METABOLITES OF RHIZOPUS ARRHIZUS 3078

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Abstract Nine compounds were isolated from the methanol extract of the mycelium of *Rhizopus arrhizus* 3078. On the basis of spectral data, they were determined to be 5α,8α-epidioxy-(20*S*,22*E*,24*R*)-ergosta-6,22-dien-3β-ol (1), (9*Z*)-glycerin-1-monooleate (2), 4-hydroxyacetophenone (3), 4-hydroxyphenylacetic acid (4), (20*S*,22*E*,24*R*)-ergosta-7,22-dien-3β,5α,6β-triol (5), (S)-3-hydroxy-3-phenylpropionic acid (6), thymine (7), uracil (8) and adenosine (9).

Key words *Rhizopus arrhizus*; mycelium; ergosterol; 4-hydroxyphenylacetic acid; (*S*)-3-hydroxy-3-phenylpropionic acid

根霉 3078 的代谢产物的研究

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摘 要 从根霉 3078 菌丝体的甲醇提取物中分离得到 9 个化合物,通过波谱分析,鉴定为 5α,8α-表二氧-(20*S*,22*E*,24*R*)-麦角甾-6,22-二烯-3β-醇(1)、甘油醇-1-单油酸酯(2)、4-羟基苯乙酮(3)、4-羟基苯乙酸(4)、(20*S*,22*E*,24*R*)-麦角甾-7,22-二烯-3β,5α,6β-三醇(5)、(*S*)-3-羟基-3-苯基丙酸(6)、胸腺嘧啶(7)、尿嘧啶(8)和腺苷(9)。

关键词 少根霉;菌丝体;麦角甾醇;4-羟基苯乙酸;(*S*)-3-羟基-3-苯基丙酸

Introduction

In the process of food fermentation, the nutrition and flavor were greatly changed due to two reasons. Firstly, some proteins were converted into small peptides and amino acid by enzymes secreted by microorganism[1,2]. Secondly, the metabolites of microorganism may affect the nutrition and flavor[3]. Fermented bean curd is a traditional Chinese food produced by fermentation, which has good flavor and nutrition. In our study, we examined the metabolites of the *Rhizopus arrhizus* 3078, which was used in the bean curd fermentation. Based on spectral data and comparison with known compounds, nine compounds isolated were determined as 5α,8α-epidioxy-(20*S*,22*E*,24*R*)-ergosta-6,22-dien-3β-ol (1), (9*Z*)-glycerin-1-monooleate (2), 4-hydroxyacetophenone (3), 4-hydroxyphenylacetic acid (4), (20*S*,22*E*,24*R*)-ergosta-7,22-dien-3β,5α,6β-triol (5), (*S*)-3-hydroxy-3-phenylpropionic acid (6), thymine (7), uracil (8) and adenosine (9). Compound 1 exhibited some cytotoxic effect[4]. Compound 5 possessed antinociceptive activity (inhibition = 47.6%, 5 mg/kg)[5]. From the results, it could be estimated that the metabolites of *Rhizopus arrhizus* 3078 used in the fermentation of bean curd may have some effect on human beings.

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Experimental

General

Melting points were measured on XRC-1 micromelting-point apparatus and uncorrected. IR spectra were recorded on a Perkin Elmer Spectrum One FT-IR spectrometer. Silica gel (200～300 mesh) for column chromatography (CC) and GF254 (30～40 μm) for TLC were purchased from Qingdao Marine Chemical Factory, Qingdao, China. NMR spectra were carried out on Bruker Advance 600 spectrometer, TMS as internal standard. Optical rotations were measured on Perkin Elmer Model 341 polarimeter.

Microorganism and culture media

Rhizopus arrhizus 3078 was obtained from Chengdu Institute of Biology, the Chinese Academy of Sci-

ences. It was maintained on potato dextrose agar slant (PDA) at 4 ℃.

