It is thought, however, to be related to the fact that northeast Louisiana is an extensively agrarian community, therefore providing more exposure for its residents to pesticides than urban communities and the nation as a whole. Future studies should show the same dropoff in levels as the nation shows, which would not explain the higher levels, but would show only that a higher level existed in this area.

Dieldrin, in this study, did not show the same higher trend when compared to national values as did DDT and heptachlor epoxide. This conflict in data presents some problems for proposing base lines for other residues. However, in examining national data for dieldrin there appears to be a definite trend of consistent levels over a period of years rather than a drop. The close correlation of Louisiana values to national values (Figure 1) might indicate that the base line level for dieldrin has been reached in northeast Louisiana and as with the nation as a whole this level will probably remain consistent. On the other hand, dieldrin may not have been used as extensively in this area as was DDT and heptachlor epoxide. Also, national data was only available through 1974 for comparison and later data comparison for 1976–1978 might prove more useful. In any event, other pesticide residues will have to be monitored before definite base line correlations for northeast Louisiana can be established. The establishment of base line pesticide levels in an agricultural environment will be very useful to agencies involved in monitoring pesticides.

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Derivatization of Several Carbamate Pesticides with Methanesulfonyl Chloride and Detection by Gas–Liquid Chromatography with the Flame Photometric Detector: Application to Residues of Carbaryl on Lentil Straw

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The mesylate derivatives of the carbamate pesticides propoxur, MCA-600 (benzo[*b*]thien-4-yl methylcarbamate), Landrin, Carbaryl, carbofuran, 3-hydroxycarbofuran, 3-oxocarbofuran, methiocarb, methiocarb sulfoxide, and methiocarb sulfone were prepared and found to be suitable for the gas chromatographic detection of these pesticides. The carbamate pesticide is hydrolyzed with methanolic KOH, and the resultant phenol reacted with methanesulfonyl chloride and pyridine to form the mesylate derivative, which is determined by a gas chromatograph equipped with a flame photometric detector in the sulfur mode. This method is highly specific and free from interference of extracted crop materials. This procedure was applied to the determination of carbaryl residues in lentil straw. Recoveries of carbaryl from lentil straw samples fortified with the pure pesticide at the rate of 0.1 ppm averaged 103.0% (range 91.0–118.0%).

The determination of carbamate pesticide residues in crops is often difficult. Because direct analysis of carbamates by gas–liquid chromatography (GLC) is difficult (since most carbamate pesticides degrade on the GLC column), the most common approach is to hydrolyze the carbamate ester linkage and derivatize the resultant phenol with a chloro, fluoro, or bromo compound to yield a product that can be readily detected by a GLC equipped with an electron-capture detector (ECD). Many of these procedures are discussed in reviews by Williams (1971) and by Dorough and Thorstenson (1975).

In general, the methods are quite sensitive, but problems may arise because of the lack of specificity of the ECD. Thus, complicated cleanup procedures of the crop extract may be necessary to remove plant materials that inhibit the derivatization of the carbamate or to remove plant materials that produce GLC peaks with the same retention times as the carbamate derivative being analyzed. In 1966, Brody and Chaney introduced the flame photometric detector (FPD), which is specific for compounds containing only phosphorus or sulfur, so many problems caused by inefficient cleanup of unwanted plant extractives were eliminated. Subsequently, Bowman and Beroza (1967) took advantage of the specificity of the FPD in the phosphorus mode and developed a method based on the thiophosphoryl derivative of several carbamate pesticides. The disadvantage was that the GLC analysis of this derivative had to be completed the same day it was prepared or an interfering compound formed that masked the derivative being determined. Later, Moye (1975) developed a procedure based on the derivatization of carbamate

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pesticides with halogenated benzenesulfonyl chlorides, followed by analysis by GLC with either the ECD or the FPD. However, the procedure based on the FPD was not demonstrated on crops. Mathur et al. (1978) also used the FPD in the sulfur mode to determine residues of carbaryl after direct derivatization of the intact carbamate with methanesulfonyl chloride. They applied the procedure to several crops that had been fortified with 5–10 ppm of carbaryl. The authors stated that their method was not applicable to the determination of carbaryl residues below 5 ppm and that the overall length of the method was excessive for routine sample analysis.

