MICROBIAL HYDROXYLATION OF ML-236B (COMPACTIN)

STUDIES ON MICROORGANISMS CAPABLE OF 3β-HYDROXYLATION OF ML-236B

NOBUFUSA SERIZAWA, SETSU SERIZAWA, KEIKO NAKAGAWA, KOUHEI FURUYA, TAKAO OKAZAKI and AKIRA TERAHARA

Fermentation Research Laboratories, Sankyo Co., Ltd.
2–58, 1-chome, Hiromachi, Shinagawa-ku, Tokyo 140, Japan

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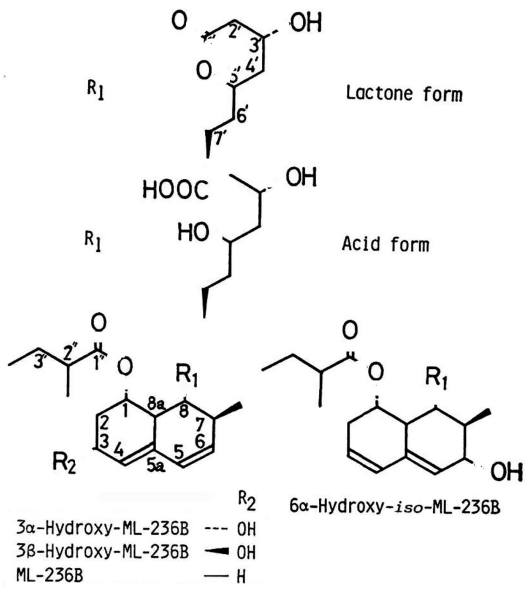
Various microorganisms were tested for capability to hydroxylate of ML-236B at the 3β-position. As the result, it was found that this ability was limited to a small group of microorganisms, mainly Zygomycetes in fungi, and Nocardia in actinomycetes.

Clinical and nutritional studies have indicated that high cholesterol levels in the blood may be one of the major causes of atherosclerosis and coronary diseases. In humans 50% or more of the total body cholesterol is derived from *de novo* synthesis. A major rate-limiting step in the cholesterol biosynthetic pathway is at the level of the microsomal enzyme, 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase, EC 1.1.1.34). This enzyme therefore is a prime target for pharmacological intervention.

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

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



As reported in the previous papers,[4,5,6] we found that only one of either optically active diastereomer, 3α- or 3β-hydroxy-ML-236B (Fig. 1), was mainly produced by hydroxylation of ML-236B by Syncephalastrum or *Mucor hiemalis*, respectively, indicating a high degree of selectivity and stereospecificity of the microbial enzymes. In addition, *Absidia coerulea* SANK 32772 catalyzed conversion of ML-236B to 6α-hydroxy-*iso*-ML-236B (Fig. 1). The sodium salts of these hydroxylation products, except 6α-hydroxy-*iso*-ML-236B, are more potent than that of the parent compound in inhibition of cholesterol synthesis.

In the present paper, we describe the various

microorganisms capable of hydroxylation of ML-236B at the 3β-position and also the method for quantitative determination of the sodium salt of 3β-hydroxy-ML-236B carboxylate in the cultured broths by high-performance liquid chromatography.

Materials and Methods

Microorganisms

Approximately 1,000 strains of the identified or isolated fungi, actinomycetes, and bacteria were used.

Chemicals

Lactone forms of ML-236B, 3α- and 3β-hydroxy-ML-236B, and 6α-hydroxy-*iso*-ML-236B were prepared as described previously.[1,4,5,6] The sodium salts of these compounds were prepared by saponification of their respective lactone forms in 0.2 N NaOH at 40°C for 2 hours.

Conversion Media

Two kinds of media, TS and F-1 media,[5,6] were used in this investigation.

Conversion Condition

A loopful amount of microorganisms was transferred from agar slant into a 100 ml-Erlenmeyer flask containing 20 ml of the medium. After cultivation at 26°C for 2~3 days on a rotary shaker (220 rpm), 500~2,000 μg/ml of sodium salt of ML-236B carboxylate or of ML-236B in lactone form were added to each flask, and cultivation was continued for an additional 2~5 days.

