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The Chemistry of *Lactarius fuliginosus* and *Lactarius picinus* °,+

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Abstract: Intact fruit-bodies of the Basidiomycetes *Lactarius fuliginosus* and *L. picinus* have been found to contain the stearic acid ester **4b**. When the mushrooms are injured **4b** is rapidly converted to the acrid free phenol **4a** which is then gradually oxidized to a mixture of red pigments, benzofuran **9** and chromenes **8**, **10-13** (or **14**), **15**, **20** and **22**. These chemical transformations are involved in the change of taste and colour of the latex and flesh of the mushrooms. Compounds **4a-b**, **8-12** and **20** were obtained also by synthesis.

INTRODUCTION

The mushrooms belonging to the genus *Lactarius* (family Russulaceae, Basidiomycotina) contain a milky-juice which can be observed if the fruit-bodies are cut or broken. The colour and taste of this latex, as well as of the flesh vary between different species, a fact that has been often exploited by mycologists as a taxonomic marker. The chemical backgrounds for such distinctions have been subjected to several investigations.

Many sesquiterpenes with different structures have been isolated and they are now considered responsible of most of these phenomena. For example the latex of the fruit bodies of *L. deliciosus* Fr. and *L. deterrimus* Gröger is first carrot-coloured, but slowly turns green; these colours have been shown to be due to guaiane sesquiterpenes[1] (for example **1**). On the contrary *L. vellereus* has a colourless latex with an intense

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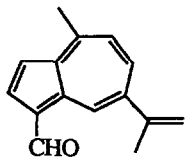
°) This paper is dedicated to Professor C. Cardani on the occasion of his 70th birthday.

+) Communication No. 28 of the series "Fungal Metabolites". For part 27 see Bocchi, M.; Garlaschelli, L.; Vidari, G.; Mellerio, G. *J. Nat. Prod.* ***1992***, *55*, 428-31.

pungent taste, owing to the presence of unsaturated dialdehyde sesquiterpenes, like the lactarane compound velleral (2).[2]

The structure of uvidin B (3) illustrates further the broad variety of compounds found in these mushrooms. Drimane sesquiterpenes like 3 have so far been found only in *L. uvidus*,[3,4] which has a white latex rapidly turning violet on exposure to the air.



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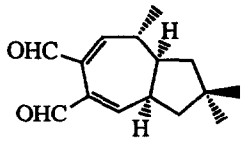

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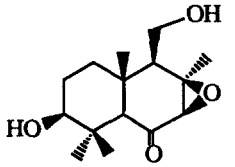


3

Recently convincing evidence has been reported[5] for the role of some of these sesquiterpenes in a chemical defence system which protects the fruit-bodies of *Lactarius* species from parasites. They would be formed enzymatically from precursor sesquiterpenoids, stored in the cells as fatty acid esters, as a response to damage to the tissues of the fruit bodies.

The possibility that a similar system has evolved also in the *Lactarius* species belonging to the Plinthogali (Burl.) Sing. section,[6] was one of the reasons for a reinvestigation of *L. fuliginosus* Fr. and *L. picinus* Fr. The results of this research are described in this paper, including a revision of the structures of some chromenes reported in a previous communication.[7]

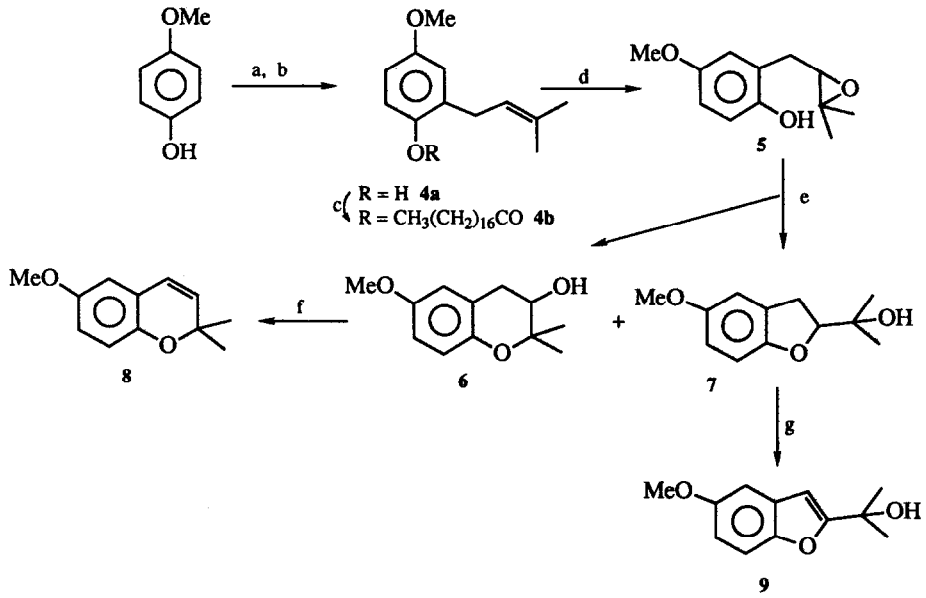
RESULTS AND DISCUSSION

Unique among the *Lactarius* species, the inedible mushrooms of the Plinthogali section have a latex and flesh which, as soon as the fruit-bodies are broken, are white and generally sweet, but turn reddish and bitterly acrid after a while. The time required for observing these changes of colour and taste varies from species to species. For example, in the case of *L. fuliginosus* the latex takes about 1 hour to turn salmon while the colour of the flesh becomes pinkish in few minutes.[8] On the contrary the milky juice of *L. acris* turns red in 20-30 seconds.[8,9] As *L. fuliginosus* in rather common in Italian woods and can be collected in reasonable amounts,[10] we studied in detail the enzymatic transformations of this mushroom, as representative of the entire Plinthogali section.

For investigating the initial contents, only young and seemingly parasite unaffected specimens of *L. fuliginosus* were collected. However, we observed that minute drops of white latex had already coagulated between the gills of the mushrooms. The specimens were frozen at -20°C and extracted still frozen with CH_2Cl_2. Only two compounds, the free phenol **4a** and the fatty acid ester **4b** were found in significant amounts (i.e. detectable with our TLC and GLC analytical systems) when such extracts were analysed immediately after preparation and separated by silica gel column chromatography. The major component was found to be the previously unknown stearic acid ester **4b**. The structures **4a** and **4b** were initially inferred by examination of the spectral data (reported in the Experimental). Thus the ^{1}HNMR spectrum of **4a** exhibits three signals (1H each) at δ 6.50, 6.61 and 6.84 showing the ortho and meta coupling constants attributable to the hydrogens of a 1,2,4-trisubstituted benzene ring. The position of the methoxy (δ 3.39) and dimethylallyl substitutents with respect to the OH group was established by NOE difference spectroscopy. Irradiation of the OMe singlet significantly (14%) enhances the intensity of the two protons mutually interacting with a meta coupling constant, while irradiation of the methylene group in the side chain affects (15%) only the proton

showing a meta coupling constant. In the case of compound **4b** the mass spectrum showed the molecular ion at *m/z* 458 and the IR (CO ester band at 1750 cm^{-1}) and ^1H NMR (signal at δ 1.30 for the methylenes of a fatty acid ester long chain) data clearly indicated **4b** to be the stearate of compound **4a**.

