since the effects are clearly manifest in the G_2 phase of the cycle, after all DNA synthesis is finished. It is doubtful, however, whether the inhibitory action of pentobarbital on protein synthesis can explain its stathmokinetic blocking action, in view of the different behaviour of cells blocked from entering prophase by cycloheximide and pederin. The G_2 blocking action of higher concentrations of pentobarbital may, however, be due to this inhibitory effect. The morphological similarity to cells treated with colchicine and colcemid suggest that a similar mode of action may be involved[12] or else some other interference with the mitotic spindle as in the case of rotenone.[5]

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Uncoupling of oxidative phosphorylation by arylhydrazono-isoxazolone fungicides

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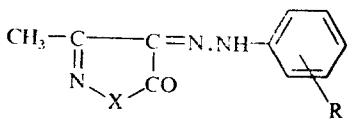
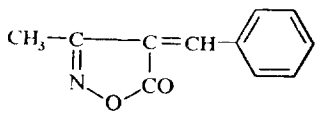
4-ARYLHYDRAZONO-3-METHYL-5-ISOXAZOLONES are effective fungicides against a number of plant pathogens[1, 2] but little is known of their mode of action. In view of their structural similarity to arylhydrazonomalononitriles, which are potent uncouplers of oxidative phosphorylation,[3, 5] it has been suggested[2] that the isoxazolone fungicides may also exert a similar uncoupling effect. To test this theory we have examined 3-methyl-4-phenylhydrazono-5-isoxazolone (I;X = 0, R = H), the *o*-chlorophenyl analogue known as drazoxolon (I;X = 0, R = 2 − Cl) and the *m*-chlorophenyl derivative (I;X = 0, R = 3 − Cl) as uncouplers of oxidative phosphorylation of rat liver mitochondria in comparison with 2,4-dinitrophenol. We included in the tests three structurally related compounds which are generally much less fungitoxic,[2] namely, 3-methyl-4-phenylhydrazono-5-pyrazolone (I; X = NH, R = H), the 1-phenyl analogue (I; X = N.Ph, R = H) and 4-benzylidene-3-



(1)



(2)

methyl-5-isoxazolone (II). Standard methods were employed for the preparation of rat liver mitochondria and for measurements of inorganic phosphate uptake.[5] All experiments took place at 37°, pH 6·8 and a final volume of 3·0 ml. The basic medium contained: 100 mM KCl, 14 mM $MgCl_2$, 1 mM EDTA, 16·7 mM glycylglycine, 30 mM sucrose. For ATP-ase experiments 2·3 mM ATP was added. For measurement of inorganic phosphate uptake the basic medium was reinforced with 2·3 mM ATP, 16·6 mM potassium phosphate, 60 mM glucose, 3·3 mg hexokinase/ml, 10 mM pyruvic acid and 1 mM fumaric acid. Mitochondria equivalent to 3 to 4 mg protein were used. The observations on respiration were made polarographically in the medium last mentioned but without ATP or hexokinase and glucose. The arylhydrazono-isoxazolones were dissolved in alcohol and added in quantities of no more than 30 μl. This amount of alcohol had no effect on the systems investigated, 2:4 Dinitrophenol (DNP) was added as required in aqueous solution. The effect of each compound on ATP-ase activity, in terms of μmole phosphate released/mg protein/min was compared with the maximal result obtained with DNP and expressed as a percentage. Phosphate uptake, in terms of μmole phosphate taken up/mg protein/min, was expressed as a percentage of the maximum uptake in control experiments. Results from both types of experiments were plotted against the negative logarithm of the concentration and thus the concentration producing a 50 per cent effect was obtained.[5]

The criteria obeyed by compounds which uncouple oxidative phosphorylation have been fully discussed elsewhere.[5, 7] The ability or lack of ability of each compound to affect oxidative phosphorylation was demonstrated by its effect upon mitochondrial phosphate uptake and ATP-ase activity.[5] The results are represented in the Table. Further confirmation of the uncoupling activity of the first three compounds was obtained by observing that these compounds stimulated mitochondrial respiration at concentrations similar to those in the Table. Also, these compounds relieved the inhibition of respiration brought about by oligomycin behaving in this as in other respects as DNP.[6]

TABLE 1. UNCOUPLING ACTIVITY OF ARYLHYDRAZONO ISOXAZOLONES AND RELATED COMPOUNDS

Compound	Concn range (μM)	Concn causing 50% inhibition of phosphate uptake* (μM)	Concn causing 50% stimulation of ATPase activity (μM)
(I;X = 0, R = H)	3·2–100	12·5	12·5
(I;X = 0, R = 2 − Cl)	1·6–50	3·0	1·25
(I;X = 0, R = 3 − Cl)	1·5–50	2·0	1·4
2·4 Dinitrophenol	—	30	30
(I;X = NH, R = H)	1·5–50	No Inhibition	No Stimulation
(I;X = N.Ph, R = H)	1·5–50	No Inhibition	No Stimulation
(II)	4·0–100	No Inhibition	No Stimulation

* Over the range of concentrations investigated no effect on oxygen consumption was discernable

It is evident that the arylhydrazono isoxazolones are more efficient uncouplers of oxidative phosphorylation than 2,4 dinitrophenol. In contrast, the absence of uncoupling effects shown by the last three compounds is striking. It seems clear that the high fungi-toxicity of the arylhydrazono isoxazolones can be attributed to a significant extent to their inhibition of oxidative phosphorylation. The

reasons for the difference in uncoupling ability of the hydrazono-isoxazolones compared with compounds (I;X = NH, R = H), (I;X = N.Ph, R = H) and (II) are not yet clear but differences in acidity and lipid solubility (compare ref. 5) *inter alia*, are almost certainly involved. These aspects are being investigated.

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