In a study to determine the anticancer qualities of some methanesulfonate derivatives of phenols, Kametani et al. (1964) described a macro procedure for preparing these compounds by derivatizing the phenol with methanesulfonyl chloride in the presence of pyridine. Their procedure is the basis for the present work. In this procedure the carbamate pesticide is hydrolyzed with methanolic potassium hydroxide, and the resultant phenol is derivatized with methanesulfonyl chloride and pyridine to form the mesylate derivative which is then analyzed by the GLC with the FPD in the sulfur mode. Described here is the preparation of the mesylate derivatives of several carbamate pesticides and the results when the procedure was applied to the determination of carbaryl residues in lentil straw. These results were compared with the results obtained by the method of Butler and McDonough (1968) which is based on derivatization with trichloroacetyl chloride and determination of the halogenated carbamate by GLC with the ECD.

This procedure was applied to the carbamate pesticides of propoxur (*o*-isopropoxyphenyl methylcarbamate), Mobam (benzo[*b*]thien-4-yl methylcarbamate), Landrin (3,4,5-trimethylphenyl methylcarbamate, 75%; 2,3,5-trimethylphenyl methylcarbamate, 18%), carbaryl (1-naphthyl methylcarbamate), and carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) as well as its metabolites consisting of 3-hydroxycarbofuran, 3-oxocarbofuran, and ethoxylated 3-hydroxycarbofuran and to methiocarb (4-(methylthio) 3,5-xylyl methylcarbamate and the sulfoxide and sulfone metabolites of methiocarb.

MATERIALS AND METHODS

Chemicals and Equipment. All solvents (dichlormethane, hexane, benzene, and methyl alcohol) were technical grade redistilled in glass. Other chemicals used were pyridine (Bakers analyzed reagent grade that was refluxed over potassium hydroxide for 1 h and then stored in a dark bottle over potassium hydroxide and magnesium sulfate), methanesulfonyl chloride (Eastman organic chemicals), potassium hydroxide (Bakers analyzed reagent grade), phosphoric acid (Allied Chemical reagent grade), cotton (washed with dichloromethane and oven dried at 110 °C), anhydrous sodium sulfate (Bakers analyzed reagent grade), Florisil A, 60/100 mesh (The Floridin Co., Tallahassee, FL), and Hyflo Supercel.

Standard solutions of propoxur, Mobam, Landrin, Carbaryl, carbofuran and its metabolites, and methiocarb and its metabolites were prepared by dissolving 0.1000 g of the pure compounds in 500 mL of dichloromethane.

The gas chromatograph was a Hewlett Packard Model 5840A equipped with a flame photometric detector fitted with a 394-mμm filter for the determination of sulfur compounds. The GLC column was 122 cm by 0.40 cm i.d., glass packed with 3% OV-101 on Gas-Chrom Q (100/120 mesh), and operated at a temperature of 190 or 210 °C with a nitrogen flow rate of 60 mL/min.

A water bath maintained at a temperature of 40–45 °C and a gentle stream of dry air (filtered through Drierite) was used for all evaporation steps of this procedure.

