MONACOLIN M, A NEW INHIBITOR OF CHOLESTEROL BIOSYNTHESIS

AKIRA ENDO, DAISUKE KOMAGATA and HIDEAKI SHIMADA

Department of Agricultural and Biological Chemistry, Tokyo Noko University, 3-5-8 Saiwaicho, Fuchu-shi, Tokyo 183, Japan

(Received for publication June 25, 1986)

Monacolin M, a new specific inhibitor of cholesterol biosynthesis structurally related to monacolin K (mevinolin), was isolated from cultures of a strain of *Monascus ruber*. The structure of monacolin M was determined to be β-hydroxybutyryl ester of monacolin J by a combination of physical techniques. It was suggested that monacolin M is derived from monacolin J *via* a synthetic pathway distinct from that for the synthesis of monacolin K, α-methylbutyryl ester of monacolin J. The inhibitory effect of monacolin M on β-hydroxy-β-methylglutaryl-CoA reductase was slightly lower than that of monacolin K.

Monacolin K (mevinolin) (Fig. 1, **3**)[1,2] and its related compounds, monacolins J (Fig. 1, **2**), L[3] and X[4] and dihydromonacolin L[4] are metabolites of *Monascus ruber*. These compounds specifically inhibit 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase, the rate-limiting enzyme in cholesterol synthetic pathway. In the present study, another metabolite of monacolin group, designated monacolin M (Fig. 1, **1**) was isolated from cultures of a strain of *M. ruber*. Its physical and chemical properties and biological activity were also studied.

Materials and Methods

Microbial Strain

Monascus ruber M4681 was employed in the present study. This strain was found to produce **1** as the minor metabolite, along with monacolins J, K and L.

Biological Assays

HMG-CoA reductase was assayed as described previously using rat liver enzyme preparation[5].

Fermentation and Isolation of Monacolin M

M. ruber M4681 was grown aerobically at 25°C for 10 days in a medium containing glycerol 7%, glucose 3%, meat extract 3%, peptone 0.8%, $NaNO_3$ 0.2% and $MgSO_4 \cdot 7H_2O$ 0.1% (pH 5). Under these conditions, this strain produced approximately 20 μg/ml of monacolin M. Culture filtrate (10 liters) was extracted at pH 6 with dichloromethane (5 liters). The water layer was pooled, adjusted to pH 2 with trifluoroacetic acid and extracted twice with 6 liters of ethyl acetate. The solvent layer was dried over Na_2SO_4 and evaporated to dryness. The residue (8.6 g) was submitted to silica gel column chromatography (Wako gel C-200, 3×42 cm). The column was developed with a solvent system of *n*-hexane - acetone (9 : 1 and 9 : 2) and fractions containing **1** were combined and evaporated to dryness, yielding 110 mg of solids. The solids were submitted to HPLC on Silica ODS column (Nihon Bunko) by using a solvent mixture of acetonitrile - water (45 : 55), yielding 8 mg of powder. This powder was further purified by HPLC on the Silica ODS column developed with a mixture of dichloromethane - 2-propanol (97: 3) and 2 mg of purified **1** was obtained.

Results and Discussion

Mass spectral analyses (CI (chemical ionization)-MS and FD (field desorption)-MS) established

Fig. 1. Structures of monacolins M (1), J (2) and K (3).

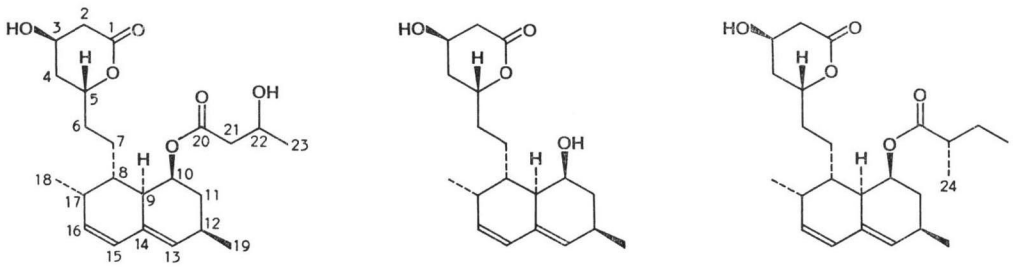
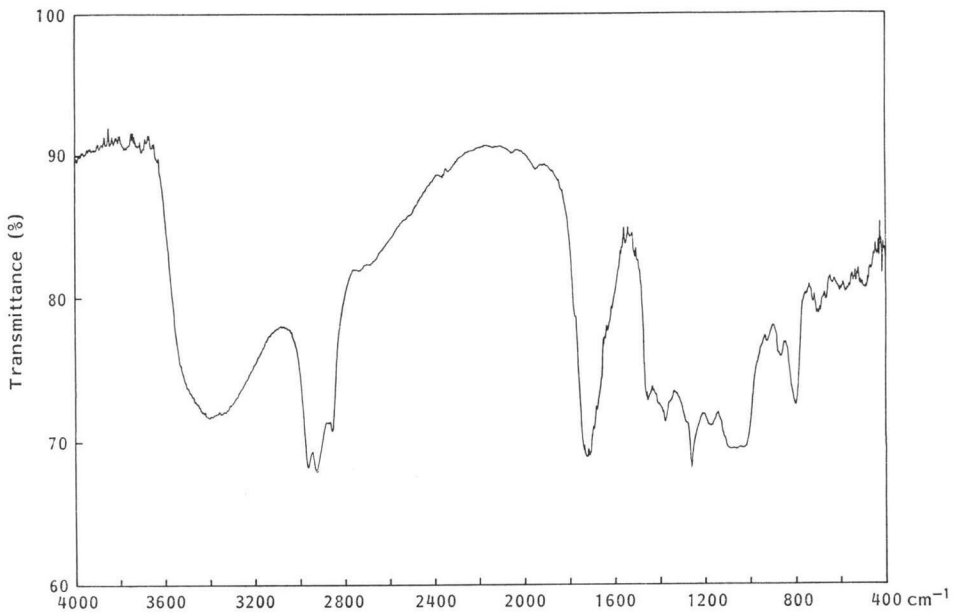


