Spectroscopic Identification of Metabolites of Carbaryl in Plants

Ralph O. Mumma,* Safy Khalifa, and Robert H. Hamilton

Carbaryl metabolism has been investigated in beans, peas, peppers, and corn. Metabolites were isolated in microgram quantities for mass and ultraviolet spectroscopic analyses. The comparison of the mass and ultraviolet spectra of the isolated metabolites with the spectra of the synthetic compounds confirms the presence of *N*-hydroxymethylcarbaryl, 4-hydroxycarbaryl, and 5-hydroxycarbaryl. The fragmentation patterns of the mass spectra are discussed. The mass spectra of 4-hydroxycarbaryl and 5-hydroxycarbaryl are sufficiently different such that identification is possible. A weak complex between a yellow plant pigment and carbaryl or its metabolites was demonstrated. This complex, which causes problems in the isolation of pure metabolites, can be decomposed with acid or separated from the uncomplexed metabolites by a Bio-Gel P2 column.

The metabolism of carbaryl (Sevin, 1-naphthyl-*N*-methylcarbamate) has been studied by many investigators in plants and animals (Abdel-Wahab *et al.*, 1966; Dorough and Casida, 1964; Knaak *et al.*, 1965; Kuhr and Casida, 1967; Oonnithan and Casida, 1966, 1968; Paulson *et al.*, 1970). In plants the aglycone metabolites have been proposed, among other products, to be 1-naphthyl *N*-hydroxymethylcarbamate (*N*-hydroxymethylcarbaryl), 4-hydroxy-1-naphthyl *N*-methylcarbamate (4-hydroxycarbaryl), and 5-hydroxy-1-naphthyl *N*-methylcarbamate (5-hydroxycarbaryl). The structures of the proposed metabolites have been based largely upon comparative R_f values with synthetic compounds on thin-layer and paper chromatography. We now report the isolation of the metabolites of carbaryl from bean and pea plants in sufficient quantity for mass and ultraviolet spectroscopic examination.

EXPERIMENTAL

Materials. All solvents were redistilled and all reagents were analyzed by tlc. Carbaryl, analytical grade, was supplied by Union Carbide Corp. Carbaryl (^{14}C-carbonyl labeled) and 1-naphthol-1-^{14}C were purchased from Nuclear Chicago Corp. Initially small quantities of 4-hydroxy-, 5-hydroxy-, and *N*-hydroxymethylcarbaryl were generously supplied by John E. Casida for tlc standards. Subsequently we synthesized these metabolites. All enzymes used in this study were purchased from Worthington Biochemical Corp.

Instruments of Analysis. Mass spectra were obtained with an LKB (Model 9000) and a Nuclide (Model GRAF 3.2) mass spectrometer. Ultraviolet spectra were obtained with a Cary Model 14 spectrophotometer. Radioactivity of samples was determined with a Packard Tri-Carb liquid scintillation spectrometer (Model 526) using the standard dioxane solution (60 g of naphthalene, 4 g of POP, 200 mg of POPOP, 20 ml of 2-ethoxyethanol, and 100 ml of methyl alcohol diluted to 1 l. with dioxane). A Nuclear Chicago thin-layer scanner (Actigraph III) was used for scanning thin-layer plates.

Pesticide Research Laboratory and Graduate Study Center, Departments of Entomology and Biology, The Pennsylvania State University, University Park, Pa. 16802

Chromatography. Thin-layer chromatography was used to follow all reactions and procedures. Plates were coated with either 0.5 or 1.0 mm thick silica gel (Supelcosil 12B or Supelcosil 12A, Supelco, Inc.). The polar gylcone metabolites were separated with the following solvent mixtures: chloroform-methanol-water (65:25:4), chloroform-methanol-water (65:50:8), chloroform-methanol-water-pyridine (65:50:8:0.5) (Mumma, 1968), and ethyl acetate-acetone-water-formic acid (5:3:1:1). The ether soluble aglycone metabolites were separated with chloroform-acetonitrile (4:1) (Kuhr and Casida, 1967), ethyl ether-hexane (4:1), and ethyl ether-benzene-ethanol-acetic acid (40:50:2:0.2) (Freeman and West, 1966). All solvent proportions mentioned here and elsewhere were made by volume.

Carbaryl and its metabolites were visualized by spraying with 15% potassium hydroxide, followed by diazotized sulfanilic acid (Krishna *et al.*, 1962). The position of the ^{14}C-labeled compounds on tlc was determined with the scanner or by radioautography (Kodak, single-coated, blue-sensitive, X-ray film for 4 to 10 days at 25 or 5° C).

