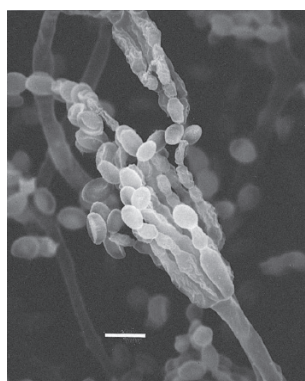


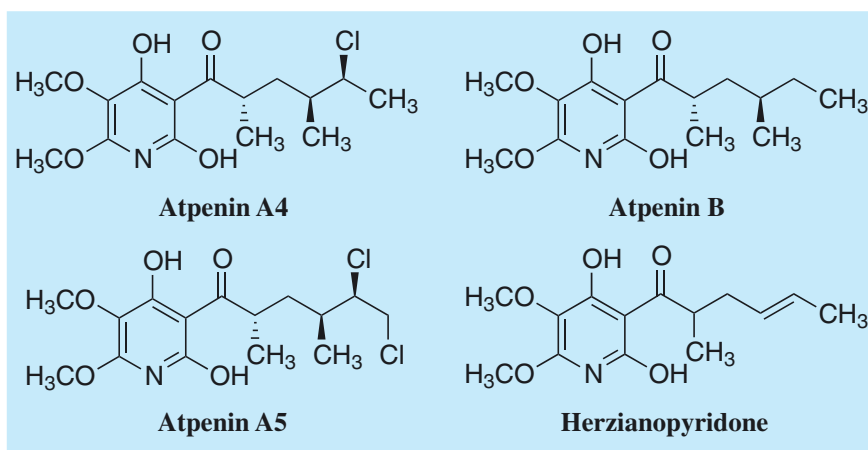
Atpenin[©]

1. Discovery, producing organism and structure¹⁻⁶⁾

Atpenins were isolated from the culture broth of *Penicillium oxalicum* FO-125. Atpenins inhibited growth of both fatty acid synthase (FAS) deficient (A-1) and acyl-CoA synthetase I (ACSI) deficient (L-7) mutants of *Candida lipolytica*, and also inhibited incorporation of long chain fatty acids into lipid fractions in Raji cells. The absolute configuration of atpenin A4 was confirmed by X-ray crystallographic analysis²⁾. The total synthesis of atpenins was reported by Quéguiner's group³⁾ and Ōmura's group^{10,11)} (See Appendix-I). Recently, atpenins and their structurally related compound, harzianopyridone, have been recognized to have potent mitochondrial complex II (succinate-ubiquinone oxidoreductase) inhibitory activity⁵⁾. The co-crystallization study of atpenin A5 and *E. coli* complex II provided a new information for the quinone-binding site.



Penicillium sp. FO-125
(*Penicillium oxalicum* FO-125)
Bar: 5 μm



2. Physical data

White powder. C₁₅H₂₁NO₅Cl₂; mol wt 366.24. Sol. in DMSO, MeOH, EtOH, CHCl₃. Insol. in H₂O.

3. Biological activity^{1,7-9)}

1) Antimicrobial activity¹⁾

Test organisms	MIC (μg/ml)		
	Atpenin A4	Atpenin A5	Atpenin B
<i>Staphylococcus aureus</i> FDA 209P	>100	>100	>100
<i>Bacillus subtilis</i> PCI 219	>100	>100	>100
<i>Micrococcus luteus</i> PCI 1001	>100	>100	>100
<i>Mycobacterium smegmatis</i> ATCC 607	>100	>100	>100
<i>Escherichia coli</i> NIHJ JC-2 IFO 12734	>100	>100	>100
<i>Pseudomonas aeruginosa</i> P-3 KB 105	>100	>100	>100
<i>Candida albicans</i> KF 1	>100	>100	>100
<i>C. lipolytica</i> L7 KF 236	6.25	0.4	1.56
<i>Saccharomyces cerevisiae</i> KF 26	>100	>100	>100
<i>Aspergillus niger</i> ATCC 6275	50	12.5	50
<i>Pyricularia oryzae</i> KF180	3.12	3.12	0.78
<i>Mucor racemosus</i> IFO 4581	>100	>100	>100
<i>Trichophyton mentagrophytes</i> KF 213	6.25	0.05	6.25
<i>T. interdigitale</i> KF 62	12.5	0.05	6.25
<i>Microsporium gypseum</i> KF 64	3.12	0.78	6.25

Bacteria: Sensitivity Disc Agar (Nissui); 37°C, 20 hrs. Fungi: Potato-glucose agar; 27°C, 72 hrs.

2) Atpenin B decreased cellular ATP levels in Raji cells with an IC_{50} value of 20 nM, suggesting inhibition of an ATP-generating system by the drug⁷⁾.

3) Atpenins inhibited nematode and mammalian mitochondrial complex II⁸⁾.

Species Activities		IC_{50} (μ M)		
		Atpenin A4	Atpenin A5	Harzianopyridone
<i>A. suum</i> Adult muscle	Complex I + II ^a	0.11	0.014	1.6
	Complex I ^b	>100	>100	>100
	Complex II (QFR) ^c	0.22	0.012	0.36
	Complex II (SQR) ^d	0.22	0.032	2
Bovine heart	Complex I+ III ^e	140	82	420
	Complex II ^d	0.011	0.0036	0.017
Rat liver	Complex I+ III ^e	>500	>500	>500
	Complex II ^d	0.024	0.0037	0.2

^a Measured by NADH-fumarate reductase activity.

