The Metabolism of Temik Aldicarb Pesticide [2-Methyl-2-(methylthio)propionaldehyde O-(methylcarbamoyl)oxime] in the Cotton Plant

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The metabolism of carbon-14 labeled Temik aldicarb pesticide in cotton plants was reinvestigated to determine the nature and toxicity of unidentified, nonorganoextractable residues. These metabolites, representing the major residue at final harvest, were found to consist of up to ten compounds. Six of these were identified by thin-layer chromatographic and mass-spectral techniques. One major metabolic pathway was reductive, leading to conjugates of 2-methyl-2-(methylsulfinyl)-propanol as the major (70 to 80%) nonorganoextractable residue. Small quantities of conjugated 2-methyl-2-(methylsulfonyl)propanol, 2-methyl-2-(methylsulfonyl)propionaldehyde oxime as well as the previously identified 2-methyl-2-(methylsulfinyl)-propionaldehyde oxime were also formed. Oxidative pathways resulted in the formation of nonconjugated 2-methyl-2-(methylsulfinyl)propionamide, 2-methyl-2-(methylsulfinyl)propionic acid and 2-methyl-2-(methylsulfonyl)propionic acid. There was no evidence of free or conjugated N-hydroxymethyl-, N-demethylcarbamate, or sulfide metabolites as residues of aldicarb. Acute oral studies on rats indicated all the newly identified metabolites have a low order of toxicity with LD_{50}'s ranging from 5700 to 16,000 mg per kg.

Temik® aldicarb pesticide [2-methyl-2-(methylthio)-propionaldehyde O-(methylcarbamoyl)oxime] is a systemic pesticide with insecticidal acaricidal and nematocidal properties (Payne *et al.*, 1966; Weiden *et al.*, 1965).

Because of its interesting biological properties and its potential use on a variety of agricultural commodities, the metabolism of aldicarb has received considerable study in plants (Bull, 1968; Coppedge *et al.*, 1967; Metcalf *et al.*, 1966), animals (Andrawes *et al.*, 1967; Dorough and Ivie, 1968; Knaak *et al.*, 1966) and insects (Bull *et al.*, 1967; Metcalf *et al.*, 1966).

These investigations have shown conclusively that the initial metabolic attack is a rapid oxidative conversion of the pesticide to aldicarb sulfoxide [2-methyl-2-(methylsulfinyl)propionaldehyde O-(methylcarbamoyl)oxime]. Further attack apparently proceeds via degradation of this carbamate to its corresponding oxime and nitrile metabolites, as well as by concurrent, but much slower, oxidation to aldicarb sulfone [2-methyl-2-(methylsulfonyl)propionaldehyde O-(methylcarbamoyl)oxime].

All studies of aldicarb metabolism to date have been concerned primarily with the less polar, organoextractable products. However, highly polar products, unextractable from aqueous solution, have also been detected (Andrawes *et al.*, 1968; Bull, 1968; Coppedge *et al.*, 1967; Metcalf *et al.*, 1966). These unextractables, which will be referred to as "water-solubles" to facilitate discussion, were of unknown nature and toxicity. They increased rapidly with time, and soon became the predominant residues. Present knowledge of biological degradation pathways of pesticides suggested these polar residues were most likely conjugates of noncholinesterase-inhibiting metabolites. However, to assure the safety of this pesticide for agricultural use, it was felt that the nature and toxicity of these unidentified residues should be ascertained.

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MATERIALS AND METHODS

Labeled Compounds. Methyl-C^{14}-thio-labeled aldicarb pesticide (specific activity 5.5 mc per mmole), the oxime sulfoxide [2-methyl-2-(methyl-C^{14}-sulfinyl)propionaldehyde oxime] (specific activity 2.6 mc per mmole), and the nitrile sulfoxide [2-methyl-2-(methyl-C^{14}-sulfinyl)propionitrile], (specific activity 2.6 mc per mmole) were prepared as previously described (Bartley *et al.*, 1966). All labeled compounds were stored at −15° C and, when required, were purified by preparative tlc prior to use.

Standards. Nonlabeled standards and potential metabolites employed in this study are summarized in Table I. All were authenticated by infrared, nmr, and elemental analyses (Durden *et al.*, 1970).

Radioactivity Determination. Radioactivity was determined with a Beckman LS-150 liquid scintillation counter utilizing an external standard channels-ratio system for quench correction. All samples were counted in a solution consisting of 1:3:3 xylene, dioxane, and ethylene glycol monoethyl ether containing 1% PPO, 0.05% POPOP, and 8% naphthalene (Bruno and Christian, 1961). Ten milliliters of the scintillation mixture were employed per sample.

Thin-Layer Chromatography. Thin-layer chromatography was carried out on 8″ × 8″ glass plates coated with a 0.35-mm layer of Silica Gel-G (Brinkmann Instruments Co.). Both one and two dimensional systems were employed utilizing the solvent systems in Table I. In practice, systems A and B were used for two dimensional chromatography of water-soluble components, whereas systems C and D (Andrawes *et al.*, 1967; Dorough and Ivie, 1968) were employed for the organo-extractable products.

Radioactive spots were located by means of radioautography utilizing Kodak NS-54T no-screen, medical x-ray film. For purposes of quantitation, the radioactive zones were scraped from the plate into a scintillation vial for direct counting. Results from at least two chromatograms were averaged to arrive at the percentage of each component.

As an aid in the identification of metabolites, the unknowns were subjected to thin-layer analyses in mixture with nonlabeled, authentic standards (Table I). Coincident spots were detected by radioautography followed by visualization of the standards with iodine vapor.

