6α-HYDROXY-*ISO*-ML-236B (6α-HYDROXY-*ISO*-COMPACTIN) AND ML-236A, MICROBIAL TRANSFORMATION PRODUCTS OF ML-236B

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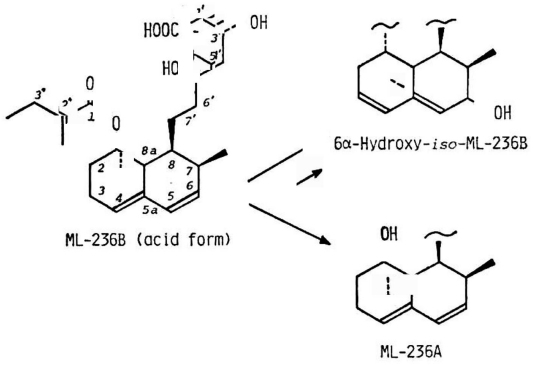
ML-236B (Fig. 1)[1,2], a competitive inhibitor of 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase, was produced by fermentation of *Penicillium citrinum* and has been shown to be active not only *in vitro* in inhibiting cholesterol synthesis but also *in vivo* in lowering serum cholesterol levels in animals and in humans. On the other hand, it was also isolated independently from the culture of *P. brevicompactum* as an antifungal antibiotic named compactin[3].

Hydroxylation of ML-236B by Syncephalastrum or *Mucor hiemalis* resulted in the production of one of either optically active diastereomer, 3α- or 3β-hydroxy-ML-236B, respectively[4,5]. Sodium salts of 3α- or 3β-hydroxy-ML-236B carboxylate were more potent than the parent compound in inhibition of cholesterol synthesis. This report describes hydroxylation of ML-236B to 6α-hydroxy-*iso*-ML-236B by *Absidia coerulea* and selective cleavage of the ester bound of ML-236B by esterase from *P. citrinum* the ML-236B producing organism and other fungi.

Fig. 1. Microbial transformation of ML-236B to 6α-hydroxy-*iso*-ML-236B, and ML-236A



A. coerulea SANK 32772 grown on an agar slant was inoculated into twenty 500-ml Sakaguchi flasks, each containing 100 ml of F-1 medium composed of 2.0% glucose, 0.15% K_2HPO_4, 0.15% $MgSO_4 \cdot 7H_2O$, 0.1% NH_4NO_3, 0.1% Polypepton (Daigo Nutritive Chemicals), 0.2% corn steep liquor, 0.1% yeast extract (Difco) and 0.001% $ZnSO_4 \cdot 7H_2O$ (pH 7.0 before sterilization).

After cultivation at 26°C for 2 days on a reciprocal shaker (120 rpm), 500 μg/ml of the sodium salt of ML-236B carboxylate was added to each flask, and cultivation was continued for additional 5 days. The progress of transformation 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 the transformation product indicated approximate Rf value of 0.44. The fermented broths of 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 for lactonization of the transformation product was added. 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 a Lobar column (Si60, Merck) using ethyl acetate as an eluant. The first part of the active eluate contained the product I, whilst the subsequent part contained the product II. These two eluates were separately collected to give 62 mg and 198 mg of the products, respectively.

The product I was identified as 3β-hydroxy-ML-236B[5], a transformation product of ML-236B by *M. hiemalis* SANK 36372, by analyses of IR, mass and ^1H NMR spectroscopic data in comparison with those of the authentic sample. On the other hand, the product II was further purified by means of Lobar column (RP-8, Merck), eluted with 35% aqueous acetonitrile, to give 82 mg of oily substance, mass spectra to $C_{23}H_{34}O_6$ (parent ion 406, calcd. 406). Mass fragmentation analysis indicated close resemblance

Fig. 2. The ^1H NMR spectrum of 6α-hydroxy-*iso*-ML-236B in lactone form (100 MHz, $CDCl_3$).

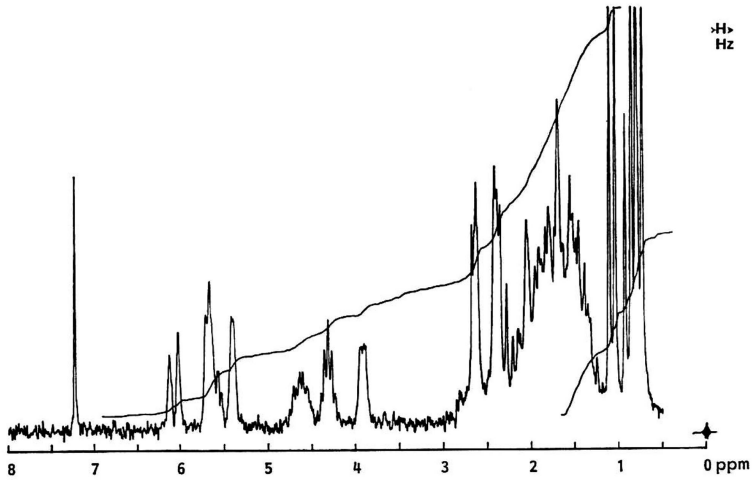


Table 1. Chemical shifts and coupling constants in ^1H NMR spectra of 6α-hydroxy-*iso*-ML-236B in lactone form (100 MHz, $CDCl_3$, TMS, δ ppm, J (Hz)).

