Residue Determination of Landrin[1] Insecticide by Trifluoroacetylation and Electron-Capture Gas Chromatography

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A glc electron-capture method has been developed to measure nanogram amounts of Landrin insecticide and related carbamates after their reaction with trifluoroacetic anhydride. Concentrations of Landrin as low as 0.02 ppm in corn can be measured by this simple procedure. The trifluoroacetylated carbamates can be separated from one another on a 6-ft column packed with Gas Chrom Q coated with either 2% Reoplex 400 or 3% OV-17. The electron capturing derivative obtained from 3,4,5-trimethylphenyl, N-methyl carbamate, the major component of an insecticide marketed under the trademark Landrin, has been identified as carbamic acid, methyltrifluoroacetyl-3,4,5-trimethylphenyl ester by infrared spectroscopy and elemental analysis.

Carbamate pesticides are important in the field of insect control. Accordingly, methods for separating and identifying microquantities of carbamates by gas chromatography have been studied and reported. These methods have usually been based upon the reaction of the phenols obtained from the hydrolyzed or altered carbamates. Gutenmann and Lisk (1965) developed a gas chromatographic method for carbaryl which depended on hydrolysis to the phenol, followed by bromination and acetylation. Likewise, Butler and McDonough (1968) hydrolyzed carbaryl and other carbamates to their respective phenols, which were then acetylated with trichloroacetyl chloride.

Unlike previous workers, we have acetylated the intact carbamate moiety, using trifluoroacetic anhydride. The trifluoroacetyl derivatives thus produced are characterized and measured on a gas chromatograph equipped with an electron-capture detector.

The determination of amines (Clarke *et al.*, 1967; Dove, 1967; McCurdy and Reiser, 1966) and amino acids (Gee, 1967; Gehrke and Stalling, 1967; Lamkin and Gehrke, 1965) by gas chromatography after converting them to trifluoroacetyl derivatives is well documented. This paper adds the carbamic acid esters to the list of compounds forming electron-capturing derivatives with trifluoroacetic anhydride.

This paper describes procedures for the determination of Landrin in corn. Landrin is the brand name for a mixture of two isomeric N-methylcarbamates: 3,4,5-trimethylphenyl, N-methylcarbamate, the major component, and its 2,3,5-trimethylphenyl isomer. Both isomers are simultaneously extracted from corn tissues with acetonitrile; the extract is cleaned up by passing through a two-component chromatographic column; and the carbamates are converted to their respective trifluoroacetyl derivatives with trifluoroacetic anhydride. The two derivatives are separated on a 6-ft glc column packed with Gas Chrom Q coated with 3% OV-17, and are quantitated with an electron-capture detector. This separation can also be achieved using a 2% Reoplex 400 column.

EXPERIMENTAL

Apparatus. The gas chromatograph initially used in these studies was a Wilkens Aerograph Hy-Fi, Model 600-B, equipped with a tritium foil electron-capture detector. The column used was 6 ft × 1/8 in. aluminum packed with 2% Reoplex 400 on 80/100-mesh Gas Chrom Q. Column and inlet temperatures were 170° and 180° C, respectively. Inlet nitrogen pressure was 14 psi. Under these conditions, the two Landrin derivatives emerged from the column at 1.5 (major peak) and 1 min. Subsequently, a Varian Model 1200 gas chromatograph, also equipped with a tritium foil electron-capture detector, was used. The column was 6 ft × 1/8 in. stainless steel packed with 3% OV-17 on 100/120-mesh Gas Chrom Q. Column and inlet temperatures were 185° and 190° C, respectively. Inlet nitrogen pressure was 40 psi. Retention times in this case were 3 min for the major component and 2 min for the minor component.

The chromatographic cleanup column used was a miniaturized glass column with sintered glass plate (Figure 1).