Macropreparation of the Mesylate Derivative of Carbaryl. Carbaryl was derivatized to its mesylate form on a macroscale and used as a standard to judge the efficiency of the micro procedure as follows: Five grams of carbaryl was placed in a 250-mL flask and hydrolyzed to its phenolic form by shaking on a wrist-action shaker for 30 min with 50 mL of 0.25 M methanolic potassium hydroxide. After shaking, 100 mL of water was added, and the solution was acidified with phosphoric acid and then transferred to a separatory funnel. The solution in the separatory funnel was extracted three times with 50 mL each of dichloromethane, and the three extracts were combined by filtering into a 250-mL flask through a funnel plugged with cotton and overlaid with sodium sulfate. The dichloromethane solution now containing 1-naphthol was evaporated to about 50 mL, and the mesylate derivative was prepared by adding 5 mL each of pyridine and methanesulfonyl chloride. The solution was allowed to stand at room temperature for 1 h and then transferred to a separatory funnel with 50 mL of dichloromethane and extracted three times with 50 mL of 0.1 N hydrochloric acid to remove any excess pyridine. Next the dichloromethane solution was then filtered through sodium sulfate and evaporated, and the resulting reddish-brown oil was dissolved in 25 mL of dichloromethane. Ten microliters of the solution was put on an Eastman chromatogram sheet (alumina 6062) and developed in a solvent system of acetone. The visible brown spot at R_f 0 and the spots at R_f 1.0 and 7.1 (visible under UV light) were removed from the chromatogram sheet and analyzed by GLC using the previously described parameters and the FPD in the sulfur mode. The only spot that produced a response was the one at R_f 1.0. Five milliliters of the original solution was then transferred onto a 1.5 by 7.5 cm liquid chromatography column of Alumina (Bakers 0536) and eluted with hexane. Several 25-mL fractions were collected and analyzed by GLC. It was found that fractions 2–5 contained the compound with the same retention time as the compound from the R_f 1.0 spot of the thin-layer chromatography. The four fractions were combined and evaporated to about 10 mL and then cooled in a refrigerator, resulting in the formation of needle-like white crystals. These crystals were then recrystallized in 10 mL of hot hexane and then dried. The crystals were then dissolved in 5 mL of dichloromethane, and 10 μL of this solution was subjected to thin-layer chromatography as above. The chromatogram showed only one spot which was at R_f 1.0. GLC analysis of this compound produced the same results as the compound at R_f 1.0 of original thin-layer chromatogram and the same as the compound found in fraction 2–5 of the liquid chromatography. The remaining dichloromethane solution was then evaporated and the white residue weighed and dissolved in dichloromethane so that 1 mL contained 200 μg of the 1-naphthol mesylate. This standard was then used to determine the efficiency of the microprocedure.

Microprocedure for the Mesylate Derivatization of Carbamate Pesticides. Each pesticide (100 μg) and its metabolite (100 μg) were placed in separate 125-mL Erlenmeyer flasks, and 1 mL of keeper solution (1 g of mineral oil in 100 mL of dichloromethane) was added. The solution was placed in the water bath and evaporated to dryness. Then 1 mL of 0.25 M methanolic potassium hydroxide was added, and the solution was allowed to stand at room temperature for 10 min. Next 2 mL of the

pyridine reagent (5 mL of pyridine dissolved in 100 mL of benzene) was added, and the solution was placed in the water bath and evaporated to dryness. Then 2 mL of the methanesulfonyl chloride reagent (0.5 mL of methanesulfonyl chloride dissolved in 50 mL of benzene) was added, and the flask was stoppered and allowed to stand at room temperature for 30 min. The solutions were returned to the water bath, evaporated to dryness, and then 25 mL of a solvent mixture of 25% dichloromethane and 75% hexane was added. The residue on the bottom of the flask was partially loosened with a glass stirring rod, and the solution was filtered through a folded filter paper. The sample flask was rinsed three times with 5 mL each of the 25–75% solvent mixture and the solution was poured through the filter. After these solutions passed through the filter, the filter paper was rinsed three times with 10 mL each of the 25–75% solvent mixture and each rinse was allowed to pass through the filter before the next was added. The solutions were placed in the water bath, evaporated to dryness, and made to a volume of 20 mL with dichloromethane (1 mL is equivalent to 5 μg of the carbamate mesylate). The solutions were stored in a refrigerator until needed.

