Constituents of Red Yeast Rice, a Traditional Chinese Food and Medicine

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Detailed analyses were undertaken of the natural constituents of red yeast rice, a traditional Chinese medicine and food known for centuries to improve blood circulation. Preparation of red yeast rice following ancient methods by fermenting the fungal strain *Monascus purpureus* Went on moist and sterile rice indicated the presence of a group of metabolites belonging to the monacolin family of polyketides, together with fatty acids, and trace elements. The presence of these compounds may explain in part the cholesterol-lowering ability associated with this traditional Chinese food.

Keywords: *Red yeast rice; traditional Chinese medicine; monacolins; constituents*

INTRODUCTION

The cereal grass, rice (*Oryzae sativa* L. Gramineae), is a staple food consumed throughout the world, particularly in Asian countries. The rice is cultivated extensively in warm climatic regions, and China has traditionally been recognized as one of the world's largest producers of this crop. There is evidence of rice production more than 11 000 years ago. Along with rice cultivation the indigenous people developed various methods of preparation and storage, and also developed alternative forms of preparation. For centuries the Chinese have used fermentation microorganisms to convert agricultural commodities into foods. This constant search for more fermented foods and flavorings has led to discoveries that form the foundation for present-day fermentation processes in food technology. These well-documented fermentation processes include the making of cheeses, bread, and alcohol of which yeast is a major ingredient. There may be hundreds of little-known fermented food products that remain relatively unknown outside of the specific and local growing region. Red yeast rice, extensively used in Chinese foods, is one example of a traditional food consumed throughout Asia (Sung, 1966). Its food and medicinal value are believed to date back more than a thousand years (Bensky and Gamble, 1986), and documented use was recorded in 800 A.D. (Leung, 1980; Stuart, 1979).

Red yeast rice, also known as red Koji or "Hongqu", consists mainly of nonglutinous rice, red yeast, and byproducts of the fermentation. In East Asia, red Koji produced by fermenting the food fungus, a *Monascus* species on steamed rice, has been used for more than 600 years for producing wines and other fermented food products. Several species of the fungus *Monascus* have also been widely used in making red wine and red soybean cheese (Chen, 1987; Hesseltine, 1979). The fungus *Monascus* first became known in Western society when van Tieghem (1884) noted the use of red powder (red yeast rice) by local populations in Java. The species that was isolated from red Koji was named *Monascus purpureus* Went in 1895 (Went, 1895), in recognition of the purple color. Today more than 30 *Monascus* strains are deposited with the American Type Culture Collection (Bethesda, MD).

The production of red yeast rice used as a foodstuff was recorded in old Chinese literature (Hu, 1982). More recently, it has been included and formulated by the Chinese Ministry of Health into their modern food additive standards (National Standard, 1982) to increase the color and delicacy of meat, fish, and soybean products as part of the Chinese diet. The naturally produced pigments from the red yeast give the food its characteristic coloration (Fabre et al., 1993), and much research has been performed on identifying the naturally produced metabolites responsible for this pigmentation (Blanc et al., 1994). Furthermore, pigments of *M. purpureus* are authorized for food use in Japan (Blanc et al., 1994).

The traditional method of making red yeast rice is based on a fermentation process (Hesseltine, 1979) whereby the yeast is allowed to ferment naturally on a bed of cooked nonglutinous whole rice kernel (Sung, 1966). This red Koji ("Hongqu") has long been recognized as a folk medicine for improving food digestion and blood circulation. "Hongqu" was described as "sweet in flavor and warm in property" by Li Shizhen (Chen, 1982), the great pharmacologist of the Ming Dynasty (1368−1644) who also reported that "Hongqu" promotes "digestion and blood circulation, can strengthen the spleen and dry the stomach." Another respected pharmacologist of the Ming Dynasty, Miao Xiyong, reported that "Hongqu" has an effect on spleen and stomach as described in the Ying system (a concept of traditional Chinese medical theory as it relates to the blood circulation), whereby blood depends on the principle of *like attracting like.*

