A new monacolin analogue from Xuezhikang capsule

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Abstract: Xuezhikang capsule (ethanol extract of red yeast rice) which produced by Beijing WBL Peking University Biotech Co., Ltd., is a traditional Chinese medication with 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibiting activity. Clinical trials indicated that Xuezhikang with lipid-lowering action could reduce the risk of cardiac events and total mortality of Chinese coronary heart disease patients. To exactly explain the clinical features of Xuezhikang, we undertook a complete study of the chemical constituents of Xuezhikang. This study resulted in the isolation of a new monacolin analogue, named α, β-dehydromonacolin L (**1**), along with two known compounds: monacolin L (**2**) and 3-(2, 6-dimethyl-1, 2, 4a, 5, 6, 7, 8, 8a-octahydronaphthalen-1-yl)propanoic acid (**3**). The chemical structures were determined by extensive 1D and 2D NMR and MS spectroscopic analysis.

Key words: monacolin; Xuezhikang; red yeast rice

CLC number: R284.1　　**Document code**: A　　**Article ID**: 0513-4870 (2011) 05-0564-04

血脂康胶囊中的一个新莫纳克林类似物

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摘要: 血脂康胶囊 (红曲醇提取物) 是北京北大维信生物科技有限公司自主研究的特制红曲霉发酵制成的调脂中药。通过血脂康调整血脂的临床观察, 发现血脂康可降低各种冠心病相关事件的危险性及各种死亡率。为了解释血脂康的许多临床表现, 对血脂康进行系统的成分研究。通过成分分析研究, 从血脂康中分离纯化了一个新的莫纳克林类化合物, 命名为 α, β-dehydromonacolin L (**1**) 及两个已知的莫纳克类化合物 monacolin L (**2**) 和 3-(2, 6-dimethyl-1, 2, 4a, 5, 6, 7, 8, 8a-octahydronaphthalen-1-yl)propanoic acid (**3**)。结合核磁共振和质谱分析确定了化合物的化学结构。

关键词: 莫纳克林; 血脂康; 红曲

Xuezhikang capsule, an ethanol extract of red yeast rice produced by Beijing WBL Peking University Biotech Co., Ltd., is a traditional Chinese medication with HMG-CoA reductase inhibiting activity, and contains a family of naturally occurring statins (monacolins), one of which is lovastatin (monacolin K, **4**, Figure 1), in addition to unsaturated fatty acids and other substances. Recent studies have demonstrated that Xuezhikang could effectively modify lipid profile (such as reducing the levels of total cholesterol, low-density lipoprotein cholesterol, and triglyceride), and reduce cardiovascular events[1−3]. Moreover, clinical trials indicated that lipid-lowering therapy with Xuezhikang could reduce the risk of cardiac events and total mortality of Chinese coronary heart disease patients[4]. To exactly explain the clinical features of Xuezhikang, we undertook a complete study of the

Received 2011-02-16.
Project supported by the National Important Special Foundation of the New Drug Development (2008ZX09202-007).
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chemical constituents of Xuezhikang. This paper reports the isolation and structure elucidation of a new monacolin analogue, which named as *α*, *β*-dehydromonacolin L (**1**), as well as the known compound monacolin L (**2**) and 3-(2, 6-dimethyl-1, 2, 4a, 5, 6, 7, 8, 8a-octahydronaphthalen-1-yl)propanoic acid (**3**) (Figure 1).

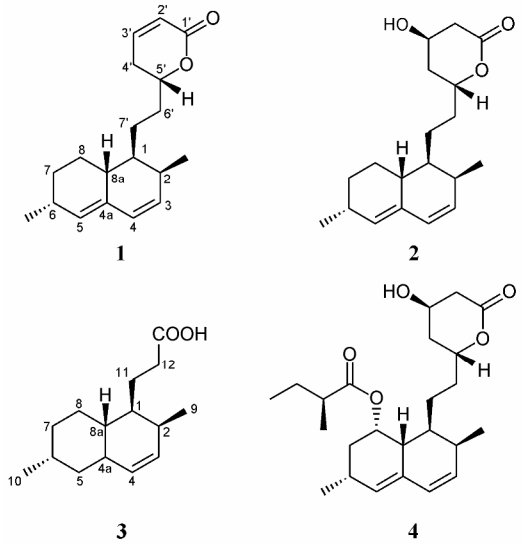

Figure 1 The chemical structures of compounds **1** − **4**

Results and discussion

Three monacolins were isolated from *n*-hexane extract of Xuezhikang capsule in sufficiently pure form for identification.

