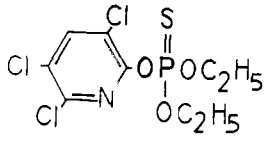
Determination of Dursban and Its Oxygen Analog in Corn and Grass by Gas Chromatography with Flame Photometric Detection

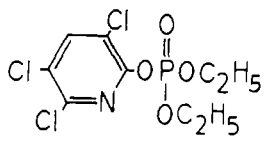
MALCOLM C. BOWMAN AND MORTON BEROZA

Dursban [*O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate] and its oxygen analog [*O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphate] were determined by gas chromatography on a glass column containing 5% w./w. DC 200 on 80- to 100-mesh Gas Chrom Q at 150° C. after the two compounds were separated by liquid chromatography on a silica gel column. The Melpar flame photometric detector, which responds with high specificity to phosphorus, was used. Conditioning of the column to the insecticides and the substrate was required. Recoveries of Dursban and its oxygen analog in the 0.1- to 5.0-p.p.m. range from grass and corn silage were 96 to 99% and 85 to 90%, respectively. Response (peak height) of the compounds was linear to at least 250 ng. Sensitivity of the method was between 0.002 and 0.010 p.p.m., with crop interference occurring only in analyses at levels at 0.010 p.p.m. or less.

Dursban [*O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate (I)] is a promising new insecticide that is active against a wide spectrum of insects (Collier and Dieter, 1965; Gray, 1965; Kenaga *et al.*, 1965; Rigterink and Kenaga, 1966). Its synthesis and its chemical, physical, and toxicological properties have been recently reported. A method was needed to determine residues of this material and its oxygen analog [*O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphate (II)] in corn and grass silage intended for use as feed for livestock.



I



II

A rapid gas chromatographic procedure suitable for the quantitative determination of nanogram amounts of I and II in corn and grass silage was developed. After extraction from the substrate, the two compounds were separated by liquid chromatography on a silica gel column and then subjected to gas chromatography on an instrument equipped with the flame photometric detector of Brody and Chaney (1966) sensitive to phosphorus. The gas chromatographic column required conditioning to Dursban and its oxygen analog before use.

Experimental

Reagents and Solvents. The silica gel, a product of J. T. Baker Chemical Co., was used as received. It lost 2.65% of its weight (presumably water) when heated overnight at 110° C.

Dursban and its oxygen analog were analytical samples (Dow Chemical Co., Midland, Mich.).

Entomology Research Division, Agricultural Research Service, U.S. Department of Agriculture, Tifton, Ga. 31794 and Beltsville, Md. 20705.

The acetone and benzene were C.P. grade solvent redistilled.

Equipment. An F & M Scientific Corp. (Avondale, Pa.) Model 700 gas chromatograph was equipped with the Melpar flame photometric detector (Brody and Chaney, 1966) and a 526-mμ interference filter (detects phosphorus) for these analyses. This flame photometric detector is at present being marketed by Micro-Tek Instruments, Inc., Baton Rouge, La.

Extraction and Liquid Chromatography. Chop the spiked or unspiked sample in a Hobart cutter and blend 50 grams with an equal weight of anhydrous sodium sulfate and 150 ml. of benzene for about 5 minutes in a Waring Blendor. Filter the product through Whatman No. 1 paper by gravity and store the extract over sodium sulfate.

Prepare the silica gel column by introducing successively, in a 2-cm. i.d. chromatographic column, a 1-cm. layer of anhydrous sodium sulfate, 10 grams of silica gel, and a 2-cm. layer of anhydrous sodium sulfate. Prewash with 50 ml. of benzene. Add 30 ml. of the extract (equivalent to 10 grams of sample), wash the extract into the adsorbent with a few small portions of benzene, and then add a total of 60 ml. of benzene to the column. Discard the first 20 ml. of eluate and collect the next 70 ml. for the Dursban analysis. Next elute the column with 60 ml. of acetone and reserve this eluate for analysis of the oxygen analog of Dursban. Concentrate the eluates at room temperature with jets of warm air and adjust the volumes with benzene in accordance with the level of residue—e.g., adjust to 2 ml. for analyses at the 0.10-p.p.m. level or to 10 ml. for analyses at the 5-p.p.m. level. (The 5-p.p.m. analysis can be made easily without concentrating the eluate.)

Gas Chromatographic Conditions. The following conditions were employed:

Column, 4-mm. i.d. × 45-cm. glass.