Recent clinical observations now clearly show that red yeast rice has the ability to lower blood-lipid levels in animal models and in humans (Shen et al., 1996; Wang et al., 1997; Li et al., 1998; Heber et al., 1999), and this observation is partly due to the presence of cholesterol synthetase inhibitors (HMG-CoA reductase inhibitors) (Mabuchi et al., 1981; Bach, 1986). A recent double-blind placebo-controlled clinical trial using a red yeast rice

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regimen clearly demonstrated the effective lowering of blood cholesterol concentrations by about 18% in 8 weeks (Heber et al., 1999). These trials demonstrate and substantiate the empirical wisdom of traditional Chinese medicine.

To understand the health-related properties ascribed to red yeast rice, we undertook a complete study of the metabolites of red yeast rice. We note that several reviews (Juzlova et al., 1996; Martinkova et al., 1995) describe the secondary metabolites of *M. purpureus*. Studies by Negishi et al., (1986) describe the production of monacolin K using 124 strains of the genus *Monascus* including many strains of *M. purpureus*. Polyketide pigments (Fabre et al., 1993; Blanc et al., 1994), saturated fatty acids (Juzlova et al., 1996), and polyketide monacolins (Endo, 1979;1985), have also been reported. Studies of pigment production using solid fermentation have been conducted (Blanc et al., 1994; Johns and Stuart, 1991; Han and Mudgett, 1992; Panitz et al., 1991; Schumacher et al., 1996). A few reports outline solid-state fermentation of rice using *M. purpureus* (Lucas et al., 1993) and *Monascus ruber* (Wang et al., 1999); however, to the best of our knowledge, no detailed analysis of the chemical constituents of red yeast rice has been undertaken. We therefore undertook a study of the metabolites formed as byproducts of the natural fermentation of *M. purpureus* on moist sterile rice. Herein we report the presence of constituents in red yeast rice and the analytical methods chosen to identify them, including high-performance liquid chromatography (HPLC), gas chromatography (GC), and liquid chromatography/mass spectrometry (LC/MS).

MATERIALS AND METHODS

Apparatus. The HPLC system consisted of a Waters 616 quaternary pump, 996 PDA photodiode array detector, and a Millenium 2010 chromatography workstation. Two columns, a Waters Nova-Pak C_{18}, 3.9 × 150 mm 5 μ column and a Phenomenex Spherisorb 3 ODS−II, 4.6 mm × 100 mm 3 μ column, were used. The mass spectrometer used was a Hewlett-Packard 5989A model. NMR spectra were obtained on a Bruker DRX 400-MHz instrument. Mineral elements were recorded on a Perkin-Elmer Optima 3000 ICP−OES spectrometer. GC analysis was performed on a HP 5890 Series II Gas Chromatograph with a HP Chemstation and HP 7673 sampler. LC/MS was performed on a HP 1090 Series II HPLC with a HP 5989 B quadrupole mass spectrometer.

Reagents. HPLC grade acetonitrile and purified distilled water were used, also reagent grades of MeOH, $CHCl_3$, cyclohexane, and ethanol. Silica gel was 200−300 mesh.

Procedures. Ingredient analyses were determined using methods provided by Official Methods of Analysis of AOAC International (16th edition).

Protein content was determined using the AOAC 990.03 method for crude protein based on total nitrogen content. The ingredients found in red yeast rice were: total sugar (73.4%), fiber (0.8%), protein (14.7%), moisture (6.0%), pigments (0.3%), ash (2.45%), phosphorus (0.4%), organic phosphorus (0.02%), monacolins (0.4%), fatty acids (2.8%), vitamin C (0.03%), vitamin A <70 IU/100 g.

Moisture data were obtained using the Karl-Fisher method.

