DIHYDROMONACOLIN L AND MONACOLIN X, NEW METABOLITES THOSE INHIBIT CHOLESTEROL BIOSYNTHESIS

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Dihydromonacolin L and monacolin X those are structurally related to monacolin K, a potent inhibitor of cholesterol biosynthesis, were isolated from cultures of a mutant of *Monascus ruber*. The structures of these two metabolites were determined by a combination of physical techniques. Data for dihydromonacolin L and monacolin X as inhibitors of hydroxymethylglutaryl-CoA reductase and sterol biosynthesis *in vitro* are also given.

Since ML-236B (compactin) was first isolated as a potent inhibitor of cholesterol biosynthesis in 1976[1], several related compounds including monacolins J[2], K (mevinolin)[3,4] and L[2] have been discovered in cultures of fungi including *Monascus ruber, Penicillium citrinum* and *Aspergillus terreus*[5~8].

In our search for new microbial metabolites having 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibition and ultimately hypocholesterolemic activities, we have discovered two new compounds, designated dihydromonacolin L and monacolin X (Fig. 1) from cultures of a mutant strain of *M. ruber*. The present communication describes the isolation, physical and chemical properties of these two compounds. The inhibition of HMG-CoA reductase is also described.

Materials and Methods

Microbial Strain

M. ruber M82121 used in this study is a mutant strain derived from the parent *M. ruber* No. 1005 by successive treatment with UV irradiation and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. The parent strain produces monacolin K as the major product, along with monacolins J and L as minor metabolites.

Biological Assays

HMG-CoA reductase was assayed as described previously using rat liver enzyme preparation[9].

Fig. 1. Structures of dihydromonacolin L (**1**), monacolin L (**2**), monacolin X (**3**) and monacolin K (**4**).

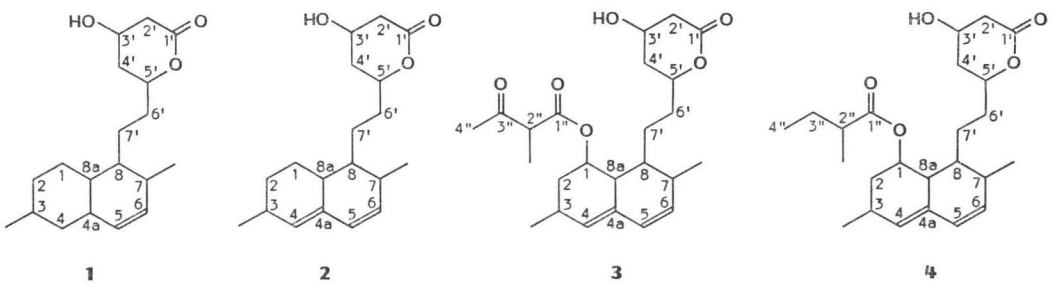


Fig. 2. Mass spectrum of dihydromonacolin L (**1**).

