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Cytotoxic Dehydromonacolins from Red Yeast Rice

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Supporting Information

ABSTRACT: Two new dehydromonacolins (**1** and **3**), together with nine known monacolins (**4**−**12**), were isolated from red yeast rice. Compounds **4**−**6** were isolated from a natural resource for the first time. Their structures were elucidated by means of NMR and mass spectroscopic analyses. The structure of dehydromonacolin N (**1**) was further confirmed by its semisynthesis from monacolin K (lovastatin) (**11**). Dehydromonacolin J (**2**), an intermediate in the semisynthesis of **1**, was obtained as a new dehydromonacolin. The structure of dehydromonacolin L (**3**) was also confirmed by an elimination reaction of monacolin L (**12**). Compound **1**, possessing a C2 side chain, is unprecedented in the natural monacolin family and exhibited moderate cytotoxic activity against Hep G2, Caco-2, and MCF-7 cancer cell lines. Dehydromonacolin K (**8**) demonstrated the most potent cytotoxicity to all three of these cell lines. The structure−activity relationship of natural and synthesized monacolins was discussed. This is the first report on the cytotoxic effects of dehydromonacolins.

KEYWORDS: *Monascus, red yeast rice, monacolins, cytotoxicity*

INTRODUCTION

Red yeast rice, produced from the fermentation of steamed rice using the fungus *Monascus purpureus*,[1] has been applied as food and medicine for improving digestion and blood circulation in China for thousands of years.[2] Recently, much attention has been focused on the family of bioactive substances named monacolins in red yeast rice, because they are inhibitors of HMG-CoA reductase, have therapeutic effects on lipid profiles of hypercholesterolemic patients,[3] and showed anticancer activities against colorectal cancer,[4] prostate cancer,[5] and breast cancer[6] etc. Since monacolin K was first reported from *Monascus ruber* by Endo in 1979[7] and, independently, by Alberts from *Aspergillus terreus*,[8] a series of monacolin compounds have been found. Monacolin J and monacolin L were isolated and reported in 1985,[9] and then dihydromonacolin L and monacolin X were found.[10] After that, monacolin M was disclosed from *M. ruber*.[11] Seven monacolins were isolated from red yeast rice.[1] Although the content of monacolins in red yeast rice is low (ca. 0.4%),[1] it is still a potential resource for discovering new natural monacolins.

In the course of our investigation on monacolins in red yeast rice, two new dehydromonacolins (**1** and **3**), together with nine known monacolins (**4**−**12**), were isolated from red yeast rice. Compounds **4**−**6** were isolated from a natural resource for the first time. Semisynthesis from monacolin K (lovastatin) (**11**) confirmed the structure of dehydromonacolin N (**1**) and generated a new compound, dehydromonacolin J (**2**), as an intermediate. The structure of dehydromonacolin L (**3**) was also confirmed by an elimination reaction of monacolin L (**12**). Some of the isolates were evaluated for their cytotoxicity against Hep G2, Caco-2, and MCF-7 cancer cell lines. Herein we report the isolation, structural elucidation, and cytotoxic activities of these monacolins.

MATERIALS AND METHODS

General Experimental Procedures. Optical rotations were obtained on a JASCO P-1010 polarimeter. UV spectra were obtained on a JASCO V-530 UV−vis spectrophotometer. NMR spectroscopy was performed on a Bruker Avance-III NMR spectrometer with tetramethylsilane (TMS) as an internal standard; chemical shifts (δ) are reported in parts per million and coupling constants (J) in hertz. Ultraperformance liquid chromatography (UPLC)-HRESIMS was performed on Waters Acquity ultraperformance liquid chromatography (UPLC) system (Waters Corp., Milford, MA) with a photodiode array (PDA) detector, hyphenated to a Bruker MicrO-TOFQ system with an electrospray ionization (ESI) interface (Bruker Daltonics, Bremen, Germany). Column chromatography (CC) was performed with silica gel (40−63 μm, Grace, USA) and Bondapak C_{18} (37−55 μm, Waters). TLC was performed on precoated Kieselgel 60 F_{254} plates (0.2 mm thick, Merk KGaA); detection was by ultraviolet (UV) illumination and by heating after spraying with 10% H_2SO_4 reagent. Semipreparative HPLC was carried out on a PerkinElmer series 200 separation system, with an Alltima C_{18} (250 × 22 mm, 10 μm) semipreparative column.

