Microbial Conversion of Mevinolin

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(Received for publication June 23, 1997)

About 3000 microorganisms (bacteria, *Actinomyces*, *Zygomyces*, *Deuteromyces*) were screened for their capacity to convert mevinolin. *Absidia coerulea* IDR 705 was found to produce two hydroxylated derivatives of mevinolin, **2** and **3**. Compound **2** is a new transformation product while compound **3** was described as a chemical modification product of mevinolin[1]. By combination of spectroscopic techniques, the structures of **2** and **3** were identified with β,δ-dihydroxy-7-(1,2-dihydro-2-hydroxymethyl-6-methyl-naphthalen-1-yl)-heptanoic acid δ-lactone and β,δ-dihydroxy-7-[1,2,3,5,6,7,8,8a-octahydro-3,5-dihydroxy-2,6-dimethyl-8-(2-methyl-butyryloxy)-naphthalen-1-yl]-heptanoic acid δ-lactone, respectively. The inhibitory effects of the two derivatives on the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase were similar to that of mevinolin.

Cardiovascular disease is one of the major causes of mortality in developed nations. There is convincing evidence that lowering plasma cholesterol levels reduces the risk of myocardial infarction[2,3]. The inhibition of the microsomal enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase, EC 1.1.1.34), the rate-limiting enzyme in cholesterol biosynthesis, reduces the total cholesterol and decreases low density lipoprotein (LDL) level in animals as well as in human[4]. Mevinolin and related compounds (compactin, pravastatin and simvastatin) are competitive inhibitors of HMG-CoA reductase[5~8].

In the present study, approximately 3000 strains of bacteria and fungi were screened for their ability for microbial conversion of mevinolin. This work has shown that a fungal strain was able to convert mevinolin (**1**) to biotransformation products. Two conversion products were isolated from the fermentation broth of *Absidia coerulea* IDR 705 by extraction and chromatographic methods, and their structures were identified by UV, IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopy. As a result of structural analysis, compound **2** was found to be a new product in which the methyl group at 2-position was hydroxylated and one of the cyclohexene moieties was aromatized.

Materials and Methods

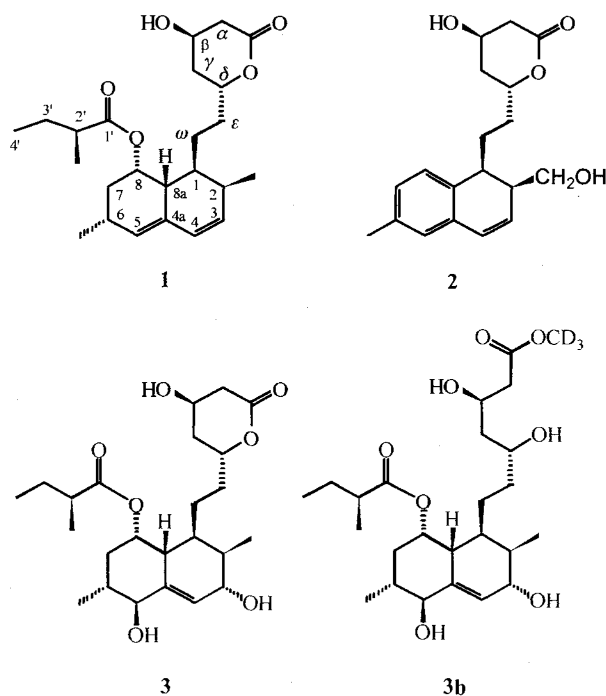
Microorganisms

Approximately 3000 strains selected from the strain collection of IDR (Institute for Drug Research Ltd., Budapest, Hungary) or isolated from soil samples originating from different sources (Israel, New Zealand, Argentina) were screened. In this study *Absidia coerulea* IDR 705 was found to convert mevinolin into two hydroxylated derivatives.

Chemicals

Mevinolin (**1**) was isolated in IDR from the fermentation broth of *Aspergillus obscurus* NCAIM(P)F 001189[9,10].

Fig. 1.



Cultivation/Conversion

Cultivation of *A. coerulea* was carried out in 500 ml Erlenmeyer flasks containing 100 ml culture medium consisting of 3% glycerol, 1% glucose, 0.6% yeast extract (Oxoid), 0.2% $NaNO_3$ and 0.1% $MgSO_4 \cdot 7H_2O$ (pH 6.5). After cultivation at 25°C for 3 days on a rotary shaker (deflection 2.5 cm, 320 rotations min^{-1}), 250 mg/litre of mevinolin in acetone solution were added to the shaken cultures, and cultivation was continued for 5 days. The microbial conversion of mevinolin was monitored by TLC on silica gel with hexane-acetone mixtures.

