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Two New *Monascus* Metabolites with Strong Blue Fluorescence Isolated from Red Yeast Rice

ZHIBING HUANG,† YANG XU,*,† LAISHENG LI,‡ AND YANPING LI†

Sino-Germany Joint Research Institute, Key Laboratory of Food Science, Ministry of Education, and Center of Analysis and Testing, Nanchang University, Nanchang, Jiangxi 330047, People's Republic of China

Red yeast rice obtained as cultures of *Monascus* AS3.4444 on rice was extracted and analyzed by high-performance liquid chromatography (HPLC). Two new *Monascus* metabolites with similar fluorescence spectra (λ_{ex} = 396 nm, λ_{em} = 460 nm) and UV absorption spectra (λ_{max} = 386 nm) were detected. They were isolated by rechromatography on a silica gel column and semipreparative HPLC, and two strong blue fluorescent compounds were obtained. Their structures were elucidated by electrospray ionization mass spectrometry (ESI−MS), electrospray ionization tandem mass spectrometry (ESI−MS/MS), intensive ESI−MS, and nuclear magnetic resonance spectroscopy (^{1}H NMR, ^{13}C NMR, COSY, and HMBC) studies. High-resolution mass spectrometry indicated the molecular formulas $C_{21}H_{24}O_5$ and $C_{23}H_{28}O_5$. The two new compounds, named monasfluore A and monasfluore B, respectively, contain a alkyl side chain, γ-lactone, and propenyl group, whereas the more lipophilic compound, monasfluore B, is a higher homologue of monasfluore A, with the more lipophilic octanoyl instead of the hexanoyl side chain.

KEYWORDS: *Monascus*; red yeast rice; metabolite; fluorescence spectra; HPLC; HR-MS; HPLC−MS/MS; NMR

INTRODUCTION

Filamentous fungi are considered to play an important role both in human lifestyle for the production of foods and health products and in environmental control for the recycling of organic compounds in the biosphere. The filamentous fungi of the genera *Monascus* are used in Asia for centuries as a source of pigments for the coloring of traditional foods. They include four major species: *M. pilosus*, *M. purpureus*, *M. ruber*, and *M. floridanus*, belonging to the class Ascomycetes and the family Monascaceae.

Red yeast rice, which is also known as koji, anka, angkak, and ben-koji, is produced by growing *Monascus* sp. on rice to produce a red-colored product. Red yeast rice was a great invention in ancient China and was used as a Chinese medicine to strengthen the spleen, promote digestion, eliminate dampness and phlegm, promote blood circulation, and remove blood stasis. It also has been widely used in the Orient to make rice wine, as a food preservative for maintaining the color and taste of fish and meat, and for its medicinal properties. *Monascus* can produce several secondary metabolites, such as the pigments, monacolins, and γ-aminobutyric acid (GABA), and dimerumic acid (*1–3*). Recently, there have been many reports on these secondary metabolites (*4–9*).

Monascus pigments are a group of fungal metabolites called azaphilones, which are synthesized from polyketide chromophores and β-keto acids by esterification. The six well-known *Monascus* pigments include yellow (monascin and ankaflavin), orange (monascorubrin and rubropunctatin), and red purple (monascorubramine and rubropunctamine). Although for years it has been known that there are six pigments and other metabolic products from *Monascus* sp., such as alcohols, organic acids, and substances with a wide range of biological and therapeutic benefits, including anticarcinogenic, antioxidative, and hypolipidemic activities, which have been described (*10–18*), in the past decade, some new pigments and metabolites have been isolated and their chemical structures have been characterized (*19–25*). However, some fluorescent compound products from *Monascus* sp. have received less attention. Therefore, characterization of the fluorescent components of red yeast rice and their beneficial and possible adverse effects are essential for a risk versus benefit assessment.

The pattern of natural products in red yeast rice is extremely complex, and it consists of a wide range of compounds of varying polarity. This study investigated the extraction, purification, and characterization of the structures of molecules involved in two fluorescent compounds in red yeast rice. The structures of two new fluorescent compounds were established by mass spectrometry and nuclear magnetic resonance (NMR) spectroscopy using a combination of 1D NMR methods (^{1}H NMR and

* To whom correspondence should be addressed. Telephone: +86-0791-8329479. Fax: +86-0791-8333708. E-mail: xuyang1951@yahoo.com.cn.

† Sino-Germany Joint Research Institute, Key Laboratory of Food Science, Ministry of Education.

‡ Center of Analysis and Testing.