Reagents. Analytical grade EtOAc, petroleum ether, $CHCl_3$, MeOH, and HPLC grade MeCN were purchased from Anaqua Chemicals Supply (USA). HCl, $NaHCO_3$, and Na_2SO_4 were obtained from BDH (Auckland, New Zealand). Triethylamine and methanesulfonyl chloride were purchased from International Laboratory (USA). Toluene was obtained from Labscan Asia (Bangkok, Thailand). LiOH, acetic anhydride, and 4-dimethylaminopyridine (DMAP) were purchased from Sigma-Aldrich (St. Louis, MO).

Material. Red yeast rice powder was purchased from Zhejiang Sanhe Bio-Tech Co. Ltd. (Quzhou, China) in March 2010. This commercial material was made by fermenting the fungus *M. purpureus*

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SW1008 on steamed rice. A voucher specimen was deposited in the School of Chinese Medicine, Hong Kong Baptist University.

Extraction and Isolation. Red yeast rice powder (2.0 kg) was extracted with EtOAc (20 L × 3) at room temperature under sonication. The extract was combined and evaporated under reduced pressure to afford a brownish residue (ca. 150 g). The residue was subjected to a silica gel CC eluted with petroleum ether/$CHCl_3$ (100:0→0:100) and then $CHCl_3$/MeOH (100:0→65:35) to obtain 16 major fractions (fractions 1−16). Fraction 6 (42 g) was chromatographed on silica gel eluted with petroleum ether/EtOAc (100:0→0:100) to give 13 fractions (fraction 6-1−6-13). Fraction 6-7 (4 g) was subjected to a silica gel CC eluted with petroleum ether/EtOAc (95:5→50:50) and then ODS eluted with MeOH/H_2O (50:50→80:20) to give compounds **3** (1 mg) and **4** (12 mg). Fraction 6-8 (300 mg) was subjected to CC on ODS eluted with MeOH/H_2O (60:40→90:10) to give compound **7** (10 mg). Fraction 6-12 (1.5 g) was subjected to a silica gel CC with $CHCl_3$/EtOAc (100:0→80:20) and then ODS eluted with MeOH/H_2O (50:50→90:10) to give compounds **1** (1 mg), **5** (20 mg), and **8** (3 mg). Fraction 6-13 (700 mg) was subjected to CC on ODS eluted with MeOH/H_2O (50:50→80:20) and finally isolated on semipreparative HPLC eluted with CH_3CN/H_2O (70:30) to give compounds **6** (5 mg), **10** (4 mg), and **9** (3 mg). Fraction 8 (23 g) was chromatographed on silica gel eluted with $CHCl_3$/MeOH (100:0→90:10) to give compound **11** (6 g). Fraction 9 (10 g) was subjected to CC on silica gel eluted with petroleum ether/EtOAc (100:0→20:80) and then purified on semipreparative HPLC eluted with CH_3CN/H_2O (70:30) to give compound **12** (70 mg).

Synthesis of Compound 1. Monacolin K (**11**) (500 mg, 1.238 mmol) was dissolved in 45 mL of CH_2Cl_2. To this solution was added 525.7 μL (378 mg, 3.77 mmol, 3 equiv) of triethylamine, followed by 145.4 μL (212 mg, 1.863 mmol, 1.5 equiv) of methanesulfonyl chloride, and the reaction was stirred at room temperature for 1 h. The reaction was diluted with CH_2Cl_2 and extracted with 0.1 M HCl (1 × 150 mL) and saturated $NaHCO_3$ (1 × 150 mL), washed with H_2O (3 × 150 mL), dried with anhydrous Na_2SO_4, and filtered to give dehydromonacolin K (**8**) (460 mg).

Compound **8** (300 mg) was suspended in 10 mL of aqueous LiOH (1 M) and refluxed overnight. The reaction was cooled to room temperature, acidified with 2 M HCl, and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic extracts were dried with anhydrous Na_2SO_4, filtered, and evaporated to give 343 mg of crude residue, which was refluxed in 10 mL of toluene for 4 h. After cooling to room temperature, the solvent was evaporated and the residue (308 mg) was purified by silica gel CC (1% MeOH in $CHCl_3$) to give compound **2** (20 mg).