Analysis of Dry Lentil Straw for Carbaryl Residues by the Mesylate Procedure. Harvest samples of dry lentil straw from plots treated by aerial applications of carbaryl at rates of 1.25 and 2.50 lb of active ingredient per acre were finely chopped in a Buffalo chopper and then stored in a freezer until analysis. Subsamples of 50 g of each sample were placed in 2-L glass containers; 500 mL of dichloromethane was added, and the solutions were allowed to stand overnight in a refrigerator. The next day the samples were tumbled in an end-over-end manner for 1 h. After tumbling, the solutions were allowed to settle and then decanted and filtered through paper into a separatory funnel containing 20 g of sodium sulfate. The solutions were shaken, filtered through paper into a glass bottle, and stored in a refrigerator until all extractions were completed.

A portion of the extract equivalent to 25 g (250 mL) was placed in a conical beaker, evaporated to about 10 mL, and then transferred to a 125-mL Erlenmeyer flask with 4–5-mL rinses of dichloromethane. Then 1 g of Hyflo Supercel was added. The solution was returned to the water bath, and the dichloromethane was evaporated. The crumbly mass on the bottom of the flask was loosened with a glass stirring rod, 4 mL of methyl alcohol was added, and the solution was warmed in the water bath. Then 20 mL of coagulating solution (1.5 g of ammonium chloride and 2 mL of phosphoric acid in 1 L of water) was added and the solution was allowed to stand in a refrigerator for at least 30 min. The cold solutions were removed from the refrigerator one at a time and filtered (into a 250-mL separatory funnel) through a funnel plugged with cotton. The stirring rod from the sample flask was used to hold the cotton in the filtering funnel in place for a few seconds during the initial filtering. This allowed the Hyflo Supercel to settle in the funnel, thus producing a clear filtrate. The sample flask was rinsed three times with 10-mL portions of a mixture of 10% methyl alcohol and 90% water (v/v), and these rinses were added to the filtering funnel. After the solution had passed through the funnel, the funnel and its contents were rinsed three times with 10-mL portions of the 10–90% methyl alcohol–water solution. The solutions in the separatory funnels were then extracted three times with 25 mL each of dichloromethane. The three extracts were combined and filtered through a funnel plugged with cotton and overlaid with sodium sulfate into a 125-mL Erlenmyer flask. After the dichloromethane solution had passed through the filtering funnel, the funnel and its contents were rinsed three times with 10 mL of dichloromethane; the solutions were evaporated; and the resultant residue was derivatized to the mesylate compound by the procedure previously described, beginning with the addition of 1 mL of the 0.25 M methanolic potassium hydroxide solution. The resultant derivative was then dissolved in 1 mL of dichloromethane for GLC analysis with the FPD.

The coagulation cleanup was efficient enough for the determination of carbaryl residues in straw. This type of cleanup may not be sufficient for determination of these residues in other crops. Then there would have to be an additional liquid chromatographic cleanup before GLC analysis. If additional cleanup were necessary, the carbaryl mesylate can be chromatographed through a Florisil column. After derivatization, the mesylate compound was dissolved in 10 mL of a solvent mixture of 25% dichloromethane and 75% hexane and then transferred onto a 15.0 by 2.0 cm column of 12 g of Florisil A with 25 mL of the 25–75% solvent mixture. As this solution reached dryness at the top of the column, an additional 40 mL of the 25–75% solvent mixture was added. As this solution reached dryness at the top of the column, the collection flask was changed, and the carbaryl mesylate was eluted from the column with 140 mL of the 25–75% solvent mixture. Recoveries of carbaryl mesylate from the column ranged from 90 to 103%.

Analysis of Dry Lentil Straw for Carbaryl Residues by the Trichloroacetyl Chloride Procedure of Butler and McDonough (1968). Samples of straw were extracted and the extracts were cleaned up by coagulation as previously described. After coagulation, the dichloromethane solutions were evaporated to about 5 mL and then transferred to test tubes and evaporated to dryness. The residue in the test tubes was trichloroacetylated by the method of Butler and McDonough (1968), and the residues were determined by GLC with an EC detector. The GLC column was a 182.0 by 0.40 cm (i.d. glass column packed with 6% DC 200 on Gas-Chrom Q (80/100 mesh) operated at an oven temperature of 200 °C and a nitrogen flow rate of 60 mL/min.