Packing, 5% w./w. DC 200 on 80- to 100-mesh Gas Chrom Q (Applied Science Laboratories, State College, Pa.) preconditioned for 2 days at 240° C.

Gases, nitrogen (carrier) 160 ml. per minute, oxygen 40 ml. per minute, hydrogen at 200 ml. per minute.

Temperatures, column 150° C., injection port 180° C., detector (external temperature) 180° C.

The column was easily conditioned for Dursban by several injections of 250-ng. amounts of insecticide in extract equivalent to 50 mg. of plant. The column was considered conditioned when several successive injections of 5-ng. amounts of the insecticide in the plant extract (0.1 p.p.m.) produced a constant response. Standards (usually 5 ng.) in plant extract were run frequently during analyses to determine the response factor and to be certain that the conditioned state was maintained. Conditioning was maintained as long as there was no prolonged stoppage in the analysis of samples and standards. When a decline in response of the standard was noted, the 250-ng. injections were resumed until test runs of the 5-ng. injections gave a constant response.

Conditioning the column for the oxygen analog was accomplished in the same manner, but it was a much slower process. Repeated injections of extract and 250-ng. amounts of the oxygen analog for 4 to 8 hours were required to achieve a constant response with 5-ng. injections (response gradually grows) when the columns were first conditioned; the conditioning time decreased as the column was used (about 1 hour on a well conditioned column). Once the column was conditioned, the day-to-day variation in response of the compounds was less than 10%.

Gas Chromatographic Analysis. Using the stated conditions, inject on the conditioned column 5 μl. of the column eluate either directly or appropriately concentrated. The retention time of Dursban was 1.95 minutes, that of its oxygen analog was 1.85 minutes.

Results and Discussion

The results of typical analyses of Dursban and its oxygen analog in spiked and unspiked samples of Coastal Bermuda grass and corn silage are given in Table I. Satisfactory analyses were obtained with both high and low levels of the compounds in the presence of each other. Dursban and its oxygen analog were also added separately to the substrates, and complete separation of the two compounds from each other in the liquid chromatography was demonstrated by gas chromatographing the Dursban and oxygen analog fractions; no Dursban was found in the oxygen analog fraction and vice versa. Response of the compounds (peak height) was proportional to concentration to at least 250 ng. Recovery of Dursban was 96 to 99%; that of its oxygen analog was 85 to 90%. The limit of sensitivity of each compound was about 2 p.p.b. (twice noise level).

A chromatogram of 2.5 ng. of each of the compounds is shown in Figure 1. As noted in the authors' previous work (Bowman and Beroza, 1966a, 1966b), the response of the oxygen analog is less than that of its thio counterpart even though the two compounds contain roughly the same amount of phosphorus.

When an attempt was made to attain sensitivities below 0.01 p.p.m., an interfering peak was found (retention time 1.90 minutes) in the Dursban fraction of grass extracts. As seen in Figure 1, the magnitude of the interference peak (solid line) was small compared with that of 2.5 ng. of Dursban run under identical conditions (broken line). This interference limited the sensitivity of the Dursban analyses in grass to 0.01 p.p.m. However, this interference caused no difficulty because it diminished as the plant matured, the interference becoming negligible as the residue dropped to low levels. Thus, when high sensitivity was needed, the interference practically disappeared. Values in Table I were corrected for the interference. In addition, small interference peaks with a retention time of

Table I. Gas Chromatographic Analyses of Dursban and Its Oxygen Analog in Coastal Bermuda Grass and Silage

Substrate	Pesticide[a]	Added		Mg. Equivalent of Crop/ Analysis	Recovered[c]	
		P.p.m.	μg.[b]		μg.[b]	%
Coastal Bermuda grass	D	0	0	25	0.50	...
	DOA	0	0	25	<0.10	...
	D	0.10	5.00	25	4.80[d]	96[d]
	DOA	0.10	5.00	25	4.41	88
	D	0.10	5.00	25	4.88[d]	98[d]
	DOA	5.00	250	5.0	226	90
	D	5.00	250	5.0	247	99
	DOA	0.10	5.00	25	4.23	85
	D	5.00	250	5.0	243	97
	DOA	5.00	250	5.0	225	90
Corn silage	D	0	0	25	<0.10	...
	DOA	0	0	25	<0.10	...
	D	0.10	5.00	25	4.90	98
	DOA	5.00	250	5.0	220	88
	D	5.00	250	5.0	240	96
	DOA	0.10	5.00	25	4.30	86

[a] D = Dursban, DOA = Dursban's oxygen analog.
[b] Per 50 grams of plant material.
[c] Mean of duplicate analyses.
[d] Corrected for apparent Dursban content (0.010 p.p.m.) of unspiked sample.