Trace Elements Analysis. One gram of red yeast rice was dissolved in 50 mL HNO_3 (10%), refluxed for 30 min, cooled to room temperature and filtered. A 10-μL sample was injected into the ICP instrument for analysis. Quantitative sample analysis required the internal standard method. Trace elements found in red yeast rice (μg/g) were: Ca (352), Mg (1092), Na (2370), Al (78), Fe (36), Mn (19), Cu (3), Zn (12), Se (<0.25).

HPLC Analyses of Monacolins. Sample Preparation. Red yeast powder (0.5 g) was dissolved in 10 mL of 75% aqueous alcohol, sonicated for 60 min, centrifuged for 10 min, and filtered through a 0.45-μ filter.

HPLC Conditions. Solvents used were A, acetonitrile; B, 0.04% diluted aqueous phosphoric acid. Gradient conditions were 0−20 min, linear, A (20 → 60%), B (80 → 40%); 20−30 min, linear, A (60 → 80%), B (40 → 20%); 30−32 min, linear, A (80 → 90%), B (20 → 10%). Detection wavelengths were 237 nm and 218 nm. The flow rate was held constant at 1 mL/min.

HPLC Analyses of Citrinin. Sample Preparation. Red yeast powder (2.5 g) was dissolved in 20 mL of absolute alcohol, sonicated for 30 min, centrifuged for 10 min at 3000 rpm, and the supernatant evaporated to dryness under reduced pressure. The residue was redissolved in 1 mL of methanol.

Standard Preparation. One milligram of citrinin (Sigma Co.) was dissolved in 100 of mL methanol.

HPLC Conditions. Solvents used were A, acetonitrile; B, 0.2% phosphoric acid. The gradient conditions included: 0−10 min, linear, A (10 → 55%), B (90 → 45%); 10−15 min, linear, A (55%), B (45%); 15−20 min, linear, A (90 → 10%), B (10 → 90%).

The fluorescence detector settings were excision wavelength at 335 nm and emission wavelength at 502 nm.

Analysis of Fatty Acids. Sample Preparation. Red yeast rice powder (0.5 g) was sonicated with 10 mL of chloroform in a screw-cap tube for 1 h; centrifuged (3000 rpm) for 10 min. Supernatant (200 μL) was transferred into a 8-mL screw cap test tube, the solvent evaporated under nitrogen flow, and 200 μg of tridecanoic acid (13:0) added as internal standard. The residue was dissolved by adding 2.00 mL of MeOH/benzene (v/v 3:1), 200 μL of acetyl chloride slowly added, tightly capped, and kept in a heating block at 100 °C for 1 h. The sample was neutralized with 4 mL of 6% potassium carbonate after cooling, mixed thoroughly by carefully releasing the pressure of the CO_2 generated, centrifuged at 1000 rpm for ca. 10 min, and the upper phase transferred to a crimp-top vial for GC analysis. GC conditions: sp2380 capillary column (Supelco, Inc.), 30 m × 0.32 mm. Carrier gas (He), ca. 3.5 mL/min; H_2, 30 mL/min; air, 300 mL/min. Oven temperature: maintain at 90 °C for 2 min, increase at 4 °C/min to 150 °C, then increase at 10 °C/min to 220 °C, hold for 6 min. Detector: FID 300 °C. injection: split 50:1, 260 °C. Lipid content (%) in red yeast rice: palmitic acid (0.56), stearic acid (0.50), oleic acid (0.62), linoleic acid (0.74), arachidic acid (0.09), linolenic acid (0.36), total unsaturated fatty acids (1.43), total fatty acid (2.84).

Preparation of Red Yeast Rice. Red yeast rice powder (2 kg) was from WPU Company, Beijing. This commercial preparation is made by traditional methods of fermenting moistened premium rice with a proprietary strain of the food fungus, *M. purpureus* for 9 days at 25 °C at a pH range of 5−6 followed by air-drying to yield red yeast rice product.

