BIOSYNTHESIS OF MONACOLINS: CONVERSION OF MONACOLIN J TO MONACOLIN K (MEVINOLIN)

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Monacolins, which include monacolins J, K (mevinolin), L, 4a,5-dihydromonacolin L and 3α-hydroxy-3,5-dihydromonacolin L (Fig. 1), are metabolites of *Monascus ruber*[1~6]. These compounds are potent inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme of cholesterol biosynthesis[6].

Previous studies have shown the conversion of 4a,5-dihydromonacolin L into 3α-hydroxy-3,5-dihydromonacolin L and monacolin L, and hydroxylation of monacolin L to monacolin J by *M. ruber*[7,8]. The present communication deals with the transformation of monacolin J to monacolin K by a cell-free extract of *M. ruber* and by living cells of *Paecilomyces viridis*. The latter fungus is known to produce compactin (ML-236B) analogs, another family of HMG-CoA reductase inhibitors[6].

M. ruber M4681 was grown aerobically in a medium containing glycerol 7%, glucose 3%, meat extract 3%, peptone 0.8%, $NaNO_3$ 0.2% and $MgSO_4 \cdot 7H_2O$ 0.1% at 25°C for 2 days. The culture broth (100 ml) was filtered and the mycelia were washed with 50 mM potassium phosphate buffer, pH 8.3, containing 2 mM EDTA. The washed mycelia (1.5 g) were ground at 0°C for 5 minutes with 3 g of alumina and 0.8 ml of 50 mM potassium phosphate buffer, pH 8.3, containing 2 mM EDTA, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride and 0.25 M sucrose (PS solution). The slurry was mixed with 6 ml of PS solution and centrifuged at 1,500 × *g* for 20 minutes. The resultant supernatant (cell-free extract) contained 15.0 mg protein/ml as determined by the method of BRADFORD[9].

A mixture (1 ml) containing 100 mM potassium phosphate buffer, pH 7.0, 0.1 mg/ml of monacolin J (acid form, obtained as described previously[4]) and 1.0 mg protein of the cell-free extract was incubated at 25°C for 6 hours with gentle shaking. Incubation was terminated with TFA (pH 2) and the mixture was extracted with 1 ml of ethyl acetate (three times). The solvent layer was evaporated to dryness under reduced pressure and submitted to HPLC using a Silica ODS column (LiChrospher RP-18, 4.0 × 250 mm), which was developed with a mixture of acetonitrile - 0.1% H_3PO_4 (55:45). The results showed that 6% of monacolin J was converted to monacolin K. No detectable monacolin K was found when incubated in the absence of monacolin J.

P. viridis L-63 was grown at 25°C for 4 days in a medium containing glycerol 7% glucose 3%, soy bean meal 3%, peptone 0.8%, $NaNO_3$ 0.2% and $MgSO_4 \cdot 7H_2O$ 0.1%. The mycelia were obtained by centrifugation and suspended in 10 ml of 50 mM phosphate buffer, pH 7.0. The mycelial suspension was incubated at 25°C with monacolin J (acid form,

Fig. 1. Monacolin analogs and their biosynthesis.

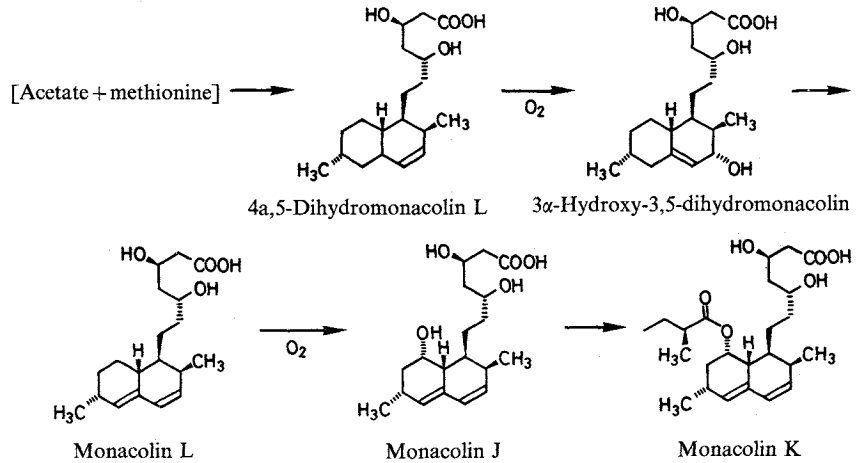
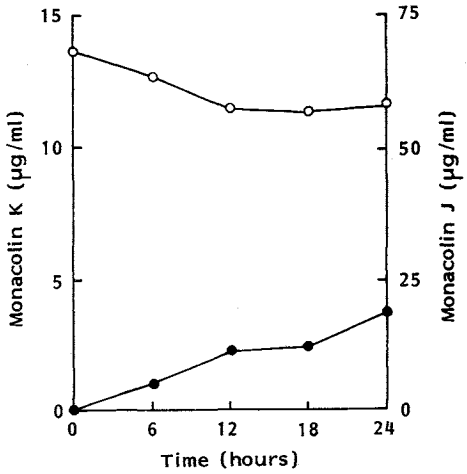


Fig. 2. Conversion of monacolin J to monacolin K by *Paecilomyces viridis* L-69.

○ Monacolin J, ● monacolin K.



Mycelia of *P. viridis* L-69 were incubated with monacolin J as described in the text and analyzed by HPLC.

65 μg/ml), and aliquots of 0.5 ml of the mixture were submitted to the analysis by HPLC as described above. The results demonstrated that ~4 μg/ml of monacolin K was produced at the expense of ~10 μg/ml of monacolin J after 24 hours (Fig. 2). No monacolin K was detectable when incubated in the absence of monacolin J.

The present experiments have shown that monacolin K is derived from monacolin J. One possible mechanism for this conversion is esterification of monacolin J with α-methylbutyryl-CoA, resulting in the formation of monacolin K. Another possibility is that monacolin J is first esterified with α-methylacetoacetyl-CoA, followed by reduction, dehydration, and reduction of the side chain ester moiety. This mechanism, which is analogous to the one for fatty acid biosynthesis, is supported by the fact that monacolin X was isolated from a strain of *M. ruber*[3]. Further study is needed to identify the nature of the conversion of monacolin J to monacolin K.

P. viridis L-69 produces ML-236A and compactin (ML-236B), which are structurally related to monacolin J and monacolin K, respectively[6]. Accordingly, it is reasonable that monacolin J was converted to monacolin K by *P. viridis* L-69, although this fungus produces no detectable amounts of monacolin K without exogenously added precursors.

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