Compounds **4a** and **4b** were synthesized from 4-methoxyphenol by the reactions reported in Scheme 1. They required no comment. In this way we obtained larger amounts of **4a** and **4b** for further investigations.



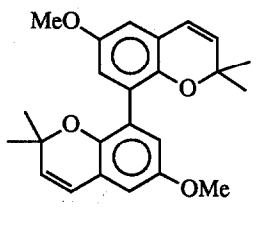
Scheme 1 - Reagents: a, Na/C_6H_6; b, $Me_2C{=}CHCH_2Br$; c, $Me(CH_2)_{16}COCl$, Py, DMAP; d, MCPBA, AcOK; e, MeONa/MeOH; f, p-TsOH/C_6H_6; g, NBS, AcONa/CCl_4

The ester **4b** was found to be tasteless, while the free phenol is strongly acrid. Therefore it appeared likely enough that **4a** and **4b** are associated with the already discussed change of the taste of *L. fuliginosus* fruit-bodies. A simple experiment was then performed to check whether after the fruit-bodies have been broken, the taste of the latex as well as the relative amounts of compounds **4a** and **4b** change with time. At various times after cutting, few drops of the milky-juice were collected with a capillary tube, suspended in CH_2Cl_2 and rapidly analyzed by TLC. This analysis was continued until the latex remained colourless. Only a single compound, the lipophilic ester **4b**, was found in specimens of the latex immediately collected after breakage of the fruit-bodies. Shortly afterwards (few seconds - one minute) **4b** was accompanied by a significant amount of free phenol **4a**, while the taste of the latex became unpleasantly acrid. Older samples of colourless latex contained traces of ester **4b**. These results clearly indicated that **4b** is enzymatically rapidly converted into **4a**. Furthermore it was evident that substrate **4b** and lipases transforming **4b** into **4a** are stored in separate compartments of intact fruit-bodies, while they are brought into contact in damaged tissues. Interestingly the chromenes previously isolated from *L. fuliginosus*[7] were not detected in this experiment.

The tissues of many living organisms, including plants, animals and fungi, redden or brown on exposure to the air. The true chemical structures of precursors, coloured compounds and enzymes required for these transformations are still rather unknown. It is generally accepted, however, that in damaged tissues

leuco compounds of phenolic nature are oxidized to reddish, brown or black pigments by some enzymes, collectively known as oxidases.[11,12] The entire process bears many similarities with the formation of melanins.[13]

We suspected that free phenol **4a** was the actual substrate for oxidizing enzymes, such as phenol oxidases, responsible for the reddening of latex and flesh of *L. fuliginosus*. These enzymes are indeed widely distributed in mushrooms.[14] Therefore, to investigate the fate of phenol **4a** in reddening fruit-bodies of *L. fuliginosus*, specimens were ground in a home mixer (without the addition of a solvent). After the colour of the flesh turned bright red, the mush was packed in a glass column for chromatography and washed exhaustively with CH_2Cl_2. The colour of the washings was first bright red-orange, then faded progressively to pale yellow as elution continued and the flesh of fruit-bodies released its initial reddish colour. The extract was concentrated and chromatographed with AcOEt over a short pad of Al_2O_3 (activity III) to remove free fatty acids. GC analysis of the eluate showed a mixture of peaks which were identified as **4a**, the chromenes **8,10-13** (or **14**) and **20**. (*vide infra*) and common methyl fatty acid esters.[7,15] The low amount of free phenol **4a** in the mixture indicated that most of its initial content had been transformed in the chromogenic reactions.



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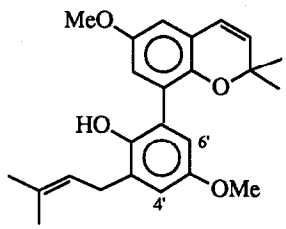

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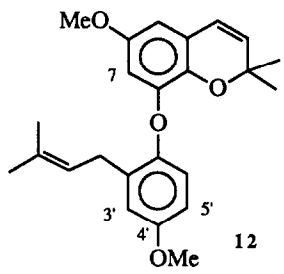

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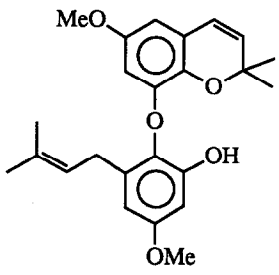

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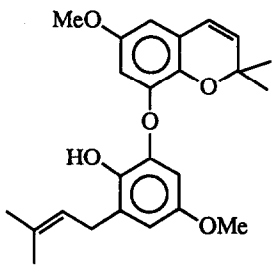

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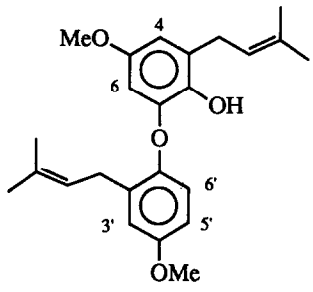


20

At present we have no evidence for the exact structure of the red pigments, as they remained absorbed irreversibly at the top of the Al_2O_3 column. Nevertheless they are undoubtedly correlated biosynthetically with free phenol **4a** and compounds **8,10-13** (or **14**) and **20**. Eventually an interesting experiment further supported the actual fate of compound **4a** in the mushrooms. Synthetic phenol **4a**, dissolved in CH_2Cl_2 (about 0.5 mg/ml), was added to the mush of fruit bodies, previously washed exhaustively with CH_2Cl_2 (vide above). Much to our satisfaction, the flesh of the mushrooms took again, in few minutes, a bright red colour, which faded away as the fruit-bodies were washed with the solvent. GC analysis of this extract, almost superimposable to the previous chromatogram, showed that reddening originated from oxidation reactions

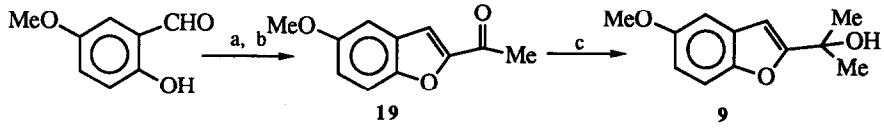
identical to those observed for endogenous phenol **4a**. Regeneration of the red colour could again be obtained by addition of new portions of **4a** to the mushrooms, its brightness decreasing considerably after two or three experiments.