Isolation of Monacolins. Red yeast rice was soaked in (3 × 4 L) MeOH at ambient temperature. The combined solvent extracts were concentrated to give a mixture that was separated further by chromatography on a silica gel column, eluting with a solvent gradient of chloroform/cyclohexane (1:1) to chloroform/methanol (99:1). Several fractions enriched in components belonging to the monacolin class were combined on the basis of TLC and HPLC analysis. Using silica gel chromatography, the mixture was separated further and the following compounds were isolated: **1** (100 mg), **2** (20 mg), **3** (15 mg), **4** (10 mg), **5** (10 mg), **6** (8 mg), and **7** (2 mg).

LC/MS Analysis. Chromatography using Zorbax C_{18}, 4.6 × 75 mm, 3.5-μ steel column; methanol−water (70:30) isocratic elution at flow rate 0.5 mL/min; temperature, 30 °C; injection volume, 10 μL. Mass range measured 150−900-μ; quadrupole temperature, 140 °C; EM, 2173 V; the spectra were acquired in the positive mode. LC/MS connection was through an i.d. 0.007" stainless steel tubing.

Sample Preparation. Preparation was the same as the HPLC analysis of monacolins.

Monacolin K (**1**), Colorless needles, mp 173−174 °C. UV λ_{max}^{MeCN}: 230, 237, 244 nm. IR ν_{max}^{KBr}: 3440, 1728, 1460, 1383 and 1192 cm^{-1}. Electron ionization (EI)/MS, 404 (M^+, $C_{24}H_{36}O_5$).

