

## Characterization of $^{14}\text{C}$ Terminal Residues in Rice Plants Treated with $^{14}\text{C}$ Ring-Labeled Benthicarb

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### ABSTRACT

The terminal  $^{14}\text{C}$  residues in rice treated with  $^{14}\text{C}$  ring-labeled benthicarb were characterized. Rice was treated at 5 lb/acre, then grown to maturity. Straw and grain were harvested and analyzed for  $^{14}\text{C}$ . The total  $^{14}\text{C}$  in the straw and the grain was 2.0 and 0.3 ppm, respectively, calculated as benthicarb. No (less than 0.01 ppm) parent compound or metabolites containing the intact thiocarbamate moiety were found in either the straw or the grain. Approximately 0.25 ppm 4-chlorobenzoic acid (0.41 ppm  $^{14}\text{C}$  calculated as benthicarb) was isolated from straw as bound, conjugated and/or free metabolites. Two other major  $^{14}\text{C}$  metabolites (0.33 ppm), both containing carboxylic acid and the 4-chlorobenzylthio moieties in the molecules, were partially identified. Approximately 0.02 ppm 4-chlorobenzyl methyl sulfone was found in the grain.

**Key words :** Thiocarbamate herbicide, Degradation products of benthicarb

### INTRODUCTION

Benthicarb [S-4-chlorobenzyl N, N-diethylthiocarbamate] is a thiocarbamate with good herbicidal activity. This chemical has been used for the last two decades to control weeds in rice paddies. Benthicarb is particularly effective against barnyard grass there. A few studies have been conducted on the metabolic and the environmental fates of this chemical. They include: animal metabolism<sup>(1-3)</sup>; plant metabolism<sup>(2, 4-5)</sup>; microbial and soil metabolism<sup>(6-8)</sup>; photodegradation<sup>(8-9)</sup>; and degradation in water environment<sup>(10-11)</sup>. The fate and Persistence of benthicarb and its potentially toxic metabolite in mature rice plant have not yet been described.

The purpose of this study was to investi-

gate the residue and the degradation products of benthicarb in mature rice. One week after seeding, rice was treated with  $^{14}\text{C}$  ring-labeled benthicarb at 5 lb/acre, then grown to maturity (5 months). Straw and grain were harvested, and the  $^{14}\text{C}$  residues in the samples were characterized.

### MATERIALS AND METHODS

#### I. Chemicals

The [phenyl- $^{14}\text{C}$ ] benthicarb was purchased from New England Nuclear (Boston, Massachusetts). The  $^{14}\text{C}$  ring-labeled benthicarb which had a specific activity of 3.68 mCi/mM (31,788 dpm/ $\mu\text{g}$ ) was used. All authentic compounds for cochromatography used in this study

were: N-ethyl-S-(4-chlorobenzyl)thiocarbamate, S-(4-chlorobenzyl)thiocarbamate, N,N-diethyl-S-(4-chloro-2-hydroxybenzyl)thiocarbamate, N-ethyl-S-(4-chloro-2-hydroxybenzyl)thiocarbamate, N,N-diethyl-S-(4-chloro-3-hydroxybenzyl)thiocarbamate, N,N-diethyl-S-(4-chlorobenzyl)-S-monoxothiocabamate, N,N-diethyl-S-(4-chlorobenzyl)-S-dioxythiocarbamate, 4-chlorobenzyl alcohol, 2-hydroxyl-4-chlorobenzyl alcohol, 3-hydroxyl-4-chlorobenzyl alcohol, 4-chlorobenzoic acid, 2-hydroxy-4-chlorobenzoic acid, 3-hydroxy-4-chlorobenzoic acid, 4-chlorobenzyl mercaptan, 4-chlorobenzyl sulfonic acid, bis(4-chlorobenzyl) disulfide, bis(4-chlorobenzyl) monoxydisulfide, bis(4-chlorobenzyl) dioxydisulfide, 4-chlorobenzyl methyl sulfide, 4-chlorobenzyl methyl sulfoxide, 4-chlorobenzyl methyl sulfone, N-4-chlorobenzoylglycine, N-4-chlorobenzoylleucine, 4-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid, 3,4,5-trihydroxybenzoic acid, 4-hydroxycinnamic acid, caffeic acid, vanillic acid, ferulic acid and syringic acid. All these authentic compounds were supplied by the Organic Synthesis Division, Chevron Chemical Company, Richmond, California.

## II. *Planting and Treatment of Rice*

A stock watering tank (6 × 8 ft.) was used for growing the rice. The tank was placed outside, uncovered at the Delta Branch Experimental Station, Mississippi State University, Stoneville, Mississippi. Soil was placed in the tank to a depth of six inches. An overflow pipe was four inches above the soil level. Rice (Nato variety) was planted at a rate of 400 lb seed/acre. Treatment with formulated  $^{14}\text{C}$  ring-labeled benthio-carb at 5 lb/acre was carried out 1 week later. The formulated material was prepared by adding 1.161 g of  $^{14}\text{C}$  ring-labeled benthio-carb 117 mg Atloz emulsifying agents, 3409F/3404F (60/40) in ether. The ether was evaporated and 23 mg of xylene added to mixture. The formulated benthio-carb was diluted to 50 ml with water, and 25 ml of this solution was applied to the tank.

