Table V. Relative Concentrations of Azodrin and Its Metabolites in Cotton Seedlings Treated with Azodrin-P^{32} and in Lepidopterous Larvae Fed Treated Seedlings

	Per Cent of Recovered Radioactivity as Azodrin-P^{32} Equivalents in Indicated Extract[a]									
	Seedling		Internal				Excreta			
Product	1	4	Z-1	V-1	Z-4	V-4	Z-1	V-1	Z-4	V-4
Phosphoric acid	1.0	3.4	2.5	3.2	8.5	8.7	1.3	1.7	4.0	3.6
Monomethyl phosphate	0.0	0.0	0.0	0.0	0.0	2.0	0.0	0.0	0.0	2.0
Dimethyl phosphate	1.3	2.3	32.7	31.9	32.9	40.0	7.8	12.2	5.9	9.9
O-Demethyl Azodrin	4.7	5.3	13.3	10.9	11.3	8.5	9.3	11.7	11.0	7.8
Unknown B	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0
Hydroxymethyl Azodrin	0.0	0.0	14.9	28.5	7.1	28.1	18.0	39.1	11.8	53.2
N-Demethyl Azodrin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.2	0.0	3.2
Azodrin	93.0	88.5	36.6	25.5	40.2	12.7	63.6	34.1	67.3	18.3
Azodrin-P^{32} μg.-equivalents:										
Extracted	17.0	12.3	4.0	3.5	4.2	4.0	9.7	9.4	6.2	8.0
Unextracted	0.8	1.8	0.7	0.5	0.7	0.7	0.2	0.6	0.4	0.5

[a] Z = *H. zea;* V = *H. virescens;* 1, 4 = number of days after seedlings were treated. Insects were allowed to feed on treated seedlings for 8 hours.

Table VI. Relative Concentrations of Azodrin and Its Metabolites in Excreta of Male Rats Following an Intraperitoneal Injection with 5 Mg./Kg. of Azodrin-P^{32}

	μg. Azodrin-P^{32} Equivalents of Indicated Products					
Hours after Treatment	H_3PO_4	Dimethyl phosphate	O-Demethyl Azodrin	Hydroxymethyl Azodrin	Azodrin	Per Cent of Dose Excreted
			URINE			
0–2	3.5	89.2	37.7	62.6	152.9	23.1
2–4	2.8	49.4	15.2	38.5	48.8	10.3
4–6	6.0	88.9	14.7	33.9	27.7	11.4
6–8	2.5	39.1	7.1	11.4	11.3	4.8
8–10	1.2	20.6	3.3	5.4	4.8	2.4
10–12	0.7	10.3	1.7	1.9	2.2	1.1
12–24	4.7	46.0	8.7	10.3	9.3	5.3
24–48	7.1	19.5	2.4	3.7	3.8	2.4
			FECES			
0–24	2.6	16.9	15.2	10.1	31.3	5.1
24–48	0.4	6.5	0.9	1.3	1.2	0.7

duction of *O*-demethyl Azodrin by an approximate 4 to 1 ratio. Only trace amounts of *N*-demethyl Azodrin were observed during the experimental period.

Acknowledgment

The authors thank E. F. Feichtmeir, P. E. Porter, John Potter, and Ken Loeffler of the Shell Development Co., Modesto, Calif., for their assistance and cooperation on this research project. The research was supported in part by a grant-in-aid from Shell Development Co.

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Received for review August 5, 1965. Accepted January 10, 1966. In cooperation with the Texas Agricultural Experiment Station, Texas A & M University, College Station, Tex.

HERBICIDE METABOLISM

Absorption, Translocation, and Metabolism of Diphenamid-1-C^{14} by Tomato Seedlings