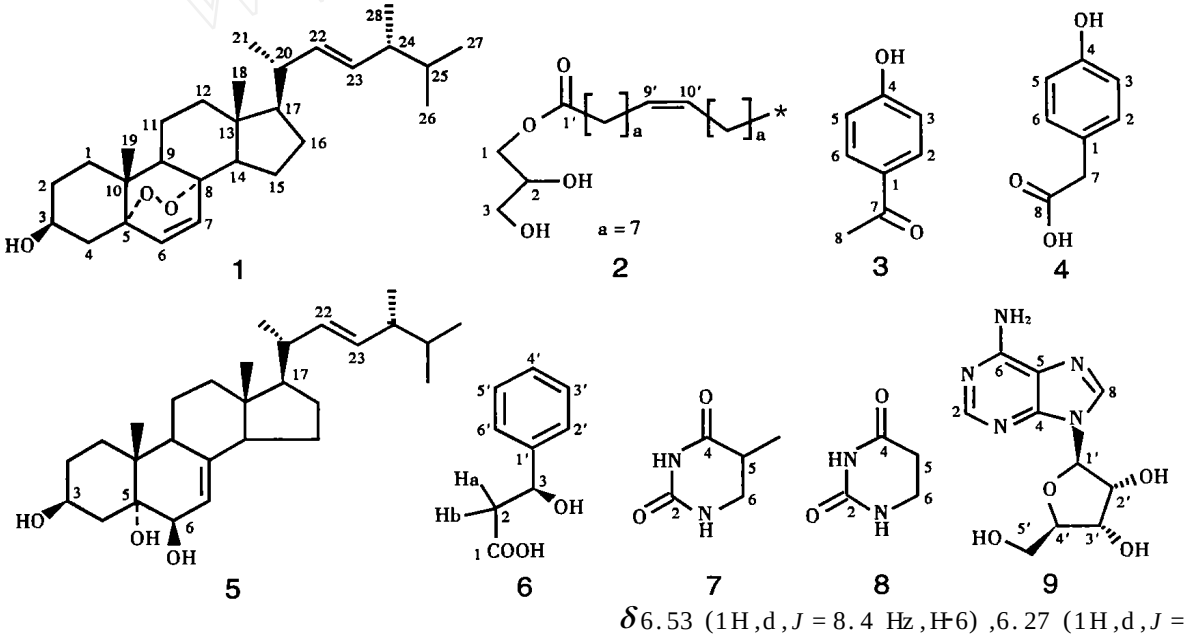
Culture medium was comprised of dextrose (20 g/L), yeast extract (1 g/L), KH_2PO_4 (3 g/L), $MgSO_4 \cdot 7H_2O$ (1.5 g/L), potato extract (200 g potato was extracted with 1 Liter boiling water for 20 min), and its pH was adjusted to 6.0 with NaOH (aq).

Fermentation

The fresh mycelium grown on PDA medium at 28 ℃ for 5 d was inoculated into 500 mL flasks containing 250 mL potato dextrose medium, which were sterilized at 121 ℃ and 15 psi for 30 min. Flasks with inoculated medium were placed in rotary shaker at 28 ℃ and incubated at 180 rpm for 7 d.

Extraction and isolation

The total mycelium was filtrated and dried at 55 ℃ in oven. The dried mycelium (180 g) was extracted (1 L × 5) exhaustively with methanol at 50 ℃ (2 h each). Evaporation of the solvent from the extract in *vacuo* gave a residue (28 g). The residue was chromatographied on a silica gel column (420 g, 5.5 cm × 31.0 cm) eluted gradiently by petroleum ether (bp. 60～90 ℃)-acetone (20∶1, 15∶1, 10∶1, 5∶1, 2 L each) and $CHCl_3$-CH_3OH (20∶1, 15∶1, 10∶1, 7∶1, 5∶1, 3∶1, 2 L each). Based on the TLC monitoring, the collected fractions were combined into nine fractions (A: 0.5 g; B: 8.5 g; C: 1.1 g; DA: 1.75 g; DB: 0.75 g; E: 0.17 g; F: 1.36 g; G: 0.3 g; H: 1.6 g). Fr. C was chromatographied over silica gel column (50 g, 2.6 cm × 19.0 cm) eluted with chloroform-acetone (100∶1, 500 mL) to give 1 (25 mg). CC (column chromatography) of DB with petroleum ether (bp. 60～90 ℃)-acetone (5∶1, 300 mL; 3∶1, 300 mL) gave 2 (35 mg). Compound 3 (7 mg) was obtained from DA by CC over silica gel column eluted with petroleum ether (bp. 60～90 ℃)-acetone (10∶1, 200 mL). CC of E over silica gel column (30 g, 2.5 cm × 14.0 cm) eluted with $CHCl_3$-CH_3OH (15∶1, 800 mL) gave EA, EB and EC. Subsequent purification of EA by CC (8 g, 0.8 cm × 20.0 cm) eluted with $CHCl_3$-CH_3OH (15∶1, 150 mL) afforded 4 (10 mg). EB was subjected to CC over silica gel (8 g, 0.8 cm × 20.0 cm) with $CHCl_3$-CH_3OH (10∶1, 220 mL) to afford 5 (5 mg) and 6 (5 mg). Further CC of EC over silica gel (15 g, 1.8 cm × 12.0 cm) eluted with $CHCl_3$-CH_3OH (12∶1, 250 mL) yield 7 (15 mg). Compound 8 (10 mg) was isolated from F by CC over silica gel C-18 (3.6 cm × 20.0 cm) with H_2O-CH_3OH (10∶1, 1100 mL). Compound 9 (25 mg) was obtained from H by CC over silica gel column (50 g, 2.4 cm × 27.0 cm) eluted with $CHCl_3$-CH_3OH (5∶1, 1000 mL).