Flood water was added three weeks after

treatment when rice was four to six inches tall. The rice was grown under flooded conditions for 5 months after planting up to harvest.

## III. *Assay for the $^{14}\text{C}$*

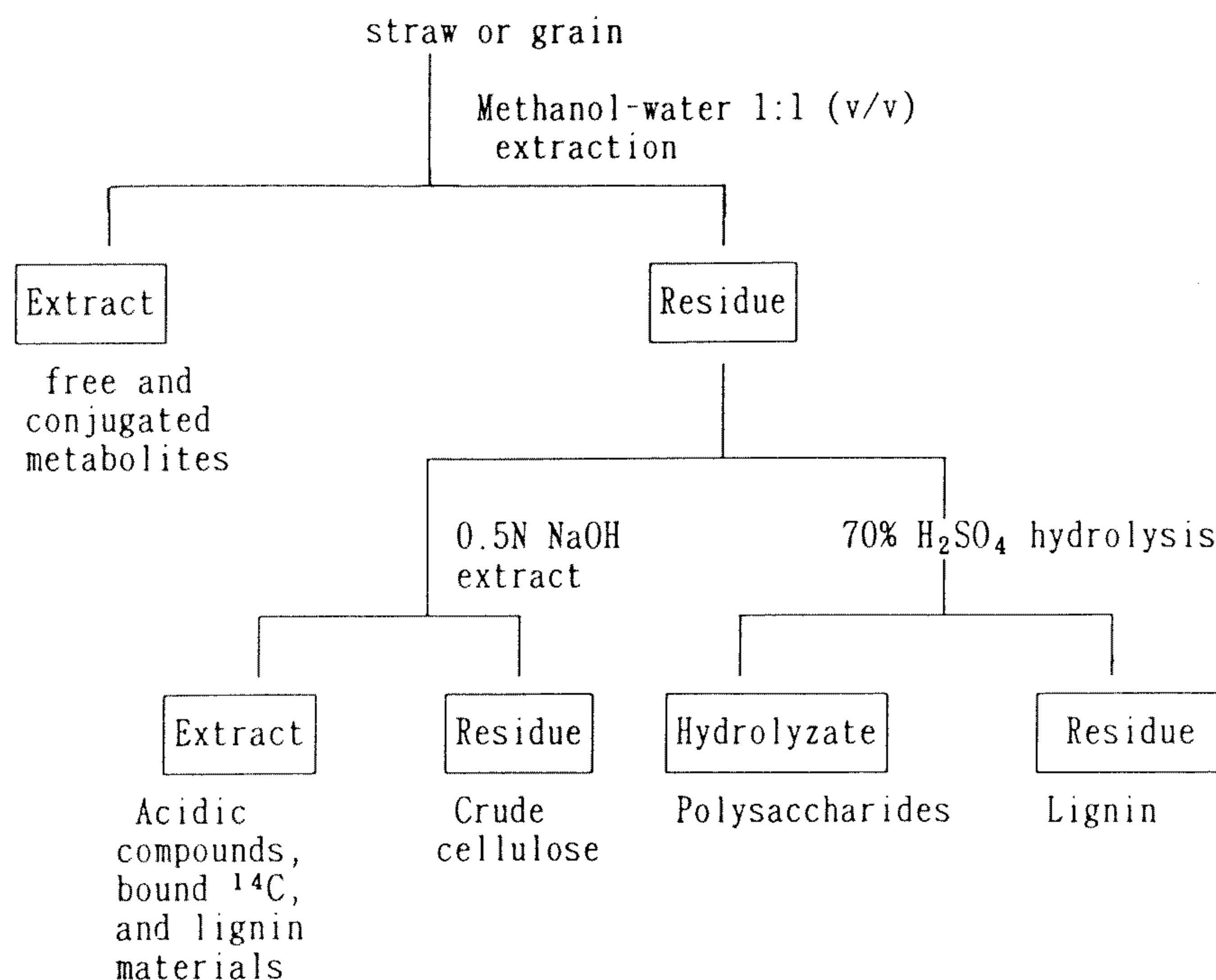
All extracts and regions scraped from thin-layer chromatoplates were counted in a Nuclear Chicago Isocap/300 liquid scintillation spectrometer. Aliquots (0.01-1 ml) of the various extracts or silica gel regions scraped from chromatoplate were dissolved in 10 ml of Scintisol (Isolab, Inc.) or 16 ml of a scintillation mixture containing 8 ml 2-methoxyethanol and 8 ml PPO (2,5-diphenyloxazole) in toluene (8 g/l). Solid samples ranging from 50 to 200 mg before and after extraction were combusted in duplicate with a Packard Model 306 sample oxidizer. The combusted samples were added to the scintillation mixture and counted in a liquid scintillation counter for determination of the  $^{14}\text{C}$ .