Reagents. Solvents were reagent grade, redistilled shortly before use; other common chemicals also were reagent grade. The alumina was obtained from Fisher Scientific Co., No. A-540, 80/200-mesh. The trifluoroacetic anhydride was

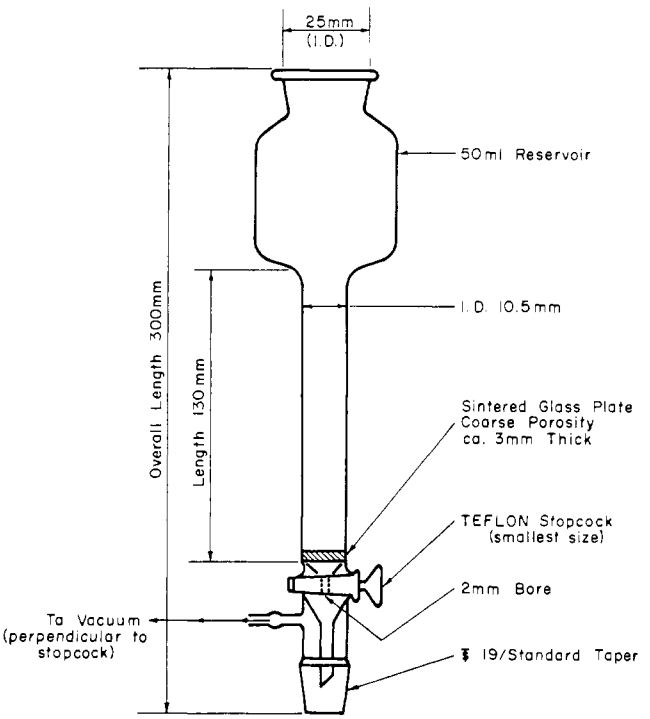


Figure 1. Miniature chromatograph column

Shell Development Co., Biological Sciences Research Center, Modesto, Calif. 95352

[1] Shell trademark

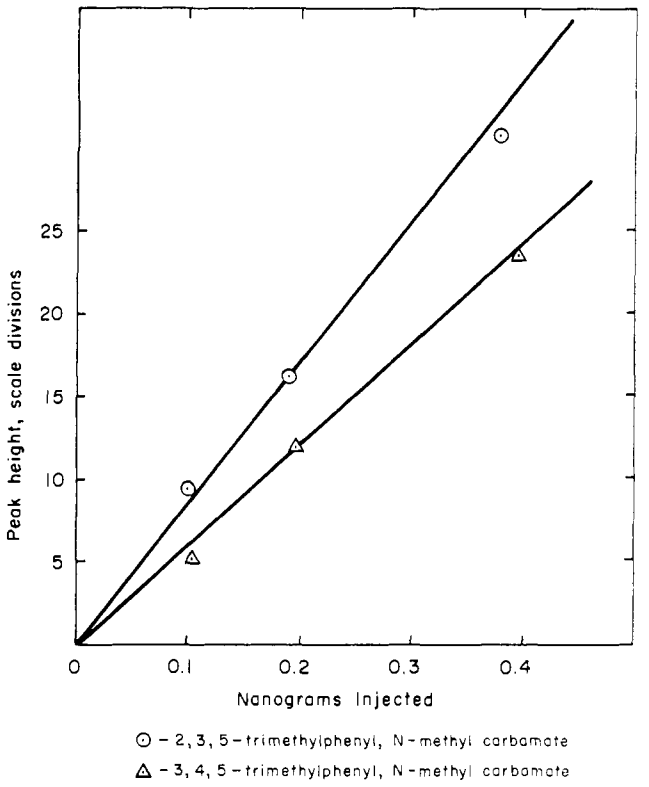


Figure 2. Standard curves for Landrin insecticide after derivatization with trifluoroacetic anhydride

reagent grade, Eastman No. 7386, 98+% pure. Solka Floc, Grade BW-40, was obtained from Brown Co., and Darco G-60 carbon, low phosphorus, was obtained from Matheson Coleman and Bell (L-1026).

Pesticide samples were analytical standards, 99+% pure.