DISCUSSION

Purified carbaryl mesylate prepared on a macroscale was used as a standard to determine the efficiency of the microprocedure. When the conditions were established for 100% reaction, the same conditions were used for the preparation of mesylate derivatives of the other carbamate pesticides in this work. Derivatives of each pesticide were also prepared several times at levels ranging from 5 to 100 μg and compared. Peak areas (determined by integrater) were within ±10% for each preparation. When 0.1 or 0.5 M methanolic potassium hydroxide was used to hydrolyze the carbamate to its respective phenol, the peak area of the mesylate compound was reduced by 25–50%. However, increasing or decreasing the concentration of the methanesulfonyl chloride in benzene over a range of 1–10% did not affect the efficiency of the procedure. This was also true of the pyridine concentration in benzene over a range of 2–10%. The methanesulfonyl chloride and the pyridine solutions were found to be stable for at least 1 week but were prepared daily as a precaution for this study. The mesylate derivatives were also prepared by using solutions of pyridine and methanesulfonyl chloride dissolved in hexane, dichloromethane, and chloroform. All of these solvent systems reduced the efficiency of the method; hexane produced the poorest results. An attempt

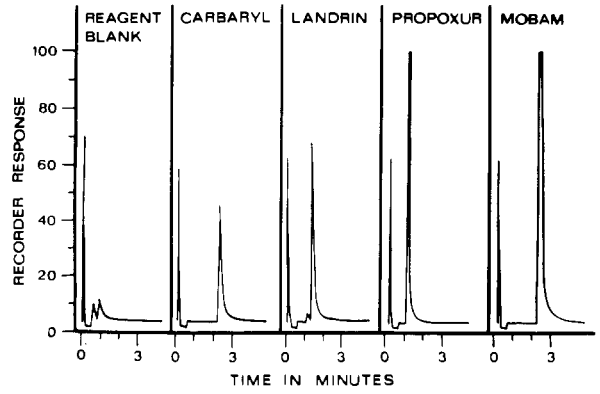

Figure 1. Chromatograms of 5 ng of the mesylate derivatives of the compounds as determined with the FPD on a 3% OV-101 GLC column at 190 °C (attenuation 1).

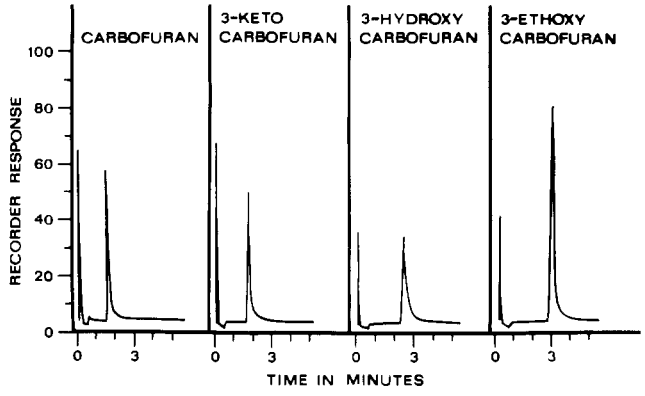

Figure 2. Chromatograms of 5 ng each of the mesylate derivatives of carbofuran and 3-oxocarbofuran and 8 ng each of 3-hydroxycarbofuran and ethoxylated 3-hydroxycarbofuran as determined with the FPD on a 3% OV-101 GLC column at 190 °C (attenuation 1).

was made to derivatize carbaryl and carbofuran by adding all of the reagents (0.25 M methanolic potassium hydroxide, the 5% pyridine solution and the 1% methanesulfonyl chloride solution) together in the reaction flask. The yields were only 20% of those produced by the described microprocedure.

