Diazinon Residues on Field-Sprayed Kale

Hydroxydiazinon—A New Alteration Product of Diazinon

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A previously unreported, cholinesterase inhibiting, alteration product of diazinon has been isolated from field-sprayed kale. This compound has retention times on two glc columns and an R_f value on tlc identical with those of a compound prepared by the ultraviolet irradiation of diazinon. The ultraviolet irradiation product has been isolated and tentatively identified by infrared, nuclear magnetic resonance, and mass spectrometry as hydroxydiazinon, O,O-diethyl-O-[2-(2'-hydroxy-2'-propyl)-4-methyl-6-pyrimidinyl] phosphorothioate.

Organophosphorus pesticides are finding increased use in the control of insect damage to crops. With this increased use there has developed a growing concern for the nature of the residues which remain when pesticides are applied to crops. One of the insecticides in common use today is diazinon, O,O-diethyl-O-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate.

Previous work by Ralls et al. (1966, 1967) demonstrated that the principal conversion products of diazinon are the oxygen analog, O,O-diethyl-O-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphate, and 2-isopropyl-4-methylpyrimidin-6-ol.

In this laboratory, a study of extracts of diazinon field-sprayed kale samples by enzyme inhibition thin-layer chromatography (El-Refai and Hopkins, 1965; Gardner et al., 1969; Mendoza et al., 1968) showed an additional product not previously reported.

Thin-layer chromatography (tlc) showed that this unknown product was not found in diazinon standards, the diazinon used to prepare the spray solution, the aqueous spray solution itself, or the control kale extracts. It was assumed that the unknown compound was a true alteration product of diazinon.

Cook (1954, 1955) and Mitchell (1961), using paper chromatographic techniques, have shown that UV irradiation of a number of pesticides leads to the formation of compounds having polarities different from the parent pesticide. By first employing UV irradiation of a diazinon standard, small amounts of a product were obtained which, after isolation, gave the same R_f by tlc and the same R_t by glc as that of the unknown compound found in the kale extracts.

This paper reports the residues found on the diazinon field-sprayed kale and the preparation and characterization of a UV-irradiation product, hydroxydiazinon, prepared from a diazinon standard and similar to the unknown compound found in the diazinon field-sprayed kale.

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EXPERIMENTAL

Crop Studies. Kale which had been grown under natural field conditions was sprayed with an aqueous emulsion of technical diazinon to give an estimated crop coverage of 2 lbs of the active material per acre. Portions of the plants were harvested 2, 7, 11, and 15 days after the single application of the pesticide. Similarly, portions of untreated kale were harvested at these same time intervals to serve as controls.

The kale from each of the harvest intervals was composited and thoroughly mixed in a Hobart food chopper. The pesticidal residues were extracted and cleaned by the method of Storherr et al. (1964) as modified by Watts et al. (1969). The final solution represented 2.0 g per ml of the kale sample.

Gas Chromatography. The concentrations of diazinon, the oxygen analog, and the alteration product in the 2.0 g per ml solutions were determined using a Barber-Colman Model 5360 gas chromatograph equipped with a potassium chloride thermionic detector, KCl-TD (Giuffrida, 1966). Two glass glc columns 6 ft × 4 mm i.d. were used. One contained 10% DC-200 and the other 2% diethylene glycol succinate (stabilized), both prepared on 80- to 100-mesh Gas Chrom Q (Watts and Storherr, 1969). The glc conditions were as follows: carrier gas, N_2 at 60 ml per min, injection port temp. 210° C; detector temp. 230° C, column temp. 185° C for the 10% DC-200 column and 175° C for the diethylene glycol succinate (DEGS) column. Air flow to the detector was 300 ml per min. The electrometer setting was 1×10^{-8} AFS (base line current set at 40-50% FSD at 1×10^{-8} AFS by adjusting hydrogen flow) for the 10% DC-200 column and 3×10^{-9} AFS (set as above before switching to 3×10^{-9} AFS) for the 2% DEGS column. Chart speed 0.5 in per min. The same columns and conditions were used to gather data on the UV irradiation product, hydroxydiazinon.

