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

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The Basidiomycete *Schizophyllum commune* was found to transform compactin (ML-236B) to 8a-hydroxycompactin. This compound was isolated by solvent extraction and column chromatography, and its structure was determined by a combination of IR, UV, $^{1}H$ NMR and $^{13}C$ NMR spectroscopy. Monacolin K was also converted to the corresponding hydroxylated analogue. Data on the inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase and sterol biosynthesis *in vitro* are presented for these hydroxylated compounds.

Compactin (ML-236B) and monacolin K (mevinolin) (Fig. 1) inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol biosynthesis, and are effective in lowering plasma cholesterol levels in human as well as in animals[1,2].

As reported in the previous paper[3], we found that *Circinella muscae* converted compactin to 5'-phosphocompactic acid. It was also reported that incubation of compactin with *Syncephalastrum nigricans, Mucor hiemalis* and *Absidia coerulea* resulted in the production of 3$\alpha$-hydroxycompactin, 3$\beta$-hydroxycompactin and 6$\alpha$-hydroxy-*iso*-compactin, respectively[4~6]. The present communication describes microbial hydroxylation of compactin and monacolin K at the 8a-position by *Schizophyllum commune* (Fig. 2). Isolation, physical and chemical properties of the compounds, and the inhibition of HMG-CoA reductase and sterol biosynthesis *in vitro* are also described.

## Materials and Methods

### Microbial Strains

All microbial strains used in the present study were obtained from the Institute for Fermentation, Osaka (IFO).

Fig. 1. Lactone forms of compactin (ML-236B) and monacolin K.

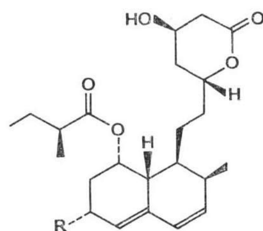
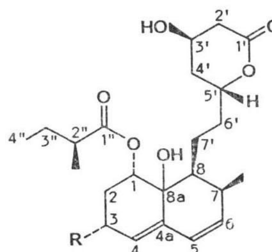



Fig. 2. Structures of 8a-hydroxycompactin and 8a-hydroxymonacolin K.



Materials

Compactin (ML-236B) and monacolin K were isolated as described previously[7,8]. These compounds were converted to their acid forms (sodium salts) by saponification prior to use.

Growth for HPLC Analysis

Test tubes (2×20 cm), each containing 10 ml of a medium consisting of 1% glucose, 0.2% peptone (Daigo Eiyo), 0.1% meat extract (Kyokuto Seiyaku), 0.1% yeast extract (Difco) and 0.3% corn steep liquor, were inoculated with agar slant cultures and shaken at 25°C and 120 strokes per minute (spm). After 3 days, 0.05% compactin (or a related compound) was added to the cultures and growth was continued for 5 days. Samples of culture were withdrawn at intervals and 2-ml portions of the culture filtrate were extracted twice at pH 3 with EtOAc (2 ml). The solvent layer was dried over $Na_2SO_4$ and evaporated. The resulting residue was dissolved in methanol (2 ml) and analyzed by HPLC (high performance liquid chromatography) on Silica ODS using 0.1% $H_3PO_4$ - acetonitrile (45: 55) as eluant. Under these conditions hydroxylated products derived from compactin and monacolin K had elution volumes of 9.8 and 11.8 ml, respectively. The products were assayed by measuring integrated absorbance at 237 nm of HPLC peaks.

