

Progress in the Chemistry of
Organic Natural Products

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Progress in the
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Natural Products

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Secondary Metabolites and the Control of Some Blue Stain and Decay Fungi

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1. Introduction

Wood discoloration has been grouped in four categories based on the cause of the stain: enzymatic and chemical reactions, changes caused by contact with chemicals, chemical reactions accompanying the early stages of decay, and color changes associated with the growth of fungi (1). Only the latter two types of discoloration (biotic stains) will be discussed in this review. The discoloration by pigmented hyphae and spores on the wood surface is not included in this discussion.

Although blue stain fungi do not cause decay of wood, they lower the quality of wood and wood products (Fig. 1). In the pulping process, blue stain reduces pulp brightness, which leads to significant increases in the

use of bleaching chemicals. It has been estimated that annual losses due to stain in the USA alone exceed \$50 million (1).

The blue, black, brown or gray, or occasionally yellow, purple, pink or green discoloration in the sapwood is caused by the pigmented hyphae or by the release of pigments from the hyphae. Since the bluish discoloration is the most predominant, this stain is referred to as blue stain, sapstain, or mineral stain. It is believed that the discoloration is due to melanin-based pigments (2). In some cases the discoloration may be attributed to the formation of complexes of iron and other metals with phenolic compounds, especially with strongly chelating siderophores, which are produced by many fungi. It has been reported that the foliage of blue stain diseased lodgepole pine shows an increased level of iron with respect to foliage of healthy trees. This increase of iron uptake by plants caused by the accumulation of fungal iron chelators is considered as an iron stress response and may be linked to the characteristic discoloration of the sapwood of diseased trees (3).

In this review we will focus mainly on the chemical aspects of the blue stain and decay, especially on conifers and aspen wood.

Decay is the result of wood digestion (both lignin and cellulose) by fungi (Fig. 1). It is the major type of loss of wood which limits the use of

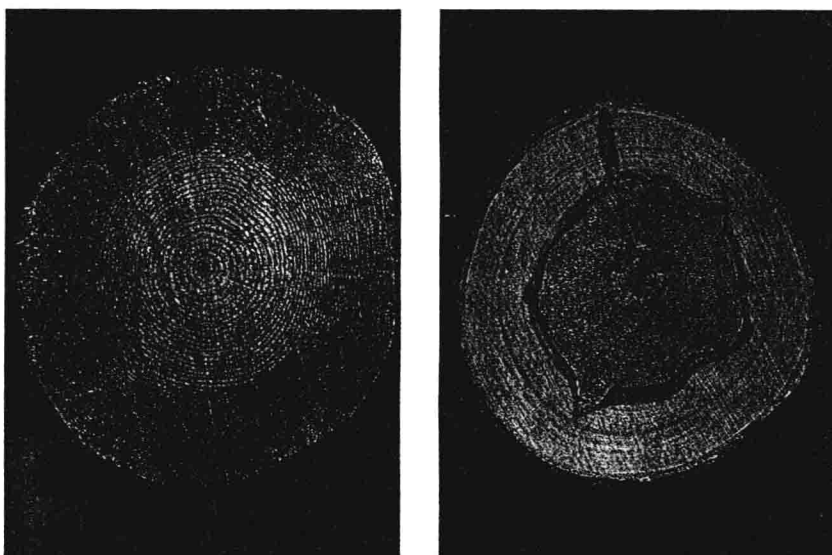


Fig. 1. Blue stained pine tree (left) and aspen decay (right)

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conifer and broad leaved trees. There are two major types of decay, brown rots (mainly the carbohydrates of the cell wall are attacked by the fungi) and white rots (both carbohydrates and lignin are attacked by the fungi). The metabolites and control of both types of fungi will be discussed in this review.