These results pointed out that phenol oxidases of *L. fuliginosus* are not inhibited by organic solvents, a property observed by Klibanov for mushroom tyrosinase,[16] while their turnover seems not very high. More interestingly, preliminary experiments indicate that these enzymes have a low substrate specificity, being active also on other unnatural exogenous phenols.[17]

The chemistry of *L. picinus* was not investigated in such details, as we could find only few specimens of this mushroom, having gills already red spotted. GC analysis of the extract, performed in the same conditions that *L. fuliginosus*, revealed the presence of phenol **4a** and aromatic compound **8-12** and **15**. Thus in tissues of *L. picinus* compound **4a** is probably involved in similar reactions than in *L. fuliginosus*.

Biomimetic synthesis confirmed the structure[7] of compound **8** (Scheme I). Epoxidation of free phenol **4a** with MCPBA in buffered (CH_3CO_2K) CH_2Cl_2 gave epoxyphenol **5** in excellent yield (97%), accompanied by traces of dihydrofuran **7**. This result was rather unexpected, as in the epoxidation of o-isopentenylphenols with peracids preliminary protection of the phenol function (as acetate) is usually necessary to prevent further cyclization to dihydrofuran and dihydropyran derivatives.[18] In the case of compound **4a** protection was superfluous, probably for the reduced acidity of the phenol having a MeO group in position para. MeONa catalyzed cyclization of epoxyphenol **5** procedeed uneventfully, according to an S_N2 mechanism, giving a mixture (10:1) of the two isomers **7** and **6**. The major compound **7**. corresponding to a 5-exo-tet process, was that expected on the basis of steric and stereoelectronic effects.[19] The free alcohols **6** and **7** had identical chromatographic properties; however, the hindered tertiary alcohol **7** was not acetylated in standard acylation conditions (Ac_2O/Py at room temperature) and could be separated easily from the acetate of compound **6**. After saponification, brief treatment with p-TsOH in C_6H_6 converted compound **6** into the aromatized benzopyran **8**, identical with the natural chromene.[7] Oxidation of dihydrofuran **7** with NBS is boiling CCl_4, buffered with AcONa, afforded benzofuran **9**, albeit in poor yields (20%).

This compound was found identical (R_f and spectral data) with a metabolite previously isolated from *L. picinus*,[7] having a structure not yet established.[20] An alternative synthesis of the same alcohol, performed in better overall yields, is shown in scheme 2. The potassium salt of commercial 2-hydroxy-5-methoxy-benzaldehyde was condensed with chloroacetone to give 2-acetyl-5-methoxybenzofuran (**19**), which was converted into **9** by a Grignard reaction.



19 **9**

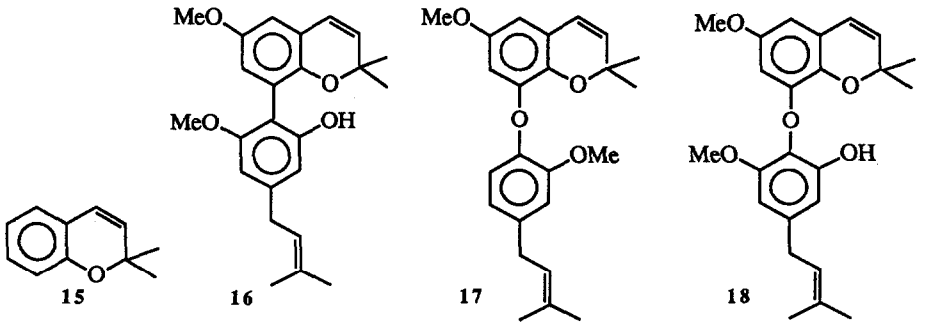
Scheme 2 - Reagents: a, KOH/EtOH; b, $ClCH_2COCH_3$; c, CH_3MgBr

Biosynthetic considerations, i.e. the common formation from the same precursor **4a**, required the structures **16**, **17** and **18**, previously assigned to three chromenes isolated from *L. fuliginosus*,[7] to be corrected as **11**, **12** and **13** or **14**.

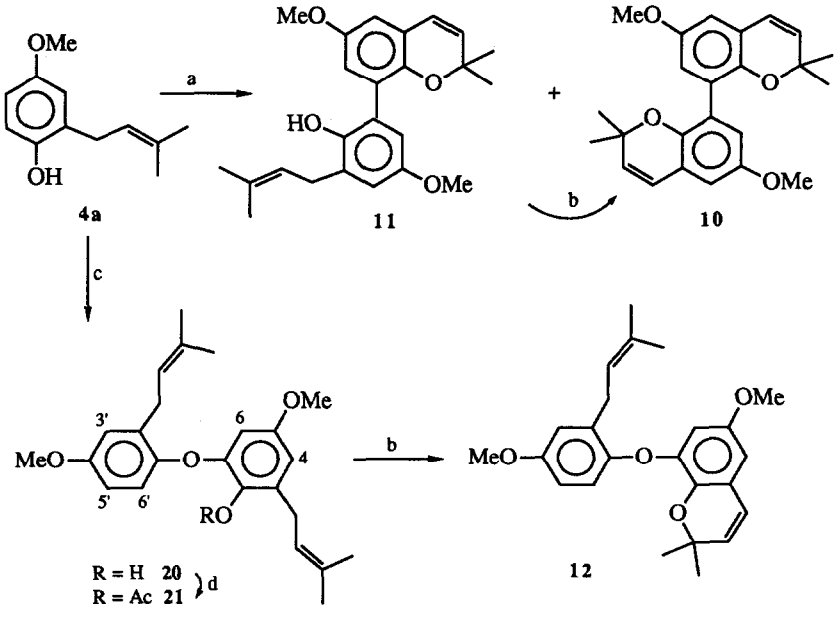
While no conclusive evidence could be achieved to discard either structure **13** or **14**, further NOEDS experiments and total synthesis firmly supported formulae **11** and **12**. For example, irradiation of the 4'-OMe group in the ^{1}H-NMR spectrum of compound **12** markedly affected the intensity of both H-3' (10%) and H-5'

(12%) signals, while irradiation of the methylene group in the side chain induced a positive NOE on H-3' (8%) and H-7 (3%) signals. The latter result clearly indicated that the C_5 chain is oriented outside the pyran ring in the preferred rotamer of compound **12** (see formula).

Chromenes **10-13** (or **14**) and **20** can be considered oxidation dimers of phenol **4a**.