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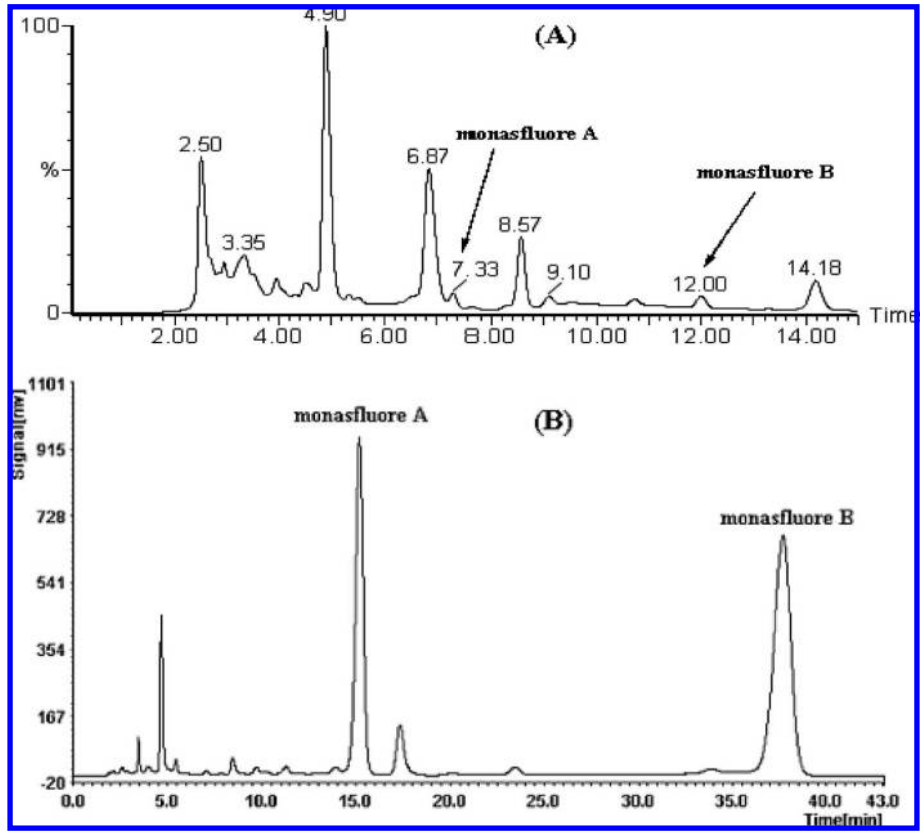

Figure 1. (**A**) HPLC chromatogram of extracts of red yeast rice obtained from *Monascus* AS3.4444 grown on rich rice medium. (**B**) Semipreparative HPLC chromatograms of the eluate from the third chromatographic silica gel column.

^{13}C NMR) and 2D shift-correlated NMR techniques [correlation spectroscopy (COSY), heteronuclear multiple-quantum coherence (HMQC)−heteronuclear single-quantum correlation (HSQC), and heteronuclear multiple-bond correlation (HMBC)], for the complete ^{1}H and ^{13}C signal assignments.

MATERIALS AND METHODS

Reagents. Acetonitrile [high-performance liquid chromatography (HPLC) grade] and methanol (HPLC grade) were obtained from Merck (Darmstadt, Germany). All other chemicals were of analytical grade from ChromTech (Shanghai, China) and were not further purified. Deionized water was obtained with an in-house Milli-Q Plus System (Millipore, Inc., Billerica, MA) at 18.2 MΩ; this deionized water is referred to as "water" hereafter.

Thin-Layer Chromatography (TLC). TLC was performed on 5 × 5 cm silica gel 60 plates (Merck, Darmstadt, Germany); petroleum/ethyl acetate/acetone (8:1:1, v/v/v) was used as the solvent. Monasfluore A and monasfluore B revealed a blue fluorescence under a ultraviolet lamp.

HPLC Analysis. The HPLC system consisted of a Waters 510 solvent delivery pump (Waters), a 7725 manual injector system equipped with a 20 μL loop, a model 2475 multi-wavelength fluorescence detector (Waters), and a UV200 detector equipped with Echrom98 chromatography manager for integration (Dalian Elite Analytical Instruments Co. Ltd.). Chromatographic separation was achieved at room temperature using a 250 × 4.6 mm i.d., 5 μm Symmetry C_{18} column (Waters), with isocratic elution of water/acetonitrile (23:77, v/v) at a flow rate of 0.8 mL/min.

HPLC−Electrospray Ionization Mass Spectrometry (ESI−MS) and HPLC−Electrospray Ionization Tandem Mass Spectrometry (ESI−MS/MS) Analysis. The HPLC−MS analyses were performed using a Waters LC/ZQ 2695/4000 HPLC−MS system (Waters). The LC/DAD/MS/MS analysis was carried out in an Agilent 1100 chromatograph equipped with a diode array detector and mass detector in series (Agilent). The HPLC was controlled by version 08.03 Chemstation software (Agilent). The mass detector was an ion-trap spectrometer equipped with an electrospray ionization interface and controlled by version 4.1 LCMSD software (Agilent). The ionization conditions were adjusted at 350 °C and 4 kV for capillary temperature and voltage, respectively. The nebulizer pressure was 65 psi, and the nitrogen flow rate was 11 mL/min. The full-scan mass covered the range from *m/z* 200 to 1000. Collision-induced fragmentation experiments were performed in the ion trap using helium as a collision gas, with voltage ramping cycles from 0.3 to 2 V. All mass spectrometry data were recorded in the positive-ion mode. Total ion chromatograms were recorded as alternating automatic events: full-scan mass spectra (MS) and MS/MS of the pseudomolecular ion and MS^n (n = 1–3) to fragment the major ions obtained in every step.

