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FUNGAL METABOLITES 10.
NEW CHROMENES FROM LACTARIUS FULIGINOSUS FRIES AND LACTARIUS PICINUS FRIES

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6-Methoxy-2,2-dimethylchromene (I) and five new chromenes (II-VI) were isolated from Lactarius fuliginosus and L.picinus (Basidiomycetes). These unusual structures have been elucidated by spectroscopic methods.2,2-Dimethylchromene (VII) was identified by GC-MS in L. picinus.

We report here for the first time the finding of chromenes in Basidiomycetes. As part of our program to isolate and characterize metabolites of Basidiomycetes, and particularly of Russulaceae, we examined the acetone and ethanolic extracts of two species belonging both, for the similarity of their morphological characters, to the same section ("fuliginosi") of the genus Lactarius: Lactarius fuliginosus Fries and Lactarius picinus Fries.[1]

The TLC analysis of the extracts, compared with that of the extracts of previously studied Lactarius species (i.e. L. scrobiculatus[2-5] and L. uvidus[6]) showed a quite different pattern of spots which appeared green when the plate was sprayed with vanillin and sulphuric acid and heated at 120°C.

The metabolites of L. fuliginosus (1 Kg) were first roughly separated by chromatography on alumina column and then isolated on a Silica gel column by elution with hexane-ethyl acetate (95:5) mixture, under pressure, using a Chromatospac (Jobin-Ivon) instrument. The order of elution was I,III,II,IV,V. Final purification could be achieved by preparative TLC.

For the sake of clarity the compounds will be discussed according to the similarity of their structures. To I (4.5 mg) we assigned the structure of 6-methoxy-2,2-dimethylchromene on the basis of the following data:
MS (12.5 eV, DIS, m/z): 190 (M^+, 23%), 175 ($M-CH_3$, 100%), 132 ($M-CH_3COCH_3$, 10%).
IR (neat, cm^{-1}): 1610, 1580, 1495 (aromatic absorptions), 1370, 1360 (gem-dimethyl group), 1210 (ether absorption). PMR (100 MHz, C_6D_6,TMS,δ): 1.26 (s,6H,CH_3), 3.32 (s,3H,CH_3O), 5.29 (d, 1H, J_{3-4} 11.0 Hz, H-3), 6.08 (d,1H, J_{3-4} 11.0 Hz, H-4), 6.52 (m,1H, H-7), 6.61 (d,1H, J_{5-7} 3.0 Hz, H-5), 6.82 (dd,1H, J_{7-8} 11.0 Hz, J_{8-4} 1H, H-8)[7].

Compound II (3,6 mg) showed spectroscopic data very similar to I. IR (neat, cm^{-1}): 1610,1592, 1460, 1435 (aromatic absorptions), 1370, 1360 (gem-dimethyl group), 1208 (ether absorption).

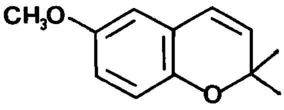
The PMR spectrum (100 MHz, $CDCl_3$,TMS) showed, besides the gem-dimethyl group (δ 1.26, s,6H), a methoxy group (δ 3.77, s,3H), a cis-disubstituted double bond (δ 5.44, d,1H, H-3 and 6.36,d, 1H, H-4, J_{3-4} 10.0 Hz) and two meta-coupled aromatic protons (δ 6.58,d,1H and δ 6.80,d,1H, J_{meta} 3.5 Hz). To explain the value of the chemical shift and the absence of any long-range

W coupling of H-4[8] (compare with I) the substituents must be located at C-6 and C-8. The M.W. (M$^+$ 378) and the symmetry of the molecule evident from the spectra indicated that II should be a dimer of a methoxychromene, the two units being linked by a C-C bond at C-6 or at C-8, depending on the relative position of the methoxy group. A fine Overhauser effect was observed for H-5 and H-7 when the methoxy group was irradiated and this allowed us to definitively assign to II the structure of 8,8'-bis(6-methoxy-2,2-dimethylchromene).
MS (75 eV, DIS, m/z): 378 (M$^+$, 28%), 363 (M-CH$_3$,100%), 174 (M-2x15^{++}, 30%), 144 (174-CH$_2$O, 12%).