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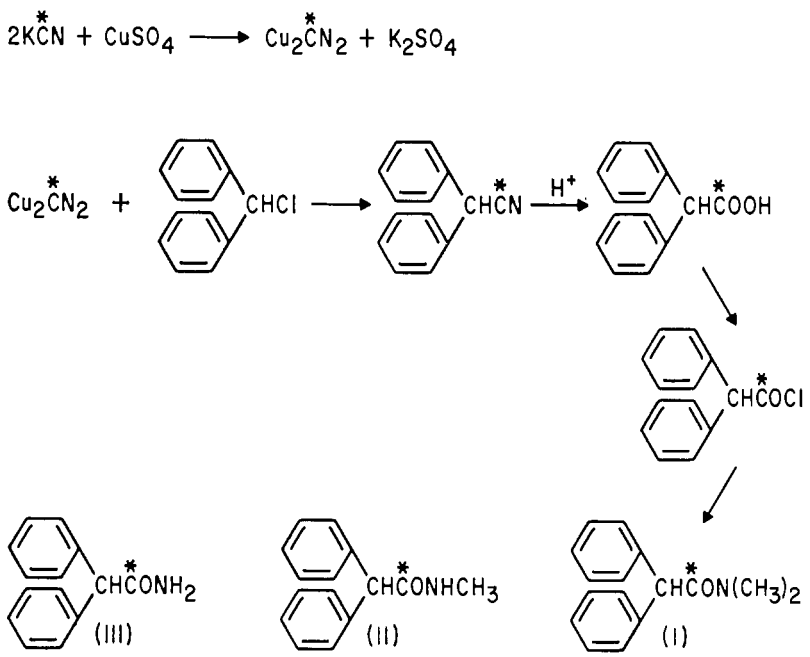
Diphenamid, 2,2-diphenyl-*N,N*-dimethylacetamide, absorbed and translocated by tomato seedlings from a nutrient solution, was dealkylated to give 2,2-diphenyl-*N*-methylacetamide and diphenylacetamide in the leaf tissue. Tomatoes grown in diphenamid-treated soil contained up to 11.8 p.p.m. of the desmethyl metabolite in the leaves. None was detected in ripe or green fruit.

DIPHENAMID is commercially useful for the pre-emergence control of weeds in tomato, pepper, potato, strawberry, tobacco, peanut, and other crops. This study was initiated to discover whether diphenamid was absorbed and translocated by a nonsusceptible plant and if translocation occurred, to discover the fate of the material.

Materials and Methods

Radioactive samples were counted in solution using a Packard Tri-Carb scintillation spectrometer, Model 314 EX-2. Paper and thin-layer chromatograms were counted using Vanguard Papergram Scanners (*1*).

Radioactive Diphenamid and Related Compounds. Diphenamid labeled with carbon-14 at the carboxyl carbon position was synthesized:



This sequence, as far as diphenyl acetyl chloride, has been described (*5*) and the addition of 2 molar equivalents of dimethylamine in benzene solution to the diphenylacetyl chloride, filtration, and evaporation of the solvent gave crude diphenamid-1-C^{14} (0.8 mc. per mmole). The radiochemical yield from the potassium cyanide was 34%. The purity of the compound and also of diphenylacetic acid 1-C^{14} retained from the reaction sequence was assessed by paper chromatography using the system 1-butanol–ammonium hydroxide–water, 100:3:18, using the descending technique on Whatman No. 1 paper. Diphenamid had R_f 0.9 and diphenyl acetic acid had R_f 0.7.

Carboxyl - labeled 2,2-diphenyl - *N*-methylacetamide (II) and 2,2-diphenylacetamide (III) were prepared by diluting 10 mg. of diphenylacetic acid-1-C^{14} (0.8 mc. per mmole) with 250 mg. of unlabeled diphenylacetic acid, converting the diluted acid to the acid chloride, then treating with either methylamine to give II or concentrated ammonium hydroxide to give III, both of specific activity 0.03 mc. per mmole. These carboxyl-labeled compounds were used as chromatography standards.

Experimental

Extraction of Tomato Seedlings Grown in Hoagland's Nutrient Solution Containing Diphenamide-1-C^{14}. In the first experiment, 20 tomato seedlings (6 inch, Rutgers variety) were grown in Hoagland's nutrient solution containing 4.062×10^4 d.p.m. per ml. (equivalent to 5.5 μg. per ml. of 0.8 mc. per mmole of diphenamid-1-C^{14} or 5.5 p.p.m. of radioactive diphenamid solution). At various time intervals two plants were removed from the solution, the roots were washed with water, the combined Hoagland's solution and washings were sampled, and the volume was measured. The plants were extracted twice each with 50 ml. of benzene by macerating the tissue in a Waring Blendor. The benzene extract was separated by centrifugation and aliquots were counted. The remaining solid residue was extracted twice with 50 ml. of water, using the same method, and aliquots of the water were counted.