Table I. Thin-Layer Chromatographic Behavior of Aldicarb and Related Standards

No.	Compound	Abbreviation	R_f Value in Indicated Solvent System[a] A	B	C	D	E	F
1	2-Methyl-2-(methylthio)propionaldehyde O-(methylcarbamoyl)oxime (Aldicarb)	T	0.94	0.90	0.61	0.83	...	...
2	2-Methyl-2-(methylsulfinyl)propionaldehyde O-(methylcarbamoyl)oxime (Aldicarb sulfoxide)	T_1	0.68	0.30	0.05	0.13	...	...
3	2-Methyl-2-(methylsulfonyl)propionaldehyde O-(methylcarbamoyl)oxime (Aldicarb sulfone)	T_2	0.80	0.72	0.10	0.59	...	...
4	2-Methyl-2-(methylsulfinyl)propionaldehyde oxime (Oxime sulfoxide)	T_1O	0.59	0.62	0.13	0.19	...	...
5	2-Methyl-2-(methylsulfonyl)propionaldehyde oxime (Oxime sulfone)	T_2O[b]	0.74	0.92	0.61	0.76	...	...
6	2-Methyl-2-(methylsulfinyl)propionitrile	T_1N[b]	0.83	0.77	0.38	0.62	...	...
7	2-Methyl-2-(methylsulfonyl)propionitrile	T_2N[b]	0.90	0.93	0.57	0.69	...	...
8	2-Methyl-2-(methylsulfinyl)propionamide	T_1Am[b]	0.61	0.40	0.06	0.11	...	...
9	2-Methyl-2-(methylsulfonyl)propionamide	T_2Am[b]	0.70	0.70	0.11	0.38	...	...
10	2-Methyl-2-(methylsulfinyl)propanol	T_1Al[b]	0.52	0.26	0.07	0.09	...	...
11	2-Methyl-2-(methylsulfonyl)propanol	T_2Al[b]	0.70	0.80	0.40	0.59	...	...
12	2-Methyl-2-(methylsulfinyl)propionaldehyde O-carbamoyloxime	DT_1[b]	0.47	0.28	0.02	0.08	...	...
13	2-Methyl-2-(methylsulfonyl)propionaldehyde O-carbamoyloxime	DT_2[b]	0.58	0.72	0.12	0.46	...	...
14	2-Methyl-2-(methylthio)propionaldehyde O-(hydroxymethylcarbamoyl)oxime	HMT[c]	0.78	0.84	0.36	0.55	...	...
15	2-Methyl-2-(methylsulfinyl)propionaldehyde O-(hydroxymethylcarbamoyl)oxime	HMT_1[c]	0.42	0.23	0.0	0.0	...	...
16	2-Methyl-2-(methylsulfonyl)propionaldehyde O-(hydroxymethylcarbamoyl)oxime	HMT_2[c]	0.62	0.67	0.07	0.29	...	...
17	2-Methyl-2-(methylsulfinyl)propionic acid	T_1Ac[b]	streaked		0.0	0.0	0.75	0.70
18	2-Methyl-2-(methylsulfonyl)propionic acid	T_2Ac[b]	streaked		0.0	0.0	0.93	0.72
19	N-hydroxy-2-methyl-2-(methylsulfinyl)-propionamide	T_1HA[b]	streaked		0.0	0.0	0.25	0.29
20	N-hydroxy-2-methyl-2-(methylsulfonyl)-propionamide	T_2HA[b]	streaked		0.0	0.0	0.40	0.51

[a] A—6:1 Chloroform-methanol; B—2:1 Dioxane-benzene; C—2:1 Ether-hexane + 20% acetone; D—3:1 Methylene chloride-acetonitrile; E—3:1:1 Ether-benzene-formic acid; F—4:1:1 Benzene-methanol-acetic acid; [b] J. A. Durden *et al.*, 1970. [c] Durden and Stollints, 1970.

DOSING AND HARVEST OF PLANTS

Cotton plants (*Gossypium hirsutum* L. var. Coker 100) were grown in a greenhouse in 3-in. pots until the second pair of leaves reached a size of about 2 in. in width. The soil was washed from the plant, and the roots immersed in 100 ml of water containing about 25 or 50 ppm, respectively, of aldicarb-C^{14} or oxime sulfoxide-C^{14}. After the plant had absorbed most of the solution, the liquid level was brought to 100 ml with distilled water, and held at this point throughout the remainder of the experiments.

Alternatively, aldicarb-C^{14} in aqueous acetone solution was applied by soil drench to potted cotton plants at a rate of 0.81 lbs per A. Sufficient plants were dosed to provide two replicates consisting of three plants each for each of the four harvest periods. The pesticide was watered into the soil, and the plants were maintained under normal greenhouse growing conditions.

At preselected intervals, the plants were harvested, weighed, and frozen for storage until analyses could be effected. Using these techniques, direct root immersion resulted in the absorption of 75% and 55%, respectively, of the dosed aldicarb and oxime sulfoxide. Soil drench experiments resulted in 25 to 30% incorporation of the applied aldicarb.

Extraction. The frozen sample was transferred to a Waring Blendor, along with 50% aqueous ethanol, utilizing approximately 5 to 10 ml of solvent per gram of sample (wet weight). The mixture was homogenized 5 min, and then filtered by suction. After washing the filter cake with 50% ethanol (5 ml per gram) the filtrate was concentrated under vacuum at 40° C to a viscous syrup. The residue was taken up into 50 ml of water and extracted successively with six 50-ml volumes of 1:1 chloroform-acetonitrile to separate organosoluble and water-soluble metabolites. The total radioactivity in each phase was determined, and the organosoluble portion was analyzed by two dimensional thin-layer chromatography using solvent systems C and D (Table I).

