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SECONDARY METABOLITES FROM *Pleurotus ostreatus*

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Pleurotus ostreatus (Jacq.: Fr.) Quel., also known as the brown-skinned lateral mushroom, is a large edible fungus of the family Pleurotaceae and genus *Pleurotus* [1] that is widely distributed in China. *Pleurotus ostreatus* has various pharmacological activities such as antitumor, antibacterial, antiviral, immunomodulatory, and as a hypoglycemic lipid [1]. Some bioactive metabolites, such as sterols, organic acids, polyphenols, and other components, have been isolated from the fruiting body of *P. ostreatus* [2, 3].

In this paper, the structures of 16 compounds were isolated and identified as ergosterol (**1**), α-dimorphecolic acid (**2**), 4-hydroxy-17*R*-methylincisterol (**3**), volemolide (**4**), diisobutyl phthalate (**5**), dipentyl phthalate (**6**), bis(2-methylheptyl) phthalate (**7**), 1,2-dihydroxymintlactone (**8**), ergost-7,9(11),22-trien-3β,5α,6α-triol (**9**), bis(2-ethylhexyl)benzene-1,2-dicarboxylate (**10**), dibutyl phthalate (**11**), leptosphaerin (**12**), phenylalanine (**13**), formamide (**14**), (1′*R*,2′*R*,3′*S*,4′*R*)-1,2,4-triazole nucleoside (**15**), and pyroglutamic acid methyl ester (**16**) based on their ^{1}H NMR and ^{13}C NMR spectral data. Compounds **2–16** were isolated and identified, to our knowledge for the first time, from the fruiting bodies of *P. ostreatus*.

The mushroom powder was extracted with 95% ethanol using the heating reflux method, and the solvent was recovered under reduced pressure to obtain the ethanol extract. The ethanol extract was suspended in water and extracted with petroleum ether (PE), EtOAc, and *n*-BuOH. The PE fraction was chromatographed on silica gel and eluted with PE–EtOAc (50:1→5:95) to give six major fractions (Frs. 1–6). Fraction 3 was recrystallized to give compound **1**. Fraction 4 was separated with PE–EtOAc (10:1→8:2) and purified by pre HPLC (50–100% MeOH) to give compounds **2–5**. The EtOAc fraction was chromatographed with PE–EtOAc–MeOH (10:1:0.1→7:3:0.1) to give three fractions (Frs. 1–3). Fractions 1–3 were separated by Sephadex LH-20 with $CHCl_3$–MeOH (1:1), respectively, and then purified by pre-HPLC (95% MeOH) to give compounds **6–11**. Using the same method, the *n*-BuOH fraction gave four fractions (Frs. 1–4; EtOAc–MeOH, 1:0→1:1). Fractions 1 and 2 were purified using Sephadex LH-20 and further purified using HPLC to give compounds **12** and **13**. Fractions 3 and 4 were purified using medium-pressure preparative chromatography (RP-18) with MeOH–H_2O (15:85→100:0), and further purified using HPLC to obtain compounds **14–16**.

α-Dimorphecolic acid (2), $C_{18}H_{32}O_3$, pale yellow oily liquid. ^{1}H and ^{13}C NMR spectral data were similar to those published [4, 5].

4-Hydroxy-17*R*-methylincisterol (3), $C_{21}H_{32}O_3$, white needle crystals. ^{13}C NMR (150 MHz, CD_3OD, δ, ppm): 173.65 (C-6), 112.62 (C-7), 173.03 (C-8), 107.07 (C-9), 36.16 (C-11), 36.45 (C-12), 49.88 (C-13), 51.71 (C-14), 22.23 (C-15), 30.14 (C-16), 56.69 (C-17), 12.07 (C-18), 41.60 (C-20), 21.53 (C-21), 136.46 (C-22), 133.81 (C-23), 44.35 (C-24), 34.35 (C-25), 20.47 (C-26), 20.10 (C-27), 18.18 (C-28) [6, 7].

Volemolide (4), $C_{22}H_{34}O_3$. ^{13}C NMR (150 MHz, CD_3OD, δ, ppm): 172.68 (C-6), 115.27 (C-7), 171.10 (C-8), 109.52 (C-9), 35.70 (C-11), 36.28 (C-12), 49.95 (C-13), 52.04 (C-14), 22.08 (C-15), 30.12 (C-16), 56.72 (C-17), 12.24 (C-18), 41.60 (C-20), 21.50 (C-21), 136.42 (C-22), 133.85 (C-23), 44.35 (C-24), 34.35 (C-25), 20.47 (C-26), 20.11 (C-27), 18.18 (C-28), 50.51 (9-OCH_3) [8].

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Diisobutyl phthalate (5), $C_{16}H_{22}O_4$. ^{1}H NMR (600 MHz, CD_3OD, δ, ppm, J/Hz): 7.73 (2H, dd, J = 6.0, 3.6, H-3, 6), 7.63 (2H, dd, J = 6.0, 3.0, H-4, 5), 4.06 (4H, d, J = 6.6, H-8, 8′), 2.03 (2H, m, H-9, 9′), 0.99 (12H, d, J = 6.6, H-10, 10′, 11, 11′) [9].

