Fate of 3-Hydroxy-*N*-methyl-*cis*-crotonamide Dimethyl Phosphate in Cotton Plants

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The nature and rate of metabolism of Azodrin (3-hydroxy-*N*-methyl-*cis*-crotonamide dimethyl phosphate) in cotton plants were studied. Oxidative conversion of Azodrin to its *N*-methylol derivative was of minor importance. The primary sites of hydrolytic degradation were at the vinyl-phosphate bond and one methyl-phosphate bond. The half life of Azodrin in cotton leaves was about 7 days. Azodrin was rapidly lost from the surface of cotton leaves following foliar application.

Azodrin (3-hydroxy-*N*-methyl-*cis*-crotonamide dimethyl phosphate, Shell SD-9129) is a substituted vinyl-phosphate insecticide that appears promising as a foliar spray (*3*) and a systemic (*3, 8*) for the control of several insect pests. The oxidative metabolism of this compound has been studied in bean plants following stem injection (*7*) and in insects and mammals (*2, 7*); these investigations showed that the metabolic fate of Azodrin was similar to that of the closely related vinyl phosphate, Bidrin (3-hydroxy-*N,N*-dimethyl-*cis*-crotonamide dimethyl phosphate). The oxidative and hydrolytic metabolism of Bidrin in plants, insects, and rats (*1*) and its systemic activity in cotton plants (*6*) have been reported. The authors therefore undertook the present study to characterize the oxidative and hydrolytic metabolism of Azodrin in cotton plants grown in the greenhouse and in the field. Both foliar and systemic treatments were used. Also, some additional information on the nature of an unidentified toxic water-soluble metabolite of Bidrin (*1*) is reported.

Experimental

The Azodrin-^{32}P (4.1 and 10 mc. per gram), Bidrin-^{32}P (12 mc. per gram), and the theoretical metabolites were supplied by Shell Development Co., Modesto, Calif., or synthesized in the authors' laboratory (*1, 2*). Previously described extraction techniques (*1, 2*) and chromatographic procedures (*1*) were used. Essentially, the plant material was extracted with a mixture of acetone and water (1 to 1 v./v.), the volume of extract reduced under vacuum, and paper chromatography used to separate the various metabolites. The developed chromatograms were exposed to x-ray film to locate areas of radioactivity. More than 5000 disintegrations per minute of radioactivity were spotted on each chromatogram; the counting efficiency of the equipment used was 45%. These spots were cut from the chromatogram and radioassayed directly with a gas-flow, thin-window Geiger-Müller counter. Each extract was chromatographed at least two times in the two systems used (*2*). Standards were cochromatographed routinely with the extracts and located colorimetrically with Hanes-Isherwood reagent (*5*). Chemical names and R_f values are shown in Table I.

Deltapine Smoothleaf variety cotton, grown either in 1-gallon cans of soil in a greenhouse at 80° to 95° F. or in the field at temperatures ranging from 65° to 95° F., was used. The leaves were characterized by age as follows: new growth, leaves that were not on the plant at time of treatment; expanding, leaves at the growing tip of the plant that were 1.5 to 2 inches in diameter; young mature, leaves from the uppermost part of the plant that were about 3 inches in diameter; and mature leaves near the middle and lower parts of the plant that were about 3 inches in diameter.

Seed Treatment. Saw-delinted cotton seeds were treated with 0.5 mg. of Azodrin-^{32}P per seed. Sufficient water was added to the Azodrin so that the solution coated all seeds rapidly and thoroughly and no excess was left. The seeds were air-dried and planted, 6 per 1-gallon can of soil, in the greenhouse. To minimize leaching of the toxicant, the 1-gallon cans were watered from the bottom. The seed meats, cotyledons, and true leaves were harvested at intervals after planting and extracted, and the extracts chromatographed.

Foliar Treatment. Experiments were conducted in the greenhouse and field to determine the fate of Azodrin-^{32}P applied to the upper surfaces of cotton leaves of different ages. Expanding, young mature, and mature leaves were treated topically with 40 μg. of Azodrin per leaf in a total volume of 50 μl. of an aqueous Azodrin solution. At intervals after treatment, the leaves were harvested, rinsed for 3 to 5 minutes in a known volume of distilled water, and then extracted. The external rinse and internal extracts were chromatographed separately. Radioassay of these extracts plus the unextractable radioactivity remaining in the plant materials gave recovery data. The difference between the amount of radioactivity applied and the amount recovered was assumed to be the amount lost by volatilization.