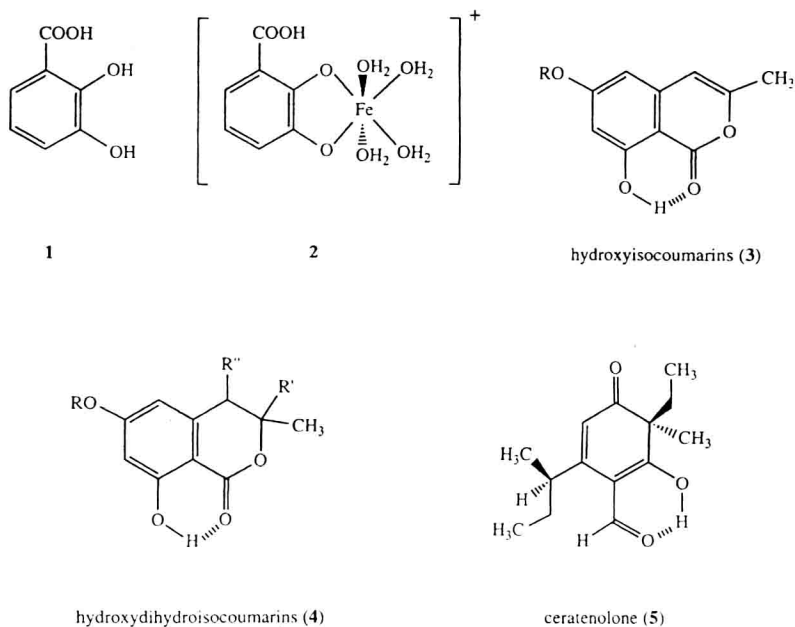
Certain chemicals have been employed for preventing stain and decay. The application of solutions of sodium carbonate, borax, chlorinated phenols, organic mercury compounds, copper compounds, organic nitrogen containing heterocyclic or quaternary ammonium fungicides have been reported (4). The use of many of these preservatives, such as mercury compounds and pentachlorophenol, has been severely restricted because of environmental and worker's exposure concerns. Currently there is a growing trend to develop acceptable alternatives for chemical prevention of blue stain development. Progress has been made in biological control of stain fungi (5, 6, 7, 8).

2. Blue Stain Fungi

2.1. Blue Stain on Conifers

The blue stain disease of conifers causes the death of more than 40 million trees a year in Western Canada where the mountain pine beetle is the vector of the disease. The microflora associated with the mountain pine beetle consists of several species of yeasts and mycelial fungi. Four species of the genus *Ceratocystis* have consistently been isolated from stained conifer wood: *C. clavigera*, *C. huntii*, *C. ips*, and *C. minor*. Fungi of this genus are also responsible for the oak wilt and Dutch elm diseases (9).

2,3-Dihydroxybenzoic acid (1), a well known iron-chelating agent (siderophore), has been identified amongst the secondary metabolites of some of these species (Chart 1). Compound 1 is involved in the iron transport systems of microbes via iron-chelating. It gives a bright blue complex with ferric ions (2) and greenish-brown complex with cobalt. In addition to a large number of ubiquitous metabolites, some simple phenolics, hydroxyisocoumarins (3), and hydroxydihydroisocoumarins (4) have been identified. Ceratenolone (5), isolated from *C. minor*, is one of the strongest fungal iron chelators (10). All these siderophores may be responsible for the staining effect, due to complexation with ferric and other ions (3, 9, 10).

Chart 1. Siderophores isolated from *Ceratocystis* species

2.2. Blue Stain on Broadleaf Trees

Aspen (*Populus tremuloides*) is the most widely distributed tree species in North America. In Canada aspen represents more than 50% of the merchantable hardwood timber. Its previously neglected utilization has increased substantially in recent years. A serious limitation to its use in the pulping process, however, is the pronounced susceptibility to blue stain caused by fungi (11).

The two most important aspen blue stain fungi are *Ophiostoma crassivaginaturn* and *O. piliferum*. The secondary metabolites of the blue stain fungus *O. crassivaginaturn* were studied in an attempt to obtain insight into the nature of the staining material. Several simple phenolic compounds were identified which, at least in part, can be responsible for the discoloration of aspen wood infected with this fungus (12).

