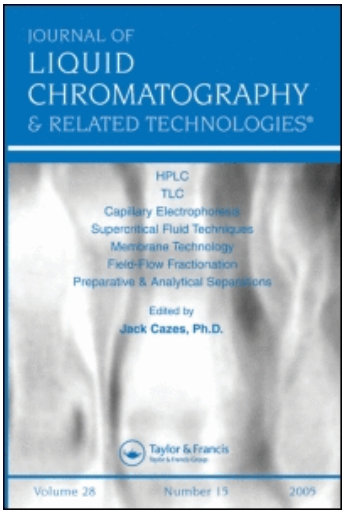

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Preparation of Mevinolinic Acid from *Monascus purpureus* Using High-Speed Countercurrent Chromatography (HSCCC)

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ABSTRACT

High-speed countercurrent chromatography (HSCCC) was applied for the separation of a crude *Monascus purpureus* extract. The separation was carried out using a two-phase solvent system composed of *n*-hexane/ethyl acetate/methanol/water (1/1/1/1, v/v) at a flow rate of 1.0 mL/min. From 250 mg of the alkaline treated extract the method yielded 40 mg of mevinolinic acid with a purity of 99% in each separation. The product

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was confirmed as mevinolinic acid by electrospray ionization multiple mass spectrometry (ESI-MS) and NMR analysis.

Key Words: *Monascus purpureus*; Mevinolinic acid; Preparation; High-speed countercurrent chromatography.

INTRODUCTION

Recent clinical observations clearly have shown that the extract of *Monascus purpureus* is effective for lowering blood-lipid level in animal models and in humans. The extract of *M. purpureus*, contains lovastatin and mevinolinic acid as the major bioactive components, which can lower cholesterol by inhibiting hydroxymethylglutaryl-coenzyme A reductase.[1–5] Also, both lovastatin and sodium salt of mevinolinic acid can inhibit bacterial luciferase in vitro.[6] The amount of mevinolinic acid in extract of *M. purpureus* is relatively lower than lovastatin.[7] Although the extract of *M. purpureus* contains numerous components, they are mostly degraded by an alkaline treatment, which also transforms lovastatin into mevinolinic acid as illustrated in Fig. 1. Therefore, it is more efficient to isolate mevinolinic acid from the alkaline-treated extract.

In the present paper, we describe the preparative separation of mevinolinic acid from the extract treated with alkali using high-speed countercurrent chromatography (HSCCC).[8,9]

EXPERIMENTAL

Reagent

n-Hexane, ethyl acetate, and methanol for HSCCC separation were of an analytical grade, while acetonitrile for HPLC analysis was of an HPLC grade.

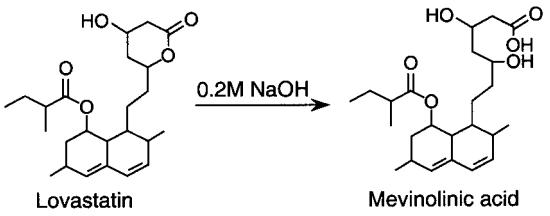

Figure 1. Transformation of lovastatin into mevinolinic acid in NaOH solution.

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Preparation of Mevinolinic Acid Extract from *Monascus purpureus*

A 100 g of dried powder of *M. purpureus* was soaked with 1.0 L of 0.1 M phosphoric acid for 1 hour at room temperature. The filtrate was extracted with an equal volume of ethyl acetate two times. The extracts were combined and evaporated in vacuum to yield 4.1 g of dried powder (lovastatin extract), which was dissolved in 500 mL of 0.2 M NaOH and then kept at 35°C for 30 min. This solution was extracted with benzene to remove impurities. The aqueous solution was acidified with 3 M phosphoric acid to pH 5 and extracted with an equal volume of ethyl acetate two times. The ethyl acetate extracts were combined and evaporated to dryness in vacuum yielding 1.5 g of mevinolinic acid extract. This sample was subjected to HSCCC separation.

HPLC Analysis of Mevinolinic Acid

HPLC analysis[10] was conducted on a Waters HPLC system composed of a quaternary pump with a degasser, a temperature-controlled column compartment, a variable wavelength detector, a manual injector, and a data processor. Separation of mevinolinic acid was performed on a Spherisorb BDS column at 30°C eluted with a mobile phase composed of acetonitrile—0.1% phosphoric acid (55/45, v/v) at a flow rate of 1 mL/min. The detection wavelength was 238 nm.