Thin-Layer Chromatography (tlc). Amounts of the 2-day diazinon cleaned up extract, calculated to contain approximately 50 ng of diazinon, were spotted on 8 × 8 in. 0.25 mm silica gel G plates for tlc. Plates were developed using a 1:3:16 mixture of ethyl ether, acetone, and chloroform, respectively. A procedure utilizing cholinesterase inhibition for

Table I. Residues Found by Glc[a] in Diazinon-Treated Kale

	Days After Application			
	2	7	11	15
Diazinon (ppm)	8.8	2.9	2.0	1.6
Diazoxon (ppm)	0.004	0.007	0.002	0.002
Alteration Product of Diazinon[b] (ppm)	0.18	0.05	0.03	0.03

[a] Column was the 10% DC-200 column. [b] Later identified as hydroxydiazinon. Quantitated using the compound prepared by UV-irradiation of diazinon.

Table II. Chromatographic Properties of Diazinon and Its Conversion Products[a]

Chromatographic System

	glc-I[b] RR_t	glc-II[c] RR_t	tlc R_f
Diazinon	1.00	1.00	0.77
Diazoxon	0.91	1.68	0.58
Hydroxydiazinon (UV Preparation)	1.53	2.92	0.70
Alteration Product of Diazinon (Kale)	1.53	2.92	0.70

[a] RR_t = retention time relative to diazinon. [b] Glc-I: 10% DC-200. Column temperature 185° C. Retention time of diazinon = 7.5 min. [c] GLC-II: 2% diethylene glycol succinate. Column temperature 175° C. Retention time of diazinon = 2.3 min.

Figure 1. Structural formulae for diazinon (1), diazoxon (2), and hydroxydiazinon (3)

detection (El Refai and Hopkins, 1965; Mendoza *et al.*, 1968; Gardner *et al.*, 1969) was used on one tlc plate.

Another thin-layer plate not subjected to oxidation or the cholinesterase inhibiting detection was used for isolation of the new inhibition product. The silica gel in the area corresponding to the unknown inhibition area in the developed plate (R_f = 0.70) was removed and extracted with ethyl acetate and acetone. This solution after concentration was chromatographed on glc using the KCl-TD detector.

Preparation of the Diazinon UV-Irradiation Product. A diazinon working solution was prepared by dissolving 4 g diazinon in ethyl acetate and diluting to 10 ml with the same solvent. A 2 ml aliquot (800 mg) of this standard was spread over the surface of a clean, uncoated, 8 × 8 in. glass plate and the ethyl acetate allowed to evaporate, leaving a film of diazinon adhering to the surface of the plate. The plate was then irradiated for 2 hr with ultraviolet light at a distance of $1\frac{1}{2}$ in. from the light source (GE Germicidal Lamp, 25 watts, No. G 25T8). The irradiated material was removed from the plate by washing with ethyl acetate. The ethyl acetate washings were collected in a Kuderna-Danish apparatus and concentrated using a Snyder column.

The product obtained by the UV irradiation process was initially separated from unreacted diazinon and several minor products by collection from a gas-liquid chromatograph using the 10% DC-200 column. For the separation and cleanup of larger amounts, a Florisil column was used with the elution schemes developed by Mills (1961) and modified by Wessel (1967). In this separation, the ethyl acetate was removed from the solution of the irradiation product by repeatedly adding isooctane and concentrating. The isooctane solution was then added to a 4-in. activated Florisil column and eluted with 200 ml each of 6%, 15%, and 50% ethyl ether in petroleum ether. The irradiated product eluted in the 50% ethyl ether fraction. A total of approximately 200 mg of product was isolated by this method.

Instrumentation. The following instruments were used for the characterization studies: a Perkin-Elmer Model 621 IR
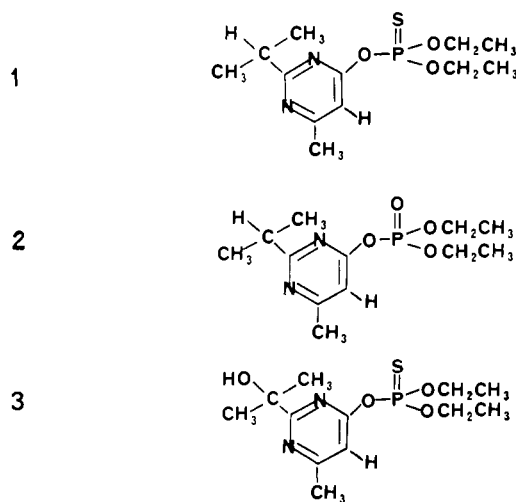
Spectrophotometer fitted with a dual 6X beam condensing unit; an Atlas CH-4B mass spectrometer equipped with a molecular beam inlet system; and a Varian A-60 nmr spectrometer. The accurate mass measurements were obtained on a CEC 21-110 mass spectrometer by peak matching. The sample was admitted through an all-glass reservoir system heated to 100° C.