Plant Growth and Treatment. Bean (*Phaseolus vulgaris* var. pinto), pea (*Pisum sativum* var. arvense), pepper (*Capsicum frutescens* var. grossum) and corn (*Zea mays*, Pa. 1812) seeds were planted in individual pots containing vermiculite and the plants were grown under greenhouse conditions for 10 to 14 days. Carbaryl (^{14}C-carbonyl labeled), 10^6 dpm (10 μg), dissolved in 10 μl of acetone-water (2:1) was injected, by means of a gas chromatography syringe, into a glass capillary tube implanted in the stem of plants. When the plant had absorbed the sample, after 10 to 20 min, another 10 μl of acetone-water containing 10 μg of unlabeled carbaryl was added to the capillary tube. In the case of bean and pea plants, where sufficient quantities of carbaryl metabolites were needed for spectroscopic analysis, several hundred plants were used for injections.

Extraction and Work Up. The sixth day after injection of carbaryl, the plant leaves and stems were cut into small pieces. Fifty grams were homogenized with a Virtis homogenizer for 5 min in 60 ml of chloroform-methanol (2:1). The homogenate was then filtered with suction and the filtrate was evaporated to dryness under nitrogen. The residue was dissolved in 4 ml of chloroform-methanol (2:1) and washed according to Folch *et al.* (1957). The carbaryl metabolites

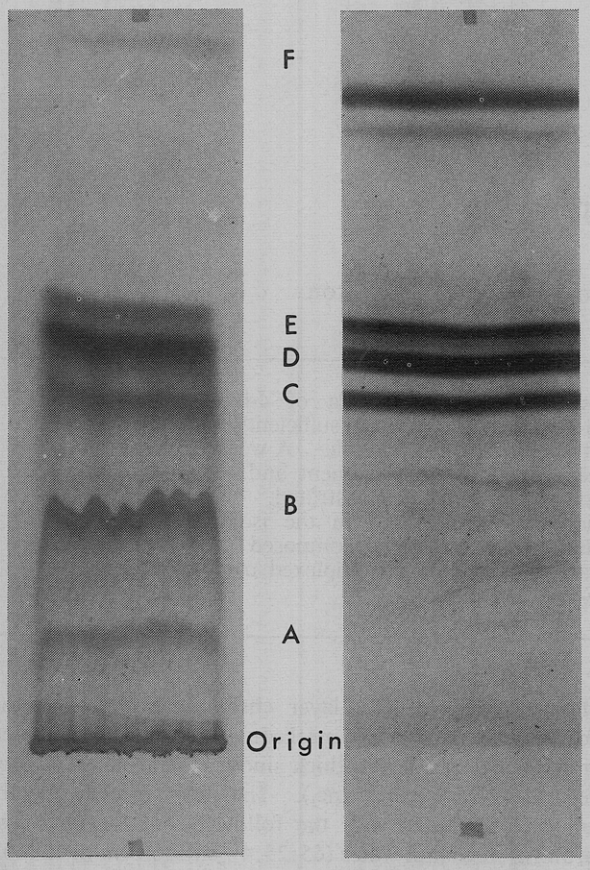

Figure 1. Radioautograms of thin-layer chromatograms of the water soluble ^{14}C-carbaryl metabolites. Left: total metabolites found in the aqueous extract of the pinto bean plant. Right: water soluble ^{14}C-carbaryl metabolites found in pooled fractions 3, 4, and 5 from a Bio-Gel P2 column of the aqueous extract. The developing solvent was chloroform-methanol-water (65:25:4)

were found in the upper water phase, while carbaryl and α-naphthol were found in the chloroform phase. The upper water phase was evaporated to a small volume, placed in a vial, and stored at 4° C until further analyzed.

The water soluble metabolites were either directly separated by one-dimensional tlc or partially purified on molecular sieve columns. The columns (3 cm × 30 cm) were prepared with Bio-Gel P2 (100–200 mesh) from a slurry in water. The water soluble metabolites were dissolved in 5 ml of H_2O and placed on the top of the column. The column was eluted with H_2O. Twenty milliliters were collected in each tube and the contents of the tubes were selectively combined, following analysis by tlc.

The water soluble glycone ^{14}C-carbaryl metabolites, from either direct extraction or following the Bio-Gel P2 column, were placed as a long band on a 20 × 20 cm tlc plate. The plates were developed with chloroform-methanol-water (65:25:4), which was found to be superior to other solvents evaluated and listed in the chromatographic section. The plates were exposed to X-ray film for approximately 1 week to locate the metabolites. Each radioactive band was scraped from the tlc plate and placed into a 50-ml centrifuge tube. The silica gel, containing the metabolites, was extracted with 70% ethanol, which was then evaporated to dryness, and the metabolites stored at 5° C in the dark prior to enzymatic degradation. The glycone metabolites obtained from the tlc plates of extracts that had not been partially purified by the Bio-Gel P2 column required rechromatography.

