N-Perfluoroacyl Derivatives for Methylcarbamate Analysis by Gas Chromatography

Methylcarbamate insecticides react rapidly and quantitatively with trifluoroacetic (TFA), pentafluoropropionic (PFP), and heptafluorobutyric (HFB) anhydrides to give *N*-perfluoroacylated derivatives. Unlike many of the parent methylcarbamates, the derivatives are stable to gas chromatographic conditions and detected at low levels by the electron-capture and alkali-flame ionization detectors. The infrared, nmr, and mass spectral properties of the compounds are characteristic and permit ready qualitative identification. The applicability of trifluoroacetylation to eight methylcarbamate insecticides and the metabolites of carbofuran was demonstrated.

The determination of *N*-methylcarbamate insecticides has presented considerable difficulties because they tend to undergo thermal dissociation under many gas chromatographic conditions, to the parent phenol and methyl isocyanate (Zielinski and Fishbein, 1965), and they lack a suitable "handle," other than nitrogen, for highly sensitive and/or specific determination using the more common glc detection systems. The use of short columns of high liquid phase load and silanized supports greatly alleviates the thermal breakdown; successful glc analyses of carbamates have thus been reported in which the microcoulometric (Cook *et al.*, 1969) or KCl-thermionic (Riva and Carisano, 1969) nitrogen detector systems were employed.

Several recent approaches to the glc determination of methylcarbamates have been based on a hydrolytic cleavage under basic or acidic conditions, with subsequent derivatization of the liberated phenol or methylamine. Examples of the former include the use of trichloroacetyl (Butler and McDonough, 1968), monochloroacetyl (Argauer, 1969), and dimethylthiophosphoryl (Bowman and Beroza, 1967) derivatization. Examples of the latter include 2,6-dinitro-4-trifluoromethylphenyl (Crosby and Bowers, 1968), 2,4-dinitrophenyl (Holden *et al.*, 1969), and *para*-bromobenzoyl (Tilden and Van Middelem, 1970) derivatization. An alternate procedure employs on-column transesterification of the methylcarbamate to yield methyl *N*-methylcarbamate (Moye, 1971; Van Middelem *et al.*, 1971).

Methylcarbamates react with acetic anhydride under acid catalysis to give *N*-acetyl derivatives (Sullivan *et al.*, 1967; Fraser *et al.*, 1968; Paulson *et al.*, 1970). The resulting derivatives are more stable to glc conditions than the carbamates themselves and are useful for characterization of hydroxy metabolites, since the hydroxy groups are acetylated under the same conditions. Lau and Marxmiller (1970) showed that formation of *N*-trifluoroacetyl derivatives produces compounds with good thermal stability and volatility, with the added advantage of being detectable in nanogram quantities by the electron-capture glc detector. Application to analysis of Landrin residues in corn, oats, and soybeans resulted in a method sensitive to 0.02 ppm.

The present work was undertaken to show the general applicability of trifluoroacetylation to methylcarbamate derivatization. The conditions of reaction and derivative properties were studied for a number of methylcarbamate insecticides. The derivatives were, in all cases studied, formed rapidly and quantitatively, were amenable to glc determination using the electron-capture and alkali-flame ionization detectors, and gave rise to characteristic infrared, nmr, and mass spectra for qualitative identification. The second purpose of this study was to investigate the applicability of pentafluoropropionyl and heptafluorobutyryl derivatives for methylcarbamate determination, particularly noting whether or not these compounds offered any distinct advantage over the trifluoroacetyl derivatives.

EXPERIMENTAL

Trifluoroacetic anhydride (Aldrich Chemical Co.) and pentafluoropropionic and heptafluorobutyric anhydrides (K&K Laboratories, Inc.) were used as received. All solvents were double distilled. Pesticide analytical grade standards were used as supplied: Baygon (*O*-isopropoxyphenyl methylcarbamate); Bux (1-ethylpropyl)phenyl and (1-methylbutyl)phenyl methylcarbamate isomer mixture; carbaryl (1-naphthyl methylcarbamate); carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate); Landrin (3,4,5-trimethylphenyl methylcarbamate); Matacil [4-(dimethylamino)-*m*-tolyl methylcarbamate]; Mesurol [4-(methylthio)-3,5-xylyl methylcarbamate]; and Mobam (4-benzothienyl methylcarbamate).

Infrared spectra were obtained as films using a Perkin-Elmer 337 grating spectrophotometer. Nmr spectra were obtained in deuteriochloroform, TMS internal standard, using a Hitachi Perkin-Elmer R-20 high-resolution nmr spectrometer. Mass spectra were obtained by direct sample inlet using a Varian M-66 mass spectrometer at 70 eV ionization potential.

