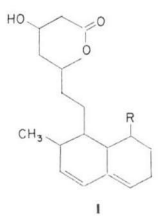
ML-236A, ML-236B, AND ML-236C, NEW INHIBITORS OF CHOLESTEROGENESIS PRODUCED BY *PENICILLIUM CITRINUM*

Sir:

Clinical and nutritional studies have indicated that high cholesterol levels in the blood may be one of the major causes of atherosclerosis and coronary heart disease. In this context the metabolism of cholesterol in liver is of paramount importance because liver seems to be the sole organ supplying serum cholesterol and because the conversion of cholesterol into bile acids in liver is quantitatively the most important pathway for its elimination from the body. These facts suggest that the inhibition of endogenous cholesterol synthesis in liver could lead to a lowering of its level in the blood and several studies have been made for this purpose.[1~4]

In a previous paper from this laboratory[5], citrinin, known as an antibiotic, was isolated from cultures of the fungus *Pythium ultimum* as an inhibitor of cholesterol synthesis in a rat liver enzyme system. Hypocholesterolemic activity of this antibiotic was shown in both liver and serum when administered orally to rats. Further work in this laboratory to detect specific inhibitors of cholesterol synthesis produced by micro-organisms has led to the isolation of three metabolites (I), ML-236A, ML-236B, and ML-236C, produced by *Penicillium citrinum*. The present communication describes the production, isolation and some biochemical and biological activities of these compounds.* Preliminary abstracts of these studies have been published.[6,7]

More recently BROWN *et al.*[8] have isolated ML-236B from cultures of *Penicillium brevicompactum* as an antifungal metabolite (designated compactin).



I

ML-236A R=-OH
ML-236B R=-OCOCH(CH$_3$)CH$_2$CH$_3$
ML-236C R=-H

P. citrinum SANK 18767 was grown aerobically in a medium containing 3% malt extract, 2% glucose and 0.1% peptone in a 6,000-liter fermentor for 96 hours. The culture filtrate (2,900 liters from 3,000-liter culture broth) was concentrated *in vacuo* to 450 liters and the active compounds were extracted with ethyl acetate at pH 4. The extract was concentrated *in vacuo* to dryness and the resultant pellet (327 g) was applied to a column of silica gel in *n*-hexane. ML-236C was first eluted from the column with *n*-hexane - acetone (95: 5) and then ML-236B with *n*-hexane - acetone (85:15), following which ML-236A was eluted with acetone. The active fractions containing the individual metabolites were separately concentrated *in vacuo* to dryness. The dried product containing ML-236A (194 g) was dissolved in ethyl acetate and washed successively with saturated Na_2CO_3 and NaCl solutions. The dried product from the ethyl acetate layer (70 g) was adsorbed on a silica gel column in benzene. After washing the column with benzene - ethyl acetate (8: 2), ML-236A was eluted with benzene - methanol (95: 5), and the active fractions were concentrated to dryness, giving 9 g of ML-236A as a white powder. The dried fraction containing ML-236B (38 g) was dissolved in benzene (500 ml) and the insoluble materials were removed by filtration. The filtrate was concentrated and allowed to stand overnight, and the resultant white crystals were collected by filtration. The compound was recrystallized from benzene and then from ethanol, giving 10.5 g of ML-236B as white crystals. The ML-236C-containing product (3.2 g) was dissolved in dichloromethane and then applied to a column of silica gel. The column was washed with dichloromethane and then developed with dichloromethane - ethyl acetate (95: 5). The active eluate was concentrated to dryness, giving 2.1 g of ML-236C as an oily substance.

Fig. 1 shows the inhibitory effects of the three metabolites on cholesterol synthesis; ^{14}C-acetate incorporation into digitonin-precipitable sterols in a rat liver enzyme system was measured by the method of KNAUSS *et al.*[9] Of the three compounds, the major metabolite ML-236B was

* The structures of these metabolites were determined by a combination of X-ray crystallographic, spectroscopic, and chemical methods by Dr. C. TAMURA (Central Research Laboratories) and Dr. A. TERAHARA (Fermentation Research Laboratories), Sankyo Co., Ltd. Details of these studies will be published elsewhere.