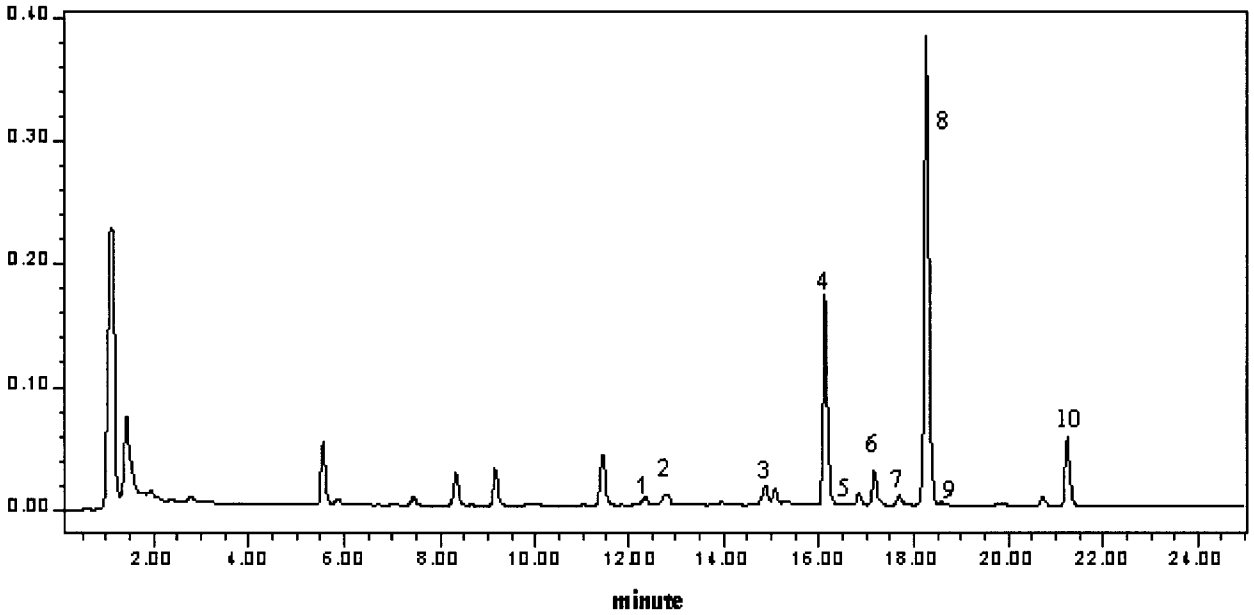


Figure 1. HPLC Traces of monacolins at 237 nm (compounds **4** and **7** can only be seen with monitoring at 218 nm). 1,2, unknowns, 3, hydroxy acid form of monacolin L (compound **5a**); 4, hydroxy acid form of monacolin K (compound **1a**); 5, methyl ester of hydroxy acid form of monacolin L (compound **6**); 6, monacolin L (compound **5**); 7, hydroxy acid form of dehydromonacolin K (compound **2a**); 8, monacolin K (compound **1**); 9, methyl ester of hydroxy acid form of monacolin K (compound **3**); 10, dehydromonacolin K (compound **2**).

Dehydromonacolin K (**2**), Colorless needles. mp, 125−128 °C. UV λ_{max}^{MeCN}: 230,237,247 nm. IR ν_{max}^{KBr}: 1720, 1454, 1379, 1254, and 1194 cm^{-1}. EI-MS: 386 (M^+, $C_{24}H_{34}O_4$).

Compound (**3**): Colorless oil. EI-MS: 436 (M^+, $C_{25}H_{40}O_6$). UV λ_{max}^{MeCN}: 228, 236, 244 nm. IR ν_{max}^{KBr}: 3440, 1728, 1460, 1383, and 1192 cm^{-1}.

Compound (**4**): Colorless gum. UV λ_{max}^{MeCN}: 217 nm. IR ν_{max}^{KBr}: 1720, 1462, 1379, 1250, 1194, and 1043 cm^{-1}. EI/MS: 287(M^+ − $C_5H_9O_2$).

Monacolin L (**5**): Colorless needles. UV λ_{max}^{MeCN}: 230, 237, 243 nm. IR ν_{max}^{KBr}: 3441, 1726, 1462, 1383, and 1192 cm^{-1}. EI/MS: 304 (M^+, $C_{19}H_{28}O_3$).

Compound (**6**): Colorless gum. UV λ_{max}^{MeCN}: 230, 237, 247 nm. IR ν_{max}^{KBr}: 3450, 1728, 1439, and 1261 cm^{-1}. EI/MS: 336 (M^+, $C_{20}H_{32}O_4$).

Dihydromonacolin K (**7**): Colorless gum. UV λ_{max}^{MeCN}: 218 nm. IR ν_{max}^{KBr}: 1720, 1462, 1379, 1250, 1194 and 1043 cm^{-1}. EI/MS: 406 (M^+, $C_{24}H_{38}O_5$).

RESULTS AND DISCUSSION

Red yeast rice consists mainly of rice and byproducts of the fermentation. The most abundant ingredient is starch shown as total sugar, accounting for more than 73% of the bulk. The crude protein content is approximately 15%, and other ingredients are found in lesser quantities. Of the trace elements, magnesium and sodium are the most abundant metal elements in the rice.

The analysis of the metabolic byproducts of the fermentation was achieved by separating the components of the aqueous methanol extract of Cholestin (Cholestin is a commercial preparation of red yeast rice) into various chemical classes. Further separation led to the isolation of individual components for further study. The various groups that were analyzed are polyketides, fatty acids, and pigments. When untreated rice was analyzed, no pigments or polyketides were detected, leading to the conclusion that these compounds are indeed secondary metabolites of *M. purpureus*.