Compound **1** was obtained as colorless gum, $[\alpha]_D^{25}$ +36.63 (*c* 0.636, CH_2Cl_2); The HR-ESI-MS of **1** showed a molecular ion peak at *m/z* 287.198 5 $[M+H]^+$ (calcd. 287.200 6) indicating a molecular formula $C_{19}H_{26}O_2$ that required 7 degrees of unsaturation. Besides one carbonyl and three double bonds, there must be three more rings in the molecule. UV absorbance at 232, 240, and 249 nm was similar to those of monacolin K[5]. The ^{1}H NMR spectrum (Table 1) showed five olefinic protons (*δ* 5.46 (1H, br s), 5.75 (1H, dd, *J* = 6.0, 9.5 Hz), 5.94 (1H, d, *J* = 10.0 Hz), 6.06 (1H, d, *J* = 10.0 Hz), 6.93 (1H, td, *J* = 4.0, 10.0 Hz)), one oxygenated methine (*δ* 4.46 (1H, m)), and two methyl groups (*δ* 0.92 (3H, d, *J* = 7.0 Hz), 1.01 (3H, d, *J* = 7.0 Hz)). The ^{13}C NMR spectrum (Table 1) indicated a total of 19 carbons, and DEPT (Table 1) spectrum displayed the presence of two methyl groups (*δ* 13.9, 21.2), ten CH groups including one oxygenated methine (*δ* 78.3) and five sp^2 CH signals (*δ* 121.5, 128.4, 130.5, 133.0, 145.0), five CH_2 groups (*δ* 22.6, 24.4, 29.3, 29.6, 32.4), two quaternary carbon signals including one carbonyl carbon signal (*δ* 164.6) and one sp^2 carbon signal (*δ* 136.6). Aforementioned NMR data indicated that compound **1** is a monacolin analogue[5, 6]. Comparing the molecular formula and ^{13}C NMR data of compound **1** with those of monacolin L (compound **2**), it was found that compound **1** was absent of one molecular H_2O, and had one extra double bond and the chemical shift of C-2' and C-3' moved obviously toward downfield. This was confirmed further by the presence of two double-bond protons (*δ* 6.06 and 6.93) in the ^{1}H NMR. In the HMBC spectrum of **1** (Figure 2), the olefinic protons *δ* 6.06 and 6.93 showed correlations with carbon at 164.6 (C-1'). Thus, the double bond should locate at C-2' and C-3'. Additionally, the optical rotation at $[\alpha]_D^{25}$ +36.63 (*c* 0.636, CH_2Cl_2) is similar to that of monacolin K, whose optical rotation at $[\alpha]_D^{25}$ +307.6 (*c* 1, MeOH)[5], suggesting that the chiral carbons C-5', C-1, C-2, C-6, C-8a of compound **3** also had *R*, *S*, *S*, *R*, *R*-configurations. Thus, the structure of compound **1** was identified as (*R*)-6-(2-((1*S*, 2*S*, 6*R*, 8a*R*)-2, 6-dimethyl-1, 2, 6, 7, 8, 8a-hexahydronaphthalen-1-yl)ethyl)-5, 6-dihydropyran-2-one.

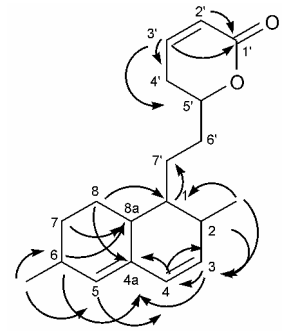

Figure 2 The key HMBC correlations of compound **1**

Compound **2** was obtained as colorless gum, $[\alpha]_D^{25}$ +164.16 (*c* 0.6, CH_2Cl_2/MeOH = 2∶1), structurally elucidated as the known compound, monacolin L[6].

Comound **3** was obtained as colorless needles, and has been previously obtained from the cultures of *Aspergillus nidulans* mutant with controlled lovastatin biosynthesis gene, and was structurally elucidated as the known compound, 3-(2, 6-dimethyl-1, 2, 4a, 5, 6, 7, 8, 8a-octahydronaphthalen-1-yl)propanoic acid[7].

