MICROBIAL HYDROXYLATION OF ML-236B (COMPACTIN) AND MONACOLIN K (MB-530B)

Sir:

ML-236B[1,2] (Fig. 1) is a competitive inhibitor of 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase produced by fermentation of *Penicillium citrinum* and has been shown to be active not only *in vitro* to inhibit cholesterol biosynthesis but also *in vivo* to lower serum cholesterol level in animals and humans. This compound was also isolated independently from culture of *P. brevicompactum* as an antibiotic named compactin[3].

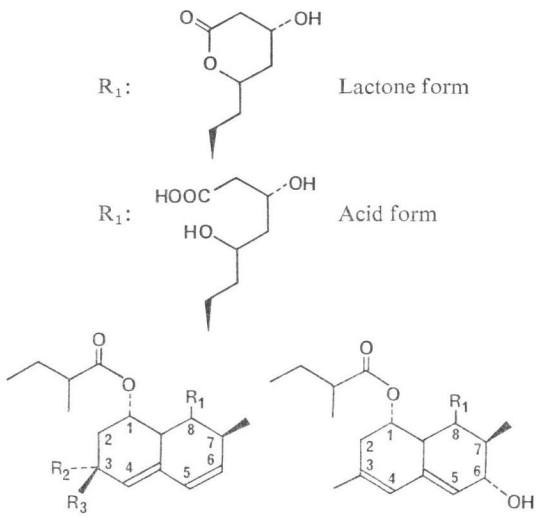
Similar structural features as well as biological activities have also been reported for MB-530B[4] (monacolin K[5] or mevinolin[6], Fig. 1) isolated from the fermentation broths of *Monascus ruber* and *Aspergillus terreus*, respectively.

Recent work has shown that sodium salt of 3-hydroxy-ML-236B carboxylate (Fig. 1) primarily isolated as a minor mammalian metabolite of ML-236B* is more potent than the parent compound in inhibition of cholesterol synthesis *in vitro*.

This result has stimulated the exploration of hydroxylations of ML-236B and MB-530B by microorganisms. A series of microorganisms have been tested for their oxidation activity at 3-position of ML-236B or MB-530B: *Mucor hiemalis* SANK 36372 was found to be one of the most potent microorganisms.

M. hiemalis SANK 36372 grown on an agar slant was inoculated into twenty 500-ml Erlenmeyer flasks, each containing 100 ml of TS medium composed of 1.0% glucose, 0.2% Polypepton (Daigo Nutritive Chemicals), 0.1% meat extract (Difco) and 0.3% corn steep liquor. After cultivation at 26°C for 3 days on a rotary shaker (220 rpm), 500 μg/ml of ML-236B was added to each flask and cultivation was continued for additional 5 days. The microbial conversion of ML-236B was monitored by thin-layer chromatography (TLC) on silica gel (Kieselgel $60F_{254}$, Merck) developed with benzene - acetone - acetic acid (50: 50: 3), on which ML-236B and a transformation product indicated approximate Rf values of 0.6 and 0.45, respectively. The fermented broth of the flasks were pooled, filtered (1.9 liters), adjusted to pH 3.0 with 2 N HCl and then extracted with three portions of 1 liter of ethyl acetate. The extract was washed with a saturated aqueous solution of sodium chloride and then a catalytic amount of trifluoroacetic acid was added for lactonization of the transformation product. The resulting mixture was then washed with a 5% aqueous solution of sodium bicarbonate, dried over anhydrous sodium sulfate and concentrated under reduced pressure to dryness. The residue was subjected to preparative liquid chromatography on Lobar column (Si60, Merck) using 70% benzene in acetone as an eluent. The fractions containing the transformation product were combined and were crystallized to give 455 mg (43.7% yield) of colorless crystals, mass spectra to $C_{23}H_{34}O_6$ (parent ion 406, calcd. 406), $[\alpha]_D^{22}+194.0°$ (c 0.51, methanol) and mp 138~142°C. UV absorption showed maxima in methanol at 230, 237 and 245 nm, indicating the presence of bicyclic diene chromophore "bis-dehydrodecalin". The ^1H NMR spectrum of

Fig. 1. Structures of 3β-hydroxy-ML-236B, ML-236B, 3-hydroxy-MB-530B, 6α-hydroxy-*iso*-MB-530B and MB-530B.



	R_2	R_3
ML-236B	H	H
3β-Hydroxy-ML-236B	H	OH
MB-530B	CH_3	H
3-Hydroxy-MB-530B	CH_3	OH
	or	
	OH	CH_3

* The details will be reported elsewhere.

Fig. 2. The ^1H NMR spectrum of 3β-hydroxy-ML-236B (90 MHz).

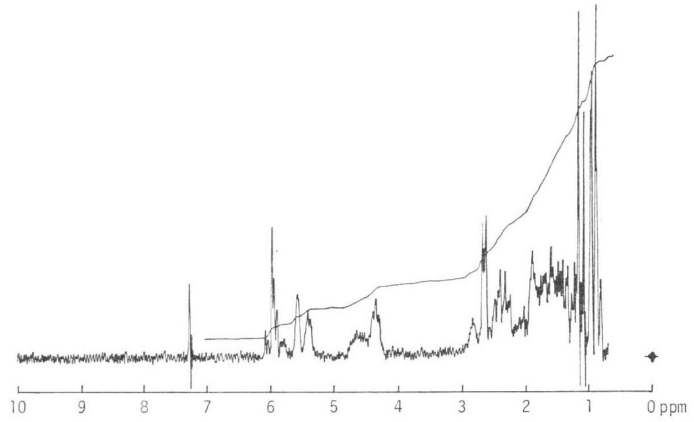
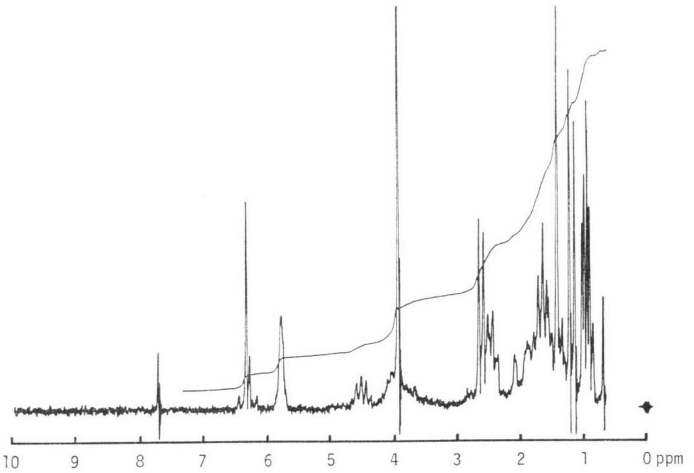