## IV. *Extraction of the $^{14}\text{C}$*

Samples of straw or grain weighing 25 to 50 g were extracted three times with 200 ml portions of methanol-water 1:1 (v/v). The extractions were carried out in a Virtis Homogenizer for 20 minutes. The extraction flask was placed in a water bath at about 50°C. The residue remaining after methanol-water extraction was further extracted with aqueous 0.5 N sodium hydroxide. The extraction procedure is shown in Figure 1. The lignin preparation was carried out according to the procedure described by Browning<sup>(12)</sup>.

## V. *Fractionation and Separation of the $^{14}\text{C}$*

The methanol was removed from the aqueous methanol extract with a rotary evaporator. The aqueous residue was acidified to pH 1 with 20% HCl, then saturated with  $(\text{NH}_4)_2\text{SO}_4$ . The  $^{14}\text{C}$  in this mixture was separated into five fractions; i.e. hexane, ether, ether-ethanol (2:1), aqueous residue and brown solid. Each of the



**Figure 1.** Extraction procedure for separation of  $^{14}\text{C}$  in straw and grain.

organo-extractable fractions was further separated into acid and neutral or phenolic fractions by extraction with 5%  $\text{KHCO}_3$ . The fractionation procedure is shown in Figure. 2.

#### VI. Isolation and Identification of the $^{14}\text{C}$

Thin-layer chromatography (TLC) utilized  $20 \times 20$  cm silica gel F-254 precoated chromatoplates (E.M. Laboratories, Inc., Elmsfor, N.Y.) with 0.25 mm or 0.5 mm gel thickness for analysis. The solvent systems used for isolation of the  $^{14}\text{C}$  degradation products and for co-chromatography with authentic compounds were as follows: (A) hexane only; (B) hexane-benzene (1:1); (C) chloroform-ether (4:1); (D) benzene-ether (1:1); (E) benzene (saturated with formic acid)-ether (3:1); (F) benzene-methanol-formic acid (100:20:1).  $R_f$  values for the authentic compounds using one dimensional development are given in Table 1. Various combinations and sequences of developing solvents were used for adequate resolution of the metabolite. Radioautography with single coated blue sensitive X-ray

film (Eastman Kodak Co., Rochester, N.Y.) was used to locate radioactive derivatives on TLC chromatoplate.

#### VII. Enzyme Hydrolysis

Samples were incubated with  $\beta$ -glucosidase (1000 units/mg, 50 mg, Calbiochem, San Diego, CA) in acetate buffer, pH 4.6, at  $34^\circ\text{C}$  overnight. The incubation mixture was acidified with HCl to pH 1 and extracted with ether. The ether extract was characterized by TLC analysis.

## RESULTS

#### Extraction Study

Various extraction mediums were tested, and showed that methanol-water 1:1 (v/v) was the most effective solvent for the extraction of the  $^{14}\text{C}$  in the straw and grain. It is sufficient for extraction of both free and conjugated metabolites. Approximately 50% (1 ppm) of the  $^{14}\text{C}$  in the straw and 10% (0.03 ppm) in the grain

were extracted with aqueous methanol.

#### *Nature of the $^{14}\text{C}$ in the Straw (2 ppm)*

About (50%) 1 ppm of the  $^{14}\text{C}$  was extracted with neutral aqueous methanol. About 0.7 ppm (35%) of the remaining  $^{14}\text{C}$  was further extracted with 0.5 N sodium hydroxide. About 0.2 ppm (10%) of the  $^{14}\text{C}$  was not extracted.

#### *Characterization of the $^{14}\text{C}$ in the Methanol-Water Extract from Straw (1.1 ppm)*

To facilitate the identification of the  $^{14}\text{C}$  by TLC, the  $^{14}\text{C}$  in the extract was separated into several fractions and characterized. The  $^{14}\text{C}$  distribution is given in Table 2. Those fractions were:

