Gas Chromatographic Analysis of Ciba C-9491 [*O*-(2,5-Dichloro-4-iodophenyl) *O,O*-Dimethyl Phosphorothioate], Its Oxygen Analog, and Its Phenolic Hydrolysis Product in Sweet Corn and Milk

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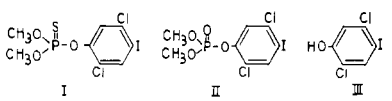
A gas chromatographic method was devised for determining residues of Ciba C-9491 [*O*-(2,5-dichloro-4-iodophenyl) *O,O*-dimethyl phosphorothioate], its oxygen analog [2,5-dichloro-4-iodophenol dimethyl phosphate], and its phenolic hydrolysis product [2,5-dichloro-4-iodophenol] in sweet corn and milk. The three compounds were separated by liquid chromatography on two columns, silica gel and alumina. The columns were operated first in tandem, which allowed the Ciba C-9491 to pass through, and then separately to elute the oxygen analog from the silica gel with acetone and the phenol from the alumina with methanol. The gas chromatography of the Ciba C-9491 and its oxygen analog fractions was carried out with a flame photometric detector sensitive to phosphorus, while that of the phenol fraction was conducted with an electron-capture detector. Other than the separation of the three compounds, cleanup was minimal. Recoveries of Ciba C-9491 were 93 to 100%, those of the oxygen analog were 82 to 93%, and those of the phenol were 53 to 71%. Sensitivity was better than 0.01 p.p.m.

Ciba C-9491 [formula I, *O*-(2,5-dichloro-4-iodophenyl) *O,O*-dimethyl phosphorothioate, Ciba Agrochemical Co., Vero Beach, Fla.] is a nonsystemic insecticide with a low mammalian toxicity (LD_{50} for the rat is 2000 mg. per kg.). It is recommended for control of lepidopterous larvae, aphids, flies, mosquito larvae, and many other insects.



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At the Georgia Coastal Plain Experiment Station (Tifton, Ga.), Ciba C-9491 was found effective against insects that attack sweet corn (Young, 1967), and a method of determining the persistence of its residues in corn intended for human consumption as well as for livestock feed was needed. Residues may consist of the insecticide itself, its oxygen analog (formula II, hereafter O-analog), and the phenol (formula III) formed on hydrolysis of the insecticide. Since the corn plant is fed to cows, the analysis of these residues in milk was also needed.

A procedure was devised for separating the three compounds by liquid chromatography on short columns of silicic acid and alumina and then determining each one by gas chromatography on appropriately conditioned columns. Ciba C-9491 and its O-analog were determined with an instrument equipped with the flame photometric detector of Brody and Chaney (1966) set up to sense phosphorus; the phenol was determined with the electron-capture detector. Aside from the separation of the three compounds, no cleanup other than a solvent partition of the milk fraction containing Ciba C-9491 was applied. Recoveries from milk and corn in the 0.05- to 5.0-p.p.m. range were: Ciba C-9491, 93 to 100%; O-analog, 82 to 93%; and the phenol, 53 to 71%. Sensitivity for the three compounds ranged between 0.002 and 0.005 p.p.m.

MATERIALS AND METHODS

Reagents and Solvents. Silica gel (J. T. Baker Chemical Co., No. 3405) and alumina (Fisher Scientific Co., A-540 adsorption alumina, 80- to 200-mesh) were used as received. The adsorbents lost 2.65 and 2.48% of their weight, respectively, after being heated overnight at 110° C.

Acetone, benzene, hexane, acetonitrile, and methylene chloride were redistilled C.P. grade solvents. Absolute methanol was used as received. Sodium sulfate was anhydrous reagent grade chemical.

Analytical samples of Ciba C-9491, its O-analog, and its phenolic hydrolysis product were furnished by Ciba Agrochemical Co.

Apparatus. Two gas chromatographs were used. One was an F & M Scientific Corp. (Avondale, Pa.) Model 700 gas chromatograph equipped with the Melpar flame photometric detector (526-mμ interference filter) of Brody and Chaney (1966). [The detector is now available from MicroTek Instruments, Inc., Baton Rouge, La.] The other was a Jarrel-Ash Model 700 instrument equipped with an electron-capture detector.