High-Speed Countercurrent Chromatography Separation of Mevinolinic Acid

A type-J HSCCC instrument was used for the separation of mevinolinic acid. It holds a separation column at a distance of 10 cm from the center of the centrifuge. The column revolves around the central axis of the centrifuge and simultaneously rotates about its own axis at the same angular velocity in the same direction. The column holder hub was 25 cm in length and 6 cm in OD. The multilayer coil was prepared by winding 50 m of 2.6 mm ID polytetrafluoroethylene (PTFE) tubing (Zeus Industrial Products, Raritan, NJ) onto the holder hub. The capacity of the column was 260 mL. The mobile phase was delivered using a Waters 510 HPLC pump (Millipore Corporation, Milford, MA). Mevinolinic acid was separated using a two-phase solvent system composed of *n*-hexane/ethyl acetate/methanol/water (1/1/1/1, v/v).

In each separation, the multiplayer coiled column was first entirely filled with the upper organic stationary phase. Then, the apparatus was rotated at a low speed of ca. 200 rpm and the sample solution containing 300 mg of mevinolinic acid extract in 20 mL of mobile phase was injected through the injection loop.

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Then, the aqueous mobile phase was pumped into the column at a flow rate of 2.0 mL/min while the rotation speed was increased to 800 rpm. The effluent was monitored at 238 nm and collected with a fraction collector.

MS and NMR Analyses

^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ on a Bruker AMX 300 spectrometer (Karlsruhe, Germany) with 300 MHz for ^{1}H- and 75.5 MHz for ^{13}C-measurements, respectively. Electrospray ionization multiple mass spectrometry (ESI-MS) experiments were performed on a Bruker Esquire LC-MS/MS in positive and negative mode using a syringe pump. Drying gas was nitrogen at a gas flow of 7 L/min (330°C) and nebulizer pressure was set to 30 psi. Parameters for negative ESI mode were as follows: capillary, −3500 V; end plate, −3000 V; capillary exit, 90 V; skim 1, 30 V; skim 2, 10 V. For positive ESI mode the parameters were as follows: capillary, 3500 V; end plate, 3000 V; capillary exit, −90 V; skim 1, −30 V; skim 2, −10 V.

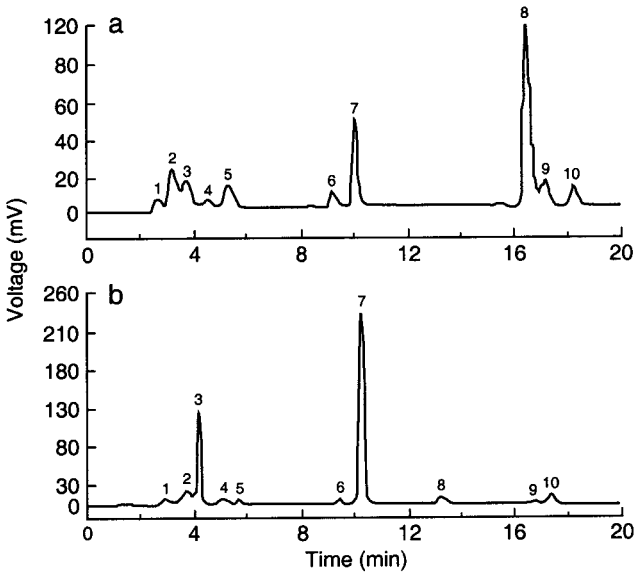

Figure 2. HPLC analysis of lovastatin extract (a) and mevinolinic acid extract (b) from *M. purpureus.* Experimental conditions: Apparatus: Waters HPLC system consisting of a quaternary pump with a degasser, a temperature-controlled column compartment, a variable wavelength detector, a manual injector, and a data processor; column: Spherisorb BDS column; Temperature: 30°C; mobile phase: acetonitrile—0.1% phosphoric acid (55/45, v/v); flow rate; 1 mL/min; detection wavelength: 238 nm.

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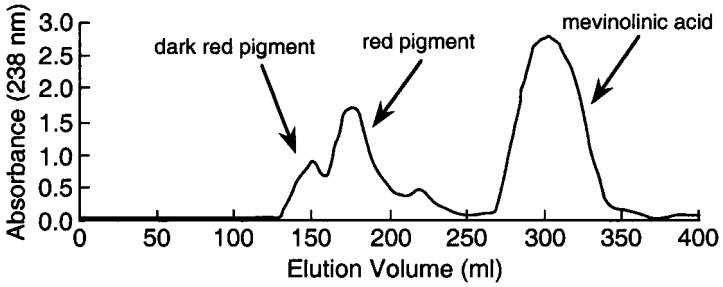

Figure 3. High-speed countercurrent chromatography separation of 250 mg of mevinolinic acid extract. Experimental conditions: Apparatus: type-J high-speed CCC centrifuge with 10 cm revolution radius; column: multiplayer coil consisting of 50 m long, 2.6 mm ID PTFE tubing wound onto a holder hub of 25 cm in length and 6 cm in OD with a total capacity of 260 mL; Solvent system: *n*-hexane/ethyl acetate/methanol/water (1/1/1/1, v/v); mobile phase: lower aqueous phase; flow rate: 2.0 mL/min; sample: 300 mg of mevinolinic acid extract in 20 mL of mobile phase; revolution: 800 rpm; detection: 238 nm.