High-performance Liquid Chromatographic Analysis

3β-Hydroxylation activities were determined by high-performance liquid chromatography (HPLC) using the following solvent system: 30% acetonitrile and 0.2% PIC-A reagent (Waters) in water for μBondapak C_{18} (Waters) with a flow rate of 1 ml/minute. The sodium salts of 3α- and 3β-hydroxy-ML-236B and of 6α-hydroxy-*iso*-ML-236B carboxylate were detected at 237 nm of the UV absorption maximum. After completion of incubation, 2 μl of the culture filtrate was injected into the column for HPLC analysis.

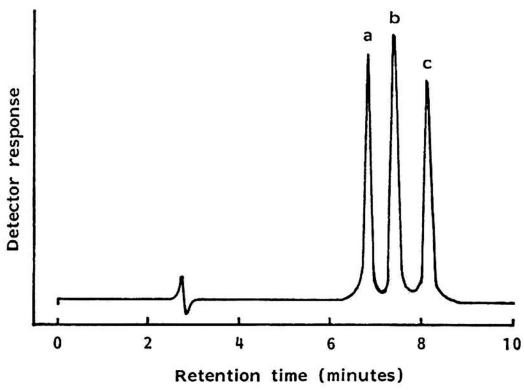
Results and Discussion

HPLC Analysis of the Sodium Salt of 3β-Hydroxy-ML-236B Carboxylate

An accurate method for routine quantitative analysis of 3β-hydroxy-ML-236B was required to assay ML-236B in the cultured broths. As a result a satisfactory resolution and the recovery of sodium salt of 3β-hydroxy-ML-236B carboxylate were obtained by HPLC analysis. High-performace liquid chromatogram of the authentic compounds is shown in Fig. 2.

Fig. 2. High-performance liquid chromatograms of the sodium salts of 3α- and 3β-hydroxy-ML-236B, and 6α-hydroxy-*iso*-ML-236B carboxylate.

a, 3α-hydroxy-ML-236B: b, 3β-hydroxy-ML-236B; c, 6α-hydroxy-*iso*-ML-236B.



Fungal Strains Capable of Hydroxylation of ML-236B at the 3β-Position

Approximately 500 strains of identified fungi including Zygomycetes, Ascomycetes, Basidiomycetes, and Fungi Imperfecti from stock culture were tested for their ability to hydroxylate ML-236B. The data in Table 1 indicate that there

Table 1. Fungal strains capable of hydroxylation of ML-236B at the 3β-position.

Microorganism	The form added of ML-236B*	Medium	Conversion ratio**
Absidia coerulea SANK 32772	Na	F-1	+2
Actinomucor elegans SANK 16380	lac	TS	+1
Circinella muscae SANK 14158	lac	TS	+1
C. rigida SANK 43875	lac	TS	trace
C. umbellata SANK 43972	lac	TS	+1
〃 SANK 44172	lac	TS	+1
〃 SANK 44272	lac	TS	+1
Cunninghamella echinulata SANK 14258	lac	F-1	+2
〃 SANK 44572	Na	F-1	+1
Gongronella butleri SANK 31072	lac	TS	+1
Mortierella isabellina SANK 46072	lac	TS	trace
Mucor bacilliformis SANK 33572	lac	TS	trace
M. circinelloides f. *circinelloides* SANK 33772	lac	TS	+1
〃 SNAK 33972	lac	TS	+1
M. circinelloides f. *griseocyanus* SANK 35672	lac	TS	+1
M. dimorphosporus SANK 34772	lac	TS	trace
M. fragilis SANK 35072	lac	TS	+1
M. genevensis SANK 35272	lac	TS	+1
M. heterosporus SANK 35772	lac	TS	trace
M. hiemalis f. *corticolus* SANK 34572	lac	TS	trace
M. hiemalis f. *hiemalis* SANK 11669	lac	F-1	+3
〃 SANK 11969	lac	TS	+2
〃 SANK 12069	lac	TS	+4
〃 SANK 34972	lac	TS	+2
〃 SANK 36172	lac	TS	+4
〃 SANK 36272	lac	TS	+4
〃 SANK 36372	lac	TS	+4
〃 SANK 36472	lac	TS	+4
〃 SANK 36572	lac	TS	+4
〃 SANK 37072	lac	TS	+4
〃 SANK 37172	lac	TS	+4
〃 SANK 37272	lac	TS	+1
M. hiemalis var. *albus* SANK 36072	lac	TS	+4
M. hiemalis var. *flavus* SANK 35972	lac	TS	+4
M. hiemalis var. *griseus* SANK 36672	lac	TS	+4
〃 SANK 36872	lac	TS	+4
M. hiemalis var. *toundrae* SANK 36772	lac	TS	+4
M. racemosus f. *sphaerosporus* SANK 35472	lac	TS	trace
M. spinosus SANK 12379	lac	TS	trace
Phycomyces blakesleeanus SANK 45172	lac	TS	trace
Rhizopus arrhizus SANK 16580	lac	TS	+1
R. chinensis SANK 12269	lac	TS	trace
R. circinans SANK 16480	lac	TS	+1
Syncephalastrum nigricans SANK 42172	Na	TS	+1
〃 SANK 42272	Na	TS	+1
〃 SANK 42372	Na	TS	+1
S. racemosum SANK 41872	Na	F-1	+1
〃 SANK 41972	Na	F-1	+1
Zygorhynchus moelleri SANK 41472	lac	TS	+2
〃 SANK 41672	lac	TS	+1
Pycnoporus coccineus SANK 11280	Na	F-1	+2
Rhizoctonia solani SANK 16776	Na	F-1	+2
〃 SANK 22972	Na	F-1	+2