Procedure. The standard curves for the two isomeric carbamates comprising Landrin are developed as follows: zero, 0.5-, 1.0-, and 2.0-μg quantities of each carbamate (dissolved in 0.2-ml of ethyl acetate) are transferred to a series of 10-ml graduated cylinders. Trifluoroacetic anhydride (0.2-ml) is added to each, the cylinders are stoppered, the contents mixed by shaking, and the cylinders left overnight at room temperature, protected from light. The derivatives are taken up in 0.3-ml of ethyl ether, and 4.5-ml of distilled hexane is added to dilute the organic phase to 5.0-ml total volume, affording test concentrations of each carbamate (derivatized) at 0-, 0.1-, 0.2-, and 0.4-μg per ml. The organic solutions are immediately washed with 3 × 5 ml of distilled water to decompose and remove excess anhydride reagent, then dried over anhydrous sodium sulfate. One microliter of each standard solution is injected into the gas chromatograph. Two linear calibration curves are obtained when the responses for the two emerging peaks are plotted *vs.* the weight of each isomer injected. Figure 2 illustrates standard curves obtained from 0-, 0.10-, 0.20-, and 0.40-ng, of each of the two isomeric carbamates after derivatization with trifluoroacetic anhydride. The procedures for the extraction, cleanup, and analysis for Landrin in crops follow.

Extraction. Fifty grams of a representative corn sample were blended with 200 ml of acetonitrile in the presence of anhydrous sodium sulfate and filtered. The filtrate was washed with hexane to remove fat-soluble interferences, and 100 ml was diluted with 1000 ml of water. This aqueous solution, 440 ml, representing 10-g of corn, was extracted with 2 × 50 ml of 3-to-1 hexane-ether. The extract was dried with anhydrous Na_2SO_4 and a 5-g aliquot was concentrated to approximately 1 ml prior to cleanup and analysis.

Table I. Percentage Recovery of Landrin Components from Crops Fortified with 0.2 ppm of the Respective Carbamates Prior to Extraction and Cleanup

Crop	Recovery, %[a] 3,4,5-trimethylphenyl, N-methyl carbamate	2,3,5-trimethylphenyl, N-methyl carbamate
Corn ears	90	90
Corn stover	85–110	100–110
Corn ensilage	70–110	75–110
Oats	85	...
Soybeans	95	...

[a] Averages of two to four values.

Table II. Chromatographic Data for Trifluoroacetyl Derivatives of Some Carbamates Related to Landrin Insecticide

	Relative Retention Time	Response,[a] nanogram
SD 8530 (3,4,5-trimethylphenyl N-methylcarbamate)	1	0.4
SD 8786 (2,3,5-trimethylphenyl N-methylcarbamate)	0.7	0.2
SD 16627 [carbamic acid, methyl-4-(hydroxymethyl)-3,5-xylyl ester]	1.3	0.6[b]
SD 17557 [carbamic acid, methyl-, 3-(hydroxymethyl)-4,5-xylyl ester]	1.5	0.7[b]

[a] Responses correspond to the quantity (nanograms) of the compound which was injected to produce a half-scale deflection, a peak 7.0 cm high.

[b] These hydroxymethyl analogs of SD 8530 and SD 8786, respectively, gave better Gaussian peaks on a 6-ft column packed with Gas Chrom Q coated with a 1 to 1 mixture of 2% Reoplex 600 and 10% QF-1. On this latter column, the sensitivity for SD 16627 is 0.3 ng and for SD 17557 0.2 ng.

