NOTES

CITRININ, AN INHIBITOR OF CHOLESTEROL SYNTHESIS

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A compound active against sterol biosynthesis was isolated from the culture filtrate of the fungus *Pythium ultimum* IAM 6073 and identified as citrinin which had been isolated as an antibiotic.[1]

The sequence of reactions by which cholesterol is formed from acetyl-CoA is one of the most complex biosynthetic pathways in eukaryotic cells. In every vertebrate species so far studied, potent activity in cholesterol synthesis can be detected in the liver which seems to be the sole organ supplying plasma cholesterol. Because of the importance of the plasma cholesterol level in atherosclerosis, several approaches have been made to control it by decreasing cholesterol synthesis in the liver.[2~4] The inhibition of endogenous cholesterol synthesis could lead to a lowering of its level in the plasma. This communication describes inhibition by citrinin of cholesterol and ergosterol synthesis and its hypocholesterolemic activity in rats.

The enzymatic synthesis of cholesterol was assayed by measuring the radioactive nonsaponifiable products derived from ^{14}C-acetate in a rat liver enzyme system by the method of KNAUSS *et al.*[5] Culture filtrates of microorganisms including fungi, bacteria, and actinomycetes were tested for inhibitory activity in this assay system, and *Pythium ultimum* was selected as one of the producers of an inhibitor.

The fungus was cultured aerobically in a medium containing 2.0% glucose, 2% peptone and 0.3% corn steep liquor in a 30-liter fermentor for 4 days. The culture filtrate was adjusted to pH 2, and the active compound was extracted with benzene. The extract was concentrated *in vacuo*, resulting in the formation of yellowish crystals of the active compound. The compound was recrystallized from hot chloroform.

Calcd.: C 62.39, H 5.64, O 31.97
Found: C 62.39, H 5.58, O 32.03

The chemical structure of 4,6-dihydro-8-hydroxy-3,4,5-trimethyl-6-oxo-3H-2-benzopyran-7 carboxylic acid was suggested by n.m.r. spectrum. The identity with citrinin was proven by comparison with authentic samples (kindly supplied by Dr. S. UDAGAWA, National Institute of Hygienic Science, Tokyo).

As shown in Fig. 1, citrinin strongly inhibited cholesterol synthesis from ^{14}C-acetate. The concentration at which cholesterol synthesis was inhibited by 50% was about 8.5 mcg/ml (3.4×10^{-5}M). Under the same conditions, ^{14}C-acetate incorporation into fatty acid fraction was also reduced by citrinin but to a lesser extent. The concentration of the inhibitor required for 50% inhibition of fatty acid synthesis was approximately 50 mcg/ml. Citrinin was also inhibitory in the sterol synthesizing system of the yeast *Saccharomyces cerevisiae* in which ^{14}C-acetate incorporation into nonsaponifiable lipids was determined as described by KAWAGUCHI (Fig. 1).[6] At a concentration of 100 mcg/ml, the inhibitor reduced the sterol synthesis by approximately 50%.

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Fig. 1. Inhibition by citrinin of ^{14}C-acetate incorporation into nonsaponifiable and fatty acid fractions in rat liver and yeast enzyme system.

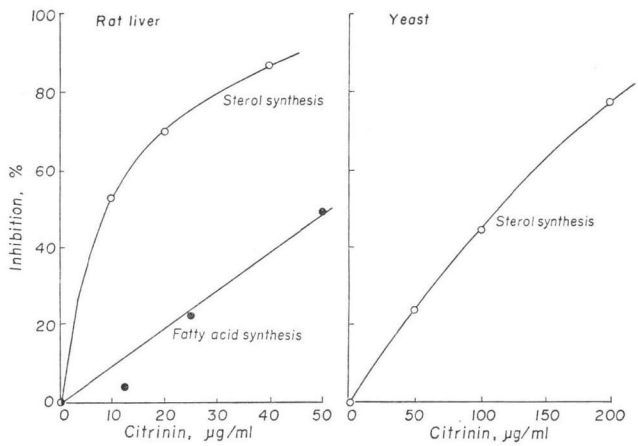

Table 1. Effect of citrinin and clofibrate on plasma cholesterol level in rats.[a)]