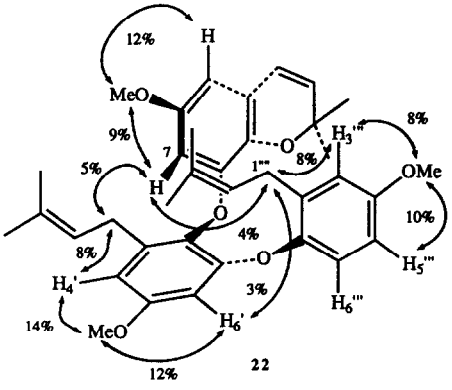
In the plant and animal kingdoms oxidative dimerization of phenolic compounds by C-C and C-O couplings are catalyzed by enzymes having iron or copper as a prosthetic group and effecting one-electron transfer processes.[21] The reactions were simulated in vitro by oxidation of **4a** with Fe^{3+} and Cu^{2+} ions (Scheme 3).[22]



Scheme 3 - Reagents: a, $Cu(NO_3)_2$ $3H_2O$, Py-H_2O; b, DDQ, Et_2O; c, $K_3Fe(CN)_6$; d, Ac_2O-Py

In the event, exposure of phenol **4a** to the complex formed between $Cu(NO_3)_2.3H_2O$ and pyridine[23] afforded compound **10** in traces and a little amount of the monocyclized product **11**. Its IR, MS and ^{1}H-NMR spectra were found identical with the natural chromene isolated from *L. fuliginosus*.[7] Furthermore cyclodehydrogenation of the 2-(3,3-dimethylallyl)phenol moiety with 2,3-dichloro-5,6-dicyano - benzoquinone (DDQ) in Et_2O[24] gave the symmetrical bis-3-chromene **10** in good yields, identical with the natural compound.[7] In another experiment,stirring a solution of phenol **4a** in Et_2O with aqueous alkaline $K_3Fe(CN)_6$[25] afforded dimer **20** in 28% yield, accompanied by traces of the cyclodehydrogenated compound **12**. The structure **20** was established by acetylation to **21** and analysis of MS (MW 382), IR (OH band at 3300 cm^{-1}) and ^{1}HNMR spectra. The latter showed, in addition to two meta coupled protons at δ 6.06 and 6.38, an ABC system assigned to three aromatic protons in 1,2,4 position and the characteristic signals of two 3,3-dimethylallyl groups. Again DDQ was the reagent of choice for the 2-allylphenol **20** cyclodehydrogenation. Chromene **12**, thus obtained, was identical with the compound endogenous to *L. fuliginosus*. Noteworthy a proper choice of the metallic species can modulate the outcome of phenol **4a** oxidative dimerization, giving either the product of C-C (→ **11**) or C-O (→ **20**) coupling.

During this investigation on *L. fuliginosus*, in addition to the compounds already discussed, we isolated trimer **22**, another interesting new oligomer of phenol **4a**. Structure **22** was established on the basis of the molecular weight (m/z 570 in the EIMS spectrum) and the ^{1}HNMR spectrum, (reported and assigned in the Experimental part) showing the signals of two 3,3-dimethylallyl side chains, three OMe singlets, one characteristic AB quartet for a 3-chromene ring, two pairs of two meta coupled aromatic protons (H-5, H-7 and H-4', H-6') and the expected cluster of signals for the hydrogens H-3"', H-5"' and H-6"'. The results of extensive NOEDS experiments, reported on formula **22**, confirmed the structure. Furthermore NOE's observed between H-7 and H-1" (5%), H-7 and H-1"" (4%), H-6' and H-1"" (3%) indicate for compound **22** the propeller like conformation shown in the formula below.



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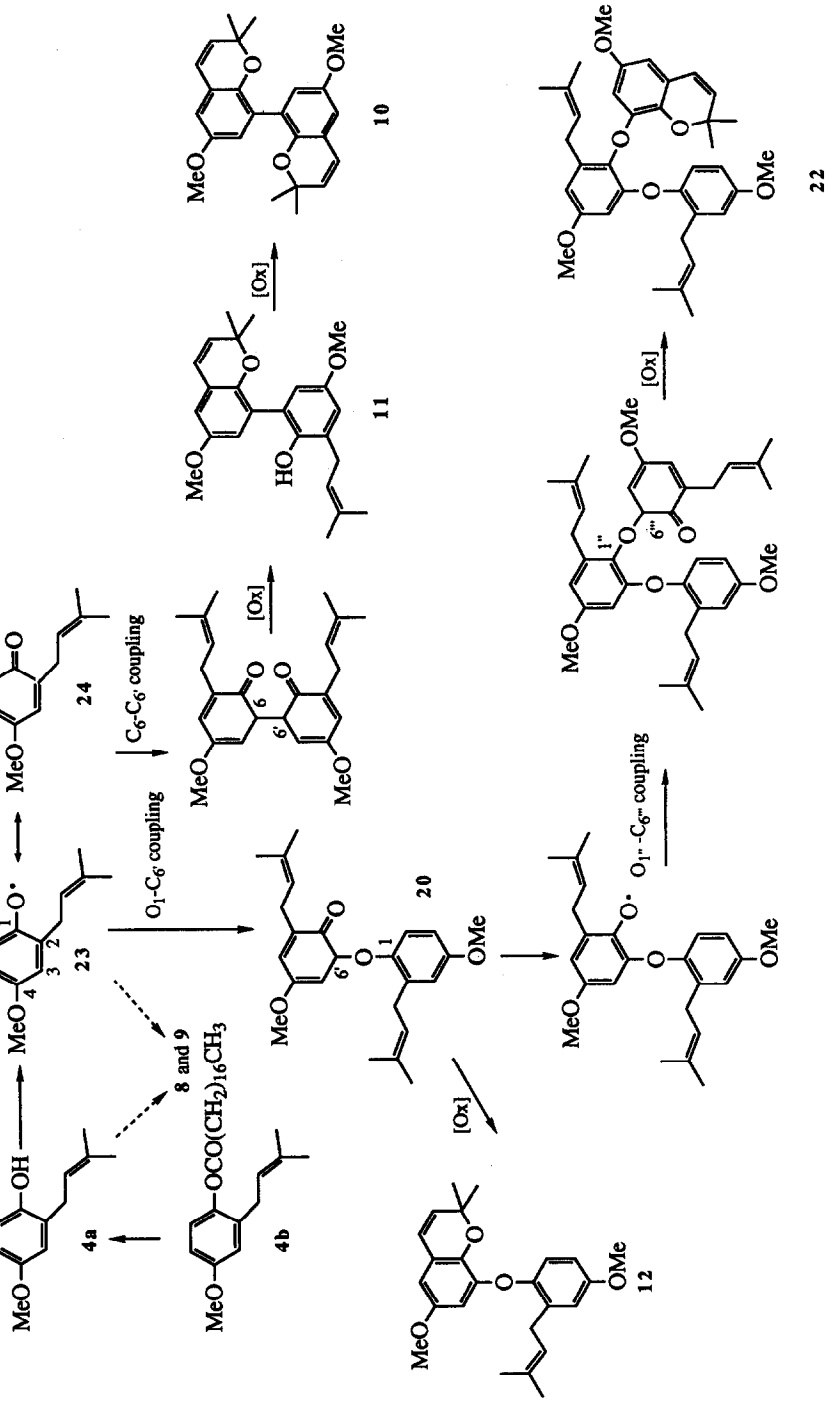