Compound **2** (5 mg, 0.016 mmol) was dissolved in 500 μL of pyridine. To this solution was added 200 μL (200 mg, 1.96 mmol, 100 equiv) of acetic anhydride, followed by 1 mg (0.008 mmol, 0.5 equiv) of 4-dimethylaminopyridine (DMAP). After the reaction was stirred at room temperature overnight, the reaction was diluted with 10 mL of H_2O and extracted with EtOAc (3 × 10 mL). The organic layer was evaporated to give compound **1** (6 mg).

Synthesis of Compound 3. Monacolin L (**12**) (10 mg, 0.033 mmol) was dissolved in 1.2 mL of CH_2Cl_2. To this solution was added 14.0 μL (10 mg, 0.010 mmol, 3 equiv) of triethylamine, followed by 3.9 μL (5.7 mg, 0.050 mmol, 1.5 equiv) of methanesulfonyl chloride, and the reaction was stirred at room temperature for 1 h. The reaction was diluted with CH_2Cl_2 and extracted with 0.1 M HCl (1 × 5 mL) and saturated $NaHCO_3$ (1 × 5 mL), washed with H_2O (3 × 5 mL), dried with anhydrous Na_2SO_4, and filtered to give dehydromonacolin L (**3**) (6 mg).

Cell Culture and Cytotoxicity Assay of Compounds 1−3, 8, and 11. Exponentially growing cells were plated in a 96-well microplate (Becton, Dickinson and Co.) at densities of 8000 cells (Hep G2), 5000 cells (Caco-2), and 2000 cells (MCF-7) per well in 100 μL of culture medium, which were allowed to adhere overnight before treatment. Compounds of different concentrations were then added, and the cells were incubated for another 48 h (Hep G2) or 72 h (Caco-2 and MCF-7) at 37 °C in a humidified incubator with 5% CO_2. Monacolin K was used as the positive control. Cytotoxicity was evaluated with the colorimetric MTT assay. Briefly, MTT solution (10 μL per well, 5 mg/mL solution) was added to each well and incubated for 4 h at 37 °C. One hundred microliters of stop solution (10% SDS in 0.01 N HCl) was then added to each well, and they were kept overnight at room temperature. The optical densities of the resulting solutions were colorimetrically determined at 570 nm using a microplate reader. Dose−response curves were generated, and results were expressed as IC_{50} values in micromolar. Tests were performed in triplicate and all experiments repeated three times (n = 3).

Dehydromonacolin N (1). **1** was obtained as a colorless oil: $[\alpha]^{22}_D$= +30.7 (c 0.30, MeOH); UV (MeOH) λ_{max} (log ε) 230 (3.86), 238 (3.82), 246 (3.72) nm; ^{1}H and ^{13}C NMR, see Tables 1 and 2 and the Supporting Information; HR-ESI-MS, 345.2063 ([M + H]$^+$, $C_{21}H_{29}O_4$, calcd 345.2060).

Table 1. ^{1}H NMR (400 MHz, $CDCl_3$) Data for Compounds 1−3 (δ in Parts per Million)[a]

position	1	2	3
1	1.73 (m)	1.82	1.41
2	2.39	2.37	2.30
3	5.79 (dd, J = 9.6, 6.1 Hz)	5.79 (dd, J = 9.6, 6.0 Hz)	5.72 (dd, J = 9.6, 6.4 Hz)
4	5.99 (d, J = 9.6 Hz)	5.99 (d, J = 9.6 Hz)	5.91 (d, J = 9.6 Hz)
5	5.53 (m)	5.55 (m)	5.43 (m)
6	2.45	2.46	2.33
7	1.97	1.88	1.71
	1.91	1.88	1.59
8	5.37 (dd, J = 5.9, 3.4 Hz)	4.24 (dd, J = 6.4, 3.2 Hz)	1.17 (d, J = 12.4 Hz)
			1.77
8a	2.24	2.16	2.03
9	0.90 (d, J = 7.0 Hz)	0.91 (d, J = 7.0 Hz)	0.89 (d, J = 7.0 Hz)
10	1.07 (d, J = 7.0 Hz)	1.19 (d, J = 7.0 Hz)	0.99 (d, J = 7.0 Hz)
2′	6.01 (ddd, J = 9.6, 2.6, 0.9 Hz)	6.02 (dt, J = 9.6, 1.6 Hz)	6.03 (dt, J = 9.6, 1.6 Hz)
3′	6.87 (m)	6.89 (m)	6.89 (m)
4′	2.34	2.36	2.35
	2.28	2.36	2.35
5′	4.38 (m)	4.43 (m)	4.43 (m)
6′	1.89	1.92	1.88
	1.41	1.56	1.52
7′	1.53	1.80	1.76
	1.38	1.52	1.40
2″	2.03 (s)		

[a]Overlapped signals are reported without designating multiplicity.

