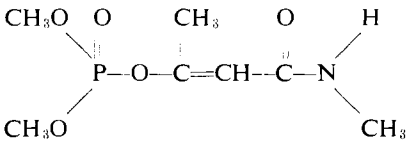
Gas Chromatographic Analysis of 3-Hydroxy-*N*-methyl-*cis*-crotonamide Dimethyl Phosphate (Azodrin) and 3-Hydroxy-*N*,*N*-dimethyl-*cis*-crotonamide Dimethyl Phosphate (Bidrin)

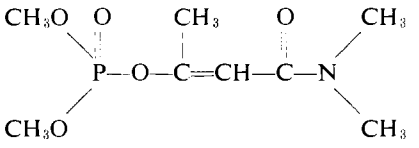
MALCOLM C. BOWMAN[1] AND MORTON BEROZA[2]

A gas chromatographic method for determining Azodrin and Bidrin utilizes a short, lightly loaded Carbowax 20M column, conditioned to Bidrin and Azodrin. The insecticides are detected with the Melpar flame photometric detector equipped with a 526-mμ filter to sense phosphorus. Peak height is proportional to concentration, and response is linear to at least 250 ng. In the analysis of extracts of sweet corn plants, almost no cleanup was required, and recoveries ranged between 94 and 101% in the 0.05- to 5.0-p.p.m. range. The method is rapid and sensitive, a fraction of a nanogram being readily detectable.

Azodrin (formerly Shell SD-9129, I) has been shown to be effective against insects that attack sweet corn (*14*) and cotton (*2*, *10*). As a part of an investigation to determine whether this insecticide could be used on sweet corn plants, a study of its persistence on field-grown corn was planned, and a rapid, sensitive analytical method was required. At the same time a method of analysis for Bidrin (formerly Shell SD-3562, II) was desired because of its similar chemical structure and its effectiveness against insects and mites that attack ornamentals and field, forage, and vegetable crops (*10*). The development of a method applicable to both pesticides seemed especially worthwhile because Bull and Lindquist (*2*, *3*), Menzer and Casida (*6*), and Hall and Sun (*4*) had reported Azodrin to be a major metabolite of Bidrin. A residue method for Bidrin should therefore include an analysis for Azodrin.



I



II

Several procedures for the analysis of the two insecticides have been reported in the past few years.

Sun and coworkers (*4*, *5*, *12*, *13*) used cholinesterase inhibition to analyze for Bidrin and Azodrin. Most recently, Lau (*5*) used this enzyme inhibition in a spectrophotometric method after differential extraction to remove interferences and partition chromatography to separate the two insecticides; the chromatographic separation and other treatments greatly improved the specificity of the method, which is sensitive to 0.1 p.p.m.

Murphy, Gaston, and Gunther (*7*) described a colorimetric method of analyzing for Bidrin. It required an acid reflux and distillation, hydrolysis with alkali, and determination of the liberated dimethylamine as the dimethyl dithiocarbamate after addition of cupric ion and carbon disulfide. Since the method responds only to dialkylamines, Azodrin was not detected. The method is sensitive to 0.2 p.p.m. in 125-gram samples.

To determine residues of Bidrin, Stevens, and Van Middelem (*11*) blended cabbage in methylene chloride with activated charcoal and magnesium sulfate, removed interferences by refluxing the macerates with 40% sodium bisulfite, reacted the methylene chloride extractive of the mixture with sodium hypoiodite, and analyzed the petroleum ether extract of the reaction product by gas chromatography with an electron-capture detector for the iodoform that was formed in the reaction. Sensitivity was 0.01 p.p.m. in 100-gram samples. This method may also be suitable for determining Azodrin but no data on this possibility were given. These authors found it possible to gas chromatograph Bidrin directly using electron-capture detection, but substrate interference was too high for a useful determination.