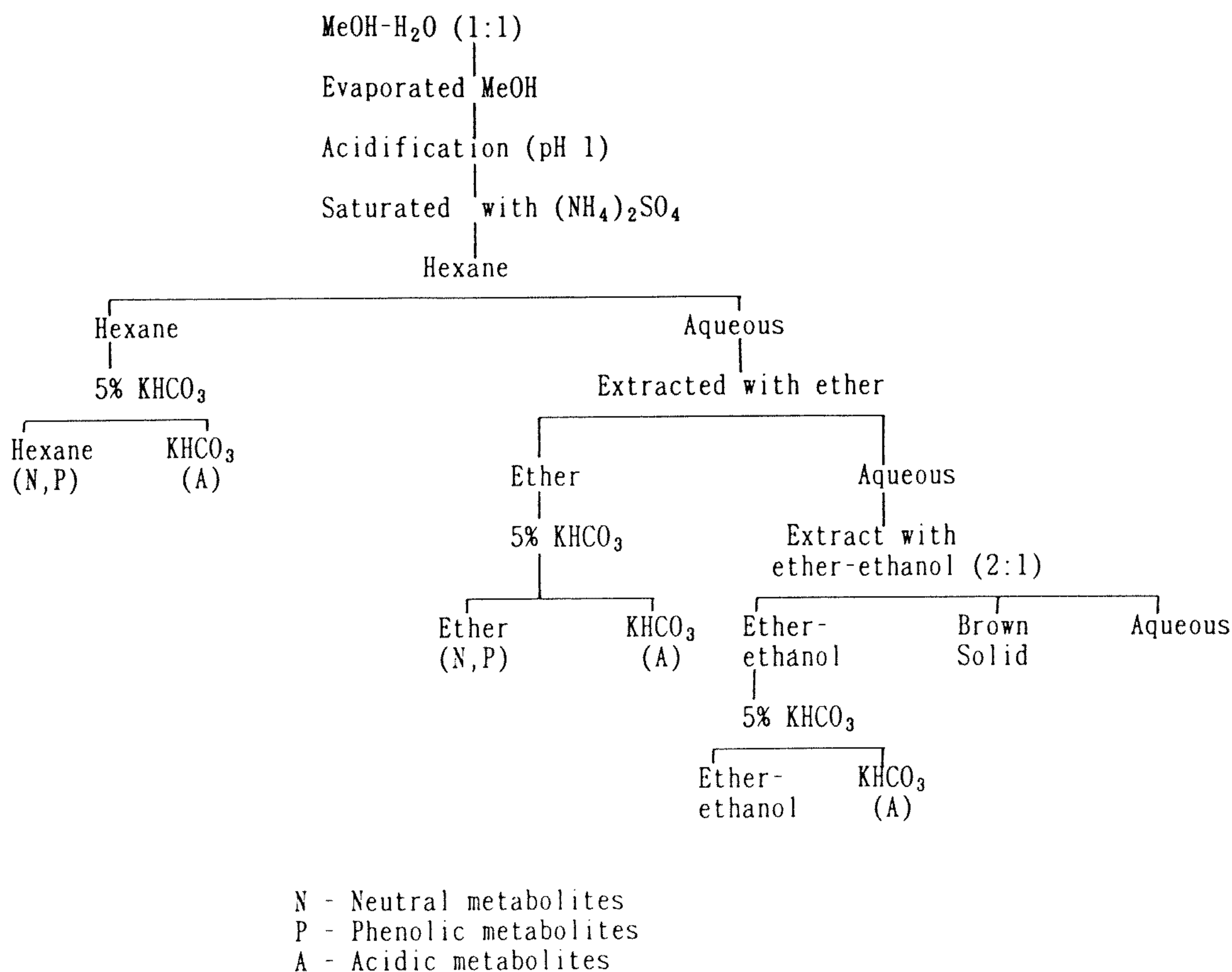
##### *1. Hexane Soluble $^{14}\text{C}$*

Over 95% (0.08 ppm) of this  $^{14}\text{C}$  were acidic compounds extractable with 5% potassium bicarbonate solution. Most (0.08 ppm) of the  $^{14}\text{C}$  was identified as 4-chlorobenzoic acid

**Table 1.** Thin-layer chromatography Rf values for standard compounds

Compound	Rf values in indicated			TLC solvent system	D	E	F
	A	B	C				
4-chlorobenzoic acid	0.00	0.00	0.12	0.50	0.49	0.38	
2-hydroxy-4-chlorobenzoic acid	0.00	0.00	0.05	0.13	0.52	0.24	
3-hydroxy-4-chlorobenzoic acid	0.00	0.00	0.05	0.24	0.32	0.29	
N-4-chlorobenzoylglycine	0.00	0.00	0.00	0.00	0.12	0.22	
N-4-chlorobenzoylleucine	0.00	0.00	0.05	0.13	0.25	0.30	
N,N-diethyl-S-(4-chlorobenzyl) thiocarbamate	0.00	0.12	0.60	0.91	0.60	0.65	
N-ethyl-S-(4-chlorobenzyl) thiocarbamate	0.00	0.07	0.53	0.88	0.55	0.57	
S-(4-chlorobenzyl)thiocarbamate	0.00	0.01	0.30	0.71	0.40	0.40	
4-chlorobenzyl alcohol	0.00	0.04	0.30	0.63	0.35	0.40	
2-hydroxy 4-chlorobenzyl alcohol	0.00	0.00	0.23	0.60	0.30	0.35	
4-chlorobenzyl mercaptan	0.22	0.45	0.65	0.94	0.67	0.67	
bis-4-chlorobenzyl disulfide	0.17	0.50	0.65	0.94	0.67	0.67	
4-chlorobenzyl methyl sulfide	0.19	0.42	-	-	-	-	
N,N-diethyl-S-(4-chloro-2-hydroxybenzyl)thiocarbamate	-	0.08	0.63	-	0.60	0.64	
N-ethyl-S-(4-chloro-2-hydroxybenzyl)thiocarbamate	-	0.00	0.50	-	0.47	0.47	
N,N-diethyl-S-(4-chloro-3-hydroxybenzyl)thiocarbamate	-	0.00	0.40	-	0.43	0.52	
N-ethyl-S-(4-chloro-3-hydroxybenzyl)thiocarbamate	-	0.00	0.35	-	0.34	0.38	
N,N-diethyl-S-(4-chlorobenzyl)-S-monoxothiobaminate	-	0.00	0.28	-	0.13	0.47	
4-chlorobenzyl methyl sulfoxide	-	0.00	0.09	-	0.09	0.37	
4-chlorobenzyl methyl sulfone	-	0.00	0.33	-	0.23	0.52	