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Table I. Chromatographic Behavior of Azodrin and Its Phosphorus-Containing Metabolites in the Presence of Biological Materials

Abbreviated Name	Chemical Name	R_f Values in System[a] A	B
Phosphoric acid	Phosphoric acid	0.00	0.00
Monomethyl phosphate	Methyl dihydrogen phosphate	0.04	0.00
Unknown A		0.08	0.00
Dimethyl phosphate	Dimethyl hydrogen phosphate	0.19	0.00
O-Demethyl Azodrin	3-Hydroxy-*N*-methyl-*cis*-crotonamide methyl hydrogen phosphate	0.24	0.00
Azodrin acid	3-Hydroxy-*cis*-crotonic acid dimethyl phosphate	0.35	0.54
Unknown B		0.42	0.00
N-hydroxymethyl Azodrin	3-Hydroxy-*N*-hydroxymethyl-*cis*-crotonamide dimethyl phosphate	0.72	0.26
N-demethyl Azodrin	3-Hydroxy-*cis*-crotonamide dimethyl phosphate	0.80	0.43
Azodrin	3-Hydroxy-*N*-methyl-*cis*-crotonamide dimethyl phosphate	0.85	0.75

[a] A = acetonitrile–water–ammonium hydroxide (40:9:1 v./v.) with uncoated Whatman No. 3 paper. B = $CHCl_3$ with Whatman No. 3 paper impregnated with ethylene glycol (15% in acetone).

Stem Treatment. Several greenhouse cotton plants with 8 to 10 true leaves were each treated with 5 mg. of Azodrin-^{32}P mixed with 95 mg. of lanolin (*6*, *8*). This mixture was spread in a 1-inch band around the stems of the plant 2 inches above the soil. At intervals after treatment, leaves of different ages were removed and extracted, and the extracts chromatographed. Aliquots of the lanolin paste from the cotton stems were dissolved in chloroform and chromatographed.

Treatment by Petiole Injection. Expanding, young mature, and mature leaves of greenhouse and field cotton were each treated by petiole injection using a previously described stem injection technique (*4*) with 70 μg. of Azodrin-^{32}P in 20 μl. of an aqueous solution. These leaves were harvested at intervals after treatment and extracted, and the extracts chromatographed.

Inert Surfaces. The bottom halves of several glass Petri dishes were each treated with 96 μg. of Azodrin-^{32}P in 100 μl. of water. The solution was distributed uniformly over each dish and the dishes were then held in the greenhouse. At intervals after treatment, one or more dishes were rinsed with a 1 to 1 mixture of acetone–water. These rinses were chromatographed.

Bidrin Unknown. Previous research indicated that an unidentified metabolite of Bidrin (unknown A) (*1*) contained nearly all the intact Bidrin molecule and that upon acidification (pH 1), *N*-hydroxymethyl Bidrin [3-hydroxy-*N*-(hydroxymethyl)-*N*-methyl crotonamide dimethyl phosphate] was recovered. The present experiment was designed to determine if *N*-hydroxymethyl Bidrin was the immediate precursor of the Bidrin unknown A. *N*-Hydroxymethyl Bidrin was obtained by biological synthesis by injecting rats subcutaneously with sublethal (10 mg. per kg.) doses of an aqueous Bidrin-^{32}P solution. Urine collected from these rats was combined, diluted with water, and extracted several times with chloroform. The radioactivity in the chloroform was distributed as follows: 53.4% *N*-hydroxymethyl Bidrin, 19.7% Azodrin, and 26.8% Bidrin. Young mature excised cotton leaves were allowed to take up 100 μg. of Bidrin-^{32}P or 100-μg. of Bidrin equivalents of the mixture. These leaves were harvested at 1, 2, and 3 days after treatment and processed as before.