Dehydromonacolin J (2). **2** was obtained as a colorless oil: $[\alpha]^{22}_D$= +82.7 (c 0.75, MeOH); UV (MeOH) λ_{max} (log ε) 230 (3.89), 238 (3.89), 246 (3.77) nm; ^{1}H and ^{13}C NMR, see Tables 1 and 2 and the Supporting Information; HR-ESI-MS, 303.1959 ([M + H]$^+$, $C_{19}H_{27}O_3$, calcd 303.1954).

Dehydromonacolin L (3). **3** was obtained as a colorless oil: $[\alpha]^{22}_D$= +3.5 (c 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 230 (2.76), 238 (2.60), 246 (2.52) nm; ^{1}H and ^{13}C NMR, see Tables 1 and 2 and the Supporting Information; HR-ESI-MS, 287.2011 ([M + H]$^+$, $C_{19}H_{27}O_2$, calcd 287.2005).

RESULTS AND DISCUSSION

Repeated column chromatography of the ethyl acetate extract of red yeast rice afforded two new dehydromonacolins (**1** and **3**), as well as nine known monacolins (**4**−**12**). The known compounds were identified as α,β-dehydrodihydromonacolin L

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Table 2. ^{13}C NMR (100 MHz, $CDCl_3$) Data for Compounds 1−3

position	1	2	3
1	32.6	36.5	41.9
2	29.9	30.9	31.4
3	133.3	133.7	132.9
4	128.5	128.6	128.3
4a	131.8	131.4	136.5
5	129.8	130.2	130.5
6	27.6	27.5	28.7
7	32.4	36.0	29.3
8	68.2	65.4	22.5
8a	37.3	38.9	34.9
9	14.1	14.1	13.8
10	22.8	24.0	21.2
1′	164.5	164.8	164.5
2′	121.6	121.5	121.4
3′	145.0	145.3	144.9
4′	29.7	29.9	29.6
5′	78.1	78.6	28.2
6′	30.9	32.3	32.4
7′	23.8	24.2	24.4
1″	171.3		
2″	21.5		

(**4**),[12] the ethyl ester of monacolin K (**5**),[13] (1*S*,2*S*,4a*R*,6*S*,8*S*,8a*S*,3′*S*,5′*R*,2″*S*)-methyl 1,2,4a,5,6,7,8,8a-octahydro-3′,5′-dihydroxy-2,6-dimethyl-8-[(2-methyl-1-oxobutyl)oxy]-1-naphthaleneheptanoate (**6**),[14] *α*,*β*-dehydrodihydromonacolin K (**7**),[1] dehydromonacolin K (**8**),[1] the methyl ester of the hydroxyl acid form of monacolin K (**9**),[1] dihydromonacolin K (**10**),[1] monacolin K (**11**),[1] and monacolin L (**12**)[9] on the basis of comparison of their NMR and HR-ESI-MS data with those reported in the literature. It is worthwhile to point out that compounds **4**−**6** were isolated from a natural resource for the first time.