The aqueous phase was treated with approximately 150 ml of 1:1 methanol-acetonitrile to selectively precipitate extraneous plant extracts. The supernatant liquid was collected by centrifugation, and the precipitate washed once with 50 ml of acetonitrile-methanol. The washings were centrifuged, and the supernatants combined and concentrated *in vacuo* at 40° C to a volume of approximately 5 ml. Loss of radioactivity during precipitation was less than 5%.

Combustion analyses of extracted plant tissues in an oxygen bomb (Andrawes *et al.*, 1967) indicated unextracted radioactivity was consistently in the range of 5%, regardless of the preharvest growing period.

Ion-Exchange Chromatography. Amberlite IRA-400, 20–50 mesh, AR grade ion-exchange resin was converted to the carbonate form as follows:

Approximately 200 g of the resin was allowed to stand overnight under 1 liter of 1M ammonium carbonate solution. The following morning the resin was transferred to a 50-mm i.d. column, and was covered with 2 liters of fresh 1M carbonate. The solution was allowed to percolate through the resin bed at rate of 3 to 5 ml per min, after which the resin was eluted successively with 1 liter each of 0.1 and 0.005M aqueous carbonate. The resin was removed from the column and stored under 0.005M aqueous carbonate solution until used.

A slurry of the resin–aqueous carbonate mixture was added to an 8-mm i.d. column to give a total height of 35 cm.

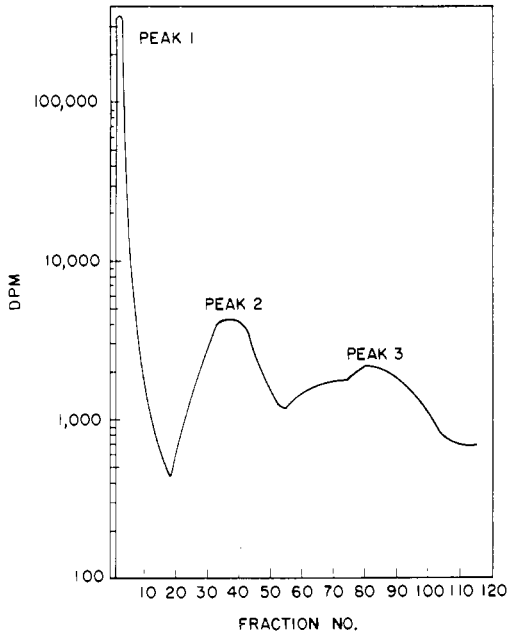


Figure 1. Ion exchange chromatography of aldicarb water-soluble metabolites

The resin was back-flushed 5–7 times in the prescribed manner (Dow Chemical Co., 1958) with 0.005M aqueous carbonate. The excess liquid was drained to approximately 1 cm above the bed, and an aliquot of the crude plant water-soluble extract containing 2–5 million dpm was added to the column.

A 0.1M aqueous carbonate solution was saturated with carbon dioxide until the pH fell to 6.8 to 7.0. A portion of this solution was diluted to give 0.05, 0.01, and 0.005M concentrations, and 200 ml of each were added in order of increasing concentration to a four-chambered, stirred, gradient apparatus (Buchler Inst., Inc.). The column was connected to the gradient device, and the metabolite mixture eluted with a continuous gradient of increasing ionic strength. Fractions were collected in 6-ml volumes at a rate of 1–1.5 ml per min. Peak boundaries were determined by counting an aliquot of every fifth fraction. Recovery of radioactivity was near quantitative.

The appropriate fractions of each peak were combined and concentrated to dryness under vacuum to remove the excess ammonium carbonate. The residual material in peaks 2 and 3 (Figure 1) was acidified to pH 1–2 with 6N hydrochloric acid, and then analyzed directly by thin-layer chromatography. Solvent systems employed were: peak 2, Systems E and F (Table I); 3:10:3 butanol-formic acid-water (upper phase); and 2:2:1 acetonitrile–chloroform-formic acid. Peak 3, Systems E and F; 10:5:5:2 butanol-ethanol-water-acetic acid.

If peak 1 contained abnormally large quantities of extraneous plant impurities, it was purified further by taking up the residue in a few milliliters of water, and treating the solution with excess, saturated lead diacetate solution. When precipitation was complete, the mixture was centrifuged and the supernatant liquid treated with carbon dioxide to precipitate excess lead ion. After removal of the lead carbonate and evaporation of the aqueous solution to dryness, the product was sufficiently pure to permit tlc analysis and enzymatic hydrolysis. Recovery of radioactivity was quantitative.

Enzymatic Hydrolysis. The purified glycoside mixture from the ion-exchange column (peak 1, Figure 1) was taken up in 5 ml of 0.1M sodium acetate buffer (pH 5.0). A mixture of 1 mg each of β-glucosidase (almonds; Sigma Chem., Inc.), and hemicellulase (Nutritional Biochem. Co.), and a drop of toluene were added, and the mixture was incubated at 37° C with magnetic stirring for 24 hr. The liberated aglycones were analyzed by two-dimensional tlc. Hydrolysis was 80 to 90% complete in one incubation. Additional hydrolysate could be obtained with a second incubation of the recovered conjugate. Blank experiments utilizing boiled enzyme liberated no aglycone.

Treatment of Cotton Plants with 2-Methyl-2-(methyl-C^{14}-sulfinyl)-propanol (Aglycone C). A radiolabeled sample of aglycone C was obtained from the enzymatic hydrolysis of mass-isolated, water-soluble conjugate extracted from aldicarb-treated cotton. The metabolite was purified by preparative tlc, and was taken up in 3 ml of water. A young cotton plant was allowed to absorb the samples over a 15-day period by root uptake. At the end of this period the leaves were homogenized with 50% aqueous ethanol, then filtered and concentrated. The solution was subjected to ion-exchange chromatography, after which the conjugated material in peak 1 was hydrolyzed enzymatically.