Identification

5α,8α-Epidioxy-(20S,22E,24R)-ergosta-6,22-dien-3β-ol (1) Colorless needles (petroleum ether, bp. 60～90 ℃), $C_{28}H_{44}O_3$, $[\alpha]_D^{20}$ -40.5 ° (c = 0.1, $CHCl_3$), mp. 175～177 ℃; ESI-MS *m/z*: 429 $[M+H]^+$ (positive mode); IR υ_{max}^{KBr} cm^{-1}: 3380 (-OH), 2955, 2871, 1631 (-CH=CH-), 1457, 1378, 1074, 967; ^1H NMR ($CDCl_3$, 600 MHz): δ 6.53 (1H, d, *J* = 8.4 Hz, H-6), 6.27 (1H, d, *J* = 8.4 Hz, H-7), 5.23 (1H, dd, *J* = 13.5, 7.6 Hz, H-23), 5.17 (1H, dd, *J* = 13.5, 7.6 Hz, H-22), 3.97 (1H, m, H-3), 1.01 (3H, d, *J* = 6.6 Hz, H-21), 0.94 (3H, d, *J* = 4.3 Hz, H-28), 0.92 (3H, s, H-19), 0.86 (3H, d, *J* = 4.2 Hz, H-26), 0.85 (3H, s, H-18), 0.83 (3H, d, *J* = 4.2 Hz, H-27); ^{13}C NMR ($CDCl_3$, 150 MHz): δ 135.6 (C-6), 135.4 (C-22), 132.5 (C-23), 131.0 (C-7), 82.4 (C-5), 79.7 (C-8), 66.7 (C-3), 56.4 (C-17), 51.9 (C-

14), 51.2 (C-9), 44.8 (C-13), 43.0 (C-24), 40.0 (C-20), 39.5 (C-12), 37.2 (C-4), 37.1 (C-10), 34.9 (C-1), 33.3 (C-25), 30.3 (C-2), 28.9 (C-16), 23.6 (C-11), 21.1 (C-21), 20.8 (C-15), 20.2 (C-26), 19.9 (C-27), 18.4 (C-19), 17.8 (C-28), 13.1 (C-18). The NMR data and optical rotation were in agreement with those reported for 5α,8α-epidioxy-(20*S*,22*E*,24*R*)-ergosta-6,22-dien-3β-ol[6].

(9*Z*)-Glycerin-1-monooleate (2) Colorless oil, $C_{21}H_{40}O_4$, ESI-MS *m/z*: 357 $[M+H]^+$, 379 $[M+Na]^+$ (positive mode); ^{1}H NMR ($CDCl_3$, 600 MHz): δ 5.36 (1H, dt, *J* = 12.0, 6.1 Hz, H-9), 5.33 (1H, dt, *J* = 12.0, 6.1 Hz, H-10), 4.19 (1H, dd, *J* = 11.9, 4.2 Hz, H-1′), 4.15 (1H, dd, *J* = 11.9, 4.2 Hz, H-1′), 3.93 (1H, m, H-2′), 3.70 (1H, dd, *J* = 11.4, 3.6 Hz, H-3′), 3.60 (1H, dd, *J* = 11.4, 5.9 Hz, H-3′), 2.32 (2H, t, *J* = 6.7 Hz, H-2), 1.98 (4H, m, H-8, 11), 1.28 (24H), 0.86 (3H, t, *J* = 6.8 Hz, H-18); ^{13}C NMR ($CDCl_3$, 150 MHz): δ 174.6 (C-1), 130.2 (C-9), 129.9 (C-10), 70.5 (C-2), 65.4 (C-1), 63.6 (C-3), 34.4, 32.1, 29.9, 29.7, 29.5, 29.4, 29.3, 27.4, 25.1, 22.9, 14.3. The ^{1}H and ^{13}C NMR data were identical with those reported for (9*Z*)-glycerin-1-monooleate[7].