When the glycone carbaryl metabolites were sufficiently pure, they were dissolved in 1 ml of buffer (pH 7) and subjected to enzymatic degradation (β-glucosidase, β-glucuronidase, peroxidase) for 20 hr. The incubation mixtures were then extracted with ethyl ether 3–4 times. The ethyl ether was concentrated to a small volume and the contents placed as a long band on a 20 × 20 cm tlc plate. The plates were developed with the ethyl ether-benzene-ethanol-acetic acid solvent (Freeman and West, 1966) which was superior to the other solvents evaluated. After exposure to X-ray film, each aglycone band was removed from the plates. The silica gel was extracted with ethanol or acetone. The solvent was concentrated to a small volume and pooled with the same aglycone metabolites from other tlc plates. When sufficient quantities were obtained, the extracts were analyzed by direct probe mass spectrometry and by ultraviolet spectroscopy.

Carbaryl-naphthyl-^{14}C. Ring-labeled carbaryl was prepared by the condensation of 1-naphthol-1-^{14}C (19.6 mCi/mM) with an excess of methylisocyanate (Krishna *et al.*, 1962). The carbaryl product was purified with tlc.

4- and 5-Hydroxy-1-naphthyl *N*-Methylcarbamates. Either 1,4 or 1,5-naphthalenediol (50 mmole) was dissolved in 50 ml of anhydrous redistilled acetone in a Teflon-lined screw cap flask. Three milliliters of methylisocyanate and five drops of the catalyst dibutyltin diacetate were added. After 2 days, the reaction mixture, which contained mainly soluble monocarbamate, unreacted naphthalenediol and insoluble biscarbamate, was filtered. The biscarbamate precipitate was saved for spectroscopic analysis. The filtrate was concentrated and immediately placed on preparative tlc plates (1.0 mm) (ethyl ether-hexane, 4:1). The desired monocarbamate band (R_f = *ca.* 0.5) was located with ultraviolet light and scraped from the tlc plates. The silica gel containing the monocarbamate was placed in a small column and eluted with acetone. The acetone was evaporated to dryness, the residue dissolved in a minimum amount of ethyl acetate, and the monocarbamate precipitated with petroleum ether (67–70° C). The compound was recrystallized until it possessed the desired melting point and was pure by tlc analysis.

***N*-Hydroxymethylcarbaryl.** *N*-Hydroxymethylcarbaryl was prepared essentially in the same manner as previously described (Durden *et al.*, 1970). Two grams of crude *N*-hydroxymethylcarbaryl was purified by preparative tlc (ethyl ether-benzene-ethanol-acetic acid, 40:50:2:0.2).

Preparation of Carbaryl Complex with Quercitrin. Quercitrin (5 mg) was dissolved in 1 ml of H_2O, and to this solution was added ^{14}C-carbaryl (5 μg) dissolved in 0.1 ml of ethanol. After 5 min an aliquot was removed and analyzed by tlc. The plates were developed with chloroform-methanol-water (65:25:4) and with chloroform-methanol-water-hydrochloric acid (65:25:4:0.05). The same chromatographic procedure was repeated after a drop of 0.1 *N* HCl was added to a quercitrin-^{14}C-carbaryl aliquot and to the water soluble metabolites from the bean plants.

RESULTS AND DISCUSSION

The metabolism of carbaryl was investigated in beans, peas, peppers, and corn. Each plant possessed essentially the same metabolites in varying amounts. Microgram quantities of the metabolites were isolated for spectroscopic examination from several hundred bean and pea plants. Carbaryl (^{14}C-carbonyl labeled and, in some experiments, ^{14}C-ring labeled) was injected into stems of young plants by means of a glass capillary. Six days later the plants were harvested and extracted. Various methods were examined for homogenizing and extracting the plants. The method proving most quanti-

Table I. Composition of Fractions from Bio-Gel P2 Column

Fraction	Number of Tubes	Composition (Bands)
1	1–4	...
2	5–7	A, B
3	8–10	C, D, E
4	11–13	D, E
5	14–17	D, E, F
6	18–22	...

tative involved homogenization of minced plant tissues, with a Virtis homogenizer, in chloroform-methanol (2:1). The plant extracts were then washed according to the method of Folch *et al.* (1957). The glycone metabolites were found entirely in the upper aqueous phase.

Thin-layer chromatography of the water soluble metabolites of carbaryl in plants has presented problems to us and other investigators. The metabolite bands were not sharply defined and contained small amounts of many other metabolites (Kuhr and Casida, 1967). Several tlc solvents were examined for potential use; chloroform-methanol-water (65:25:4) gave the best separation. Figure 1 shows the separation obtained for the total water soluble metabolites of carbaryl in beans. Six bands or regions can be found: A, B, C, D, E, and F. The silica gel containing the individual bands was removed and the metabolites were eluted. Upon rechromatography of these bands, an unusual observation was made. Bands C, D, and E were not pure, but contained small amounts of metabolites with greater R_f values. For example, band C contained small amounts of D, E, and F. Band D contained small amounts of E and F, but no C. When the intensely yellow bands A or B were rechromatographed, no A or B bands were found, only bands C, D, E, and F. Upon additional rechromatography, bands C, D, E, and F were pure.