RESULTS

Crop Studies. The levels of diazinon, the oxygen analog, and the alteration product in the field-sprayed kale are tabulated in Table I. The results show that the concentration of the alteration product is substantially lower than the parent compound. It is at a much higher concentration, however, than the oxygen analog, which has been reported to be the major cholinesterase inhibiting metabolite of diazinon. In all cases, the oxygen analog was found at a concentration substantially less than 0.01 ppm.

The previously reported conversion product, 2-isopropyl-4-methyl pyrimidin-6-ol, was also found on the kale, but because this product is not a true inhibitor, quantitative determinations are not reported.

Chromatography. Chromatographic properties of diazinon, its oxygen analog, the unknown compound isolated from kale, and hydroxydiazinon are presented in Table II. Hydroxydiazinon, the product isolated from the UV-irradiation of diazinon, appeared to be identical to the alteration or unknown compound found in the sprayed kale (see Table II for R_t and R_f values). Hydroxydiazinon was not found in the samples of untreated kale nor in any of the diazinon standards or spray solutions.

Since the spectroscopic evidence necessary for structural proof could not be obtained on the cleaned up kale extracts because of crop interferences, the UV-irradiation product (hydroxydiazinon), identical to the unknown compound in R_f and R_t values, served as the test compound.

Figure 1 gives the structural formulas for diazinon, the oxygen analog of diazinon, and the proposed hydroxydiazinon.

STRUCTURAL DETERMINATIONS

Infrared Spectroscopy. The infrared spectra of diazinon and of the irradiation product (hydroxydiazinon) are shown in Figures 2a and 2b. Table III summarizes the characteristic frequencies of these spectra (Bellamy, 1958; Rao, 1963).
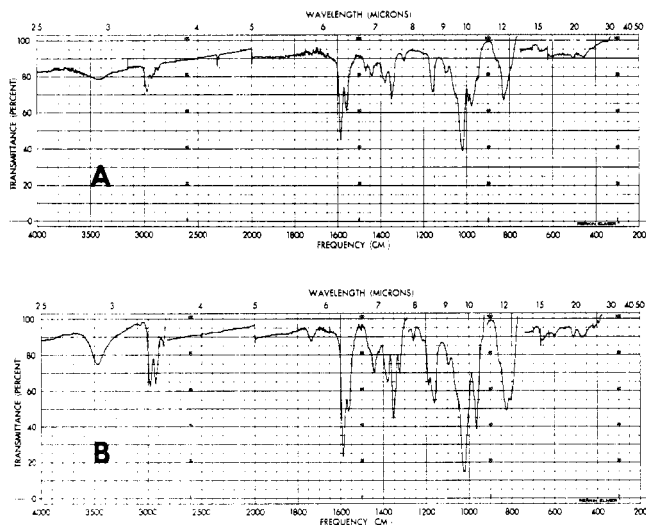
Figure 2. Infrared spectra of (A) diazinon and (B) hydroxydiazinon

Figure 3. Mass spectra of diazinon (top) and hydroxydiazinon (bottom)

The spectrum of the irradiation product is enhanced by the presence of three new absorption bands. The first of these bands of medium intensity appears at 3468 cm^{-1} and is attributed to OH stretching; the second, at 1323 cm^{-1}, arises from OH in-plane deformation, and the third, at 1187 cm^{-1}, from C—O stretching in the tertiary alcohol (Colthup, 1964).

In brief, the significant structural difference between these two compounds lies in the tertiary H in the isopropyl group of diazinon which is oxidized to a tertiary alcohol in the diazinon irradiation product.

