Gas Chromatographic Separation of the Aglycone Metabolites of Carbaryl

Safy Khalifa[1] and Ralph O. Mumma*

Trifluoroacetyl, heptafluorobutyryl, and chloroacetyl derivatives of carbaryl and various aglycone metabolites (4-hydroxy-1-naphthyl *N*-methylcarbamate, 5-hydroxy-1-naphthyl *N*-methylcarbamate, 6-hydroxy-1-naphthyl *N*-methylcarbamate, 7-hydroxy-1-naphthyl *N*-methylcarbamate, and 1-naphthyl *N*-hydroxymethylcarbamate) were prepared and analyzed by gas–liquid chromatography (glc). Derivatives of 1-naphthol and various naphthalenediols, resulting from basic hydrolysis of the aglycones, were also examined, including the chloromethyldimethylsilyl and trimethylsilyl derivatives. Trifluoroacetyl and heptafluorobutyryl derivatives of the carbamate aglycones were sufficiently thermally stable to be analyzed by glc. The trifluoroacetyl derivatives of the carbamates were most desirable because of their ease of purification, their short retention time, and their high sensitivity to electron affinity detectors. Of the reagents evaluated, the chloromethyldimethylsilyl derivatives of the naphthalenediols gave the best separation on glc.

Carbaryl (1-naphthyl *N*-methylcarbamate), an economically important insecticide, is metabolized by plants and animals to several glycosidic derivatives (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; Mumma *et al.*, 1971). In plants, these derivatives exist as glucosides while in animals they are present chiefly as glucuronides and sulfates. Soil microorganisms also convert carbaryl to similar aglycone metabolites (Liu and Bollag, 1971).

The *N*-methylcarbamate pesticides are in general not capable of being directly analyzed by gas–liquid chromatography (glc). They are thermally unstable at temperatures usually needed for glc and decompose to an alcohol (1-naphthol in the case of carbaryl) and *N*-methylisocyanate. However, Riva and Carisano (1969) have directly analyzed carbaryl by glc employing low temperatures, short columns, and a low concentration (0.5%) stationary phase. Also, pesticidal carbamates have been gas chromatographed as their trimethylsilyl derivatives (Fishbein and Zielinski, 1965).

The analytical procedures usually employed for quantifying carbaryl are based upon quantifying either 1-naphthol or *N*-methylamine, both being derived from basic hydrolysis of carbaryl. 1-Naphthol can be detected by a colorimetric method employing a *p*-nitrobenzenediazonium fluoroborate derivative (Johnson, 1964) and this technique has been used to quantify carbaryl at the 0.01 ppm levels. Several investigators have brominated 1-naphthol and detected the brominated derivative by gas chromatography employing an electron affinity detector (Rolls and Cortes, 1964; Van Middelem *et al.*, 1965; Gutenmann and Lisk, 1965). This technique has proved useful at the 0.1 ppm level. Bowman and Beroza (1967) developed a sensitive method employing derivatization of the alcohol (1-naphthol in the case of carbaryl) with thiophosphoryl chloride, followed by gas chromatography and flame photometric detection of the thiophosphoryl derivatives. Methylamine also can be quantified as an amide (Tilden and Van Middelem, 1970). Moye (1971) recently described a new method to quantify *N*-methylcarbamates, an on-column transesterification of *N*-methylcarbamates to methyl *N*-methylcarbamate with subsequent detection by a Rb_2SO_4 pellet alkali flame ionization detector. Clark *et al.* (1966) has examined the glc properties of various fluoroacyl derivatives of amines and phenolic amines. Argauer (1969) has reported an analytical procedure for Banol utilizing chloroacetylation of the phenolic hydrolysis products and subsequent detection by electron capture. Recently Seiber (1971) described the perfluoroacylation of some carbamates.