Mevinolin and the hydroxylated compounds were measured by HPLC under the following conditions: an eluent consisting of 30% acetonitrile and 70% 0.1 M NaH_2PO_4 aqueous solution (pH 4.2) was applied on Nucleosil C18 columns (guard: 5 μ, 40 × 4 mm; measuring: 10 μ, 240 × 4 mm) with a flow rate of 1 ml/minute. Column temperatures were 22°C. Wavelengths of detection were 265 and 190 nm, respectively. Sample preparation: 2.0 ml aliquots of the fermentation broth and 2.0 ml ethanol were mixed for 5 minutes by ultrasonification and centrifuged at 4000 *g* for 20 minutes, 20 μl of the supernatant was injected for HPLC analysis[11].

Spectroscopic Methods

Specific optical rotation values ($[\alpha]_D$) have been determined with a JASCO J-720 spectropolarimeter. UV spectra were recorded on a Varian Cary 3E UV-VIS spectrophotometer in MeOH. IR spectra were recorded on a Bruker Vector-22 FT-IR spectrometer in film. NMR spectra were measured on a Bruker AC-250 and a Bruker DRX-400 spectrometer, respectively. Mass spectra were taken on a Finnigan MAT 8430 instrument under the following conditions: resolution 1250, ion acceleration voltage 3 kV, ion source temperature 250°C, EI E_e = 70 eV, I_e = 0.5 mA, evaporation temperatures 90°C (**2**) and 130°C (**3**), CI reagent gas *i*-butane, evaporation temperature 80°C. High resolution mass measurements were made at R = 10000, using perflourokerosene (PFK) as the reference standard.

Biological Assay

HMG-CoA reductase inhibitory effect was determined by Panlabs Inc. Pharmacology Services using rat liver enzyme[14].

Results

The preliminary screening revealed that some fungal species transformed **1** into known compounds and one fungus strain *A. coerulea* IDR 705 generated two hydroxylated derivatives **2** and **3**, besides some known compounds (*e.g.* monacolin J). The pooled fermented broths (1.0 litre) were adjusted to pH 3.0 with 1 N H_2SO_4 and then extracted three times with 300 ml of EtOAc. The extract was washed with a 5% aqueous $NaHCO_3$, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to dryness. The residue (0.8 g) was chromatographed on a silica gel column prepared of 15 g Kieselgel 60 (Reanal Ltd., Budapest). The column was developed with 45% acetone in hexane to yield 15 mg of **2** (oil, $[\alpha]_D = +13.01°$, *c* 0.15, acetonitrile), and further developed with 60% acetone in hexane to yield 10 mg of **3** (oil, $[\alpha]_D = +22.33°$, *c* 0.12, acetonitrile). Biotransformation as well as isolation and purification of the transformation products were monitored by TLC on silica gel (Kieselgel 60 F_{254}, Merck) developed with acetone-hexane (50 : 50), in which the transformation products gave Rf values of 0.3 (**2**) and 0.2 (**3**), respectively. The purity of the products was also checked by HPLC. Retention times of the lactones **2** and **3** were 15.7 minutes and 7.8 minutes, respectively.

Characteristic Spectral Data

Compound **2**:

UV: λ_{max} = 216 and 262 nm

IR: ν_{OH} = 3399; ν_{CH} = 2924; ν_{CO} = 1709; 1257; 1069; 912; 732 cm^{-1}

Mass spectra:

EI (70 eV) $M^{+\cdot}$ = 316 (3.8%) (M, $C_{19}H_{24}O_4$), *m/z* 298 (6.5%, $[M-H_2O]^{+\cdot}$), *m/z* 181 (50%), *m/z* 173 (10.7%, $[M-\text{side chain}]^+$), *m/z* 155 (100%, $[173-H_2O]^+$).

CI (*i*-butane) $[M+H]^+$ = 317 (17%), *m/z* 299 (42%, $[M+H-H_2O]^+$), *m/z* 281 (54%, $[M+H-2H_2O]^+$), *m/z* 174 (69%, $[M+H-\cdot\text{side chain}]^{+\cdot}$), *m/z* 155 (70%, $C_{12}H_{11}^+$), *m/z* 145 (100%).

