stumps of hardwoods, particularly those of white oak, beech, maple, and birch in the southeastern United States. It grows in overlapping clusters, usually with *Stereum ostrea* (Blume et T.Nees) Fr., mostly in the fall months (September–November). *M. incarnatus* is a rather small mushroom (2–5 cm in diameter), with a very characteristic bright coral pink color of the upper cap—hence, the common name Coral Woodcrust. The fruiting body is irregularly shaped, elliptical or semicircular, slightly convex without stem. The pore surface is whitish and veined. The spores are white, elliptical, and 2–4

µm in diameter. The mushroom gives the yellow to orange test reaction with KOH.

MATERIALS AND METHODS

We collected *M. incarnatus* in the fall of 2001, 2002, and 2003 at Duke Forest in Durham, North Carolina. Fruiting bodies were extracted fresh with 95% ethanol and subjected to chromatographic separation, using standard and argentated column chromatography followed by HPLC on Waters Delta Prep 4000 system using reverse phase Symmetry-C₈, 5 µm column, eluting with isocratic solution of MeOH-H₂O (85/15 v/v). Antimicrobial assays were conducted at the microbiological laboratories of the National Center for Natural Products Research against following pathogens: *Candida albicans*, *C. glabrata*, *C. krusei*, *Cryptococcus neoformans*, *Aspergillus fumigatus*, *Mycobacterium intracellulare*, *Staphylococcus aureus*, and methacillin resistant *S. aureus* (MRSA).

RESULTS

A crude extract of *Merulius incarnatus* exhibited significant activity against several microorganisms, particularly *Staphylococcus aureus* and methacillin resistant *S. aureus* (MRSA). The most active fraction showed IC₅₀ = 3.5 µg/mL against MRSA. These results prompted us to do further studies on isolation and

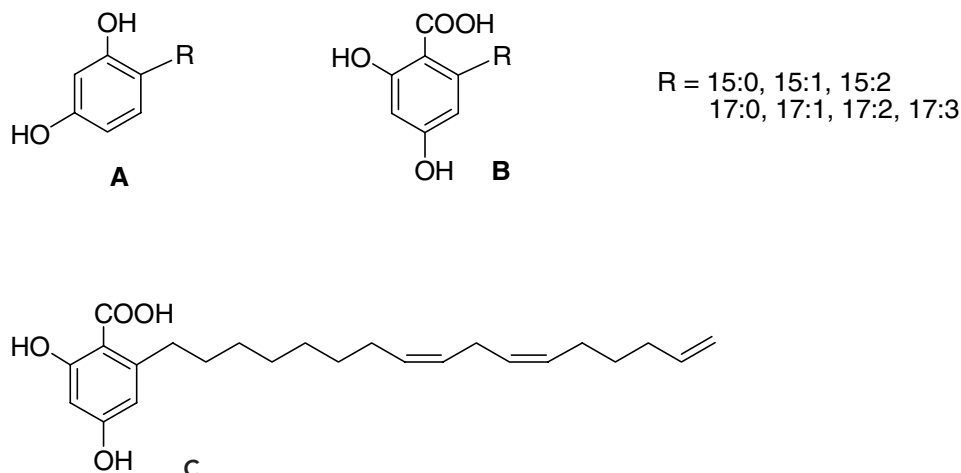


FIGURE 21. (A) Compounds with unsaturated aliphatic chains have a very specific pattern of the double bonds characteristic for resorcinolic lipids isolated from other plants, fungi, and bacteria (Kozubek and Tyman, 1999). They have either one or two double bonds in a *cis* configuration, and in some cases, also the terminal double bond. **(B)** The compounds with *cis-trans* conjugated system in place of isolated *cis-cis* were also isolated **(C)**.

structure elucidation of active secondary metabolites from this mushroom. The series of resorcinol (A) and resorcinolic acid (B) derivatives substituted with a long (C15 and C17) saturated and unsaturated aliphatic chain were obtained. For each series of derivatives (A and B) we were able to isolate seven compounds with 0, 1, 2, or 3 double bonds in the side chain.

DISCUSSION

Isolation of series of resorcinol and resorcinolic acid derivatives substituted with a long saturated and unsaturated aliphatic chain makes it the first and yet not published example of work on identification of secondary metabolites from *Merulius incarnatus*. Five compounds isolated are new and have never been found in any species. Other compounds isolated such as merulinic acid A, D, and E have been found in other mushroom species such as *Merulius tremellosus* Schrad. and *Hapalopilus mutans* (Peck) Gilb. et Ryverden (Giannetti et al., 1978; Sontag et al., 1999). Some resorcinol derivatives isolated from *M. incarnatus* are also known as the components of cereal grains (Kozubek and Tyman, 1999). All resorcinolic acid derivatives exhibited selective antimicrobial activity against methacillin resistant *Staphylococcus aureus*.

The structural similarity of all isolated compounds

within a particular series made the separation and isolation process extremely challenging. It required the use of vacuum liquid chromatography (VLC), argentated column chromatography (ACC), and reverse phase high performance liquid chromatography (HPLC), and in the cases of resorcinolic acid also a chemical modifications. The fact of the isolation of compounds with *cis-trans* conjugated system is unprecedented. We believe that these compounds are formed by enzymatic or photochemical isomerization *in vivo*. Further studies to understand the mechanism of formation and biological role of these compounds are needed.

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