Isolation of 8a-Hydroxycompactin

Fourteen 500-ml Sakaguchi flasks, each containing 100 ml of a medium composed of 2% malt extract and 0.2% yeast extract, were inoculated with agar slant cultures of strain IFO 4928 and shaken at 25°C and 120 strokes per minute. After 3 days, 0.05% of ML-236B (Na salt) was added to each flask and cultivation was continued for an additional 5 days. The broth was filtered (1,200 ml), adjusted to pH 2 with trifluoroacetic acid and extracted with three 1,200-ml portions of EtOAc. The solvent layer was dried over anhydrous sodium sulfate and evaporated. The resulting oily residue, redissolved in 200 ml of EtOAc, was washed with an equal volume of 5% aqueous sodium bicarbonate. The solvent layer was dried over anhydrous sodium sulfate and evaporated to yield 700 mg of oily residue. This was chromatographed on a silica gel column (Wako gel C-200, 2×15 cm) developed with *n*-hexane - acetone (9: 1~8: 2). Fractions containing the conversion product were combined and evaporated to yield 167 mg of residue. Fractionation of this product on a preparative HPLC column of Fine-SIL $C_{18}$-10 (Nihon Bunko) using 0.1% $H_3PO_4$ - acetonitrile (45: 55) as an eluent yielded 64 mg of 8a-hydroxycompactin as an oil.

Isolation of Monacolin K Product

Strain IFO 4928 was grown at 25°C in ten 500-ml Sakaguchi flasks containing 100 ml of 2% malt extract - 0.2% yeast extract medium for 3 days and then incubated for further 5 days in the presence of 0.05% monacolin K (Na salt) and the culture filtrate was processed as described above. The oily residue (15 mg) obtained after rechromatographed on a preparative HPLC column of Fine-Sil-5 (Nihon Bunko) using dichloromethane - 2-propanol (97: 3) as an eluant, yielding 8 mg of 8a-hydroxymonacolin K as an oil.

Biological Assay

HMG-CoA reductase was assayed as described previously using a rat liver enzyme preparation[9]. The synthesis of non-saponifiable lipids from [$^{14}$C]acetate was determined using a rat liver enzyme system by the method described previously[10].

## Results and Discussion

### Biotransformation

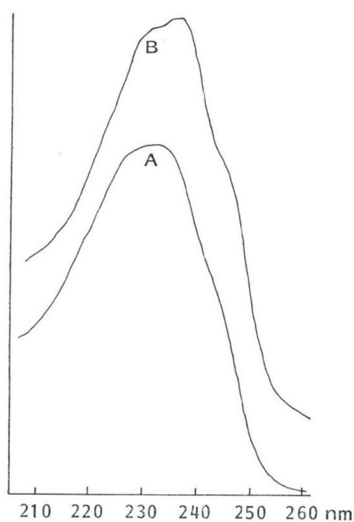
Approximately 2,000 strains of microorganisms were tested for their ability to modify compactin. *S. commune* strains (IFO 4928, IFO 4929, IFO 6503, IFO 6504 and IFO 6505) were found to be capable of hydroxylating compactin at 8a-position. Strain IFO 4928 was used in this study. Under the growth conditions adopted for HPLC analysis, 70~80% of the compactin added was converted to 8a-hydroxycompactin, while 10~20% of the monacolin K was transformed to 8a-hydroxy-

monacolin K.

## Structure Determination

Elemental analysis (calcd: C 67.96, H 8.43, found: C 67.86, H 8.50) and mass spectral data (SIMS: $(M+H)^+$ 407, $(M+Li)^+$ 413) for the compactin product established $C_{23}H_{34}O_6$ as the molecular formula. The UV spectrum in ethanol ($\lambda_{max}$ 234 nm, $\varepsilon$ 21,400) (Fig. 3) differed slightly from that of compactin[7], indicating a modification close to the bicyclic diene chromophore, bisdehydrodecaline. The IR spectrum (KBr) of the product (Fig. 4) was similar to that of compactin. The $^{13}$C NMR spectrum of the new compound resembled that of compactin in showing 23 lines; thus the carbon skeleton remained intact. However, one of the seven methine carbons presented as a doublet in the spectrum of compactin was converted to oxygen bearing carbon and appeared as a singlet in the new product. Since the $^{13}$C NMR spectrum of compactin has been completely assigned[11], comparison of the spectra allowed all carbons of the product to be assigned (Table 1). Assignment of signals in the $^{1}$H NMR spectrum is shown in Table 2. Irradiation of H-7 at 2.46 ppm resulted in partial collapse of the multiplet (H-8) at 1.83 ppm as well as conversion of the double-doublet (H-6) at 5.77 ppm to a doublet and the doublet (H-7$\beta$-$CH_3$) at 1.15 ppm to a singlet. From these findings, it was concluded that C-8a was converted to an oxygen bearing carbon in the new compound (Fig. 2).