Metabolites were determined by thin-layer chromatography.

Purification and Mass-Spectral Analysis. A mixture of the unknown aglycones was obtained by enzymatic hydrolysis of mass-isolated, water-soluble cotton metabolites. The metabolites were separated by preparative tlc (6:1 chloroform-methanol) and were purified for mass-spectral identification by successive thin-layer chromatography in four solvent systems: 6:1 chloroform-methanol; 2:1 dioxane-benzene; 2:1:1 ethyl ether-chloroform-methanol; and 8:1 ethyl ether-methanol.

To assure analytical samples free of unnecessary contamination, all solvents were freshly redistilled prior to use, and each silica-gel plate was preswept with the appropriate solvent system prior to spotting of the sample.

Between chromatographic steps, the radioactive zone was scraped from the plate, and the sample was eluted from the gel by five successive washings with 2 to 5 ml of methanol. Aliquots of the washings were counted to follow the course of extraction. The combined extracts were reduced to dryness at 35° C under a gentle nitrogen stream.

Samples consisting of 25 to 100 μg of the purified aglycone metabolites were analyzed in an Associated Electronics Industries, MS-902b high-resolution mass spectrometer. Samples were introduced by direct probe insertion and fragment identities were established by accurate mass measurement (peak matching) at a resolution of 16.000.

RESULTS

Cotton plants readily convert both aldicarb and the oxime sulfoxide to highly polar products not extractable from aqueous solution with chloroform-acetonitrile (Table II).

Table II. Metabolism of Aldicarb and Oxime Sulfoxide to Water-Soluble Products

Days After Treatment	Organic/Aqueous Partition Value
Aldicarb	
14	4.92
30	1.55
60	0.53
Oxime sulfoxide	
30	0.42

Unlike the organoextractable metabolites which were analyzed directly by thin-layer chromatography, the nonorganoextractables comprised a complex mixture which required additional cleanup before satisfactory analyses could be effected.

In brief, the water-solubles were analyzed by first passing the aqueous mixture over an Amberlite IRA-400, anion-exchange column, eluting with a continuous gradient of aqueous ammonium carbonate of increasing ionic strength. The metabolite mixture was separated into three distinct bands (Figure 1). The major peak 1, comprising 70 to 80% of the applied radioactivity, was nonacidic, and was eliminated with the void volume. The balance of the metabolite mixture eluted from the column as acids in two incompletely separated peaks, 2 and 3. The fractions composing the individual peaks were combined and the ammonium carbonate was removed by concentration to dryness under vacuum.

The acid eluting as peak 2 was analyzed by tlc and was found to be chromatographically identical in four solvent systems with 2-methyl-2-(methylsulfinyl)propionic acid. Small amounts of the corresponding sulfone acid, 2-methyl-2-(methylsulfonyl)propionic acid, were also detected in peaks 2 and 3. The structure of the latter acid was confirmed by co-chromatography as above.

Careful tlc analysis of the metabolite mixture prior to and after ion-exchange suggested no degradation of metabolites was occurring on the strongly basic column. However, the resin was capable of partial hydrolysis of aldicarb sulfoxide, and it was found important that all carbamates be removed by extraction prior to the ion-exchange step. The acids could be detected by tlc in the crude residue, prior to ion exchange in quantities similar to those found on the column. It must be concluded, therefore, that the acids exist in the free form in the extracts, and did not result through hydrolysis of conjugates on the basic anion-exchange resin.

The second acidic peak, 3, was further resolved into two highly polar unknown components present in a roughly 2:1 ratio. Oxidation of this mixture with 1:1 acetic acid-30% hydrogen peroxide resulted in an apparent conversion of the major, more polar metabolite to the less polar product, suggesting the two unknowns were possible sulfoxide-sulfone analogs. Attempted enzymatic hydrolyses to an identifiable aglycone with peptidase or carboxypeptidase were unsuccessful. Boiling the mixture in 2N hydrochloric acid for 2 hr gave only ill-defined products which streaked nearly to the solvent front.

Thin-layer chromatography of peak 1 indicated it contained 60 to 85% highly polar products which remained near the origin. The same chromatogram also indicated minor amounts of three apparently unconjugated, but heretofore unidentified, metabolites designated A, B, and C, and small amounts (4 to 8%) of known organosoluble metabolites, aldicarb sulfoxide, oxime sulfoxide, and nitrile sulfoxide not removed by the chloroform-acetonitrile extraction (Figure 2). The highly polar unknown products were probably glycoside conjugates by virtue of the fact that enzymatic hydrolysis resulted in the liberation of large quantities (80 to 90% conversion) of unknown C.

Partial conversion of C to A was often noted during isolation and purification steps. Oxidation of aldicarb and its metabolites has often been observed during workup and tlc, suggesting C and A might be sulfoxide and sulfone analogs of the same aglycones. This hypothesis was confirmed by the fact that oxidation of C with 1:1 acetic acid–30% hydrogen peroxide resulted in its quantitative conversion to A.