In order to simplify the isolation procedure it was found desirable to pass the total aqueous soluble metabolite through a molecular sieve column (Bio-Gel P2). This treatment completely separated bands A and B from bands C, D, E, and F, as summarized in Table I. Fractions 3, 4, and 5 were pooled, concentrated, and the contents analyzed on tlc (Figure 1). The tlc showed good separation with very little tailing between the bands. When these bands C, D, and E were rechromatographed, only one band was obtained. The Bio-Gel P2 column simplified the purification of the glycone metabolites and was used routinely. Band F is carbaryl.

The intensely yellow bands A and B suggested a π complex between the plant flavonoid pigments and the aromatic ring of carbaryl and its metabolites. The weak complex was evidently continually decomposing on tlc, yielding all the metabolites. Such complexes are well known in organic chemistry, but to out knowledge have not been proposed for pesticide metabolites. To evaluate this possibility, quercitrin, a purchased flavonoid, and ^{14}C-carbaryl were mixed and the resulting aqueous solution analyzed by tlc (Figure 2). ^{14}C-labeled areas appeared at two spots on the tlc, one spot being carbaryl and the other slower moving spot being bright yellow. The ^{14}C-labeled yellow spot was removed, rechromatographed, and only carbaryl was obtained; the weak complex had decomposed. A complex was also formed between quercitrin and the ^{14}C-carbaryl metabolite (band E). Because of the problems the weak complex creates in studying carbaryl metabolism, conditions were sought that would decompose the complex and not affect the structure of the metabolites. The complex was shown to be unstable under

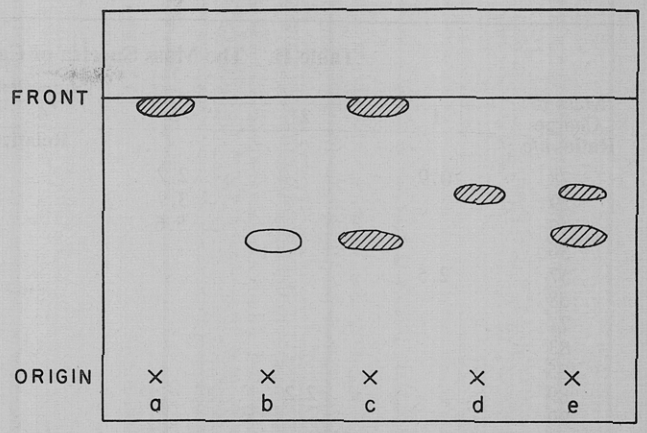

Figure 2. Thin-layer chromatogram demonstrating a complex between quercitrin and carbaryl and its metabolites. The origins are as follows: a, ^{14}C-carbaryl; b, quercitrin; c, quercitrin plus ^{14}C-carbaryl; d, compound from band E (aglycone of *N*-hydroxymethyl carbaryl); e, quercitrin plus compound from band E. The hatched areas represent radioactive areas. The developing solvent was chloroform-methanol-water (65:25:4)

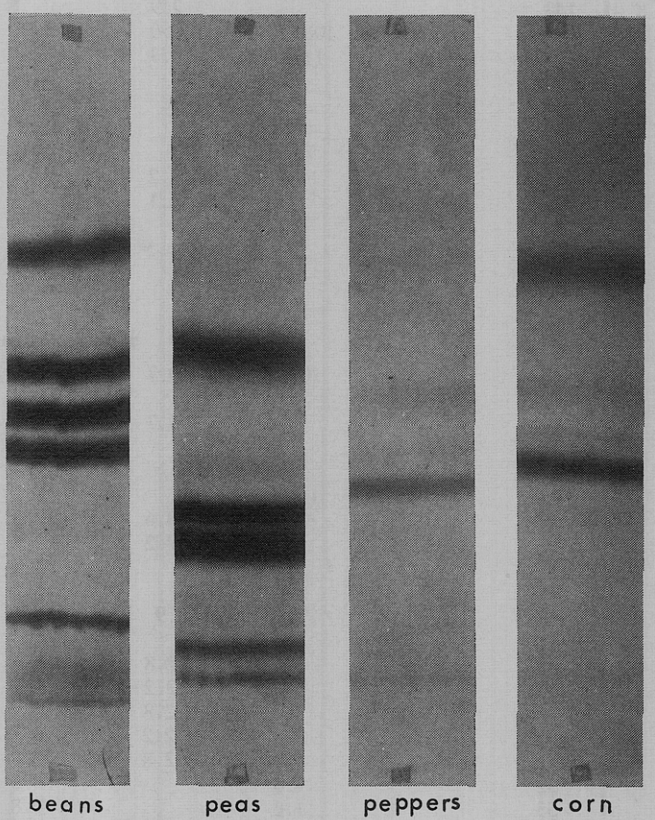

Figure 3. Radioautogram of thin-layer chromatograms of the ethyl ether soluble aglycone metabolites of carbaryl in beans, peas, corn, and peppers. The developing solvent was ethyl ether-benzene-ethanol-acetic acid (40:50:2:0.2)

acidic conditions, and can be decomposed by acidifying either the aqueous extract or the tlc developing solvent.

