Photodegradation of two Dinitrophenolic Pesticide Chemicals, Dinobuton and Dinoseb, Applied to Bean Leaves

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Dinitrophenols and their esters are used as insecticides, acaricides, ovicides, herbicides, and fungicides. Residues of these pesticide chemicals are generally determined as the parent phenol, after saponification when necessary, by methods which normally do not detect many related compounds, such as those that might form by biological or photochemical oxidation or reduction. Since the photochemistry of dinitrophenolic pesticide chemicals is poorly understood, it is important to develop the information needed to interpret the data obtained in studies on the persistence and toxicology of residues on or in food and feed. Accordingly, C^{14}-labeled preparations of 2-sec-butyl-4,6-dinitrophenol (dinoseb, DNBP) and its isopropyl carbonate (dinobuton, Acrex®, Dessin®) were exposed to sunlight on growing bean foliage in order to determine the nature of the persisting residues.

Materials and Methods

Dinobuton-C^{14}, as provided by Union Carbide Corp., South Charleston, West Va., was a ring-labeled preparation of greater than 99% radiochemical purity with a specific activity of 3.5 mc/mmole. Dinoseb-C^{14}, of 0.59 mc/mmole specific activity and of greater than 99% radiochemical purity, was obtained by hydrolysis of dinobuton-C^{14} with methanolic ammonium hydroxide at 25°C., purification on a Florisil column with benzene and chloroform-methanol (4:1) mixture, successively, for elution, and recrystallization from hexane. Authentic preparations of 6-hydroxybutyl- and 6-butenyl-2,4-dinitrophenol were provided by Union Carbide Corp.

Dinobuton-C^{14} (5.5 μg) or dinoseb-C^{14} (4.9 μg) in 20 μl of ethanol was applied uniformly, by a described procedure (1,2), to the upper surface (25-30 cm^2) of each primary leaf

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(0.6 g) of garden snapbean seedlings (Contender variety). Immediately after treatment, the plants were exposed outside to full sunlight for 6 hours on the first day, 10 hours on the second day, and 4 hours on the third day, and stored inside in the dark during the intervening periods of 14 hours or less. These studies were made in Berkeley, California, during August, 1968, in a period of no rainfall.

Leaves treated with dinobuton-C^{14} were rinsed 3 times with acetone (3 ml) to recover surface residues and homogenized 3 times in methanol (3 ml) to recover penetrated residues. A 2/5-aliquot was used for the separation and radiodetermination of the surface residues by direct thin-layer chromatography (TLC); alternatively, a 3/5-aliquot was hydrolyzed with methanolic ammonium hydroxide prior to TLC analysis. The penetrated residues were determined by TLC only after hydrolysis with ammonium hydroxide. Dinoseb-C^{14}-treated leaves were cut into small pieces and homogenized 3 times in methanol (3 ml) and the extracts were analyzed by TLC without hydrolysis. In each case, the TLC analysis involved the use of 1) silica gel F-254 chromatoplates (Merck) of 0.25-mm gel thickness, which were developed, in the first direction, with benzene and, in the second direction, with ethyl acetate, 2) radioautography, and 3) transferring, by scraping, the radioactive gel regions into scintillation vials for direct counting (1,2). The results reported here are averages of duplicate analyses on separate leaves.

Different ammonium hydroxide hydrolysis procedures were used for the surface- and the penetrated residues from dinobuton treatment. After evaporation of the acetone extract to dryness under nitrogen, the surface residues were hydrolyzed by addition of 1.0 ml each of methanol and concentrated ammonium hydroxide, and digesting for 24 hours at 25°C. The resulting solution was evaporated under nitrogen at 25°C. to 0.5-1.0 ml, 1 ml of concentrated hydrochloric acid and 50 mg of sodium chloride were added, and the reaction mixture was extracted 3 times with ether (3 ml). Radiocarbon contents of aliquots of the ether- and water-soluble phases were determined and the remaining ether extract was subjected to TLC analysis. The penetrated residues in 9.8 ml of methanol were hydrolyzed by addition of 3 ml of 10% ammonium hydroxide, digestion for 24 hours at 25°C., evaporation under nitrogen to a volume of 1-2 ml, addition of 2 ml of concentrated hydrochloric acid and 200 mg of sodium chloride, and extraction 3 times with ether (3 ml), yielding water-soluble and ether-soluble extractives which were analyzed as above.

