MONACOLIN K, A NEW HYPOCHOLESTEROLEMIC AGENT PRODUCED BY A *MONASCUS* SPECIES

Sir:

In previous papers from our laboratory, ML-236B, a metabolite of *Penicillium citrinum* that was isolated as an inhibitor of cholesterol synthesis[1], was shown to have hypocholesterolemic activity in several animal species[2,3]. Further work in this laboratory to search for microbial metabolites having cholesterol-lowering activity led to the isolation of a new active compound (designated as monacolin K) produced by a *Monascus* species. The present paper describes isolation, physical and chemical properties and hypocholesterolemic effects of monacolin K.

The strain of *Monascus* employed in the production of monacolin K, which was isolated from a food sample collected in Thailand, was classified as *Monascus ruber* and designated as *Monascus ruber* No. 1005.

M. ruber No. 1005 was grown aerobically at 28°C in a medium containing 6% glucose, 2.5% peptone, 0.5% corn steep liquor (Corn Products Co., U.S.A.) and 0.5% ammonium chloride for 10 days. From the culture filtrate (5 liters), monacolin K was extracted with 5 liters of ethyl acetate at pH 3 and the extract was concentrated *in vacuo* to dryness. The resultant pellet was dissolved in 100 ml of benzene and the insoluble materials were removed by filtration. The filtrate was washed twice with 100 ml of 5% $NaCO_3$ and then mixed with 100 ml of 0.2 N NaOH with stirring at room temperature for 2 hours. The aqueous layers were pooled, adjusted to pH 3 with 6 N HCl and extracted twice with 100 ml of ethyl acetate. The solvent layer was collected and evaporated to dryness, giving 260 mg of an oily substance. This material was dissolved in a small volume of benzene, from which monacolin K was obtained as crystals. The compound was recrystallized from aqueous acetone, giving 87 mg of monacolin K as colorless crystals.

Monacolin K melted at 157~159°C (dec.) and had a $[\alpha]_D^{25}$ value of +307.6° (*c* 1, methanol). The molecular formula, $C_{24}H_{36}O_5$ (Mw 404), was obtained by elemental analysis (Calcd.: C 71.31, H 8.91, O 19.78%; Found C 71.56, H 8.85, O 19.59%) and high resolution mass spectroscopy.

The UV spectrum (methanol) showed maxima at 229, 237 and 246 nm ($E_{1cm}^{1\%}$ 550, 650 and 430, respectively) (Fig. 1). The IR spectrum (KBr) showed absorption bands at 3550, 2970, 1696 and 1220 cm^{-1} (Fig. 2). The ^{13}C-NMR spectrum (CD_3OD) indicated the presence of 2 ester carbonyl carbons (δ 173.29 and 178.16), 4 methyl carbons (δ 12.18, 14.13, 16.62 and 23.39) and methylene and methine carbons (Fig. 3). In addition to peaks at (*m/e*) 404 (M^+), 302 (M−102), 284 (M−120) and 224 (M−180), prominent peaks in the mass spectrum of monacolin K were observed at 198 (M−206), 172 (M−232), 159 (M−245) and 157 (M−247) (Fig. 4). Monacolin K was soluble in methanol, ethanol, acetone, chloroform and benzene but not soluble in *n*-hexane and petroleum ether. The Rf value in TLC (Merck, Kieselgel $60F_{254}$) was 0.47 in dichloromethane - acetone (4: 1).

The LD_{50} of monacolin K in mice (oral administration) was over 1,000 mg/kg. Hypocholesterolemic activity of monacolin K was demonstrated in the two experiments described below. Male Wistar-Imamichi rats (240~280 g) were injected intraventrically with 400 mg/kg of the detergent Triton WR-1339 (Ruger Chemical Co., U.S.A.) and intraperitoneally with 10 mg/kg of monacolin K suspended in saline. Control animals received Triton and saline alone. After 14 hours, the animals were sacrificed and plasma

Fig. 1. UV spectrum of monacolin K (in methanol).

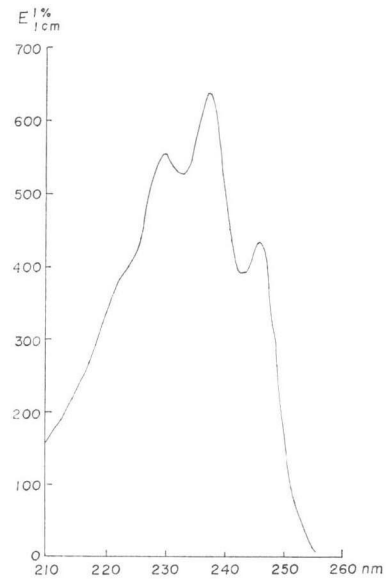


Fig. 2. IR spectrum of monacolin K (KBr).