Dehydromonacolin N (**1**), obtained as a colorless oil, exhibited the molecular ion at *m*/*z* 345.2063 $[M + H]^+$ in the HR-ESI-MS spectrum, which was consistent with the molecular formula $C_{21}H_{28}O_4$. The UV absorptions of **1** at λ_{max} 230 (3.86), 238 (3.82), and 246 (3.72) nm, indicating the presence of a conjugated double bond at the naphthalene moiety, are typical triplet absorptions in the UV spectra of monacolins.[1] The ^1H NMR spectrum of **1** displayed three methyl signals at δ_H 0.90 (3H, d, *J* = 7.0 Hz, H-9), 1.07 (3H, d, *J* = 7.0 Hz, H-10), and 2.03 (3H, s, H-2″) and five olefin signals at δ_H 5.79 (1H, dd, *J* = 9.6, 6.1 Hz, H-3), 5.99 (1H, d, *J* = 9.6 Hz, H-4), 5.53 (1H, m, H-5), 6.01 (1H, ddd, *J* = 9.6, 2.6, 0.9 Hz, H-2′), and 6.87 (1H, m, H-3′). Comparison of the overall ^1H NMR data revealed high similarities between **1** and dehydromonacolin K (**8**).[1] The only difference was that the methyl signal at δ_H 2.03 (3H, s, H-2″) in the side chain of **1** was replaced with signals for a *sec*-butyl group in **8**, in agreement with the molecular weight of **1** ($C_{21}H_{28}O_4$) that was 42 (C_3H_6) mass units less than that of **8** ($C_{24}H_{34}O_4$). Furthermore, the ^1H NMR data of **1** closely resembled those of the synthesized monacolin possessing a C2 side chain,[15] with the exception that signals of the oxygenated methine [δ_H 4.39 (1H, m, H-3′)] and the vicinal methylene [δ_H 2.67 (2H, d, *J* = 4.0 Hz, H-2′)] in the known compound disappeared, and a pair of olefinic proton signals at δ_H 6.87 (1H, m, H-3′) and δ_H 6.01 (1H, ddd, *J* = 9.6, 2.6, 0.9 Hz, H-2′) were observed in **1**. Therefore, it was elucidated to be the dehydration derivative of the known compound and named dehydromonacolin N. This elucidation was supported by the molecular weight difference of 18 (H_2O) mass units between **1** and the known compound. Assignment of all the proton signals (Table 1) and CH−CH linkage of **1** was confirmed by the ^1H−^1H COSY spectrum (Figure 1).

A transformation from monacolin K (**11**) to compound **1** was undertaken as described in Scheme 1 to confirm the chemical structure of compound **1**.

Elimination from the methanesulfonates of monacolin K (**11**) gave dehydromonacolin K (**8**). Basic hydrolysis of **8** by reflux overnight with lithium hydroxide solution and then relactonization by reflux in toluene afforded dehydromonacolin J (**2**). The ^1H (Table 1) and ^{13}C (Table 2) NMR spectra of **2** showed a close relationship with those of **8**,[1] except for the difference at the ester-linked side chain. The disappearance of an ester carbonyl signal at δ_C 176.9 (C-1″), as well as the signals for a *sec*-butyl group at the side chain in **2**, indicated the loss of the side chain. It was supported by its molecular weight ($C_{19}H_{26}O_3$), which is 84 (C_5H_8O) mass units less than that of **8** ($C_{24}H_{34}O_4$). The upfield shift of H-8 from δ_H 5.39 to δ_H 4.24 and of C-8 from δ_C 67.8 to δ_C 65.4 also confirmed that the ester-linked side chain at C-8 in **8** was replaced by the hydroxyl in **2**. Therefore, compound **2** was elucidated as a new dehydromonacolin and named dehydromonacolin J, bearing the structure as illustrated in Figure 1.

Finally, acetylation of **2** yielded a new compound, which was identified to be **1** after comparison of the retention time (9.2 min) in UPLC with mobile phase using A (0.1% formic acid in H_2O; 0→12 min, 80%→20%) and B (0.1% formic acid in CH_3CN; 0→12 min, 20%→80%), accurate molecular mass, and ^1H NMR data with that isolated from red yeast rice. The ^{13}C NMR (Table 2) spectrum of synthesized **1** showed 21 carbon signals, including 3 methyl, 4 methylene, 11 methane, and 3 quaternary carbons. It also closely resembled that of dehydromonacolin K (**8**),[1] with the exception of a difference at the ester-linked side chain. A methyl signal at δ_C 21.5 (C-2″) appeared in **1** instead of signals for the *sec*-butyl group in **8**, further confirming the structural elucidation of **1**.

On the basis of the above evidence, the structure of **1** was confirmed as demonstrated in Figure 1. This is the first example bearing the acetyl group in the natural monacolin family.