Experiment	Compound	Time after administration (hrs.)	Dose (mg/kg)	Plasma cholesterol	
				mg/100 ml[b)]	% Reduction
1	Citrinin	3	2.0	83.5±6.7	
			5.0	85.8±8.1	
	Control			84.9±6.5	
2	Clofibrate	3	100	63.4±5.2	8.1 (P>0.05)[c)]
			200	63.0±6.6	8.9 (P>0.05)
	Control			69.0±4.6	
3	Citrinin	18	2.0	75.1±4.9	8.4 (P>0.05)
			5.0	61.8±6.3	24.6 (P<0.001)
	Clofibrate		100	62.1±5.1	24.4 (P<0.001)
			200	46.4±1.8	43.4 (P<0.001)
	Control			82.0±4.4	

a) Groups of 5 animals (Wistar-Imamichi male rats), weighing about 230 g (range, 220~240 g) were fasted for 3 hours and the test compound was then administered orally 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 plasma cholesterol was determined by a conventional method.

b) Values are mean ±standard deviation.

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

Table 2. Hypolipidemic activity of citrinin in rats[a)].

Experiment	% Citrinin in diet	Body weight[b)] (g)	Liver weight[b)] (g)	% Reduction in plasma		% Reduction in liver	
				Cholesterol	Triglycerides	Cholesterol	Triglycerides
1	0.01	161±11.3	10.2±1.5	13.1 P<0.01	5	5	55.5 P<0.001
	Control	159±10.3	10.0±1.1				
2	0.025	156±6.6	9.7±1.4	13.5 P<0.01	36.0 P<0.01	19.0 P<0.01	42.3 P<0.01
	Control	171±8.0	9.6±0.8				

a) Groups of 5 animals (Wistar-Imamichi male rats) weighing about 120 g at the start of the experiments were used. Citrinin was mixed with ground commercial rat chow (Oriental Yeast Co., Tokyo) containing 38% corn starch, 10% wheat starch, 25% milk casein, 8% powdered filter paper, 6% salt mixture, 6% salad oil (Benibana), 5% sucrose and 2% vitamin mixture. A control group received ground chow without citrinin. All animals were allowed free access to diet and water. After 7 days, the animals were fasted for 6 hours and blood samples were then obtained by cardiac puncture. The plasma and liver were analyzed for total cholesterol and triglycerides by conventional methods.

b) At the end of experiment. Values are mean ± standard deviation.

citrinin in rats, clofibrate (*p*-chlorophenoxyisobutyrate) was used as the reference drug. This drug is most widely used today in the treatment of hyperlipidemia but its mechanism of action is not clear.[7] Table 1 shows the effect of citrinin on the serum cholesterol level in rats after a single oral administration. Citrinin showed no cholesterol-lowering effect at 3 hours after its administration at the dose of either 2.0 and 5.0 mg/kg. However, the citrinin administration was effective at 18 hours. Hypocholesterolemic activity of citrinin at 5.0 mg/kg was comparable to the effect of clofibrate at 100 mg/kg.

It is evident from Table 2 that citrinin administration to normal rats for 7 days considerably lowered the plasma and liver cholesterol levels. The effect (on the triglyceride level) was much more pronounced both in plasma and liver. The

body weight gain in rats was not affected at 0.01% citrinin in the diet but was slightly reduced at 0.025%. Unlike clofibrate which is known to be hepatomegalic[7], citrinin did not cause an increase in liver weight (Table 2). The decrease in plasma and liver cholesterol levels can probably be attributed to the inhibition of hepatic cholesterogenesis by citrinin.

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