An Isomer of Hydroxydiazinon Formed by Metabolism in Sheep

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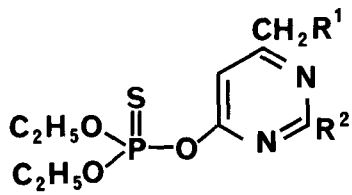
Hydroxydiazinon (Ib) was first detected (1) as a product of the ultraviolet irradiation of diazinon (Ia), and later (2) as a metabolite in sheep fed with diazinon. This communication reports the identification of another metabolite in sheep as an isomer of hydroxydiazinon, diethyl 6-hydroxymethyl-2-isopropyl-4-pyrimidinyl phosphorothionate (Ic).



Ia $R^1 = H$ $R^2 = CH(CH_3)_2$
Ib $R^1 = H$ $R^2 = C.OH(CH_3)_2$
Ic $R^1 = OH$ $R^2 = CH(CH_3)_2$
Id $R^1 = H$ $R^2 = COCH_3$

Experimental

Four sheep were dosed with diazinon (800 or 1000 mg/kg) by stomach tube. One was killed after 48 hours and its tissues extracted with acetone. Urine was collected from the others for 3 days after dosing and extracted with ethanol-free chloroform. The tissue extract, purified by column chromatography and tlc as described previously (2), except that elution of the column was continued with 1:1 acetone-chloroform to desorb the new metabolite, gave a sample of (Ic) which was still impure. The metabolite (Ic) was also recovered from the urine extract (after drying) by adsorption on a column of silica gel (Brockman grade II) and elution with 1:40 methanol-chloroform. Repeated tlc with 1:4 acetone-hexane and 2:3 ethyl acetate-hexane gave a product pure enough for spectroscopic identification.

This sample from the urine was examined by nmr (in $CDCl_3$ containing tetramethylsilane as internal standard, and using signal averaging from 272 accumulated scans), by ms (by direct insertion into the ion source, at 70eV), and by ir (as a KBr disc, or in CS_2 or CCl_4).

Results and Discussion

The compound gave a red spot on tlc plates with 2,6-dichloro-p-benzoquinone-4-chloroimine, and inhibited

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cholinesterase after, but not before, oxidation with bromine, showing it to be a fully esterified phosphorothionate. (Cf. reference 3.)

The nmr spectrum shows peaks at 8.65 (d,6H, J 7Hz), 8.59 (t,6H), 6.8 (m, 1H), 5.61 (dq, 4H, J 10, 7Hz), 5.26 (s, 2H) and 3.20 (s, 1H) in addition to peaks from impurities at 7.82 (Me_2CO?) and 8-9 (long-chain alkyl compounds?). Compared with diazinon, the isopropyl signals (at 8.68 and 6.9 in Ia) are almost unchanged, as are the peaks from the diethoxyphosphorus group (at 8.62 and 5.63 in Ia). The ring CH_3 at 7.51 in diazinon is replaced by a 2H peak at 5.26 and the pyrimidine H shifts from 3.32 to 3.20, consistent with the conversion of this CH_3 to CH_2OH. Further, addition of trifluoroacetic anhydride to the solution shifted the 2H peak downfield to 4.41 (-0.85 p.p.m.; the corresponding shift on trifluoroacetylation of benzyl alcohol is -0.75 p.p.m.), demonstrating the presence of an hydroxyl *a* to the CH_2 group.

The mass spectrum, with important peaks at 320 (M^+) and 195, confirms the molecular weight for the suggested structure (Ic) and provides further evidence of the importance of the isopropyl CH in the Damico rearrangement (4). Thus, the rearrangement peak is large for diazinon (at m/e 179) and in the present case (at m/e 195) but not for hydroxydiazinon (Ib) or the acetyl derivative (Id) formed by uv irradiation of diazinon (3), when the important CH is absent. The trimethyl silyl derivative of the new metabolite shows a major peak at 392 (M^+), confirming the presence of a single reacting group (hydroxyl).

The ir spectrum generally resembles that of diazinon, but bands at 2930 and 2850 cm^{-1} are now stronger than those at 2970 and 2870 cm^{-1}, showing that the ratio of methylene to methyl groups has increased. The hydroxyl group gives a weak, broad band at ca. 3450 cm^{-1}. Strong peaks at 1590 and 1570 cm^{-1} (pyrimidine ring) and 830 cm^{-1} (P=S) confirm that the molecule is still a substituted pyrimidinyl phosphorothionate.

Metabolites formed by hydroxylation on C-1' or C-2' of the isopropyl group of diazinon or the derived pyrimidinol (5) have been reported previously, but hydroxylation of the C-6 methyl has not. The metabolites (Ib) and (Ic) were found in all the tissues examined (fat, liver, muscle, and brain) and in blood and urine, but the proportion of (Ic) was much higher in urine than in the tissues. This ready excretion would decrease its hazard as a residue, but it may be toxicologically important as an intermediate metabolite.

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