Seven monacolins were isolated from methanol extracts of red yeast rice in sufficiently pure form for identification. The other compounds are presumed to be monacolin-related based on HPLC and total UV profiles (see Figure 1). Each compound was identified based on literature comparison (Endo, 1980; Endo et al., 1985; Alberts et al., 1980; Houck et al., 1993; Albers-Schonberg et al., 1981; Araki and Konoike 1997; Bartmann et al., 1986). For compounds **1**−**3**, **5**−**6**, the UV spectra exhibit peak maxima at ca. 230, 237, and 246 nm, profiles typical of the monacolins (Endo et al., 1985); compounds **4** and **7** exhibit maxima only at 217 nm, because there is no double bond between carbons C-4a and 5. This was confirmed further by the absence of the H-5 double-bond proton in the H NMR. This H-5 proton resonates in compounds **1**−**3**, **5**−**6** at 5.43 ppm. Furthermore in **2** and **4** there is a conjugated lactone and the H-2′ and H-3′ protons resonate at 6.03 and 6.86, respectively. Both protons are absent in compounds **1**, **3**, **5**−**7**. The same profiles were found for the compounds in the HPLC window eluting from 5 to 13 min. Stereochemical aspects are based solely on comparative NMR chemical shift and coupling constant data with stereochemical literature assignments. Compounds **1**, **2**, **5**, and **7** were identified as monacolin K (Endo, 1980; Alberts et al., 1980), dehydromonacolin K (Houck et al., 1993), monacolin L (Endo et al., 1985), and dihydromonacolin K (Albers-Schonberg et al., 1981), respectively. Compound **3** was identified as the methyl ester of lactone ring-opened monacolin K (Araki and Konoike, 1997). Compound **6** was the methyl ester, of lactone ring-opened monacolin L. Because both **3** and **6** appear as methyl esters we checked to see if these were artifacts of the extraction. Indeed these compounds are present after ethanol extraction so we deduced that they are naturally occurring. Although a molecular ion for **4** was not seen, EI/MS of **4** revealed a quasi-MW at 287 $(M-101)^+$, which suggested the loss of an ester side chain ($C_5H_9O_2$), corresponding to a molecular weight of 388, which implies a molecular formula of $C_{24}H_{36}O_4$.

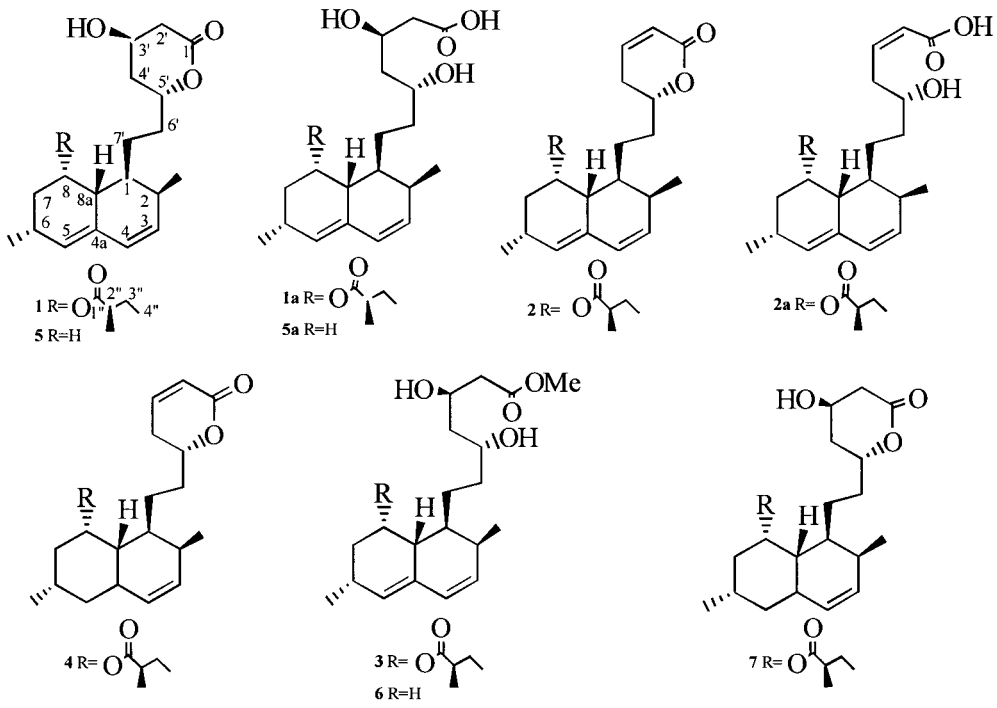


Figure 2. Monacolins from red yeast rice.

