# 3α-HYDROXY-ML-236B (3α-HYDROXYCOMPACTIN), MICROBIAL TRANSFORMATION PRODUCT OF ML-236B (COMPACTIN)

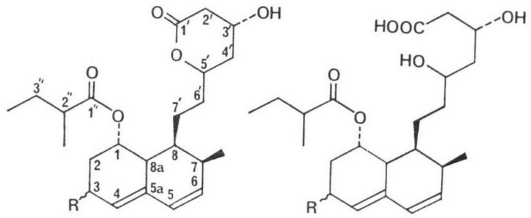
Sir:

As reported in the previous paper, we found that *Mucor hiemalis* catalyzed the 3β-hydroxylation of ML-236B (Fig. 1)[1]. Sodium salt of 3β-hydroxy-ML-236B carboxylate is more potent than the parent compound in inhibition of cholesterol synthesis.

This report describes diastereomeric 3α-hydroxylation of ML-236B by the strains belonging to the genus Syncephalastrum.

*Syncephalastrum nigricans* SANK 42372 grown on an agar slant was inoculated into twenty 500-ml Erlenmeyer flasks, each containing 100 ml of TS medium[1]. 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 the transformation product indicated approximate Rf values of 0.6 and 0.46, respectively. The fermented broth 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 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 180 mg (17.3% yield) of colorless crystals, mass spectra to $C_{23}H_{34}O_6$ (parent ion 406, calcd. 406), $[\alpha]_D^{22}$ +310.9° (*c* 0.66, methanol) and mp 141~143°C. The mass spectral fragmentation pattern of this transformation product closely resembles that of 3β-hydroxy-ML-236B. 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 the transformation product in $CDCl_3$ is shown in Table 1 and Fig. 2.

Fig. 1. Structures of 3α- and 3β-hydroxy-ML-236B and ML-236B.




Lactone form Acid form

| | R |
|---|---|
| 3α-Hydroxy-ML-236B | ---OH |
| 3β-Hydroxy-ML-236B | ◀OH |
| ML-236B | —H |

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

Further screening of the microorganisms capable of 3α-hydroxylase activity of ML-236B

Table 1. Chemical shifts and coupling constants in $^1H$ NMR spectra of 3α-hydroxy-ML-236B in lactone form (200 MHz, $CDCl_3$, TMS, δ ppm, *J* (Hz)).

| Assignment | δ ppm | Multiplicity |
|---|---|---|
| 3″-$CH_3$ | 0.89 | 3H, t, 7.6 |
| 7β-$CH_3$ | 0.91 | 3H, d, 7.0 |
| 2″-$CH_3$ | 1.13 | 3H, d, 6.8 |
| 3α-OH | 1.74 | 1H, d, 10 |
| 3′α-OH | 1.87 | 1H, d, 3 |
| 2β | 1.91 | 1H, ddd, 15~16, 5, *ca.* 2 |
| 8aβ | 2.26 | 1H, dm, 12, --- |
| 2″ | 2.35 | 1H, sex, 7 |
| 7α | 2.2~2.5 | 1H, m |
| 2α | 2.40 | 1H, ddt, 15~16, 4, 1~2 |
| 2′α | 2.64 | 1H, ddd, 17~18, 4, 1.5 |
| 2′β | 2.74 | 1H, dd, 17.5, 5 |
| 3β | 4.22 | 1H, dtbr, 10, 5, --- |
| 3′β | 4.38 | 1H, sex, 3~4 |
| 5′α | 4.62 | 1H, m |
| 1β | 5.51 | 1H, td, 3~4, *ca.* 2 |
| 4 | 5.76 | 1H, dt, 5, *ca.* 2 |
| 6 | 5.95 | 1H, dd, 10, 5.5~6 |
| 5 | 6.03 | 1H, d, 9.5 |

Fig. 2. The $^1H$ NMR spectrum of 3$\alpha$-hydroxy-ML-236B in lactone form (200 MHz, $CDCl_3$).