Results

Seed Treatment. The metabolism of Azodrin following seed treatment (Table II) was not so rapid as that of Bidrin (*1*). One week after planting, 50% of the radioactivity from plants grown from treated seed was identified as Azodrin. In a similar experiment with Bidrin, less than 25% of the radioactivity recovered was Bidrin plus oxidative metabolites after 1 week (*1*).

The detoxification of Azodrin applied as a cotton seed treatment resulted in large amounts of radioactivity being incorporated into plant constituents which were not extracted by the solvent used. Since *O*-demethyl Azodrin was recovered in all extracts, the cleavage of a methyl phosphate probably was one of the initial detoxification steps. Dimethyl phosphate, probably formed by hydrolysis of the vinyl-phosphate bond of Azodrin, was recovered in increasing amounts with time. *N*-Hydroxymethyl Azodrin was observed at only the 21-day harvest. Unknown B, which had an R_f value very similar to Bidrin unknown A (*1*), was recovered in increasing amounts with time, and was the major metabolite extracted at 14 and 21 days.

Foliar Treatment. The loss of Azodrin from cotton leaves following foliar treatment (Table III) was, for the most part, probably a result of volatilization. Usually, and particularly with plants grown in the field, nearly all of the loss occurred during the first 2 days after treatment. In the greenhouse, less volatilization of Azodrin occurred from the oldest leaves, but this decreased loss was not correlated with penetration because penetration rates were inversely proportional to leaf age.

Because of the large loss of radioactivity from leaves of field-grown cotton, precise information could not be obtained on the fate of the remaining Azodrin. The data in Table III indicate that Azodrin was metabolized

Table II. Metabolism of Azodrin Following Cotton Seed Treatment[a]

Compound	Days after Seed Planted							
	2[b]	3[b]	4[c]	5[c]	7[c]	10[c]	14[d]	21[d]
Phosphoric acid	6.5	15.7	24.0	10.0	31.4	30.2	1.6	6.4
Dimethyl phosphate	1.6	1.1	1.4	2.1	2.9	3.5	5.8	6.9
O-Demethyl Azodrin	5.8	2.1	2.8	4.1	3.5	2.3	2.6	1.2
Unknown B	0	0	1.0	2.0	2.5	5.0	10.4	13.4
N-Hydroxymethyl Azodrin	0	0	0	0	0	0	0	4.2
Azodrin	83.2	77.1	65.5	61.6	50.5	38.7	50.3	19.9
Unextractable	2.9	4.0	5.0	11.0	9.0	20.0	29.0	48.0
% of applied dose recovered	13.3	14.7	20.0	19.6	26.0	8.5	14.0	11.3

[a] Data based on radioactivity recovered from plants grown from cotton seed treated with 0.5 mg. Azodrin-^{32}P per seed. Results expressed as per cent of radioactivity found in the plants. Figures are averages from 3 plants per sample date.
[b] Seed meat only.
[c] Cotyledons only.
[d] Cotyledons plus true leaves.

Table III. Fate of Azodrin-^{32}P Applied Topically to Cotton Leaves[a]