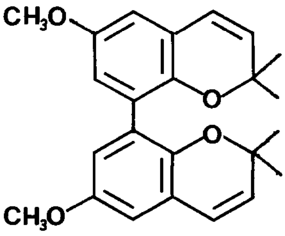
Compound III (18 mg) even if presented some analogy with I and II in the IR spectrum (see Figure 1), showed more complicated PMR data. We could establish that the molecule contained the following groups: 6,8-disubstituted-2,2-dimethylchromene, two methoxyls, 3-methyl-2-butenyl and 1,2,4-trisubstitued phenyl. Overhauser effects observed on the aromatic protons by irradiation of the methylene and the methoxy groups allowed us to assert that one methoxyl was on the chromene ring B in 6 and the second in position 2 of the aromatic ring with the 3-methyl-2-butenyl chain in position 4. The mass spectrum indicated a molecular weight 380 (M$^+$) corresponding to the formula $C_{24}H_{28}O_4$.The fourth oxygen atom could be only between the chromene and the phenyl ring forming a diaryl ether, as demonstrated by some fragmentation ions (see data on the formula III) and the ^{13}C-NMR spectrum which was in agreement with the proposed structure showing five singlets for oxygenated aromatic quaternary carbon atoms. On the basis of this data we assigned to III the structure of 6-methoxy-8[2'-methoxy-4'(3"-methyl-2"-butenyl)phenoxy]2,2-dimethylchromene.

Compound IV (0.7 mg) had M.W. 396 (M$^+$) which corresponds to the formula $C_{24}H_{28}O_5$ containing one more oxygen atom than III. The presence of a hydroxyl absorption in the IR spectrum (3470 cm^{-1}) and in the PMR spectrum (δ 6.58, s,1H, disappearing by addition of D_2O) together with the presence of a 8-substituted-6-methoxy-2,2-dimethylchromene ring and a 1,2,4,6-tetrasubstituted phenyl ring led us to assign to IV the structure of 6-methoxy-8[6'-hydroxy-2'-methoxy-4'(3"-methyl-2"-butenyl)phenoxy]2,2-dimethylchromene. Data are reported in Figure 1.

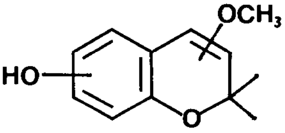
Compound V (4.3 mg) was an isomer of III (M.W. 380, $C_{24}H_{28}O_4$) but with the same 1,2,4,6-tetrasubstituted ring as IV; therefore V must be lacking in the oxygen atom linking the chromene and the aromatic rings. On these spectroscopic data (see Figure 1) we assigned to V the structure of 6-methoxy-8[6'-hydroxy-2'-methoxy-4'(3"-methyl-2"-butenyl)phenyl] 2,2-dimethylchromene though we could not record the ^{13}C-NMR spectrum because of the small amount of V.