The aspen fungi *Peniophora polygonia* and *Sporormiella similis*, the former showing decay and the latter showing blue-stain antifungal activity, were studied for production of secondary metabolites. In both cases isobutyric and isovaleric acid were amongst the most active metabolites inhibiting the growth of decay and stain fungi (5, 13).

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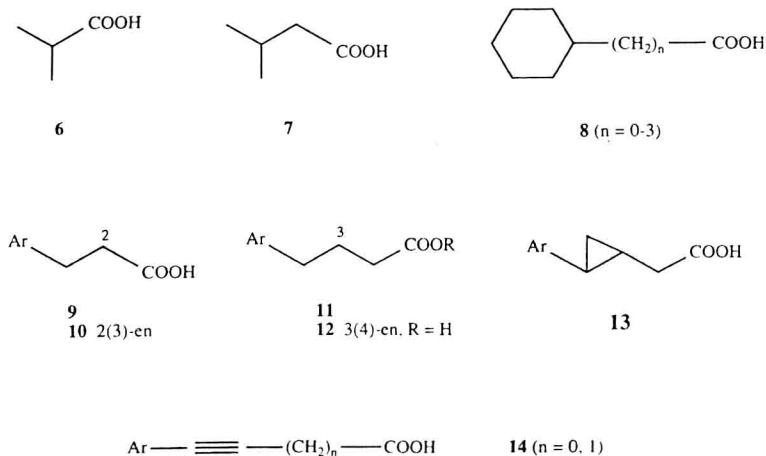


Chart 2. Some antifungal homologs and analogs of isobutyric acid

Following this natural lead, several homologs and analogs of isobutyric (6) and isovaleric acid (7), notably, the known enzyme inhibitor 4-phenyl-3-butenic acid (12) were tested (Chart 2). Of 38 compounds tested, strong inhibitory activity, superior to that of some commercial fungicides, was observed for several cyclohexyl aliphatic acids (8) and for the enzyme inhibitor 12 and its triple bond analogs 14, while the cyclopropane analogs 13, the cinnamic acids 9 and 10, and 11 proved less active (14). The acids 12 and 14 prevented colonization of aspen wood chips by the two stain fungi at concentrations as low as 1 $\mu\text{g}/\text{ml}$.

In a similar approach, senecioic acid (15a) and tiglic acid (16), minor, but very active anti-blue stain fungi compounds isolated from *S. similis*, were the natural leads (5) (Chart 3). The antifungal activities of 25 derivatives and analogs were studied. Senecioic acid, tiglic acid, 2,4-dihydroxyseneciophenone (17a) and the analogs cyclohexylidene acetic acid (18) and cyclohexene-1-carboxylic acid (19) completely prevented colonization of aspen wood chips at 10 $\mu\text{g}/\text{ml}$. Consequently, it was suggested that some of these active compounds are good candidates for chemical protection of aspen wood and wood products (15).

Stachybotris cylindrospora often has been isolated from clean xylem tissue of *P. tremuloides*. The metabolites produced by *S. cylindrospora* cultured in liquid medium were subjected to bioassay guided separation on XAD-16 non-ionic resin, followed by Sephadex LH-20 and silica gel chromatography to afford two known antifungal sesquiterpenes, trichodermin (20) and trichodermol (21) (Chart 4). Both compounds

