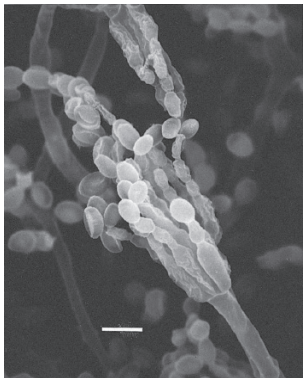
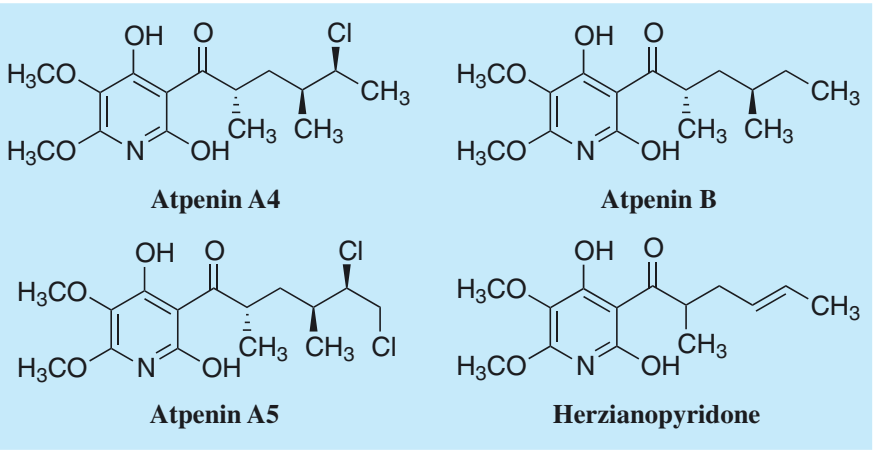
Atpenin ©

1. Discovery, producing organism and structure[1-6)]

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 analysis[2)]. The total synthesis of atpenins was reported by Quéguiner's group[3)] 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 activity[5)]. The co-crystallyzation 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. $C_{15}H_{21}NO_5Cl_2$; mol wt 366.24. Sol. in DMSO, MeOH, EtOH, $CHCl_3$. Insol. in H_2O.

3. Biological activity[1,7-9)]

1) Antimicrobial activity[1)]

Test organisms	MIC (μg/ml)		
	Atpenin A4	Atpenin A5	Atpenin B
Staphylococcus aureus FDA 209P	>100	>100	>100
Bacillus subtilis PCI 219	>100	>100	>100
Micrococcus luteus PCI 1001	>100	>100	>100
Mycobacterium smegmatis ATCC 607	>100	>100	>100
Escherichia coli NIHJ JC-2 IFO 12734	>100	>100	>100
Pseudomonas aeruginosa P-3 KB 105	>100	>100	>100
Candida albicans KF 1	>100	>100	>100
C. lipolytica L7 KF 236	6.25	0.4	1.56
Saccharomyces cerevisiae KF 26	>100	>100	>100
Aspergillus niger ATCC 6275	50	12.5	50
Pyricularia oryzae KF180	3.12	3.12	0.78
Mucor racemosus IFO 4581	>100	>100	>100
Trichophyton mentagrophytes KF 213	6.25	0.05	6.25
T. interdigitale KF 62	12.5	0.05	6.25
Microsporum gypseum 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 drug[7].

3) Atpenins inhibited nematode and mammalian mitochondrial complex II[8].

Species	Activities	IC_{50} (μM)		
		Atpenin A4	Atpenin A5	Harzianopyridone
A. suum	Complex I + II[a]	0.11	0.014	1.6
Adult muscle	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 inhibitors[8].