In this way, since the volume of the Hoagland's nutrient taken up was known and the d.p.m. of the solid residue was determined by Schöniger combustions (*7*), a total recovery of radioactivity was found. Typically (7-day harvest) it was ca. 90%, distributed as follows: recovered nutrient 39.7%, benzene extract 45.3%, aqueous extract 14.6%, unextracted residue 0.4%. By separation on Dowex 50 (H^+) resin and paper chromatography, it was shown that the aqueous extracts contained labeled carbohydrates which were naphthoresorcinol-positive and labeled amino acids, which were ninhydrin-positive (*6*). All further work was carried out using the benzene extracts.

The single paper chromatography system yielding the best resolution of the standard compounds, diphenamid (I), methyldiphenylacetamide (II), diphenylacetamide (III), and diphenylacetic acid was prepared by developing the Whatman No. 2 paper in the upper phase of a mixture of 687 ml. of Skellysolve C, 333 ml. of benzene, 800 ml. of methanol, and 200 ml. of water at 34° C. after equilibration overnight in a tank containing this mixture. The four compounds had the following R_f's: diphenylacetic acid 0.25, diphenylacetamide 0.4, diphenyl *N*-methylacetamide 0.55, and diphenamid 0.8. Several other paper and thin-layer systems were investigated without obtaining improved resolution. When the benzene extracts from the tomato seedlings grown for 6 hours in diphenamid-1-C^{14} solution were chromatographed using the Skellysolve C–benzene–aqueous methanol system, only diphenamid was detected. After 12 hours, 24 hours, and 7 days, however, other peaks were evident on the scans of the paper chromatograms. Thus, in the benzene extract from the 7-day plants, diphenamid constituted ca. 59% of the radioactivity when calculated from the areas of the peaks and diphenyl-*N*-methylacetamide (36%), diphenylacetamide (5%), and a small peak corresponding with diphenylacetic acid were detected. The R_f's of the peaks shifted when co-chromatographed with the cold standards, but they were not separated. In a few instances, the diphenyl-*N*-methylacetamide peak was split into two peaks when co-chromatographed with cold standards, but the separation was not reproducible.

To confirm the presence of the desmethyl compound, a portion of the benzene extract from the 24-hour treatment containing 1.037×10^5 d.p.m. (20% of the extract) was mixed with 11.2 mg. of cold diphenyl-*N*-methylacetamide. The solution was evaporated to dryness and the residue crystallized from Skellysolve B–acetone. After recrystallization from acetone–Skellysolve B again, the specific activity of the recovered compound was determined; it was 0.1076 μc. per mmole.

Two further crystallizations, one from carbon tetrachloride and one from acetone-Skellysolve B failed to change the specific activity—i.e., after the third crystallization the specific activity was 0.1099 μc. per mmole and after the fourth crystallization it was 0.1081 μc. per mmole. Using this inverse dilution method, it was concluded that 2,2-diphenyl-*N*-methylacetamide was present in the extract. From the foregoing data, it was calculated that 11.4% of the radioactivity in the sample was the desmethyl compound.

A second experiment using 36 tomato seedlings grown in a 10 p.p.m. solution of diphenamid-1-C^{14} in Hoagland's nutrient for 6 days gave the same results as before. The scan of the benzene extract is shown in Figure 1. When the benzene extract was co-chromatographed with diphenyl-*N*-methylacetamide-1-C^{14} there was no separation of the slower moving material (R_f 0.53) in the extract from the labeled standard.

Soil Treatment with Diphenamid-1-C^{14}. Four tomato seedlings grown in 8-inch diameter pots were treated with 50 grams per pot of dry soil containing 14.525 mg. of diphenamid-1-C^{14}. The treated soil was spread evenly over the existing surface of the soil and each pot was watered with 300 ml. of water. The plants were thereafter kept in the greenhouse and watered twice daily. After 21 days, an upper and a lower leaf from each plant were removed. The upper leaves were combined and extracted with benzene. After aliquots

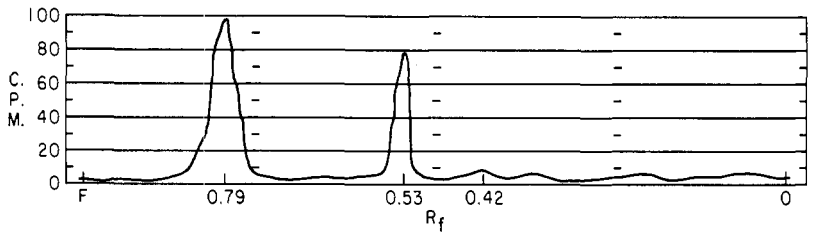


Figure 1. Scan of paper chromatogram of benzene extract from tomato seedlings grown in 10 p.p.m. diphenamid-1-C^{14} solution

were counted, the solvent was removed by distillation and portions of the residue were paper chromatographed using the Skellysolve C–benzene–aqueous methanol system. The lower leaves were treated in a similar manner.