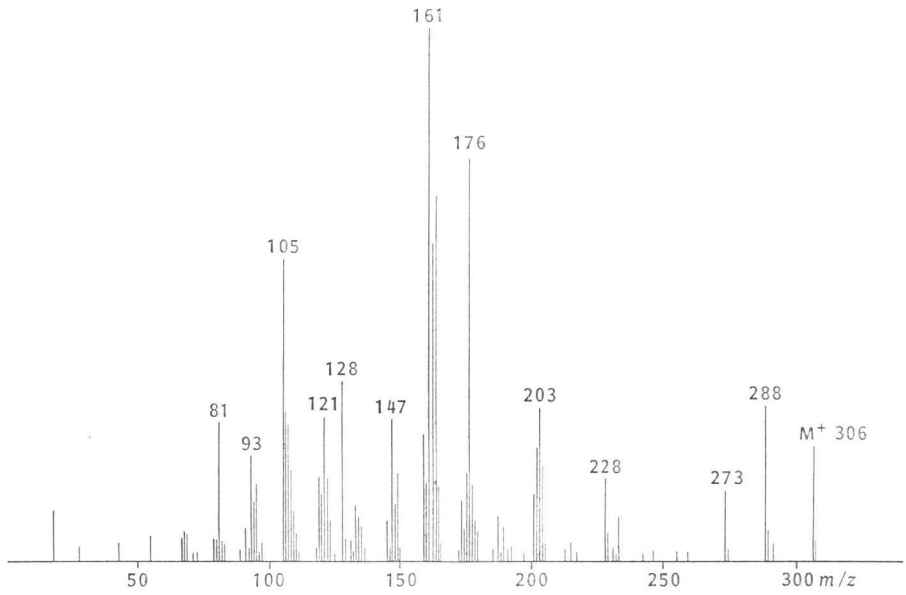
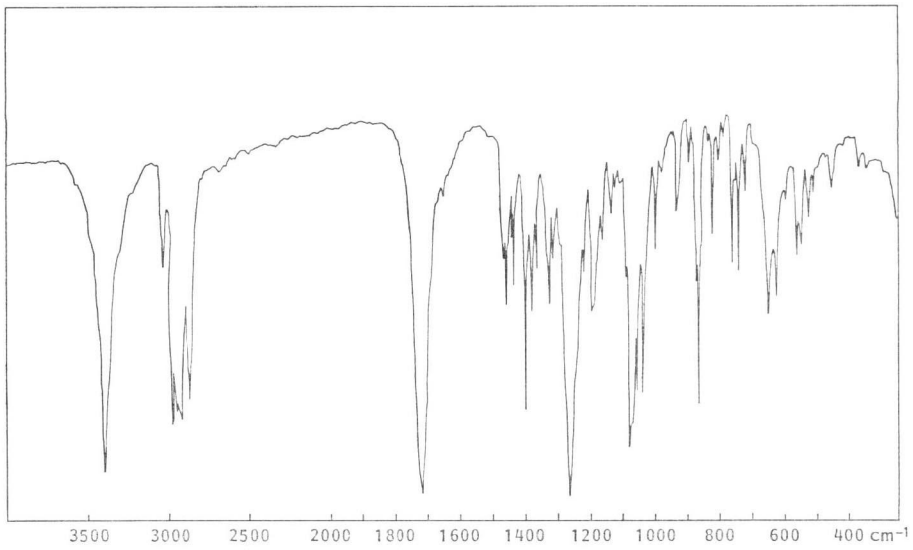


Fig. 3. IR spectrum of dihydromonacolin L (**1**).



The synthesis of non-saponifiable lipids from [^{14}C]acetate was determined using rat liver enzyme system by the method described previously[10].

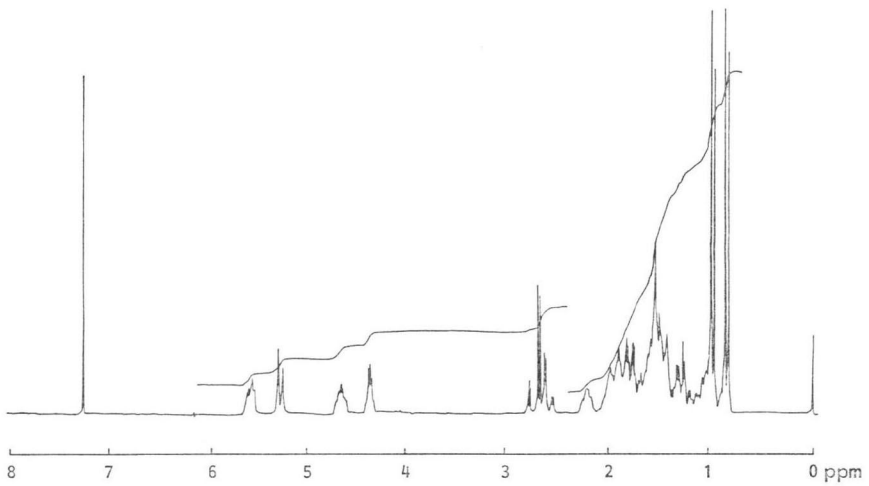
Fermentation and Isolation of Dihydromonacolin L and Monacolin X

M. ruber M82121 was grown aerobically at 25°C for 11 days in a medium containing glycerol 11%, glucose 1%, soy bean powder 5%, peptone 0.8%, $NaNO_3$ 0.1%, $Zn(NO_3)_2$ 0.05% and olive oil 0.5% (pH 6.5). Under these growth conditions, *M. ruber* M82121 produced 300~500 μg/ml of dihydromonacolin L along with monacolin L (600~700 μg/ml), monacolin X (300~400 μg/ml) and monacolin K (10~20 μg/ml). Culture filtrate (1 liter) was acidified to pH 3 with trifluoroacetic acid and extracted with an equal volume of ethyl acetate. The ethyl acetate extract was dried over Na_2SO_4 and evaporated

Table 1. ^{13}C NMR data for dihydromonacolin L (**1**) and monacolin L (**2**).