Whether the carbaryl metabolites are complexed with a flavonoid pigment within the plant is not known. However, our experiments indicate that a portion of the carbaryl metabolites is weakly complexed with a plant pigment after homogenization and extraction. The concentration of the complex is undoubtedly a function of the structure and of the concentration of both the plant pigment and the metabolites. The complex continuously decomposes on tlc and results in

Table II. The Mass Spectra of Carbaryl and Derivatives at 20 and 70 eV

Mass to Charge Ratio *m/e*	Numerical Designation of Compounds[a]								
	1	2	3	4	5	6	7	8	9
	Relative Intensity of Ions at 20 eV								
28	0.9		2.2			1.2	1.4	2.0	2.9
29			3.7						
43			5.6						4.8
56						1.7		3.5	3.7
57	2.5					7.3	2.2	11.8	12.0
58							6.7		3.3
71						4.1			
83						4.1			
85						3.5			
89		2.2							
99						1.6			
103				5.6	2.7				
104				11.7	4.7	1.5	1.2		
105				10.8		3.5		2.3	
114		3.5							
115	6.1	50.0	14.7						
116	10.0	30.0	16.2						
130				2.9					
131				25.1	38.7	8.3	16.5	5.9	14.5
132				12.6	11.3	5.7	4.8	3.7	4.2
143			2.9						
144	100.0	100.0[b]	100.0						
145	11.4	11.0	10.3						
158				4.0					
159				4.7				1.8	
160				100.0[b]	100.0[b]	100.0	100.0	100.0	100.0
161				9.0	14.0	11.4	10.2	11.4	10.8
187			2.2						
199			2.1						
201	5.7[b]								
217			1.5[b]			7.0[b]	6.7[b]	7.7	6.9
218						1.0		1.0	
274								1.7[b]	1.2[b]
	Relative Intensity of Ions at 70 eV								
15	2.5	0.9	0.7			4.9	2.7	4.1	4.6
17							3.0		
18			1.7	5.3	21.3		9.7		1.7
26		1.7		3.4		1.0			
27		2.0		5.6		2.8		4.1	4.4
28	2.6	11.1		4.2	3.8	7.0		13.3	16.8
29		1.6	10.6	2.3		1.9		1.9	
30			7.2						
37		1.2		2.2					
38		2.8		4.7					
39	2.3	7.8	3.9	6.4	23.4	1.9		1.1	
41						1.2			
42			2.8						
43			12.2			2.8	2.1	1.9	9.6
44			2.8						
50		5.4	2.2	19.7		4.5	2.7	2.3	
51	1.5	5.2	2.8	22.0	18.1	6.4	6.4	3.1	
52		1.0		5.3	3.1	1.2			
53		1.6		6.8	2.9	2.3			
54			1.4	4.4					
55				2.3		2.4		2.3	
56						2.4	3.9	6.7	
57	3.2	3.0	3.1			10.3	3.1	17.8	18.4
57-1/2			3.6						
58	4.0	4.2	3.6			6.6	3.6	6.3	
58-1/2			0.3						
61		2.2							
62	1.6	6.3		4.7	2.8				
63	4.2	11.7	7.2	7.6	6.9	2.8	2.7		
64		2.1							
65	2.0	4.0	2.8	2.8	3.4				
65-1/2			1.0	3.6	3.6				
66				7.3	3.8				
71						1.6			
72		4.8	4.4						
74	1.3	4.3	2.5	9.8	5.6	2.6	2.1		

(*Continued on next page*)

Table II. (Continued)

Mass to Charge Ratio m/e	Numerical Designation of Compounds[a]								
	1	2	3	4	5	6	7	8	9
	Relative Intensity of Ions at 70 eV								
75	1.5	3.7	2.8	9.1	5.8	3.5	2.4		
76				23.5	7.5	7.1	3.0	4.4	
77	1.3	2.1		42.8	22.5	20.7	10.9	11.5	8.8
78				11.4	6.9	3.0	9.7		
80				4.5	8.6		3.0		
85		1.3							
86		2.8							
87	1.3	4.5	1.9						
88	1.5	3.2	1.9						
89	4.7	10.9	6.7						
90		2.2							
102				12.5	9.4	6.6	4.8	3.7	3.6
103				24.2	19.4	15.0	9.4	8.5	8.0
104				33.3	10.0	7.0	3.9	4.8	2.9
105				27.3		14.8		9.6	
113	1.5	3.1	1.9						
114	2.9	7.2	4.4	2.3					
115	36.7	78.3	55.6	5.3	7.5	4.2	3.6	2.6	3.6
116	22.8	39.1	30.0						
117	17.0	3.7							
126	1.1								
127	3.0		2.8						
130				4.2		2.6			
131				58.3	68.8	30.4	45.5	19.3	37.6
132				25.0	20.0	13.9	10.6	8.9	8.8
143	2.3	2.6	5.6			1.0			
144	100.0	100.0[b]	100.0						
145	11.4	11.3	11.1						
158				4.2					
159				8.3	5.0	7.1	5.8	7.0	
160				100.0[b]	100.0[b]	100.0	100.0	100.0	100.0
161				11.8[b]	10.9	11.8	10.9	11.1	10.4
187			2.2						
199			2.2						
201	5.4[b]								
217			1.0[b]			5.0[b]	5.2[b]	4.8	4.7
274								1.0[b]	0.6[b]