Compound	% of Applied Radioactivity Recovered at Indicated Days after Treatment[b]											
	Greenhouse Plants						Field Plants					
	2		4		7		2		4		7	
	E	I	E	I	E	I	E	I	E	I	E	I
EXPANDING LEAVES												
Phosphoric acid	1.1	2.2	1.5	10.6	3.4	5.5	0	1.8	0.4	2.4	0.2	3.5
Unknown A	1.7	0	1.3	0	2.0	0	0	0	0	0	0	0
Dimethyl phosphate	10.6	2.5	9.2	4.9	8.9	8.6	0.2	0.6	1.8	2.0	0.5	1.9
O-Demethyl Azodrin	4.9	1.9	4.2	4.1	4.9	1.3	0.2	0.6	1.1	2.2	0.3	0.9
Unknown B	0	0	0	0	0	0	0	0	0	0	0	0
Azodrin plus *N*-hydroxymethyl Azodrin	4.6	29.4	1.8	38.4	0.8	27.5	0.6	9.0	0.6	10.5	0	6.7
Unextractable	...	5.0	...	6.0	...	11.0	...	2.0	...	3.0	...	6.0
Total	23	41	18	64	20	54	1	14	4	20	1	19
Loss[c]	36		18		26		85		76		80	
YOUNG MATURE LEAVES												
Phosphoric acid	2.8	0.6	2.9	2.2	6.8	4.5	0	1.2	0.4	2.2	0.8	1.7
Unknown A	1.8	0	1.6	0	4.4	0	0	0	0	0	0	0
Dimethyl phosphate	20.6	4.5	12.5	7.1	15.4	13.3	1.4	0.7	1.4	1.1	2.1	2.1
O-Demethyl Azodrin	7.7	1.3	7.5	1.5	9.3	1.2	0.2	1.4	0	0.4	0.5	1.3
Unknown B	0	0	0	0	0	0.8	0	0	0	0	0	0
Azodrin plus *N*-hydroxymethyl Azodrin	23.1	29.6	7.4	31.2	2.2	13.2	4.4	9.8	1.2	8.2	0.6	6.9
Unextractable	...	2.0	...	4.0	...	9.0	...	2.0	...	2.0	...	4.0
Total	56	38	32	46	38	42	6	15	3	14	4	16
Loss[c]	5		22		20		79		83		80	
MATURE LEAVES												
Phosphoric acid	9.7	0.9	7.8	3.3	19.2	1.4	...	...	...	...	...	...
Unknown A	3.9	0	2.9	0	9.0	0	...	...	..	...	...	...
Dimethyl phosphate	25.0	2.4	20.6	4.0	25.5	6.6	...	...	...	...	...	...
O-Demethyl Azodrin	10.6	0.8	18.6	1.5	13.4	2.4	...	...	...	...	...	...
Azodrin plus *N*-hydroxymethyl Azodrin	26.7	15.8	15.2	15.5	1.7	5.2	...	...	...	...	...	...
Unextractable	...	2.0	...	3.0	...	5.0	...	...	...	...	...	...
Total	76	22	65	28	69	24	...	...	...	...	..	...
Loss[c]	2		7		7							

[a] 40 μg. Azodrin applied to upper surface of each leaf.
[b] E = external H_2O rinse of leaves. I = extract of rinsed leaves.
[c] Loss determined by difference between amount applied and recovered.

both on the surface and inside treated leaves. Except for unknown B, the same metabolites were recovered from these leaves, both external and internal, as from the previously described seed treatment experiment. Extensive metabolism of Azodrin on the surface of greenhouse plants is indicated in Table III. The occurrence of unknown A on the surface of the treated leaves was the only departure from the seed treatment data. Extracts of the expanding leaves contained as much as or more intact Azodrin than extracts of the young mature and mature leaves.

Stem Treatment. The fate of Azodrin applied to cotton stems in lanolin (Table IV) indicated that the insecticide was stable in lanolin. More than 90% of the radioactivity in the lanolin removed from the stems 21 days after treatment was Azodrin. Metabolism of Azodrin in the various age leaves was similar. More unknown B was found in mature leaves than in expanding leaves, but none was found in new growth. The per cent radioactivity associated with Azodrin in the new growth reached a peak at 10 days after treatment and then decreased; however, the increasing total amount of radioactivity in the new growth more than compensated for this. Movement of noninsecticidal Azodrin metabolites from old growth to new growth probably accounted for some of the apparent rapid degradation of the insecticide.

Treatment by Petiole Injection. Since little difference was found in the metabolism of Azodrin by leaves of different ages in the petiole injection experiment, only data from expanding leaves are shown (Table V). These data show that Azodrin is stable inside the cotton plant with a half life of about 8 days for greenhouse cotton and about 7 days for field-grown cotton. Very small amounts of unknown B and *N*-hydroxymethyl Azodrin were found. Somewhat larger concentrations of unknown B were found in extracts of older leaves, up to a maximum of nearly 5%.

Inert Surface. The half life of Azodrin on a glass surface held in the greenhouse (Table VI) was about 5.5 days. Nearly one half of the loss resulted from volatilization; of the remainder, hydrolysis of the vinyl-phosphate bond to form dimethyl phosphate and of the methyl-phosphate bond to form *O*-demethyl Azodrin appeared to be the major change. No oxidation occurred.