Carbon No.	**1** (ppm)	**2** (ppm)	Carbon No.	**1** (ppm)	**2** (ppm)
1	23.1	23.0	8	39.1	39.5
2	31.8	29.8	8a	35.3	35.4
3	26.9	29.1	1′	170.2	171.2
3-CH_3	17.6	22.1	2′	39.5	39.9
4	38.4	129.1	3′	61.3	62.2
4a	38.5	134.0	4′	36.7	36.2
5	131.1	130.8	5′	75.5	76.5
6	132.6	137.2	6′	32.6	33.3
7	31.4	31.8	7′	23.2	25.0
7-CH_3	14.6	35.4			

Fig. 4. ^{1}H NMR spectrum of dihydromonacolin L (**1**).



to dryness. The residue (4.2 g) was dissolved in 300 ml of benzene and the solution was washed with 5% $NaHCO_3$ (200 ml). The solvent layer was concentrated to dryness and the residue was dissolved in 0.1 M NaOH (300 ml) by stirring at 40°C for 2 hours. After filtration, the solution was acidified with trifluoroacetate (pH 3) and extracted with an equal volume of ethyl acetate. The solvent layer was concentrated to dryness and the residue (3.1 g) submitted to high performance liquid chromatography (HPLC) on a preparative column filled with Nucleosil $_5C_{18}$ using 0.1% H_3PO_4 - acetonitrile (50: 50) as eluent. Under these conditions, monacolin X, monacolin L, dihydromonacolin L and monacolin K appeared in eluate in this order. The fractions containing dihydromonacolin L were concentrated *in vacuo* to a small volume and extracted with dichloromethane. The extract was evaporated to dryness and the residue dissolved in a small volume of aqueous solution of ethanol, from which dihydromonacolin L was obtained as crystals (200 mg). The HPLC eluate containing monacolin X was treated as above and 162 mg of purified monacolin X was obtained as crystals.

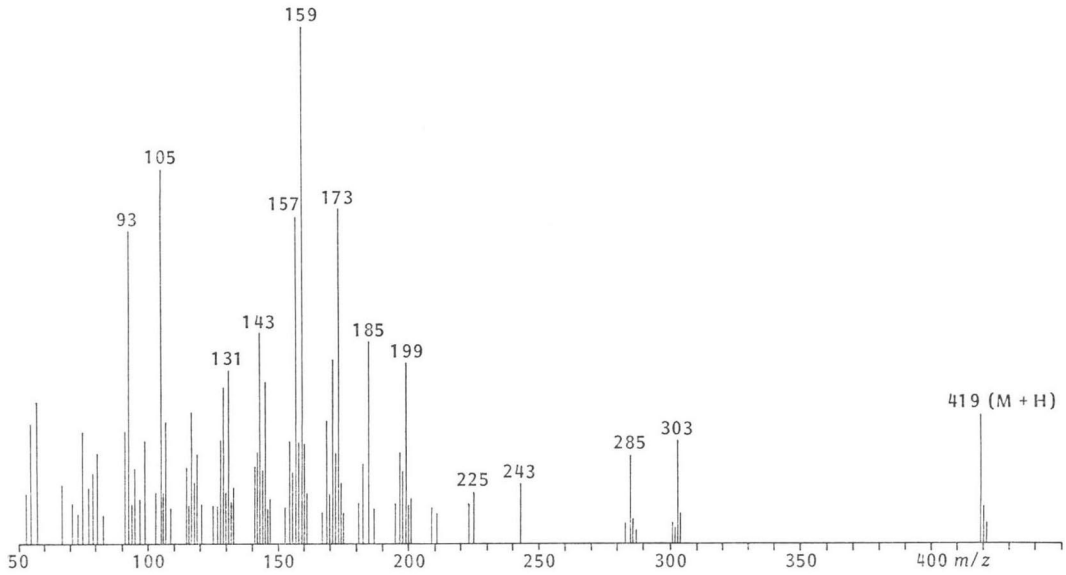
Results and Discussion

Structure Determination

Dihydromonacolin L

Elemental analysis, mass spectrum and ^{13}C NMR data for dihydromonacolin L (**1**) (mp 163~

Fig. 5. Mass spectrum of monacolin X (3).