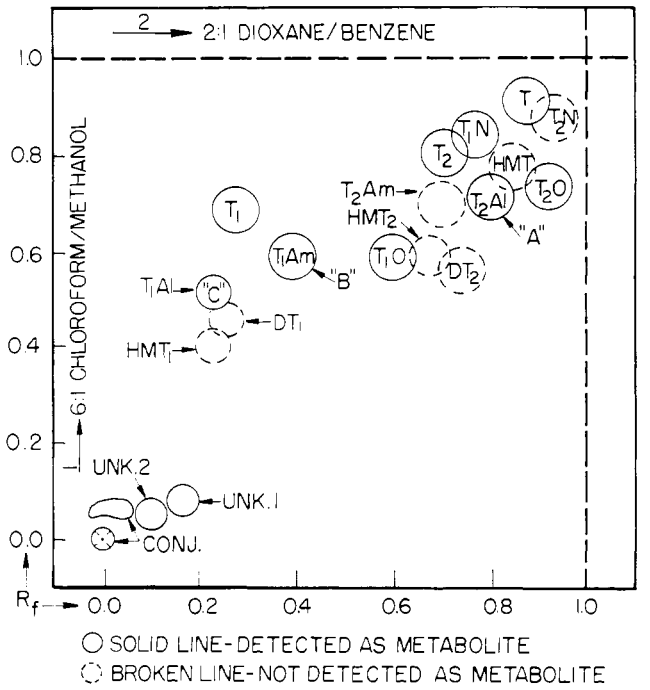


Figure 2. Two-dimensional tlc of aldicarb, its metabolites, and related standards

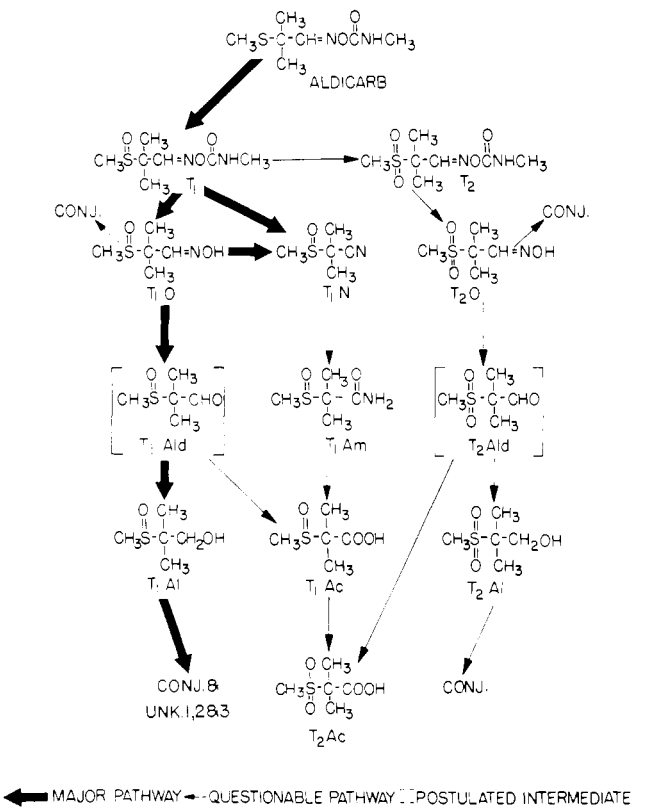


Figure 3. A metabolic pathway of aldicarb in cotton plants

Metabolite B occurred unconjugated and was detected in approximately equal amounts in both the organic and aqueous fractions.

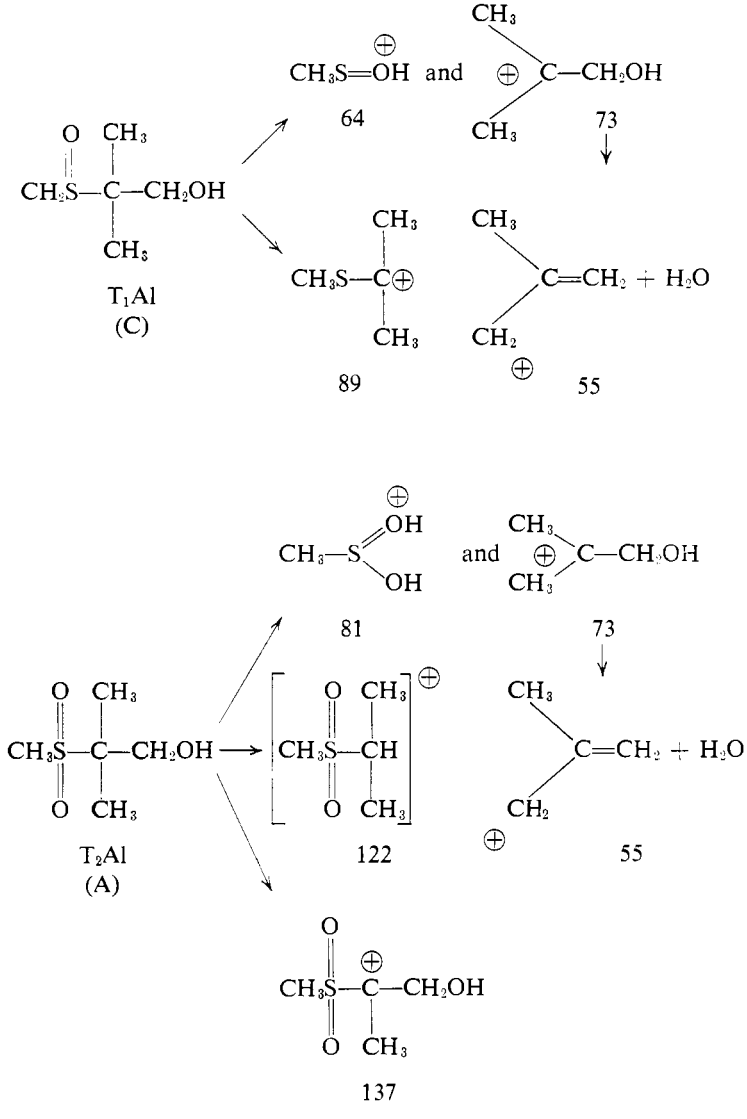
Metabolites B and C were mass isolated and purified by preparative tlc in sufficient quantity (25 to 100 μg) for high resolution mass-spectral analyses. During the purification of C, sufficient A was also formed by air oxidation to allow its collection in adequate quantity and purity for analysis.