The present paper reports the details of a rapid, sensitive gas chromatographic method that can be used for the analysis of the two insecticides. The highly selective response to phosphorus of the Melpar flame photometric detector described by Brody and Chaney (*1*) made it possible to analyze extracts of sweet corn plants with a sensitivity of about 0.002 p.p.m. (twice noise) for each insecticide with virtually no cleanup. The compounds are analyzed directly rather than through derivatives.

Experimental

Apparatus. An F & M Scientific Corp. (Avondale, Pa.) Model 700 gas chromatograph equipped with the Melpar flame photometric detector (526-mμ interference filter) described by Brody and Chaney (*1*) was used.

Solvent. Chloroform was distilled C.P. grade.

[1] Entomology Research Division, Agricultural Research Service, U. S. Department of Agriculture, Tifton, Ga.

[2] Entomology Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Md.

Procedure

Sample Preparation and Extraction. Chop whole sweet corn plants (or the separated parts—e.g., stalks, leaves, husks, ears) in a Hobart cutter and mix well for uniformity. Add 50 grams of the chopped plant material to a Waring Blendor containing 50 grams of anhydrous sodium sulfate and 150 ml. of chloroform and blend for about five minutes. Filter the slurry through Whatman No. 1 paper and store the filtrate over anhydrous sodium sulfate at 0° C. until analysis.

Gas Chromatographic Analysis. Set up the gas chromatograph with the following operational parameters:

COLUMN. Glass, 45 cm. × 4 mm. i.d. (6 mm. o.d.)

PACKING. Carbowax 20M (w./w.), 1%, on 80- to 100-mesh Gas Chrom Q (Applied Science Laboratories, State College, Pa.)

CARRIER GAS. Nitrogen at 160 ml. per minute

OTHER GASES. Oxygen at 40 ml. per minute; hydrogen at 200 ml. per minute

COLUMN TEMPERATURES. 170° C. for Azodrin; 150° C. for Bidrin.

OTHER TEMPERATURES. Injection port 180° C.; detector (external) 180° C.

After conditioning the column overnight at 170° C., condition the column further by injecting extract and insecticide until the latter gives a constant response. Run standards frequently, such standards being made up of an untreated extract and known amounts of the insecticides.

Inject into the gas chromatograph 5 μl. of the chloroform filtrate without cleanup, either directly, diluted, or concentrated (with a jet of dry air at room temperature), as appropriate. Determine insecticide content by comparing peak heights of unknowns with those of the standards.

Results and Discussion

Samples of sweet corn plants, either untreated or spiked with the insecticides at various levels, were extracted and analyzed. The results are shown in Table I. Recoveries were between 94 and 101%. Chromatograms of the pesticides alone and those of the corn extracts with and without pesticides at column

Table I. Gas Chromatographic Analysis of Azodrin and Bidrin in Raw (No Cleanup) Chloroform Extracts of Whole Sweet Corn Plants

Pesticide	Added		Milligram Equivalents of Corn per Analysis	Recovered	
	P.p.m.	μg.[a]		μg.[a]	%
Azodrin	0.00	0.00	16.7	<0.1	...
				<0.1	...
	0.05	2.50	16.7	2.35	94
				2.42	97
	0.50	25.0	16.7	24.7	99
				24.4	98
	5.00	250	1.67	253	101
				248	99
Bidrin	0.00	0.00	16.7	<0.1	...
				<0.1	...
	0.05	2.50	16.7	2.40	96
				2.37	95
	0.50	25.0	16.7	24.6	98
				24.4	98
	5.00	250	1.67	248	99
				251	100
Azodrin[b] plus	0.50	25.0	16.7	24.1	96
				24.5	98
Bidrin[b]	0.50	25.0	16.7	24.6	98
				24.3	97
Azodrin[b] plus	5.00	250	1.67	247	99
				251	100
Bidrin	0.10	5.00	16.7	4.70	94
				4.75	95
Azodrin[b] plus	0.10	5.00	16.7	4.70	94
				4.80	96
Bidrin[b]	5.00	250	1.67	253	101
				250	100

[a] Per 50 grams of plant material.
[b] Single sample containing both insecticides.

temperatures of 150° and 170° C. are presented in Figures 1 and 2. One or 2 ng. of the pesticides gave good-sized peaks. With short, lightly loaded columns, the column temperature could be kept low enough to avoid decomposing the phosphates. Single peaks were obtained with both insecticides, and there was no evidence of degradation during chromatography with either compound. After the column was conditioned, peak height was proportional to concentration over three decades of concentration and to at least the 250-ng. level.