Fig. 2. IR spectrum of monacolin M (1).



molecular weight and elemental composition of **1** for 406 and $C_{23}H_{34}O_6$ (calcd 406.518), respectively. UV spectrum in methanol was identical to those of **2** and **3**[2,3,6]. IR spectrum (KBr) (Fig. 2) was essentially the same with those of **2** and **3**[2,3,6]. Although the molecular peak was not seen in EI (electron impact)-MS, peaks were seen at *m/z* 302 (M−104), 284 (M−122), 269 (M−137), 224 (M−182), 198 (M−208), 183 (M−223), 172 (M−234), 159 (M−247) and 157 (M−249). This fragmentation pattern was identical to that for **2** with a molecular weight of 302[2]. Further, **1** was converted to **2** by alkaline hydrolysis and treatment with a fungal esterase that catalyzed conversion of **3** to **2** (data for esterase treatment, unpublished).

Data for complete assignment of ^1H and ^{13}C NMR of **2** and **3**[3,4,7] and for ^1H NMR of **1** (Fig. 3) indicated the presence of one hydroxyl group on the ester-linked side chain of **1**. Those results, as well as data for multiplicity of ^1H and chemical shift, indicated the presence of β-hydroxybutyl ester. Further, irradiation of the methyl proton at 1.23 ppm caused collapse of the methine proton (multiplet) at 4.18 ppm to doublet and the former proton (doublet) collapsed to singlet by the irradia-

Fig. 3. ^{1}H NMR spectrum of monacolin M (**1**).

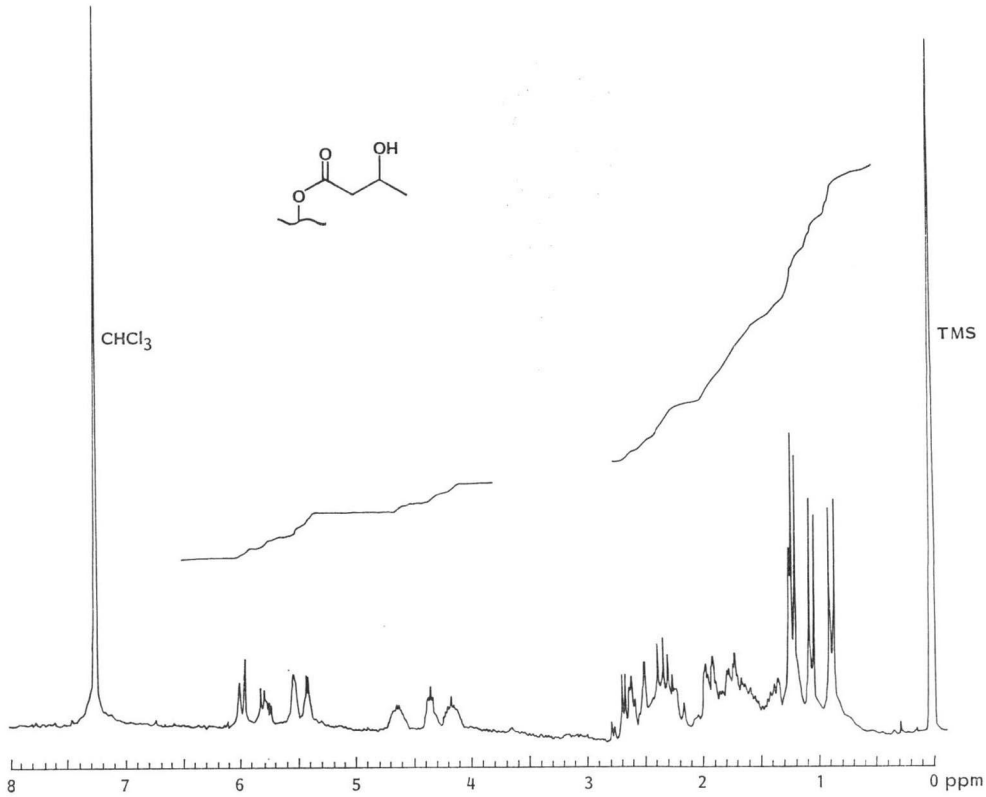


Fig. 4. ^{1}H and ^{13}C NMR chemical shifts of ethyl β-hydroxybutyrate.