A: hexane only; B: hexane-benzene (1:1); C: chloroform-ether (4:1); D: benzene-ether (1:1); E: benzene saturated with formic acid-ether (3:1), F: benzene-methanol-formic acid (100:20:1)



**Figure 2.** Scheme for fractionation and separation of  $^{14}\text{C}$  from extracts.

and 3% ( $<0.01$  ppm) as 2-hydroxy-4-chlorobenzoic acid. Approximately 5% ( $<0.01$  ppm) of the  $^{14}\text{C}$  in the hexane extract was neutral compound. TLC analysis showed one major spot which corresponded to bis-(4-chlorobenzyl) monodisulfide. This degradation product was probably derived from 4-chlorobenzyl mercaptan by oxidation during the separation process.

## 2. Ether Soluble $^{14}\text{C}$

About 95% (0.38 ppm) of the  $^{14}\text{C}$  in the ether soluble fraction was extractable with 5% potassium bicarbonate solution indicating that most of the  $^{14}\text{C}$  in the ether fraction was also in nature similar to carboxylic acid derivatives. About 30% (0.12 ppm) of the  $^{14}\text{C}$  was isolated and identified as 4-chlorobenzoic acid, and 3% (0.01 ppm) as 2-hydroxy 4-chlorobenzoic acid. There

were two major acidic unknown metabolites designated as "Unknown A" (43%, 0.17 ppm) and "Unknown B" (10%, 0.04 ppm). Unknown A and Unknown B were more polar than 4-chlorobenzoic acid. The  $R_f$  values of Unknown A and Unknown B in the benzene (saturated with formic acid)-ether 3:1 (v/v) solvent system were 0.08 and 0.15, respectively, while the  $R_f$  value of 4-chlorobenzoic acid was 0.48.

Both Unknown A and Unknown B could be easily methylated with diazomethane in ether solution at  $0^\circ\text{C}$  to each form a less polar product. The methylated Unknown A had an  $R_f$  value of 0.2 and the methylated Unknown B had an  $R_f$  value of 0.46 when developed with chloroform-ether 4:1 (v/v). In this solvent system, both Unknown A and Unknown B (before treatment with diazomethane) stayed at the origin. Unknown A was stable in both 1 N hydro-

chloric acid and 1 N aqueous ethanolic potassium hydroxide solutions at room temperature overnight. However, when Unknown A was heated with 1 N potassium hydroxide in aqueous ethanol solution at 80°C for three hours, it degraded into many radioactive materials which were less polar than the parent compound. The chromatogram was developed with chloroform-ether 4:1 (v/v) for 5 cm followed by hexane-benzene 1:1 (v/v) for 10 cm. The degradation products identified were 20% bis (4-chlorobenzyl) disulfide plus 4-chlorobenzyl mercaptan ( $R_f$  0.7), 12% bis (4-chlorobenzyl) dioxysulfide ( $R_f$  0.45), 50% bis (4-chlorobenzyl)-monoxysulfide ( $R_f$  0.35), and about 20% stayed at the origin. Mass spectral analysis by electron impact ionization showed no parent (molecular ion) peak in either methylated Unknown A or B, but the base peak of the fragmentation ion at mass 125 (m/e) of the 4-chlorobenzyl fragment ion was present. These experimental facts indicate that both unknown metabolites contain 4-chlorobenzylthio and carboxylic acid groups in their molecules. Therefore, it is reasonable to propose that the two metabolites are probably fatty acid derivatives containing the 4-chlorobenzylmercapto group in their molecules.

### 3. Ether-Ethanol Soluble $^{14}\text{C}$

Metabolites in this fraction were mostly conjugated. The  $^{14}\text{C}$  was separated into two fractions with dilute potassium bicarbonate so-

lution. Approximately 80% (0.12 ppm) of the  $^{14}\text{C}$  was extracted with 5%  $\text{KHCO}_3$  solution, indicating that most of the  $^{14}\text{C}$  in the ether-ethanol fraction was also acidic in nature. Based on chemical analysis (methylation and hydrolysis) and the  $R_f$  values, two major metabolites in this acidic fraction correspond to Unknowns A and B in ether soluble fraction.

The bicarbonate insoluble  $^{14}\text{C}$  (20%) was neutral ether-ethanol soluble  $^{14}\text{C}$ . Studies with acid and  $\alpha$ -glucosidase enzyme hydrolysis indicated that about 90% of this  $^{14}\text{C}$  was hydrolyzable. The radioactive products released from this conjugated fraction after enzyme or acid hydrolysis were ether soluble. 4-Chlorobenzoic acid was identified as a major, and 4-chlorobenzyl disulfide was identified as a minor product.