Compound **3**:

UV: λ_{max} = 199 nm

IR: ν_{OH} = 3435; ν_{CH} = 2965; ν_{CO} = 1717; 1261; 1071; 1003 cm^{-1}

Mass spectrum:

EI (70 eV) $M^{+\cdot}$ = 438 (1%) (M, $C_{24}H_{38}O_7$), *m/z* 420 (6%, $[M-H_2O]^{+\cdot}$), *m/z* 336 (11%, $[M-i\text{-valeric acid}]^+$), *m/z* 318 (80%, $[M-i\text{-valeric acid}-H_2O]^{+\cdot}$), *m/z* 175 (100%, $[318-\text{side chain}]^+$), *m/z* 157 (30%, $[175-H_2O]^+$).

NMR spectra of compounds **2** and **3**:

^1H and ^{13}C NMR, DEPT 135, NOE difference, ^1H-^1H COSY and heterocorrelation spectra of compound **2**, and ^1H, ^{13}C and DEPT 135 spectra of

Table 1. ^{1}H NMR data of compounds **2**, **3** and **3b**.

	2 in $CDCl_3$	**3** in $CDCl_3$	**3** in MeOH-d_4	**3b** in MeOH-d_4
H-1	2.75 (ovl)	~1.75 (ovl)	1.78 (ovl)	~1.75 (ovl)
H-2	2.85 (ovl)	~2.0 (ovl)	1.95 (ovl)	1.95 (ovl)
2-CH_2OH	3.70~3.95 (m)			
2-Me		0.84 (d)	0.87 (d)	0.86 (d)
H-3	5.68 (dd, 9.5 and 1.8)	3.88 (m)	3.85 (t, 4.1)	3.85 (t)
H-4	6.45 (dd, 9.5 and 2.5)	5.90 (dd)	5.81 (dd, 4.1 and 2.0)	5.80 (dd)
H-5	6.85~6.96 (ovl)	3.98 (ovl)	3.92 (d, 2.0)	3.92 (d)
H-6	—	~2 (ovl)	1.98 (ovl)	1.98 (ovl)
6-Me	2.28 (s)	1.00 (d)	1.02 (d)	1.03 (d)
H-7	6.85~6.96 (ovl)			
H_2-7		~1.6 (ovl) and ~2.2 (ovl)	1.65 (ovl) and 2.20 (ddd, 14.8; 5.1 and 3.1)	1.65 (ovl) and 2.20 (ovl)
H-8	6.85~6.96 (ovl)	5.28 (m)	5.25 (q, 2.5)	5.24 (m)
H-8a	—	~2.4 (ovl)	2.43 (m)	2.40 (m)
H_2-α	2.50~2.75 (ovl)	2.55~2.75 (m)	2.55 (ddd, 17.4; 3.1 and 1.5) 2.73 (dd 17.4 and 4.5)	2.36 (dd) and 2.50 (dd)
H-β	4.30 (qi, 3.7)	4.35 (qi, ~3.5)	4.28 (qi, ~3.5)	4.20 (qi, ~7)
H_2-γ	1.64 (ovl) and 1.88 (ovl)	~1.8 (ovl) and ~2.0 (ovl)	1.75 (ovl) and 1.95 (ovl)	~1.6 (ovl)
H-δ	4.62 (m)	4.65 (m)	4.64 (m)	3.68 (qi, ~7)
H_2-ε	1.30~1.70 (ovl)	1.2~1.8 (ovl)	1.55 (ovl) and 1.75 (ovl)	1.4~1.8 (ovl)
H_2-ω	1.40~1.80 (ovl)	1.2~1.8 (ovl)	1.40 (ovl) and 1.70 (ovl)	1.4~1.8 (ovl)
H-2′	—	~2.4 (ovl)	2.38 (sx 7.2)	2.35 (ovl)
2′-Me	—	1.10 (d)	1.14 (d)	1.12 (d)
H_2-3′	—	1.4~1.8 (ovl)	1.45 (ovl) and 1.68 (ovl)	1.45 (ovl) and 1.68 (ovl)
H_3-4′	—	0.86 (t)	0.90 (t)	0.88 (t)

Chemical shifts are given relative to TMS as internal reference. Coupling patterns and coupling constants [Hz] are shown in parentheses.

compound **3** were recorded in $CDCl_3$ using a Bruker AC-250 NMR spectrometer. In addition, ^{1}H, ^{13}C, DEPT 135 spectra and a TOCSY spectrum with a short mixing time (quasi COSY) of compound **3** in MeOH-d_4 solution were measured on a Bruker DRX-400 spectrometer†.

A full assignment of the ^{1}H and ^{13}C NMR spectra of compounds **2** and **3**—based on the above measurements and the literature[12,13]—is given in Tables 1 and 2.