Table 1. Monacolins Identified and Yields in Red Yeast Rice (wt %/wt Rice)[a]

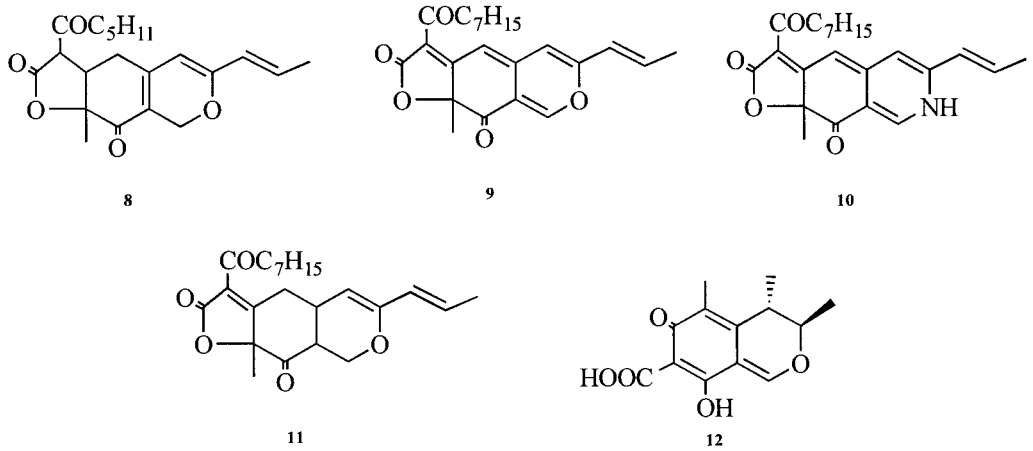
monacolin K (**1**)	0.2
monacolin K (hydroxy acid form)	0.1
dihydromonacolin K (**7**)	trace
dehydromonacolin K (**2**)	0.03
monacolin (**3**)	0.02
monacolin (**4**)	0.02
monacolin (**6**)	0.01
monacolin L (**5**)	0.02
total monacolins	0.4

[a] Values are stated as percentages. The detection limit of monacolins is approximately 1 ng/g.

A typical HPLC trace showing the profile of the monacolin mixture in red yeast rice is shown in Figure 1. Using the HPLC conditions described with single-wavelength detection at UV wavelengths of 237 and 218 nm, baseline resolution of eight related compounds was achieved. The UV−HPLC peaks were identified as the following compounds: monacolin K (**1**), 18.3 min; dehydromonacolin K (**2**), 21.2 min; methyl ester of monacolin K hydroxy-acid form (**3**), 18.5 min; hydroxy acid form of monacolin K (lactone ring-opened form **1a**) 16.2 min; monacolin L (**5**), 17.7 min; methyl ester of monacolin L hydroxy acid form (**6**), 17.2 min. Structures of all these compounds are shown in Figure 2. Monacolin K is most often present with its ring-opened lactone congener form. These two compounds are produced in a ratio that varies from 1:2 to 2:3, respectively, and they account for approximately 90% of the "total monacolin fraction". The other components are usually observed at levels ranging from trace quantities to 0.02 wt %/wt rice (Table 1). The relative levels of metabolites in the red yeast rice were calculated by measuring the respective UV response factors for these monacolins at 237 nm, and the results are given in Table 1. These were calculated using a three-point calibration for the hydroxy acid; the peak area for this compound in all preparations was within the calibrated range in all tests.