164°C, $[\alpha]_D^{25}$ +123.9° (*c* 0.5, methanol)) established $C_{19}H_{30}O_3$ as the molecular formula. Except for the obvious difference in mass units, the mass spectrum of **1** (Fig. 2) compared well with that of monacolin L[2]. Significant peaks were seen at *m/z* 306 (M^+), 288 (M−18), 273 (M−33), 176 (M−130) and 161 (M−145). The IR spectrum of **1** (Fig. 3) compared well with that of monacolin L. The decoupled ^{13}C NMR spectrum of **1** (Table 1) revealed the presence of 19 carbon atoms and the existence of 1 carbonyl carbon at 170.2 ppm. The signals at 132.6 and 131.1 ppm appeared as doublets in the off-resonance decoupled spectrum, which indicated the presence of –CH=CH– group. The signals at 75.5 and 61.3 ppm also appeared as doublet in the off-resonance decoupled spectrum, which indicated two >CH–O– groups. The signals at 17.6 and 14.6 ppm were assigned to be $-CH_3$ groups. The assignment of each signals of ^1H NMR spectrum (Fig. 4) of **1** were 5.59 ppm (1H, m, H-6), 5.30 (1H, m, H-5), 4.69 (1H, m, H-5′), 4.40 (1H, m, H-3′), 2.69 (2H, m, H-2′), 2.23 (1H, m, H-7), 2.04~1.02 (17H, m, CH_2, CH and OH), 0.98 (3H, d, J=7 Hz, 3-CH_3) and 0.84 (3H, d, J=7 Hz, 7-CH_3). Irradiation of H-7 at 2.23 ppm caused collapse of the signal at 5.59 ppm to doublet and the doublet at 0.84 ppm to a singlet, which indicated the position of a double bond in the molecule of **1** is between C-5 and C-6. Based on the above informations, the structure of dihydromonacolin L as that shown in Fig. 1 (**1**) was assumed.

Fig. 6. UV spectrum of monacolin X (3).

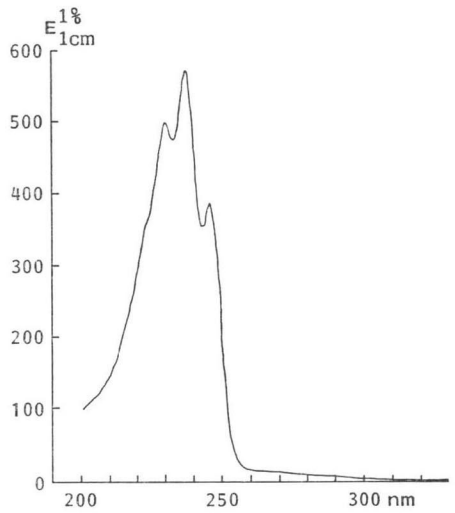


Fig. 7. IR spectrum of monacolin X (3).

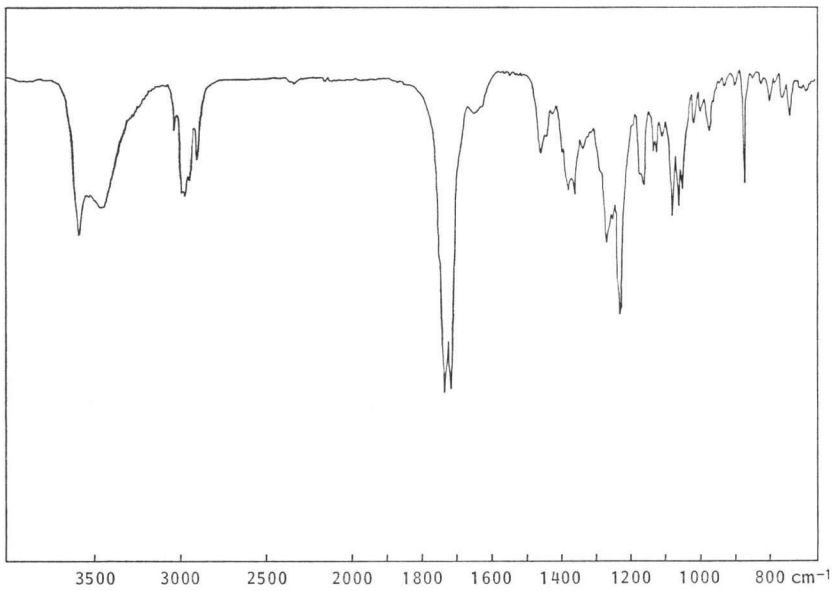
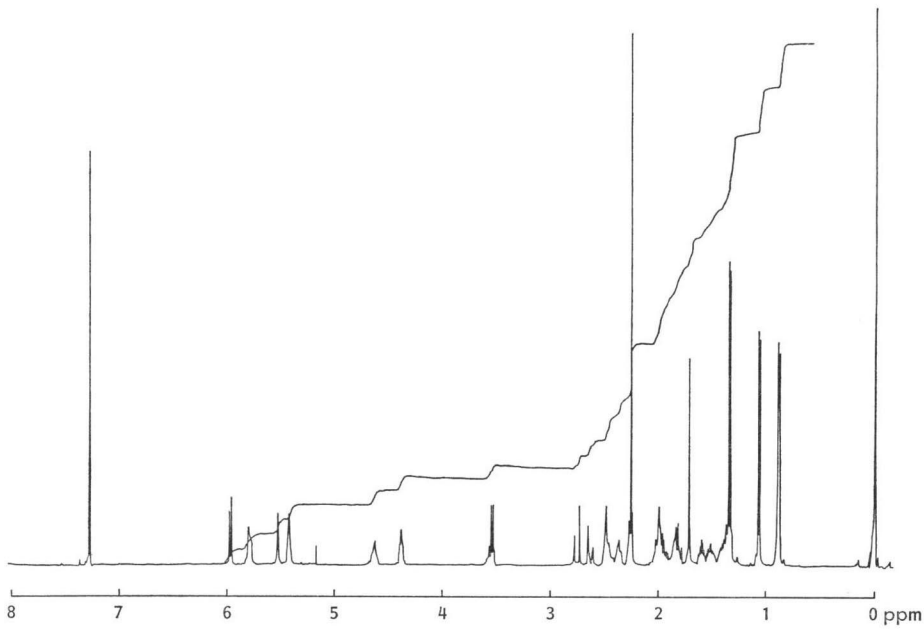