Scheme 4 - Proposed Biosynthetic pathway to compounds **8-12, 20** and **22**

CONCLUSION

A thorough investigation of the constituents of two inedible mushrooms, *L. fuliginosus* and *L. picinus*, showed that these two representative species of the Plinthogali section are chemically very well differentiated from the other *Lactarius* species investigated so far. In fact, instead of sesquiterpenes we found phenol and chromene compouds. Only the tasteless fatty acid ester **4b** is contained in the white latex of intact fruit-bodies. Injuring the mushrooms tissues triggers two enzymatic transformations of substrate **4b**, causing the flesh and latex to become unpleasantly acrid and reddish. An enzymatic lipase quickly releases free phenol **4a** from ester **4b**, then **4a** is slowly oxidized to red pigments by phenol oxidases. In the first step of this process phenoxy radicals with structures **23** and **24** are formed. These give rise to colourless oligomers **10-13** (or **14**), **20** and **22** by oxidative C-C and C-O intermolecular couplings (scheme 4). Chromene **8** and benzofuran **9** can as well arise from **4a** by intramolecular oxidative cyclization of radical **23**. Of course other biosynthetic pathways, ionic in character, can in principle be proposed for the latter two compounds.

At this stage it is impossible to support or discard the hypothesis that compound **4b** and its transformation products **4a**, **8-15**, **20** and **22** constitute a chemical defense system which becomes active in injured fruit-bodies, protecting the mushrooms against parasites. In this respect it is very interesting to note that, while protected phenol **4b** is tasteless and biologically inactive, the free phenol **4a** is acrid and show strong antifungal activity against *Candida albicans*. The mixture of chromenes has only a weak antifeedant activity against *Spodoptera littoralis*.[26]

EXPERIMENTAL

Melting points were determined on a Fisher-Johns hot plate and are uncorrected. IR spectra were recorded (film or KBr pellets) with a Perkin-Elmer Model 257 spectrometer; ^{1}HNMR spectra were recorded in $CDCl_3$ solution, unless otherwise indicated, using a Bruker WP80SY, a Bruker CXP300 or a Varian XL-100 spectrometer. Chemical shifts are reported in δ units with Me_4Si as internal standard; the abbreviations s=singlet, d=doublet, t=triplet, q=quartet, qu=quintet, m=multiplet and b=broad are used throughout. Coupling constants (J) are reported in Hz. Mass spectra were determined with a Du Pont 21-492B mass spectrometer at 70 eV using a direct inlet system. Specific optical rotations were determined with a digital Perkin-Elmer 241 polarimeter. Woelm Al_2O_3 (activity III) and Merck Kieselgel 60 (0.043-0.060 mm) were used for column chromatography. Medium pressure CC was performed employing a Miniprep 100 (Jobin-Yvon) instrument. Analytical GF_{254} TLC plates (250 μm) were obtained from Merck. The spots were visualized under UV light or by spraying the plates with an EtOH sulphuric acid-vanilline solution and then heating at 120°C for few minutes. Compounds **4a-b**, **8**, **10-15**, **20** and **22** gave olive-green spots; benzofuran **9** appeared as a blue spot. For GC analysis a Perkin-Elmer Sigma 3B gas chromatography with a FID and a 1.5% Dexsil 300 GC on Chromosorb W AWDMCS (80-100 mesh; Supelco) packed glass silanized column, 2m x 2mm I.D. was used. The column temperature was increased from 80° to 280°C at 5°C/min, the carrier gas (N_2) flow-rate was 30 cc/min, the injector temperature was 250°C and the detector temperature was 300°C. GC-MS was carried out on a DuPont 21-492B mass spectrometer equipped with a DuPont data system and coupled to a Varian 2700 gas chromatograph. The column was a 1.5% Dexsil 300 GC as above, but of dimensions 4m x 2mm I.D. The column temperature was increased from 80 to 320°C at 8°C/min, the carrier gas (He) flow-rate was 45 cc/min and the temperatures were: injector 250°C, jet separator 330°C and MS source 250°C. A FID was used. All solvents were purified and dried by standard techniques just before use. All reactions were routinely carried out under an inert atmosphere of dry, oxygen free N_2 or argon. All

organic solutions were washed with brine, then dried with $MgSO_4$ and filtered prior to rotary evaporation at water aspirator pressure. Residual solvent was removed under vacuum, usually at less than 1 torr. Reactions yields are for TLC homogeneous compounds.

Extraction and isolation of the compounds. L. fuliginosus Fr. was collected in different woods of Italian Appenninines, while *L. picinus* Fr. was found in coniferous forest near Champoluc (Aosta Valley). For the isolation of compounds **4a** and **4b**, fresh and apparently intact specimens of *L. fuliginosus* were frozen at -20°C and soaked still frozen in distilled (P_2O_5) CH_2Cl_2. After 10 min the extract was dried, concentrated and separated on a silica gel column with a hexane-Me_2CO gradient (from 99:1 to 10:1) to give **4b** and **4a**. In a separate experiments few specimens of *L. fuliginosus* were ground at room temperature in a home mixer without addition of any solvent. After 40-50 min the mush was washed exhaustively with CH_2Cl_2. After separation of H_2O, the organic layer was dried and concentrated. Column chromatography over Al_2O_3, using EtOAc as eluent, gave a mixture, enriched in chromenes, which was subjected to GC and GC-MS.[15] For preparative purposes about 2.5 Kg of *L. fuliginosus* were ground and extracted with Me_2CO for few hours. The organic solvent was removed under vacuum and the aqueous layer was extracted with EtOAc, then with n-BuOH. Column chromatography of the EtOAc extract (4.5 g) over Al_2O_3 (100 g) gave three fractions, eluted with $CHCl_3$ (0.88 g), AcOEt (0.138 g) and MeOH (0.218 g) respectively. Individual compounds **8**, **10-13** (or **14**), **22** were isolated from the first two fractions according to procedures described in reference 7.

Spectroscopic data for the revised structures[7] **11** and **12** are again reported in full with the corrected assignments.

