Metabolism of 2-Chloro-4,5-dimethylphenyl *N*-Methylcarbamate in Bean Plants

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The insecticide Banol, 2-chloro-4,5-dimethylphenyl *N*-methylcarbamate, is absorbed from solution and metabolized to a water-soluble metabolite by bean plants. This metabolite was shown to have the carbamate side chain intact and to have the ring modified by hydroxylation and conjugation with glucose. The synthesis of Banol-4-methyl C^{14} is described.

The insecticidal activity of 2-chloro-4,5-dimethylphenyl *N*-methylcarbamate (Banol) has been described by Lemin, Boyack, and MacDonald (*6*). Studies in this laboratory and by Wang (*9*) and coworkers have shown that C^{14}-labeled Banol is absorbed readily from solution by both bean and tomato plants and that the label is translocated rapidly. It has also been shown in our laboratory and by Casida and coworkers (*1*) that Banol, which is only sparingly soluble in water, is converted rapidly by plants to water-soluble metabolites which are stable within the plant.

This paper describes experiments showing that C^{14}-Banol labeled on the *N*-methyl, carbonyl, or 4-methyl position is converted to the same water-soluble metabolite. This metabolite represents the major fate of Banol in bean plants. The characterization of this metabolite is the major objective of this communication.

Materials and Methods

Carbon-14–labeled 2-chloro-4,5-dimethylphenyl *N*-methylcarbamates labeled in the *N*-methyl or carbonyl position were purchased from the New England Nuclear Corp., Boston, Mass., and had specific activities of 6.10 and 5.75 mc. per mmole, respectively. Banol 4-methyl-C^{14} was synthesized in our laboratory as described below, and had a specific activity of 4.76 mc. per mmole. Radioactivity was counted with a Packard Tricarb liquid scintillation counter (Model 314Ex) using internal standardization for counting efficiency correction. Nonextractable plant fiber was assayed by Schoeniger combustion (*7*), absorption of the carbon dioxide in hyamine hydroxide, and counting an aliquot in Diotol (*5*).

Thin-layer plates and paper chromatograms were scanned for radioactivity with a Vanguard Model 880D dual-channel auto-scanner with a Model 885 glass plate scanner attachment. Mass spectra were determined using an Atlas Model CH-4 mass spectrometer equipped with a TO-4 source at an ionizing current of 70 e.v.

Beta-glucosidase was purchased from the Mann Research Laboratory, New York, N.Y., and had an activity of 350 units per mg. per minute. Ferric chloride–potassium ferricyanide reagent was prepared by mixing equal volumes of aqueous 15% ferric chloride and 1% potassium ferricyanide solutions immediately before use.

p-Dimethylaminobenzaldehyde reagent was prepared by dissolving 1 gram of the aldehyde in 100 ml. of ethanol containing 5 ml. of concentrated hydrochloric acid. Whatman No. 2 paper and descending development were used for paper chromatography. Silica gel plates were purchased from Custom Service Chemicals Co., Wilmington, Del. Cellulose plates were purchased from Brinkman Instrument Co., Westbury, N.Y.

Synthesis of Banol 4-Methyl-C^{14}. The reaction sequence for the preparation of Banol 4-methyl-C^{14} is shown in Figure 1.

4-Bromo-3-methylphenol (III) was prepared by the method of Walther and Zipper (*8*), and recrystallized from cyclohexane. Colorless needles, m.p. 62–64° C. (lit. 62° C.). Analysis calculated for C_7H_7BrO: C, 44.95; H, 3.77; Br, 42.73. Found: C, 45.07; H, 3.55; Br, 42.86.