4-Hydroxyacetophenone (3) Colorless needles (petroleum ether, bp. 60～90 ℃), $C_8H_8O_2$, mp. 107～108 ℃, ESI-MS *m/z*: 135 $[M-H]^-$ (negative mode); ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.90 (2H, brd, *J* = 6.6 Hz, H-3, 5), 6.91 (2H, brd, *J* = 6.6 Hz, H-2, 6), 2.56 (3H, s, H-8); ^{13}C NMR ($CDCl_3$, 150 MHz): δ 198.2 (C-7), 161.1 (C-4), 131.3 (C-2, 6), 130.1 (C-1), 115.6 (C-3, 5), 26.5 (C-8). The ^{1}H and ^{13}C NMR data were identical with those reported for 4-hydroxyacetophenone[8].

4-Hydroxyphenylacetic acid (4) Colorless needles (CH_3OH), $C_8H_8O_3$, mp. 148～150 ℃, ESI-MS *m/z*: 153 $[M+H]^+$ (positive mode); ^{1}H NMR (CD_3OD, 600 MHz): δ 7.08 (2H, brd, *J* = 8.4 Hz, H-2, 6), 6.72 (2H, brd, *J* = 8.4 Hz, H-3, 5), 3.48 (2H, s, H-7); ^{13}C NMR (CD_3OD, 150 MHz): δ 175.1 (C-8), 156.2 (C-4), 130.1 (C-2, 6), 125.6 (C-1), 115.0 (C-3, 5), 39.9 (C-7). The ^{1}H and ^{13}C NMR data were in agreement with those reported for 4-hydroxyphenylacetic acid[8].

(20S, 22E, 24R)-Ergosta-7, 22-dien-3β, 5α, 6β-triol (5) Colorless needles ($CHCl_3$), $C_{28}H_{46}O_3$, $[\alpha]_D^{20}$ -60.2° (c = 0.1, C_5H_5N), mp. 250～252 ℃; ESI-MS *m/z*: 431 $[M+H]^+$ (positive mode); IR υ_{max}^{KBr} cm^{-1}: 3380 (-OH), 2955, 2871, 1631 (-CH=CH-), 1457, 1378, 1074, 967; ^{1}H NMR (C_5D_5N, 600 MHz): δ 5.72 (1H, brs, H-7), 5.24 (1H, dd, *J* = 15.2, 7.2 Hz, H-22), 5.17 (1H, dd, *J* = 15.2, 7.2 Hz, H-23), 4.83 (1H, m, H-3), 4.30 (1H, brs, H-6), 1.50 (3H, s, H-19), 1.03 (3H, d, *J* = 6.7 Hz, H-21), 0.92 (3H, d, *J* = 6.7 Hz, H-28), 0.83 (6H, d, *J* = 4.9 Hz, H-26, H-27), 0.63 (3H, s, H-18); ^{13}C NMR (C_5D_5N, 150 MHz): δ 141.5 (C-8), 136.1 (C-22), 132.0 (C-23), 120.4 (C-7), 76.1 (C-5), 74.2 (C-6), 67.5 (C-3), 56.1 (C-17), 55.2 (C-14), 43.7 (C-9, C-13), 43.1 (C-24), 41.9 (C-4), 40.8 (C-20), 39.8 (C-12), 38.0 (C-10), 33.8 (C-2), 33.3 (C-25), 32.6 (C-1), 28.4 (C-16), 23.4 (C-15), 22.4 (C-11), 21.3 (C-27), 20.1 (C-26), 19.8 (C-21), 18.8 (C-19), 17.8 (C-28), 12.5 (C-18). The NMR data and optical ratation were identical with those reported for (20*S*,22*E*,24*R*)-ergosta-7,22-dien-3β,5α,6β-triol[9].