[a] The compounds are as follows: (1) 1-naphthyl *N*-methylcarbamate (carbaryl); (2) α-naphthol; (3) 1-naphthyl *N*-hydroxymethylcarbamate (*N*-hydroxymethylcarbaryl); (4) 1,4-naphthalenediol; (5) 1,5-naphthalenediol; (6) 4-hydroxy-1-naphthyl *N*-methylcarbamate (4-hydroxycarbaryl); (7) 5-hydroxy-1-naphthyl *N*-methylcarbamate (5-hydroxycarbaryl); (8) naphthyl-1,4-bismethylcarbamate; (9) naphthyl-1,5-bismethylcarbamate. [b] Indicates molecular ion.

the appearance of excessive tailing and overlapping bands. The π complexes are probably not specific for carbaryl and its metabolites, but probably are formed with other aromatic pesticides. For pesticide metabolism studies, plants that possess high concentrations of flavonoids perhaps should be avoided. However, if the complexes do pose problems, we have shown that they can be decomposed with acid or separated with a Bio-Gel P2 column. This weak complex formation evidently explains why the previous investigators also have experienced considerable tailing and overlap of bands on tlc.

The compounds from bands C, D, and E from the tlc plates of the water soluble metabolites were eluted and treated with glycosidic enzymes. β-Glucosidase gave the best cleavage, approximately 85%, while β-glucuronidase and horseradish peroxidase gave considerably less cleavage. The ethyl ether-soluble aglycone products from the glycosidic cleavage were analyzed by tlc. Although a number of solvent systems were evaluated, the Freeman-West solvent gave the best separation. When the total water soluble metabolites were treated with enzyme and analyzed by tlc, five bands or areas were produced (bands 1, 2, 3, 4, and 5). The same number of bands were found in each plant examined (Figure 3). However, the chromatograms were not performed at the same time and demonstrate the variability of thin-layer chromatographic separations. The relative concentrations of these bands varied greatly with each plant and condition of growth.

The results of the glycosidic cleavage of the water soluble metabolites are as follows. Band F gave rise to the ethyl ether soluble band 5, which was identified as carbaryl. Band E gave rise to band 2, which cochromatographed with synthetic *N*-hydroxymethylcarbaryl, a proposed metabolite. Band D produced two ether soluble metabolites that corresponded to bands 3 and 4, which cochromatographed with standard 4-hydroxycarbaryl and 5-hydroxycarbaryl, respectively. Band C gave rise to band 1, usually present in low quantities and presumably corresponding to the proposed 5,6-dihydro-5,6-dihydroxycarbaryl (Kuhr and Casida, 1967). Figure 4 summarizes the interrelationships between the glycones and aglycones. Bands 2, 3, and 4 were scraped from the tlc plates, eluted, and the extract was concentrated and stored at −20° C in the dark prior to analysis by mass and ultraviolet spectroscopy.

In order to facilitate the identification and interpretation of the mass spectra of the isolated metabolites, the mass spectra of carbaryl, its proposed metabolites, and related synthetic compounds were obtained. Table II shows the