Dehydromonacolin L (**3**) was obtained as a colorless oil and exhibited the molecular ion at *m*/*z* 287.2011 $[M + H]^+$ in the HR-ESI-MS spectrum, which was consistent with the molecular formula $C_{19}H_{26}O_2$. The UV absorbance at λ_{max} 230 (2.76), 238 (2.60), and 246 (2.52) nm is identical to that of **2**, suggesting they have the same skeleton. The ^1H NMR spectroscopic data of **3** were almost identical to those of **2**, except that H-8 observed at δ_H 5.37 in **2** shifted to the upfield region (δ_H 1.17) in **3**, suggesting that the hydroxyl at C-8 in **2** was absent in **3**. This change was consistent with the observed molecular weight difference of 16 mass units between **2** and **3**. Furthermore, the ^1H NMR data of **3** were closely related to those of monacolin L (**12**),[9] except that signals of the oxygenated methine [δ_H 4.38 (1H, m, H-3′)] and the vicinal methylene [δ_H 1.75 (1H, m, H-2′$_a$), δ 1.98 (1H, m, H-2′$_b$)] in monacolin L disappeared, and a pair of olefinic proton signals at δ 6.89 (m, H-3′) and δ 6.03 (dt, *J* = 9.6, 1.6 Hz, H-2′) were observed in **3**. Therefore, it was elucidated to be the dehydration derivative of monacolin L (**12**) and named dehydromonacolin L. This elucidation was supported by the molecular weight difference of 18 (H_2O) mass units between **3** and monacolin L. Assignment of all the

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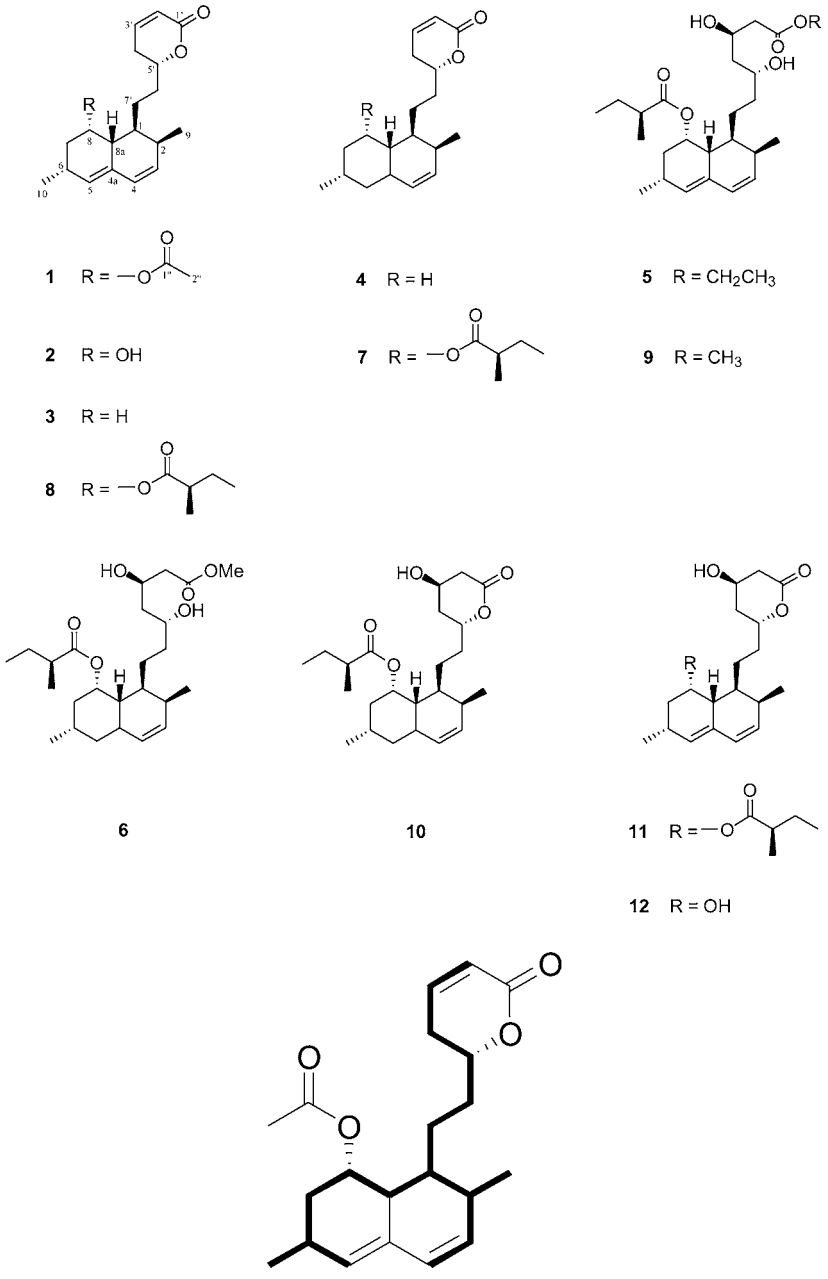