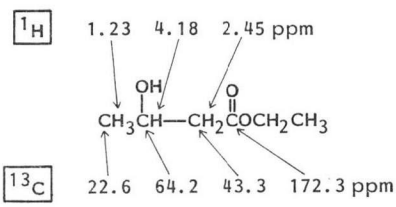


Table 1. Assignment of ^{1}H NMR spectrum of monacolin M (**1**) (200 MHz in $CDCl_3$).

ppm	Assignment
0.91	H-18 (3H, d)
1.09	H-19 (3H, d)
1.24~2.72	(19H)
4.18	H-22 (1H, m)
4.37	H-3 (1H, m)
4.63	H-5 (1H, m)
5.42	H-10 (1H, m)
5.54	H-13 (1H, m)
5.80	H-16 (1H, dd)
5.99	H-15 (1H, d)

Table 2. Assignment of ^{13}C NMR spectrum of monacolin M (**1**) (50 MHz in $CDCl_3$).

No.	ppm	Multiplicity	Assignment
1	173.0	s	C-20
2	170.0	s	C-1
3	133.2	d	C-16
4	131.8	s	C-14
5	129.6	d	C-13
6	128.3	d	C-15
7	75.7	d	C-5
8	68.6	d	C-10
9	64.5	d	C-22
10	62.7	d	C-3
11	43.4	t	C-21
12	38.6	t	C-2
13	37.3	d	C-9
14	36.4	d	C-8
15	36.1	t	C-4
16	32.5	t	C-11
17	32.5	t	C-6
18	30.7	d	C-17
19	27.4	d	C-12
20	23.5	t	C-7
21	22.7	q	C-23
22	22.7	q	C-19
23	13.9	q	C-18

tion of the latter proton.

Based on the above information, we assumed the structure of monacolin M as that shown in Fig. 1 (**1**). The absolute configulation of the hydroxyl group on the side chain was not determined in the present experiments.

On the bases of the data for complete assignment of ^{1}H and ^{13}C NMR of **3**, together with those for β-hydroxybutyrate ethyl ester (Fig. 4), protons and carbons of **1** were assigned as shown in Tables 1 and 2.

Monacolin M inhibited HMG-CoA reductase activity by 50% at a concentration of 2.9 μM. Under the same conditions, monacolin K inhibited reductase activity by 50% at 0.8 μM.

Of the three metabolites, monacolins J, K and L, monacolin L is first synthesized from 9 molecules of acetate, which is converted to monacolin J by hydroxylation. Monacolin K is then derived from monacolin J[7]. α-Methyl-β-ketobutyryl ester of monacolin J (monacolin X) is accumulated in the culture of a mutant strain of *M. ruber* that produces no detectable amount of monacolin K. Inversely, the parental strain produces monacolin K, while no monacolin X is detectable in its culture[4]. These observations suggest that monacolin J is converted to monacolin X which is then transformed to monacolin K. Further, it is likely from their structure that monacolin M is derived from monacolin J *via* a synthetic pathway distinct from that for monacolins X and K.

References

1) ENDO, A.: Monacolin K, a new hypocholesterolemic agent that specifically inhibits 3-hydroxy-3-methylglutaryl coenzyme A reductase. J. Antibiotics 33: 334~336, 1980
2) ALBERTS, A. W.; J. CHEN, G. KURON, V. HUNT, J. HUFF, C. HOFFMAN, J. ROTHROCK, M. LOPEZ, H. JOSHUA, E. HARRIS, A. PATCHETT, R. MONAGHAN, S. CURRIE, E. STAPLEY, G. ALBERS-SCHONBERG, O. HENSENS, J. HIRSHFIELD, K. HOOGSTEEN, J. LIESCH & J. SPRINGER: Mevinolin, a highly potent competitive inhibitor of hydroxymethylglutaryl-coenzyme A reductase and cholesterol-lowering agent. Proc. Natl. Acad. Sci. U.S.A. 77: 3957~3961, 1980
3) ENDO, A.; K. HASUMI & S. NEGISHI: Monacolins J and L, new inhibitors of cholesterol biosynthesis produced by *Monascus ruber*. J. Antibiotics 38: 420~422, 1985
4) ENDO, A.; K. HASUMI, T. NAKAMURA, M. KUNISHIMA & M. MASUDA: Dihydromonacolin L and monacolin X, new metabolites those inhibit cholesterol biosynthesis. J. Antibiotics 38: 321~327, 1985
5) KURODA, M. & A. ENDO: Inhibition of *in vitro* cholesterol synthesis by fatty acids. Biochim. Biophys. Acta 486: 70~81, 1977
6) ENDO, A.: Monacolin K, a new hypocholesterolemic agent produced by a *Monascus* species. J. Antibiotics 32: 852~854, 1979
7) ENDO, A.; Y. NEGISHI, T. IWASHITA, K. MIZUKAWA & M. HIRAMA: Biosynthesis of ML-236B (compactin) and monacolin K. J. Antibiotics 38: 444~448, 1985