The labeled compounds not recovered from the insoluble portion by the above procedures were determined by combustion (3). They account for the following amounts of the applied radiocarbon: dinobuton - 1% at each of 0.5 and 4 hours, and 0.5% at 16 hours; dinoseb - 10% at 0.5 hour, 8% at 8 hours, and 3% at 16 hours.

Results

Table 1 gives the total recovery of radioactive products found in and on bean leaves following the application of ring-C^{14}-labeled dinobuton or dinoseb and exposure to sunlight for various lengths of time. Fig. 1 shows illustrative TLC chromatograms for the radioactive products derived from dinobuton and dinoseb on exposed bean leaves, corresponding to the regions designated in Table 1 as dinobuton, dinoseb, products of intermediate R_f values (or ester unknown and phenol unknowns A and B), and products at or near origin.

Volatilization is one mechanism of residue dissipation for both compounds because there is a progressive loss of the applied radiocarbon with time. A portion of the labeled materials is not extracted from the plant, accounting for another source of loss. The products persisting for 2 hours or longer after treatment mostly are degradation products, not dinobuton or dinoseb.

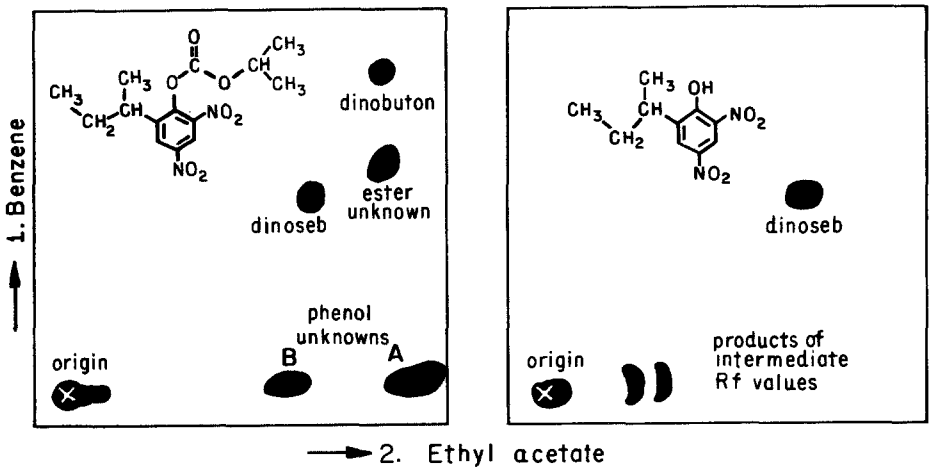

Fig. 1. Illustrative chromatograms showing TLC characteristics of labeled products recovered from bean foliage treated with ring-C^{14}-labeled preparations of dinobuton (left) or dinoseb (right) and exposed to sunlight.

TABLE 1

The Effect of Exposure to Sunlight on Total Recovery of Radioactive Residues from Bean Leaves following Application of Ring-C^{14}-labeled Dinobuton and Dinoseb

Hours of exposure to sunlight after applic.	Recovery[a] of radioactive compounds in residue, %, after application of Dinobuton						Dinoseb			
	Dino-buton	Dino-seb	Products of intermediate R_f values	Products at or near origin	Water-soluble products	Total	Dino-seb	Products of intermediate R_f values	Products at or near origin	Total
0	96	4	0	0	0	100	100	0	0	100
0.5	56	10	13	8	14	101	78	12	4	94
1	37	10	20	13	14	94	40	13	9	62
2	27	12	20	14	14	87				
4	33	4	13	16	11	77	8	11	15	34
6	27	3	15	16	12	73				
8	24	3	10	17	8	62	6	4	18	28
12	11	2	9	16	10	48				
16	5	2	7	20	8	42	4	0	16	20
20	3	1	7	18	8	37				

[a] Sum of surface- and penetrated residues.

Regardless of the time after treatment with dinobuton (0.5 to 20 hours), the surface material rinsed from the leaf with acetone in each instance accounts for 71 to 73% of the total radiocarbon recovered in the surface plus penetrated residues. Dinobuton appears only in the surface material but, even if present, it would not be detected in the penetrated material because the analysis of this fraction involves hydrolysis as a preliminary step. The dinoseb residue that forms from dinobuton application appears in approximately equal amounts in the surface and penetrated residue fractions; in the acetone rinse of the surface of leaves, it occurs as free dinoseb but, in the penetrated residues, it possibly is present as dinoseb or as some derivative (such as dinobuton) which degrades to dinoseb on hydrolysis.