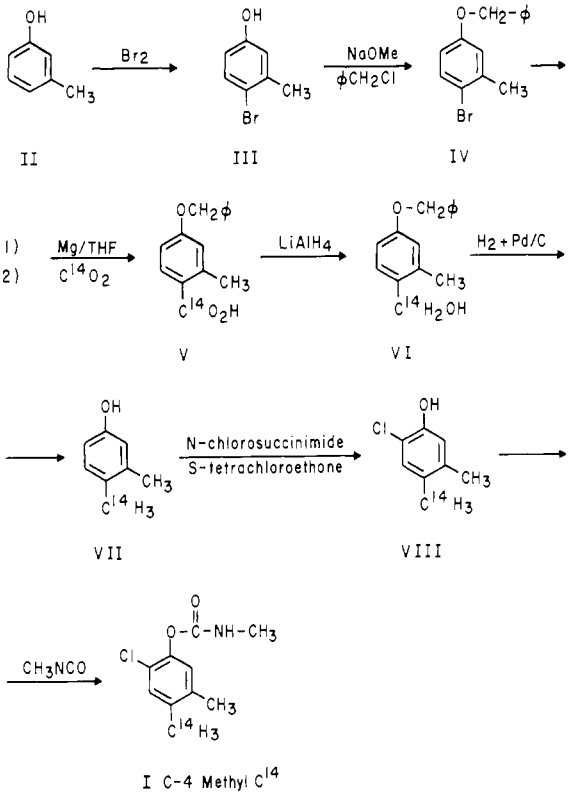

Figure 1. Synthesis of 2-chloro-4,5-dimethylphenyl *N*-methylcarbamate 4-methyl C^{14}

The Upjohn Co., Kalamazoo, Mich.

4-Bromo-3-methylphenyl Benzyl Ether (IV). Sodium methoxide (8.1 grams) was dissolved in methanol (100 ml.) and III (28.0 grams) was added. Benzyl chloride (18.9 grams) was added and the mixture was refluxed for 4 hours and cooled to room temperature, and water (300 ml.) and methylene chloride (300 ml.) were added. The lower phase was removed and the upper phase extracted with two 250-ml. portions of methylene chloride. The methylene chloride extracts were combined, dried, and evaporated under reduced pressure to give IV (36.5 grams, 86%), m.p. 67–70° C. An analytical sample was recrystallized from methanol, m.p. 70.5–71.5° C. Calculated for $C_{14}H_{13}BrO$: C, 60.66; H, 4.73; Br, 28.84. Found: C, 60.56; H, 4.87; Br, 28.58.

4-Carboxy-C^{14}-3-methylphenyl Benzyl Ether (V). Barium carbonate-C^{14} purchased from the Oak Ridge National Laboratory was used to generate $C^{14}O_2$. Prior to use the carbonate with a specific activity of 57 mc. per mmole was diluted to 16.2 mc. per mmole with inactive barium carbonate. The carbonation was run in two equal batches using a modification of an apparatus reported by Eberson (*4*). Magnesium (189 mg.) was placed in a dry 250-ml. three-necked flask, equipped with a stirrer, reflux condenser, addition funnel, and an inlet for dry nitrogen. Anhydrous ether (2 ml.) and methyl iodide (1 drop) were added to initiate Grignard formation. IV (2.14 grams) in dry tetrahydrofuran (40 ml.) was added slowly with stirring and refluxing. After 3 hours of refluxing, Grignard formation was complete. Barium carbonate (612 mg., 50 mc.) was placed in the carbonation apparatus. The cooled Grignard solution was introduced and the apparatus sealed with a rubber septum. The Grignard solution was frozen with liquid nitrogen and the system evacuated. It then was degassed by freezing and thawing and finally refrozen and evacuated; subsequently it was warmed to −15° C. with a methanol-ice bath. The barium carbonate was decomposed with 40% perchloric acid and the magnetically stirred Grignard solution maintained at −15° C. for 5 more minutes. The excess Grignard reagent was decomposed by the immediate addition of 10% hydrochloric acid (10 ml.). Ether (50 ml.) and 5% hydrochloric acid (50 ml.) were added. The organic phase was separated and the aqueous phase extracted with three 50-ml. portions of ether. The combined ether extracts were extracted four times with 50 ml. of 5% sodium bicarbonate. Long extraction periods were necessary for complete extraction of sodium salt of the acid into the aqueous phase. The combined bicarbonate extracts were acidified with HCl and extracted four times with 50-ml. portions of ether. The combined ether extracts were dried over anhydrous sodium sulfate and evaporated under reduced pressure to give V. The combined yield for two runs (1142 mg., 76%) was recrystallized from methylene chloride–cyclohexane to give 934 mg., m.p. 130.5–31.5° C. An analytical sample of unlabeled material had a melting point of 131.5–32° C. Calculated for $C_{15}H_{14}O_3$: C, 74.36; H, 5.83. Found: C, 74.05; H, 5.82.