Dipentyl phthalate (6), $C_{18}H_{26}O_4$, yellow powder. ^{1}H NMR (600 MHz, CD_3OD, δ, ppm, J/Hz): 7.73 (2H, dd, J = 5.4, 3.0, H-3, 6), 7.62 (2H, dd, J = 5.4, 3.0, H-4, 5), 4.29 (4H, t, J = 6.6, H-8, 8′), 1.73 (4H, m, H-9, 9′), 1.45 (8H, dd, J = 15.6, 7.8, H-10, 10′, 11, 11′), 0.98 (6H, t, J = 7.2, H-12, 12′) [10].

Bis(2-methylheptyl)phthalate (7), $C_{24}H_{38}O_4$, colorless oily liquid. ^{1}H NMR (600 MHz, CD_3OD, δ, ppm, J/Hz): 7.72 (2H, dd, J = 5.4, 3.6, H-4, 5), 7.62 (2H, dd, J = 5.4, 3.0, H-3, 6), 4.21 (4H, m, H-1′, 1″), 1.68 (2H, m, H-2′, 2″), 1.42 (16H, m, H-3′, 3″, 4′, 4″, 5′, 5″, 6′, 6″), 0.96 (6H, d, J = 7.2, H-2′, CH_3-2″), 0.92 (6H, m, H-7′, 7″) [11].

1,2-Dihydroxymintlactone (8), $C_{10}H_{14}O_4$. ESI-MS *m/z* 199.0 $[M + H]^+$. ^{1}H and ^{13}C NMR spectral data were similar to those published [12].

Ergost-7,9(11),22-trien-3β,5α,6α-triol (9), $C_{16}H_{22}O_4$. ^{1}H NMR (600 MHz, CD_3OD, δ, ppm, J/Hz): 5.60 (1H, d, J = 6.6, H-11), 5.26 (1H, dd, J = 15.0, 7.2, H-23), 5.20 (1H, dd, J = 15.0, 7.8, H-22), 5.09 (1H, s, H-7), 4.01 (1H, s, H-6), 3.92 (1H, m, H-3), 1.07 (3H, s, H-19), 1.03 (3H, d, J = 6.6, H-21), 0.94 (3H, d, J = 6.6, H-28), 0.86 (3H, d, J = 6.6, H-26), 0.84 (3H, d, J = 7.2, H-27), 0.58 (3H, s, H-18) [13].

Bis(2-ethylhexyl)benzene-1,2-dicarboxylate (10), $C_{24}H_{38}O_4$. ^{1}H NMR (600 MHz, CD_3OD, δ, ppm, J/Hz): 7.72 (2H, dd, J = 5.4, 3.0, H-2, 5), 7.62 (2H, dd, J = 5.4, 3.0, H-3, 4), 4.21 (4H, m, dd, J = 5.4, 1.2, H-1′, 1″), 1.68 (2H, m, H-2′, 2″), 1.43 (4H, m, H-7′, 7″), 1.38 (4H, m, H-5′, 5″), 1.33–1.37 (8H, m, H-3′, 3″, 4′, 4″), 0.94 (6H, t, J = 6.9, H-8′, 8″), 0.91 (6H, t, J = 6.0, H-6′, 6″) [14].

Dibutyl phthalate (11), $C_{16}H_{22}O_4$. ^{1}H NMR (600 MHz, CD_3OD, δ, ppm, J/Hz): 0.96 (6H, t, J = 7.5, H-11, 11′), 1.42 (4H, m, H-10, 10′), 1.69 (4H, m, H-9, 9′), 4.27 (4H, t, J = 6.6, H-8, 8′), 7.60 (2H, m, H-3, 4), 7.70 (2H, m, H-2, 5) [15].

Leptosphaerin (12), $C_8H_{11}NO_5$, needles. ^{1}H NMR (600 MHz, CD_3OD, δ, ppm, J/Hz): 7.56 (1H, d, J = 2.0, H-3), 5.13 (1H, dd, J = 5.3, 2.0, H-4), 3.78 (1H, dd, J = 5.2, 5.2, H-5), 3.67 (2H, d, J = 5.2, H-6), 2.16 (3H, s, OCH_3) [16].

Phenylalanine (13), $C_9H_{11}NO_2$, white powder. ESI-MS *m/z* 166.0 $[M + H]^+$ [17].

Formamide (14), CH_3NO, white needles. ^{1}H NMR (600 MHz, CD_3OD, δ, ppm, J/Hz): 5.72 (1H, t, J = , H-1), 5.21, 4.68 (2H, t, J = , N-H). ^{13}C NMR (150 MHz, CD_3OD, δ, ppm): 163.8 (C-1) [18].

(1′*R*,2′*R*,3′*S*,4′*R*)-1,2,4-Triazole nucleoside (15), $C_7H_{11}N_3O_4$, white powder. ^{1}H NMR (600 MHz, CD_3OD, δ, ppm, J/Hz): 8.40 (1H, s, H-5), 8.28 (1H, s, H-3), 6.05 (1H, d, J = 6.6, H-1′), 4.83 (1H, dd, J = 6.6, 5.4, H-2′), 4.42 (1H, dd, J = 5.4, 2.4, H-3′), 4.25 (1H, dd, J = 5.4, 2.4, H-4′), 3.96 (1H, dd, J = 12.6, 2.4, H-5′), 3.84 (1H, dd, J = 12.6, 3.0, H-5′) [17].

Pyroglutamic acid methyl ester (16), $C_6H_9NO_3$. ESI-MS *m/z*144.0 $[M]^+$ [19].

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