The efficiency of the derivatization reaction was also examined in relation to time and temperature. The reaction of all carbamates except methiocarb and its sulfoxide and sulfone metabolites and 3-hydroxycarbofuran was completed after standing at room temperature for 5 min. The reaction time was examined at 5, 15, 30, 45, and 60 min, and the peak areas of methiocarb and its metabolites and 3-hydroxycarbofuran did not increase after 30 min. Subjecting these compounds to temperatures of 45 and 100 °C for 10 min did not enhance the reaction. In fact, the 100 °C temperature produced lower yields.

As little as 0.05 mL of water could reduce the efficiency of the derivatization as much as 30%; therefore care should be taken to use dry air in the evaporation steps, and solutions should be filtered through sodium sulfate where specified. The effects of using a rotary evaporater for the evaporation steps of this method were not investigated.

The mesylate derivatives of the carbamates and their metabolites, except 3-oxocarbofuran, were stable for at least 30 days. The 3-oxocarbofuran mesylate began deteriorating after 7 days and was reduced by 50% after 14 days.

Figures 1, 2, and 3 show chromatograms of the mesylate derivatives of the various carbamate pesticides tested. The peaks are not symmetrical, indicating that the GLC col-

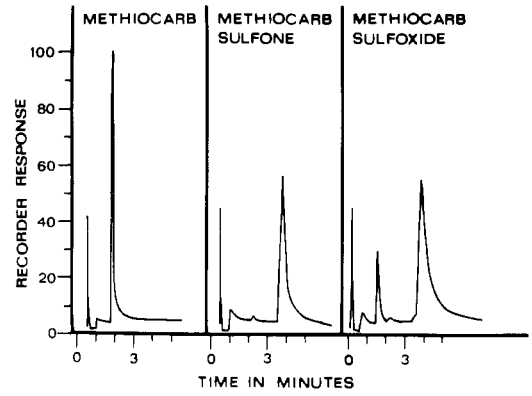

Figure 3. Chromatograms of 8 ng each of the mesylate derivatives of the compounds as determined with the FPD on a 3% OV-101 GLC column at 210 °C (attenuation 1).

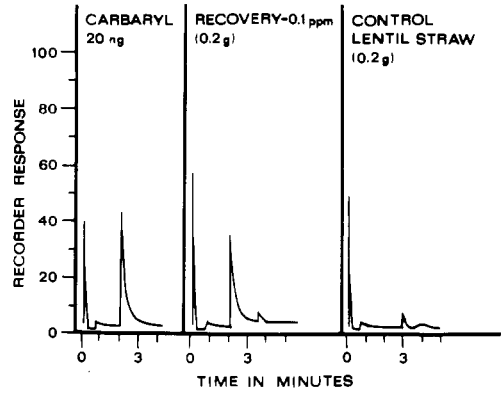

Figure 4. Chromatograms of a control sample and 0.1 ppm recovery of carbaryl from lentil straw as determined by the methanesulfonyl method (recovery 90.0%) (attentuation 4).

umn used may not have been the best choice. However, other GLC columns such as 3% SE 30, 10% DC-200, and 5% Carbowax 20M were tried, and the 3% OV-101 column gave the best efficiency and sensitivity. These chromatograms shown in the figures were prepared from a new GLC column, and as time in use increased, so did the sensitivity, especially in the case of the methiocarb sulfoxide and methiocarb sulfone. Cook et al. (1977) incorporated an acid hydrolysis ethoxylation step into their procedure to convert 3-hydroxy- and (3-hydroxy-7-phenol)carbofuran to their respective ethoxy compounds. These ethoxylated compounds were more easily handled and produced better recoveries than the parent hydroxy compounds. Figure 2 shows that ethoxylated 3-hydroxycarbofuran can be readily derivatized to the mesylate compound and that when this is done greater sensitivity is achieved. Since the basis for this mesylate procedure is conversion of the parent carbamate to its phenol and then mesylation of the phenol, phenolic compounds too could be efficiently mesylated. This was found to be true with the phenolic compounds of carbofuran, 3-hydroxycarbofuran, 3-oxocarbofuran, and the ethoxylated 3-hydroxycarbofuran. The chromatograms for these compounds were the same as the ones shown in Figure 2 for the mesylated parent compounds.