Mass Spectrometry. The mass spectra of a number of organophosphorus pesticidal compounds, including diazinon, have been published (Damico, 1966). The mass spectral correlations and nomenclature delineated in that work were utilized in the present study. However, differences in instrumentation necessitated the recording of the spectrum of diazinon on the Atlas CH4B mass spectrometer to insure
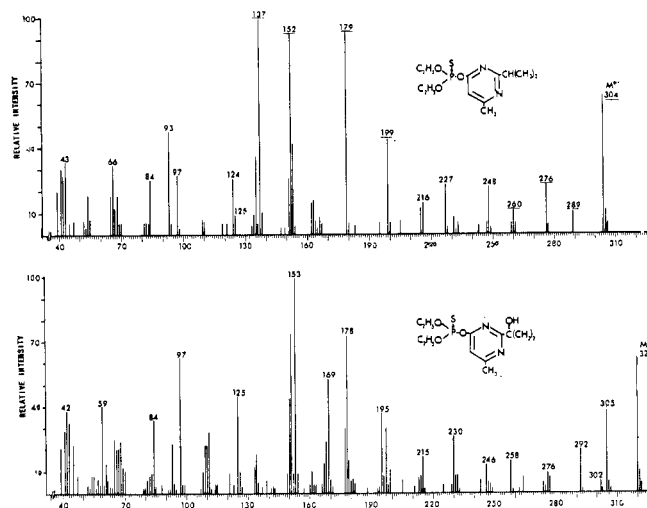
Table III. Characteristic IR Frequencies of Diazinon and Its Irradiation Product

Group	Diazinon	Diazinon UV Product
OH stretching		3468 (M)
CH$_3$ asym. st. in C$_2$H$_5$	2970 (M)	2976 (M)
CH$_3$ asym. st. to ring and CH$_2$ asym. stretching	2928 (W)	2924 (M)
CH st. (tertiary)	2904 (W)	
CH$_3$ & CH$_2$ sym. st	2868 (W)	2852 (W)
C = N skeletal vibration in pyrimidyl ring (Rao. 1963)	1584 (S)	1586 (S)
	1557 (M)	1562 (S)
	1468 (W)	1468 (Sh)
	1438 (W)	1442 (W)
CH$_3$ & CH$_2$ asym. i.p.d.	1468 (W)	1468 (Sh)
	1438 (W)	1442 (W)
CH$_3$ sym. i.p.d.	1377 (W)	1378 (W)
	1347 (W)	1353 (W)
OH i.p.d.		1323 (M)
C-O st. in tertiary alcohol		1187 (M)
p-O-C$_2$H$_5$	1157 (M)	1160 (M)
p-O-C$_{aliphatic}$ asym. st.	1018 (VS)	1022 (VS)
1 H o.p.d. on ring	860 (Sh)	858 (Sh)
p-O-C$_{aliphatic}$ sym. st.	826 (M)	824 (M)
p = s st.	648 (W)	654 (W)

S = strong, M = medium, W = weak; st. = stretching; i.p.d. = in plane deformation; o.p.d. = out of plane deformation.

that its comparison with the irradiation product (hydroxydiazinon) would be valid (see Figure 3).

Briefly, diazinon undergoes two characteristic rearrangements, α-cleavage of the molecular ion with migration of the ethyl group to the oxygen atom of the Z moiety with the loss of a hydrogen to give the intense peak at m/e 179, and α-cleavage of the molecular ion with hydrogen rearrangement to yield the peak at m/e 152. The base peak at m/e 137 is formed by loss of a methyl group from the ion at m/e 152. Unpublished metastable data indicate this is the pathway for the formation of the ion at m/e 137.

The $\begin{array}{c} C_2H_5O \\ \quad \quad \quad \quad \\ C_2H_5O \end{array}$ $\overset{S}{\underset{}{\uparrow}}$ P— group is characterized (Damico. 1966) by intense peaks at m/e 153, 125, and 97.

The mass spectral data of the irradiation product showed a molecular weight of 320. Also, a number of peaks shifted 16 amu above the corresponding peaks in the diazinon spectrum. (See Biemann, 1962, for a discussion of the Mass Spectrometric Shift Technique.) The major peaks shifted are indicated by an underlined mass number on the diazinon spectrum. The peak at m/e 302, attributed to the loss of water in the irradiation product (absent in diazinon) suggested that the 16 amu shift was due to the incorporation of an oxygen atom into the molecule. With the fragmentation patterns of both compounds exhibiting such great similarities, indicating no change in the basic skeleton of the molecule, some type of alcohol was suspected. Infrared studies confirmed a tertiary alcohol. In order to fulfill these criteria, the only position for such a moiety is on the isopropyl group. The peak at m/e 59 then could result by simple cleavage of the isopropyl alcohol moiety with charge retention on the oxygen.

