COMMUNICATIONS

Uncoupling of Oxidative Phosphorylation in Intact Mites and in Isolated Mite Mitochondria by a New Acaricide, 5,6-Dichloro-1-phenyloxy-carbonyl-2-trifluoromethylbenzimidazole

By J. R. CORBETT and B. J. WRIGHT. (*Fisons Agrochemical Division, Chesterford Park Research Station, Saffron Walden, Essex, U.K.*)

The new acaricide is hydrolysed by water to give 5,6-dichloro-2-trifluoromethylbenzimidazole, which is an uncoupler of mammalian and insect mitochondria (Büchel, Korte & Beechey, 1965; Beechey, 1966; Jones & Watson, 1967; Ilivicky & Casida, 1969). The acaricide would therefore be expected to act as an uncoupling agent. The following evidence suggests that this is the mode of action of the compound:

(a) The acaricide and its hydrolysis product uncoupled rat liver mitochondria with the same activity (the minimum uncoupling concentration was approx. $2\mu\text{M}$ with 2mg of mitochondrial protein present in an oxygen electrode assay), but the acaricide itself only exerted its full stimulatory effect on oxygen uptake after a lag period.

(b) Mitochondria were isolated with difficulty from the greenhouse red spider mite (*Tetranychus telarius* L.) essentially by the method developed for insects by Ilivicky, Chefurka & Casida (1967). This is believed to be the first reported isolation of mite mitochondria. Using pyruvate as substrate, we have established that the isolated mite mitochondria show a respiratory control value of approx. 2, and that they are uncoupled by both the acaricide and its hydrolysis product. As with the rat liver mitochondria, the minimum uncoupling concentration was about $2\mu\text{M}$, and the acaricide only exerted its full uncoupling effect after a lag period.

(c) If an acaricide kills mites through an uncoupling effect on oxidative phosphorylation, the mites should show an increase in respiratory rate before dying. This was tested by immobilizing mites by sticking them to the outside of a roll of sticky tape, which was then placed over the centre well of a Warburg flask. After the initial rate of oxygen uptake had been measured the rolls were removed and the mites treated with chemical. Both the acaricide and its hydrolysis product stimulated respiration by approx. 70%. Suitable controls did not show this stimulation.

In agricultural practice the acaricide is greatly superior to its hydrolysis product in mite control and in its safety to crops. Presumably this is because the lipophilic nature of the acaricide causes it to be concentrated in the plant cuticle, which is

eaten by mites. The ensuing hydrolysis of the acaricide inside the mites will cause death by uncoupling.

Beechey, R. B. (1966). *Biochem. J.* **98**, 284.

Büchel, K. H., Korte, F. & Beechey R. B. (1965). *Angew. Chem. int. Ed.* **4**, 788.

Ilivicky, J. & Casida, J. E. (1969). *Biochem. Pharmac.* **18**, 1389.

Ilivicky, J., Chefurka, W. & Casida, J. E. (1967). *J. econ. Ent.* **60**, 1404.

Jones, O. T. G. & Watson, W. A. (1967). *Biochem. J.* **102**, 564.

The Mode of Action and Metabolism of 6-Chloro-2-trifluoromethylimidazo[4,5-b]pyridine, an Experimental Herbicide

By C. P. BOND and J. R. CORBETT. (*Fisons Agrochemical Division, Chesterford Park Research Station, Saffron Walden, Essex, U.K.*)

The title compound, and a close relative, 2-*tert*-butyl-6-chloroimidazo[4,5-*b*]pyridine, are powerful photosynthetic inhibitors *in vitro*. The Hill reaction was measured with maize and pea chloroplasts by using (i) the oxygen electrode and (ii) ferri-cyanide reduction measured spectrophotometrically. The compounds caused 50% inhibition of the Hill reaction at 1.5 and $2.4\mu\text{M}$ respectively. Preliminary experiments suggest that there is no uncoupling of either cyclic or non-cyclic photophosphorylation at these concentrations. The obvious corollary that the compounds are herbicidal by inhibiting photosynthetic electron flow was tested by keeping the treated plants in the light, or in the dark, by using a simple home-made growth cabinet. The herbicidal effect on mustard, maize and tomato was the same in the light as in the dark for both compounds. We conclude that the herbicidal action of the compounds was not due to interference with photosynthesis.

Tests with mitochondria (rat liver, pea and cauliflower) by using (i) Warburg manometry and (ii) the oxygen electrode showed that the title compound was a moderately active uncoupler of oxidative phosphorylation (minimum uncoupling concentration was about $50\mu\text{M}$), but that the *tert*-butyl compound was not an uncoupler at $100\mu\text{M}$. Since it is probable that both compounds act in the same way, uncoupling of oxidative phosphorylation must also be ruled out as a herbicidal mechanism.

Preliminary metabolic studies have been carried out on the title compound labelled with ^{14}C in the

trifluoromethyl group. In the rat monohydroxylation of the compound appears to occur in the 5-, 6- and 7-positions, and conjugates of these materials are found in the urine. Similar hydroxy metabolites were found in maize growing in soil treated with the labelled compound, both free and in conjugation. In 9-week-old maize 25% of the ^{14}C was present as trifluoroacetic acid; this was confirmed by co-chromatography and isotope dilution as the benzylthiuronium salt. Since the proportion of 6-hydroxy- to the 5- and 7-hydroxy derivatives increased with time it is possible that it is these latter compounds that give rise to trifluoroacetic acid, although no intermediates were detected.