2-(3-Methyl-2-butenyl)-4-methoxyphenol (**4a**). Mp 56-59°C; IR (KBr) cm^{-1}. 3400, 1600, 1500, 1430, 1375, 1345, 1280, 1200, 1180, 1150, 1105, 1040, 920, 870, 845, 805, 785, 730, 715; EIMS, m/z (% rel. int.): 192 (M^+, 87), 177(M-Me,26), 163(12), 161(11), 149(17), 137(100), 136(90), 121(24), 115(18), 109(29), 108(71), 105(19), 91(35), 79(38), 77(54), 65(41), 55(34), 43(32), 41(67); ^1H NMR (300 MHz, C_6D_6) δ:1.54 (3H, bs, MeC=), 1.57(3H, bs, MeC=), 3.30(2H, bd, $J_{1'-2'}$= 7.2, $J_{1'-3}$= 0.7, Ar-CH_2), 3.39 (3H, s, OMe), 5,34(1H, tqu, $J_{1'-2'}$=7.2, $J_{2'-4'}$=1.5, *CH*=C(Me)$_2$), 6.50(1H, d, J_{5-6}=8.5, H-6), 6.61 (1H, dd, J_{5-6}=8.5, J_{3-5}=3.0, H-5), 6.84(1H, bd, J_{3-5}=3,0, $J_{3-1'}$=0.7, H-3).

2-(3-Methyl-2-butenyl)-4-methoxyphenyl stearate (**4b**). Mp 31-32°C; IR (film) cm^{-1}: 2910, 2850, 1750, 1605, 1585, 1490, 1415, 1375, 1290, 1275, 1190, 1180, 1135, 1110, 1040, 935, 920, 720; EIMS, *m/z* (% rel. int.): 458(M^+, 7), 193(22), 192(100), 137(11), 69(8), 57(10), 43(16), 41(10);^{1}HNMR (80 MHz) δ:0.92 (3H, bt, ω-Me of stearate chain), 1.3 (28H, bs, $(CH_2)_{4-17}$ of stearate chain), 1.72 (3H, bs, MeC=), 1.77 (3H bs, MeC=), 2.57 (2H, t, J=7.0, -CH_2-COOAr), 3.20(2H, bd, J=7.0, Ar-CH_2), 3.80(3H, s, OMe), 5.25 (1H, bt, J=7.0, *CH*=C(Me)$_2$, 6.62-7.0 (3H, m, H-3, H-5 and H-6).

2-(1-Hydroxy-1-methylethyl)-5-methoxybenzofuran (**9**). Mp 44-45°C; IR(KBr) cm^{-1}. 3420, 2970, 2830, 1610, 1595, 1470, 1445, 1360, 1300, 1203, 1170, 1135, 1080, 1025, 955, 930, 845, 795, 750; EIMS, *m/z* (% rel. int.): 206 (M^+, 34), 191(M-Me, 100), 148(8), 91(14), 77(13), 43(85); ^{1}HNMR (300 MHz) δ:1.66(6H, s, Me_2C-O), 3.83(3H, s, OMe), 6.52(1H, d, J_{3-7}=0.9, H-3), 6.87(1H, dd, J_{6-7}=8.8, J_{6-4}= 2.6, H-6), 7.01(1H, dd, J_{4-6}=2.6, J_{4-7}=0.6, H-4), 7.35(1H, d of distorted triplets, J_{6-7}=8.8, J_{7-4}=0.6, J_{7-3}=0.9, H-7).

6-Methoxy-8-[2-hydroxy-3-(3-methyl-2-butenyl)-5-methoxyphenyl]-2,2-dimethyl-2H-chromene (**11**). IR (film) cm^{-1}: 3380, 1590, 1465, 1390, 1380, 1205, 1165, 1155, 1135, 1050, 950, 910, 840, 790; EIMS, *m/z* (% rel. int.): 380 (M^+, 30), 365 (M-Me, 100), 309(18), 281(18), 191(15), 175(16), 91(22), 55(20); ^{1}HNMR (100 MHz, C_6D_6) δ: 1.20(6H, s, Me_2C-O), 1.77 (6H, bs, Me_2C=), 3.36 (3H, s, OMe), 3.49(3H, s, OMe), 3.79(2H, bd, J=7.0, Ar-CH_2). 5.26 (1H, d, J_{3-4}=10.0, H-3), 5.66(1H, m, *CH*=C(Me)$_2$), 6.10(1H, d, J_{3-4}=10.0, H-4), 6.58(1H, s, OH), 6.63(1H, d, J_{5-6}=3.0, H-5 or H-7), 6.93 (1H, d, J_{5-7}=3.0, H-7 or H-5), 7.00 (1H, d, $J_{4'-6'}$=3.5, H-4' or H-6'), 7.09(1H, d, $J_{4'-6'}$=3.5, H-6' or H-4').

6-Methoxy-8-[2-(3-methyl-2-butenyl)-4-methoxyphenoxy]-2,2-dimethyl-2H-chromene (**12**). IR (film) cm^{-1}: 1610, 1575, 1480, 1435, 1385, 1375, 1285, 1255, 1200, 1148, 1125, 1045, 955, 900; $UV_{\lambda max}^{MeOH}$ (log ϵ): 271(3.76), 275(3.76), 332(3.45); EIMS, *m/z* (% rel. int.): 380 (M^+, 49), 365(M-Me, 100), 325(M-Me_2C=CH, 3), 205(3), 191(8), 190(M^{++}, 6), 175(22), 161(9), 144(17); ^{1}HNMR(100 MHz, C_6D_6) δ:1.24 (6H, s, Me_2CO), 1.59 (3H, bs, J=1.5, MeC=), 1.64(3H, bs, J=1.5, MeC=), 3.28(3H, s, OMe), 3.34(3H, s, OMe), 3.59(2H, bd, J=7.0, $ArCH_2$), 5.35(1H, d, J_{3-4}=10.0, H-3), 5.50(1H, tqu, J=7.0 and 1.5, C*H*=C($Me)_2$), 6.12(1H, d, J_{3-4}=10.0, H-4), 6.33(1H, d, J_{5-7}=3.0, H-7). 6.54(1H, dd, $J_{5'-6'}$= 9.0, $J_{5'-3'}$=3.0, H-5'), 6.57(1H, d, J_{5-7}=3.0, H-5), 6.85(1H, d, $J_{5'-6'}$=9.0, H-6'), 6.95(1H, d, $J_{5'-3'}$= 3.0, H-3'); ^{13}CNMR(25.2 MHz) δ:17.7 and 25.7(2xq, *Me*$_2$C=), 27.4(q, *Me*$_2$C-O), 28.5(t, CH_2), 55.5(q, OMe), 75.7(s, Me_2C-O), 105.0(d, C-5 and C-7), 111.3(d, C-3), 120.1[a](d, C-6'), 122.1[a](d, C-5'), 122.3[a](d, $ArCH_2$*C*H=), 122.9(s, C-4a), 132.2(d, C-4), 132.7(s, Me_2*C*=), 134.0[b](s, C-2'), 137.0[b](s, C-8a), 144.9[c](s, C-8), 148.4[c](s, C-1'), 153.3[d](s, C-6), 155.7[d](s, C-4'). Attributions of the signals marked with the same letter can be interchanged.

