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William A. Ayer, and Latchezar S. Trifonov

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AROMATIC COMPOUNDS FROM LIQUID CULTURES OF *LACTARIUS DELICIOSUS*

WILLIAM A. AYER* and LATCHEZAR S. TRIFONOV

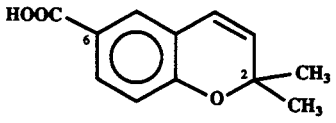
Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2

ABSTRACT.—The metabolites produced when the fungus *Lactarius deliciosus* is grown in liquid culture are reported. Previously only the metabolites of the fruiting bodies (mushrooms) of the fungus have been reported. The liquid cultures produce different metabolites. Anofinic acid [**1**], a new chroman-4-one [**2**], and 3-hydroxyacetylindole [**4**] were obtained, along with known cyclic dipeptides, ergosterol, and a mixture of fatty acids.

Two types of metabolites have been reported from the fruiting bodies of members of the genus *Lactarius* (family Russulaceae, Basidiomicotina subdivision) namely, sesquiterpenes [guaianes (1,2), marasmanes (3,4), lactaranes, secolactaranes, isolactaranes (2,5), and drimanes (7,8)] and aromatic compounds [2,2-dimethylchromene, 6-methoxy-2,2-dimethylchromene, and derivatives thereof (6), as well as simple esters of isoprenylated hydroquinones (9)]. It has been shown that the metabolites responsible for the color change of the injured fruiting body of *L. deliciosus* are azulene-type sesquiterpenes (1,2). There are no reports on the metabolites produced when *L. deliciosus* is grown in liquid culture. Herein we report on the metabolites isolated from the liquid cultures of this fungus (malt extract and modified malt extract) as well as those produced in a malt extract agar medium.

Lactarius deliciosus is a mycorhizal fungus that grows extremely slowly in liquid media. We found that it grows best in malt extract liquid medium containing 1% added glucose, but several months' growth is required to produce even small amounts of metabolites. The filtered culture broth of *L. deliciosus* was extracted with CH_2Cl_2 or EtOAc and the extract was subjected to flash chromatography on Si gel. Pure compounds were obtained by repeated column chromatography and/or prep. tlc.

Compound **1** was obtained as a colorless solid with physical and spectroscopic properties identical with those of anofinic acid, previously isolated from the plant *Anodendron affine* (10,11). It has not been reported before as a fungal metabolite.



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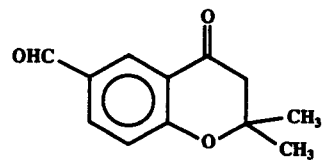
Compound **2** was obtained as colorless needles, mp 87.0–91.0°, possessing a pleasant odor. It has the same empirical formula as **1** ($C_{12}H_{12}O_3$) as shown by hreims. However, instead of the C-3–C-4 olefinic system present in **1**, compound **2** has a CH_2CO fragment, characterized by an ^{1}H-nmr signal at δ 2.79 (2H, singlet) and ^{13}C-nmr signals at δ 48.6 (t) and 191.4 (s). The CHO group (s at 9.93 ppm in the ^{1}H-nmr spectrum) and the CO group are responsible for the very low-field chemical shift of H-5 (d, J=2.1 Hz at 8.36 ppm) and of H-7 (dd, J=8.6 and 2.1 Hz at 8.04 ppm). The ^{13}C-nmr spectrum was consistent with the proposed structure that we designate as lactarochromal. This compound [**2**] is a



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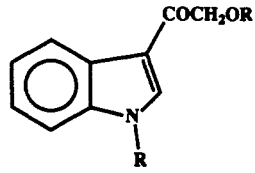
new representative of the small group of fungal chroman-4-ones (12).

In addition to compounds **1** and **2**, cyclo-Pro-Val, indole-3-carboxaldehyde, and a trace amount of cyclo-Pro-Leu were isolated, as well as a small amount of an indole derivative, 3-hydroxyacetylindole [**4**], isolated as its *N,O*-diacetyl derivative, **3**. The structure of the latter compound is based on the ^{1}H-nmr spectrum, showing two acetyl signals, one two-proton singlet at 5.23 ppm (CH_2), and aromatic signals very similar to those of indole-3-carboxaldehyde. The hreims showed the molecular ion ($C_{14}H_{13}NO_4$) as well as fragments formed by loss of ketene and $AcOCH_2$ (see Experimental). Neither **3** nor the parent compound **4** have been reported previously.