* Na: sodium salt, lac: lactone form, substrate concentration: 0.05%.

** trace: below 0.5%, +1: 0.5~5%, +2: 5~10%, +3: 10~30%, +4: 30~90%.

Table 2. Actinomycetes strains capable of hydroxylation of ML-236B at the 3β-position.

Microorganism	Medium	Conversion ratio*
Nocardia sp. SANK 62781, Soil isolate	TS	+3
〃 SANK 62881, 〃	TS	+3
〃 SANK 62981, 〃	TS	+4
Nocardia autotrophica SANK 91272	TS	+1
N. asteroides SANK 62065	TS	+1
N. coeliaca SANK 63665	TS	trace
N. farcinica SANK 64265	TS	trace
Streptomyces roseochromogenus SANK 61972	F-1	trace

* Substrate concentration: 0.2% sodium salt of ML-236B carboxylate. +1, +3, +4, and trace: see to Table 1.

are 53 fungal strains in 26 species of 13 genera capable of hydroxylation of ML-236B at the 3β-position: these are shown in Table 1. It is interesting to know that all of these fungi, except for *Pycnoporus coccineus* and *Rhizoctonia solani*, belong to the Zygomycetes.

Actinomycetes Strains Capable of Hydroxylation on ML-236B at the 3β-Position

The fungi capable of hydroxylation of ML-236B at the 3β-position were found not to tolerate increase in the amount of ML-236B added to the culture medium probable because of its antifungal activity. Actinomycetes and bacteria which cover approximately 500 strains of identified cultures and isolates from soil samples were therefore tested for their ability to hydroxylate ML-236B.

As shown in Table 2, it was found that the isolates from soil samples indicated have a potent ability to hydroxylate of ML-236B at the 3β-position. These strains were identified as Nocardia. Details of the taxonomic studies of these strains will be reported elsewhere. Because of the identification of these soil isolates as Nocardia, some strains of this genus from the stock culture were tested. As shown in Table 2, it was found that *Nocardia autotrophica, N. asteroides, N. farcinica,* and *N. coeliaca* possess hydroxylation activity of ML-236B.

As was expected, contrary to fungi, actinomycetes tolerated a higher amount of ML-236B in the culture medium.

It is well-known that many microorganisms possess hydroxylation activity of various organic compounds, especially of steroids. The present investigation, however, indicates that the organisms capable of hydroxylation of ML-236B are somewhat different kinds from those already known for their hydroxylation activity and are rather limited to a small group of microorganisms, such as Zygomycetes as well as *P. coccineus,* and *R. solani* in fungi, and Nocardia in actinomycetes and *Streptomyces roseochromogenus.*

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