Fig. 3. UV spectra of 8a-hydroxycompactin (A) and 8a-hydroxymonacolin K (B) (EtOH).




The molecular formula of the monacolin K product was established as $C_{24}H_{36}O_6$ by ele-

Fig. 4. IR spectrum of 8a-hydroxycompactin (KBr).

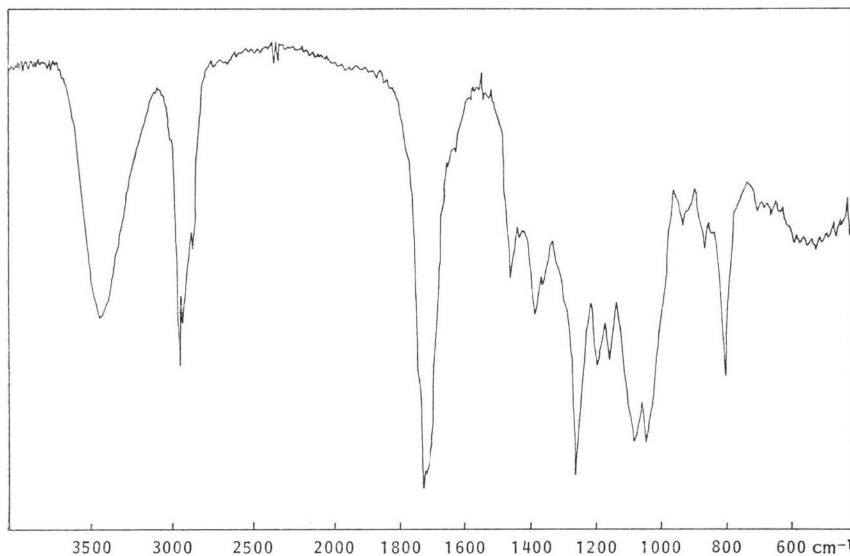


Table 1. $^{13}C$ NMR assignments for compactin, 8a-hydroxycompactin and 8a-hydroxymonacolin K (50 MHz, $CD_3OD$, TMS, ppm).

| Carbon No. | Compactin[11] | 8a-Hydroxy-compactin | 8a-Hydroxy-monacolin K | Carbon No. | Compactin[11] | 8a-Hydroxy-compactin | 8a-Hydroxy-monacolin K |
|---|---|---|---|---|---|---|---|
| 1 | 69.2 d | 72.0 d | 72.4 d | 1′ | 173.4 s | 173.3 s | 173.4 s |
| 2 | 27.2 t | 22.1 t | 28.5 t | 2′ | 39.2 t | 39.2 t | 39.2 t |
| 3 | 21.9 t | 22.0 t | 28.8 d | 3′ | 63.3 d | 63.3 d | 63.3 d |
| 3-$CH_3$ | — | — | 23.3 q | 4′ | 36.6 t | 36.6 t | 36.7 t |
| 4 | 124.4 d | 127.6 d | 134.0 d | 5′ | 78.0 d | 78.1 d | 78.2 d |
| 4a | 135.2 s | 136.7 s | 135.0 s | 6′ | 34.1 t | 34.5 t | 34.5 t |
| 5 | 129.4 d | 127.4 d | 127.6 d | 7′ | 24.9 t | 21.1 t | 21.2 t |
| 6 | 133.6 d | 133.5 d | 133.7 d | 1″ | 178.2 s | 177.5 s | 177.5 s |
| 7 | 32.1 d | 32.5 d | 32.4 d | 2″ | 43.1 d | 42.9 d | 42.8 d |
| 7-$CH_3$ | 14.1 q | 15.2 q | 15.2 q | 2″-$CH_3$ | 17.4 q | 17.2 q | 16.5 q |
| 8 | 38.2 d | 40.8 d | 40.7 d | 3″ | 27.9 t | 27.8 t | 27.9 t |
| 8a | 38.7 d | 71.1 s | 70.9 s | 4″ | 12.2 q | 12.2 q | 12.2 q |

s: Singlet, d: doublet, t: triplet, q: quartet.