6-Methoxy-8-{2-[2-(3-methyl-2-butenyl)-4-methoxyphenoxy]-4-methoxy-6-(3-methyl-2-butenyl)phenoxy}-2,2-dimethyl-2H-chromene (**22**). EIMS, *m/z* (% rel. int.): 570(M^+, 100), 555(M-Me, 79), 378(15), 365(20), 363(12); ^{1}HNMR (300 MHz) δ:1.40(s, 6H, Me_2C-O), 1.58, 1.61, 1.65 and 1.69(4xs, 4Me, 2 *Me*$_2$C=), 3.03(2H, bd, J=8.0, H_2-1""), 3.33(2H, bd, J=7.0, H_2-1"), 3.61(3H, s, MeO-6), 3.67 (3H, s, MeO-5'), 3.76 (3H, s, MeO-4"'), 5.09(1H, bt, J=8.0, H-2""), 5.28(1H, bt, J=7.0, H-2"), 5.64(1H, d, J=9.7, H-3), 6.08(1H, d, J=3.0, H-4'), 6.12(1H, d, J=2.8, H-7), 6.17(1H, d, J=2.8, H-5), 6.27(1H, d, J=9.7, H-4), 6.44(1H, d, J=3.0, H-6'), 6.64(1H, dd, $J_{5'''-6'''}$=8.8, $J_{5'''-3'''}$=3.0, H-5"'), 6.69(1H, d, $J_{3'''-5'''}$=3.0, H-3"'), 6.79(1H, d, $J_{5'''-6'''}$=8.8, H-6"').

Synthesis of compounds **4a** *and* **4b**. A suspension of sodium phenoxide, prepared by refluxing in 40 mL of anhydrous benzene for 4h 3.7 g (33 mmol) of 4-methoxyphenol and 0.76 g (33 mmol) of metallic Na, was cooled to 0°C and to it was added, with good stirring, 4.92 g (33 mmol) of 4-bromo-2-methyl-2-butene over a period of 30 min. After stirring for 5 h longer and then standing overnight, benzene was removed under reduced pressure and H_2O (50 mL) and petroleum ether (50 mL) were added to the residue. The mixture was extracted with three 25 mL portions of 10% NaOH solution and with 25 mL of "Claisen alkali". To make the latter, a mixture of KOH (30 g) in H_2O (25 mL) is diluted to 100 mL with MeOH. The combined alkaline extracts were acidified with dilute H_2SO_4, extracted with Et_2O and dried over $MgSO_4$. After removal of volatiles, the residue was separated by chromatography on a silica gel column, under medium pressure. Elution with cyclohexane-EtOAc (85:15) mixture gave phenol **4a** (1.96 g) in 31% yield, identical with the natural compound. Commercial stearoyl chloride (363 mg, 1.2 mmol) and a catalytic amount of DMAP were added to phenol **4a** (76 mg, 0.4 mmol) in dry pyridine (1.5 mL) and the mixture was left overnight. The solution was diluted with Et_2O (20 mL), washed with 5% NaOH, 10% HCl, brine and dried ($MgSO_4$). Cc of the residue, using cyclohexane-EtOAc (99:1) as eluent, afforded pure ester **4b** (136 mg, 74%) identical (R_f, IR and ^{1}HNMR spectra) with the natural compound.

2-(2,3-Epoxy-3-methylbutyl)-4-methoxyphenol (**5**). Solid 80% *m*-chloroperbenzoic acid (115.8 mg, 0.54 mmol) and KOAc (20 mg) were added to phenol **4a** (79.3 mg, 0.41 mmol) in CH_2Cl_2 at 0°C. After completion of the reaction, the solution was quenched with ice cold H_2O and estracted with Et_2O. The organic layer was washed with 5% $Na_2S_2O_5$, 5% $NaHCO_3$, brine and dried. After removal of solvent, the residue was purified by silica gel cc. Elution with cyclohexane-EtOAc, 85:15, afforded pure **5** (76.6 mg, 89%); IR (film) cm^{-1}: 3360, 2960, 2815, 1610, 1500, 1430, 1380, 1210, 1040, 925, 895, 845, 810, 770, 755, 725, 710; ^{1}HNMR (80 MHz) δ:1.35(3H, s, Me), 1.47(3H, s, Me), 2.63-3.25 (3H, m, CH_2Ar and CH-O), 3.77 (3H, s, OMe), 6.50-7.0(3H, m, Ar-H); EIMS, *m/z* (% rel. int.): 208 (M^+, 100), 190(M-H_2O, 17), 175(36), 150(27), 137($ArCH_2^+$, 98), 123(19), 108(18), 91(13), 77(18), 71(34), 59(19), 43(55), 41(18).

2,2-Dimethyl-3-hydroxy-6-methoxychromene (**6**) *and 2-(1-hydroxy-1-methylethyl)-5-methoxy-2,3-dihydrobenzofuran* (**7**). A solution of epoxyphenol **5** (57 mg, 0.27 mmol) in MeOH (2 mL) and MeONa (14.8

mg) was stirred at room temperature until TLC indicated disappearance of starting material. The mixture was diluted with ice-cold H_2O and extracted with Et_2O. Removal of solvent gave a residue (46 mg) which was immediately exposed to standard acetylation conditions. Usual work up gave a mixture of **7** and **6** acetate which were separated by silica gel cc. Elution with cyclohexane-EtOAc, 85:15, gave **7** (40 mg) and **6** acetate (3.8 gm). Compound **7**: IR (film) cm^{-1}: 3430, 2975, 2815, 1605, 1480, 1375, 1300, 1230, 1200, 1135, 1025, 1000, 980, 960, 945, 860, 810, 735; EIMS, *m/z* (% rel. int.): 208(M^+, 100), 190(M-H_2O, 37), 175(75), 150(79), 149(53), 137(68), 136(24), 135(53), 121(24), 91(21), 77(15), 71(22), 59(63), 43(53); ^{1}HNMR (80 MHz) δ:1.23(3H, s, Me), 1.35(3H, s, Me), 2.02(1H, bs, OH), 3.12(2H, d, J=9.0, H_2-3), 3.75(3H, s, OMe), 4.53(1H, t, J=9.0, H-2), 6.50-6.80 (3H, m, Ar-H). **6** Acetate: IR (film) cm^{-1}: 2980, 2958, 1725, 1490, 1450, 1428, 1375, 1255, 1220, 1150, 1035, 950, 920, 900, 850, 815; EIMS, m/z (% rel. int.): 250 (M^+, 45), 208(15), 190(M-AcOH, 36), 175(100), 149(18), 137(40), 136(22), 77(11), 71(24), 43(65); ^{1}HNMR (80 MHz) δ:1.30(3H, s, Me), 1.42(3H, s, Me), 2.06(3H, s, $MeCO_2$), 2.72(1H, dd, $J_{4\text{-}4'}$=17.0, $J_{4\text{-}3}$=5.0, H-4), 3.15(1H, dd, $J_{4\text{-}4'}$=17.0, $J_{4'\text{-}3}$=4.8, H-4'), 3.73 (3H, s, OMe), 4.98(1H, t, J=5.0, H-3), 6.50-6.75(3H, m, Ar-H). On exposure to methanolic K_2CO_3 **6** acetate was converted into the corresponding free alcohol **6**, mp 112-115°C; IR(KBr) cm^{-1}: 3400, 1490, 1375, 1365, 1245, 1225, 1205, 1140, 1035, 950, 840, 810; EIMS, *m/z* (% rel. int.): 208 (M^+, 91), 190(26), 175(60), 150(29), 138(69), 137(100), 136(39), 123(30), 109(18), 108(24), 91(13), 78(19), 77(27), 71(63), 65(20), 59(18), 43(95); ^{1}HNMR (80 MHz) δ:1.28 (3H, s, Me). 1.33 (3H, s, Me). 2.71(1H, dd, $J_{4\text{-}4'}$= 17.0, $J_{4\text{-}3}$=5.8, H-4), 3.13(1H, dd, $J_{4\text{-}4'}$=17.0, $J_{4'\text{-}3}$=5.0, H-4'), 3.76(3H, s, OMe), 3.82(1H, t, J=5.5, H-3), 6.55-6.81 (3H, m, Ar-H).