Cleanup. Partially deactivated alumina was prepared by spraying a fine mist of water onto tumbling 80/200-mesh alumina until the moisture content was 10 ± 1%, as measured by Karl Fischer. Solka Floc and Darco G-60 (5 to 1 w/w) were thoroughly mixed, washed with boiling acetone, then dried overnight at 100° C. Three grams of the conditioned alumina (bottom layer) and 0.2-g of the Solka-Floc Darco (top layer) were used to load a chromatographic column (Figure 1), which was then carefully calibrated so that a volume of interference-containing forecut could be discarded and a discrete Landrin-containing band collected for analysis. This was accomplished by obtaining elution profiles of Landrin and of the corn extract on passing through the column. Under these conditions, and using 3-to-1 hexane-ether as eluant, it was found that the first 10 ml of eluate contained no Landrin, but most of the corn extractives. This forecut was discarded. The Landrin emerged in the next 18 ml of eluate, and this plus an extra 2 ml was collected for analysis. Since different lots of adsorbents can vary, the chromatographic cleanup column should be checked with known mixtures of standards and adjustments should be made in elution volumes, as necessary. The ideal column should show a carbamate-free forecut of 10 ± 2-ml and a following carbamate-containing fraction of 20 ± 4 ml. To accomplish this, the ether-hexane mixture used for developing the column may need to be varied slightly.

Analysis. The Landrin-containing fraction emerging from the chromatographic column was collected and concentrated to near dryness under a jet of dry air; the residue was taken up in 0.2 ml of ethyl acetate and determined as with the standards. Table I lists percentage recovery of Landrin com-

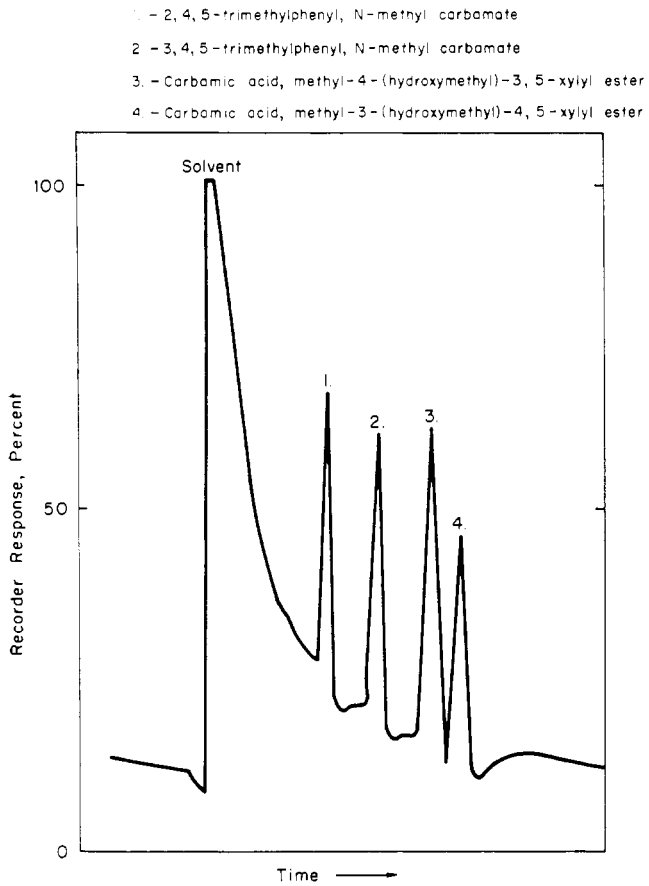


Figure 3. Chromatogram of trifluoroacetyl derivatives of carbamates related to Landrin insecticide

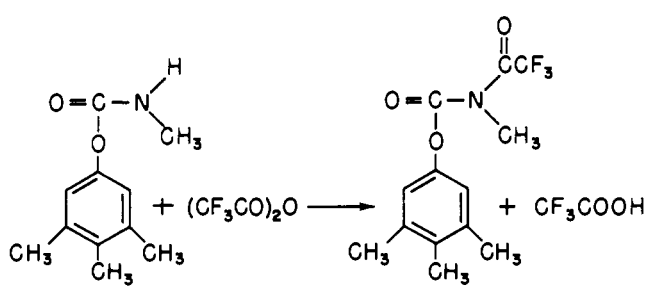
ponents from crops fortified with 0.2 ppm of the respective carbamates prior to extraction and cleanup.