4-Hydroxymethyl C^{14}-3-Methylphenyl Benzyl Ether (VI). Lithium aluminum hydride (185 mg.) was added to dry tetrahydrofuran (50 ml.). V (930 mg.) in dry tetrahydrofuran (50 ml.) was added and the mixture was refluxed for 2 hours, at which time the reaction was complete as seen by thin-layer chromatography (benzene–acetic acid, 9 to 1 on silica gel GF). The excess hydride was decomposed with water and the reaction mixture acidified with 2% HCl (50 ml.). The tetrahydrofuran was removed under reduced pressure and the aqueous mixture extracted with three 50-ml. portions of ether. The combined solution was washed with 1% sodium bicarbonate (25 ml.) and water (25 ml.), dried over anhydrous sodium sulfate, and evaporated under reduced pressure to give VI (989 mg.) (some solvent still present) of oil which crystallized upon seeding. An analytical sample of unlabeled material recrystallized from methylene chloride–Skellysolve F had a melting point of 54–55° C. Calculated for $C_{15}H_{16}O_2$: C, 78.92; H, 7.06. Found: C, 78.79; H, 7.27.

3,4-Dimethylphenol (4-Methyl C^{14}) (VII). VI (989 mg.) was dissolved in 95% ethanol (30 ml.) and 5% palladium on carbon (800 mg.) was added. The reaction mixture was hydrogenated at atmospheric pressure for 18 hours, during which time 192 ml. of hydrogen was absorbed. The catalyst was removed by filtration and 2% aqueous potassium hydroxide (50 ml.) added to the filtrate. The ethanol was removed under reduced pressure and the basic aqueous layer extracted with benzene (25 ml.). The basic solution was acidified with hydrochloric acid, saturated with sodium chloride, and extracted with four 50-ml. portions of methylene chloride. The methylene chloride extract was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The resulting oil crystallized upon seeding with an authentic sample of 3,4-dimethylphenol.

2-Chloro-4,5-dimethylphenol (4-Methyl-C^{14}) (VIII). *N*-Chlorosuccinimide (515 mg.) and VII from the previous reaction were dissolved in *s*-tetrachloroethane (50 ml.). The reaction mixture was heated on a steam bath under nitrogen for 3 hours and cooled, and ether (100 ml.) and water (25 ml.) were added. The phases were mixed, the aqueous phase was discarded, and the organic phase extracted with six 25-ml. portions of 5% sodium hydroxide. The alkaline extracts were combined, acidified with hydrochloric acid, and extracted with four 40-ml. portions of methylene chloride. The methylene chloride solution was dried over anhydrous sodium sulfate and the solvent evaporated under reduced pressure. The crude 2-chloro-4,5-dimethylphenol was purified by the following procedure, developed by C. Y. Yoon, The Upjohn Co. The residue was dissolved in benzene (30 ml.) and concentrated sulfuric acid (0.5 ml.) was added. The reaction mixture was refluxed for 2 hours using a Dean-Stark trap to remove any water formed, and then cooled to room temperature. Water (30 ml.) was added and the layers were separated. The aqueous extract was washed with two 25-ml. portions of benzene and the combined benzene extracts were washed with water (25 ml.). The organic layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure to give a brown oil (219 mg.) which was

steam-distilled. The steam distillate was extracted with four 20-ml. portions of methylene chloride. The methylene chloride extract was dried over anhydrous sodium sulfate and the solvent evaporated under reduced pressure to give VIII (137 mg., 23% yield from V). The VIII obtained was diluted with 137 mg. of inactive VIII to a specific activity of 8 mc. per mmole.

2-Chloro-4,5-dimethylphenyl *N*-Methylcarbamate 4-Methyl-C^{14} (I). VIII (specific activity 8 mc. per mmole) (246 mg.) was dissolved in anhydrous ether (10 ml.) and triethylamine (1 drop) added. Methyl isocyanate (125 mg.) was added and the mixture was stoppered and allowed to stand overnight. The ether was evaporated under reduced pressure to give crude I, 4-methyl-C^{14} (282 mg., 84%). Recrystallization from ether-cyclohexane gave 134 mg. of I, 4-methyl-C^{14} (m.p. 125–8° C.). Further dilution with unlabeled I (60 mg.) followed by recrystallization gave 179 mg. (m.p. 128.5–30.5° C.), with a specific activity of 4.76 mc. per mmole, representing a 4% over-all radiochemical yield from barium carbonate. Thin-layer chromatography on silica gel GF using either benzene or chloroform as the developing solvent showed the radiochemical purity of I, 4-methyl-C^{14}, to be 98%.