Synthesis of 2-(1-hydroxy-1-methylethyl)-5-methoxybenzofuran (**9**).

a) *By NBS oxidation of dihydrobenzofuran* **7**. Freshly crystallized NBS (11.2 mg, 0.063 mmol), benzoyl peroxide (1 mg) and solid KOAc (5 mg) were added to a solution of compound **7** (12 mg, 0.058 mmol) in CCl_4 (10 mL). The mixture was heated to reflux for 90 min, cooled, filtered and taken to dryness. The residue was purified with a silica gel column chromatography. Elution with cyclohexane-EtOAc, 85:15, afforded, further a mixture of unidentified brominated compounds, benzofuran **9** (2 mg), identical (IR and ^{1}HNMR spectra, TLC mobility) with the natural compound.

b) *From 2-hydroxy-5-methoxybenzaldehyde.* A solution of KOH (425 mg) and 2-hydroxy-5-methoxybenzaldehyde (1 g, 6.6 mmol) in EtOH (4 mL) was heated at 50°C for 1 hour. Chloroacetone (608 mg, 6.6 mmol) was slowly added, then the mixture was stirred at room temperature for 3 hours. Cold H_2O was added, MeOH was evaporated under reduced pressure and the residue was taken up in Et_2O. The organic layer was washed with brine, dried ($MgSO_4$) and evaporated. Crystallization of the residue from MeOH gave 2-acetyl-5-methoxy-benzofuran (**19**) (600 mg, 48%) as colorless crystals, mp 84-85°C; IR (KBr) cm^{-1}: 3075, 2825, 1670, 1610, 1545, 1470, 1350, 1280, 1210, 1185, 1145, 1015, 968, 908, 845, 825, 750; EIMS, *m/z* (% rel. int.) 190(M^+, 94), 175(M-Me, 100), 119(31), 76(11), 43(32); ^{1}HNMR (80 MHz) δ:2.62(3H, s, Me), 3.90(3H, s, OMe), 7.0-7.25(2H, m, H-4 and H-6), 7.47 (1H, s, H-3), 7.50(1H, d, J=8.5, H-7). To an Et_2O solution of MeMgI, prepared in the standard way from MeI (178 mg, 1.25 mmol) and metallic Mg (30 mg, 1.25 mmol), was slowly added over 15 min. a solution of compound **19** (180 mg, 0.95 mmol) in dry Et_2O (3 mL). The mixture was stirred for 2 hours at room temperature, washed with saturated aqueous NH_4Cl solution, brine, and dried ($MgSO_4$). Cc of the residue, using C_6H_6-Me_2CO, 12:1, as eluent afforded benzofuran **9** (161 mg, 82%), identical with the natural compound.

$K_3Fe(CN)_6$ oxidation[25] of phenol **4a**. A solution of phenol **4a** (100 mg, 0.52 mmol) in Et_2O (20 mL) was vigorously stirred at room temperature with a solution of $K_3Fe(CN)_6$ (206 mg, 0.62 mmol) in 0.2 N KOH (8 mL). After 50 min, the two layers were separated and the aqueous phase was extracted twice with Et_2O. The combined Et_2O extracts were dried and evaporated. Silica gel cc of the residue, using hexane-Et_2O 12:1 as eluent, gave dimer **20** (28 mg, 28%), mp 48-50°C; IR(KBr) cm^{-1}: 3450, 1600, 1485, 1435, 1370, 1195, 1135, 1100, 1040, 985, 945, 920, 835; *m/z* (% rel. int.): 382 (M^+, 100), 192(21), 137(17); ^{1}HNMR (80 MHz) δ.1.61, 1.67 and 1.74 (12H, 3s, 2xMe_2C=), 3.31 (4H, bt, J=7.0, 2x$ArCH_2$), 3.63(3H, s, OMe), 3.80 (3H, s,

OMe), 5.0-5.6(2H, m, 2xCH=), 6.06(1H, d, J=3.0, H-4), 6.38(1H, d, J=3.0, H-6), 6.50-7.12 (m, 3H, H-3', H-5', H-6').

DDQ oxidation[24] of dimer **20**. DDQ (6.5 mg, 0.029 mmol) in Et_2O (0.5 mL) is added to a stirred solution of the *o*-allylphenol **20** (10 mg, 0.026 mmol) in Et_2O (1 mL). After stirring the solution for 1 h, the organic solvent was evaporated and the residue purified by chromatography on silica gel. Elution with hexane-Et_2O, 12:1, gave 3-chromene **12** (8.5 mg. 85%) identical (R_f, IR and ^{1}HNMR spectra) with the natural compound.

$Cu(NO_3)_2.3H_2O$ oxidation[23] of phenol **4a**. A solution of phenol **4a** (50 mg, 0.26 mmol) in MeOH (1.2 mL) was added to a solution of the complex formed from $Cu(NO_3)_2.3H_2O$ (63 mg, 0.26 mmol) and Py (62 mg, 0.78 mmol) in H_2O (3 mL). The mixture was stirred at room temperature for 24 h, then more Et_2O and aqueous 5% HCl were added and the organic layer was separated, washed with brine and dried. Removal of solvent gave a residue which was purified by chromatography on silica gel. Elution with hexane-Et_2O, 12:1, gave a trace of dimer **10**, free phenol **11** (5 mg, 10%) and an unidentified yellow compound (8 mg), which is a trimer of phenol **4a** (MS and ^{1}HNMR spectra). DDQ oxidation of compound **11**, as above, gave the 3-chromene dimer **10** in quantitative yield.

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