In view of the many metabolites of carbaryl, it is important to develop analytical procedures for their analysis, especially since the aglycone metabolites exhibit anticholinesterase activity (Kuhr and Casida, 1967). Therefore, the object of this investigation was to develop a gas chromatographic method for the separation of carbaryl and its aglycone metabolites employing electron affinity detection. Two approaches were used: first, to derivatize the phenols resulting from hydrolysis of the carbamates; and second, to derivatize directly the carbamates with reagents possessing halogen atoms. These derivatives were then analyzed by glc and evaluated for their ease of separation and sensitivity to electron affinity detection. The following carbaryl aglycone metabolites or potential metabolites were considered: 4-hydroxy-1-naphthyl *N*-methylcarbamate (4-hydroxycarbaryl), 5-hydroxy-1-naphthyl *N*-methylcarbamate (5-hydroxycarbaryl), 6-hydroxy-1-naphthyl *N*-methylcarbamate (6-hydroxycarbaryl), 7-hydroxy-1-naphthyl *N*-methylcarbamate (7-hydroxycarbaryl), and 1-naphthyl *N*-hydroxymethylcarbamate (*N*-hydroxymethylcarbaryl). The following derivatives were investigated: chloroacetyl, trifluoroacetyl, heptafluorobutyryl chloromethyldimethylsilyl (CMDMS), and trimethylsilyl (TMS).

EXPERIMENTAL

Materials and Reagents. All solvents were redistilled and all reagents were analyzed by thin-layer chromatography. Carbaryl, analytical grade, was supplied by Union Carbide Corp. and 1-naphthol was purchased from Fisher Scientific Co. *N*-Hydroxymethylcarbaryl, and 4- and 5-hydroxycarbaryl were synthesized as previously reported (Mumma *et al.*, 1971). Hydroxycarbaryl (6 and 7) and 1,6-naphthalenediol were supplied by J. A. Durden. Chloroacetic anhydride, trifluoroacetic anhydride, and 1,4- and 1,5-naphthalenediol were purchased from Eastman Organic Chemicals. *N,O*-Bis(trimethylsilyl)acetamide (BSA), *N*-trifluoroacetylimidazole, *N*-heptafluorobutyrylimidazole, bis-chloromethyltetramethyldisilazane, and chloromethyldimethylchlorosilane were obtained from Supelco, Inc.

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

[1] Present address: University of California, Division of Entomology, Berkeley, California 94720.

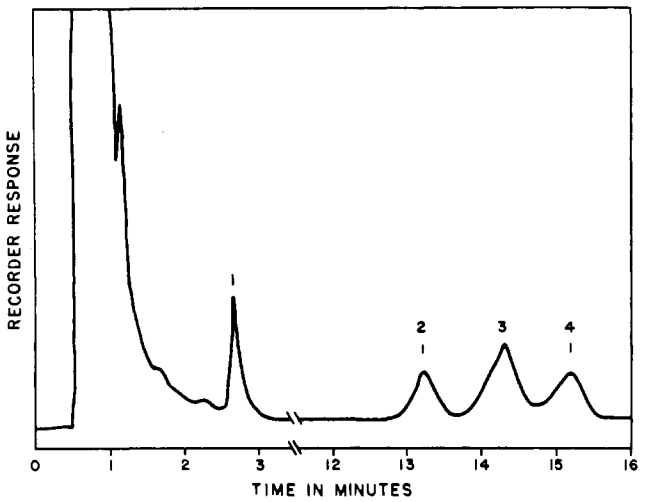


Figure 1. Gas chromatograph of CMDMS derivatives. The peak identities are: (1) 1-naphthol; (2) 1,4-naphthalenediol; (3) 1,5-naphthalenediol; and (4) 1,6-naphthalenediol

Gas Chromatography. A Barber-Colman Model 5000 (flame ionization detector) and a Micro Tek Model 200 (electron affinity detector, ^{63}Ni) gas chromatograph (4-mm × 180-mm U tube column, 3% SE-30 on 80–100 mesh Supelcoport) were used. The column temperature was as described in the results and discussion section. The injection inlet and detector temperatures were 20°C higher than the column temperature. The nitrogen flow rate was 80 ml/min.

Preparation of Reagents and Derivatives. The reagents for derivatization were prepared as follows: 0.5 ml of purchased derivatization reagent was diluted in 5 ml of benzene. Bischloromethyltetramethyldisilazane and chloromethyldimethylchlorosilane were mixed (3:1, by vol) and then diluted with benzene as above.