Several other peaks appearing in the spectrum of the irradiation product may be attributed to the influence of the alcohol group. The molecular ion at m/e 320 loses water to form the ion at m/e 302. The latter may subsequently undergo α-cleavage with ethyl migration to produce the intense ion at m/e 178. A similar process, α-cleavage with ethyl migration, plus the loss of a hydrogen, could take place from the molecular ion of diazinon to produce an ion at m/e 179, from the molecular ion of the irradiation product to produce an ion at m/e 195, and from the M-18 ion of the irradiation product to produce an ion at m/e 177.
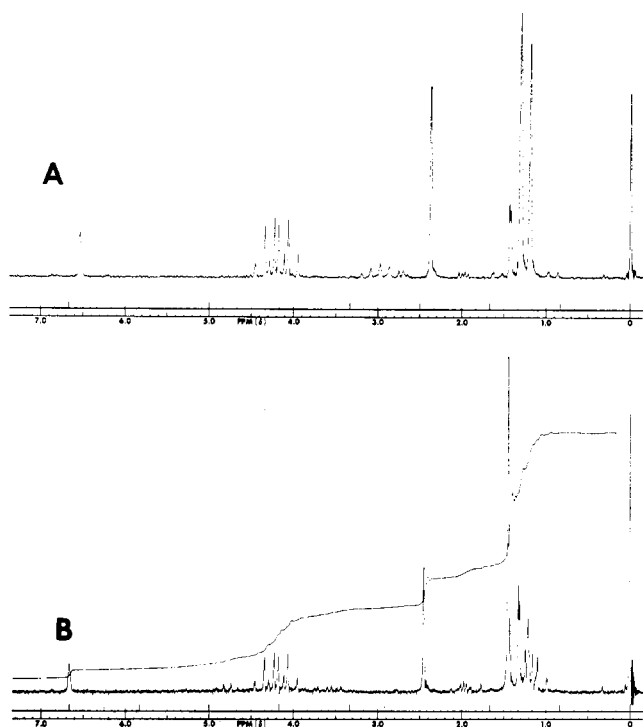
Figure 4. 60 MHz nmr spectra of acetone-d₆ solutions of (A) diazinon and (B) hydroxydiazinon

Similarly, the ion at m/e 246 may arise by two successive losses of 28 amu from the M-18 ion or possibly by loss of the isopropyl alcohol moiety from the M-15 ion at m/e 305. Metastable transitions were not observed for any of these processes, and since the product ions of both processes would have identical elemental compositions, high resolution measurements would not be helpful in distinguishing between them. However, two successive losses of 28 amu from the molecular ion and the concomitant metastable peaks were observed for diazinon, lending support for the former mechanism in the irradiation product.

An intense ion is observed in the metabolite at m/e 151 with the elemental composition of $C_8H_{11}N_2O$, as determined by accurate mass measurement. It probably forms by β-cleavage of the molecular ion with the charge being stabilized by the heterocyclic ring system. The ion at m/e 169 is a doublet with elemental compositions of $C_8H_{13}N_2O_2$ and $C_7H_9N_2OS$. The former most likely arises by α-cleavage of the molecular ion with a double hydrogen rearrangement. The latter, if arising from the metabolite, obviously involves migration of the sulfur atom and rearrangement, for which there are several possibilities. However, in light of the inlet system used, decomposition must be suspected.

Nuclear Magnetic Resonance Spectroscopy. The evalua-

tion of the nmr spectra of diazinon and its UV irradiation product (Figures 4a and 4b) further supports the proposed structure for the product as hydroxydiazinon, O,O-diethyl-O-[2-(2'-hydroxy-2'-propyl)-4-methyl-6-pyrimidinyl] phosphorothioate. In the spectrum of diazinon there is a doublet at 1.2 ppm (δ) for the protons of the two isopropyl methyl groups, and a septet at 3.0 ppm for the methine proton. The spectrum of the irradiation product is quite similar to that of the parent compound. However, the doublet corresponding to isopropyl methyl protons has collapsed to a singlet (1.4 ppm), and the multiplet for the methine proton is absent. This indicates that the methine proton of diazinon has been replaced by another group whose nmr-active nuclei do not produce splittings of the isopropyl methyl absorption, and that the remainder of the molecule has not been altered by exposure to UV light.

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