Table 2. $^{1}H$ NMR assignments for 8a-hydroxycompactin and 8a-hydroxymonacolin K (200 MHz, $CDCl_3$, TMS, ppm).

| Assignment | 8a-Hydroxy-compactin | 8a-Hydroxy-monacolin K | Assignment | 8a-Hydroxy-compactin | 8a-Hydroxy-monacolin K |
|---|---|---|---|---|---|
| 1β | 5.16 1H d | 5.22 1H d | 3′β | 4.36 1H | 4.36 1H |
| 2 | 1.95 1H | 1.77 1H | 4′ | 1.70 1H | 1.65 1H |
| | 2.36 1H | 2.34 1H | | 2.02 1H | 1.98 1H |
| 3 | 2.14 2H | — | 5′α | 4.66 1H | 4.63 1H |
| 3β | — | 2.46 1H | 6′ | 1.32 1H | 1.33 1H |
| 3α-$CH_3$ | — | 1.08 3H d | | 1.90 1H | 1.88 1H |
| 4 | 5.69 1H | 5.64 1H | 7′ | 1.38 1H | 1.39 1H |
| 5 | 5.99 1H d | 6.00 1H d | | 1.57 1H | 1.55 1H |
| 6 | 5.77 1H dd | 5.85 1H dd | 2″ | 2.38 1H | 2.36 1H |
| 7α | 2.46 1H | 2.47 1H | 2″-$CH_3$ | 1.11 3H d | 1.11 3H d |
| 7β-$CH_3$ | 1.15 3H d | 1.12 3H d | 3″ | 1.45 1H | 1.42 1H |
| 8α | 1.83 1H | 1.83 1H | | 1.68 1H | 1.65 1H |
| 2′α | 2.60 1H ddd | 2.65 1H ddd | 4″ | 0.88 3H t | 0.88 3H t |
| 2′β | 2.74 1H dd | 2.73 1H dd | | | |

mental and mass spectral analysis (SIMS: $(M+H)^+$ 421, $(M+Na)^+$ 443). The UV spectrum in ethanol showed $\lambda_{max}$ at 231 and 237 nm (Fig. 3). The IR spectrum (KBr) closely resembled that of 8a-hydroxycompactin. The carbons and protons of the product were assigned as shown in Tables 1 and 2. As with compactin, the data indicated that C-8a was hydroxylated in the new compound.

### Biological Activity

Concentrations required for 50% inhibition of the incorporation of [$^{14}$C]acetate into non-saponifiable lipids were 22.9 nM and 25.7 nM for 8a-hydroxycompactin and 8a-hydroxymonacolin K, respectively, while those for compactin and monacolin K were 17.1 nM and 6.9 nM, respectively. HMG-CoA reductase was inhibited 50% by 8a-hydroxycompactin at 1.7 μM and by 8a-hydroxymonacolin K at 0.54 μM. Under the same condition, compactin and monacolin K inhibited reductase 50% at 0.71 μM and 0.22 μM, respectively. Hypocholesterolemic activity of the 8a-hydroxylated compounds in animals will be reported elsewhere.

When determined by disc diffusion assay, both 8a-hydroxycompactin and 8a-hydroxymonacolin K (at 100 $\mu$g/ml) showed no detectable inhibitory effects on the growth of *Escherichia coli* 0111, *Proteus vulgaris* W-091, *Staphylococcus aureus* Oxford, *Streptococcus faecalis* I, *Saccharomyces cerevisiae* IFO 1346 and *Aspergillus oryzae* IFO 30103.

In the present study, approximately 2,000 fungal strains including 596 genera (1,489 species) were assayed for their ability to transform compactin and monacolin K. Among them *S. commune* was the only species that was able to hydroxylate compactin and monacolin K at C-8a.

In a study of microbial degradation of aromatic amino acids by fungi, MOORE and TOWERS showed that *S. commune* could convert phenylacetic acid to 2-oxyphenylacetic acid by hydroxylation[12]. Transfomation of alkaloids and steroids by *S. commune* has, however, not yet been reported.

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