The carbamates (carbaryl, 4-, 5-, 6-, and 7-hydroxycarbaryl) and the phenols (1-naphthol, 1,4-, 1,5-, and 1,6-naphthalenediol) were dissolved in anhydrous benzene (1 mg per 6 ml) and placed in a centrifuge tube. To this was added 0.2–0.4 ml of anhydrous pyridine and 0.3 ml of the above derivative reagents. The contents of the centrifuge tube were mixed with a Vortex and then let stand for 15 min at 25°C, after which time each tube was washed with three 5-ml portions of water. Higher reaction temperatures and longer times were evaluated. The benzene solution was directly analyzed or diluted prior to gas chromatography, depending upon which detector was employed.

RESULTS AND DISCUSSION

Phenols. The trifluoroacetyl, heptafluorobutyryl, chloroacetyl, CMDMS, and TMS derivatives of 1-naphthol, 1,4-, 1,5-, and 1,6-naphthalenediol were prepared. The trifluoroacetyl and the heptafluorobutyryl derivatives were formed with either the corresponding anhydride or imidazole reagents. Pryidine was helpful as a catalyst in all cases. Since the reactions were essentially complete at room temperature in 15 min, no beneficial effect was observed when higher temperatures or longer reaction times were employed. Excess halogenated reactants were removed from the reaction mixture by washing with water to prevent desensitization of the electron affinity detector. Some problem was experienced with completely removing the silyl reactants and additional washings were necessary.

Table I. Gas Chromatographic Retention Time of Derivatives of 1-Naphthol, 1,4-, 1,5-, and 1,6-Naphthalenediol on a 3% SE-30 Column

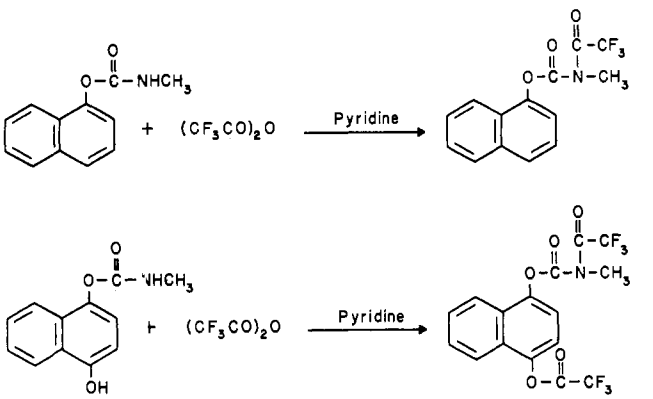
Compound	Retention time (min) of derivatives[a] (190°C)					
	1	2	3	4	5	6[b]
1-Naphthol	1.90	1.27	1.53	2.08	2.23	2.70
1,4-Naphthalenediol		1.53	2.25	5.57	5.57	13.2
1,5-Naphthalenediol	4.35	1.53	2.25	5.57	6.05	14.30
1,6-Naphthalenediol	4.53	1.70	2.55	5.70	6.50	15.20

[a] The derivatives are as follows: 1, nonderivative; 2, trifluoroacetyl; 3, heptafluorobutyryl; 4, chloroacetyl; 5, trimethylsilyl; 6, chloromethyldimethylsilyl. [b] Column temperature of 210°C.

Derivatives of the phenols were thermally stable and easily analyzed by glc employing an SE-30 column at 190°C (Table I). Column temperatures of 165 and 200°C were also evaluated. Stationary phases of OV-17 and OV-210 gave undesirably long retention times. The trifluoroacetyl and heptafluorobutyryl derivatives of the phenols gave poor separations at all temperatures. The CMDMS derivatives gave the best separation, as shown in Figure 1. The TMS derivatives do not contain halogen atoms; however, they do provide comparable retention times.

Using an analytical method based upon quantifying the phenols resulting from hydrolysis of the carbamates, 1-naphthol, *N*-hydroxymethylcarbaryl, and carbaryl would be quantified simultaneously as derivatives of 1-naphthol. Fortunately, the metabolites of carbaryl exist usually as glycosides, and these glycosides can easily be separated from carbaryl. The nuclear substituted metabolites, 4-, 5-, and 6-hydroxycarbaryl, can easily be quantified from their corresponding naphthalenediols.

