Effect of Inhibitors of Cholesterol Biosynthesis on Yeast Growth (34581)

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The results obtained in several laboratories have demonstrated that the steps involved in the formation, from simple precursors, of cholesterol in mammalian systems and sterols in yeasts are very similar (1, 2). For this reason it seems logical that potential inhibitors of cholesterol biosynthesis may be studied in many ways for biological activity. One of the primary screening methods used to detect substances which inhibit cholesterol synthesis employs a rat liver homogenate system. In this procedure the effect of the test compound on the incorporation of radioactive acetate or mevalonate into labeled cholesterol is measured (3). Microorganisms have also been used as a screening device for detecting potential inhibitors of cholesterol synthesis. Wright (4) employed *Lactobacillus acidophilus* and Stewart and Woolley (5) *L. acidophilus* and *Saccharomyces cerevisiae* as test systems for screening compounds as possible antimetabolites of mevalonic acid. More recently Johnson and Jasmin (6) have described a procedure in which *Tetrahymena pyriformis* were used as assay organisms. Aaronson *et al.* (7) have also studied the effect of cholesterol synthesis inhibitors in *T. pyriformis*, as well as in other protozoa, and have suggested that such organisms may be useful for the screening of compounds as inhibitors of sterol synthesis. There is only limited information available, however, regarding the comparative activities of compounds in these various test systems. The purpose of the present work was to directly compare the activities of several compounds as inhibitors of sterol synthesis, using an *in vitro* rat liver homogenate system, and as inhibitors of yeast growth.

Methods. Five compounds with widely differing activities as inhibitors of the synthesis of cholesterol by rat liver homogenates were selected for testing as possible inhibitors of yeast growth. These compounds were 2, 3-dimethyl-5-phenyl-2-*trans*-4-*trans*-pentadienoic acid, 3-methyl-5-phenyl-2-*trans*-4-*trans*-pentadienoic acid, 3β-dimethylsulfonio-5-cholestene chloride (8), Pimaricin, and 3-hydroxy-3,7,11-trimethyldodecanoic acid (9).

The activities of the compounds as inhibitors of cholesterol biosynthesis were determined using an *in vitro* rat liver homogenate system similar to that described by Bucher and McGarrahan (10). The complete system contained, in a total volume of 9.5 ml, the test compound or a control solution, 5 ml of liver homogenate, 0.1 *M* potassium phosphate buffer (pH 7.2), 50 μmoles of Mg^{2+}, 255 μmoles of niacinamide, 1.8 μmoles of ATP, 1.5 μmoles of DPN, and 2 μCi ($\leq$ 1 μmole) sodium acetate-1-^{14}C. Following incubation under a gas phase of 100% oxygen at 37° for 4.5 hr, the flask contents were hydrolyzed with base, the nonsaponifiable materials extracted using petroleum ether and the sterol digitonides prepared. The digitonides were dissolved in glacial acetic acid and aliquots were taken for cholesterol determination using the reagents suggested by Abell *et al.* (11), and for ^{14}C assay using a Packard Instrument Company Tri-Carb liquid scintillation spectrometer. The specific activity (cpm/mg of cholesterol) was calculated and the effect of the test compound was expressed as the percentage change from the control value. The procedures used for determining inhibition of yeast growth were described previously (12). All four yeast species, *Hansenula anomala, Candida lipolytica, Saccharomyces cerevisiae* and *Geotrichum candidum,* were selected on the basis of their containing significant quantities of lipids.

Results and Discussion. The relative activity of the test compounds as inhibitors of *in vitro* cholesterol synthesis is shown by the results presented in Table I. As indicated previously, the compounds were selected so that structures having a wide range of activi-

TABLE I. Assay of Test Compounds for Inhibition of Cholesterol Biosynthesis.

Test compound	Amount (μmoles/flask)	Inhibition[a] (%)
2,3-Dimethyl-5-phenyl-2-*trans*-4-*trans*-pentadienoic acid	0.12	51
	0.25	84
3β-Dimethylsulfonio-5-cholestene chloride	0.25	68
	0.50	80
3-Hydroxy-3,7,11-trimethyldodecanoic acid	0.5	45
	1.0	64
3-Methyl-5-phenyl-2-*trans*-4-*trans*-pentadienoic acid	1.3	31
	2.7	67
Pimaricin	2.5	7
	5.0	11

[a] Group means of cholesterol digitonide specific activity from control flasks ranged between 10,000 and 15,000 cpm/mg.

ty would be represented. The most effective inhibitor, 2, 3-dimethyl-5-phenyl-2-*trans*-4-*trans*-pentadienoic acid, was approximately 20 times as active as the 3-methyl acid and 100 times as active as Pimaricin, which had the least activity in this test system.

