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Cytotoxic monacolins from red yeast rice, a Chinese medicine and food

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ABSTRACT

Seven new monacolins, monacolins Q–S (**1–3**), α,β-dehydromonacolin S (**4**), 3α-hydroxy-3,5-dihydromonacolin L (**5**), 3β-hydroxy-3,5-dihydromonacolin L (**6**), and α,β-hydromonacolin Q (**7**) were isolated and characterized from the methanol extract of red yeast rice. In addition, six known monacolins, α,β-dehydrodihydromonacolin K (**8**), dehydromonacolin K (**9**), dehydromonacolin L (**10**), monacolin K (**11**), dihydromonacolin K (**12**), dihydromonacolin L (**13**) and two compounds other than monacolins (**14**, **15**) were also isolated. Structure elucidation of the isolates was achieved by means of NMR and mass spectroscopic data analyses. Compounds **1–5**, **8**, **9**, **11**, and **13** were evaluated for their cytotoxic activity against four cancer cell lines (SK-MEL, KB, BT-549, SK-OV-3) and two noncancerous kidney cell lines (LLC-PK1 and Vero). Monacolin Q (**1**), monacolin R (**2**) α,β-dehydrodihydromonacolin K (**8**), dehydromonacolin K (**9**), and monacolin K (**11**) showed cytotoxicity to most of these cell lines in terms of inhibition of cell proliferation. The cytotoxicity of monacolin K (**11**) was the most potent among all the tested monacolins.

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1. Introduction

Red yeast rice, which is also known as koji, anka, angkak and ben-koji, is produced from the fermentation of steamed rice using the fungus *Monascus purpureus*. It has been utilized for hundreds of years for colouring food, wine and preserving meat (Fabre et al., 1993). It has also been used as a folk medicine for improving food digestion and blood circulation in East Asia for centuries (Ma et al., 2000). Several types of secondary metabolites, including pigments, monacolins, citrinin, dimerumic acid and γ-aminobutyric acid, have been reported from red yeast rice (Akihisa et al., 2005; Hsu, Hsu, Liang, Kuo, & Pan, 2010; Jongrungruangchok et al., 2004; Juzlova, Martinkova, & Kren, 1996; Loret & Morel, 2010; Patakova, 2013; Zhu et al., 2012). Monacolins are inhibitors of HMG-CoA reductase, the rate-limiting enzyme of cholesterol biosynthesis (Endo, Komagata, & Shimada, 1986; Zhu et al., 2012). Clinical observations clearly showed that red yeast rice can lower blood lipid levels (Heber et al., 1999; Wang et al., 1997). Recently, a double blind placebo controlled randomized clinical trial demonstrated that red yeast rice supplements have the ability to lower total cholesterol by 15% in 8 weeks (Verhoeven et al., 2013). In the United States, some physicians recommend the use of red yeast rice to patients with statin intolerance (Becker et al., 2009). Besides cholesterol-lowering effects, monacolins have been reported to possess anticancer properties against colon (Poynter et al., 2005; Zhu et al., 2012), liver (Wei & Popovich, 2013; Zhu et al., 2012), prostate (Shannon et al., 2005), and breast cancer (Campbell et al., 2006; Zhu et al., 2012) and have antioxidant activities (Dhale, Divakar, Kumar, & Vijayalakshmi, 2007).

In this paper, we report the isolation and characterization of seven new monacolins (**1–7**), together with six known monacolins (**8–13**) and two other known compounds (**14**, **15**), from red yeast rice. Since the cytotoxicity of monacolins isolated from red yeast rice has been reported earlier (Zhu et al., 2012) and statins, such as lovastatin (monacolin K), were shown to exhibit breast cancer growth prevention (Campbell et al., 2006), we investigated the *in vitro* anti-cell proliferative activity of the isolated monacolins in order to determine their anticancer potential and to see if any of the new isolates would be more promising.

2. Materials and methods

2.1. General materials

Agilent Technologies 6200 series mass spectrometer was employed for MS. 1D- and 2D-NMR spectra were recorded on Varian Unity Inova 600 MHz spectrometer or Bruker DRX-500 spectrometer using $CDCl_3$ or CD_3OD as solvents, with solvent peaks as internal standards. The specific rotation was measured on