Gas–Liquid Chromatography. A Varian Aerograph Model 1700 gas chromatograph equipped with tritium foil electron-capture and Rb_2SO_4 alkali-flame ionization detectors was used with the following columns: (1) 4 ft 3% SE-30 on 60/80 A/W, DMCS treated Chromosorb G; (2) 7 ft 6% SE-30 on 70/80 A/W, DMCS treated Chromosorb G; (3) 6 ft 3% SE-30 on 80/100 Varaport 30; and (4) 6 ft 5% AN 600 on 60/80 A/W, DMCS treated Chromosorb G. All columns were 3 mm i.d. glass. Nitrogen carrier gas flow rate was 30 ml/min for electron-capture and 20 ml/min for alkali-flame ionization. Air and hydrogen flow rates were 200 and 25 ml/min for alkali-flame ionization. Injector and detector temperatures were 240 and 215°C, respectively; column temperatures are noted with the chromatograms. Gas chromatography of underivatized methylcarbamates intact using alkali-flame ionization detection was facilitated by first conditioning the column at the operating temperature with several 1-μl injections of trifluoroacetic anhydride.

Derivative Preparation. A solution of 10 mg of the methylcarbamate, 4 ml of benzene, and 0.5 ml of anhydride was heated in a sealed 2- or 4-dram screw cap vial with Teflon cap liners at 100°C for 2 hr in an oil bath. The colorless oily product was isolated by evaporation of solvent and excess reagent using a rotary evaporator and taken immediately for spectral analysis. Alternatively, the benzene solution was diluted with 4 ml of hexane, washed with water (three 5-ml portions), and diluted with hexane to an appropriate volume for gas chromatographic analysis. Both spectral

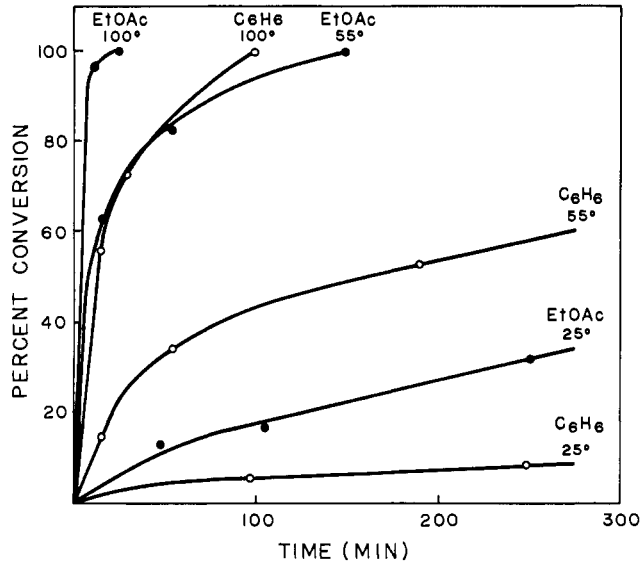

Figure 1. Rate of conversion of carbaryl to its TFA derivative; dependence on temperature and solvent

and gas chromatographic analyses indicated complete conversion for all methylcarbamates studied. To insure that the same path was followed on the microgram scale, and that no interference from solvent or reagent would be encountered, the reaction was carried out as above using 10 μg of carbaryl. The peak height and retention time of the resulting product in benzene–hexane was identical with that obtained from the corresponding amount of derivative from reaction of 10 mg of carbaryl. No interferences were observed in the electron-capture chromatograms of derivatives or reagent blanks.

Effect of Temperature and Solvent on Rate of Derivatization. The reaction was carried out as above using 4 mg of carbaryl and carbofuran; sealed vials were removed at different time intervals for bath temperatures of 25, 55, and 100°C. Percent conversion was calculated by monitoring both the unreacted carbamate and carbamate TFA derivative by direct injection of 1-μl portions of the reaction mixture, using alkali-flame ionization gas chromatography and column 1, for periods of time up to 16 hr. The procedure was repeated using ethyl acetate and tetrahydrofuran as solvents in place of benzene.

Derivative Stability. Storage at 10°C in benzene–hexane solvent resulted in no measurable decomposition after 2 days. In the absence of solvent the derivatives were partially hydrolyzed by traces of atmospheric moisture after 2 weeks, even when stored at −10°C. The product formed was the corresponding methylcarbamate, confirmed by infrared analysis. No decomposition was observed in the gas chromatographic systems employed, even at a column temperature of 250°C.