Extraction of Corn. Chop stalks and leaves of sweet corn plants (forage portion) in a Hobart cutter and mix well. Blend 50 grams with an equal weight of anhydrous sodium sulfate and 150 ml. of benzene for 5 minutes in a Waring Blendor. (Fortify samples with one or more of the three compounds in 1 ml. of benzene prior to blending.) Filter the product through Whatman No. 1 paper by

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gravity, and store over sodium sulfate. The extract contains the equivalent of 1 gram of corn per 3 ml.

Extraction of Milk. Shake the sample to disperse the cream uniformly, and add 100 grams to a Waring Blendor. [If the sample is to be fortified, add the compound(s) in 1 ml. of acetone and blend for 1 minute.] Add 300 ml. of acetone, blend for 3 minutes, and filter through Whatman No. 1 paper on a Büchner funnel. Wash the blender and filter funnel with an additional 25 ml. of acetone. Extract the filtrate with 200 and then 100 ml. of methylene chloride, and percolate each methylene chloride extract successively through a plug of sodium sulfate about 4 cm. in diameter and 5 cm. thick. Evaporate the percolate almost to dryness under a Snyder column on a steam bath, and then just to dryness at room temperature under water pump vacuum (ca. 35 mm. of Hg). Add 10 ml. of benzene to dissolve the fatty residue, and reserve the solution for the liquid chromatographic separation.

Liquid Chromatographic Separation of C-9491, Its O-Analog, and Its Phenol. The separation is most conveniently accomplished with two columns as shown schematically in Figure 1. Prepare one of the columns (2-cm. I.D., Shell type) by adding successively 5 grams of sodium sulfate, 5 grams of silica gel, and 10 grams of sodium sulfate. Prepare the other in a filtering tube (Corning No. 9480, 2.5-cm. I.D.) by adding successively a small plug of glass wool, 10 grams of sodium sulfate, 5 grams of alumina, and 10 grams of sodium sulfate. Position the tube containing alumina directly beneath the silica gel column, and add 50 ml. of benzene to prewash the adsorbents in both the column and the filter tube. Discard the filtrate. Add 60 ml. of the extract of corn (equivalent to 20 grams) or the 10-ml. benzene extract of milk (equivalent to 100 grams) to the silica gel column, and allow it to percolate into the column. Use small portions of benzene to a total of 10 ml. to wash the container, and then wash the extract into the adsorbent. Elute the column with 80 ml. of benzene allowing the eluate to pass through the filtering tube. Collect this eluate; it contains the Ciba C-9491.

Remove the filtering tube, and elute it with 50 ml. of absolute methanol. This fraction contains the phenol.

Elute the silica gel column with 50 ml. of acetone. This fraction contains the O-analog.

Ciba C-9491 in Corn. Evaporate the benzene eluate to near dryness by using a water pump vacuum (ca. 35 mm. of Hg) and a warm water bath (ca. 60° C.). Adjust the volume with benzene as appropriate, and inject a 5-μl. aliquot directly into the gas chromatograph with the flame photometric detector. For high sensitivity, adjust the volume to 2 ml. (5 μl. is equivalent to 50 mg. of corn).

Ciba C-9491 in Milk. Evaporate the benzene eluate as described for corn, but bring it completely to dryness. Transfer the fatty residue with 5 ml. each of pre-equilibrated hexane and acetonitrile to a 30-ml. separatory funnel, and shake for 1 minute. After allowing the layers to separate, remove and reserve the acetonitrile layer. Extract the hexane layer with another 5-ml. portion of pre-equilibrated acetonitrile, and combine the two acetonitrile extracts adjusting their volume to 10 ml. if required. Discard the hexane layer. [Theoretically, 93% of the Ciba C-9491 is recovered because its p-value is 0.26—i.e., $1 - (0.26)^2 = 0.93$ (Equation 4 of Beroza and Bowman, 1966). Accordingly, a correction was applied to the recoveries of Ciba C-9491 for the 7% loss in the partitioning step.] Inject 5 μl. of the acetonitrile solution (equivalent to 50 mg. of milk) directly into the gas chromatograph equipped with the flame photometric detector.