Experimental

General NMR spectra were run on Bruker Avance III 600 MHz and Bruker Avance-500 MHz

spectrometers. EI-MS and HR-ESI-MS were measured on Bruker APEX II FT-ICR and Bruker microTOF-QII mass spectrometers. IR spectrum was recorded on a Nicolet Nexus-670 Fourier transform infrared spectrometer. UV spectra were measured on a Shimadzu UV2401PC UV/vis spectrophotometer. The optical rotation was measured on a Perkin Elmer 341LC polarimeter. Column chromatography (CC) was carried out using silica gel (200−300 mesh), and thin-layer chromatography (TLC) was performed on silica gel GF_{254} from Qingdao Haiyang Chemical Group Co., China. RP-C_{18} silica gel (YMC) was purchased from Greenherbs Science & Technology Development Co., Ltd., China. Sephadex LH-20 (Pharmacia) was purchased from H&E Co., Ltd., China. All organic solvents were of analytical purity and were purchased from Beijing Chemical Reagent Co., Ltd., China. HPLC was performed on a Shimadzu 6AD apparatus using 5C18-MS-II column (ODS, 250 mm × 10 mm) and monitored with a UV detector.

Extraction and isolation The powder content of Xuezhikang capsule (1 kg) was ultrasonically extracted 3 times with *n*-hexane, CH_2Cl_2, ethyl acetate, *n*-butanol and 70% MeOH successively. The *n*-hexane extract was then concentrated under reduced pressure to give a residue (84 g), 50 g of which was subjected to silica gel column chromatography eluted with a gradient mixture of petroleum ether-ethyl acetate (75∶25 to 0∶100) and ethyl acetate-MeOH (100∶0 to 50∶50) to yield 9 fractions (H1-H9). Fr. H2 (5.2 g) was further subjected to a silica gel column eluted with petroleum ether-ethyl acetate (85∶15) to yield 6 fractions (H2A-H2F). Fraction H2B (4.1 g) was chromatographed over a Sephadex LH-20 column (CH_2Cl_2-MeOH 1∶1), and afforded five subfractions (H2B1-H2B5). H2B5 was purified by reversed-phase semi-preparative HPLC and yielded compound **1** (10 mg). Fr. H5 (3.0 g) was further subjected to a Sephadex LH-20 column chromatography (CH_2Cl_2-MeOH 1∶1) to yield 8 fractions (H5A-H5H). Fraction H5E (370 mg) was chromatographed over C_{18} column to give 6 fractions (H5E1-H5E6). H5E3 was purified by reversed-phase semi-preparative HPLC and yielded compound **2** (50 mg). Fraction H5F was further purified on silica gel column to afford compound **3** (14 mg).

Identification

Compound 1 colorless gum, $[\alpha]_D^{25}$ +36.63 (*c* 0.636, CH_2Cl_2); HR ESI-MS *m/z*: 287.198 5 $[M+H]^+$ (calcd. 287.200 6); UV (CH_2Cl_2) λ_{max}: 232, 240, 249 nm; IR ν_{max} cm^{-1} (KBr) 2 926, 1 717; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data see Table 1.

Table 1 NMR spectral data of compound **1** ($CDCl_3$)

No.	^1H NMR	^{13}C NMR	HMBC (^1H to ^{13}C)
1	1.34 (1H, m)	28.7 (d)	C7'
2	2.07 (1H, m)	34.9 (d)	C3
3	5.75 (1H, dd, *J* = 6.0, 9.5 Hz)	133.0 (d)	C4, C4a
4	5.94 (1H, d, *J* = 10.0 Hz)	128.4 (d)	C3, C4a
4a		136.6 (s)	
5	5.46 (1H, br s)	130.5 (d)	C4
6	2.34 (1H, m)	31.5 (d)	C4a, C8a
7	1.44/1.73	24.4 (t)	C8a
8	1.20/1.80	22.6 (t)	C4a, C1
8a	1.44 (1H, m)	41.9 (d)	
1'		164.6 (s)	
2'	6.06 (1H, d, *J* = 10.0 Hz)	121.5 (d)	C1'
3'	6.93 (1H, td, *J* = 4.0, 10.0 Hz)	145.0 (d)	C5', C4', C1'
4'	2.38 (2H, m)	29.6 (t)	C2', C3', C1'
5'	4.46 (1H, m)	78.3 (d)	
6'	1.54 (1H, m), 1.91 (1H, m)	32.4 (t)	
7'	1.61 (1H, m), 1.71 (1H, m)	29.3 (t)	
2-Me	1.01 (3H, d, *J* = 7.0 Hz)	21.2 (q)	C1, C3
6-Me	0.92 (3H, d, *J* = 7.0 Hz)	13.9 (q)	C6, C5