RESULTS AND DISCUSSION

Conditions for carrying out trifluoroacetylation reactions were defined by reacting carbaryl and carbofuran with excess anhydride using benzene, ethyl acetate, and tetrahydrofuran as solvents, at three temperatures. Results for carbaryl using benzene and ethyl acetate for periods to 5 hr are shown in Figure 1. Derivatization rates for carbofuran were virtually identical to those for carbaryl, as were those using tetrahydrofuran in place of ethyl acetate. The conversion for carbaryl and carbofuran at 25°C after 16 hr was approximately 80% in ethyl acetate and tetrahydrofuran, but only 20% in benzene.

The results indicate that two factors, solvent polarity and temperature, govern the rate of reaction and suggest three conditions for practical use: ethyl acetate at room temperature for 16 hr or more (the procedure of Lau and Marxmiller, 1970); ethyl acetate at 55°C for 2 hr; and benzene at 100°C for 2 hr. Using microgram quantities of carbamate under the

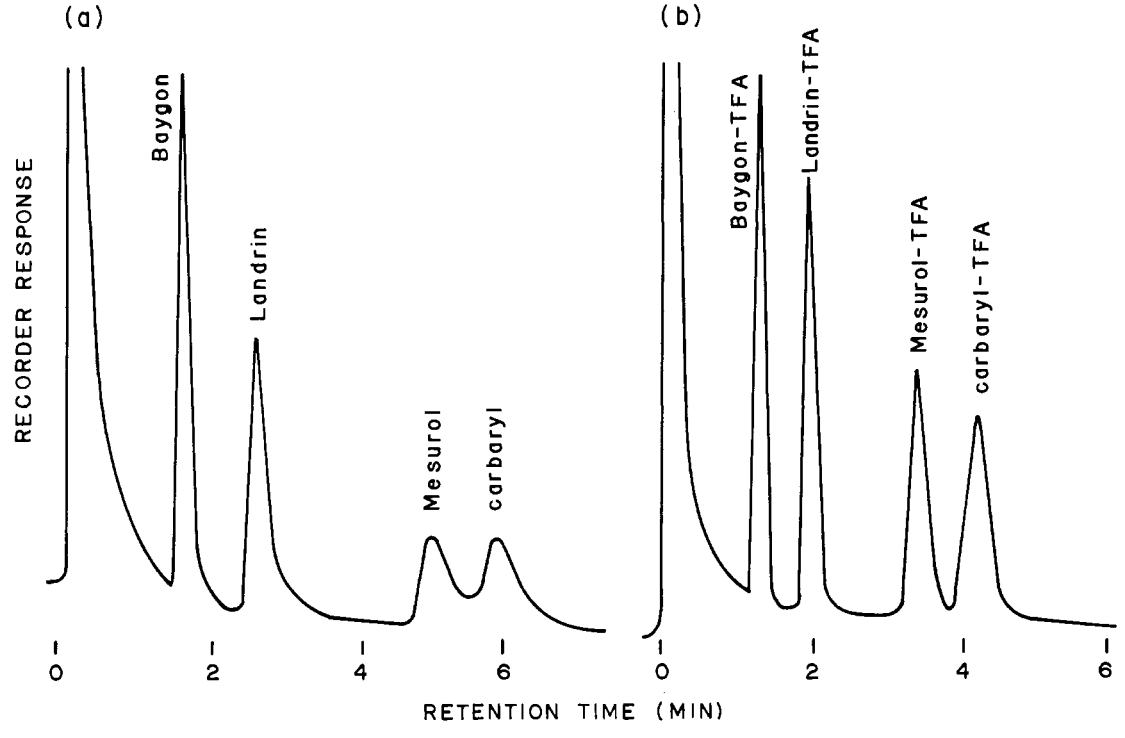

Figure 2. Gas chromatograms of a mixture of four methylcarbamates (a) before derivatization with trifluoroacetic anhydride, 200 ng of each injected, and (b) after derivatization, 100 ng of each injected, using column 1 at 180°C and alkali-flame ionization detector

conditions described, electron-capture background was consistently lower using the third method. Choice of conditions for a particular application, however, will depend on the type of sample (residue or formulation), detection system employed, and interferences generated from the sample substrate, particularly in residue analyses.

Some of the practical results of trifluoroacetylation are shown in Figure 2. On the left is shown a chromatogram of 200 ng each of four methylcarbamates before treatment, and on the right is shown a chromatogram of 100 ng each of the same four carbamates after trifluoroacetylation. The retention time of each compound was shortened by approximately 25% after derivatization and the peak shape was greatly improved, resulting in at least a twofold increase in sensitivity. The situation is thus reminiscent of the improvements in sensitivity reported for primary and secondary amines upon trifluoroacetylation (McCurdy and Reiser, 1966).