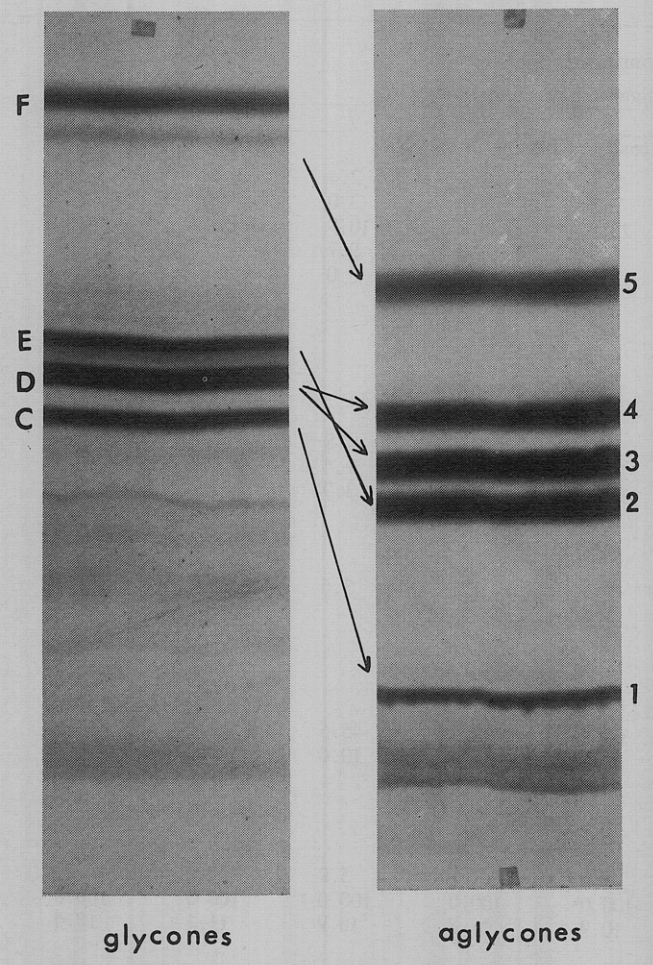

Figure 4. Radioautograms of thin-layer chromatograms of metabolites of carbaryl from a bean plant showing the interrelationship between the glycones and the aglycones. Left: glycones (chloroform-methanol-water; 65:25:4). Right: aglycones obtained after enzymatic treatment of the glycones (ethyl ether-benzene-ethanol-acetic acid; 40:50:2:0.2). Bands 2, 3, 4, and 5 correspond to the R_f values of synthetic *N*-hydroxymethylcarbaryl, 4-hydroxycarbaryl, 5-hydroxycarbaryl, and carbaryl, respectively

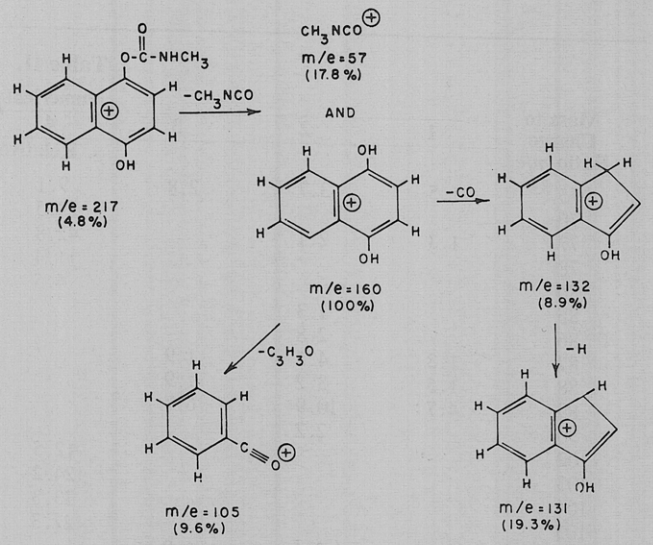

Figure 5. Mass spectral fragmentation of 4-hydroxycarbaryl

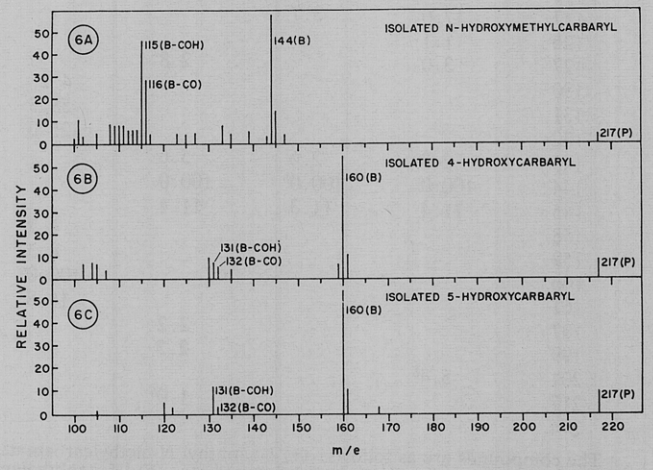

Figure 6. The mass spectra of the isolated ethyl ether soluble aglycone metabolites of carbaryl in bean and pea plants. Spectra 6A is the isolated metabolite from band 2 (70 eV), 6B the isolated metabolite from band 3 (20 eV), and 6C the isolated metabolite from band 4 (20 eV)

mass spectra at 20 eV and 70 eV. Although the mass spectra of carbaryl, α-naphthol (Damico and Benson, 1965; Mumma, 1969) and *N*-hydroxymethylcarbaryl (Durden *et al.*, 1970) at 70 eV have been reported previously, we have presented the spectra of these same compounds obtained under our experimental conditions for comparison.

All the compounds studied, including the biscarbamates, possessed molecular ions of low intensity and contained *m/e* 144 (naphthol) or *m/e* 160 (hydroxynaphthol) as the base ion. The second most intense ion corresponds to the loss of –CO– or –COH from naphthol (*m/e* 115, 116) or hydroxynaphthol (*m/e* 131, 132). In general, very few other characteristic ions exist, except for the molecular ions. Detailed explanations of the fragmentation of carbaryl, α-naphthol, and *N*-hydroxymethylcarbaryl have been published (Damico and Benson, 1965; Durden *et al.*, 1970). One additional characteristic of the spectra of carbaryl, α-naphthol, and *N*-hydroxymethylcarbaryl is that they all possess dipositive ions (70 eV), of which the most intense is *m/e* 57.5. This ion is of considerable importance because very few contaminants possess an ion at this value. The *m/e* 57.5 ion can be recognized easily when carbaryl or *N*-hydroxymethylcarbaryl represents only a few percent of the total sample, and this characteristic was very useful in the isolation of *N*-hydroxymethylcarbaryl. The 20 eV spectra of these three compounds do not possess the *m/e* 57.5 ion.