Carbamates. Most *N*-methylcarbamates are thermally unstable and unsuitable for analysis by glc. The proton attached to the nitrogen is readily transferable to the phenolic oxygen when the carbamate is subjected to elevated temperatures or under mass spectroscopic conditions (Damico and Benson, 1965). When the proton attached to the nitrogen is replaced, the resulting compound has greater thermal stability (Fishbein and Zielinski, 1965). Consequently, carbaryl and its metabolites were subjected to derivatization with the previously mentioned acylating reagents.



The carbamates readily formed trifluoroacetyl and heptafluorobutyryl derivatives at room temperature with either the anhydride or imidazole reagents. Chloroacetic anhydride did not acylate the amide nitrogen. The formation of *N*-trifluoroacetyl carbaryl and *N,O*-bis(trifluoroacetyl) 4-hydroxycarbaryl are illustrated as follows.

The trifluoroacetyl and heptafluorobutyryl derivatives of carbaryl, 4-hydroxycarbaryl, 5-hydroxycarbaryl, 6-hydroxy-

Table II. Gas Chromatographic Retention Time of Derivatives of Carbaryl and its Metabolites on a 3% SE-30 Column

Compound	Retention time (min) of derivatives (200°C)[a] 1	2
1-Naphthol	2.00[b]	
N-Hydroxymethylcarbamate	2.00[b,c]	
Carbaryl	3.33	5.00
4-Hydroxycarbaryl	4.50	8.00
5-Hydroxycarbaryl	4.50	8.00
6-Hydroxycarbaryl	4.83	9.17
7-Hydroxycarbaryl	3.92	7.00

[a] The derivatives are as follows: 1, trifluoroacetyl; 2, heptafluorobutyryl. [b] At 165°C. [c] Determined to be trifluoroacetyl 1-naphthol by mass spectrometry.

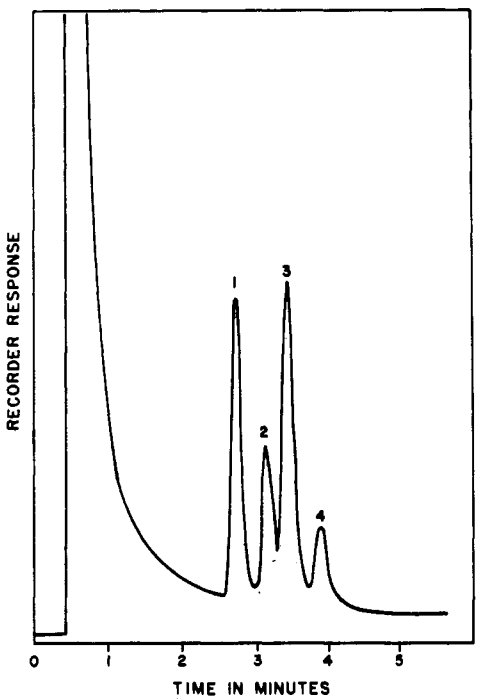


Figure 2. Gas chromatograph of trifluoroacetyl derivatives of carbaryl and its aglycone metabolites. The peak identities are: (1) carbaryl; (2) 7-hydroxycarbaryl; (3) 4- and 5-hydroxycarbaryl; and (4) 6-hydroxycarbaryl

carbaryl, and 7-hydroxycarbaryl were sufficiently thermally stable for analysis by glc. Structures of the derivatives have been confirmed by mass spectroscopy. Retention times of these derivatives on an SE-30 column at 200°C are presented in Table II. Unfortunately, *N*-hydroxymethylcarbaryl did not form the same products as the other aglycones. *N*-Hydroxymethylcarbaryl is more sensitive to hydrolysis than the other carbamates, and under the derivatization conditions, only trifluoroacetyl 1-naphthol or heptafluorobutyryl 1-naphthol was formed. Thus, *N*-hydroxymethylcarbaryl can be quantified as a 1-naphthol derivative. The trifluoroacetyl derivatives have relatively short retention times on glc (Figure 2), good sensitivity to electron affinity detectors, and the derivatives are easily isolated in relatively pure forms. Thirty-five picograms of carbaryl gives a half-scale response with an electron affinity detector (5×10^{-13} A per full scale, 2.5×10^{-9} A standing current). Under these glc conditions the 4- and 5-hydroxycarbaryl derivatives are not well separated. The heptafluorobutyryl derivatives have undesirably long retention times at 200°C on the SE-30 column and consequently do not give sharp peaks.