The analysis of both insecticides in the same injected sample may be carried out with the column temperature at 150° C. (Figure 1); but the Azodrin peak is much broader and shorter than the peak obtained with the column at 170° C. (Figure 2). If the analysis of both insecticides is carried out at 170° C., the Bidrin peak does not separate sufficiently from early peaks produced by the corn extractives (Figure 2). The authors preferred to analyze Bidrin with the column at 150° C. (retention time, t_R, 1.5 minutes) and Azodrin at 170° C. (t_R, 2.0 minutes).

Extraneous peaks that appeared in the analysis of low residues in the corn extracts did not interfere with the determinations, but it was necessary to allow them to elute before another sample was injected. For example, in the chromatogram of 0.833 ng. of Azodrin in a raw—i.e., with no cleanup—chloroform extract of sweet corn shown in Figure 2, the Azodrin peak at 2.0 minutes is cleanly separated from the interference, but the peak between 2.6 and 5.0 minutes must be allowed to elute before one proceeds with the next analysis. The latter peak disappeared as the plant matured.

Initially, attempts were made to extract the pesticides with acetone and with water. With acetone (sodium sulfate used), recoveries were low; and with water, almost no response was obtained. In later experiments,

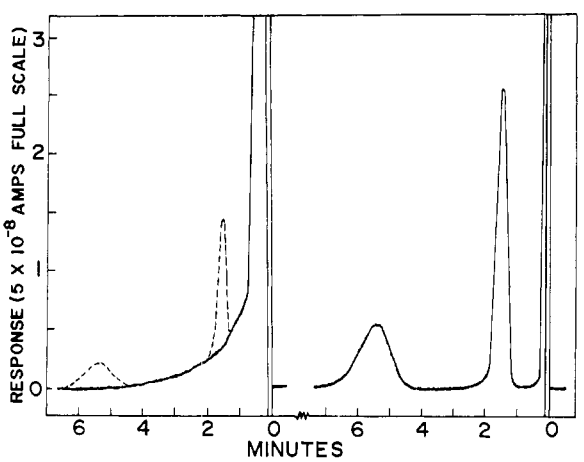


Figure 1. Gas chromatographic analysis of Azodrin (t_R, 5.40 minutes) and Bidrin (t_R, 1.50 minutes) with column temperature at 150° C.

Right, 2 ng. of each injected in chloroform; left, 0.833 ng. (0.05 p.p.m.) of each in a chloroform extract equivalent to 16.67 mg. of sweet corn (corn extract, solid line; insecticide peaks, dotted lines). The interfering peak from corn has not yet emerged

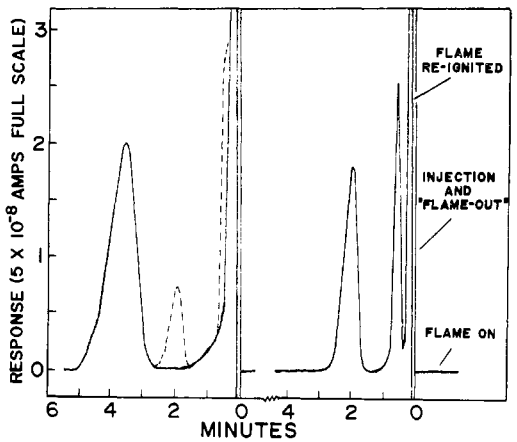


Figure 2. Gas chromatographic analysis of Azodrin (t_R, 2.00 minutes) and Bidrin (t_R, 0.60 minute) with column temperature at 170° C.