The mass spectra of aglycones C and A (Table III) indicated they were the alcohols, 2-methyl-2-(methylsulfinyl)-propanol, T_1Al, and 2-methyl-2-(methylsulfonyl)propanol, T_2Al, respectively. Although neither compound gave a parent ion, both fragmented to give a m/e = 73 as the major (100%) peak. This ion, established by mass measurement as an isobutyl alcohol fragment, dissociated by loss of water giving a second large peak at m/e = 55. The postulated pathway was confirmed by a large metastable transition at 41.4. The sulfoxide-sulfone relationship of C and A was established by intense peaks at m/e = 64 (CH_4SO^+) and 89 ($C_4H_9S^+$) in C, and peaks at 81 ($CH_5SO_2^+$), 122 ($C_4H_{10}SO_2^+$), and 137 ($C_4H_9SO_3^+$) in A.

ment with that of metabolite B. The structure was further confirmed by co-chromatography in six tlc solvent systems.

DISCUSSION

The metabolism of aldicarb and the oxime sulfoxide in cotton plants is summarized in Tables IV–VI, and a postulated metabolic pathway is presented in Figure 3. In agreement with the results of earlier investigators (Bull, 1968; Coppedge *et al.*, 1967; Metcalf *et al.*, 1966) it is apparent that the initial metabolic attack on aldicarb is both oxidative and hydrolytic in nature, yielding known organo-soluble products. These metabolites, for the most part, are transient in nature and are further converted to the water-soluble residues. These



The postulated structures of A and C were confirmed by comparison of their spectra with those of the authentic standards. Additional confirmation was provided by co-chromatography of the radiolabeled metabolites with their respective standards in six, two-dimensional tlc systems.

Unknown B was found by mass spectrometry to be the amide, 2-methyl-2-(methylsulfinyl)propionamide, T_1Am. Mass measurements for the parent ion at m/e = 149, as well as peaks at 44, 58, 86, and 89 (Table III) were in agreement with the proposed structure.

The mass spectrum of an authentic standard, including the m/e = 58 (C_3H_8N+) rearrangement fragment, was in agreement with that of metabolite B. The structure was further confirmed by co-chromatography in six tlc solvent systems.

Surprisingly, the chief metabolic pathway in the formation of water-solubles is one of reduction, leading to the alcohol sulfoxide, T_1Al, in high conversion. Only minor quantities of the alcohol occur free in the aqueous and organic fractions, the bulk of the metabolite being in the form of a highly polar glycoside conjugate.

The recovery of oxime sulfoxide either free or conjugated was remarkably low. A separate feeding study with this residues are undoubtedly identical to the unknown water-soluble and highly polar unknowns referred to in earlier studies by Bull, 1968, Coppedge *et al.*, 1967, and Metcalf *et al.*, 1966.

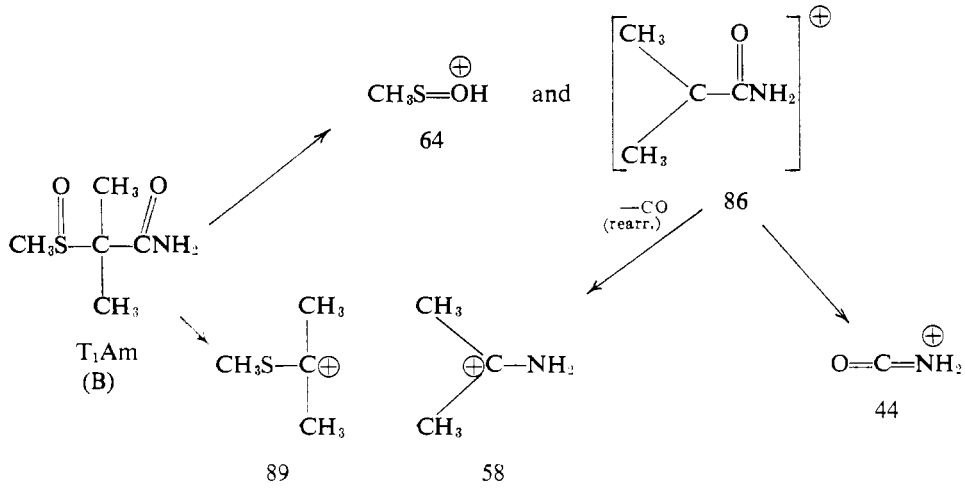


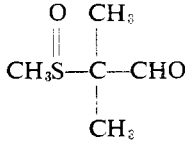
Table III. Mass-Spectral Data from Aldicarb Water-Soluble Metabolites

Metabolite	*m/e*	% Rel. Intensity	Mass Found	Mass Calculated	Fragment
$CH_3S-C(CH_3)-CH_2OH$[a]	55	87	55.05509	55.05477	C_4H_7+
	57	33	57.07097	57.07042	C_4H_9+
	64	33	63.99985	63.99828	CH_4SO+
	73	100	73.06572	73.06534	C_4H_9O+
	89	20	89.04208	89.04250	C_4H_9S+
		Metastable 41.4 73+ → 55− + H_2O			
$CH_3S-C(CH_3)(OCH_3)-CH_2OH$[a]	55	69	55.05509	55.05477	C_4H_7+
	57	10	57.07097	57.07042	C_4H_9+
	73	100	73.06558	73.06534	C_4H_9O+
	81	13	81.00088	81.00101	CH_3SO_2+
	122	17	122.04011	122.04015	$C_4H_{10}SO_2+$
	137	2	137.02716	137.02724	$C_4H_9SO_3+$
		Metastable 41.4 73+ → 55+ + H_2O			
$CH_3S-C(CH_3)(CH_2)-CNH_2$[b]	44	80	44.01295	44.01363	CH_2NO+
	58	50	58.06628	58.06567	C_3H_8N+
	86	100	86.06089	86.06059	C_4H_8NO+
	89	13	89.04281	89.04250	C_4H_9S+
	149 (M+)	3[c]	149.04987	149.05105	$C_5H_{11}NO_2S+$