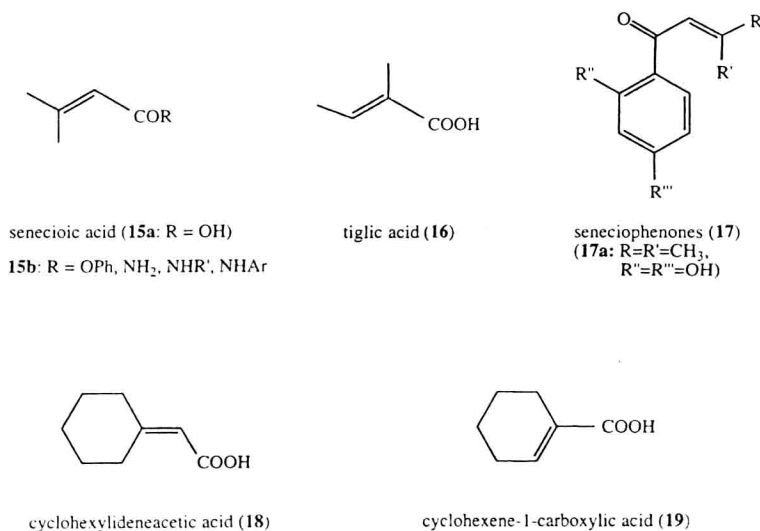


Chart 3. Antifungal derivatives and analogs of senecioic and tiglic acid

significantly reduced the growth of the aspen blue stain fungus *O. crassivaginat* at 10 µg/ml and completely inhibited the growth at 100 µg/ml. The novel sesquiterpene metabolites stachybotrydial (**22**) and stachybotramide (**23**), as well as the isochromane **24** showed no inhibitory activity. Based on the strong antagonism between *S. cylindrospora* and *O. crassivaginat*, as well as on the innocuous nature of *S. cylindrospora*, it was suggested that this fungus could be used as a bioprotectant of *P. tremuloides* wood against staining (6).

An unidentified *Zythiostroma* species, isolated from aspen, was also found to be antagonistic to *O. crassivaginat*. Zythiostromic acids A (**25**) and B (**26**), as well as zythiostromolide (**27**) isolated from the culture broth of this fungus, although new natural compounds, were inactive against the blue stain fungi (16) (Chart 5).

The fungus *Lecythophora hoffmannii* has been shown (17) to be strongly antagonistic to some blue stain fungi. Liquid cultures of the fungus show weak and variable activity against those blue stain fungi. The C-glucoside lecytoside (**28**) was isolated from the liquid culture but showed very weak activity *in vitro* against the target fungi (18) (Chart 6). However, when the fungus was grown on solid rice medium, a very active compound called lecythophorin was obtained (19). Extensive NMR studies, combined with some degradation studies, showed that lecythophorin possesses structure **29**, closely related to the known antibiotic chaetiacandīn (**30**). In fact, beginning with the benzylic

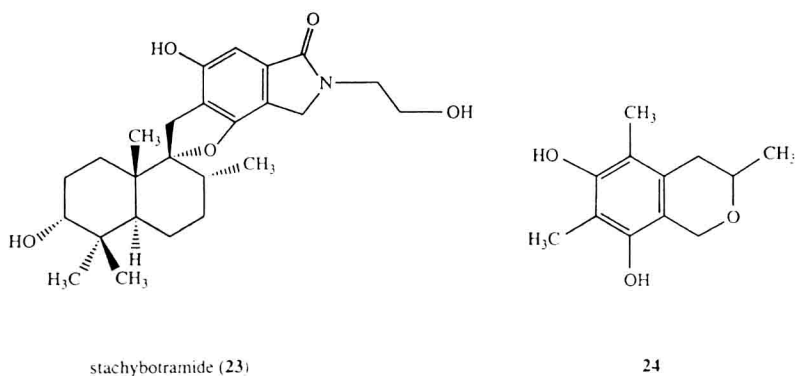
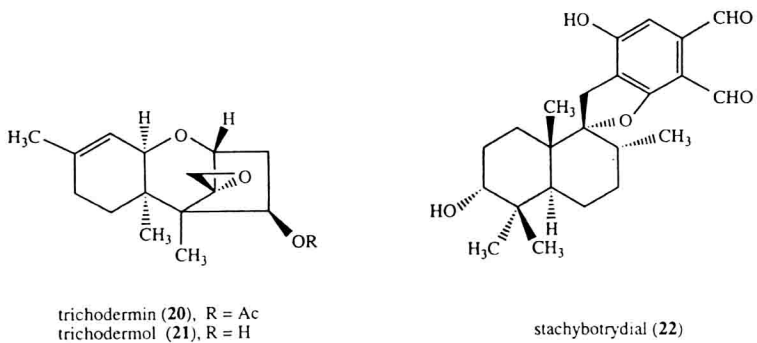