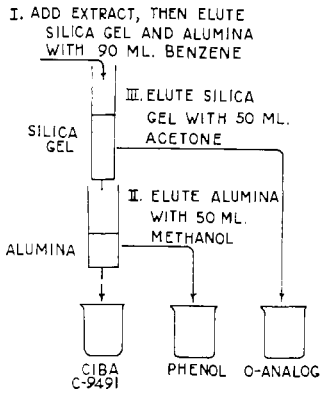

Figure 1. Means of separating Ciba C-9491, its O-analog, and its phenol by liquid chromatography on silica gel and alumina

O-Analog in Corn or Milk. Evaporate the acetone eluates from the silica gel columns to near dryness under a Snyder column on a steam bath. Adjust to the appropriate volume with acetone, and inject 5 μl. of the solution directly into the gas chromatograph with the flame photometric detector. For high sensitivity, adjust the volume of the corn extract to 2 ml. (5 μl. is equivalent to 50 mg. of corn) and the milk extract to 5 ml. (5 μl. is equivalent to 100 mg. of milk).

Phenol in Corn or Milk. Transfer the 50 ml. of methanol eluate from the alumina column to a 250-ml. separatory funnel containing 25 ml. of a saturated solution of sodium chloride and 50 ml. of distilled water. Extract twice with 50-ml. portions of benzene, and successively percolate the extracts through a plug of sodium sulfate (ca. 2.5 × 3 cm. thick). Evaporate the benzene extract to near dryness with a water pump vacuum and a water bath at 60° C. Make the residue up to an appropriate volume, and inject 5 μl. of the solution into the gas chromatograph equipped with an electron-capture detector. Adequate sensitivity was obtained by adjusting the volume of the corn extract to 5 ml. (5 μl. is equivalent to 20 mg. of corn) and the milk extract to 10 ml. (5 μl. is equivalent to 50 mg. of milk).

Gas Chromatography. Use the following conditions for analyses with the flame photometric detector: column, 50-cm. × 4-mm. I.D. glass; packing, 10% w./w. DC 200 on 80- to 100-mesh Gas Chrom Q (Applied Science Lab., State College, Pa.) preconditioned overnight at 230° C.; gases, nitrogen (carrier) 160 ml. per minute, oxygen 40 ml. per minute, hydrogen 200 ml. per minute; temperatures, column 160° C., injection port 180° C., detector (external temperature) 180° C.

The column was easily conditioned for Ciba C-9491 by a few 250-ng. injections of the insecticide in extract equivalent to 50 mg. of corn or milk. When several successive trials with 5-ng. amounts of insecticide in the same amount of extract produced a constant response, the column was considered conditioned (Shuman and Collie, 1963; Bowman and Beroza, 1966). Conditioning of the column for the O-analog was similarly accomplished but was much slower; extracts containing 250-ng. amounts of O-analog were injected for 4 to 8 hours to attain a constant response with

5-ng. amounts of the compound (response grows gradually until constant). The conditioned state maintained itself as long as no prolonged stoppage in analysis occurred and was readily regained by repeating the conditioning; however, the time required for conditioning decreased as the column was used (about 1 hour on a well conditioned column). Standards were checked frequently. Response of the compounds (peak height) was linear over at least three decades of concentration and varied less than 10% from day to day. With these conditions, the retention time of Ciba C-9491 was 4.40 minutes, and that of its O-analog was 3.50 minutes.

Use the following conditions for analyses with the electron-capture detector: column, 50-cm. × 4-mm. I.D. glass; packing, 20% w./w. SE-30 on 80- to 100-mesh Gas Chrom Q preconditioned overnight at 230° C.; carrier gas, nitrogen at 200 ml. per minute; temperatures, column 190° C., injection port (on column) 190° C., detector 200° C.; range, 10^{-9} amp. full scale, attenuation × 2.5 with 10-mv. recorder.

With these conditions, the retention time of the phenol was 1.00 minute. (Retention times of Ciba C-9491 and its O-analog were 6.70 and 5.20 minutes, respectively.)

Conditioning of the column for maximum response to the phenol and extracts as described required several hours, but once the column was conditioned, response remained satisfactory, even when the column was not used for 16 hours (overnight). Peak height of the phenol was linear with concentration to about 5 ng. per 5-μl. injection.

RESULTS AND DISCUSSION

Figure 2 shows typical chromatograms of Ciba C-9491 and its O-analog both in pure form (standards) and in corn extracts. No interferences were observed in the analysis of the corn extracts or the extracts from milk (accordingly, no chromatogram of the milk extract is shown). Figure 3 shows chromatograms of the phenol in pure form and in the milk and corn extracts. Interference is present but is very minor.