Experimental

Two reactions, one chemical and one enzymatic, plus the use of mass spectroscopy led to the characterization of the major metabolite as a glucoside of ring-hydroxylated Banol. Treatment of Banol with a solution of ammonia results in ammonolysis to *N*-methyl urea plus the parent phenol. Treatment of the water-soluble metabolite of Banol labeled on either the *N*-methyl or carbonyl position gave labeled *N*-methyl urea, indicating that the carbamate side chain was intact.

Enzymatic hydrolysis of the water-soluble metabolite with beta-glucosidase gave an organosoluble compound, more polar than Banol. This enzymatically hydrolyzed product was subjected to mass spectroscopy, which indicated that it was hydroxylated Banol.

Treatment with C^{14}-Labeled Banol and Extraction of Bean Seedlings. Banol labeled with C^{14} on the *N*-methyl group (2.15 mg., 13.5×10^7 d.p.m.), labeled on the carbonyl carbon (2.31 mg., 13.7×10^7 d.p.m.), and labeled on the 4-methyl carbon (2.33 mg., 10.9×10^7 d.p.m.) was dissolved in acetone (4 ml.) and the solutions were diluted with deionized water to 400 ml. Thirty-six sand-grown, 12-day-old Pinto bean seedlings were divided into three groups of 12 plants each and placed in beakers containing gently aerated solutions of each labeled Banol. The plants were grown under an 18-hour photoperiod using three Sylvania F-40 Grolux lamps at a distance of about 18 inches from the leaf surface. Water was added daily to the 400-ml. mark. After 7 days, the plants were removed from the feeding solutions and the roots rinsed with water. The rinse was combined with the residual feeding solution and aliquots were counted.

The plants were macerated in methanol (200 ml. for each three plants) with the aid of a Vir-Tis 45 homogenizer. The combined methanolic extracts from each treatment were separated from the insoluble material by centrifugation and passed through a column of anhydrous sodium sulfate to remove some of the water. The extracts then were evaporated under reduced pressure at 40° C. and the residues partitioned between benzene and water (50 ml. of each). The phases were separated and aliquots of each phase counted. Both phases then were evaporated under reduced pressure. The benzene extracts were redissolved in acetone (10 ml.) and the aqueous extracts redissolved in water (5 ml.) for thin-layer chromatography.

The distribution of radioactivity in various fractions from the three C^{14}-labeled Banols is shown in Table I.

Thin-Layer Chromatography. Each extract (25 μl.) was chromatographed on a 250-micron thick 2 × 8 inch silica gel GF plate using two systems: methyl ethyl ketone–acetone–water (9.3:2.6:1.0) (the MAW system) and chloroform. The plates were dried and scanned for radioactivity. The results are shown in Tables II and III. Metabolite B, present in the benzene extract, was found by rechromatography (MAW) of the metabolites present at the origin after chloroform development. The radioactive zones of the metabolite mixture after chloroform chromatography were individually removed from the plate, eluted with methanol, and rechromatographed using the MAW system. Thus the zone with R_f 0.5 using chloroform development had R_f 1.0 in the MAW system but the origin

Table I. Distribution of Radioactivity in Extracts of Bean Seedlings Grown for 7 Days in C^{14}-Labeled Banol Solutions

Position of C^{14} Label	*N*-Methyl		Carbonyl		C-4-Methyl	
	D.p.m. (10^7)	Activity applied, %	D.p.m. (10^7)	Activity applied, %	D.p.m. (10^7)	Activity applied, %
Feeding solution (time 0)	13.5	100	13.7	100	10.9	100
Feeding solution (7-day)	0.32	2.4	0.30	2.2	0.65	6.0
Benzene extract	0.89	6.4	0.78	5.7	0.59	5.4
Aqueous extract	3.63	27.0	4.16	30.3	3.28	30.1
Insoluble solids	1.69	12.5	0.69	5.0	2.62	24.0
Recovery		48.3		43.2		65.5

Table II. R_f's of Metabolites and Unchanged (I) when Chromatographed on Two Thin-Layer Systems

Metabolite	System	R_f
A (unchanged Banol)	$CHCl_3$	0.50
B + C + X	$CHCl_3$	0.0
A + B	MAW	1.0
C	MAW	0.65
X	MAW	0.0

zone from the chloroform-developed plate had three radioactive zones at R_f's 0.0, 0.65, and 1.0 when rechromatographed using the MAW system.