Chart 4. Secondary metabolites of *Stachybotrys cylindrospora*

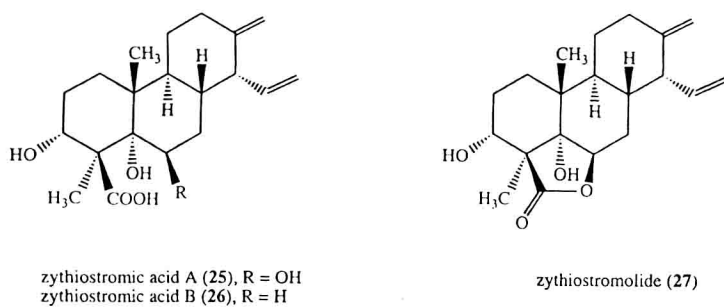
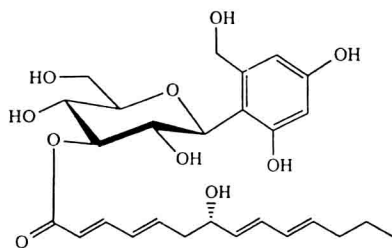
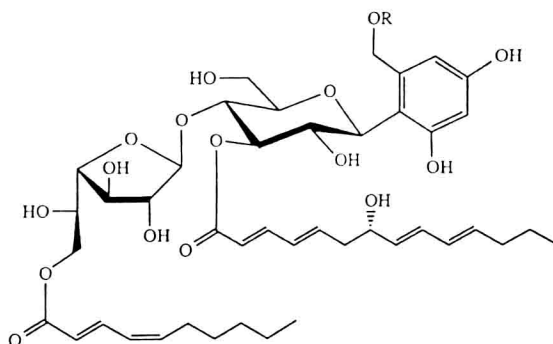


Chart 5. Diterpenes from an unidentified *Zythiostroma* species



lecythoside (28)

lecythophorin (29) R = SO₃Na

chaetiacandin (30) R = H

Chart 6. C-glucosides of *L. hoffmannii*

methylene group, it was possible to correlate all the C's and H's in the aromatic ring and the glucose and galactose portions of the structure. The presence of the galactose ring was verified by alkaline hydrolysis followed by methanolysis with HCl to give O-methylgalactopyranose. The furanoside ring, not unexpectedly, was transformed to the pyranoside by this operation.

Lecythophorin (29) shows very good activity at 1 µg/ml against the blue stain fungi (20), but the fact that the fermentation yield is very low and synthetic preparations of this compound will, presumably, not be possible on a commercial scale, precludes the use of this compound in the field.

References, pp. 17-20

3. Decay Causing Fungi

3.1. Decay on Conifers

A list of common decay fungi on coniferous trees is presented in Table 1 (21).

Anisomyces odoratus has been reported to produce odoriferous simple aromatic compounds, terpenes and lanostadienoic acids (22). The unusual amino acid 2-amino-4-N-ureidopropionic acid and its oxalyl derivative were recently isolated from *Coniophora puteana* (23).

Only steroids have been isolated from *Echinodontium tinctorium* and *Fomitopsis pini* (22). The long chain 2-hydroxy- and 2,3-dihydroxy fatty acids of ceramide, the n-phytosphingosines and the antitumor polysaccharides of *Fomitopsis pinicola* have been studied (24, 25). *Fomitopsis officinalis* yielded ergosterol-related steroids, officinalic acid, polyacetylenes and agaricic acid (22), as well as so-called agaric acid. The latter has been separated by TLC into three steroidal components (26).