Methiocarb, its sulfoxide, and sulfone metabolites each contain an atom of sulfur. Thornton and Dräger (1973) showed that these compounds could be determined with the FPD, but must be silylated to obtain good GLC properties. Mesylating methiocarb and its metabolites not only eliminates the need for silylation but also increases sensitivity by adding an additional sulfur atom to the molecule. Determination of residues of methiocarb and

Table I. Comparison of Residues of Carbaryl Found in Dry Lentil Straw as Determined by the Methanesulfonyl Chloride and the Trichloroacetyl Chloride Methods

sample	treatment rate, active ingredient per acre	plot no.	residues found, ppm: methanesulfonyl chloride method	residues found, ppm: trichloroacetyl chloride method
untreated control	0	6	ND[a]	ND
recovery[b] (1.0 ppm)	0	6	0.97	0.99
			1.05	1.08
recovery (0.1 ppm)	0	6	0.090	0.095
			0.118	0.102
treated samples	1.25	10	1.11	0.88
		4	0.79	0.81
		7	0.12	0.10
		11	11.00	12.90
		5	4.61	4.50
	2.50	8	1.07	1.07
		2	1.29	0.93
		14	0.81	0.75
		1	4.40	4.33
		10	0.07	0.08
		4	0.49	0.53

[a] ND (none detected) indicates that the residues in these samples were below the lower limit of detection for these samples which was 0.05 ppm. [b] Recoveries were prepared by fortifying control samples of straw with pure carbaryl prior to extraction.

its sulfoxide and sulfone metabolites by the mesylate procedure in crops of spinach, peas, celery, rhubarb, and strawberries has been completed and will be reported elsewhere.

Results in Table I show that the residues of carbaryl in dry lentil straw as determined by our methanesulfonyl chloride method and by the trichloroacetyl chloride method of Butler and McDonough (1968) are comparable. The chromatogram of the control sample of lentil straw, in Figure 4, is quite free of interfering peaks. This, generally, is not the case when residues of carbamate pesticide are determined with a method involving the use of the ECD. Also, high background peaks made it difficult to determine low carbaryl residues in lentil straw when the trichloroacetyl chloride method was used.

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Volatile Constituents of the Chestnut Flower

Kenji Yamaguchi and Takayuki Shibamoto*[1]

The volatile constituents of the chestnut flower (*Castanea creata* Sieb et Zucc), which have not been studied prior to this report, have been investigated by gas chromatography–mass spectrometry. Fifty-four compounds were positively identified in the oil which was obtained using a simultaneous distillation–extraction apparatus. The compounds identified include 20 aliphatic compounds, 10 monoterpenes, 1 sesquiterpene, 16 aromatic compounds, and 7 miscelleneous compounds. The main constituents of this oil were 1-phenylethyl alcohol and 2-phenylethyl alcohol, which comprise 44% of this oil.

Castanea grows wild over Europe, Asia, North America, and North Africa. The nuts are edible and very popular. *Castanea creata* Sieb et Zucc (Japanese chestnut) grows wild throughout Japan. The flower (catkin form) blooms in June and possesses a characteristic sweet odor. In this study, the volatile constituents of the chestnut flower were isolated and identified by means of gas chromatography–mass spectrometry.

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EXPERIMENTAL SECTION

Taxonomic identification of this plant was made by Professor Masao Arai, Tokyo Agricultural University, Tokyo, Japan. Samples were collected near Konosu City on June 15, 1978, by K. Yamaguchi. Freshly collected flowers were carefully sorted on white paper to remove any kind of foreign materials (barks, twigs, soil, etc.). Five hundred grams of flowers were subjected to simultaneous distillation and extraction (SDE) (Likens and Nickerson, 1964). The extracting solvent was methylene chloride and steam distillation–extraction (water/methylene chloride = 1200 mL/200 mL) was continued for 16 h. The extract was dried over anhydrous sodium sulfate for 12 h and the

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