An electron-capture composite chromatogram of seven TFA derivatives is shown in Figure 3 for injections of 2 ng of each derivative. The high column temperature at which this analysis was carried out testifies to the thermal stability of the products. Response of the TFA derivative of carbaryl to the electron-capture detector was linear over the range 0.5 to 10 ng and approximately one-fifth that of lindane.

The spectral properties of the derivatives were found to be quite characteristic. A comparison of the infrared, nmr, and mass spectral properties of carbaryl and its TFA derivative serves to illustrate this point. The infrared spectra of carbaryl before and after derivatization show the appearance in the latter of a second carbonyl absorption, at 5.7 μ, of nearly identical intensity to that of the carbamate carbonyl, at 5.85 μ. The ratio of these two could, in fact, be used as an assay of derivative purity. The loss of N–H absorption at 3.0 μ and appearance of C–F stretching modes at 7–8 μ further serve to characterize the infrared spectra. The nmr spectra showed collapse of the broad N-methyl doublet present in the carbamate to a sharp singlet, at 3.3 ppm, in the derivative. This pattern was followed by all the methylcarbamates studied and, like the infrared information, could be used to assess product purity.

The major path followed in the mass spectral fragmentation involved, first, loss of neutral methyl isocyanate from the molecular ion with rearrangement to the acylated phenol radical cation. This species represented the base peak in most of the derivatives studied. Subsequent fragmentation involved loss of the trifluoroacetyl radical and then loss of carbon monoxide. The fragmentation pattern for trifluoroacetylated carbaryl was thus analogous to that reported by Paulson *et al.* (1970) for acetylated carbaryl. In some of the derivatives where there existed an alkyl side chain on the aromatic ring, for example Bux or Baygon, fragmentation of the alkyl substituent preceded the loss of methyl isocyanate and led to a more complicated fragmentation pattern. Still the base peak could be interpreted as due to an acylated phenol, same as with carbaryl. One further noteworthy feature in the mass spectra of the derivatives studied was the enhanced relative abundance of the parent peak in the derivative compared with the parent methylcarbamate. The parent peak relative abundance for carbaryl–TFA was, for example, 35% in contrast with 7% observed by us and others (Damico and Benson, 1965) for the underivatized carbamate.

One of the purposes in undertaking this study, as noted previously, was to investigate the properties of the pentafluoropropionyl and heptafluorobutyryl derivatives of methylcarbamates as possible alternates to the trifluoroacetyl derivatives. In short, it was found that the rate of formation, product stability, and infrared, nmr, and mass spectral properties of these compounds were nearly identical to those of the TFA derivatives. Glc examination revealed that the retention times of the three types of derivatives were nearly

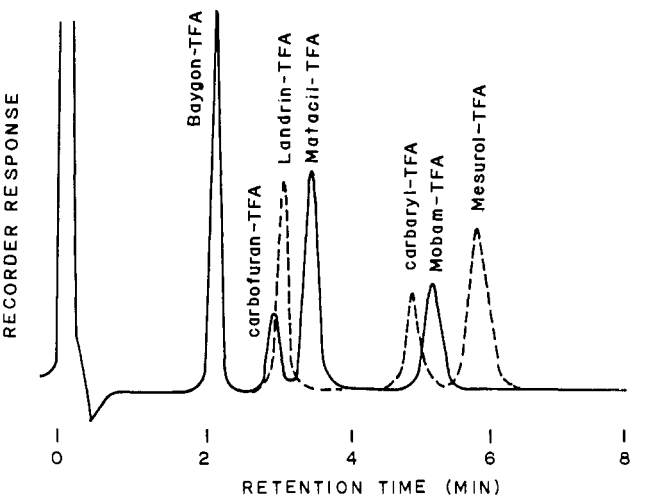

Figure 3. Composite gas chromatogram of seven methylcarbamate TFA derivatives, 2 ng of each injected, using column 2 at 230°C and electron-capture detector