A variety of benzoquinones, phenols and steroids have been isolated from the conifer decay fungus *Gloeophyllum saepiarium* (22).

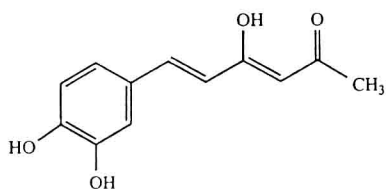
Inonotus hispidus is a decay fungus rarely occurring on conifers but predominantly affecting deciduous trees. Recently it was reported that the ethanolic extract of fruiting bodies of *I. hispidus* contains a new pigment, hispolon (31) (Chart 7) which is accompanied by the known

Table 1. Some Common Decay Fungi of Coniferous Trees

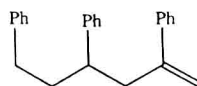
<i>Anisomyces odoratus</i> (Wulf.:Fr.) Pat. = <i>Trametes odoratus</i> (Wulf.:Fr.) Fr.
<i>Armillaria ostoyae</i> (Romag.) Herink = <i>A. obscura</i> (Pers.) Herink
<i>Coniophora puteana</i> (Schum.:Fr.) Karst
<i>Echinodontium tinctorium</i> (Ell. & Ev.) Ell. & Ev. = <i>Fomes tinctorium</i> Ell. & Ev.
<i>Fomitopsis officinalis</i> (Vill.:Fr.) Bond. & Sing. = <i>Fomes officinalis</i> (Vill.:Fr.) Newman
<i>Fomitopsis pinicola</i> (Sw.:Fr.) Karst. = <i>Fomes pinicola</i> Sw.:Fr.
<i>Gloeophyllum saepiarium</i> (Wulf.:Fr.) Karst. = <i>Lenzites saepiarium</i> (Wulf.:Fr.) Fr.
<i>Haematostereum sanguinolentum</i> (Alb. & Schw.:Fr.) Pouzar = <i>Stereum sanguinolentum</i> Alb. & Schw.:Fr.
<i>Hirschioporus abietinus</i> (Dicks.:Fr.) Donk = <i>Polyporus abietinus</i> Dicks.:Fr.
<i>Inonotus hispidus</i> (Bull. ex Fr.) Karst.
<i>Inonotus tomentosus</i> (Fr.) Gilbertson = <i>Polyporus tomentosus</i> Fr.
<i>Peniophora pseudo-pini</i> Weres & S. Gibson
<i>Phaeolus schweinitzii</i> (Fr.) Pat. = <i>Polyporus schweinitzii</i> Fr.
<i>Phellinus pini</i> (Thore.:Fr.) Pil. = <i>Fomes pini</i> (Thore.:Fr.) Lloyd.
<i>Pholiota alnicola</i> (Fr.) Sing. = <i>Flammula alnicola</i> (Fr.) Quél.
<i>Serpula himantoides</i> (Fr.) Bond. = <i>Merulius himantoides</i> Fr.

styryl pyrone, hispidin. Both compounds have immunomodulatory and antiviral activity (27).

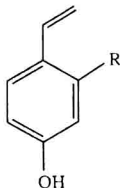
Phellinus pini is the most economically important conifer decay fungus which causes white pocket rot in many species of pine. Antagonism between *P. pini* and several other wood pathogens have been reported. When the fungus was grown in liquid medium several aromatic and benzoquinone metabolites were isolated of which 4-hydroxystyrene (33) and the pentaenone 35 showed activity against



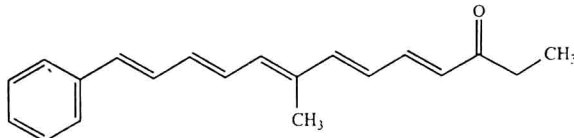
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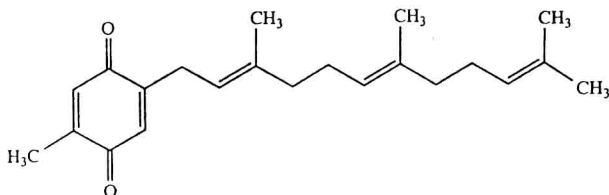
32



33, R = H
34, R = OH



35



36

Chart 7. Secondary metabolites produced by *Inonotus hispidus* and *Phellinus pini*

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several wood stain and decay fungi while compounds **32**, **34**, and **36** were inactive (28) (Chart 7).

