COMPETITIVE INHIBITION OF SQUALENE SYNTHETASE BY SQUALESTATIN 1

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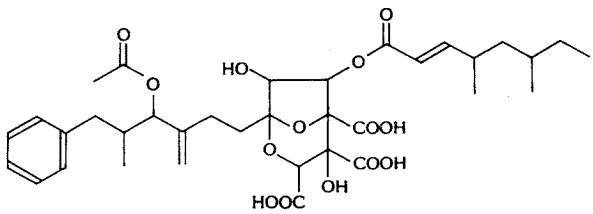
Recently, 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase inhibitors have received great attention as safe and effective cholesterol-lowering drugs[1]. However, cholesterol is synthesized from acetyl-CoA *via* a series of more than 20 enzymatic reactions. Among these, squalene synthetase, as well as HMG-CoA reductase, play key roles in the regulation of the pathway[2,3]. Squalene synthetase (EC 2.5.1.21) is a microsomal enzyme which catalyzes the two sequential reactions: head-to-head condensation of farnesyl pyrophosphate (FPP) to form presqualene pyrophosphate (PSPP, Fig. 1) and reduction of PSPP to squalene, thus yielding one molecule of squalene from two molecules of FPP[4].

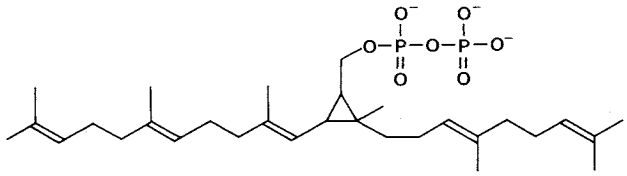
Recently, several compounds that inhibit squalene synthetase (named squalestatins) have been isolated from *Phoma* sp. by DAWSON *et al.*[5]. One of these metabolites, squalestatin 1 (Fig. 1), was demonstrated to be effective in lowering plasma cholesterol in marmosets[6]. While screening for squalene synthetase inhibitors of microbial origin we have isolated the same compound from *Setosphaeria khartoumensis*. This communication deals with the isolation and mechanism of action of squelestatin 1.

Setosphaeria khartoumensis L1685 was grown aerobically at 25°C for 14 days in a medium containing 2% corn strach, 0.5% glucose, 2.5% soybean meal, 0.5% Farmamedia (Procter & Gamble Oilseed Products Co., U.S.A.), 0.1% Staminol (Sapporo Beer Co., Japan) and 1% $CaCO_3$ in flasks. Culture filtrate (8 liters) was mixed with 400 ml of Diaion HP-20 and adjusted to pH 3 with HCl. After stirring overnight at 4°C, the resin was packed in a column (36 × 390 mm) and washed with 2 liters of 25 mM potassium phosphate (pH 8.0). Active compound was eluted with 400 ml of 50% aq methanol. The active fractions were concentrated and resultant oily residue was suspended in 1 liter of water. The suspension was adjusted to pH 3 and extracted with ethyl acetate. The organic phase was dried over Na_2SO_4 and concentrated to give 1.72 g of residue. After dissolving this material in 1.7 ml of methanol, active substance (22 mg) was isolated by a preparative HPLC (Inertsil PREP-ODS (30 × 250 mm, GL Sciences Co, Japan) developed

Fig. 1. The structures of squalestatin 1 and PSPP.