Bidrin Unknown. The chromatographic behavior of the radioactivity extracted from the excised leaves treated with Bidrin or the mixture of 26.8% Bidrin, 53.4% *N*-hydroxymethyl Bidrin, and 19.7% Azodrin (Table VII) strongly suggests that *N*-hydroxymethyl Bidrin is the percursor of Bidrin unknown A. Three days after treatment, 30.9% of the radioactivity extracted from the leaf treated with the mixture was Bidrin unknown A; only 4.5% of the radioactivity extracted from the Bidrin-treated leaf was Bidrin unknown A.

Discussion

Oxidative *N*-demethylation of Azodrin to its *N*-hydroxymethyl and *N*-demethyl derivatives appears to

Table IV. Metabolism of Azodrin Following Stem Treatment of Cotton Plants[a]

Compound	% of Applied Radioactivity at Indicated Days after Treatment				
	3	7	10	14	21
			Mature Leaves		
Phosphoric acid	1.0	4.9	6.2	3.1	8.8
Dimethyl phosphate	1.5	2.9	5.6	3.6	10.5
O-Demethyl Azodrin	3.0	4.6	5.2	5.5	5.4
Unknown B	0	0.1	2.6	3.8	4.2
Azodrin plus *N*-hydroxymethyl Azodrin	84.4	80.3	67.3	68.6	49.6
Unextractable	10.5	7.0	13.1	15.2	22.0
μg. Azodrin equiv./leaf	11.2	60.0	...	142.2	113.0
			Young Leaves		
Phosphoric acid	5.4	10.0	17.2	13.2	7.5
Dimethyl phosphate	5.3	3.6	6.5	4.5	7.5
O-Demethyl Azodrin	5.4	3.8	5.1	5.3	3.0
Unknown B	0	0.1	0	0.4	1.5
Azodrin plus *N*-hydroxymethyl Azodrin	79.5	74.0	58.1	60.1	55.5
Unextractable	4.0	8.0	13.1	16.5	25.0
μg. Azodrin equiv./leaf	14.6	123.5	...	94.3	181.2
			New Growth		
Phosphoric acid	27.4	3.7	1.4	15.8	15.0
Dimethyl phosphate	4.2	5.6	0.2	6.1	6.7
O-Demethyl Azodrin	1.7	1.9	0	1.5	1.1
Unknown B	0	0	0	0	0
Azodrin plus *N*-hydroxymethyl Azodrin	49.0	50.8	72.4	40.0	29.9
Unextractable	17.0	38.0	26.0	36.5	42.0
μg. Azodrin equiv./leaf	2.4	7.3	...	18.3	46.4
			Azodrin-Lanolin Mixture from Plant		
Phosphoric acid	...	0.7	...	1.1	3.0
Dimethyl phosphate	...	0.9	...	3.7	5.1
O-Demethyl Azodrin	...	0.2	...	1.4	0.8
Azodrin[b]	...	98.0	...	93.9	90.7

[a] Leaves only harvested; greenhouse cotton plants treated with 5 mg. Azodrin-^{32}P in lanolin per plant. [b] No *N*-hydroxymethyl Azodrin found.

Table V. Fate of Azodrin-^{32}P in Expanding Cotton Leaves[a]

Compound	% of Applied Radioactivity at Indicated Days after Treatment							
	Greenhouse Plants				Field Plants			
	3	7	14	21	3	7	14	21
Phosphoric acid	1.5	0.5	3.8	1.8	3.3	1.0	4.1	4.6
Dimethyl phosphate	1.5	2.6	2.8	0.6	1.6	2.0	5.6	6.2
O-Demethyl Azodrin	4.6	3.9	4.7	1.5	4.1	5.0	5.1	1.5
Unknown B	0	0	1.4	0.2	0	0	1.0	0.5
N-Hydroxymethyl Azodrin	0	0	0	0	0	0	0	0.8
Azodrin	69.4	57.3	33.4	26.2	72.2	41.3	34.7	12.8
Residue	3.6	10.4	12.4	15.4	2.8	6.3	12.1	14.1
Loss[b]	19.3	24.4	40.4	55.1	15.0	43.8	36.7	60.1

[a] 70 μg. Azodrin-^{32}P administered per leaf via petiole injection.
[b] Loss figured by difference between amount applied and recovered.