The secondary metabolites of the remaining decay fungi listed in Table 1 have not been studied so far.

3.2. Decay on Broadleaf Trees

A list of common decay fungi on broadleaf trees is presented in Table 2 (21).

The production of aryl sesquiterpene metabolites (**37–39**) (Chart 8) by *Armillaria ostoyae*, a highly forest-pathogenic fungus, is enhanced up to 400-fold when the fungus is grown in the presence of an antagonist. Under these conditions several new metabolites are also produced. A detailed structure-antibiotic activity study of these metabolites has been conducted (29, 30). The odorous substances of *Bjerkandera adusta* have been studied (31). The white rot fungus *Coriolus versicolor* (32) produces highly oxygenated cytotoxic steroids (**40**, **41**).

Fomes fomentarius is a parasitic fungus of beech trees from which several benzotropolone pigments (**42–45**) and an unusual dicarboxylic acid (**46**) have been reported (33–36) (Chart 9).

A series of highly oxygenated bitter triterpenoids, ganoderenic acids **47–51** and furanoganoderic acid (**52**) (Chart 10) have been found in

Table 2. *Some Common Decay Fungi of Broadleaf Trees*

<i>Armillaria ostoyae</i> (Romag.) Herink = <i>A. obscura</i> (Pers.) Herink
<i>Bjerkandera adusta</i> (Willd.:Fr.) Karst = <i>Polyporus adusta</i> Willd.:Fr.
<i>Cerrena unicolor</i> (Bull.:Fr.) Murr. = <i>Daedalea unicolor</i> Bull.:Fr.
<i>Coriolus hirsutus</i> (Wulf.:Fr.) Quél. = <i>Polyporus hirsutus</i> Wulf.:Fr.
<i>Coriolus versicolor</i> (L.:Fr.) Quél. = <i>Polyporus versicolor</i> L.:Fr.
<i>Flammulina velutipes</i> (Curt.:Fr.) Sing = <i>Collybia velutipes</i> Curt.:Fr.
<i>Fomes fomentarius</i> (L.:Fr.) Kickx = <i>Polyporus fomentarius</i> L.:Fr.
<i>Ganoderma applanatum</i> (Pers. ex Wallr.) Pat. = <i>Elfvingia applanatum</i> (Pers. ex Wallr.) Karst.
<i>Gymnopilus spectabilis</i> (Fr.) Sing. = <i>Pholiota spectabilis</i> (Fr.) Gill
<i>Inonotus hispidus</i> (Bull. ex Fr.) Karst.
<i>Hirschioporus paragamenus</i> (Fr.) Bond & Sing. = <i>Polyporus paragamenus</i> Fr.
<i>Lyophyllum ulmarium</i> (Bull.:Fr.) Kuehn. = <i>Pleurotus ulmarium</i> (Bull.:Fr.) Kumm.
<i>Peniophora polygonia</i> (Pers. Fr.) Bond = <i>Corticium polygonia</i> Pers.:Fr.
<i>Phellinus tremulae</i> (Bond.) Bond & Boriss. = <i>Fomes igniarius</i> (L.:Fr.) Kickx.
<i>Pholiota destruens</i> (Brond.) Quél.
<i>Pholiota squarrosa</i> (Pers.:Fr.) Kumm.
<i>Piptoporus betulinus</i> (Bull.:Fr.) Karst. = <i>Polyporus betulinus</i> Bull.:Fr.
<i>Radulodon americanus</i> Ryv. = <i>Radulum casearium</i> (Morg.) Lloyd