The results of analyzing corn, both unfortified and fortified with the three compounds at various levels, are given in Table I; the samples were carried through the entire procedure. Recoveries of Ciba C-9491 were close to quantitative (97 to 100%) and those of the O-analog were at least 90% (90 to 93%). Recoveries of the phenol were low (53 to 60%). When the phenol was carried through the procedure without the substrate—i.e., separation by liquid chromatography and gas chromatographic analysis—recovery was quantitative. Thus the phenol appears to conjugate with ingredients in the substrate and thereby prevent complete recovery. In the analysis of several other phenols derived from insecticides (Bowman and Beroza, 1967a, b), recoveries have also been low. Since the phenol is not considered a toxicant, the determination of its residue was not obligatory. However, the analysis does provide an estimate of the amount of phenol present. Completeness of extraction should be checked on the product analyzed.

Should it be desirable to analyze only the parent compound in corn, the analysis may be readily accomplished by injecting into the gas chromatograph the raw benzene extract appropriately concentrated or diluted. The phenol and oxygen analog do not interfere.

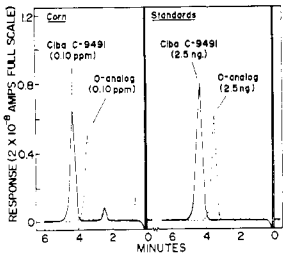

Figure 2. Chromatograms of Ciba C-9491 (I) and its O-analog (II) obtained with the flame photometric detector

Right, 2.5 ng. of each injected in 5 μl. of benzene and acetone, respectively; left, extract equivalent to 20 mg. of corn fortified with 0.10 p.p.m. (2 ng.) of C-9491 or O-analog injected in 5 μl. of benzene or acetone, respectively. Small peaks before the main ones were caused by solvent interference

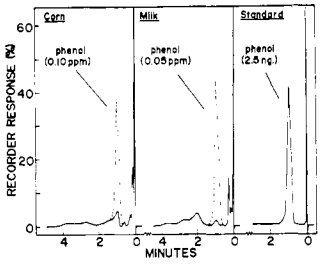

Figure 3. Chromatograms of phenol (III) obtained with the electron-capture detector

Right, in 5 μl. of benzene; center, in 5 μl. of benzene containing extract equivalent to 50 mg. of milk fortified with 0.05 p.p.m. (2.5 ng.) of phenol; left, in 5 μl. of benzene containing extract equivalent to 20 mg. of corn fortified with 0.10 p.p.m. (2 ng.) of phenol

Table I. Gas Chromatographic Analysis of Ciba C-9491, Its Oxygen Analog, and Phenol in Sweet Corn Plants

Trial	Compound	Added P.p.m.	Added μg.[b]	Mg. Equivalent of Crop/ Analysis	Recovered[a] μg.[b]	Recovered[a] %
1	C-9491	0	0	50	<0.15	...
	O-analog	0	0	50	<0.20	...
	Phenol	0	0	20	<0.24[c]	...
2	C-9491	0.50	25	20	24.7	99
	O-analog	0.50	25	20	23.2	93
	Phenol	0.50	25	5	14.9	60
3	C-9491	5.00	250	5	249	100
	O-analog	0	0	50	<0.13	...
	Phenol	0	0	20	<0.24[c]	...
4	C-9491	0	0	50	<0.10	...
	O-analog	0.50	25	20	23.0	92
	Phenol	0	0	20	<0.24[c]	...
5	C-9491	0	0	50	<0.10	...
	O-analog	0	0	50	<0.13	...
	Phenol	0.50	25	5	14.2	57
6	C-9491	5.00	250	5	248	99
	O-analog	0.10	5.0	20	4.50	90
	Phenol	0.10	5.0	20	2.65	53
7	C-9491	0.05	2.5	50	2.42	97
	O-analog	0.05	2.5	50	2.28	91
	Phenol	0.05	2.5	20	1.32	53

[a] Mean of duplicate analyses.
[b] Per 50 grams of plant material.
[c] Based on twice the interference in the unfortified sample.

Although the stalks and leaves of whole sweet corn plants—i.e., forage—were analyzed, results were essentially identical in typical trials—e.g., No. 2 of Table I—that included the ears, husks, and silks excluded from forage.