### 4. Water Soluble $^{14}\text{C}$

A small amount of  $^{14}\text{C}$  (4%) remained in the aqueous residue. It was composed of very polar materials, and no further investigation was done.

### 5. Brown Solid Material $^{14}\text{C}$

The brown solid material isolated from the aqueous methanol extract of the straw had high radioactivity. It was obtained as precipitates by saturation of aqueous methanol extract with ammonium sulfate. The concentration of the  $^{14}\text{C}$  in this solid was 17 ppm (554 dpm/mg), about eight times higher than the  $^{14}\text{C}$  concentration in

**Table 2.** Nature of  $^{14}\text{C}$  in the aqueous methanol extract from straw

Fraction	% of $^{14}\text{C}$ in extract	% of $^{14}\text{C}$ in straw	ppm of $^{14}\text{C}$ calculated as benthocarb
Hexane soluble	7.1	4.4	0.088
Ether soluble	36.1	20.2	0.404
Ether-ethanol soluble	13.2	7.4	0.148
Brown solid material	35.9	20.1	0.402
Aqueous residue	7.1	4.0	0.080
Total	99.4	56.1	1.122

the original straw. This solid material was not soluble in benzene, ether, acetone, dioxane or dimethyl sulfoxide, but it was sparingly soluble in hot alcohol. Elemental and spectral analyses were carried out. Infrared spectra indicated that it contained (A)  $\text{SiO}_2\text{H}_2\text{O}$  (3100-3650, 1100 and  $800\text{ cm}^{-1}$ ); (B) alkyl (2920 and  $2860\text{ cm}^{-1}$ ); (C) aromatic and unsaturated ( $1640$ ,  $1540$ ,  $1460$  and  $1400\text{ cm}^{-1}$ ). Elemental analysis showed that this solid was composed of 35.6% of carbon, 5.4% of hydrogen and 4% of nitrogen. Spectrochemical analysis showed that the solid contained 11.3% of silicon. The analytical results indicated that the  $^{14}\text{C}$  in the brown solid appeared to be natural constituents mainly composed of lignin and silicates derivatives.

*Characterization of Alkaline Extractable  $^{14}\text{C}$  from the Straw (0.70 ppm) after the Use of Aqueous Methanol*

The aqueous alkaline extracts were acidified and fractionated according to the procedure used in the separation of the  $^{14}\text{C}$  in the methanol-water extracts (Figure 2). The fractionation data (Table 3) indicate that approximately 18% (0.13 ppm) of the  $^{14}\text{C}$  in the extract was hexane soluble; about 14% (0.10 ppm) was extractable with ether; about 17% (0.12 ppm) was extractable with ether-ethanol; and less than 1% ( $< 0.01\text{ ppm}$ ) remained in aqueous residue. Approximately 50% (0.35 ppm) of the  $^{14}\text{C}$  in the straw isolated as a brown solid material was a lignin derivative. TLC analysis of the hexane and ether

soluble fractions indicated that about 90% (0.11 ppm) of the  $^{14}\text{C}$  in hexane extract, and about 70% (0.07 ppm) of the  $^{14}\text{C}$  in the ether extract, were isolated as 4-chlorobenzoic acid.

*Characterization of  $^{14}\text{C}$  in the Final Residue (0.18 ppm) of Straw after Successive Extractions with Aqueous Methanol and Alkaline Solutions*

Less than 10% of the  $^{14}\text{C}$  (0.18 ppm) remained in the residue after successive extraction with aqueous methanol and alkaline solution. Most of this  $^{14}\text{C}$  could be removed by boiling with 20% sodium bisulfite solution followed by 20% potassium hydroxide solution. The cellulose isolated by this pulping procedure contained about 1% (0.02 ppm) of the total  $^{14}\text{C}$  in the straw, indicating that the majority of the  $^{14}\text{C}$  in the residue was incorporated into the lignin instead of the cellulose fraction.

*Nature of  $^{14}\text{C}$  in the Rice Grain (0.3 ppm)*

The majority of the  $^{14}\text{C}$  in the grain could not be extracted with organic solvents. Only 3% of the  $^{14}\text{C}$  in the grain was extracted with benzene, 4% with acetone, 8% with methanol and 10 to 12% with aqueous methanol. The nature of the  $^{14}\text{C}$  in the aqueous methanol extract is shown in Table 4. Separation and identification by TLC showed that the  $^{14}\text{C}$  in the hexane and the ether fractions was the same product. It was identified as 4-chlorobenzyl methylsulfone. Therefore, over 60% (0.02 ppm) of the  $^{14}\text{C}$  in

**Table 3.** Nature of  $^{14}\text{C}$  in the alkaline extract from straw

Fraction	% of $^{14}\text{C}$ in extract	% of $^{14}\text{C}$ in straw	ppm of $^{14}\text{C}$ calculated as benthocarb
Hexane soluble	18.3	6.4	0.128
Ether soluble	14.3	5.0	0.100
Ether-ethanol soluble	16.6	5.8	0.116
Brown solid material	50.3	17.6	0.354
Aqueous residue	0.6	0.2	0.004
Total	100.0	35.0	0.702

the aqueous methanol extract was isolated as 4-chlorobenzyl methyl sulfone. No 4-chlorobenzoic acid was found from grain samples.