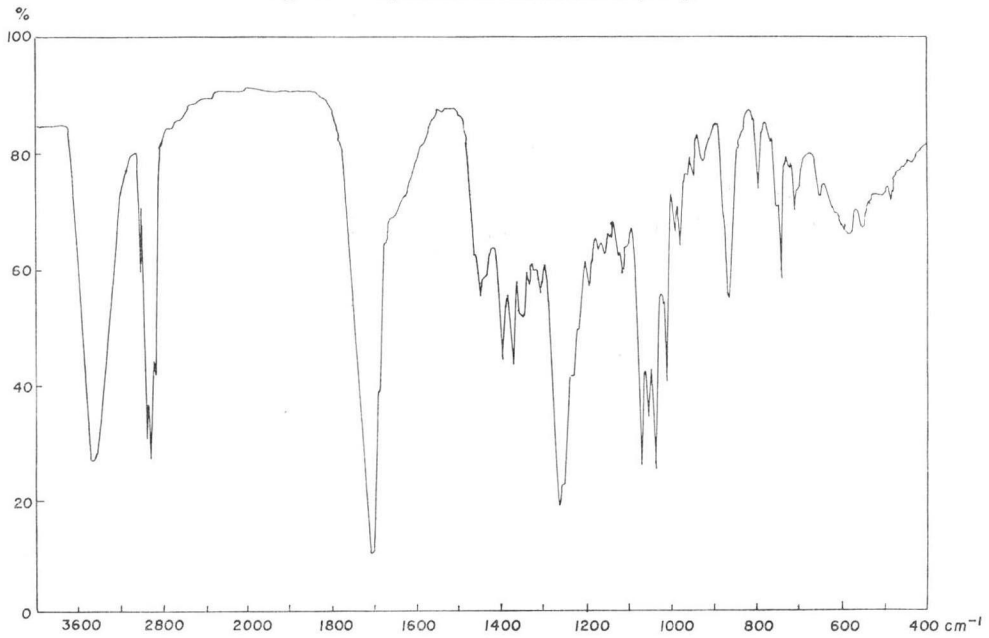
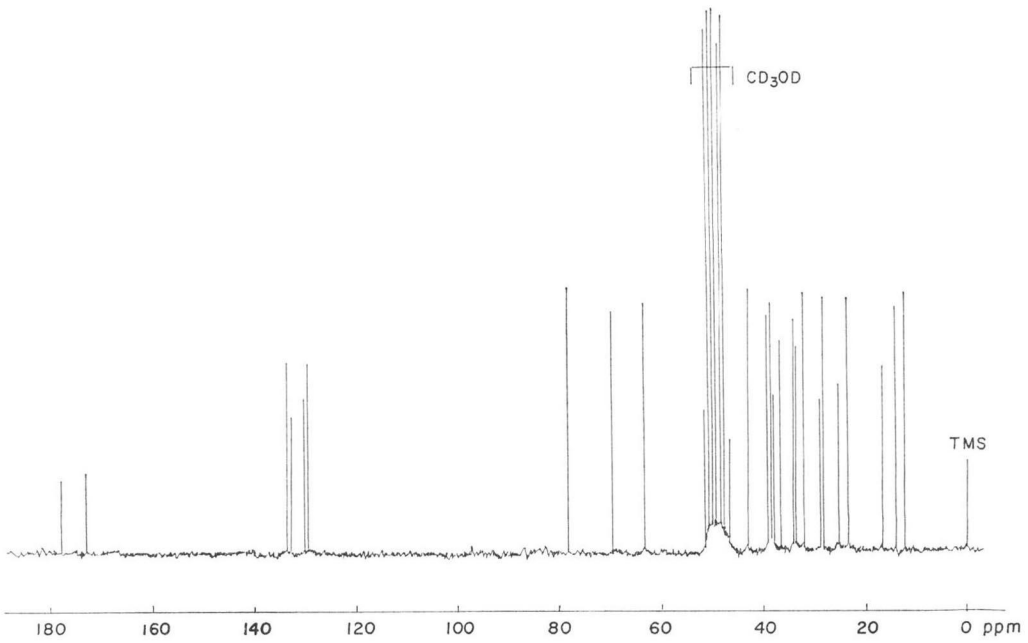


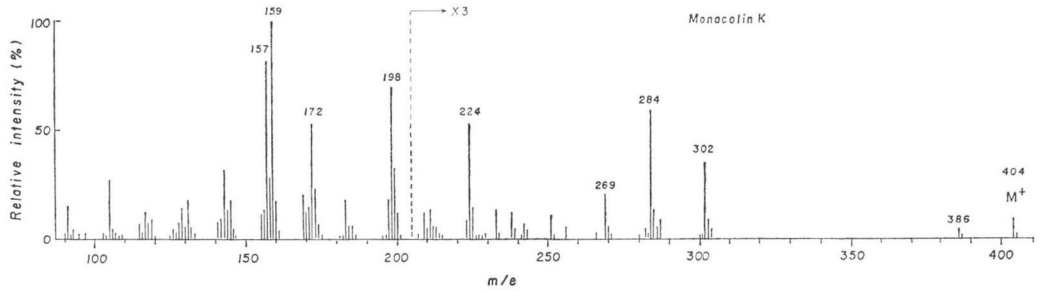
Fig. 3. ^{13}C-NMR spectrum of monacolin K.



cholesterol was determined by a conventional method using Determiner TC 5 (Kyowa Hakko Kogyo Co., Tokyo). Under these conditions, plasma cholesterol levels of the treated animals were reduced by 23.9% (mean for 5 animals, $P < 0.01$).

When monacolin K was orally given to 2 rabbits (New Zealand White, 2.7~2.9 kg) twice a day (at 9 a.m and 5 p.m.) at a dose of 1 mg/kg (2 mg/kg/day) for 5 days, reductions of plasma

Fig. 4. Mass spectrum of monacolin K.



cholesterol levels obtained after 3 and 5 days were 12.3 and 21.6%, respectively, in one animal and 10.1 and 26.2% in the other.

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AKIRA ENDO

Fermentation Laboratory
Tokyo Noko University
Saiwaicho, Fuchu-shi
Tokyo, 183 Japan

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