Assignment	δ ppm	Multiplicity
7β-CH_3	0.78	3H, d, 7.0
3''-CH_3	0.87	3H, t, 7~8
2''-CH_3	1.09	3H, d, 6.8~7
8, 4', 6', 7', 3''	1.3~2	9H, m
7α	2.03	1H, m
8aβ	2.09	1H, dbr, 10
2''	2.32	1H, sex, 7
2	2.40	2H, m
2'α	2.61	1H, ddd, 17.5, 4, 1.5
2'β	2.68	1H, dd, 17.5, 5
	2.8	1H, br, OH
6β	3.93	1H, dbr, 5
3'β	4.33	1H, qu, *ca.* 5
5'α	4.63	1H, m
1β	5.42	1H, td, 4, *ca.* 1
3	5.65	1H, dt, 10, *ca.* 4
5	5.71	1H, dbr, 5, —
4	6.09	1H, dbr, 10, —

of the product II to 3α- or 3β-hydroxy-ML-236B. It showed UV absorption maxima in methanol at 229 (sh), 234.8 and 244.5 (sh) nm, indicating the presence of bicyclic diene chromophore "bis-dehydrodecalin". The ^1H NMR spectra of the product II in $CDCl_3$ is shown in Table 1 and Fig. 2. From the data described above, the structure of the product II in lactone form was assigned to 6α-hydroxy-*iso*-ML-236B (Fig. 1).

Meanwhile, *P. citrinum* SANK 18767 grown on an agar slant was inoculated into twenty 500-ml Sakaguchi flasks, each containing 100 ml of the F-1 medium. After cultivation at 26°C for 2 days on a reciprocal shaker (120 rpm), 500 μg/ml of the sodium salt of ML-236B carboxylate was added to each flask and cultivation was continued for additional 4 days.

The transformation of ML-236B was monitored by a TLC on the same condition described above, on which ML-236B and the product III indicated approximate Rf values of 0.6 and 0.35, respectively.

The cultured broth was filtered to remove the mycelial cake, and the filtrate (1.9 liters) was adjusted to pH 3.5 with 6 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 a catalytic amount of trifluoroacetic acid for lactonization of the product III was added. 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 silica gel column chromatography (Wako gel C-200, 10 g) using *n*-hexane (100 ml), *n*-hexane - acetone (85: 15, 200 ml) and acetone. The eluate with acetone was concentrated under reduced pressure to dryness. Thus, the product III was obtained as white powder (435 mg).

The product III showed UV absorption maxima at 230, 236, and 245 nm, indicating the presence of a bicyclic diene chromophore, "bis-dehydrodecalin". It was identified as ML-236A[1] (Fig. 1), one of the minor components produced by the ML-236B-producing strain of *P. citrinum*, by Rf value on silica gel TLC, and IR and ^{1}H NMR spectroscopic data in comparison with those of known ML-236A.

In addition to the ML-236B producer, the enzyme activity was detected in some other fungi, such as *P. chrysogenum* SANK 12768, *Fusarium solani* SANK 23023, *F. solani* SANK 11876, *F. lini* SANK 10561, *Helminthosporium sativum* SANK 11558, *Mucor griseocyanus* SANK 35672, *Rhizopus nigricans* SANK 15076 and *R. nigricans* SANK 14458.

Inhibitory activity of the sodium salt of 6α-hydroxy-*iso*-ML-236B carboxylate against cholesterol synthesis *in vitro* was about the same as that of the parent compound.

The oxidation mechanism of ML-236B to 6α-hydroxy-*iso*-ML-236B has not been clarified. Previously, we reported that 3β-hydroxy-MB-530B carboxylic acid was rapidly isomerized to 6α-hydroxy-*iso*-MB-530B carboxylic acid under acidic condition because of the instability of the tertiary hydroxyl group of 3β-hydroxy-MB-530B carboxylic acid[5]. Under acidic conditions, 3β-hydroxy-ML-236B carboxylic acid was also gradually isomerized to 6α-hydroxy-*iso*-ML-236B carboxylic acid. However, ML-236B was converted to 6α-hydroxy-*iso*-ML-236B even under neutral condition in which the culture fluid was adjusted to pH 6.0~7.5 with 20% sodium carbonate according to the change in color indicated by bromothymol blue. Hydroxylation of ML-236B to 6α-hydroxy-*iso*-ML-236B by *A. coerulea* SANK 32772 has not yet been clarified to be catalyzed by enzymatic reaction because the enzyme concerned has not been isolated.

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