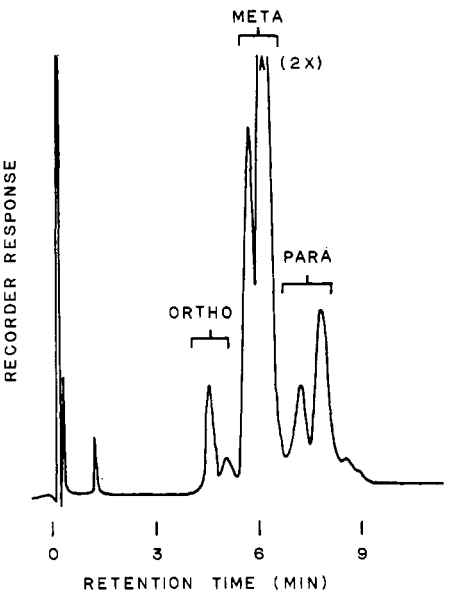

Figure 4. Gas chromatogram of technical Bux after derivatization with trifluoroacetic anhydride, 10 ng of mixture injected, using column 3 at 160°C and electron-capture detector

Table I. Electron-Capture glc Properties of TFA, PFP, and HFB Derivatives of Carbaryl and Carbofuran

	Relative ec response	Retention time[a]
Carbaryl TFA	1.0	2.80 min
Carbaryl PFP	1.7	2.65
Carbaryl HFB	2.2	3.00
Carbofuran TFA	1.0	1.50
Carbofuran PFP	3.8	1.45
Carbofuran HFB	5.2	1.55

[a] Column 1, 190°C.

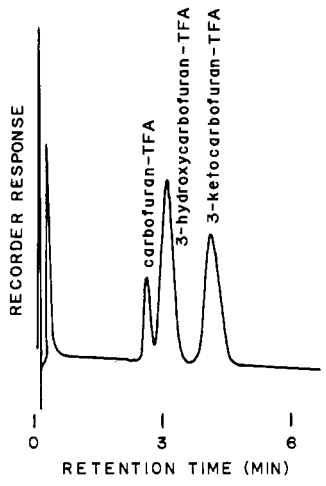

Figure 5. Gas chromatogram of mixture of TFA derivatives of carbofuran, 3-hydroxycarbofuran, and 3-ketocarbofuran, 10 ng of each injected, using column 4 at 195 °C and electron-capture detector

identical, as illustrated in Table I for carbaryl and carbofuran. Similar observations have been made previously with homologous perfluoroacylated amines (Vanden Heuvel *et al.*, 1964). Relative electron-capture response increased only twofold for carbaryl and fivefold for carbofuran on passing from the trifluoroacetyl to the heptafluorobutyryl derivatives. Increases of several thousandfold have been reported for homologous series of perfluoroacylated aliphatic amines (Clarke *et al.*, 1966).

Analysis of technical products can be done rapidly and conveniently by the direct derivatization procedure, as illustrated in Figure 4 for technical Bux insecticide. Bux is a mixture of six major structural isomers, the *ortho*-, *meta*-, and *para*-2- and 3-pentyl-substituted phenyl methylcarbamates. Resolution of the individual isomers by direct gas chromatography is not satisfactory; improved resolution was effected, however, by chromatography of the TFA derivatives.

Hydroxy metabolites of methylcarbamates are readily converted to nonpolar *N*- and *O*-trifluoroacetylated derivatives with trifluoroacetic anhydride. Thus, determination of metabolites by this method appears to offer some real advantages worth pursuing further. The electron-capture gas chromatogram of carbofuran and its hydroxy and keto metabolites (Figure 5) serves to illustrate this point. In addition to the convenient peak shape and retention time, the trifluoroacetylated hydroxy metabolite offers greater sensitivity to electron-capture, owing apparently to the presence of two trifluoroacetyl groups.

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A Glc Assay for Microsomal Thioether Oxidation

p-Chlorothioanisole has been investigated as a substrate for microsomal thioether oxidation. In both mouse and housefly microsomal preparations, it is oxidized to *p*-chlorophenyl methylsulfinyl ether. The ease and accuracy of the assay procedure suggest that *p*-chlorothioanisole may have wide application for the measure of thioether oxidation.

In vitro assays for microsomal thioether S-oxidation commonly use chlorpromazine as a substrate (Salzman and Brodie, 1956; Hart and Fouts, 1965; Rubin *et al.*, 1964). Chlorpromazine may, however, also undergo side-chain oxidation, ring hydroxylation, and/or N-oxidation. Furthermore, chlorpromazine is neither a simple nor a readily available substrate. Since insects both activate (Metcalf *et al.*, 1957, 1963, 1966) and detoxify (Kapoor *et al.*, 1970) insecticides through thioether oxidation, a simple substrate may have more utility for insecticide studies in both insects and higher animals.

We present here an assay method encompassing a readily available substrate whose metabolites are limited to thioether oxidation products.

METHODS AND MATERIALS

Model Metabolites. *p*-Chlorothioanisole, mp 17–19 °C, was obtained from Matheson Coleman and Bell, Norwood,