The Conversion of Diazinon to Hydroxydiazinon in the Guinea-Pig and Sheep

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Pardue et al (1) recently identified hydroxydiazinon (HOD) among the products of the ultra-violet irradiation of diazinon, and showed that it was also formed in or on kale that had been sprayed with diazinon in the field. The work reported here shows that HOD is also a metabolite of diazinon in the guinea-pig and sheep.

Experimental

Irradiation product. Diazinon was irradiated at 254 nm, substantially as described by Pardue et al (1). The products were separated by thin-layer chromatography (TLC) on fluorescent silica gel with 1:4 acetone-hexane as solvent. The zone thought to contain HOD was eluted, examined by gas chromatography (GLC) and further purified by TLC until the eluate showed only a single GLC peak. GLC was on XE-60 stationary phase with thermionic detection: details have been published previously (2).

Metabolism by guinea-pig liver *in vitro*. Previous experiments had shown that a metabolite (M), thought to be HOD, was formed when guinea-pig liver slices were incubated with diazinon and that its yield was highest after about 15 minutes. The livers from two guinea-pigs (total weight 45 gm) were sliced and incubated separately for 15 minutes with 100 ml of continuously oxygenated Krebs Ringer bicarbonate containing 50 p.p.m. of diazinon. The livers were combined, dried by suction, triturated with an equal weight of anhydrous sodium sulphate, and extracted with 2 x 100 ml acetone. The acetone was evaporated, the aqueous residue extracted with hexane, and the hexane solution applied to a 10 gm column of silica gel of Brockman grade III activity. The column was eluted successively with 100 ml hexane, 100 ml 50% chloroform-hexane and 15 x 10 ml chloroform. GLC of the eluates after evaporation and transfer to acetone showed that most of the M was in the 7th-10th chloroform fractions. These were combined and concentrated for characterization.

Metabolism by sheep *in vivo*. A sheep was dosed by stomach tube with diazinon (800 mg/kg) and killed after 48 hours. Extraction and clean-up of M from liver, kidney, muscle, fat and brain (50 gm of each) followed the procedure used for guinea-pig liver. Most of the lipid was removed from fat and brain extracts by chilling and centrifugation. The relative concentrations of M in the original tissues were estimated by the method of Machin & Quick (2).

Characterization. The specimens of M from the tissue eluates were compared with the irradiation product by TLC in 1:4 acetone-

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hexane and by GLC on 2% XE-60 and 2% DEGA + 0.2% phosphoric acid. The infra-red spectrum of the irradiation product in carbon tetrachloride was recorded with a Perkin-Elmer 237 spectrometer.

Results and Discussion

The infra-red peaks of the irradiation product agreed closely with those of HOD (1) in position, shape and relative intensity. The agreement was considered sufficient to identify the product as HOD although no other spectroscopic facilities were available.

The samples of M from the different tissues all showed identical chromatographic behavior. The retention times of diazoxon, HOD and M, relative to diazinon, were 1.66, 2.46 and 2.46 on XE-60 and 1.38, 2.53 and 2.53 on DEGA-phosphoric acid. Rf values of the compounds by TLC in 1:4 acetone-hexane were 0.21, 0.36 and 0.36 respectively. Although M could not be identified positively, the chromatographic results provide virtually conclusive evidence that it is HOD.

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All the tissues from the dosed sheep showed substantial GLC peaks for HOD: although these could not be related directly to absolute quantities, they probably represented concentrations of a few p.p.m. in the tissues if the thermionic detector was as sensitive to HOD as to diazoxon. The highest residue was found in fat and the proportions in fat, liver, muscle, kidney and brain were about 6:2:2:1:1. Residues of diazinon were clearly much higher than those of HOD in all the tissues.

HOD seemed to be detected so sensitively by its inhibition of cholinesterase after oxidation as to suggest that its oxygen analogue is a stronger inhibitor than diazoxon. If this is so, the formation of HOD is likely to be an important pathway in the metabolism of diazinon.

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References

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