Table VI. Fate of Azodrin-^{32}P on Glass Surface in Greenhouse

Compound	% of Applied Dose at Indicated Days after Treatment				
	1	3	7	14	21
Phosphoric acid	0.2	1.3	5.7	10.8	3.4
Dimethyl phosphate	1.8	4.0	10.3	16.5	19.7
O-Demethyl Azodrin	4.7	11.9	9.6	10.8	21.6
Azodrin	93.3	68.8	36.4	15.6	7.4
Loss[a]	0	14.0	38.0	46.3	47.9

[a] Figured by difference between amount applied and recovered.

Table VII. Comparative Fate of Bidrin and *N*-Hydroxymethyl Bidrin in Excised Cotton Leaves[a]

Compound	% of Applied Dose at Indicated Days after Treatment[b]					
	1		2		3	
	B	M	B	M	B	M
Phosphoric acid	3.3	5.0	9.0	11.1	18.3	17.2
Dimethyl phosphate	1.1	3.2	1.9	6.1	4.1	7.3
O-Demethyl Bidrin[c]	3.0	2.8	3.6	4.2	5.4	5.1
Bidrin acid[d]	0	4.7	0.8	6.9	0	9.9
Bidrin unknown A	0	15.5	2.5	23.5	4.5	30.9
Bidrin plus oxidation products	92.6	68.7	82.1	48.1	67.7	29.4

[a] *N*-hydroxymethyl Bidrin = 3-hydroxy-*N*-(hydroxymethyl)-*N*-methyl crotonamide dimethyl phosphate. Paper chromatographic system A used; see (*5*).
[b] B = 100 μg. of Bidrin-^{32}P; M = 100 μg. Bidrin-equivalents of mixture of 53.5% *N*-hydroxymethyl Bidrin, 19.7% Azodrin, and 26.8% Bidrin.
[c] 3-Hydroxy-*N*,*N*-dimethylcrotonamide methyl hydrogen phosphate.
[d] 3-Hydroxycrotonic acid dimethyl phosphate.

be of minor importance in cotton plants. No *N*-demethyl derivative was found in extracts of cotton plants treated with Azodrin. The lack of very high specific activity Azodrin in the present study may have accounted for this apparent lack of *N*-demethyl Azodrin, since traces of *N*-demethyl Azodrin have been reported in bean plants (*7*). Small quantities of *N*-hydroxymethyl Azodrin were found in some extracts of Azodrin-treated cotton, but only after extended periods of time. *N*-Hydroxymethyl Azodrin was recovered only after a relatively high concentration of unknown B had been found (Table II) or the concentration of unknown B decreased (Table V).

When cotton leaves were treated with Bidrin unknown A, significant quantities of *N*-hydroxymethyl Bidrin were formed (*1*). The authors believe, but have no direct evidence, that the small amounts of *N*-hydroxymethyl Azodrin found in cotton plants are very rapidly hydrolyzed (*1*) or converted to Azodrin unknown B, thus reducing the chances of recovering intact *N*-hydroxymethyl Azodrin. It is believed that unknown B eventually is degraded, releasing either hydrolytic products or *N*-hydroxymethyl Azodrin. This degradation may take place in the plant or could possibly occur during the extraction procedures.

The major products resulting from hydrolysis of Azodrin-P^{32} in cotton were dimethyl phosphate, *O*-demethyl Azodrin, and phosphoric acid. The latter, which remained at the origin of the chromatograms, probably included some normal plant products which had incorporated some of the radioactivity. *O*-demethyl Azodrin was formed from Azodrin by cleavage of one of the methyl-phosphate bonds. The absence of monomethyl phosphate, a logical metabolite of *O*-demethyl Azodrin, suggests that the compound was converted very rapidly to phosphoric acid. Dimethyl phosphate and probably acetoacetic acid methylamide were formed by cleavage of the vinyl-phosphate bond of Azodrin. Dimethyl phosphate also could have been formed by hydrolysis of the vinyl-phosphate bond of *N*-hydroxymethyl Azodrin. No Azodrin acid or *O*-demethyl Azodrin acid was found, which suggests lack of hydrolysis of the amide bond. With Bidrin, significant hydrolysis of the amide bond occurred (*1*). Whether the occurrence of the *N*-hydroxymethyl derivative is correlated in any way with the occurrence of the acid derivative is not known. However, the *N*-hydroxymethyl compound may be an intermediate of the acid. The data in Table VII indicate that this