Fig. 3. The ^1H NMR spectrum of the methyl ester of 3-hydroxy-MB-530B carboxylate (90 MHz).

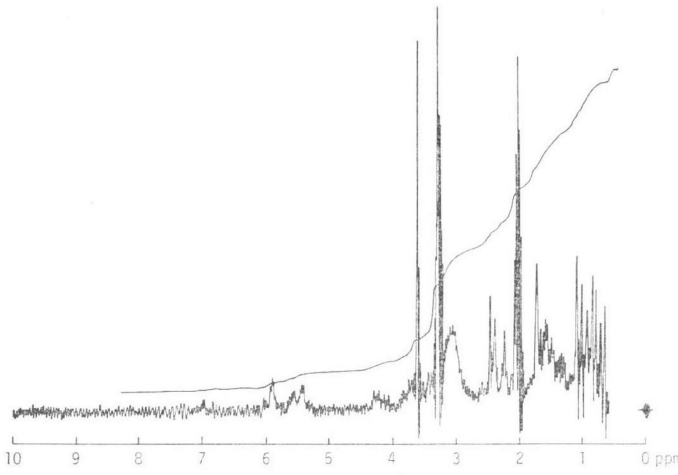


the transformation product in $CDCl_3$ is shown in Fig. 2.

From the data described above, the structure of the transformation product in lactone form was assigned to 3β-hydroxy-ML-236B (Fig. 1).

Furthermore, hydroxylation of MB-530B was carried out as follows: *M. hiemalis* SANK 36372 grown on an agar slant was inoculated into twenty 500-ml Erlenmeyer flasks, each containing 100 ml of TS medium. After cultivation at 26°C for 4 days on a rotary shaker (220 rpm), 500 μg/ml of MB-530B was added to each flask and cultivation was further continued for 6 days. The culture broth was filtered and the resulting 1.9 liters of the filtrate were adsorbed on a column of Diaion HP-20 (Mitsubishi Chemical Ind. Co.). The column was eluted with 70% aqueous methanol, and the active eluate was concentrated under reduced pressure. The residue was adjusted to pH 3.0 with trifluoroacetic acid and extracted with two portions of 1 liter ethyl acetate. Although the transformation products from MB-530B indicated only a single spot with Rf value of 0.47 on the TLC under the same conditions as above, it was later found that the 3-hydroxy-MB-530B carboxylic acid produced was rapidly isomerized to 6α-hydroxy-*iso*-MB-530B carboxylic acid under acidic conditions. To avoid this isomerization, 3-hydroxy-MB-530B carboxylic acid was purified further as its methyl ester. The ethyl acetate extract was immediately added to a stoichiometric excess of an ethereal solution of diazomethane. The reaction mixture was allowed to stand for 30 minutes, then

Fig. 4. The ^1H NMR spectrum of the methyl ester of 6α-hydroxy-*iso*-MB-530B carboxylate (90 MHz).



was washed with a saturated aqueous solution of sodium chloride and concentrated to dryness under reduced pressure. The resulting residue was subjected to high-performance liquid chromatography on a μBondapak C_{18} (Waters) column eluted with 53% aqueous methanol. The first part of the active eluates contained methyl ester of 3-hydroxy-MB-530B carboxylate, whilst the subsequent part contained methyl ester of 6α-hydroxy-*iso*-MB-530B carboxylate. These two eluates were separately collected and purified to give 180 mg (17.3% yield) and 170 mg (16.3% yield) of methyl esters of 3-hydroxy-MB-530B and 6α-hydroxy-*iso*-MB-530B carboxylate, respectively. Methyl ester of 3-hydroxy-MB-530B carboxylate showed UV absorption maxima in methanol at 230, 236 and 244.5 nm. It formed a tris (trimethylsilyl) derivative; $C_{34}H_{64}O_7Si_3$ (parent ion 668, calcd. 668). The ^1H NMR spectrum of this compound in $CDCl_3$ is shown in Fig. 3. Methyl ester of 6α-hydroxy-*iso*-MB-530B carboxylate showed UV absorption maxima in methanol at 238.3 and 250 (sh) nm. It formed a tris (trimethylsilyl) derivative; $C_{34}H_{64}O_7Si_3$ (parent ion 668, calcd. 668). The ^1H NMR spectrum of this methyl ester in $(CD_3)_2CO$ is shown in Fig. 4.

The results of measurement of the inhibitory activity against cholesterol synthesis *in vitro* of these transformation products both in sodium salt and methyl ester form indicates that the addition of hydroxyl group at 3-position confers 2- to 3-fold enhancement of the activity in comparison with their parents. However, the sodium salt of 6α-hydroxy-*iso*-MB-530B carboxylate is less active than that of MB-530B.

The details of structural elucidation and biological activity of these transformation products will be reported elsewhere.

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