The major metabolite, C, appeared in the largest concentration in the aqueous extracts and was separated easily from the unchanged Banol and minor metabolites using the MAW system. The percentage of the radioactivity fed was based on the area under the radioactive scanner curves. That A was indeed unchanged Banol was shown by cochromatography experiments when the metabolite did not separate from authentic samples of Banol.

Paper Chromatography of Banol with an Ammoniacal Solvent. Banol (100 μg.) and *N*-methylurea were paper-chromatographed using the system 2-propanol–concentrated ammonia–water (7:1:2) (IAW). After drying, the area near the solvent front was masked and the remainder of the paper sprayed with *p*-dimethylaminobenzaldehyde reagent. The area near the solvent front was unmasked and sprayed with ferric chloride–potassium ferricyanide reagent. The results are seen in Table IV.

Chromatography of tracer amounts of Banol *N*-methyl, carbonyl, and 4-methyl in IAW gave results also consistent with the following reaction:

$$ArO\text{-}\overset{O}{\overset{\|}{C}}\text{-}NH\text{-}CH_3 + NH_3 \rightarrow ArOH + NH_2\text{-}\overset{O}{\overset{\|}{C}}\text{-}NH\text{-}CH_3$$

Ammonolysis and Thin-Layer Chromatography of Metabolite C from *N*-Methyl and Carbonyl-C^{14}-Labeled Banol. Aliquots of the aqueous extracts from the plants treated with Banol *N*-methyl-C^{14} and Banol carbonyl-C^{14} were chromatographed using the MAW system. The radioactive area (R_f 0.65) was removed from each plate and eluted with methanol. The eluates were reduced in volume under nitrogen and spotted on cellulose plates. The origins were lightly oversprayed with IAW solvent and then incubated for 1 hour in an atmosphere saturated with IAW. The plates were dried, spotted with methyl urea (20 μg.), and developed in IAW. The plates were dried, scanned for radioactivity, and then sprayed with *p*-dimethylaminobenzaldehyde reagent. The results are shown in Table V.

The above results indicate that although metabolite C is not homogeneous, it consists primarily of a material with an unaltered side chain which upon ammonolysis is converted to methylurea. Further proof that the ammonolysis product is methylurea was obtained as follows.

Table III. Distribution and Occurrence of Metabolite in Benzene and Aqueous Extracts

Metabolite	C^{14}-Label Position	Extract of Plant	% of Radioactivity Fed
A (unchanged Banol)	N—CH_3	Benzene	1.1
	C=O	Benzene	1.3
	4—CH_3	Benzene	1.3
B	N—CH_3	Benzene	1.6
	C=O	Benzene	0.2
	4—CH_3	Benzene	1.2
C	N—CH_3	Benzene	4.0
	C=O	Benzene	4.2
	4—CH_3	Benzene	2.6
C	N—CH_3	H_2O	20.3
	C=O	H_2O	26.3
	4—CH_3	H_2O	23.2
X	N—CH_3	H_2O	6.7
	C=O	H_2O	3.6
	4—CH_3	H_2O	6.9

Table IV. Chromatographic Behavior of Banol and *N*-Methylurea in IAW System

Material Applied	R_f	*p*-Dimethylaminobenzaldehyde	$FeCl_3$–$K_3Fe(CN)_6$
Methylurea	0.67	Yellow	...
Banol	0.70	Yellow	...
	0.90	...	Blue

Table V. Ammonolysis and Thin-Layer Chromatography (IAW System) of Metabolite C

Material Applied	R_f	%
Metabolite C *N*-methyl-C^{14}	0.81	88
	0.55	12
Metabolite C carbonyl-C^{14}	0.81	71
	0.48	29
Methylurea	0.81	...

Inverse Dilution of Ammonolysis Product of "C" *N*-Methyl C^{14} with Methylurea. Metabolite C, *N*-methyl-C^{14}, was purified partially by chromatography and elution as above. The methanolic eluate (3 ml.) was treated with IAW solvent (1 ml.) and incubated at 25° C. for 1 hour and methylurea (54.7 mg.) was added. The solvents were removed under nitrogen and the residue was recrystallized three times from acetone, once from acetonitrile, and once again from acetone. A sample of each recrystallization was counted, with the results shown in Table VI.