ESI−HRMS. High-resolution mass spectra were measured on FT-MS-Bruker Apex IV (7.0 T). The main advantage of the instruments is that they provide accurate mass and high resolution and have a high dynamic range of qualities critical for resolving individual peptides from complex samples. They have an extremely accurate mass up to 0.5 ppm and resolution of more than 100 000 [full width at half-maximum, (fwhm) definition].

NMR Spectroscopy. NMR spectra were recorded in $CDCl_3$ at room temperature using a Bruker Avance 400 spectrometer [400 MHz (^{1}H NMR) and 100 MHz (^{13}C NMR)]. Chemical shifts are given on the δ scale. The pulse programs of the following 2D experiments were taken from the Bruker software library, and the parameters were as follows: 400/100 MHz gradient-selected HMQC spectra, relaxation delay D1 = 1.5 s; 400/100 MHz gradient-selected HMBC spectra, relaxation delay D1 = 1.5 s, evolution delay D2 = 3.45 ms, and delay for evolution of long-range coupling D6 = 50 ms; 400 MHz gradient-selected ^{1}H,^{1}H COSY spectra, relaxation delay D1 = 1.5 s; and 100 MHz DEPT-135 spectra, relaxation delay D1 = 2.0 s and evolution delay D2 = 3.45 ms.

Organism, Preparation, and Extraction of Red Yeast Rice. The strains *Monascus* AS3.4444 were obtained from the Institute of Microbiology, Chinese Academy of Sciences (Beijing, China). After 5.0 mL of distilled water was put into a strain slant and vortexed, the

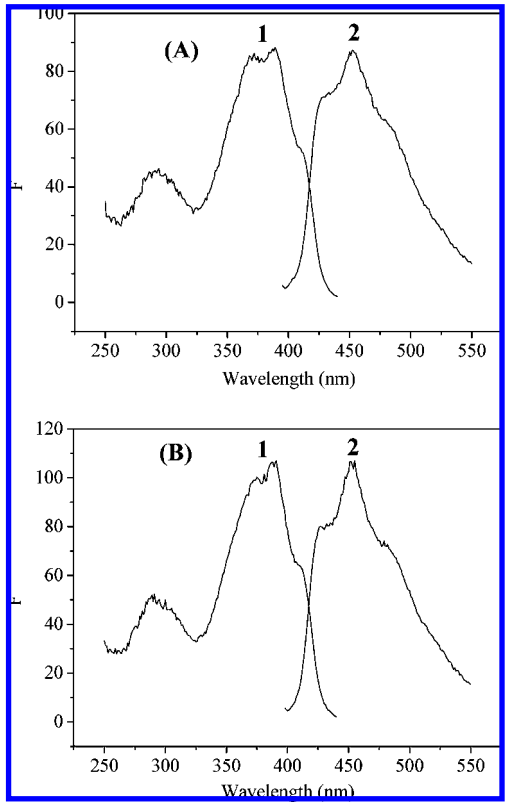

Figure 2. Excitation (1) and emission (2) spectra of (**A**) monasfluore A and (**B**) monasfluore B. (1, λ_{ex} = 396 nm; 2, λ_{em} = 460 nm).

spore solutions were collected. Seed cultures were grown in 500 mL baffled flasks containing 75 mL of malt extract agar medium that had been inoculated with 5 mL of the spore solutions. Flasks were then cultivated for 36 h at 30 °C and 200 rpm on a rotary shaker. The composition of the malt extract agar medium was as follows: maltose extract, 20 g/L; peptone, 1 g/L; glucose, 20 g/L; and agar, 15 g/L. For fermentation cultures, after the 500 mL baffled flasks containing 100 g of ground rice had been inoculated with 2.0 mL of the seed cultures, they were cultivated for 2 weeks at 30 °C and dried at 40 °C.

A 0.1 g aliquot of the red yeast rice thus obtained was extracted for analytical purposes by sonication with 2.0 mL of methanol and centrifugation to obtain a clear supernatant, which was directly used for HPLC.