Figure 1. $^{1}H-^{1}H$ COSY correlations in compound **1**.

proton signals (Table 1) and CH−CH linkage of **3** was confirmed by the $^{1}H-^{1}H$ COSY spectrum (Figure 2).

A transformation from monacolin L (**12**) to compound **3** was undertaken as described in Scheme 2 to confirm the chemical structure of compound **3**. Elimination from the methanesulfonates of monacolin L (**12**) yielded a compound that was identified to be **3** after comparison of the retention time (11.0 min) in UPLC with mobile phase using A (0.1% formic acid in H_2O; 0→12 min, 80%→20%) and B (0.1% formic acid in CH_3CN; 0→12 min, 20%→80%), accurate molecular mass, and ^{1}H NMR data with those isolated from red yeast rice. The ^{13}C NMR (Table 2) spectrum of synthesized **3** showed 19 carbon signals, including 2 methyl, 5 methylene, 10 methane, and 2 quaternary carbons, which closely resemble those of dehydromonacolin J (**2**), except that a methylene signal at δ_C 22.5 (C-8) appeared in **3** instead of an oxygenated methane signal [δ_C 65.4 (C-8)] in **2**. This further confirmed the absence of hydroxyl at the C-8 position of **3**. On the basis of the above evidence, the structure of **3** was identified as demonstrated in Figure 2.

Monacolins are gaining popularity as anticancer agents against various cancers including colorectal cancer, skin cancer (melanoma), prostate cancer, and breast cancer. Their antitumor effects maybe due to inhibition of cell proliferation, promotion of apoptosis, inhibition of angiogenesis, prevention of metastasis, improvement of immunity, or possibly the targeting of the CSC population.[16] However, the cytotoxicity of dehydromonacolins remains unknown.

In this study, we focused on the preliminary screening of dehydromonacolins for their cytotoxicity. All four of these dehydromonacolins (**1**−**3** and **8**) obtained in our research share the same structure with only difference in the length of side chains, so we wish to see the relationship between the cytotoxity and the side chain. Monacolin K (**11**), the

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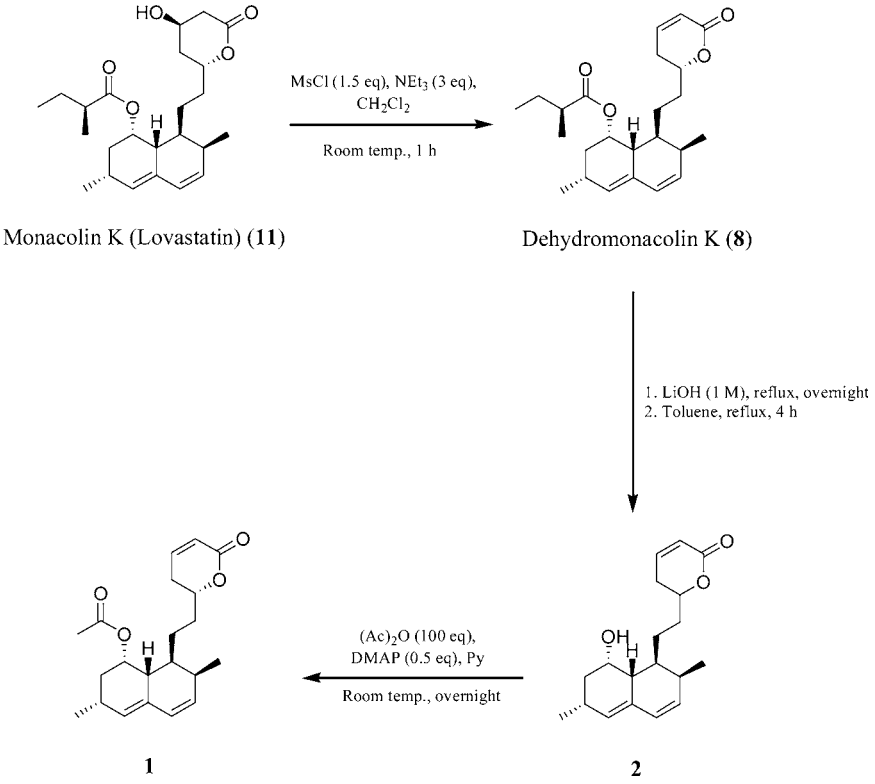
Scheme 1. Semisynthesis of 1 from Monacolin K (11)