Butanol–Acetic Acid–Water (60:15:25) (BAW) Chromatography of Metabolite C from *N*-Methyl-C^{14}-Labeled Banol. BAW chromatography of metabolite

Table VI. Specific Activity of Recrystallized Methylurea

Solvent	Specific Activity, D.P.M./Mg. × 10^3
Acetone	
1X	1.95
2X	1.94
3X	1.85
Acetonitrile	
1X	2.56
Acetone	
1X	2.46

C *N*-methyl-C^{14} showed that it was not methylurea. In this system the intact metabolite C had an R_f of 0.80, while methylurea had an R_f of 0.68.

β-Glucosidase Hydrolysis of Metabolite C. Samples of metabolite C from *N*-methyl-C^{14}, carbonyl-C^{14}, and 4-methyl-C^{14}–labeled Banol were prepared by chromatography and elution as above. The eluates were taken to dryness under nitrogen, and redissolved in water (3.0 ml.) and 0.1-ml. aliquots counted. Portions of each were treated as follows:

CONTROLS, 1.0 ml. of extract, 3.0 ml. of 0.1*N* sodium acetate buffer (pH 5.1), and 1.0 ml. of deionized water.

REACTION MIXTURE, 1.0 ml. of extract, 3.0 ml. of buffer, and 1.0 ml. of β-glucosidase solution (prepared by dissolving β-glucosidase) (2.0 mg. in deionized water, 5.0 ml.) were added to each tube and the tubes were mixed thoroughly. An aliquot of each benzene extract was counted. The results are shown in Table VII.

Metabolite C appears, therefore, to be a glucoside which upon enzymatic hydrolysis is converted to an organosoluble aglycone.

Only one radioactive peak was obtained upon chromatography of either the benzene extract or the remaining radioactive material in the buffered enzyme solution with the MAW system or with chloroform. The aglycone of metabolite C was chromatographically more polar than Banol but less polar than C and was chromatographically similar to metabolite B.

Mass Spectroscopy of Aglycone of Metabolite C. A 20-mm. i.d. column was prepared from 30 grams of silica gel slurried in MAW solvent. A 1.0-ml. portion of the aqueous extract from plants treated with Banol carbonyl-C^{14} was mixed with silica gel (2.5 grams) and placed on the column. The column was eluted with MAW and 10-ml. fractions were collected (Figure 2). Tubes 8 and 9 were combined, evaporated under reduced pressure, and rechromatographed with MAW solvent on a 750-micron thick silica gel plate. The silica gel around the radioactive area at R_f 0.65 was removed and eluted with methanol and the methanol evaporated under reduced pressure. The residue was dissolved in acetate buffer (4.0 ml.) and 1.0 ml. of β-glucosidase solution (2 mg. of β-glucosidase per 5.0 ml. of water) was added. The mixture was incubated at 37° C. for 18 hours and then extracted three times with 5 ml. of benzene. The solvent was removed under nitrogen and the residue redissolved in acetone

Table VII. Radioactivity of Benzene Extracts from β-Glucosidase Hydrolysis of Metabolite C

	N-Methyl-C^{14} Label, D.P.M. × 10^4	Carbonyl-C^{14} Label, D.P.M. × 10^4	4-Methyl-C^{14} Label, D.P.M. × 10^4
Theory	3.65	4.40	3.03
Enzyme	2.04	2.14	1.93
Control	0.02	0.03	0.09

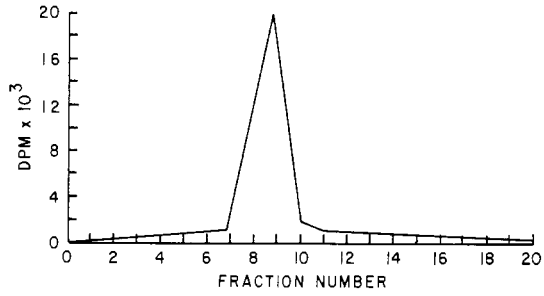

Figure 2. Column profile of metabolite C from Banol–carbonyl C^{14} using MAW as developing solvent

(100 μl.) to facilitate introduction into the mass spectroscopy. The results are shown in Figure 3.