Recoveries of 2,3,5-trimethylphenyl, N-methyl carbamate were usually somewhat higher than the 3,4,5-isomer. This may be due to the shorter retention time of the former on the gas chromatographic column and to its lower adsorptivity on the cleanup column. Table II lists four carbamates for which derivatives were successfully prepared, their approximate sensitivities by electron-capture gas chromatography, and their relative retention times.

Figure 3 shows a chromatogram of trifluoroacetyl derivatives of the carbamate isomers.

Figure 4 illustrates a typical glc chromatogram of residues in crops and the corresponding control sample.

Discussion. Trifluoroacetic anhydride reacts with N-methyl carbamates according to the following equation



The N-trifluoroacetyl ester structure was verified by two carbonyl bands at 1738 and 1783 cm^{-1}, respectively, and the $-CF_3$ band at 2110 cm^{-1}. Elemental analysis for nitrogen served to confirm the empirical formula, as the theoretical and actual nitrogen values were identical—*i.e.*, 4.8% nitrogen.

Preparation of the derivatives is simple, but requires a

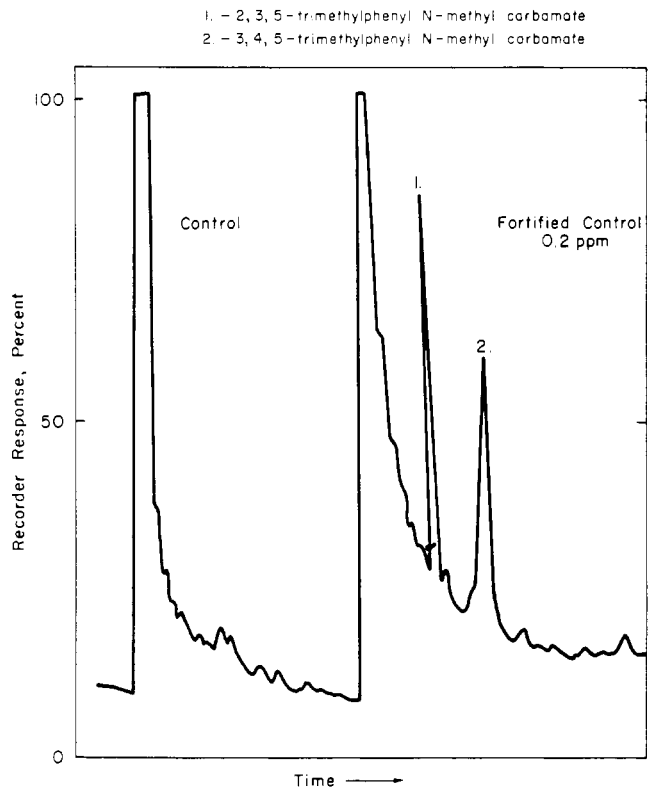


Figure 4. Chromatogram of corn ear extracts

reaction time at room temperature of 16 hr or more for quantitative conversion. A reaction time greater than this has no deleterious effect, and retention times of up to 3 days were used successfully. The time required for complete reaction could be shortened by raising the temperatures. However, at higher temperatures, results were not as reproducible as with the 16-hr-room-temperature procedure. At higher temperatures, the background "noise" was multiplied and accentuated, especially when crop extracts were analyzed.

The amount of trifluoroacetic anhydride reagent used per test was varied from 0.1 to 0.3 ml without significantly altering the extent of conversion. However, quantities less than 0.1 ml per test frequently led to low recoveries, while quantities more than 0.3 ml sometimes produced higher crop background values.

The conversion of the carbamates to their trifluoro-derivatives was carried out in ethyl acetate, because of consistent high yields. Other solvents such as acetonitrile, ether, and hexane were examined, but yields of derivatized products were lower.

For the cleanup of crop materials, it was found that alumina partially deactivated with water could best separate the phenyl methylcarbamates from crop coextractives. Each carbamate may be eluted from such a column as a tight and discrete band. Careful calibration of such a column leads to the desired separation of the carbamates to be analyzed.

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Received for review September 22, 1969. Accepted March 18, 1970.