The dried red yeast rice (0.5 kg) was extracted twice with 1.0 L of 95% ethanol at room temperature for 30 min under sonication. The mixture was suction-filtered, and the residue was washed with 200 mL of 95% ethanol. The extracts were combined and concentrated under reduced pressure. The residue was suspended in a petroleum (60–90 °C)/ethyl acetate mixture, subjected to a silica gel (70–230 mesh) column in the mixture of petroleum and ethyl acetate, and eluted with increasing concentrations of ethyl acetate and acetone to increase polarities. The fractions eluted with the solvents of less than 25% ethyl acetate and acetone in petroleum were collected and concentrated under reduced pressure to obtain the crude extract.

Isolation of Monasfluore A and Monasfluore B from Crude Extracts. A part of the crude extract was coated on silica gel (1:5 sample/adsorbent, w/w) and subjected to dry flash chromatography. Sufficient petroleum was passed through the column to remove the oily hydrophobic materials. Extensive gradient elution was then employed using a solvent of increasing polarity (20:1:1, 15:1:1, 10:1:1, and 8:1:1 petroleum/ethyl acetate/acetone, v/v/v) to yield six fractions. Similar fractions were combined to give two main fractions according to TLC, and the solvent was removed under reduced pressure. The fraction with strong blue fluorescence was further separated by rechromatography on a silica gel column to give 5 mL fractions. These resultant fractions were further analyzed by HPLC, and then fractions with a similar single-peak profile were combined, respectively.

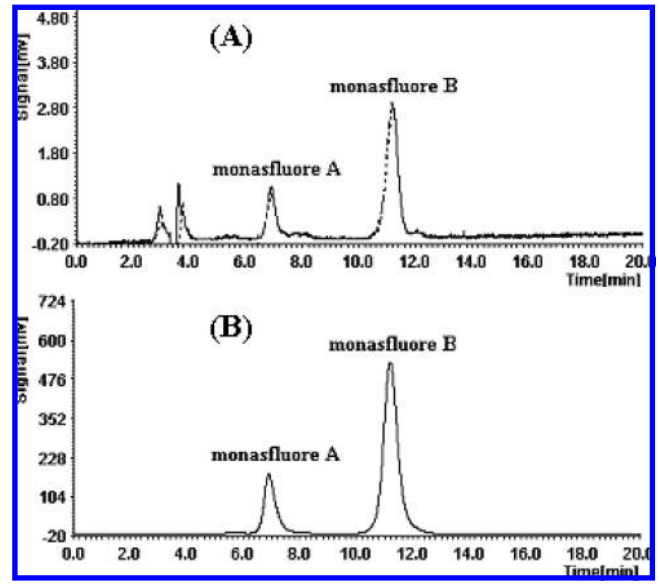

Figure 3. HPLC profile detected by a (**A**) ultraviolet detector and (**B**) fluorophotomeric detector connected in series.

To further purify the two new fluorescence materials, the methanol solution was fractionated to obtain monasfluore A and monasfluore B by semipreparative HPLC on a 150 × 9.4 mm i.d., 5 μm Zorbax SB-C_{18} column (Agilent) using a isocratic elution corresponding to that described above but using water/methanol (30:70, v/v) instead of water/acetonitrile (23:77, v/v) and a flow of 2.5 mL/min. These resultant fractions were further analyzed by HPLC, and then fractions with a similar single-peak profile were combined, respectively. After repeated purification by HPLC, it formed green oils upon evaporation of methanol and water.

Monasfluore A. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.85–0.89 (3H, t, H-19), 1.19–1.22 (6H, m, H-16, H-17, and H-18), 1.62 (3H, s, H-10), 1.89–1.92 (3H, m, H-13), 2.45–2.53 (1H, m, H-15a), 3.06–3.12 (1H, dt, H-15b), 3.72–3.75 (1H, d, J = 12.0 Hz, H-9), 3.85–3.88 (1H, d, J = 12.1 Hz, H-9a), 5.41 (1H, s, H-5), 5.96 (1H, s, H-4), 5.99 (1H, s, H-11), 6.51–6.52 (1H, m, H-12), and 7.36 (1H, s, H-1).

^{13}C NMR ($CDCl_3$, 100 MHz) δ: 13.83 (C-19), 18.41 (C-13), 22.74 (C-10), 23.44 (C-16), 29.68 (C-17), 31.29 (C-18), 42.91 (C-15), 43.50 (C-9a), 56.56 (C-9), 82.81 (C-6a), 106.47 (C-5), 106.69 (C-4), 114.19 (C-6b), 122.82 (C-11), 135.12 (C-12), 144.41 (C-4a), 146.82 (C-1), 155.96 (C-3), 168.58 (C-8), 191.25 (C-6), and 202.31 (C-14).

ESI−MS, m/z (%): 357.4 (50), $[M + H]^+$, 379.3 (100), $[M + Na]^+$, 395.3 (50), $[M + K]^+$, 709.6 (5), [2M], 735.6 (80), $[2 M + Na]^+$, 751.5 (10), $[2 M + K]^+$. ESI−MS/MS, m/z (%): 311.1 (10), $[M^+$-46], 285.1 (100), $[M^+$-72], 259 (15), $[M^+$-98], 241.1 (30), $[M^+$-116], 215.1 (20), $[M^+$-142], 185.1 (20), $[M^+$-172]. HRMS, m/z: 357.16909, $[M + H]^+$, 379.15115, $[M + Na]^+$ (calculated for $[M + H]^+$, $C_{21}H_{25}O_5$: 357.16965 and for $[M + Na]^+$, $C_{21}H_{24}NaO_5$: 379.15159).

Monasfluore B. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.85 (3H, t, H-21), 1.26 (10H, m, H-16, H-17, H-18, H-19, and H-20), 1.58 (3H, m, H-10), 1.92 (3H, s, H-13), 2.45–2.53 (1H, m, H-15a), 3.06–3.12 (1H, dt, H-15b), 3.72–3.75 (1H, d, J = 12.1 Hz, H-9), 3.85–3.88 (1H, d, J = 12.2 Hz, H-9a), 5.41 (1H, s, H-5), 5.95 (1H, s, H-4), 5.98 (1H, s, H-11), 6.50–6.56 (1H, s, H-12), and 7.35 (1H, s, H-1).

^{13}C NMR ($CDCl_3$, 100 MHz) δ: 13.98 (C-21), 18.44 (C-13), 22.52 (C-10), 23.12 (C-20), 23.18 (C-16), 28.87 (C-17), 28.98 (C-19), 31.60 (C-18), 42.84 (C-15), 42.96 (C-9a), 56.56 (C-9), 82.79 (C-6a), 106.49 (C-5), 106.67 (C-4), 114.21 (C-6b), 122.83 (C-11), 135.07 (C-12), 144.36 (C-4a), 146.78 (C-1), 155.94 (C-3), 168.55 (C-8), 191.22 (C-6), and 202.34 (C-14).

ESI−MS, m/z (%): 385.4 (30), $[M + H]^+$, 407.3 (80), $[M + Na]^+$, 423.3 (30), $[M + K]^+$, 769.8 (5), [2M], 791.7 (100), $[2 M + Na]^+$, 807.7 (15), $[2 M + K]^+$. ESI−MS/MS, m/z (%): 367.1 (8), $[M^+$-18], 339.1 (18), $[M^+$-46], 313.2 (100), $[M^+$-72], 241.1 (31), $[M^+$-144], 215.1 (20), $[M^+$-170], 185.1 (20), $[M^+$-200]. HRMS, m/z: 385.20088, [M +

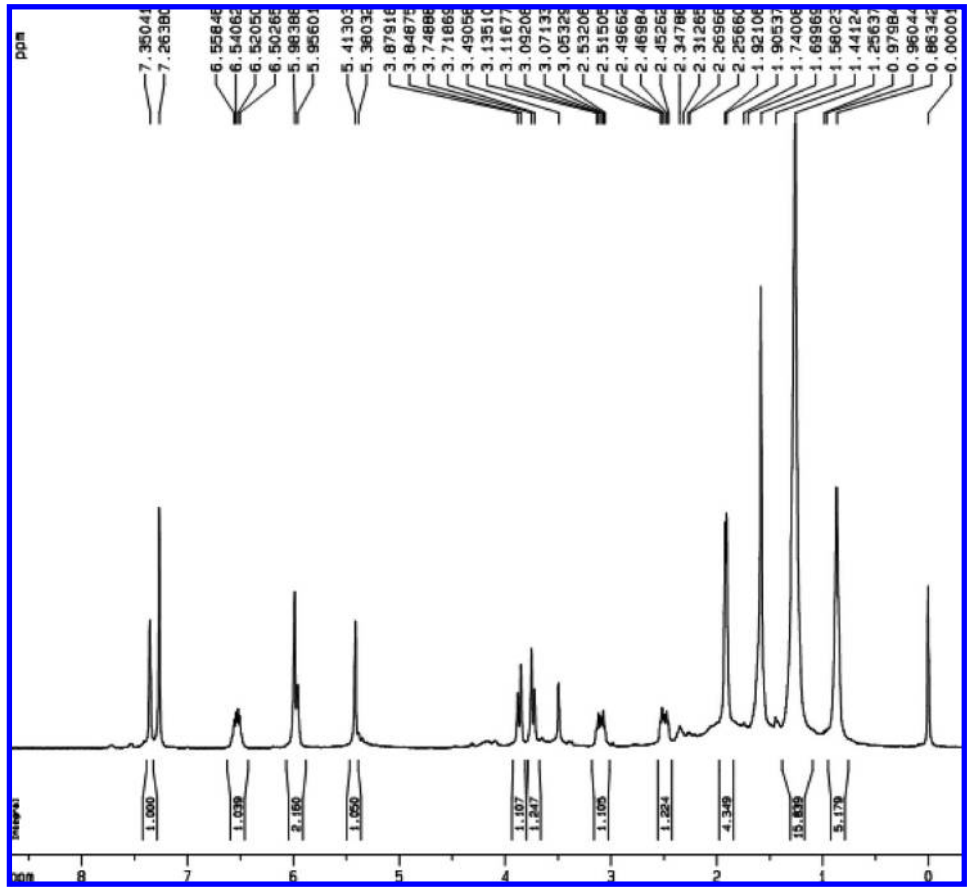

Figure 4. ^{1}H NMR spectrum of monasfluore B.

H]$^+$, 407.18252, [M + Na]$^+$ (calculated for [M + H]$^+$, $C_{23}H_{29}O_5$: 385.20095 and for [M + Na]$^+$, $C_{23}H_{28}NaO_5$: 407.18290).

RESULTS AND DISCUSSION

TLC Analysis of Monasfluore A and Monasfluore B. Crude extracts of red yeast rice were analyzed by TLC. After ethanol extracts had been applied to plates, the silica gel 60 F254 plates were developed with a solution of petroleum/ethyl acetate/acetone (8:1:1, v/v/v). There was only one spot with strong blue fluorescence under exposure to a ultraviolet lamp ($\lambda = 365$ nm), and the R_f value was about 0.43. The results showed that the monasfluore A and monasfluore B were not completely separated.

Chromatographic Separation. In the course of HPLC analyses of extracts of red yeast rice obtained with *Monascus* AS3.4444, the chromatogram of this particular culture also contains several peaks of as yet unidentified compounds (**Figure 1A**). The peaks of monasfluore A and monasfluore B with retention times of 7.33 and 12.0 min, respectively, were seen in samples of red yeast rice obtained with the strain AS3.4444 on a medium consisting of ground rice only. As shown in **Figure 1A**, the complexity of the pigment mixture produced by *Monascus* sp. on rice interferes with analysis of monasfluore A and monasfluore B. To reduce the interference, it is necessary that the extract solution of red yeast rice was chromatographed on silica gel. The adsorption column was eluted with petroleum/ethyl acetate/acetone (20:1:1, 15:1:1, and 10:1:1, v/v/v) to elute less-polar products containing free pigments, such as monascin and ankaflavin, and then the fluorescence materials were eluted with petroleum/ethyl acetate/acetone (8:1:1, v/v/v).

Purification of Monasfluore A and Monasfluore B. The eluate from the third chromatographic silica gel column was collected in 5.0 mL fractions, and the contents of monasfluore A and monasfluore B were analyzed by HPLC with a photodiode array detector. The collected eluate was then combined into three main fractions according to the results of HPLC detection. On the basis of the elution volume, they were respectively monasfluore A concentrate, monasfluore A and monasfluore B mixture, and monasfluore B concentrate in that order. Both the monasfluore A and monasfluore B concentrates were collected and further purified by semipreparative HPLC. As shown in **Figure 1B**, a good peak shape and satisfactory resolution of monasfluore A and monasfluore B can be achieved in semipreparative HPLC and the retention time of monasfluore A and monasfluore B were 15.3 and 38.0 min, respectively.

Fluorescence Spectra of Monasfluore A and Monasfluore B. The fluorescence spectra of monasfluore A and monasfluore B were determined, and they had similar fluorescence spectra, with maximum excitation and emission at 396 and 460 nm, respectively (**Figure 2**). A solution of red yeast rice chromatographed on silica gel showed retention times of monasfluore A and monasfluore B as 6.9 and 11.2 min, respectively, using water/methanol (15:85, v/v) instead of water/acetonitrile (23:77, v/v). When response values of monasfluore A and monasfluore B were compared by ultraviolet and fluorescence connected in series, the response values of the latter were more than 100-fold higher than that of the former (**Figure 3**).

NMR Analysis. One-dimensional NMR represents a simple method for obtaining global information about complex samples and provides a fingerprint of the monasfluore A and monasfluore B analyzed. The partial structure was established using ^{1}H COSY to determine the connectivities between protons on the basis of geminal and vicinal couplings. The determination of the chemical shifts of the protons and protonated carbons was