The paper chromatograms showed only one radioactive peak at R_f 0.50, which corresponded with the position of diphenyl-*N*-methylacetamide. There were several small traces in the scan at R_f's smaller than 0.5. Hence, it was concluded that there was no diphenamid in the foliage but that the radioactivity was due to the presence of the metabolite diphenyl-*N*-methylacetamide. From the counts made on the aliquots of the initial extracts, it was calculated that there were 10.0 p.p.m. in the upper and 11.8 p.p.m. of the compound in the lower leaves.

After 77 days a ripe tomato (41 grams) was harvested from a treated plant and extracted twice with benzene (100 ml.). Aliquots of this benzene solution did not contain any radioactivity when counted in the usual way using the scintillation counter. After 97 days another ripe tomato and a green tomato were harvested. Benzene extracts of these did not contain radioactivity.

Discussion

The *N*-demethylation of methylamines and methylamides by both plants and animals has been described. For example, Menzer and Casida (*4*) described the demethylation of Bidrin [3 - (dimethoxyphosphinyloxy) - *N*,*N*-dimethyl-*cis*-crotonamide] by animals, insects, and plants. McMahon (*3*) demonstrated that rat liver microsome fractions monodemethylated *N*,*N*-dimethyldiphenyl - acetamide, whereas rabbit microsomes demethylated both the *N*,*N*-dimethyl and the *N*-monomethyldiphenylacetamide. A proposed mechanism involves direct hydroxylation of the *N*-alkyl group with the production of formaldehyde:

$$\text{>N-CH}_3 \rightarrow \text{>N-CH}_2\text{OH} \rightarrow \text{>N-H} + \text{CH}_2\text{O}$$

In the 21-day experiment only the monomethyl compound was observed together with a trace of a material corresponding to diphenylacetamide (R_f 0.42) in the chromatogram scan. Diphenamid was not observed, leading to the hypothesis that the tomato seedlings were resistant to the herbicidal action of diphenamid because of the ability to convert the compound into the much less phytotoxic monomethyl amide. Tomato fruit, harvested from plants which showed high concentrations of the radioactive monomethyl compound in the leaves, did not contain any detectable radioactive residue, confirming the results of extensive residue determinations of commercially treated tomatoes using conventional detection methods (*2*).

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Received for review August 13, 1965. Accepted December 7, 1965.

FORMATION AND EVALUATION OF DERIVATIVES

Preparation and Insecticidal Evaluation of Alcoholic Analogs of Kepone

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Preparation and insecticidal evaluation of 61 new alcoholic derivatives of Kepone (I) are reported. All the compounds resemble Kepone in being active on chewing insects, but 18 show 80% or better kill on aphids, on which Kepone is inactive. Follow-up tests on three of the compounds against seven insects showed three to 20 times the activity of Kepone in several cases. One compound is more active than any other known toxicant on Colorado potato beetle larvae.

The ketonic insecticide-fungicide Kepone (I) [decachloro-octahydro-1,3,4 - metheno - 2*H* - cyclobuta(*cd*) pentalen-2-one, or decachloropentacyclo(5.3.0.$0^{2,6}$.$0^{4,10}$.$0^{5,9}$) decan-3-one] has undergone commercial development (*2, 5*) as a stomach insecticide effective on chewing insects. It has shown excellent control of 17 species, and good to fair control of 63; outstanding results have been noted on potato and fruit insects and on roaches and ants. Fair to good fungicidal activity has been observed on 20 plant diseases. Kepone, shown as an anhydrous material in formula I, easily undergoes hydration, and is ordinarily used as a mono- to trihydrate.

The purpose of this study was the preparation and insecticidal evaluation of a series of secondary (II) and tertiary (III) alcoholic derivatives of Kepone, all involving reaction with the carbonyl group. Compound IIIb ($R = C_6H_5-$) has been prepared by Earle (*3*). Compounds IIa and IIIa were reported (*6*) subsequent to completion of the present study, although no details of the method of preparation or the properties of the compounds were given. The materials