The biosynthetic pathway for monocolins has been described from filamentous fungi, for example, *Aspergillus*, *Penicillium*, and (the oyster mushroom) *Pleurotus ostreatus* (Kennedy et al., 1999). Monacolin K contains two polyketide chains C-18 and C-14 synthesized from incorporation of acetate and methionine. The C-18 chain is cyclized, oxidized at the 8-carbon, and esterified by the side chain. Monacolin L (**5**) is considered the precursor of monacolin J. Therefore it is expected that a set of biosynthetically related monocolins is produced by red yeast rice (Kennedy et al., 1999). When prepared appropriately in the traditional manner using the *M. purpureus* strain a set of monacolins is always present and this preparation has been shown to be clinically effective. There are, however, several commercially available purportedly red yeast rice preparations that show very little monacolin content (Heber, personal communication).

In the properly prepared red yeast rice the ratios between **1** and the ring-opened form vary between 1:2 and 2:3. Analysis of the total monacolin content indicates these two compounds together are present as 0.3% wt/wt of the red yeast rice. The total monacolin content is usually in the range of 0.4% wt/wt rice. Figure 1 is a complete red yeast rice monacolin fraction "fingerprint". An equilibrium exists between monacolin K and the lactone ring-opened congener form. To confirm this equilibrium in the presence of base the equilibrium is clearly shifted to the lactone ring-opened form. Thus, when **1** was treated with 0.05 N NaOH, base hydrolysis of the lactone ring takes place, and total conversion to the hydroxy acid occurs. This conversion was confirmed by LC/MS experiments. The pseudo molecular ion MH^+ of **1** was observed at *m*/*z* 405 with a small sodiated-adduct peak at *m*/*z* 427. When **1** was mixed with 0.05 N NaOH immediately before injection, lactone ring-opening occurred and the pseudo-molecular ion shifts to $M^+ + H_2O + Na^+$, observed at *m*/*z* 446, as expected for the ring-opened form. Thus, when the whole of a red yeast rice extract containing the monacolin mixture was



8 9 10 11 12

Figure 3. Pigments in red yeast rice. **8**, monascidin A, **9**, monascorubrine, **10**, monascorubramine, **11**, ankaflavin, **12**, citrinin.

treated in the same manner, some changes in the chromatogram were observed, with the appearance of new peaks and a reduction of peak size for compounds **1**, **2**, and **5**. Lactone ring-opening has occurred in both **2** and **5**, resulting in the formation of their respective congeners, **2a** and **5a**.

The contents of the fatty acids are given in Materials and Methods. Two sets of acids are represented, the saturated acids consisting mainly of palmitic, stearic, and arachidic acids, and the unsaturated acids mostly represented by oleic, linoleic, and linolenic acids.

The last set of compounds analyzed includes the known polyketide pigments, monascidin A, ankaflavin, monascorubrine, and monascorubramine (Blanc et al., 1994; Martinkova, 1995), shown in Figure 3. These pigments were detected in the organic layer after extraction into chloroform and could be visualized by TLC using reported conditions. No further studies were performed on these pigments; however, we evaluated the pigment fraction for citrinin content. Citrinin is a mycotoxin, a known microbiological pigmented metabolite associated with some strains of *Monascus* (Saito, 1971; Sabater-Vilar et al., 1999). In all the tested batches of red yeast rice that were used for the commercial preparation of Cholestin, no detectable levels of citrinin were found. An HPLC method, coupled to a highly sensitive fluorescence detector (see Materials and Methods), was developed to determine trace amounts of citrinin in red yeast rice, and the absence (beyond the detection limit of 0.1 ng/g) of this toxic compound.

In summary, to the best of our knowledge no reports on any secondary metabolites have appeared from red yeast rice. Further, no reports indicating the presence of monacolin compounds in solid-state fermentation of *Monascus* have been found. These compounds specifically inhibit 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase (Juzlova et al., 1996; Endo, 1985). In this study, we report the isolation and identification of seven monacolin analogues from red yeast rice. The presence of these compounds may explain in part the cholesterol-lowering ability associated with this traditional Chinese food.

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Supporting Information Available: NMR data for compounds **1**−**7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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