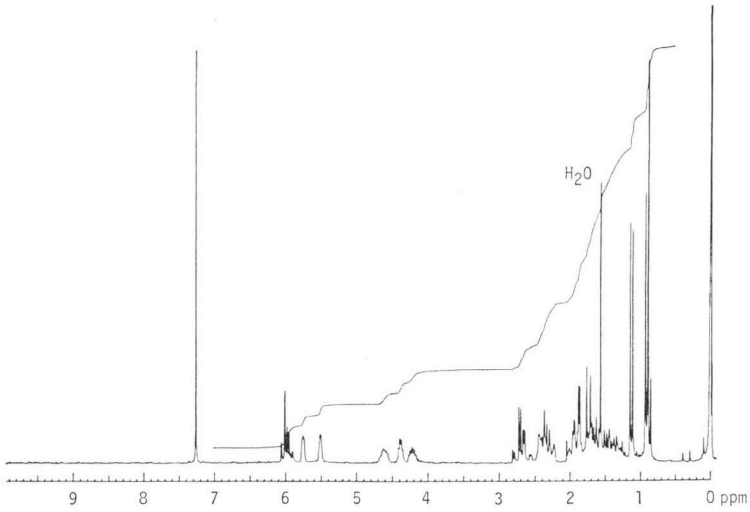



Table 2. Conversion ratio to 3$\alpha$- and 3$\beta$-hydroxy-ML-236B from ML-236B by the strains of Syncephalastrum or *Mucor hiemalis*.

| Microorganism | 3$\alpha$-Hydroxy (%) | 3$\beta$-Hydroxy (%) |
|---|---|---|
| *Syncephalastrum nigricans* SANK 42372 | 26.0 | 2.9 |
| 〃 〃 SANK 42172 | 18.3 | 0.3 |
| 〃 〃 SANK 42272 | 17.1 | 0.5 |
| *S. racemosum* SANK 41872 | 16.2 | 1.8 |
| 〃 〃 SANK 41972 | 15.8 | 1.5 |
| *Mucor hiemalis* SANK 36372 | 4.2 | 72.0 |

was carried out using the type cultures and the cultures with hydroxylation activity of ML-236B. Microorganisms on agar slant were inoculated into 100-ml Erlenmeyer flask, each containing 20 ml of TS medium. Cultivation conditions were as described above. 3$\alpha$- and 3$\beta$-hydroxylation activities were determined by high-performance liquid chromatography (HPLC) using the following solvent systems: 30% acetonitrile and 0.2% PIC-A reagent (Waters) in water for $\mu$Bondapak $C_{18}$ (Waters) with a flow rate of 1 ml/minute. Sodium salts of 3$\alpha$- and 3$\beta$-hydroxy-ML-236B carboxylates were detected at 237 nm of UV absorption maximum. After completion of incubation 2 $\mu$l of the culture filtrate was injected into the column for HPLC analysis. The results are given in Table 2.

We found that 3$\alpha$-hydroxylation of ML-236B was performed by *S. nigricans* and *S. racemosum*, but only a trace amount of 3$\beta$-hydroxy-ML-236B was produced by these organisms. It was indicated by precise analysis that 3$\alpha$-hydroxy-ML-236B was also detected as a minor transformation product of ML-236B by *Mucor hiemalis* as shown in Table 2.

Interestingly, the hydroxylation of ML-236B by *Syncephalastrum* or *M. hiemalis* produces primarily one optically active diastereomer, 3$\alpha$- or 3$\beta$-hydroxy-ML-236B, respectively. This indicates a high degree of selectivity and stereospecificity of the microbial enzymes.

Inhibitory activity of 3$\alpha$-hydroxy-ML-236B carboxylate against cholesterol synthesis *in vitro* was more potent than that of 3$\beta$-hydroxy derivative of the same compound.

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