^b Measured by NADH-quinone reductase activity. Ubiquinone 1, ubiquinone 2, or decyl-rhodoquinone was used as electron acceptor.

^c Measured by rhodoquinol-fumarate reductase activity.

^d Measured by succinate-UQ reductase activity.

^e Measured by NADH-cytochrome *c* reductase activity.

QFR, quinol-fumarate reductase; SQR, succinate-ubiquinone reductase.

4) Atpenin A5 inhibited bovine heart succinate-ubiquinone reductase much more potently than known complex II inhibitors⁸⁾.

	Atpenin A5	Carboxin	TTFA	HQNO
IC_{50} (μ M) against bovine heart complex II	0.0036	1.1	5.8	> 100

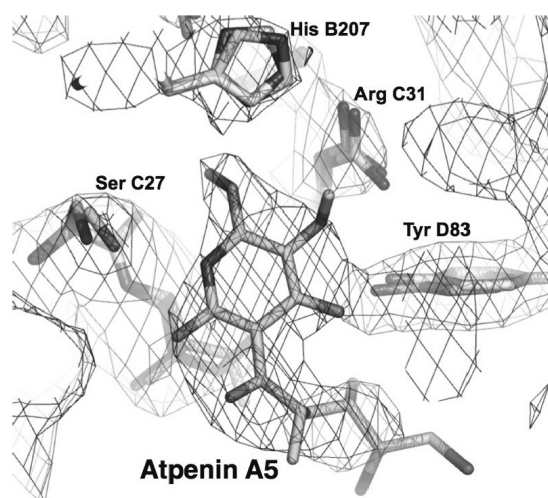
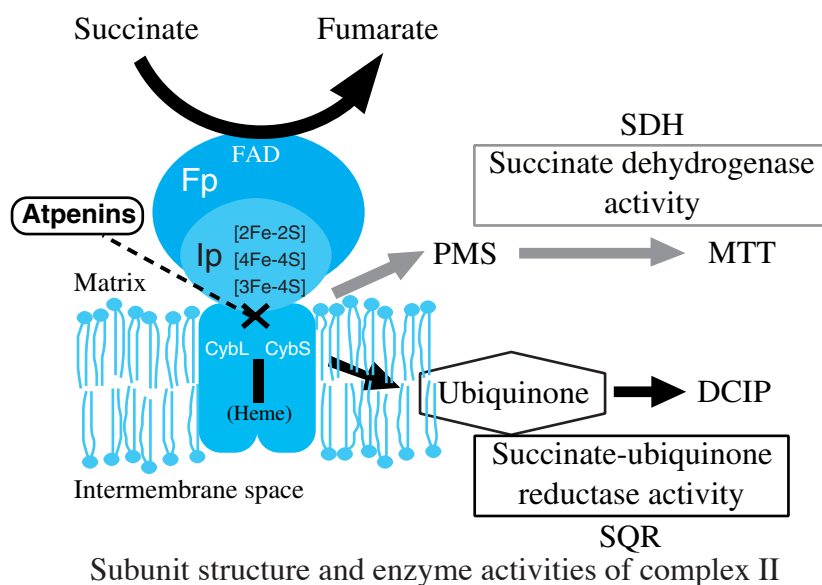
Double-reciprocal plots showed mixed inhibition of atpenins with ubiquinone 2. This indicates that atpenins may block electron transfer between the enzyme and ubiquinone by binding to a region that partially overlaps with the physiological ubiquinone binding site. The kinetic analyses showed that K_i values for atpenin A4, atpenin A5, and harzianopyridone were 6.9, 1.0, and 7.0 nM, respectively.

5) Studies of carboxin resistant fungi indicate that amino acid residues close to the [3Fe-4S] center in Ip, as well as a residue in CybS, located in the cytoplasmic loop between transmembrane segments, are important for carboxin binding and possibly for ubiquinone recognition. Thus, ubiquinone is thought to bind to complex II at the interface between Ip and the membrane-anchor domain. This explains the observation that atpenins affected not only succinate-ubiquinone reductase (SQR) activity but also succinate dehydrogenase (SDH) activity of the bovine heart complex II. Inhibition of SDH by atpenins was less potent than that of SQR, and complete SDH inhibition was not achieved even at high concentrations. These discoveries of mixed type inhibition of SQR activity are very similar to those reported for carboxin.

6) Atpenin A5-SQR complex⁹⁾

The structure of atpenin A5 in complex with *E. coli* SQR was resolved. Atpenin A5 was located within the same hydrophobic pocket as ubiquinone at, however, a different position within the

pocket. Atpenin A5 was bound deeper into the site prompting further assessment using protein-ligand docking experiments *in silico*. The initial interpretation of the quinone-binding site (Q-site) was re-evaluated in the light of this complex structure and protein-ligand docking data. Two binding positions, the Q₁-site (formerly presumed Q-site) and Q₂-site (atpenin A5-binding site), are proposed for the *E. coli* SQR quinone-binding pocket to explain these data. At the Q₂-site, the side chains of a Ser and His are suitably positioned to provide hydrogen bonding partners to ubiquinone. This allows to propose a mechanism for the reduction of ubiquinone during the catalytic turnover of the enzyme.



5. References

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