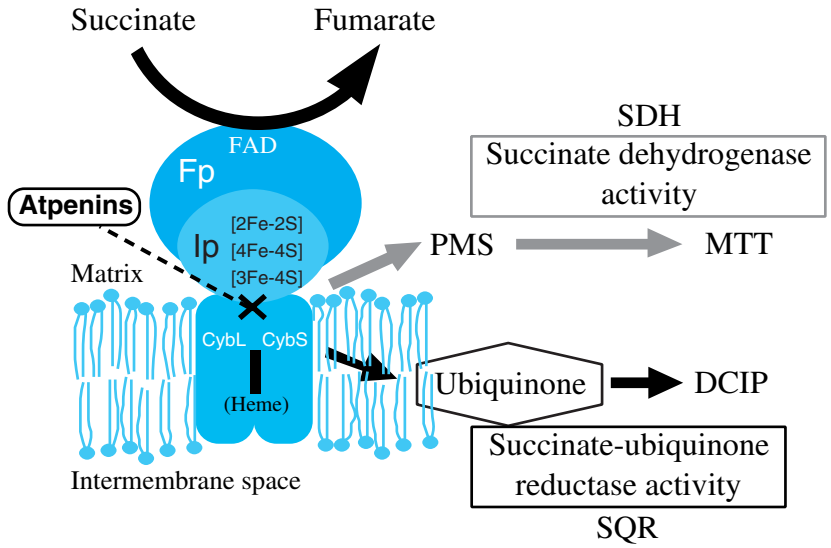
	Atpenin A5	Carboxin	TTFA	HQNO
IC_{50} (μM) against bovine heart complex II	0.0036	1.1	5.8	> 100

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 *Ki* values for atpenin A4, atpenin A5, and harzianopyridone were 6.9, 1.0, and 7.0 nM, respectively.

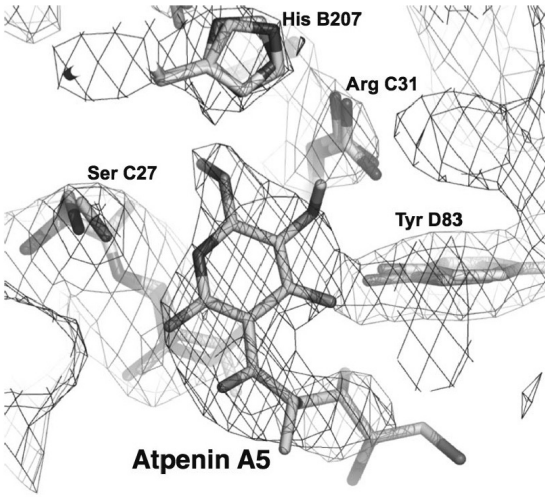
5) Studies of carboxin resistant fungi indicate that amino acid residues close to the [3Fe-4S] center in Ip, as well as a residue in CybS, located in the cytoplasmic loop between transmembrane segments, are important for carboxin binding and possibly for ubiquinone recognition. Thus, ubiquinone is thought 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 discoverys of mixed type inhibition of SQR activity are very similar to those reported for carboxin.

6) Atpenin A5-SQR complex[9]
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 Q_1-site (formerly presumed Q-site) and Q_2-site (atpenin A5-binding site), are proposed for the *E. coli* SQR quinone-binding pocket to explain these data. At the Q_2-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.



Subunit structure and enzyme activities of complex II



5. References

1. [405] S. Ōmura *et al.*, *J. Antibiot.* **41**, 1769-1773 (1988)
2. [454] H. Kumagai *et al.*, *J. Antibiot.* **43**, 1553-1558 (1990)
3. F. Trécourt *et al.*, *J. Org. Chem.* **59**, 6173-6178 (1994)
4. [868] K. Shiomi & S. Ōmura, *Proc. Jpn. Acad., Ser. B* **80**, 245-258 (2004)
5. [946] K. Kita *et al.*, *Trends Parasitol.* **23**, 223-229 (2007)
6. [947] S. Ōmura & K. Shiomi, *Pure Appl. Chem.* **79**, 581-591 (2007)
7. [446] K. Oshino *et al.*, *J. Antibiot.* **43**, 1064-1068 (1990)
8. [823] H. Miyadera *et al.*, *Proc. Natl. Acad. Sci. USA* **100**, 473-477 (2003)
9. [910] R. Horsefield *et al.*, *J. Biol. Chem.* **281**, 7309-7316 (2006)
10. [1121] M. Ohtawa *et al.*, *Chem. Pharm. Bull.* **60**, 898-906 (2012)
11. [1032] M. Ohtawa *et al.*, *J. Antibiot.* **62**, 289-294 (2009)