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AUTOPOL IV Automatic Polari meter (Rudolph, Hackettstown, NJ, USA). UV spectra were recorded on a Varian Cary 50 Bio UV–visible spectrophotometer. IR spectra were recorded on an Agilent Technologies Carry 630 FTIR. Column chromatography (CC) was performed over flash silica gel (32–63μ, Dynamic Adsorbents Inc), reversed-phase C-18 silica (Polar bond, J. T. Baker), RP HyperSep C_{18} cartridge (3 × 2 cm, SUPLECO-10 g), and Sephadex LH-20 (Sigma). Preparative TLC was performed on Silica gel GF plate with UV_{254} (500 μm, 20 × 20 cm, Uniplate-Analtech). Analytical TLC was performed on Silica gel F_{254} aluminum sheet (20 × 20 cm, Fluka) or Silica 60 RP-18 $F_{254}S$ aluminum sheet (20 × 20 cm, Merck). The detection was performed at UV-254 nm. Spots were visualized by spraying with 0.5% vanillin (Sigma) solution in conc. H_2SO_4–EtOH (5:95) followed by heating. Analytical grade solvents (Fisher Chemicals) were used for extraction and purification. All cell lines were obtained from ATCC Manassas, VA.

2.2. Plant material

Red yeast rice powder was purchased from PureBulk, Inc. (1640 Austin Rd Roseburg, OR 97471, purebulk.com). A reference (No. 15043) has been deposited at National Center for Natural Products Research, School of Pharmacy, University of Mississippi, MS, USA.

2.3. Extraction and isolation

Red yeast rice powder (800 g) was soaked in MeOH (3 l × 1, 2 l × 3 and 1 l × 3) at room temperature under sonication. The soluble part was concentrated to afford a residue (60 g), which was subjected to column chromatography (cc) over silica gel (50 × 10 cm), using gradients of hexanes/$CHCl_3$ [50:1 (1.5 l), 20:1 (1.5 l), 10:1 (1.5 l), 1:1 (1.5 l), 1:2 (1.5 l), and 0:1 (2.0 l)] and $CHCl_3$/MeOH [50:1 (1.5 l), 20:1 (1.5 l), 10:1 (2.5 l), 8:1 (1.5 l), 5:1 (1.5 l), 3:1 (2.0 l), 2:1 (3.0 l), and 0:1 (3.0 l)] to obtain 14 fractions (A–N). Fr. B (8.0 g) was chromatographed on a silica gel column (80 × 6 cm) using gradients of hexanes/acetone [40:1 (2.0 l), 20:1 (1.5 l), 15:1 (1.5 l), 10:1 (2.5 l), 9:1 (2.0 l), 4:1 (2.5 l), 7:3 (4.0 l), and 3:2 (1.5 l)] to obtain 8 fractions (B1–B8). Fr. B6 (400 mg) was subjected to repeated cc [silica gel (80 × 2.5 cm), $CHCl_3$/MeOH mixtures (1:0, 2.5 l) and (1:1, 0.5 l)] and [reversed phase C-18 silica (55 × 2 cm), MeOH/H_2O mixtures (4:1, 300 ml) and (17:3, 800 ml)] to give compounds **1** (13.0 mg) and **8** (59.1 mg). Compound **9** (300.8 mg) was purified from Fr. B7 (700 mg) by cc on reversed phase C-18 silica (55 × 2 cm), using MeOH/H_2O mixtures (4:1, 600 ml) and (17:3, 1 l). Fr. B5 (438 mg) was applied to repeated cc [silica gel column (80 × 2 cm), hexanes/acetone mixtures (9:1, 500 ml) and (4:1, 1 l)] and [reversed phase C-18 silica (55 × 2 cm), MeOH/H_2O mixtures (4:1, 1 l) and (17:3, 1 l)] to give compound **10** (6.0 mg). Fr. H (340 mg) was subjected to cc on a reversed phase C-18 silica (55 × 2 cm) using MeOH/H_2O mixture [4:1 (1.2 l)] to give compounds **11** (159.7 mg) and **12** (15.3 mg). Fr. F (1.2 g) was chromatographed on a silica gel column (85 × 2 cm), eluted with hexanes/acetone [4:1 (2 l)] to obtain 7 fractions (F1–F7). Fr. F6 (22 mg) was purified by preparative thin-layer chromatography (PTLC) ($CHCl_3$/MeOH, 20:1) to purify **6** (6.6 mg). Fr. F3 (40 mg) was purified by PTLC (hexanes/ethyl acetate, 2:1) to give **15** (8.1 mg). Fr. F5 (100 mg) was chromatographed on a reversed phase C-18 column (55 × 2 cm), eluted with MeOH/H_2O mixture [4:1 (800 ml)] to give compound **2** (23 mg). Compound **13** (4.1 mg) was purified from Fr. F4 (39 mg) by PTLC ($CHCl_3$/MeOH, 30:1). Compounds **3** (6.2 mg) and **5** (8.3 mg) from Fr. J (300 mg), **4** (3.6 mg) from Fr. K (400 mg), and **7** (9.0 mg) from Fr. M (900 mg) were obtained by repeated cc individually [Sephadex LH-20 (150 × 2 cm) using MeOH] and [reversed phase C-18 silica using MeOH/H_2O mixture (7:3, 1 l)]. Compound **14** (5.2 mg) was purified from Fr. D (271.5 mg) by cc on silica gel (80 × 2 cm) using hexanes/acetone mixture (9:1, 1 l) and then by PTLC (hexanes/acetone 7:3).