Mechanism of Inhibition of Glycollate Oxidase by Aldehyde Bisulphite Addition Compounds, and by Related Structures

By J. R. CORBETT and B. J. WRIGHT. (*Fisons Agrochemical Division, Chesterford Park Research Station, Saffron Walden, Essex, U.K.*)

The mechanism of inhibition of pea glycollate oxidase by aldehyde bisulphite addition compounds has been investigated by using the oxygen electrode. We agree with Zelitch (1957) that inhibition by $\text{HO}\cdot\text{CH}_2\cdot\text{SO}_3\text{Na}$ is due to simple competition with glycollate, but with higher members of the series we find that inhibition is due to the formation of glyoxylate bisulphite, $\text{NaO}_2\text{C}\cdot\text{CH}(\text{OH})\cdot\text{SO}_3\text{Na}$, by reaction of enzymically produced glyoxylate with the inhibitor. Our evidence for this is as follows.

(a) Nine aldehyde bisulphite compounds of widely varying structure, and including glyoxylate bisulphite, all caused 50% inhibition of pea glycollate oxidase within the range $0.8\text{--}2.0\mu\text{M}$. Compounds derived from dialdehydes were significantly more inhibitory than those derived from monoaldehydes.

(b) Spectrophotometric evidence with the addition compound of pyridine-2-aldehyde, or that of $\text{Ph}\cdot\text{C}:\text{C}\cdot\text{CHO}$, showed that the spectrum changed in minutes to that of the free aldehyde when glyoxylate was added. This also occurred when glycollate oxidase generated the glyoxylate from glycollate.

(c) The kinetic behaviour of all inhibitors, except the formaldehyde compound, was not straightforward, and resembled that of glyoxylate bisulphite. Inhibition decreased with time, probably owing to the progressive increase in the concentration of glyoxylate, which may interfere with the binding of glyoxylate bisulphite to the enzyme.

An investigation of 37 compounds structurally related to molecules known to bind to glycollate oxidase showed that some aminomethanesulphonates are also powerful inhibitors of glycollate oxidase. Although we have not investigated this inhibitory

mechanism in detail we suspect that the aminomethanesulphonates are unstable and are converted into a simpler compound, possibly the formaldehyde bisulphite addition compound: (a) With compounds of the type $\text{R}\cdot\text{NH}\cdot\text{CH}_2\cdot\text{SO}_3\text{Na}$, the best inhibitors are those in which $\text{R} = \text{CH}_3$, $\text{CH}_3\cdot\text{CH}_2$, or $\text{Ph}\cdot\text{CH}_2$ (50% inhibition of glycollate oxidase at about $3\mu\text{M}$), whereas compounds in which R is Ph or $p\text{-ClC}_6\text{H}_4$ had to be present at $>333\mu\text{M}$ to cause 50% inhibition. Therefore the most inhibitory compounds are those substituted with electron-donating groups, which will make the nitrogen atom liable to protonation, with subsequent hydrolysis to a simpler compound. (b) The hydrolysis rate of $\text{CH}_3\cdot\text{CH}_2\cdot\text{NH}\cdot\text{CH}_2\cdot\text{SO}_3\text{Na}$ was tested conductometrically at pH 7 and room temperature and the half-life was found to be a few minutes.

Zelitch, I. (1957). *J. biol. Chem.* **224**, 251.

The Effects of 1,1-Di-(*p*-chlorophenyl)-2,2,2-trichloroethane (DDT) and 1,1-Di-(*p*-chlorophenyl)-2,2-dichloroethylene (DDE) on Hepatic Microsomal Oxidase in the Rat and Japanese Quail

By P. J. BUNYAN, A. TAYLOR* and M. G. TOWNSEND. (*Biochemistry Section, Pest Infestation Control Laboratory, Ministry of Agriculture, Fisheries and Food, Tolworth, Surrey, U.K.*)

The metabolic conversion of DDT into DDE and DDD [1,1-di-(*p*-chlorophenyl)-2,2-dichloroethane] has been demonstrated in a number of vertebrates including rats (Peterson & Robison, 1964) and pigeons (Bailey, Bunyan, Rennison & Taylor, 1969a). DDT and DDE give rise to similar increases in both liver weight and microsomal drug-metabolizing enzyme activities in rats when administered at 100mg/kg for 3 days (Hart & Fouts, 1965). DDE was both more toxic than DDT and led to more rapid and larger mean liver-weight increases when fed to pigeons at 1000p.p.m. (Bailey, Bunyan, Rennison & Taylor, 1969b). The latter suggested that increases in avian hepatic microsomal enzymes after DDT administration (Gillette, Chan & Terriere, 1966) could also be primarily due to metabolically produced DDE. A similar pattern may also have been overlooked in rats owing to the high dose rates often employed to produce microsomal enzyme induction.

Here we report the measurement of liver and heart residue concentration (Taylor, Rea & Kirby, 1964) together with hepatic microsomal protein content (Lowry, Rosebrough, Farr & Randall, 1951), cytochrome *P*-450 concentration (Omura & Sato, 1964) and aniline oxidase activity (Kato & Gillette, 1965) after feeding with diets containing various

* Deceased 27 February 1970.