RESULTS AND DISCUSSION

Figure 2 shows the HPLC analysis of the lovastatin extract (a) and the mevinolinic acid extract (b) from *M. purpureus.* Obviously, lovastatin [peak 8 in Fig. 2(a)] in the lovastatin extract was transformed into mevinolinic acid [peak 7 in Fig. 2(b)] after the treatment with 0.2 M NaOH.

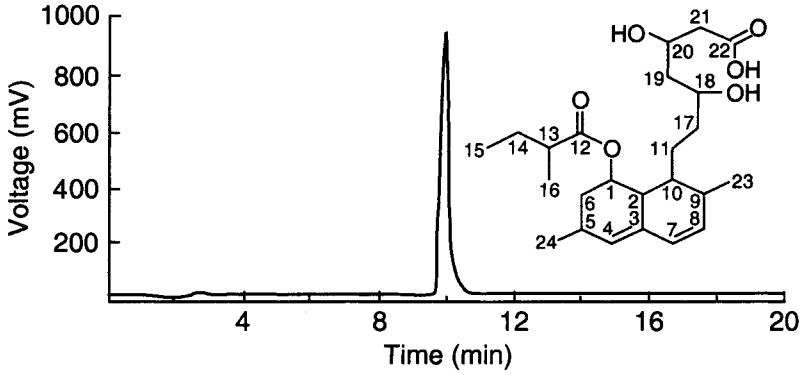

Figure 4. HPLC analysis of fractions corresponding to the last peak of the HSCCC separation. Experimental conditions are described in the Fig. 2 caption.

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Figure 3 indicates the HSCCC separation of 250 mg of mevinolinic acid extract with solvent system *n*-hexane/ethyl acetate/methanol/water (1/1/1/1, v/v). Four components were obtained in 200 min of elution. HPLC analysis demonstrated that only the last peak was pure (Fig. 4). The fractions corresponding to this pure component were collected and evaporated to dryness, yielding 79.2 mg of mevinolinic acid with a purity of 99.1% measured by HPLC. This sample was subjected to ESI-MS and NMR analysis to confirm its chemical structure as follows:

Electrospray ionization multiple mass spectrometry with a negative mode of the sample obtained from HSCCC separation shows m/z 421.6, which can be indicated as [M-H], while ESI-MS with a positive mode of the sample shows m/e 427.3, which is consistent with $[M\text{-}H_2O + Na^+]$. Therefore, the

Table 1. Chemical shift (δ) of ^{13}C-NMR of the sample obtained from HSCCC separation.

		DEPT 135	
Carbon	At 75 MH δ (ppm)	$CH_3 + CH$	CH_2
1	67.9	67.9	
2	37.4	37.4	
3	131.6	131.6	
4	129.7	129.7	
5	30.7	30.7	
6	32.8		32.8
7	128.4	128.4	
8	133.0	133.0	
9	30.7	30.7	
10	36.7	36.7	
11	24.3		24.3
12	176.7	176.7	
13	41.5	41.5	
14	26.8		26.8
15	11.7	11.7	
16	16.2	16.2	
17	33.0		33.0
18	76.3	76.3	
19	36.3	36.3	36.3
20	62.7	62.7	
21	38.7		38.7
22			
23	13.9	13.9	
24	22.8		

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Table 2. Chemical shift (δ) ^{1}H-NMR of the sample obtained from HSCCC separation.

Proton	δ (ppm)	Proton	δ (ppm)
H-10	1.72 (1H)	H-20	4.36 (1H)
H-9	2.38 (1H)	H-19	1.98 (1H)
H-9-CH_3	0.90 (3H)	H-19	1.65 (1H)
H-8	5.79 (1H)	H-18	4.35 (1H)
H-7	5.99 (1H)	H-17	1.29 (1H)
H-4	5.53 (1H)		1.89 (1H)
H-5	2.45 (1H)	H-11	1.38 (1H)
H-5-CH_3	1.08 (3H)		1.48 (1H)
H-6	1.95 (2H)	H-13	2.36 (1H)
H-1	5.38 (1H)	H-16-CH_3	1.11 (3H)
H-2a	2.27 (1H)	H-14	1.42 (1H)
H-21eq	2.63 (1H)		1.63 (1H)
H-21ax	2.72 (1H)	H-15	0.90 (3H)
18-OH	7.22 (1H)	20-OH	7.22 (1H)

molecular weight of the sample should be 422. Chemical shift (δ) of ^{13}C-NMR and ^{1}H-NMR of the sample obtained from HSCCC separation is listed in Tables 1 and 2. The data of DEPT 135 show that six carbons belong to CH_2 and 16 carbons belong to $CH + CH_3$. The full chemical shift (δ) of ^{13}C-NMR and ^{1}H-NMR can be interpreted in Tables 1 and 2 comparing to the NMR data of lovastatin.[11]

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