Compound 2 colorless gum, $[\alpha]_D^{25}$ +164.16 (*c* 0.6, CH_2Cl_2∶MeOH = 1∶1), HR-ESI-MS *m/z*: 327.193 1 $[M+Na]^+$; UV (CH_3OH) λ_{max}: 232, 239, 247 nm. ^1H NMR (600 MHz, CD_3OD) δ: 5.87 (1H, m, H-4), 5.72 (1H, m, H-3), 5.39 (1H, m, H-5), 4.20 (1H, m, H-3'), 3.76 (1H, m, H-5'), 2.55 (1H, dd, *J* = 15.0, 4.8 Hz, H-2'ax), 2.44 (1H, dd, *J* = 15.0, 8.4 Hz, H-2'eq), 2.32 (1H, m, H-6), 2.31 (1H, m, H-2), 2.05 (1H, m, H-8a), 1.81 (1H, m, H-8 or 7), 1.78 (1H, m, H-4'eq), 1.73 (1H, m, H-6' or 7'), 1.65 (1H, m, H-7' or 6'), 1.62 (1H, m, H-4'ax), 1.60 (1H, m, H-7' or 6'), 1.53 (1H, m, H-7 or 8), 1.46 (1H, m, H-7 or 8), 1.40 (1H, m, H-1), 1.30 (1H, m, H-6' or 7'), 1.20 (1H, m, H-8 or 7), 0.99 (3H, dd, *J* = 7.2, 1.8 Hz, 2-Me), 0.90 (3H, dd, *J* = 7.2, 3.0 Hz, 6-Me); ^{13}C NMR (125 MHz, CD_3OD) δ: 43.7 (C-1), 30.2 (C-2), 14.4 (2-Me), 134.2 (C-3), 129.7 (C-4), 138.4 (C-4a), 131.0 (C-5), 32.8 (C-6), 21.8 (6-Me), 35.9 (C-7 or 8), 23.8 (C-8 or 7), 36.5 (C-8a), 174.0 (C-1'), 43.2 (C-2'), 68.2 (C-3'), 30.7 (C-4'), 71.2 (C-5'), 26.0 (C-6' or 7'), 44.9 (C-7' or 6'). These data were in basic agreement

with those of monacolin L[6], thus compound **2** was elucidated as monacolin L.

Compound 3 colorless needle, EI-MS: *m/z* 236 $[M]^+$; UV (MeOH/CH_2Cl_2 = 1∶1) λ_{max}: 231, 239, 247 nm. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 11.48 (1H, s, -COOH), 5.59 (1H, m, H-3), 5.30 (1H, d, J = 9.6 Hz), 2.45 (1H, ddd, J = 15.6, 9.6, 4.8 Hz, H-2), 2.25 (2H, m, H-2 and H-12), 2.02 (1H, m, H-6), 1.94 (2H, m, H-4a and H-11), 1.59 (2H, m, H-7 and H-8), 1.53 (2H, m, H-7 and H-8a), 1.38 (1H, m, H-11), 1.27 (1H, td, J = 12.6, 4.8 Hz, H-5), 1.13 (1H, qd, J = 12.0, 3.6 Hz, H-8), 1.01 (1H, m, H-1), 0.99 (3H, d, J = 7.2 Hz, H-10), 0.85 (3H, d, J = 7.2 Hz, H-9); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 40.1 (C-1), 32.0 (C-2), 132.6 (C-3), 131.8 (C-4), 37.4 (C-4a), 39.1 (C-5), 27.7 (C-6), 32.4 (C-7), 23.8 (C-8), 41.2 (C-8a), 15.1 (C-9), 18.4 (C-10), 24.0 (C-11), 32.2 (C-12), 180.4 (-COOH). These data were in basic agreement with those of 3-(2, 6-dimethyl-1, 2, 4a, 5, 6, 7, 8, 8a-octahydronaphthalen-1-yl)propanoic acid[7], thus compound **3** was elucidated as 3-(2, 6-dimethyl-1, 2, 4a, 5, 6, 7, 8, 8a-octahydronaphthalen-1-yl)propanoic acid.

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