[a] Inlet temperature—130° C. [b] Inlet temperature—200° C. [c] Value from spectrum of authentic standard. This transition in the unknown was masked by a large peak due to pump oil.

initial aldicarb metabolite (Table V) suggests that the direct conjugative pathway is of minor importance, and that rapid metabolism to T_1Al and other water-soluble products is favored. The striking similarity between the polar metabolic products of aldicarb (Tables IV and VI) and those of the oxime sulfoxide (Table V) suggest that the latter metabolite is an important intermediate in the transformation of the pesticide to water-soluble residues.

As yet, the pathway between the oxime sulfoxide and the alcohol, T_1Al, has not been precisely defined. However, the aldehyde, T_1Ald



T_1Ald

which presumably could arise via hydrolysis of the oxime is a logical intermediate in this transformation. It is well known that in the presence of the enzyme alcohol dehydrogenase, aldehydes are in equilibrium with their corresponding alcohols (Parke, 1968). Conjugation of the alcohol would be expected to shift the equilibrium, yielding, in the case of T_1Ald, substantial quantities of the alcohol (T_1Al) glycoside.

An aldehyde intermediate is also in keeping with the observed formation of the acids T_1Ac and T_2Ac. The latter metabolites, which were formed in reasonable quantities, would arise via enzymatic oxidation of T_2Ald, a transformation have considerable precedent in the biological literature (Parke, 1968; Williams, 1959).

The detection of the amide, T_1Am, was unanticipated. This product, formed in the metabolism of both aldicarb and the oxime sulfoxide, was found unconjugated in both the organoextractables, and among the water-soluble residues. The fact that the amide was detected among the extractable products precludes the possibility that it might be an artifact arising through reaction of the acid, T_1Ac, with ammonium ion on the ion-exchange column.

Table IV. Metabolism of $C^{14}H_3S$-Labeled Aldicarb Administered by Root Uptake to Cotton Plants

Metabolite	Percent of Recovered Radioactivity in the Organic and Aqueous Extracts at Indicated Days After Treatment					
	14		30		60	
	Organic	Aqueous	Organic	Aqueous	Organic	Aqueous
T	1.0	...	1.7	...	...	...
T_1	66.5	...[a]	30.2	...[a]	12.3	...[a]
T_2	5.0	...	5.7	...	5.4	...
T_1O	1.6	1.7[b]	2.1	2.0[b]	...	1.0[b]
T_2O	...	...	0.5	...[a]	4.6	...[a]
T_1N	7.7	...[a]	15.3	...[a]	10.3	...[a]
T_1Al (C)	1.0	6.2[b]	1.3	18.8[b]	...	28.5[b]
T_2Al (A)	...	...	0.9	...	0.7	...
T_1Am (B)	...	0.4	2.3	1.4	0.7	1.4
T_1Ac	0.3	0.8	0.8	4.4	...	7.5
T_2Ac		...		0.8	...	2.3
Unk. 1 and 2	...	0.9	...	1.4	0.6	4.6
Unk. 3[c]	...	...	...	3.8	...	7.4
Unhyd. conj.	...	6.9	...	6.6	...	12.7
Total	83.1	16.9	60.8	39.2	34.6	65.4

[a] Minor amount remaining after extraction—value combined with organic. [b] As glycoside conjugate. [c] Peak 3, from ion exchange—two components, 2:1 ratio.

Table V. Metabolism of Aldicarb Oxime-S-$C^{14}H_3$ Sulfoxide in Cotton Plants After 30 Days

Metabolite	Percent of Recovered Radioactivity in Organic and Aqueous Extracts	
	Organic	Aqueous
T_1O	6.9	5.1[a]
T_1N	19.9	...[b]
T_2O	2.1	...
T_1Al	0.8	40.2[c]
T_2Al	...	0.7[c]
T_1Am	...	0.4
T_1Ac & T_2Ac	...	2.1
Unk. 1, 2[d]	...	1.1
Unk. 3[d,e]	...	8.4
Unhyd. conj.	...	12.3
Total	29.7	70.3

[a] 3.4% as glycoside conjugate. [b] Minor amount remaining after extraction–value combined with organic. [c] As glycoside conjugate. [d] Chromatographically identical to corresponding aldicarb derived unknowns. [e] Peak 3, from ion exchange—two components, 2:1 ratio.

The pathway to T_1Am is uncertain. Although there is some literature precedent for *in vivo* hydrolysis of nitriles (Parke, 1968; Williams, 1958), this pathway is generally minor, and there is no assurance that T_1Am arises from T_1N via such a source. This conclusion is based on the observation that while oxime sulfoxide yielded as much nitrile sulfoxide as did aldicarb, the oxime gave much less amide (Tables IV and V).

In keeping with the known slow oxidation of the various aldicarb sulfoxide metabolites (Table V; see also Coppedge *et al.*, 1967), only minor quantities of sulfones T_2O, T_2Al, and T_2Ac, were detected. No T_2N was observed, presumably because it was formed in quantities below the sensitivity of the analytical method employed.