Approximately 90% of the  $^{14}\text{C}$  remained in the residue after aqueous methanol extraction. This would be considered as incorporated  $^{14}\text{C}$ . To determine the nature of this  $^{14}\text{C}$ , the grain residue was subjected to drastic alkaline or acid hydrolysis (six hours reflux in 10% KOH or 10% HCl). In the alkaline hydrolysis, the hydrolysate was acidified and extracted with ether-ethanol 1:1 (v/v). About 40% (0.13 ppm) of the  $^{14}\text{C}$  was organo-extractable and about 50% (0.14 ppm) was organo-unextractable. Only 30% (0.04 ppm) of this organo soluble  $^{14}\text{C}$  was acidic derivatives (potassium bicarbonate extractable), and 70% (0.09 ppm) were neutral or phenolic products (potassium bicarbonate unextractable). In the acid hydrolysis, 40% (0.12 ppm) of the  $^{14}\text{C}$  was organo-extractable and 50% (0.15 ppm) was organo unextractable. In the organo extractable  $^{14}\text{C}$  from the acid hydrolysate, about 20% (0.03 ppm) of the organo soluble  $^{14}\text{C}$  was extracted with dilute potassium bicarbonate and 80% (0.1 ppm) was not. Therefore, approximately 0.03 ppm  $^{14}\text{C}$  acidic materials and 0.1 ppm  $^{14}\text{C}$  were neutral or phenolic materials.

## DISCUSSION

Degradation fate and terminal residues of  $^{14}\text{C}$  ring-labeled benthioncarb in mature rice plant are discussed. Samples of mature straw

and grain were used for analysis and characterization. In the straw, the  $^{14}\text{C}$  levels calculated as benthioncarb were summarized as follows: free 4-chlorobenzoic acid 0.22 ppm; carboxylic acidic compounds containing the 4-chlorobenzylthio moiety 0.33 ppm; bound  $^{14}\text{C}$  released as 4-chlorobenzoic acid after treatment with alkali 0.23 ppm; glucose conjugate of 4-chlorobenzoic acid 0.03 ppm; lignin derivative 1.03 ppm; cellulose fraction 0.02 ppm; very polar materials which remained in aqueous residue 0.08 ppm; 2-hydroxy-4-chlorobenzoic acid 0.02 ppm and glucose conjugate of 4-chlorobenzyl mercaptan < 0.01 ppm. The  $^{14}\text{C}$  levels calculated as benthioncarb in the grain were determined to be 4-chlorobenzyl methyl sulfone 0.02 ppm; and bound  $^{14}\text{C}$  (mostly incorporated into lignin derivative) 0.28 ppm. This finding made it possible to suggest tentative metabolic pathways for this herbicide (Figure 3). Benthioncarb probably undergoes hydrolysis of the thiocarbamate ester by hydrolase or esterase to form 4-chlorobenzyl mercaptan as an intermediate. This intermediate is further oxidized by mixed function oxidases and/or dehydrogenase to form 4-chlorobenzoic acid, which is further transformed into a glucose conjugate or incorporated into lignin. The 4-chlorobenzyl group could also be conjugated with amino acids. The glycine conjugate of 4-chlorobenzoic acid which was found as major metabolite in mice and rats was not found in rice<sup>(13)</sup>.

In the grain, most of the  $^{14}\text{C}$  appeared to be incorporated materials because about 90%

**Table 4.** Nature of  $^{14}\text{C}$  in aqueous methanol extract from grain

Fraction	% of $^{14}\text{C}$ in extract	% of $^{14}\text{C}$ in straw	ppm of $^{14}\text{C}$ calculated as benthioncarb
Hexane soluble	45.7	4.6	0.014
Ether soluble	25.1	2.5	0.008
Ether-ethanol soluble	9.1	0.9	0.003
Brown solid material	15.4	1.5	0.005
Aqueous residue	4.7	0.5	0.002
Total	100.0	10.0	0.032

was unextractable with aqueous methanol. In the aqueous methanol extract, almost all of the  $^{14}\text{C}$  was identified as 4-chlorobenzyl methyl sulfone indicating that biological methylation and oxidation of 4-chlorobenzyl mercaptan occurred in rice grain. 4-chlorobenzyl methyl sulfone has also been found as a major metabolite in rat and mouse tissues<sup>(13)</sup>.

The uptake, translocation and metabolism of benthicarb in rice seedlings have been studied. Benthicarb was translocated and possibly degraded easily in the rice seedling. After eight days treatment, only 1% of the parent compound was recovered, and only small amounts of ring hydroxylation of benthicarb were found. However, in this study no parent compound or other thiocarbamate metabolites were found.