Fig. 8. ^{1}H NMR spectrum of monacolin X (3).



Monacolin X

Elemental analysis, mass spectrum and ^{13}C NMR data for monacolin X (3) (mp 145°C, $[\alpha]_D^{25}$ +273.8° (*c* 0.5, methanol)) established $C_{24}H_{34}O_6$ as the molecular formula. Except for the peak at *m/z* 419 (M+H), the mass spectrum of **3** (Fig. 5) compared well with that of monacolin K[3,4]. Thus, peaks were observed at *m/z* 303 (M+H−116), 285 (M+H−134), 225 (M+H−194), 199 (M+H−220), 173 (M+H−246), 159 (M−259) and 157 (M−261). The UV (Fig. 6) and IR (Fig. 7) spectra of **3** also com-

Table 2. ^{13}C NMR data for monacolin X (**3**) and monacolin K (**4**).

Carbon No.	**3** (ppm)	**4** (ppm)	Carbon No.	**3** (ppm)	**4** (ppm)
1	69.5	68.5	1′	170.7	170.7
2	32.5	32.8	2′	38.6	38.7
3	27.4	27.5	3′	62.5	62.6
3-CH_3	22.8	22.9	4′	36.0	36.2
4	129.3	129.6	5′	76.6	75.7
4a	131.6	131.6	6′	32.7	33.0
5	129.3	129.6	7′	24.1	24.3
6	133.3	133.1	1″	170.0	171.6
7	30.6	30.7	2″	53.4	41.6
7-CH_3	13.8	13.9	2″-CH_3	12.7	16.3
8	36.6	36.7	3″	204.7	26.8
8a	37.3	37.4	4″	29.4	11.7

pared well with those of monacolin K (**4**) suggesting that **3** is very close in chemical structure to **4**. ^1H NMR spectrum of **3** (Fig. 8) suggested the presence of one $-COCH_3$ group (2.24 ppm, 3H, s) and one $-COCH(CH_3)CO-$ group (3.53 ppm, 1H, q). The structure was further studied by comparing ^{13}C NMR signals of **3** with those of **4** (Table 2). The methylene signal at 26.8 ppm of C-3″ in **4** disappeared and a carbonyl signal at 204.7 ppm was seen in **3**. The methyl signal at 11.7 ppm of C-4″ in **4** shifted to an acetoxymethyl signal at 29.4 ppm in **3**. Based on the above informations, we assumed the structure of monacolin X as that shown in Fig. 1 (**3**).

Biological Activity

The inhibition of HMG-CoA reductase *in vitro*, assayed as described previously[10], was approximately 50% by dihydromonacolin L at 4.1 μM and by monacolin X at 2.1 μM, respectively. Concentrations required for 50% inhibition of the incorporation of [^{14}C]acetate into non-saponifiable lipids were 66 nM for dihydromonacolin L and 61 nM for monacolin X, respectively.

Acknowledgment

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