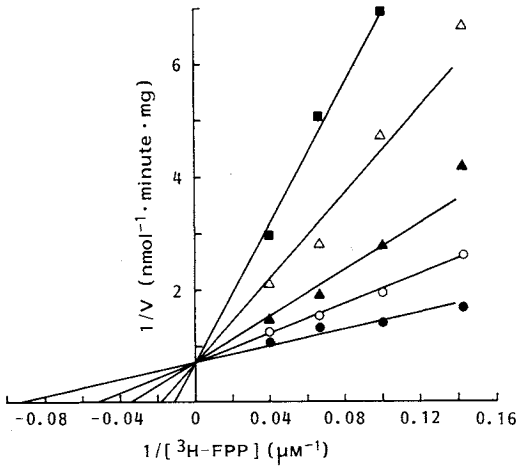
Squalestatin 1



Presqualene pyrophosphate

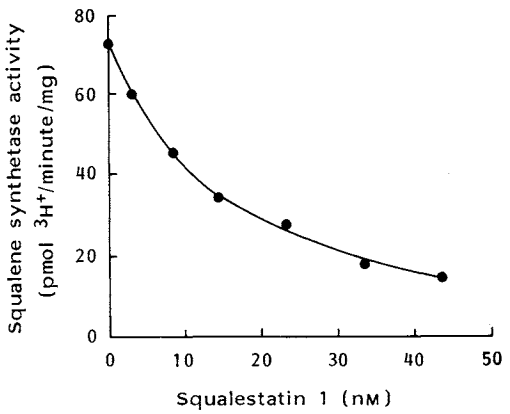
Fig. 2. Lineweaver-Burk plot for the inhibition of the overall squalene synthetase reaction by squalestatin 1.

Concentrations of squalestatin 1 were 0 (●), 1.45 (○), 2.9 (▲), 5.8 (△) and 10.1 nM (■).



Overall squalene synthetase reaction was determined by the formation of squalene from varying concentrations of [^{3}H]FPP[8]. Reaction was started by adding 15 μg of microsomal protein and continued at 37°C under nitrogen for 20 minutes. Each value represents the average of duplicate determinations.

Fig. 3. Inhibition of the first partial squalene synthetase reaction by squalestatin 1.



The first partial squalene synthetase reaction was determined by measuring $^3H^+$ released in the absence of NADPH[4] using 10 μM [^{3}H]FPP (20 Ci/mol) and 50 μg of microsomal protein. Each value represents the average of duplicate determinations.

with acetonitrile - 0.1% phosphoric acid (3 : 2)). The active compound was identified to be squalestatin 1 by a combination of chemical and spectroscopic analyses (MW 690, $C_{35}H_{46}O_{14}$) (Fig. 1).

Table 1. Effects of squalestatin 1 on the conversion of [^{3}H]FPP and [^{3}H]PSPP to squalene.

Substrate	Squalestatin 1 (nM)	Squalene formed (pmol/minute/mg)
[^{3}H]FPP	0	23.4
	33.4	1.1
[^{3}H]PSPP	0	11.7
	33.4	1.0

The second partial squalene synthetase reaction was determined using 50 μg microsomal protein and [^{3}H]PSPP[7] (0.56 μM, 30 Ci/mol) as a substrate[4]. Other conditions were identical to those in Fig. 2. Where indicated, [^{3}H]FPP (0.56 μM, 20 Ci/mol) was incubated in place of [^{3}H]PSPP to assay overall reaction. Each value represents the average of duplicate determinations.

Squalestatin 1 inhibited squalene synthetase (overall reaction) by 50% at 3.0 nM under the conditions employed in Fig. 2 (10 μM [^{3}H]FPP). The inhibition was competitive with respect to [^{3}H]FPP with a *Ki* value of 1.6 nM (Fig. 2). Effects of squalestatin 1 on the two partial squalene synthetase reactions were determined. The first partial reaction, assayed by measuring $^3H^+$ released from [^{3}H]FPP in the absence of NADPH, was inhibited 50% by squalestatin 1 at 14 nM (Fig. 3). The second partial reaction, determined by measuring squalene formation from [^{3}H]PSPP in the presence of NADPH, was also found to be sensitive to squalestatin 1; inhibition was >90% at 33.4 nM (Table 1).

In summary, squalestatin 1 was isolated from *Setosphaeria khartoumensis*. Consistently with the observation by others[6], squalestatin 1 was shown to be a potent inhibitor of squalene synthetase. This inhibition was competitive with respect to the substrate FPP. Both the first and the second steps in the reaction appeared to be inhibited by squalestatin 1. As shown in Fig. 1, squalestatin 1 is structurally related to PSPP, the product of the first step, in that each compound consists of one hydrophilic moiety connected with two hydrophobic chains. Detailed mechanisms for the inhibition of these two partial reactions are to be studied further.

Acknowledgments

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