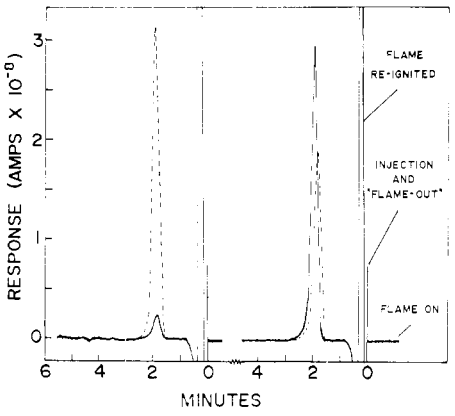


Figure 1. Chromatograms of Dursban and its oxygen analog

Right. 2.5 ng. each of pure Dursban (solid line) and its oxygen analog (broken line). Left. Unspiked extract (25 mg. equivalents) of Coastal Bermuda grass (solid line) compared with that of the extract plus 2.5 ng. of pure Dursban (broken line) run under identical conditions

13.5 minutes were observed in the Dursban fraction of grass extracts and the oxygen analog fractions of grass and corn extracts. In analyses at high sensitivity, these zones should be allowed to emerge from the column to avoid interference in subsequent runs. Several analyses can be run, and the interfering zones can then be allowed to emerge.

The authors (Bowman and Beroza, 1966a, 1966b) have noted that the need for column conditioning to obtain maximum response parallels the polarity of the compounds being chromatographed, which accounts for the conditioning requirements in the gas chromatography of a thiophosphate (P=S) being much less than in that of its oxygen analog. This finding holds true for Dursban and its oxygen analog. The repeated injections of compound and substrate undoubtedly satisfy or cover reactive or adsorptive sites and thereby allow the compound to migrate through the column with minimum alteration or adsorption. Since the conditioned state of the column diminishes on prolonged stoppage of analysis, the authors concluded that the saturation of reactive or adsorptive sites is reversible, and that conditioning sets up a steady-state condition with constant bleeding of pesticide and extract; the bleed probably causes no more than a slightly elevated base line that is not noticeable. Column conditioning, as used in this paper, has been recommended by Shuman and Collie (1963) in their analysis of pesticides.

After Dursban and its oxygen analog were separated by liquid chromatography, the highly specific response of the flame photometric detector toward the phosphorus in the two insecticidal molecules allowed the analyses to be made with high sensitivity and with no further cleanup. Because of the high specificity of the detector, the method will undoubtedly be applicable to the analysis of the two compounds in other substrates with little or no modification.

Possible interference of other phosphorus-containing pesticides may be useful information. As part of a study now in progress, 20 of these pesticides were gas chromatographed with temperature programming on the packing used in this study. Only malathion and parathion had retention times close to that of Dursban. However, Dursban is eluted much earlier than malathion or parathion on a 5% QF-1 column.

Acknowledgment

The authors thank the Dow Chemical Co. for providing the insecticides used in this study.

Literature Cited

Bowman, M. C., Beroza, M., *J. Assoc. Offic. Anal. Chemists* **49,** 1046 (1966a).
Bowman, M. C., Beroza, M., *J. Assoc. Offic. Anal. Chemists* **49,** 1154 (1966b).
Brody, S. S., Chaney, J. E., *J. Gas Chromatog.* **4,** 42 (1966).
Collier, B. L., Dieter, C. E., *Down Earth* **21**(3), 3 (1965).
Gray, H. E., *Down Earth* **21**(3), 2 (1965).
Kenaga, E. E., Whitney, W. K., Hardy, J. L., Doty, A. E., *J. Econ. Entomol.* **58,** 1043 (1965).
Rigterink, R. H., Kenaga, E. E., J. AGR. FOOD CHEM. **14,** 304 (1966).
Shuman, H., Collie, J. R., *J. Assoc. Offic. Agr. Chemists* **46,** 992 (1963).

Received for review November 25, 1966. Accepted April 12, 1967. Mention of proprietary products is for identification only and does not necessarily imply endorsement of these products by the U.S. Department of Agriculture.