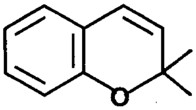
I



II



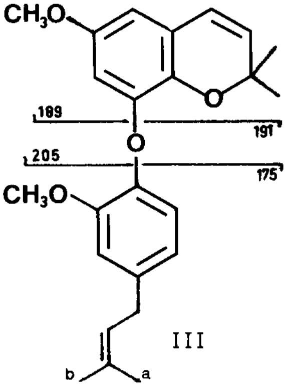
VI



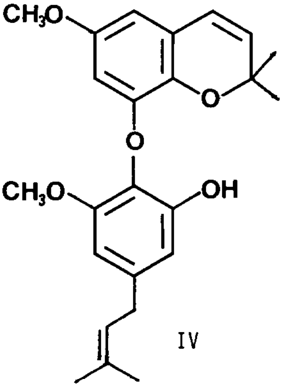
VII

The acetone and ethanolic extract of <u>L. picinus</u> was examined by GC and GC-MS[9]. Besides small amounts of fatty acid esters, we could establish the presence of six chromenes with M.W. 160, 190, 378, 380, 380 and 206 respectively. The compound with M.W. 160 could be identified as 2,2-dimethylchromene (VII) by comparison of the mass spectrum with the literature data[10]. By the retention time (GC) and comparison of their mass spectra the next four compounds were identified as I, II, III and V. By chromatographic separation, with the same procedure described for L. fuliginosus, we could isolated the main products: I, III and the compound VI of M.W. 206 (1.5 mg). VI contained : an OH group (IR: 3470 cm^{-1}), a $(CH_3)_2C<$ group (δ 1.77,s,6H), a methoxy group (δ 3.83,s,3H), a 1,2,4-trisubstituted phenyl(δ 6.90, dd,1H, J_{orto} 9.0 Hz, J_{meta} 3.0 Hz, δ 7.03,d,1H,J_{meta} 3.0 Hz, and δ 7.35,d,1H,J_{orto} 9.0 Hz), but surprisingly only one vinylic proton (δ 6.53,s,1H) which must be on the chromene A ring. Therefore the structure of a hydroxy-methoxy-2,2-dimethylchromene can be assigned to VI, one of the oxygenated substituents being on the A ring (in 3 or 4) and the other on the B ring (in 6 or 7). The definitive structure of VI will be reported elsewhere when more data will be available.

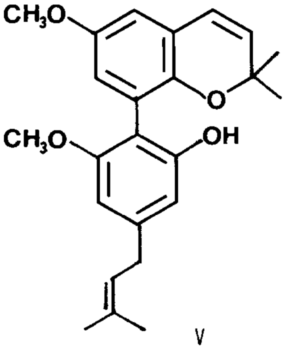
We must stress that L.fuliginosus and L. picinus differ dramatically for their content in chromenes from all the previously studied Russulaceae species which are characterized by sesquiterpenoid metabolites and they represent the first example of Basidiomycetes containing this class of natural products. Furthermore some features of the structures of II, III, IV, V and VI are quite unusual in the natural chromenes,i.e. the presence of substituents in 6 and in 8 and particularly of an aryl group in 8, the oxygenation pattern of both the chromene and the phenyl ring and the isoprenic chain on the phenyl ring. I and VII, which have simpler structures are not widespread in Nature. To our knowlegde VII was identified here for the first time and I has been isolated recently from Ageratina aromatica (Compositae)[11].



III



IV



V

FIGURE 1. SPECTROSCOPIC DATA OF III, IV AND V.

III.IR(neat,cm^{-1}): 1610,1580,1480 (aromatic absorptions), 1395,1385 (gem-dimethyl group),1200-1250(C-O-C absorptions), 1125,1148,1050,900. UV (MeOH,λ_{max}): 271(3.76), 275(3.76), 332(3.45). PMR(100 MHz,C_6D_6,TMS,δ): 1.24(s,6H,$(CH_3)_2C-O$), 1.59(br s,3H,J 1.5 Hz,CH_3), 1.64(br s J 1.5 Hz,CH_3), 3.28(s,3H,CH_3O), 3.34(s,3H,CH_3O), 3.59(br d,2H,J_{vic} 7.5 Hz,CH_2), 5.35(d,1H,J_{3-4}10.0 Hz,H-3), 5.50(m,1H,=$\underline{CH}$-CH_2), 6.12(d,1H,J_{3-4} 10.0 Hz,H-4), 6.33(d,1H,J_{meta} 2.5 Hz,H-5 or H-7), 6.54(dd,1H,J_{orto} 9.0 Hz,J_{meta} 2.5 Hz,H-5'), 6.57(d,1H,J_{meta} 2.5 Hz,H-7 or H-5), 6.85(d,1H, J_{orto} 9.0 Hz,-H-6'), 6.95(d,1H,J_{meta} 2.5 Hz,H-3').^{13}C-NMR (25.2 MHz,$CDCl_3$,ppm): 17.7(q,aCH_3), 25.7(q,bCH_3), 27.4(q,$(CH_3)_2C-O$), 28.5(t,CH_2), 55.5(q,CH_3O), 75.7(s,$(CH_3)_2\underline{C}-O$), 105.0(d,=CH-, C-7 and C-5),111.3(d,=CH-,C-3), 120.1^{a}(d,=CH-,C-6'), 122.1^{a}(d,=CH-,C-5'), 122.3^{a}(d,=CH- in the chain), 122.9(s,-$\overset{|}{C}$-,C-4 bis), 132.2(d,=CH-,C-4), 132.7(s,-$\overset{|}{C}$- in the chain), 134.0^{b}(s,-$\overset{|}{C}$=, C-4'), 137.0^{b}(s,=$\overset{|}{C}$-O,C-8 bis), 144,9^{c}(s,=$\overset{|}{C}$-O,C-8), 148.4^{c}(s,=$\overset{|}{C}$-O,C-1'), 153.3^{d}(s,=$\overset{|}{C}$-O,C-6), and 155.7^d(s,$=\overset{|}{C}$-O,C-2'). Attributions of the signals marked with the same letter can be reversed. MS(75 eV,DIS, m/z,%): 380(M^+,49), 365(M-CH_3,100), 325(M- $(CH_3)_2C=CH$,3), 205(3),191 (8), 190(M^{++},6), 189(10), 182.5(M-$CH_3{}^{++}$,4), 175(22), 161(191-CH_2O,9), 144(175-CH_3O,17).