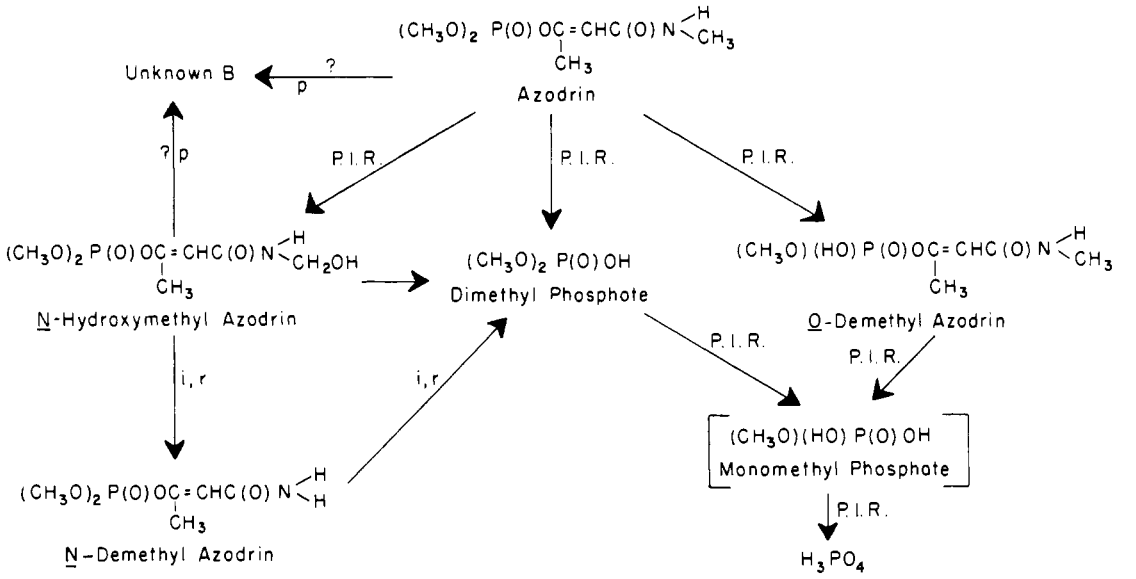

Figure 1. Proposed composite pathway of Azodrin metabolism

P, p. In cotton plants
I, i. In insects
R, r. In rats
Capital letters indicate major pathway; lowers case letters indicate minor pathways.

may be the case with Bidrin, since the leaves treated with *N*-hydroxymethyl Bidrin contained much more Bidrin acid than leaves treated with Bidrin.

Because only small amounts of unknown B were found, extensive characterization was not possible. However, sufficient data were obtained to indicate its similarity to Bidrin unknown A. Isolation and purification of unknown B were attempted by paper and thin layer chromatography. The final preparation had a pH of 5 and consisted of 90% unknown B, 2% *N*-hydroxymethyl Azodrin, and 8% hydrolytic products. When the pH was adjusted to 1 and chromatographed, the mixture contained 21% unknown B, 49% *N*-hydroxymethyl Azodrin, 22% phosphoric acid, and 8% other hydrolytic products. These data indicate that unknown B consists of more than one compound, and that the major portion may be a conjugate of *N*-hydroxymethyl Azodrin. Sufficient tests were conducted with Azodrin-^{14}C (*O*-methyl and *N*-methyl) to show that the unknown B consisted of nearly all of the parent molecule, as was the case with Bidrin (*1*).

A proposed composite pathway of Azodrin metabolism is shown in Figure 1. Degradation in cotton plants by the initial oxidation of Azodrin is much less important than in insects (*2*) or animals (*2*, *7*). After initial oxidation of Azodrin to *N*-hydroxymethyl Azodrin in insects and rats, hydrolysis is very rapid. In cotton, oxidation of Azodrin is of minor importance and the rate of hydrolysis of the parent molecule is slow, which results in an extended biological half life of the insecticide.

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