Application of dinobuton yields, in addition to dinoseb and material remaining at or near the origin of the chromatoplate, an unknown compound with a high R_f value in benzene and two other unknown products having lower R_f values. Ammonium hydroxide destroys the former but not the latter two unknowns; so, they are designated, respectively, as the ester unknown and phenol unknowns A and B. These phenol unknowns are not adequately resolved for precise determination, except in the case of the surface residue (acetone rinse) hydrolyzed with ammonium hydroxide, a procedure which destroys the ester unknown but minimizes the interference of unlabeled extractives in separation of unknowns A and B. Unknowns A and B are important penetrated - as well as surface residues. There is evidence that, at each time interval of sunlight used (0.5 to 20 hours), each of the three components making up the mixture of products having intermediate R_f value (i.e., ester unknown, and phenol unknowns A and B) comprise 10 to 40% of the total radiocarbon chromatographing in this region.

Polar materials in the surface rinse appear at or near the origin in the TLC chromatogram while those in the penetrated residue contribute about 20% of the amount recovered as materials that chromatograph at or near the origin, the remainder appearing in the water-soluble fraction which arises only from the penetrated material (because of the extraction method used). A small portion of the water-soluble materials possibly comes from decomposition of dinobuton, dinoseb, or the products of intermediate R_f values, as the result of the treatment with ammonium hydroxide, because the hydrolysis conditions used give rise to, in the presence of plant extractives, about 25% degradation of dinobuton to water-soluble products. Based on studies made without hydrolysis, each of the products at

or near the origin, the ester unknown, and phenol unknowns A and B appear, once formed, to be more persistent than dinobuton.

Under the photoconditions used, dinoseb converts to more persistent products of lower R_f values. The time-sequence involved suggests that, as with dinobuton, the products remaining at or near the origin are the terminal products found by the analytical procedure employed. Differentiation of intermediate products, and intercomparison of these products with unknowns A and B derived from dinobuton, are not possible because the large amounts of interfering plant extractives present disturb the TLC patterns.

Discussion

It is clear that the major persisting residues of dinobuton and dinoseb are degradation products which are more polar than the compounds applied. In agreement with studies on related compounds (4), conversion of dinobuton to dinoseb occurs but not in a major way. Difficulties are encountered in reproducing the rate or extent of photodegradation when a large-scale experiment is attempted in order to prepare the amount of residue desired for product isolation. After exposure for several days to strong ultraviolet light as a thin film of solid material or as an oxygenated methanol or chloroform solution, dinobuton or the corresponding acetate converts, in less than 1% yield, to photodegradation products, other than dinoseb, or to resinous insoluble materials. The photodecomposition yields are not greatly increased by the addition of selected compounds which might have photosensitizer activity. In similar studies involving photodegradation under ultraviolet light, dinoseb acetate as a thin film or as an oxygenated methanol solution yields trace amounts of degradation products, several of which, by ultraviolet and mass spectroscopy, appear to be 2,4-dinitrophenols containing modifications on the sec-butyl grouping. The chromatographic position (TLC) of phenol unknown B is generally that associated with isomeric 6-hydroxybutyl- and 6-butenyl-2,4-dinitrophenols, based on comparison with authentic compounds. Interfering materials in the plant extractives make it difficult to compare the R_f values of the products with those of authentic compounds or with the products of dinoseb acetate photolysis. Amino and diamino derivatives of dinoseb chromatograph at or near the origin in the solvent systems used. With some compounds other than dinobuton and dinoseb, photoreduction of nitro groups takes place when the irradiation is done under anaerobic conditions (4,5).

It is possible that ester hydrolysis, nitro reduction, alkyl oxidation, and hydroxyalkyl dehydration are involved in dinobuton photodegradation. Also, a portion of the degradation following the application to bean leaves may result from plant metabolism of the penetrated materials; dinoseb and certain esters of dinoseb are known to undergo nitro reduction and side chain oxidation on metabolism in mammals (6). In any case, analyses made by methods which are specific for dinobuton and dinoseb, or for other dinitrophenolic pesticide chemicals, probably do not give a reliable measure of the residue content of plants treated with the respective pesticide chemical.

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