The recoveries of the three compounds from milk are given in Table II. Respective recoveries of Ciba C-9491 at 0.5 and 0.05 p.p.m. were 96 and 93%, those of the O-analog at 0.1 and 0.05 p.p.m. were 86 and 82%, and those of the phenol at 0.1 and 0.05 p.p.m. were 71%.

The minimum detectable quantity (based on twice the noise level) of Ciba C-9491 injected with extract equivalent to 50 mg. of milk or corn was 0.003 p.p.m., and similar values for the O-analog injected with extract equivalent to 50 mg. of corn and 100 mg. of milk were 0.004 and 0.002 p.p.m., respectively. The minimum detectable amount of phenol in the trials listed in Tables I and II was 0.005 p.p.m.

Although the three compounds respond to electron-capture detection (each contains three halogen atoms in its molecule), the flame photometric detector was greatly preferred for the analysis of Ciba C-9491 and the O-analog. Unlike the electron-capture detector, the flame photometric detector is not easily contaminated by essentially unclean extracts, and its response is much more specific; thus, the need for a time-consuming cleanup is avoided. Other advantages are its linear response with concentration over a wide range, the capability of attenuating its response, the lesser interference from crop extracts, and the more stable base line.

Since the phenol contains no phosphorus or sulfur, the flame photometric detector does not respond to it, and the electron-capture detector had to be used for its determination. The phenol could not be chromatographed satisfactorily on the column used to analyze C-9491 and the O-analog, because it emerged too quickly and tailed badly. Satisfactory results were obtained with a heavily loaded (20%) column of SE-30 which increased the retention time and reduced the tailing of the phenol peak.

Although the three compounds have different retention times, their separation prior to analysis was considered advantageous (or necessary) for a number of reasons. The retention times of Ciba C-9491 and its O-analog are less than a minute apart, and large amounts of the parent compound could mask the presence of small amounts of O-analog. Since both Ciba C-9491 and its O-analog respond to electron-capture detection, it would be necessary to wait until they emerged from a phenol analysis before another sample could be injected. With these compounds removed, the phenol determinations are rapid, and large amounts of the insecticide and extracts do not have to be put through the electron-capture detector. Finally and most important, the separation of C-9491 and its O-analog removes the bulk of the electron-capturing interferences and provides an excellent cleanup for the phenol determination, which is highly desirable and probably necessary to avoid contaminating the electron-capture detector.

Separation of the three compounds on a single column of silica gel and alumina could not be readily accomplished, because the O-analog does not elute from the alumina with a reasonable volume of acetone. Both the O-analog and phenol could be eluted with methanol.

Table II. Gas Chromatographic Analysis of Ciba C-9491, Its Oxygen Analog, and Phenol in Milk

Trial	Compound	Added P.p.m.	Added μg.[b]	Mg. Equivalent of Milk/ Analysis	Recovered[a] μg.[b]	Recovered[a] %
1	C-9491	0	0	50	<0.15	...
	O-analog	0	0	100	<0.10	...
	Phenol	0	0	50	<0.26[c]	...
2	C-9491	0.50	50	50	47.8[d]	96
	O-analog	0.10	10	50	8.60	86
	Phenol	0.10	10	25	7.13	71
3	C-9491	0.05	5	50	4.63[d]	93
	O-analog	0.05	5	50	4.12	82
	Phenol	0.05	5	50	3.55	71

[a] Mean of duplicate analyses.
[b] Per 100 grams of milk.
[c] Based on twice the interference in the unfortified sample.
[d] Corrected for 7% loss in the partitioning cleanup.

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LITERATURE CITED

Beroza, M., Bowman, M. C., *Anal. Chem.* **38,** 837 (1966).
Bowman, M. C., Beroza, M., J. AGR. FOOD CHEM. **15,** 894 (1967a).
Bowman, M. C., Beroza, M., *J. Assoc. Offic. Anal. Chemists* **49,** 1154 (1966).
Bowman, M. C., Beroza, M., *J. Assoc. Offic. Anal. Chemists* **50,** 926 (1967b).
Brody, S. S., Chaney, J. E., *J. Gas Chromatog.* **4,** 42 (1966).
Shuman, H., Collie, J. R., *J. Assoc. Offic. Agr. Chemists* **46,** 992 (1963).
Young, J. R., U.S. Department of Agriculture, Tifton, Ga., private communication, 1967.

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