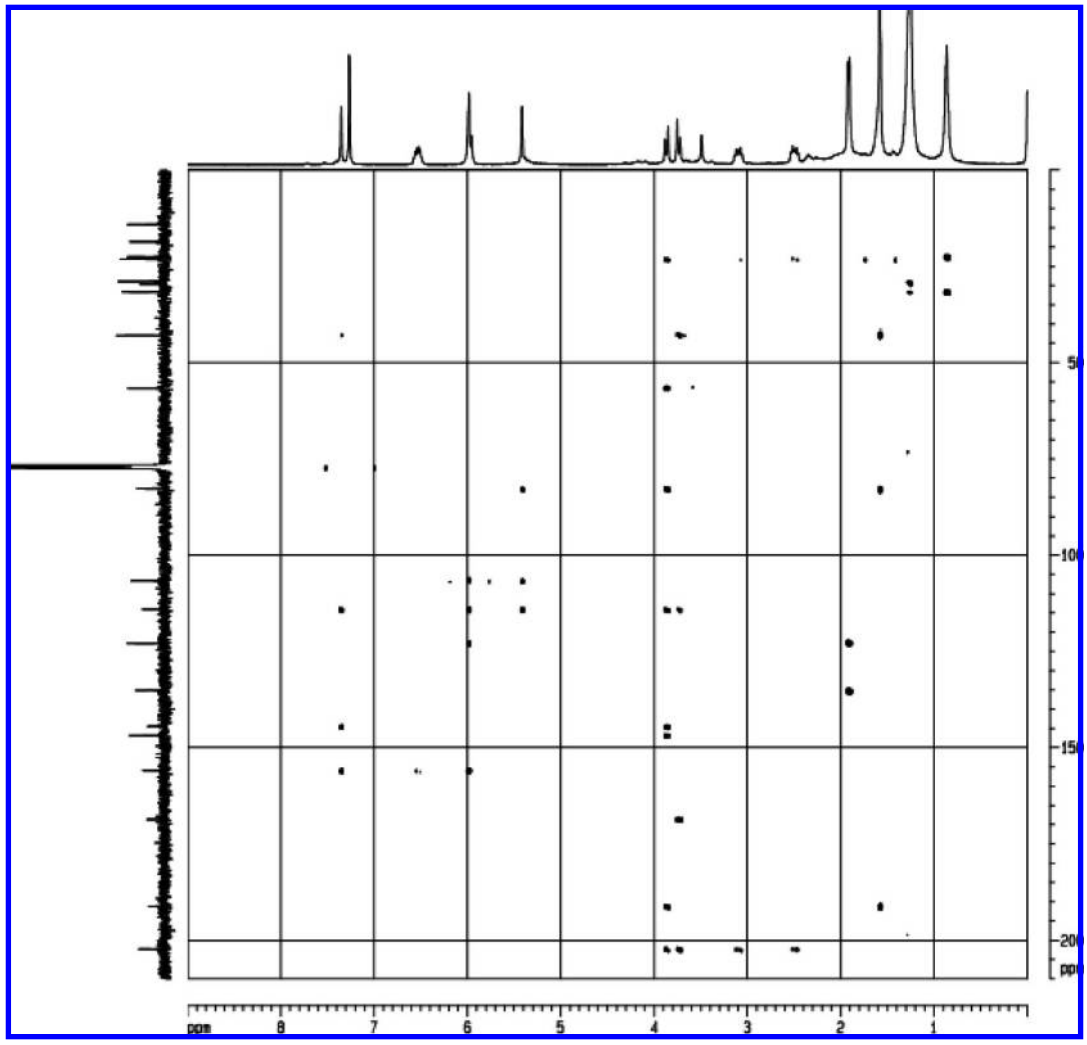

Figure 5. HMBC spectrum of monasfluore B.

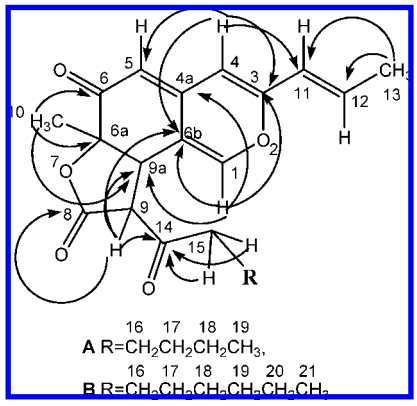

Figure 6. Structure of (**A**) monasfluore A and (**B**) monasfluore B and the long-range $^{1}H,^{13}C$ correlations found in the HMBC spectrum.

made on the basis of the study of the HMQC−HSQC spectrum. The complete structure of compounds monasfluore A and monasfluore B were established by the HMBC spectrum, which shows correlations between protons and carbons for two and three bonds.

For monasfluore A and monasfluore B structural elucidation, NMR signal assignment, ^{1}H, ^{13}C, DEPT-135, H,H-COSY, H,C-HMQC, and H,C-HMBC spectra were recorded. In the ^{13}C NMR spectrum, the signals at δ 202.34, 191.22, and 168.55 were characteristic for ketone C═O, conjugated ketone, and ester (lactone) groups, respectively. The peaks at δ 146.78, 135.07, 122.83, 106.49, and 106.67 were assigned to ═CH, and the signals at δ 155.94, 144.36, and 114.21 were assigned to quaternary sp^2 carbon atoms. In the sp^3 range of the ^{13}C and DEPT-135 spectra, the peaks revealed the presence of three CH_3, six CH_2, two CH, and one quaternary carbon atoms. The latter carbon atom is bonded to oxygen.