The configuration of C-3 and C-5 chirality centers of compound **3** were determined using NOE difference spectra. Upon saturation of H-3 signal (3.85 ppm) intensity enhancement (4.7 and 1.3%, respectively) was observed on the H-2 and 2-Me signals, indicating that the relative configuration of the 2-Me and 3-OH groups is *trans*. Upon saturation of H-5 signal (3.92 ppm) intensity enhancement (6.1 and 1.1%, respectively) occured on H-6 and 6-Me signals, indicating that the relative configuration of 5-OH and 6-Me groups is also *trans*. The absolute configurations of the chirality centers of mevinolin are known and the change of these configurations is not probable upon microbial transformation.

According to these spectroscopic data, the structures of **2** and **3** were identified with β,δ-dihydroxy-7-(1,2-dihydro-2-hydroxymethyl-6-methyl-naphthalen-1-yl)-heptanoic acid δ-lactone and β,δ-dihydroxy-7-[1,2,3,5,6,7,8,8a-octahydro-3,5-dihydroxy-2,6-dimethyl-8-(2-methyl-butyryloxy)-naphthalen-1-yl]-heptanoic acid δ-lactone, respectively.

The inhibitory effects of the derivatives **2** and **3** on HMG-CoA reductase are shown in Table 3. The biological activities of the two new compounds are similar to that of mevinolin ($IC_{50}=0.012\,\mu M$).

Discussion

In the present study, 3000 microorganisms (bacteria and fungi) were screened for their ability to convert lactone form of mevinolin. Only one strain, *A. coerulea* IDR 705 was active in transformation of mevinolin into

† When keeping at ambient temperature in MeOH-d_4 solution for a week, compound **3** was transformed quantitatively into **3b**. ^{1}H, ^{13}C, DEPT 135, NOE difference and heterocorrelation spectra of compound **3b** were measured by a Bruker AC-250 NMR spectrometer (see Tables 1 and 2).

Table 2. ^{13}C NMR data of compounds **2**, **3** and **3b**.

	2 in $CDCl_3$	**3** in $CDCl_3$	**3** in MeOH-d_4	**3b** in MeOH-d_4
C-1	38.4	29.2	29.5	29.5
C-2	41.1	35.6 (br)	37.1	37.2 (br)
2-CH_2OH	62.7			
2-Me		12.2 (br)	12.8 (br)	12.9 (br)
C-3	128.2	69.4	70.4	70.5
C-4	128.1	127.6	129.1	129.2
C-4a	135.3[a]	139.3	139.5	139.4
C-5	127.2	77.2	78.3	78.3
C-6	136.3[a]	34.2	35.7	35.7
6-Me	21.6	19.0	19.6	19.6
C-7	127.3[b]	30.6	31.6	31.7
C-8	127.4[b]	71.5 (br)	73.5 (br)	73.6 (br)
C-8a	133.4[a]	37.8	39.2	39.3
CO lact.	170.7	171.0	173.6	173.9
C-α	38.5	38.5	39.1	43.0
C-β	62.6	62.3	63.8	68.1
C-γ	36.0	35.9	36.5	44.7
C-δ	76.5	76.3	78.3	71.3
C-ε	33.5	33.5	35.0	36.6
C-ω	22.5	24.2	25.0	25.3
C-1′	—	176.3	178.1	178.2
C-2′	—	41.7	43.0	43.0
2′-Me	—	16.7	17.1	17.1
C-3′	—	26.5	27.7	27.7
C-4′	—	11.8	12.2	12.2

[a,b]: Alternative assignments.

Table 3. The inhibitory effects of the two derivatives.

Compound	Percent inhibition (%)			
	0.03 μM	0.3 μM	3 μM	30 μM
2	45	67	83	95
3	47	75	88	96

a new compound. Presumably, under aerobic condition, this microorganism is able to hydroxylate methyl group on the 2 position of the fused ring and to aromatize A ring of naphthalene moiety eliminating the 2-methylbutyryloxy moiety of the molecule to afford a new hydroxylated derivative **2**. On the other side, 3,5-dihydroxy derivative (**3**) is formed by hydration through an 4a,5-epoxy intermediate by a known mechanism. This compound has been described as a synthetic product[1]. Biological activities of the transformation products are comparable to that of mevinolin.

The above results suggest that microbial transformation is a useful tool to generate new products from mevinolin or from other related compounds, such as compactin, affording lead compounds for new drugs and further data for structure activity relationship in this type of compound.

Acknowledgments

We thank Dr. J. HALÁSZ (Chinoin Pharmaceutical and Chemical Works Co., Budapest) for the NMR spectra recorded on a Bruker DRX-400 spectrometer, and to Dr. J. BÉRDY for the critical review of the manuscript. Financial support (94-97-48-0685) from the National Committee for Technological Development is gratefully acknowledged.

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