3 R=Ac
4 R=H

EXPERIMENTAL

ISOLATION OF METABOLITES.—Cultures of *L. deliciosus* (UAMH 5548) were obtained from L. Sigler, University of Alberta Microfungus Herbarium. The fungus was first grown on malt extract agar medium at 22° for 6 months. The medium was blended, extracted with Et_2O, the extract dried over $MgSO_4$, and the solvent removed to afford ca. 100 mg of a red oil. The fungus was also grown on malt extract (20 g of malt extract, 1.5 g of yeast extract, and 1 liter of distilled H_2O) and on modified malt extract medium (20 g of malt extract, 20 g of D-glucose, 1.5 g of yeast extract, and 1 liter of H_2O) in still cultures at 18° in the dark for 9 and 4 months, respectively. The mycelium was filtered and the broth extracted with CH_2Cl_2 to give the crude extract as an oil (170 mg from 10 liters and 230 mg from 11 liters, respectively.).

In a typical procedure, the crude extract (170 mg) was subjected to flash chromatography on Si gel with petroleum ether-EtOAc-CH_2Cl_2-MeOH (81:8:8:3, 240 ml), (63:16:16:5, 250 ml), (45:40:11:4, 150 ml), and (31:58:8:3, 150 ml), with fractions of 15 ml being collected. Fractions 9–20 (1.1 mg) were purified by prep. tlc with petroleum ether-EtOAc (83:17, threefold development) to give pure lactarochromal (**2**, 0.7 mg).

Fractions 37–46 (19 mg) were subjected to prep. tlc with petroleum ether-EtOAc-CH_2Cl_2-MeOH (63:16:16:5, fivefold development). The uv active zone at R_f 0.60 was eluted with CH_2Cl_2-MeOH (90:10) and the crude material acetylated with Ac_2O (0.2 ml) and pyridine (0.2 ml) at room temperature for 40 h. Toluene (1 ml) was added and the solvent was removed *in vacuo*. The residue was chromatographed on one prep. tlc plate (10×20 cm) with petroleum ether-EtOAc (83:17, threefold development) and the uv-active zone eluted with CH_2Cl_2 to give pure *N*-acetylindole-3-carboxaldehyde (1.2 mg) as a colorless solid.

Fractions 52–80 (55 mg) were separated on four prep. tlc plates (Si gel Aldrich 28,854-3, freshly prepared 20×20 cm plates) with petroleum ether-EtOAc-CH_2Cl_2-MeOH-AcOH (67.4:16:16:0.6). The uv active zone at R_f 0.82 was eluted with CH_2Cl_2-MeOH (90:10) to give pure anofinic acid [**1**], 13.1 mg. The zone at R_f 0.50–0.53 gave 2-(*p*-hydroxyphenyl)ethanol (1.2 mg). The zone at R_f 0.36–0.40 gave a crude product (7.6 mg) that was acetylated with pyridine/Ac_2O at room temperature for 40 h. Prep. tlc of the acetylated material with petroleum ether-EtOAc (71:29, sixfold development) and extraction of the zone at R_f 0.25 afforded 1-acetyl-3-acetoxyacetylindole as a colorless oil (0.5 mg), which solidified at 0°.

The crude CH_2Cl_2 extract from the modified malt extract broth (230 mg) was treated in the same way to afford **1** (50 mg) and **2** (1.7 mg). The fraction following that contained **1** was recrystallized from EtOAc/Et_2O to give cyclo-Pro-Val (4.6 mg). The mother liquor was subjected to prep. tlc with toluene-EtOAc-MeOH (82:13:5, threefold development). The zone at R_f 0.40 was eluted with CH_2Cl_2-MeOH (90:10) to afford a colorless solid, a mixture of cyclo-Pro-Val and cyclo-Pro-Leu, according to ^{1}H-nmr and hreims spectra.