In the ^{1}H NMR spectrum (**Figure 4**), the methyl signals at δ 0.85, 1.58, and 1.92 showed triplet, singlet, and doublet multiplicities, respectively, in accordance with the presence of CH_2, quaternary C, and CH neighbors. Using the $^{1}H,^{1}H$ COSY experiment, the correlations starting from the CH_3 signal at δ 0.85 allowed for the identification of a $CH_3(CH_2)_6$ moiety. The methyl group at δ 1.92 also formed a separate spin system together with the protons of a *trans*-(*E*)-disubstituted carbon−carbon double bond, and this allowed the identification of a propenyl substituent. The remaining two methine groups (δ 3.72–3.75 and 3.85–3.88) formed the two spin system. One rather conspicuous signal of the ^{1}H NMR spectrum was the singlet at δ 7.35 (H-1). This extreme chemical shift can be well-explained by the strong deshielding effect of a coplanar, *peri*-positioned carbonyl group. The $^{1}H,^{1}H$ COSY of the monasfluore B spectrum established the connectivity from H-11 to H-13 and the coupling between H-9 and H-9a and also demonstrated the allylic coupling between H-11 and H-4.

The two and three bond proton−carbon connectivities of the building elements of the molecule were detected by the HMBC. The HMBC spectrum of monasfluore B (**Figure 5**) provided

its gross structure; key long-range $^{1}H,^{13}C$ correlations were observed from H-1 to C-3, C-4a, and C-6b; H-4 to C-3, C-5, C-6b, and C-11; H-5 to C-4, C-6a, and C-6b; 10-Me protons to C-6, C-6a, and C-9a; H-9 to C-6b, C-8, C-9a, and C-14; H-15 to C-14; H-9a to C-1, C-4a, C-6, C-6a, C-6b, C-9, and C-14; H-11 to C-4 and C-3; H-15 to C-14; and both H-11 and H-12 to C-3.

The HMQC spectrum of monasfluore B enabled assignment of the directly bonded C−H moieties. The results showed that H−C correlations signals were 0.85/13.98, 2.45–2.53/42.84, 3.06–3.12/42.84, 3.72–3.75/56.56, 3.85–3.88/42.96, 5.41/106.49, 5.95/106.67, 5.98/122.83, 6.50–6.56/135.07, and 7.35/146.78, respectively. The 5.95/106.67 cross-peak proves that the HC-5 methine is connected to the C-4a atom of the 4*H*-pyran ring, and the 7.35/191.22 correlation shows that the C-6 keto group is attached to C-6a in the same ring. From the HMBC response between the singlet methyl at δ 1.58 and the C-6 signal, we can conclude that these protons are located in three bonds distant from C-6. The H_3C-10 protons correlate with one methine carbon atom, and in this way, the latter can be assigned to C-9a. The doublet at δ 3.72–3.75 (H-9) correlates with the carbon atoms C-5 and C-9a and the lactone carbonyl C-8. This allows for the determination of the position of the lactone ring. On the basis of the cross-peaks between H-9/C (14)═O, the position of the C_7H_{15}═O moiety is also obvious.

The structure of monasfluore B was therefore 9-octanoyl-3-(2-propenyl)-6a-methyl-9,9a-dihydro-6-furo[2,3]isochromene-6,8(6a)-dione (**Figure 6**). This is an azaphilone structure, in which an eight-carbon β-keto acid is condensed with the carbonyl group at carbon 9a of the chromophore instead of the typical condensation at position 6.

Monasfluore A, an analogue of monasfluore B, revealed a molecular weight of 356. This is 28 units less than that of monasfluore B, suggesting two missing methylene groups. The identity of the UV and fluorescence spectrum with that of monasfluore B indicates the presence of the same chromophore. The NMR data confirmed that monasfluore A is a lower homologue of monasfluore B, with the less lipophilic hexanoyl instead of octanoyl side chain (**Figure 6**). This results in a less lipophilic character of monasfluore A and is consistent with the shorter retention time on RP-HPLC. The pairwise formation of metabolites with a hexanoyl or octanoyl substituent is well-known from the pigments of *Monascus*.

In general, this is an azaphilone structure in which the monasfluore A and monasfluore B have a related γ-lactone attached to a cyclohexenone ring and $H_3CCRR-O$ group. A similar $H_3CCRR-O$ group is found in the *Monascus* pigments rubropunctamin, monascorubramin, monascin, rubropunctatin, ankaflavin, and monascorubrin. The ring skeletons of monasfluore A and monasfluore B and the main pigments of *Monascus* are remarkably similar; however, the eight-carbon β-keto acid is condensed with the carbonyl group at carbon 9a of the chromophore instead of the typical condensation at position 6.

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