2.4. Monacolin Q (*1*)

Yellow solid. $[\alpha]_D^{22}$ −47.9 (*c* 0.25, $CHCl_3$); UV (MeOH) λ_{max}(nm) (log ε) 229 (3.88); IR (KBr) ν_{max} 3362, 2925, 2856, 1722, 1458, 1383, 1251, 1155, 1077, 817, 722 cm^{-1}; HRESIMS *m/z* 283.1669 [M+H]$^+$ (calcd. for $C_{19}H_{23}O_2$, 283.1698); ^1H and ^{13}C NMR data, see Tables 1 and 2.

2.5. Monacolin R (*2*)

Colourless oil. $[\alpha]_D^{22}$ −37.9 (*c* 1.0, $CHCl_3$); UV (MeOH) λ_{max}(nm) (log ε) 204 (1.52); IR (KBr) ν_{max} 3419, 2920, 1716, 1453, 1381, 1252, 1067, 1033, 853, 734 cm^{-1}; HRESIMS *m/z* 327.1890 [M+Na]$^+$ (calcd. for $C_{19}H_{28}NaO_3$, 327.1936); ^1H and ^{13}C NMR data, see Tables 1 and 2.

2.6. Hydromonacolin S (*3*)

Colourless solid. HRESIMS *m/z* 461.2493 [M+Na]$^+$ (calcd. for $C_{24}H_{38}NaO_7$, 461.2515). ^1H and ^{13}C NMR data, see published data (Jekkel et al., 1997).

2.7. α,β-Dehydromonacolin S (*4*)

Colourless oil. $[\alpha]_D^{22}$ −4.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max}(nm) (log ε): 202 (2.37); IR (KBr) ν_{max} 3429, 2927, 1717, 1457, 1385, 1256, 1154, 1189, 1072, 1009, 817, 753 cm^{-1}; HRESIMS *m/z* 443.2369 [M+Na]$^+$ (calcd. for $C_{24}H_{36}NaO_6$, 443.2410); ^1H and ^{13}C NMR data, see Tables 1 and 2.

2.8. 3α-Hydroxy-3,5-dihydromonacolin L (*5*)

White solid. $[\alpha]_D^{22}$ 4.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max}(nm) (log ε) 201 (3.79); IR (KBr) ν_{max} 3385, 2923, 2860, 1717, 1256, 1424, 1349, 1075, cm^{-1}; HRESIMS: *m/z* 345.2010 [M+Na]$^+$ (calcd. for $C_{19}H_{30}NaO_4$, 345.2042); ^1H and ^{13}C NMR data, see Tables 1 and 2.

2.9. 3β-Hydroxy-3,5-dihydromonacolin L (*6*)

Colourless solid. $[\alpha]_D^{22}$ 24.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max}(nm) (log ε) 201 (4.27); IR (KBr) ν_{max} 3401, 2925, 2860, 1721, 1584, 1404, 1329, 1259, 1065, 1015, 756 cm^{-1}; HRESIMS: *m/z* 345.2049 [M+Na]$^+$ (calcd. for $C_{19}H_{30}NaO_4$, 345.2042); ^1H and ^{13}C NMR data, see Tables 1 and 2.

2.10. α,β-Hydromonacolin Q (*7*)

Colourless solid. $[\alpha]_D^{22}$ 24.0 (*c* 0.10, $CHCl_3$); UV ($CHCl_3$) λ_{max}(nm) (log ε) 201 (2.31); IR (KBr) ν_{max} 3413, 2924, 2361, 1712, 1386, 1255, 1159, 1056, 819, 767 cm^{-1}; HRESIMS: *m/z* 301.1790 [M+H]$^+$ (calcd. for $C_{19}H_{25}O_3$, 301.1804); ^1H and ^{13}C NMR data, see Tables 1 and 2.

2.11. Biological assay

The cytotoxicity of the isolated compounds was determined against four human cancer cell lines (SK-MEL, KB, BT-549, SK-OV-3) and noncancerous pig kidney epithelial (LLC-PK_1) and monkey kidney fibroblast (VERO) cells in terms of their anti-cell proliferation activity during a 48 h incubation with the cell lines. The assay was performed in 96-well tissue culture-treated microplates. Cells were seeded to the wells of the plate (25,000 cells/well) and incubated for 24 h before treating with the test samples for 48 h. Cell viability was determined using the supravital dye Neutral

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