In a separate experiment designed to ascertain the metabolic precursor of the unknowns 1–3 (Tables IV to VII), a sample of methyl-C^{14}-sulfinyl labeled alcohol (T_1Al) obtained biosynthetically was dosed to a cotton plant by root-uptake (Table VII). It was subsequently found after 15 days that

Table VI. Metabolism of $C^{14}H_3S$-Labeled Aldicarb Administered by Soil Application to Cotton Plants

Metabolite	PPM of Recovered Radioactivity in the Organic and Aqueous Extracts at Indicated Days after Treatment							
	7		14		21		32	
	Organic	Aqueous	Organic	Aqueous	Organic	Aqueous	Organic	Aqueous
T	0.02	...	0.09	...	0.02	...	0.07	...
T_1	18.06	...[a]	4.37	...[a]	1.49	...[a]	0.74	...[a]
T_2	3.10	...[a]	1.33	...[a]	0.64	...[a]	0.47	...[a]
T_1O	0.37	0.34[b]	0.20	0.29[b]	0.07	0.10[b]	0.05	0.01[b]
T_2O	0.09	...	0.11	0.03[b]	0.03	0.05[b]	0.03	0.02[b]
T_1N	0.21	...	0.48	...	0.24	...	0.27	...
T_1Al (C)	0.22	0.33	...	1.21[b]	0.01	0.83[b]	<0.01	0.43[b]
T_2Al (A)	0.07	0.05[b]	0.06	0.05[b]	0.04	0.07[b]	0.08	...
T_1Am (B)	...	0.06	0.13	0.09	0.03	0.07	0.05	0.03
T_1Ac and T_2Ac	0.46	0.16	0.02	0.08	...	0.06	0.02	0.05
Unk. 1 and 2	0.17	0.12	...	0.26	<0.01	0.15	<0.01	0.06
Unk. 3[c]	...	0.43	...	0.30	...	0.25	...	0.16
Unhyd. conj.	...	3.02	...	0.69	...	0.28	...	0.46
TOTAL	22.77	4.51	6.79	3.00	2.57	1.86	1.78	1.21
Recovered Aldicarb equivalents (μg)	48.4		39.2		39.2		35.9	

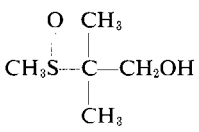
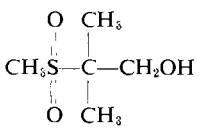
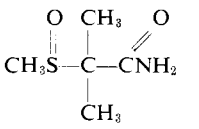
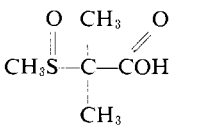
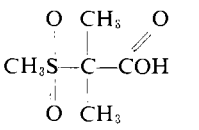
[a] Minor amount remaining after extraction—value combined with organic. [b] As glycoside conjugate. [c] Peak 3, from ion exchange—two components, 2:1 ratio.

Table VII. Metabolism of 2-Methyl-2-(Methyl-C^{14}-Sulfinyl)-Propanol (T_1Al) in Cotton Plants at 15 Days

Metabolite	Percent
T_1Al	84.9[a]
T_2Al	1.6[a]
Unk. 1, 2	1.9
Unk. 3[b]	10.0
Unhyd. conj.	1.6

[a] As glycoside conjugate. [b] Peak 3, from ion exchange—two components, 2:1 ratio.

Table VIII. Acute Oral Toxicities of Aldicarb Water-Soluble Metabolites in Rats

Metabolite	Abbreviation	Acute Oral LD_{50} (mg/kg)
	T_1Al	11,300
	T_2Al	11,300
	T_1Am	16,000
	T_1Ac	7,500
	T_2Ac	5,700

the alcohol was converted to substantial quantities of unknown 3, as well as minor quantities of unknowns 1 and 2. The three products were chromatographically identical to the corresponding unknowns detected with aldicarb and with oxime sulfoxide. Although unknowns 1–3 were not identified, the fact that they are formed from T_1Al indicates they are merely further degradation products and/or conjugates of the alcohol.

A similar feeding study utilizing the labeled acid sulfoxide, T_1Ac, resulted only in recovery of the parent acid, with no evidence of further degradation or conjugation.

Introduction of aldicarb into cotton plants by root absorption from aqueous solution proved to be an efficient and simple means of generating large quantities of water-soluble metabolites for identification purposes. To be certain that this somewhat artificial means of application did not significantly change the metabolic picture, a second study utilizing soil application of the pesticide was conducted (Table VI). As might be anticipated, the two methods of dosing gave identical metabolite spectra. However, soil application appeared to give greater quantities of sulfone metabolites, particularly T_2, than did root absorption.

By analogy with the known metabolism of other carbamate pesticides (Andrawes *et al.*, 1968; Kuhr and Casida, 1967; Weiden and Wiggins, 1969) conjugates of the N-hydroxymethylcarbamates 14–16 (Table I) were anticipated. However, we were unable to find evidence of these compounds or their anticipated N-demethylated carbamate breakdown products (12 and 13, Table I) in spite of the fact that authentic standards were available (Durden *et al.*, 1970). A similar result has been reported by Coppedge and coworkers (1967), working with the N-demethylcarbamate standards.

Oximes are chemically oxidizable to hydroxamic acids (Henecka and Kurtz, 1952), and it is possible that a similar transformation might occur biologically (Mabry, 1968). However, tlc comparisons of aldicarb cotton metabolites with the hydroxamic acid standards (19 and 20, Table I) gave no evidence that the latter were formed in the plant.

The data presented in Table VIII indicates all of the metabolites are relatively nontoxic in acute studies. The major metabolite, 2-methyl-2-(methylsulfinyl)propanol, T_1Al, had no demonstrable effect on rats when included in their daily diet for 7 days at concentrations as high as 20,000 ppm.

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