The air-dried mycelium (18 g) from the culture grown on modified malt extract was ground and extracted with Me_2CO (3×250 ml) by stirring at room temperature to give 360 mg of a red oil. This was flash chromatographed on a Si gel column with petroleum ether-EtOAc (91:9, 500 ml), (83:17, 500 ml), and (60:40, 250 ml). Fractions 1–25 (88 mg) were flash chromatographed with petroleum ether-EtOAc (99.5:0.5, 100 ml) and (98:2, 100 ml) to afford a chromatographically homogeneous fraction (50 mg), which was shown by ^{1}H nmr to consist of a mixture of glycerides. Fractions 50–82 (23 mg) gave pure ergosterol. Fractions 90–120 afforded a pale yellow solid (67 mg), a mixture of linolenic, oleic, and palmitic acids, as shown by ^{1}H-nmr and hreims spectra.

Anofinic Acid (2,2-dimethyl-2H-1-benzopyran-6-carboxylic acid) [**1**].—Colorless crystals, mp 150.0–156.0° [lit. (10) mp 158.5–160.0°]; ir, uv, and ^{1}H-nmr spectra were identical with those reported in (10); hreims *m/z* [M^+] 204.0790 (14) ($C_{12}H_{12}O_3$ requires 204.0786), 190 (12), [$M-CH_3$] 189 (100), 149 (4), 115 (5), 91 (2); ^{13}C nmr (75 MHz, $CDCl_3$) δ 28.5 (2×CH_3), 77.7 (C-2), 116.4, 121.7, 128.8, 131.1, 131.9 (C-3, C-4, C-5, C-7, C-8), 120.8, 121.6 (C-4a, C-6), 158.0 (C-8a), 171.8 (COOH).

3,4-Dihydro-2,2-dimethyl-2H-1-benzopyran-4-one-6-carboxaldehyde [**2**].—Colorless needles, possessing a pleasant odor, mp 87.0–91.0°; R_f 0.20 (petroleum ether-EtOAc, 83:17); ir ν max (CH_2Cl_2) 2977, 1698, 1609, 1570, 1487, 1459, 1265, 1185 cm^{-1}; uv λ max (MeOH, ε) 241 (22250), 274 (12800), 322 (2640) nm; hreims *m/z* [M^+] 204.0788 (65) ($C_{12}H_{12}O_3$ requires 204.0786), [$M-CH_3$] 189 (100), 148 (48), 119 (13); ^{1}H nmr (360 MHz, $CDCl_3$) δ 1.51 (6H, s, 2×CH_3), 2.79 (2H, s, CH_2), 7.06 (1H, d, J=8.6 Hz, H-8), 8.04 (1H, dd, J=8.6 and 2.1 Hz, H-7), 8.36 (1H, d, J=2.1 Hz, H-5), 9.93 (1H, s, CHO); ^{13}C nmr (125.7 MHz, $CDCl_3$) δ 26.6 (2×CH_3), 48.6 (C-3), 80.7 (C-2), 119.7 (C-8), 119.9 (C-4a), 129.9 (C-6), 131.2 (C-7), 134.8 (C-5), 164.4 (C-8a), 190.2 (CHO), 191.2 (C-4).

1-Acetyl-3-acetoxyacetyl-indole [**3**].—Colorless oil that solidified at 0°; ^{1}H nmr (360 MHz, $CDCl_3$) δ 2.26 (3H, s, $OCOCH_3$), 2.75 (3H, s, $NCOCH_3$), 5.23 (2H, s, CH_2), 7.40 (2H, t, J=8.0 Hz, H-6, H-7), 8.16 (1H, s, H-2), 8.28 and 8.39 (each 1H, d, J=8.0 Hz, H-4, H-7); hreims *m/z* [M^+] 259.0846 (15) ($C_{14}H_{13}NO_4$ requires 259.0847), [M^+-CH_2CO] 217.0742 (2) ($C_{12}H_{11}NO_3$ requires 217.0734), [$M^+-CH_2O-COCH_3$] 186.0555 (29) ($C_{11}H_8NO_2$ requires 186.0558), [$M^+-CH_2OCOCH_3-CH_2CO$] 144.0452 (100) (C_9H_6NO requires 144.0455), [$M^+-CO-OCOCH_3-CH_2CO$] 130.0657 (4) (C_9H_8N requires 130.0656), 116 (13), 89 (10).

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