(S)-3-Hydroxy-3-phenylpropionic acid (6) Colorless needles (CH_3OH), $C_9H_{10}O_3$, $[\alpha]$ -30.5° (c = 0.1, CH_3OH), mp. 117-119 ℃, ESI-MS *m/z*: 189 $(M+Na)^+$ (positive mode), 165 $(M-H)^-$ (negative mode); IR υ_{max}^{KBr} cm^{-1}: 3295 (-OH), 2965, 2654, 1698 (-C=O), 1496, 1456, 765, 703; ^{1}H NMR (CD_3OD, 600 MHz): δ 7.38 (2H, t, *J* = 7.4 Hz, H-3′,5′), 7.33 (2H, d, *J* = 7.4 Hz, H-2′,6′), 7.26 (1H, t, *J* = 7.4 Hz, H-4′), δ 5.08 (1H, dd, *J* = 8.8, 5.0 Hz, H-3), 2.69 (1H, dd, *J* = 15.2, 8.8 Hz, H-2a), 2.63 (1H, dd, *J* = 15.2, 5.0 Hz, H-2b); ^{13}C NMR (CD_3OD, 150 MHz): 173.7 (C-1), 144.0 (C-1′), 128.2 (C-2′,6′), 127.4 (C-4′), 125.7 (C-3′,5′), 70.5 (C-3), 43.8 (C-2). The NMR data and optical rotation were identical with those reported for (*S*)-3-hydroxyl-3-phenylpropionic acid[10].

Thymine (7) White crystals (CH_3OH), $C_5H_6O_2N_2$, mp. > 300 ℃, ESI-MS *m/z*: 149 $[M+Na]^+$, 275 $[2M+Na]^+$ (postitive mode); IR υ_{max}^{KBr} cm^{-1}: 3260, 3062, 1730, 1679, 1212; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 10.97 (1H, d, *J* = 5.5 Hz, H-1), 10.56 (1H, s, H-3), 7.22 (1H, d, *J* = 5.5 Hz, H-6), 1.71 (3H, s, -CH_3); ^{13}C NMR (DMSO-d6, 150 MHz): δ 165.6 (C-4), 152.2 (C-2), 138.4 (C-6), 108.3 (C-5), 12.5 (CH3). The ^{1}H and ^{13}C NMR data were identical with those reported for thymine[11].

Uracil (8) White powder (H_2O + CH_3OH), $C_4H_4N_2O_2$, mp. > 300 ℃, ESI-MS *m/z*: 135 $[M+Na]^+$; IR υ_{max}^{KBr} cm^{-1}: 3410, 3110, 1768, 1738, 1676, 1650, 1450, 1420, 1235; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 11.12 (1H, s, H-1), 10.85 (1H, s, H-3), 7.48 (1H, d, *J* = 7.7 Hz, H-6), 5.75 (1H, d, *J* = 7.7 Hz, H-5); ^{13}C NMR (DMSO-d_6, 150 MHz): δ 165.0 (C-4), 152.2 (C-2), 142.8 (C-6), 100.9 (C-5). The ^{1}H and ^{13}C NMR data were identical with those reported for uracil[12].

Adenosine(9) White powder ($CHCl_3$ + CH_3OH), $C_{10}H_{13}N_5O_4$, $[\alpha]_D^{20}$ -50.6° (c = 0.1, H_2O), mp. 235-236 ℃, IR υ_{max}^{KBr} cm^{-1}-1: 3330, 2935, 2850, 1684, 1608, 1595,

1455 ,1422 ,1382 ,1338 ,1130 ; ESI-MS *m/ z* :268 [M + H] $^{+}$, 557 (2M + Na) $^{+}$ (positive mode) ; ^{1}H NMR (C_5D_5N ,600 MHz) : δ 8.71 (1H ,s ,H-8) ,8.60 (1H ,s , H-2) ,8.32 (2H ,s ,NH_2) ,6.70 (1H ,d ,*J* = 6.0 Hz ,H-1′) ,5.49 (1H ,t ,*J* = 4.8 Hz ,H-2′) ,5.05 (1H ,dd ,*J* = 4.8 ,3.0 Hz ,H-3′) ,4.74 (1H ,m ,H-4′) ,4.30 (1H ,dd , *J* = 12.3 ,2.4 Hz ,H-5′a) ,4.13 (1H ,dd ,*J* = 12.3 ,2.4 Hz ,H-5′b) ; ^{13}C NMR (C_5D_5N ,150 MHz) : δ 157.7 (C-6) ,153.3 (C-2) ,149.9 (C-4) ,140.5 (C-8) ,121.5 (C-5) ,90.8 (C-1′) ,87.8 (C-4′) ,75.5 (C-3′) ,72.4 (C-2′) ,63.0 (C-5′) The NMR data and optical rotation were identical with those reported for adenosine[13].

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