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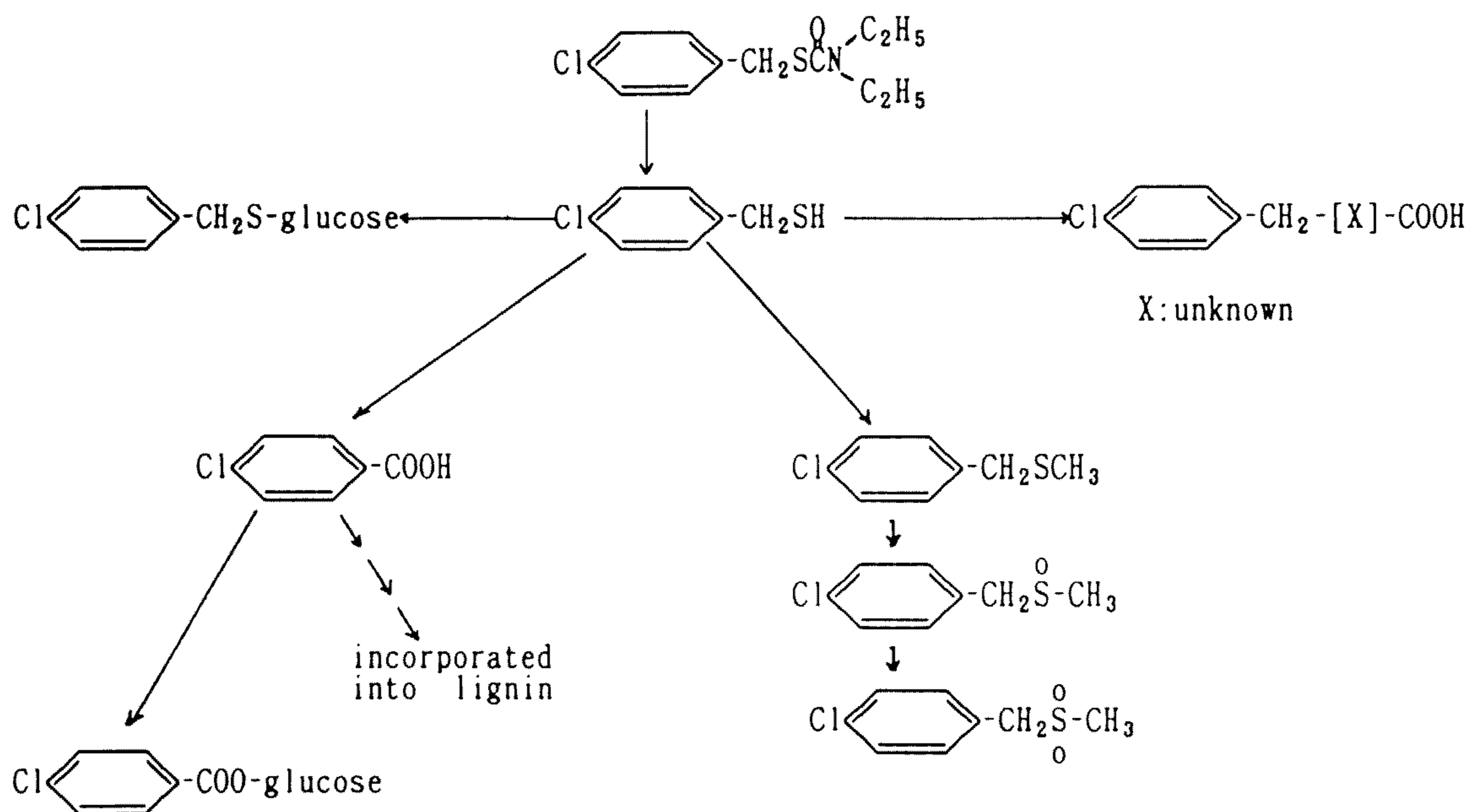


Figure 3. Proposed metabolic pathway of benthicarb in rice plants.

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## 除草劑農藥殺丹(Benthioncarb)在水稻植物中 最終產物之分離鑑定

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### 摘 要

除草劑農藥benthioncarb是植物萌前之選擇性農藥,近二十年來被廣泛使用於水稻田以除雜草,為知benthioncarb在水稻中之命運,乃以5磅/英畝之量撒佈 $^{14}\text{C}$ -benthioncarb於水槽缸,缸內種植水稻成熟收割後,採集稻草和稻米來分析 $^{14}\text{C}$ 量,得知稻草和稻米之 $^{14}\text{C}$ 全量(以benthioncarb計算)各為2.0和3.0 ppm。其中均未檢出含有母體benthioncarb和具有thiocarbamate構造體之代謝產物(<

0.01 ppm)存在。稻草中之主要benthioncarb產物為4-chlorobenzoic acid之自由型和結合型的代謝產物(0.25 ppm),其次為含有carboxylic acid和4-chlorobenzylthio構造體之代謝產物(0.33 ppm)。Benthioncarb在稻米中之主要產物為木質素(lignin)結合物(0.28 ppm)和4-chlorobenzyl methyl sulfone (0.02 ppm)。