CONCLUSIONS

1-Naphthol, 1,4-, 1,5-, and 1,6-naphthalenediol, resulting from hydrolysis of carbaryl and its metabolites, are well separated and quantified as their CMDMS derivatives on glc. Carbaryl, 4-hydroxycarbaryl, 5-hydroxycarbaryl, 6-hydroxycarbaryl, and 7-hydroxycarbaryl form thermally stable trifluoroacetyl and heptafluorobutyryl derivatives which can be easily separated and quantified by glc with high sensitivity for electron affinity detection. *N*-Hydroxymethylcarbaryl can be quantified in the same manner, in the form of its 1-naphthol derivative. This method of forming thermally stable *N*-methylcarbamate derivatives capable of being analyzed by glc will permit the direct analysis of plant and animal tissues for carbaryl and its metabolites. Perhaps other carbamate pesticides and possibly the glycosides can be analyzed also in this manner. The actual levels at which these metabolites could be detected in tissues using this procedure depend on a number of factors, such as stability of the derivatives, isolation of the metabolites, removal of the sugar with enzymes, method of work-up, and interfering material within the plant. These factors will be investigated further.

LITERATURE CITED

Abdel-Wahab, A. M., Kuhr, R. J., Casida, J. E., J. AGR. FOOD CHEM. **14,** 290 (1966).
Argauer, R. J., J. AGR. FOOD CHEM. **17,** 888 (1969).
Bowman, M. D., Beroza, M., *J. Ass. Offic. Agr. Chem.* **50,** 926 (1967).
Clark, D. D., Wilk, S., Gitlow, S. E., *J. Gas Chromatogr.* **4,** 310 (1966).
Damico, J. N., Benson, W. R., *J. Ass. Offic. Agr. Chem.* **48,** 344 (1965).
Dorough, H. W., Casida, J. E., J. AGR. FOOD CHEM. **12,** 294 (1964).
Fishbein, L., Zielinski, W. L., *J. Chromatogr.* **20,** 9 (1965).
Gutenmann, W. H., Lisk, D. J., J. AGR. FOOD CHEM. **13,** 48 (1965).
Johnson, D. P., *J. Ass. Offic. Agr. Chem.* **47,** 282 (1964).
Knaak, J. B., Tallant, M. J., Bartley, W. J., Sullivan, L. J., J. AGR. FOOD CHEM. **13,** 537 (1965).
Kuhr, R. J., Casida, J. E., J. AGR. FOOD CHEM. **15,** 814 (1967).
Liu, S., Bollag, J., J. AGR. FOOD CHEM. **19,** 487 (1971).
Moye, H. A., J. AGR. FOOD CHEM. **19,** 452 (1971).
Mumma, R. O., Khalifa, S., Hamilton, R. H., J. AGR. FOOD CHEM. **19,** 455 (1971).
Oonnithan, E. S., Casida, J. E., *Bull. Environ. Contam. Toxicol.* **1,** 59 (1966).
Oonnithan, E. S., Casida, J. E., J. AGR. FOOD CHEM. **16,** 28 (1968).
Paulson, G. D., Zaylskie, R. G., Zehr, M. V., Portnoy, E. E., Feil, V. J., J. AGR. FOOD CHEM. **18,** 110 (1970).
Riva, M., Carisano, A., *J. Chromatogr.* **42,** 464 (1969).
Rolls, J. W., Cortes, A., *J. Gas Chromatogr.* **2,** 132 (1964).
Seiber, J. N., 161 National Meeting Amer. Chem. Soc., Los Angeles, Calif. Abst. PEST 78 March–April (1971).
Tilden R. L., Van Middelem, C. H., J. AGR. FOOD CHEM. **18,** 154 (1970).
Van Middelem, C. H., Norwood, T. L., Waites, R. E., *J. Gas Chromatogr.* **3,** 310 (1965).

Received for review October 6, 1971. Accepted November 29, 1971. Authorized for publication as paper No. 4040 in the Journal Series of the Pennsylvania Agricultural Experiment Station. Supported in part by Northeastern Regional Research Project NE-53 and Regional Research Funds.