The molecular ion of *N*-hydroxymethylcarbaryl (*m/e* 217) is of low intensity (1–1.5%) and significantly lower than the molecular ions of carbaryl and its 4- and 5-hydroxy derivatives (*m/e* 217) (4.7–7.7%). Experimentally it was not necessary to heat the probe for the spectrum of *N*-hydroxymethylcarbaryl and, if one was not careful, the sample completely volatilized before the spectrum was obtained. With carbaryl and its 4- and 5-hydroxy derivatives, a probe heat of 80° C was necessary for a sufficient vapor pressure.

A summary of the fragmentation of 4-hydroxycarbaryl is shown in Figure 5, and this fragmentation is typical of that found for 5-hydroxycarbaryl, 1,5- and 1,4-naphthalenediol, and naphthyl-1,4- and naphthyl-1,5-bismethylcarbamate. The major ions of 4- and 5-hydroxycarbaryl are the following: *m/e* 217 (P, parent), *m/e* 160 (P-CH_3NCO) (B, base), *m/e* 132 (B-CO), *m/e* 131 (B-COH), *m/e* 105 (B-C_3H_3O), and *m/e* 57 (CH_3NCO). Interestingly, the spectra of the synthetic 1,4- and 1,5-biscarbamates are almost identical to the spectra of 4- and 5-hydroxycarbaryl, except for the molecular ions.

Two minor but significant differences are noted in the

Table III. Ultraviolet Absorption of Isolated Carbaryl Metabolites and Synthetic Compounds

Compound	λ_{max}^{EtOH}
Carbaryl	290(sh),[a] 280, 270(sh), 260(sh), and 222 nm
N-Hydroxymethylcarbaryl	290(sh), 280, 270(sh), 260(sh), and 222 nm
4-Hydroxycarbaryl	327(sh), 313(sh), 300, 236, and 212 nm
5-Hydroxycarbaryl	325(sh), 312(sh), 298, and 222 nm
Compound from band 2 (*N*-hydroxymethylcarbaryl)	290(sh), 280, 270(sh), and 222 nm
Compound from band 3 (4-hydroxycarbaryl)	327(sh), 313(sh), 300, 236, and 213 nm
Compound from band 4 (5-hydroxycarbaryl)	325(sh), 311(sh), 298, and 222 nm

[a] sh = shoulder.

spectra of 4- and 5-hydroxycarbaryl. 4-Hydroxycarbaryl has a relatively intense ion at *m/e* 105 (9.6%), while 5-hydroxycarbaryl has no significant ion of this mass. Another fragment ion which is characteristic is *m/e* 131. This ion is twice as intense in the 5-hydroxycarbaryl spectrum as it is in the 4-hydroxycarbaryl spectrum. For identification purposes it would be best to have an internal comparison. For example, *m/e* 131 is always about four times as intense as the *m/e* 132 in all the 5-hydroxy derivatives investigated, and only one to two times as intense in all the 4-hydroxy derivatives investigated. Dipositive ions, resulting from fragmentation of naphthalenediol and derivatives, are present in low intensity, the largest being *m/e* 65.5.

The spectra of isolated metabolites often contain a small number of ions due to impurities. The background can be reduced considerably if the spectrum can be run at 20 eV. The spectra of carbaryl and its derivatives at 20 eV are relatively simple, include only the major ions found at 70 eV, and are sufficiently characteristic of the structure to identify the compound. The molecular ions are also more intense and more easily recognized at 20 eV than at 70 eV. Figure 6 shows the mass spectra of the isolated ether soluble metabolites of carbaryl, compounds from bands 2, 3, and 4. Since the characteristic fragment ions of the carbaryl metabolites are all greater than *m/e* 100, only these ions are presented in Figure 6. The compound representing band 2 gave a mass spectrum identical to that of the proposed structure *N*-hydroxymethylcarbaryl. The compounds representing bands 3 or 4 both gave spectra corresponding to hydroxycarbaryl, and if one examines the relative intensity of the *m/e* 105 ion and the ratio of *m/e* 131 to 132, the metabolites from bands 3 and 4 represent, respectively, 4- and 5-hydroxycarbaryl as was suggested by tlc.

The ultraviolet spectra of the isolated metabolites are identical with those of the proposed metabolites, *N*-hydroxymethylcarbaryl, 4-hydroxycarbaryl, and 5-hydroxycarbaryl, and are summarized in Table III.

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