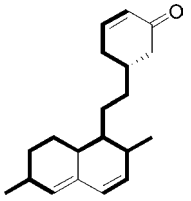
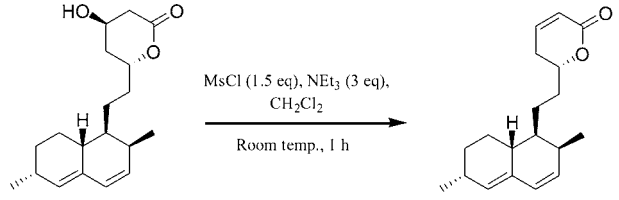


Figure 2. $^{1}H-^{1}H$ COSY correlations in compound **3**.

Scheme 2. Semisynthesis of 3 from Monacolin L (12)



cytotoxicity[17] and anticancer effects[18,19] of which have been reported in many papers, was used as a positive control in the cytotoxicity evaluation. They were evaluated for their cytotoxicity against Hep G2, Caco-2, and MCF-7 cancer cell lines using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

Among the four dehydromonacolins (**1**−**3** and **8**), compound **8** with a C5 side chain at C-8 exhibited the most potent cytotoxic activity, with IC_{50} values ranging from 3.95 ± 0.48 to 10.83 ± 0.14 μM (Table 3). Compound **1**, possessing a C2 side chain, was less cytotoxic than **8**, showing moderate cytotoxicity. Compound **2**, with a hydroxyl group at the side chain, showed weak cytotoxicity against both Hep G2 and Caco-2 cancer cell lines, with IC_{50} > 100 μM. Compound **3**, without a side chain, exhibited weak cytotoxicity toward all three cell lines used, with IC_{50} > 100 μM. The MCF-7 cell line seems to be more sensitive than the Hep G2 and Caco-2 cell lines for all of the compounds tested. From a structure−activity point of view, the cytotoxicity increased as the side chain lengthened (compare **8** with **1**−**3**), thus suggesting that extension of the alkyl function group, which in turn resulted in the increased lipophilicity, may contribute to the activity. Besides, it was found that dehydromonacolin K (**8**) had much stronger cytotoxicity than monacolin K (**11**) toward the cancer cell lines, which was in agreement with the studies revealing that more lipophilic monacolins could exert direct anticancer activity in vitro because their lipophilicity allowed them to directly permeate the cell membrane and affect cell proliferation, survival, and motility.[20]

This study demonstrated that dehydromonacolins with extended alkyl side chain exerted more potent cytotoxic effects toward cancer cells. This is the first report on the cytotoxic effects of dehydromonacolins.

Table 3. IC_{50} Values (n = 3) of Compounds 1−3, 8, and 11 for Three Cancer Cell Lines

	IC_{50} values (μM ± SD) for cell line[a]		
compound	Hep G2	Caco-2	MCF-7
dehydromonacolin N (**1**)	51.84 ± 4.44	56.98 ± 8.41	11.78 ± 0.65
dehydromonacolin J (**2**)	>100	>100	51.95 ± 1.68
dehydromonacolin L (**3**)	>100	>100	>100
dehydromonacolin K (**8**)	7.62 ± 0.97	10.83 ± 0.14	3.95 ± 0.48
Mmonacolin K (**11**)	57.62 ± 2.22	48.55 ± 3.18	27.82 ± 1.67

[a]Cell lines: Hep G2, human liver cancer; Caco-2, human colon cancer; MCF-7, human breast cancer.

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■ ASSOCIATED CONTENT

Supporting Information

1D and 2D NMR spectra of compounds **1**−**3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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