IV. IR (neat,cm^{-1}): 3470 (OH), 1590,1460 (aromatic absorptions), 1385,1375(gem-dimethyl group), 1200 (C-O-C absorption), 1165, 1155, 1130, 1049, 948, 925, 905, 840, 785. PMR(100 MHz,C_6D_6, TMS,δ):1.22(s,6H,$(CH_3)_2C$-O), 1.77(d,6H,J$<$1.0 Hz,$(CH_3)_2C=$), 3.50(s,6H,CH_3O), 3.80(d,2H,J_{vic} 7.0 Hz,CH_2), 5.28(d,1H,J_{3-4} 11.0 Hz,H-3), 5.68(m,1H,-CH=), 6.12(d,1H,J_{3-4} 11.0 Hz,H-4), 6.58 (s,1H,OH), 6.64(d,1H,J_{meta} 3.5 Hz,H-5 or H-7), 6.93(d,1H,J_{meta} 3.5 Hz, H-7 or H-5), 7.00(d, 1H,J_{meta} 4.0 Hz, H-3' or H-5'), 7.11(d,1H,J_{meta} 4.0 Hz, H-5' or H-3'). MS(75 eV, DIS,m/z,%): 396(M^+,36), 381(M-CH_3,90), 366(M-2xCH_3,100), 205(6), 189(9), 175(42).

V. IR (neat, cm^{-1}): 3380(OH), 1590,1465 (aromatic absorptions), 1390,1380 (gem-dimethyl group), 1205 (C-O-C absorption), 1165, 1155, 1130, 1050, 950, 910, 840, 790. PMR(100 MHz,C_6D_6,TMS,δ): 1.20(s,6H,$(CH_3)_2C$-O), 1.77(br s,6H,$(CH_3)_2C=$), 3.36(s,3H,CH_3O), 3.49(s,3H,CH_3O), 3.79(br d, 2H, J_{vic} 7.0 Hz,CH_2), 5.26(d,1H,J_{3-4} 11.0 Hz,H-3), 5.66(m,1H,-$\underline{CH}$-CH_2), 6.10(d,1H,J_{3-4} 11.0 Hz,H-4), 6.58(s,1H,OH), 6.63(d,1H,J_{meta} 3.5 Hz,H-5 or H-7), 6.93(d,1H,J_{meta} 3.5 Hz, H-7 or H-5), 7.00(d,1H,J_{meta} 3.5 Hz, H-3' or H-5'), 7.09(d,1H,J_{meta} 3.5 Hz,H-5' or H-3').MS(75 eV, DIS, m/z,%): 380 (M^+,30), 365(M-CH_3,100), 309(18), 281(18), 191(15), 175(16),91(22),55(20).

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9. GC was carried out on a 3% Dexil 300 glass column, T 100°C then 10°/min to 280°, N_2 flow 32 ml/min, injector T 250°C,detector T 300°C, Sigma 3 Perkin Elmer instrument.
   GC-MS was carried out on HP 5985B instrument, fused silica column (25 m x 0.2 mm ID) OV 1; He flow 1 ml/min, T 40°C(1 min) then 20°C/min to 300°C, injector (splitless) T 250°C. Mass spectra 70 eV, 300 μA.
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