Minor peaks in the mass spectrum of the aglycone could not be identified because of the high background from the extraneous plant materials not removed in the cleanup procedure. It was not possible to pick out a weak molecular ion $M=229$ (Cl = 35) in the spectrum. The molecular ion of Banol was weak and the prominence of the 2-chloro-4,5-dimethylphenol peak at $M=156$ (Cl = 35) bears out the ready loss of the elements of methyl isocyanate from the molecule. The mass spectrum of Banol as well as several other carbamates has been published by Damico and Benson (*3*).

The most prominent peak in the mass spectrum of the aglycone was at mass 172 with a peak 1/3 its intensity at mass 174. The 3 to 1 ratio of the 172:174 peaks indicates that the molecule still contains chlorine, since the natural chlorine ratio $Cl^{35}:Cl^{37}$ is 3 to 1. These peaks were shifted 16 mass units from the corresponding 156 and 158 peaks of the chlorodimethylphenol, which indicated the presence of an additional oxygen atom in the molecule. Both spectra contained a peak generated by the loss of chlorine from the corresponding phenols at mass 121 in the case of Banol and 137 in the case of the aglycone, the difference again, mass 16, being due to oxygen. The peak in the spectrum of the aglycone at mass 154 (172−18) possibly was due to loss of water from the 172 peak. The 154 peak in the spectrum of the aglycone had an intensity of only one half that of the 172 peak. This indicated that the added oxygen was phenolic rather than benzylic, since Aczel and Lumpkin (*2*) found the peak *M*-18 (loss of water) to be the most prominent peak in the mass spectra of *o*-methylbenzyl alcohols.

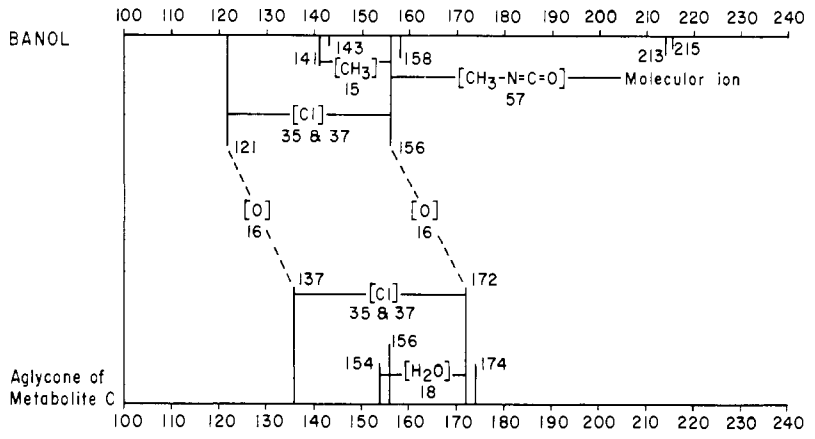

Figure 3. Pertinent mass peaks of Banol and the aglycone of metabolite C

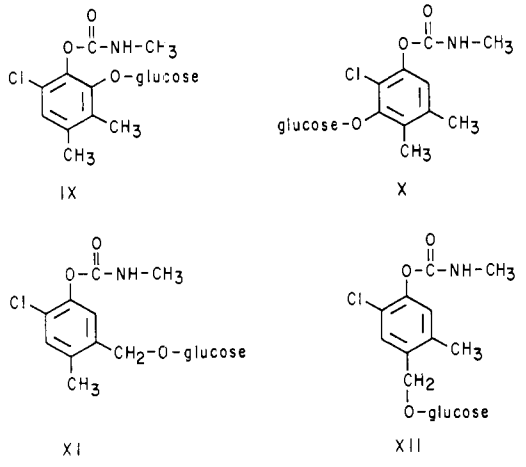

Figure 4. Structures possible for metabolite C

Discussion and Conclusions

2-Chloro-4,5-dimethylphenyl *N*-methylcarbamate, Banol, was absorbed readily from aqueous solution and metabolized by bean plants. After 7 days, only from 2.2 to 3.1% of the metabolic residue in the plant was unchanged Banol. The major metabolite, a water-soluble material, 44 to 74% of the radioactivity in the plant, was shown to have the *N*-methyl carbamate side chain intact. Enzymatic hydrolysis of this metabolite with β-glucosidase liberated an organosoluble aglycone. Mass spectroscopic evidence showed that the metabolite had another oxygen introduced to the ring and that the chlorine atom was retained. The mass spectral evidence indicated that the added oxygen was phenolic rather than benzylic, although this point was merely an indication. The structures which fit metabolite C are shown in Figure 4. Structure IX or X is preferred over XI or XII.

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