In Table II are presented the results of the yeast growth inhibition studies. 3β-Dimethylsulfonio-5-cholestene chloride had the greatest activity in this assay. Pimaricin was slightly less active and 3-hydroxy-3,7,11-trimethyldodecanoic acid had a very low order of growth inhibitory activity. Some differences were noted in the susceptibility of the different strains of yeasts studied to the inhibitory action of the various compounds. The two pentadienoic acids were most effective in reducing the growth of *C. lipolytica*, while Pimaricin was least effective against

TABLE II. Assay of Test Compounds for Inhibition of Yeast Growth.[a]

Test compound	Amount (μg/ml)	Growth inhibition (%)			
		G. candidum	*C. lipolytica*	*H. anomala*	*S. cerevisiae*
2,3-Dimethyl-5-phenyl-2-*trans*-4-*trans*-pentadienoic acid	2.5	38	0	7	0
	5.0	31	54	24	0
	10.0	48	82	42	48
3β-Dimethylsulfonio-5-cholestene chloride	2.5	0	100	7	100
	5.0	33	100	24	100
	10.0	91	100	56	100
3-Hydroxy-3,7,11-trimethyldodecanoic acid	2.5	0	0	0	0
	5.0	0	0	0	0
	10.0	1	29	0	0
3-Methyl-5-phenyl-2-*trans*-4-*trans*-pentadienoic acid	2.5	15	21	14	0
	5.0	24	86	22	0
	10.0	49	88	58	44
Pimaricin	2.5	0	0	0	96
	5.0	0	15	77	100
	10.0	90	79	100	100

[a] Control flasks had the following levels of growth (expressed as optical density readings): *G. candidum*, 0.930; *C. lipolytica*, 0.440; *H. anomala*, 1.00; and *S. cerevisiae*, 0.490.

TABLE III. Comparative Activity of Test Compounds as Inhibitors of Cholesterol Synthesis and Yeast Growth.[a]

Test compound	Relative inhibition of	
	Cholesterol synthesis	Yeast growth
2,3-Dimethyl-5-phenyl-2-*trans*-4-*trans*-pentadienoic acid	100	45
3β-Dimethylsulfonio-5-cholestene chloride	70	100
3-Hydroxy-3,7,11-trimethyldodecanoic acid	20	5
3-Methyl-5-phenyl-2-*trans*-4-*trans*-pentadienoic acid	5	50
Pimaricin	1	80

[a] The relative activity of the test compounds has been interpolated on a scale of 0 to 100.

this strain. The most active growth inhibitor, 3β-dimethylsulfonio-5-cholestene chloride, was most effective against *C. lipolytica* and *S. cerevisiae*, and least active with *H. anomala*.

In evaluating the relative effectiveness of the test compounds as inhibitors of yeast growth, consideration was given to the average inhibition observed with each compound against all four strains of yeasts. The relative effectiveness of the five test compounds as inhibitors of both cholesterol synthesis and yeast growth is compared in Table III, with the respective inhibitions being graded on a scale of 0 to 100. On this comparative basis 2, 3-dimethyl-5-phenyl-2-*trans*-4-*trans*-pentadienoic acid was approximately 20 times as active as the monomethylpentadienoic acid analogue in inhibiting cholesterol biosynthesis, but was actually slightly less active than the latter compound in reducing yeast growth. Likewise, the dodecanoic acid analogue was four times as active as 3-methyl-5-phenyl-2-*trans*-4-*trans*-pentadienoic acid in reducing cholesterol synthesis but had only one-tenth as much activity in inhibiting yeast growth. On the other hand Pimaricin, which was essentially devoid of cholesterol synthesis inhibitory activity, was very effective in reducing yeast growth.

From these results, it is apparent that there is no correlation between the inhibitory activities of the compounds tested in these two systems. While it is possible that different results might have been obtained with other microorganisms, it would seem that caution should be exercised in attempting to relate the effectiveness of a compound in reducing the growth of yeasts to its potential effect on cholesterol synthesis in mammalian systems.

Summary. No correlation was found between the ability of compounds to inhibit cholesterol synthesis by rat liver homogenates and their inhibiting effect on the growth of yeasts.

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