PIERICIDIN A: A NEW INHIBITOR OF MITOCHONDRIAL ELECTRON TRANSPORT

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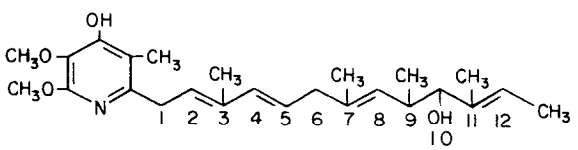
Piericidin A resembles coenzyme Q in its structure in that it has an aromatic nucleus (pyridine ring) with two adjacent methoxyl groups, nuclear methyl and oxygen groups, and an isoprenoid-like side chain. The isolation, structure and action of the compound as an insecticide have been reported by Takahashi et al. (1965). (cf. fig. 1)

STRUCTURE OF PIERICIDIN A



Because of the apparent structural relationship to coenzyme Q we have examined the inhibitory effects of Piericidin A on mitochondrial electron transport and we find differential effects on the DPNH oxidase and succinoxidase systems of beef heart mitochondria. Piericidin A is an especially potent inhibitor of DPNH oxidase activity and shows complete inhibition at 0.036 μmoles per mg mitochondrial protein. On the other hand, it is a relatively weak inhibitor of succinoxidase activity with 50% inhibition at 0.33 mμmoles per mg protein and inhibition of 80% at 2.0 μmoles per mg protein.

Reduction of mitochondrial cytochromes when succinate or DPNH are added to mitochondria is inhibited at the levels of piericidin which inhibit the respective activities. Thus the primary site of inhibition would be between the flavoprotein dehydrogenases and the cytochromes. This is consistent with the fact that both DPNH and succinate cytochrome c reductase activities are inhibited by piericidin.

Succinic ferricyanide reductase activity is partially inhibited by piericidin and the reduction of phenazine methosulfate by succinate is inhibited by piericidin. Since we have previously shown a partial require-

TABLE I

COMPARATIVE PIERICIDIN INHIBITION OF DIFFERENT ACTIVITIES OF THE MITOCHONDRIAL ELECTRON TRANSPORT CHAIN

Activity	Amount of Piericidin A Added μmoles/mg Protein	Percent Inhibition
DPNH oxidase	3.6×10^{-5}	100
	1.8×10^{-5}	44
DPNH menadione reductase*	6.6×10^{-3}	100
	6.6×10^{-4}	43
Succinoxidase	0.33	55
Succinic ferricyanide reductase	1.3	55
Succinic phenazine methosulfate	1.3	78
Cytochrome oxidase	60	36

*Enzyme prepared by ether treatment as described by Cunningham et al. (1965) and purified by chromatography on DEAE cellulose (Hall 1966).

ment for coenzyme Q in mitochondrial succinic ferricyanide reductase activity (Crane and Ehrlich 1961) it would appear that piericidin acts after the dehydrogenase and in the region of coenzyme Q. (cf. Table I)

Addition of coenzyme Q_2 to mitochondria in which succinoxidase activity has been inhibited by piericidin restores the activity. Equimolar levels of α tocopherol do not give the same restoration as coenzyme Q_2, but do give a partial reversal which may be related to reversal of non-specific inhibition by the fatty part of the molecule as in the reversal of isooctane inhibition by α tocopherol. (Pollard and Bieri 1958). The additional increment of activity restored by coenzyme Q_2 over the effect of α tocopherol could be a specific reversal of inhibition at the coenzyme Q site. Coenzyme Q_{10} is also effective in restoration of activity. (cf. Table II)

TABLE II

REVERSAL OF SUCCINOXIDASE INHIBITION BY COENZYME Q_2

Additions	Succinoxidase Activity - Piericidin	Succinoxidase Activity + Piericidin
Piericidin at 1.28 μmoles/mg protein		
None	0.35	0.11
Coenzyme Q_2 0.11 μmoles/mg	0.34	0.16
Coenzyme Q_2 0.22 μmoles/mg	0.32	0.21
Coenzyme Q_2 0.56 μmoles/mg	0.30	0.24
Coenzyme Q_2 0.22 μmoles/mg + antimycin A		0
Piericidin at 0.76 μmoles/mg		
None	0.58	0.21
Coenzyme Q_{10} 1.2 μmoles/mg	0.47	0.37
α tocopherol 4.1 μmoles/mg	0.46	0.29

Piericidin A inhibition of DPNH oxidase activity shows evidence of stoichiometric titration of a component present in mitochondria in lower amounts than any of the known redox agents. (King et al. 1964). In this respect its effect resembles rotenone inhibition and the amount required for complete inhibition is strikingly similar to saturating levels of rotenone (Ernster 1963), which has been shown to act at the level of the DPNH dehydrogenase. The DPNH ferricyanide reductase activity is not inhibited by piericidin. A purified DPNH menadione reductase from mitochondria (Crane et al. 1965) is inhibited by both rotenone and by piericidin and we feel that this enzyme may represent the primary DPNH dehydrogenase system associated with the factor which is sensitive to rotenone and piericidin. In no case does coenzyme Q_2 reverse piericidin inhibition of DPNH dehydrogenase or oxidase activity.

Cytochrome oxidase is not inhibited by piericidin at levels which inhibit succinoxidase activity. We do observe some inhibition of cytochrome c oxidase at levels 50 to 100 times greater than the levels which inhibit succinoxidase but inhibition at these levels is of doubtful significance.

From the evidence, Piericidin A inhibits the mitochondrial electron transport system in two places. (1) It specifically reacts at very low levels at a site related to DPNH dehydrogenase which may be identical with the site of rotenone action. (2) It reacts at high levels and in a reversible fashion around the area where coenzyme Q interacts with succinic dehydrogenase. The γ-pyridone nature of Piericidin A in contrast to the nitrogen-free coenzyme Q signifies that this pyridone could be absorbed and displaced very differently in different enzyme systems.

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