Table 1. Hypocholesterolemic activity of ML-236A, ML-236B and ML-236C in rats after single oral administration[a)]

Experiment	Compound	Dose mg/kg	Time after administration, hrs	Serum cholesterol	
				mg/100 ml[b)]	% Reduction
1	ML-236A	5	3	61.7±4.1	14.1 (P<0.01)[c)]
		20		58.9±4.3	17.4 (P<0.01)
	Control			71.3±4.0	
2	ML-236A	5	18	61.7±5.6	13.0 (P<0.05)
		20		60.8±6.2	14.2 (P<0.02)
	Control			70.9±4.9	
3	ML-236B	5	3	51.3±5.1	26.3 (P<0.001)
		20		48.7±4.7	30.0 (P<0.001)
	Control			69.6±4.9	
4	ML-236B	5	18	57.9±4.6	19.0 (P<0.01)
		20		56.6±5.2	20.8 (P<0.01)
	Control			71.5±4.8	
5	ML-236C	5	3	57.0±4.2	16.8 (P<0.01)
		20		54.1±2.8	21.0 (P<0.001)
	Control			68.5±6.1	

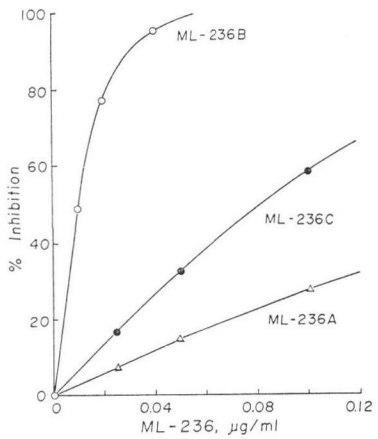
a) Groups of 5 animals (Wistar-Imamichi male rats, weighing about 230 g), fed a commercial rat chow (Oriental Yeast Co., Tokyo), received orally the test compounds suspended in 0.5 ml of saline. A control group received 0.5 ml of saline. The animals were fasted and blood was taken after 3 or 18 hours by cardiac puncture. The total serum cholesterol was determined by a conventional method.

b) Values are mean ± standard deviation.

c) P value compared to the control in the same experiments.

Fig. 1. Inhibition by ML-236A, ML-236B and ML-236C of ^{14}C-acetate conversion into digitonin-precipitable sterols in a rat liver enzyme system.

Experiments were carried out as described previously[5)] by the method of KNAUSS *et al.*[9)]



the most inhibitory, reducing cholesterol synthesis to 50% of control at a concentration of 0.01 μg/ml (2.6×10^{-8}M). ML-236A was the least effective of the three metabolites. The concentrations required for 50% inhibition were 0.08 μg/ml (2.8×10^{-7}M) for ML-236C and 0.18 μg/ml (5.9×10^{-7}M) for ML-236A. Preliminary experiments showed that these compounds had no detectable effect on the conversion of ^{14}C-mevalonate to digitonin-precipitable sterols at concentrations which inhibited sterol synthesis from acetate by more than 90%, indicating the site(s) of their action to be between acetate and mevalonate in the pathway of cholesterol synthesis.

Acute toxicity of these three substances was 500 mg/kg of body weight or more when administered intraperitoneally to mice and was more than 2,000 mg/kg when administered orally.

Table 1 shows the effects of ML-236A, B and C on the serum cholesterol level in normal rats after a single oral administration. At doses of 5 and 20 mg/kg, all three compounds produced reduction of serum cholesterol levels at 3 hours after their administration. ML-236B lowered the levels of serum cholesterol by approximately 30% at a dose of 20 mg/kg. The data in Table 1 also show that the hypocholesterolemic effect of ML-236A and ML-236B lasted at least for 18 hours under the conditions described. Details of the lipid-lowering activity of these metabolites in several species of animals includ-

ing rats, hens and dogs will be reported elsewhere.

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