Right, 2 ng. Azodrin and 1 ng. Bidrin injected in chloroform; left, 0.833 ng. (0.05 p.p.m.) of each in a chloroform extract equivalent to 16.67 mg. of sweet corn (corn extract, solid line; insecticide peaks, dotted lines). Peak between 2.6 and 5.0 minutes is interference from corn

the authors discovered that the injection of fresh aqueous solutions of the pesticides also produced no response, a result which leads them to believe that in the presence of water the insecticide decomposes in the gas chromatograph, probably by hydrolysis.

The number of reports dealing with Bidrin and Azodrin that have appeared in the past few years indicates considerable interest in these insecticides. Data on residues of Bidrin have been reported on and in mature oranges and in laboratory-processed citrus pulp cattle feed (*7*, *8*), in alfalfa (*9*), in cabbage (*11*), and in cotton seed and seedling plants (*2*). The persistence of Azodrin in cotton seedlings has also been reported (*3*). Sesamex inhibits the formation of Azodrin from Bidrin in houseflies, *Musca domestica* L., and increases the toxicity of Bidrin (*4*, *6*). The metabolism of Azodrin and Bidrin in plants, insects, and mammals has been studied in detail by radiometric analysis (*2*, *3*, *4*, *6*).

Acknowledgment

The assistance of F. G. Crumley, scientific aide, is gratefully acknowledged. We thank the Shell Chemical Company for the analytical grade samples in Azodrin and Bidrin used in these studies.

Literature Cited

(1) Brody, S. S., Chaney, J. E., *J. Gas Chromatog.* **4,** 42 (1966).
(2) Bull, D. L., Lindquist, D. A., J. AGR. FOOD CHEM. **12,** 310 (1964).
(3) *Ibid.*, **14,** 105 (1966).
(4) Hall, W. E., Sun, Y. P., *J. Econ. Entomol.* **58,** 845 (1965).
(5) Lau, S. C., J. AGR. FOOD CHEM. **14,** 145 (1966).
(6) Menzer, R. E., Casida, J. E., *Ibid.*, **13,** 102 (1965).,
(7) Murphy, R. T., Gaston, L. K., Gunther, F. A., *Ibid.*, **13,** 242 (1965).
(8) Murphy, R. T., O'Neal, J. R., Ewart, W. H., Gunther, F. A., *Ibid.*, **13,** 550 (1965).

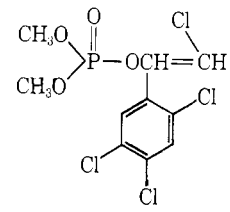
(9) Shaw, F. R., Callahan, R. A., Holbrook, F. R., *J. Econ. Entomol.* **59,** 487 (1966).
(10) Shell Chemical Co., Agricultural Chemicals Division, New York, N. Y., "Summary of Basic Data for Technical Bidrin Insecticide," May 1963, and "Summary of Basic Data for Azodrin Insecticide," April 1965.
(11) Stevens, R. D., Van Middelem, C. H., J. AGR. FOOD CHEM. **14,** 149 (1966).
(12) Sun, Y. P., Johnson, E. R., *J. Econ. Entomol.* **58,** 838 (1965).
(13) Sun, Y. P., Lau, S. C., Johnson, E. R., *J. Assoc. Offic. Agr. Chemists* **48,** 938 (1965).
(14) Young, J. R., Entomology Research Division, Tifton, Ga., unpublished data.

Received for review October 6, 1966. Accepted January 11, 1967. Mention of proprietary products is for identification only and does not constitute endorsement of these products by the U. S. Department of Agriculture.

Correction

GAS CHROMATOGRAPHIC DETERMINATION OF COMPOUND 4072 AND SHELL SD-8447 BY ELECTRON-CAPTURE AND FLAME PHOTOMETRIC DETECTION

In this article by Morton Beroza and M. C. Bowman [J. AGR. FOOD CHEM. **6,** 625 (1966)], Shell SD-8447 should be a dimethyl ester rather than a diethyl ester as indicated. The correct nomenclature and structural formula for SD-8447 are:



2-Chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate