GAS CHROMATOGRAPHIC IDENTIFICATION OF CHLORINATED INSECTICIDES BASED ON THEIR U.V. DEGRADATION*,**

K. A. BANKS AND D. D. BILLS
Department of Food Science and Technology, Oregon State University, Corvallis, Ore. (U.S.A.)
(Received October 20th, 1967)

SUMMARY

Certain chlorinated insecticides can be identified by electron capture gas–liquid chromatographic (EC-GLC) analysis of the prepared sample before and after treatment with ultraviolet irradiation. Upon U.V. treatment, characteristic degradation products arise from the insecticides investigated. Comparison of the degradation pattern obtained by EC-GLC of unknown and authentic insecticides provides an adequate identification when coupled with the p-value (partition ratio between two immiscible solvents) of the unknown and authentic insecticides and degradation products.

INTRODUCTION

A problem often encountered by the residue chemist is the confirmation of the identity of a chlorinated insecticide (CI) detected by gas–liquid chromatography (GLC). At present, identification is frequently based on the coincidence of retention time of the authentic and unknown compound on each of two or more dissimilar GLC columns, by obtaining a response for the unknown compound with both the electron capture and microcoulometric detector, and by comparison of the thin-layer chromatographic R_F values of the authentic and unknown compound. Although these methods are useful, they cannot be considered as a rigorous means for the identification of a compound. Infrared spectroscopy can yield a conclusive identification, but it is usually difficult to isolate sufficient CI from a biological sample for an analysis. Mass spectrometry provides an excellent means for the identification of relatively small quantities of a compound, but adequate pre-purification of a CI isolated from a sample and the introduction of compounds with such low vapor pressures into the mass spectrometer present difficulties.

Recently, BEROZA AND BOWMAN[1] proposed a procedure to aid in the identification of pesticide residues. Their method involved the partitioning of the pesticide between two immiscible liquid phases and the GLC analysis of each phase at equilibrium. The ratio of the quantities of a given pesticide in the two phases was referred to as the p-value (partition value).

* Technical Paper No. 2374, Oregon Agricultural Experiment Station.
** Supported in part by a PHS Pesticides Toxicology Training Grant No. 5 TOL ES 55-03 to the senior author.

Since the advent of pesticide chemistry, several reports have dealt with the degradation of CIs by ultraviolet irradiation[2–10].

This study was designed to evaluate the usefulness of the U.V. degradation patterns (as detected by electron capture GLC) as a means of identification of some of the common CIs. In addition, the p-values of the parent compounds and U.V. degradation products were determined.

EXPERIMENTAL METHODS

The CIs used in this study were analytical standards dissolved in hexane (free of interfering substances) in concentrations of 1.0, 2.5, 5.0 and 10.0 p.p.m. For irradiation, 2.5 ml of the insecticide solution was placed in a quartz cuvette (Beckman Standard Silica 1 mm absorption cell) fitted with a Teflon cap. The cuvette was positioned near the center of the U.V. beam and at a distance of 14 cm from the front of the lamp (Hanovia Utility Ultraviolet Quartz Lamp). The intensity of radiation of wavelengths of 3130 Å and shorter produced by this lamp was approximately 250 microwatts per cm^2 at a distance of 50 cm. Radiation of 1849 to 4000 Å wavelengths was transmitted by the quartz lens. Following irradiation, the solutions were analyzed by GLC. The analytical instrument was an F & M Model 810, equipped with an electron capture detector. The analyses were conducted under the following conditions: carrier gas, 95 % argon–5 % methane; injection port, 205°; detector, 205°; column, 190°; column flow, 70 ml/min. The GLC column was constructed of 4 mm I.D. by 120 cm borosilicate glass packed with 10 % DC-200 on Diatoport S. GLC peak areas were computed throughout this study by the method proposed by CARROLL[11].

To study the effect of irradiation time on the degradation patterns, solutions of 1.0 p.p.m. of each CI were analyzed after being irradiated for various lengths of time. The length of irradiation required to yield the most characteristic degradation pattern for a given insecticide was termed the optimum irradiation time (OIT). The influence of the concentration of CI in hexane solution on the degradation pattern when irradiated for the OIT was determined by irradiating five concentrations ranging from 0.2 to 10.0 p.p.m. of CI.

The p-values for all parent and degradation compounds were determined in a hexane–acetonitrile system by the method of BEROZA AND BOWMAN[1]. Equilibration of the insecticide between the two phases was carried out at 27.5°.

RESULTS AND DISCUSSION

Heptachlor epoxide, heptachlor, dieldrin, aldrin and DDD were 50 % degraded after 42, 57, 35, 23 and 52 min of irradiation, respectively (Fig. 1). The OIT selected for these compounds was 60 min since at this time all, except DDD, yielded at least one degradation peak which was approximately equivalent in size to the remaining, undegraded parent peak. At 60 min, DDD yielded two degradation peaks which were about one-fourth as large as the remaining parent peak. Further irradiation of DDD for periods of as long as 120 min did not increase the size of the degradation peaks in relation to the parent peak and only resulted in a further reduction in the amount of undegraded parent compound and its degradation products.

The OITs selected for DDE and DDT were 6 and 15 min, respectively. After

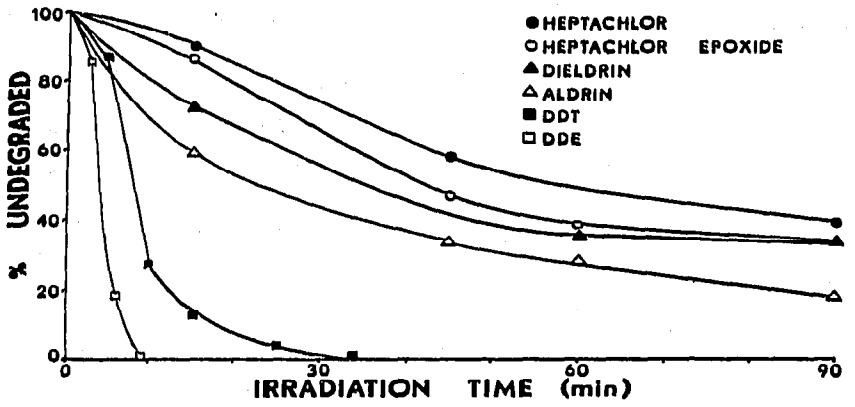

Fig. 1. Per cent of insecticide remaining undegraded after exposure to U.V. irradiation for various times. DDD (not shown) at 15, 30, 60 and 90 min was 12, 26, 56 and 63 % degraded, respectively.

U.V. irradiation for 9 min the parent DDE peak was completely absent in the chromatogram. DDT required 35 min for complete destruction.

Except for aldrin and dieldrin, the above compounds yielded more than one degradation peak at their chosen OIT (Table I). The degradation peaks are numbered in order of elution from the GLC column in all tables.

Tables I and II show the GLC elution positions and size of the degradation

TABLE I

RETENTION TIMES OF DEGRADATION PEAKS RELATIVE TO RETENTION TIME OF PARENT PEAK

Compound	*Degradation peaks*					
	1	*2*	*3*	*4*	*5*	*6*
Dieldrin	0.755					
Aldrin	0.752					
Heptachlor	0.760	0.812	0.900	1.160	1.285	1.400
Heptachlor epoxide	0.706	0.840				
DDT	0.303	0.513	0.594	0.751		
DDD	0.406	0.500				
DDE	0.552	0.779	1.118			

TABLE II

RATIO OF PEAK AREA OF DEGRADATION PRODUCTS TO PEAK AREA OF REMAINING PARENT COMPOUND AT OPTIMUM IRRADIATION TIME FOR ANALYSIS

Compound	*Irradiation time (min)*	*Ratio*[a]					
		1	*2*	*3*	*4*	*5*	*6*
Dieldrin	60	1.978					
Aldrin	60	0.405					
Heptachlor	60	0.539	0.579	0.021	0.015	0.051	0.071
Heptachlor epoxide	60	0.016	1.092				
DDT	15	0.255	0.018	0.038	0.061		
DDD	60	0.018	0.082				
DDE	6	0.575	0.149	0.605			

[a] Ratio: peak area of degradation product/peak area of parent compound.

peaks relative to the parent compound peaks when irradiated at the selected OITs. The following degradation peaks were small compared to their parent compound peak, and were not apparent on the chromatogram unless sufficient sample was injected to yield a full-scale response for the remaining, undegraded parent peak: heptachlor epoxide: No. 1; DDT: Nos. 2, 3, 4 and 5; DDD: No. 1; and heptachlor: Nos. 3 and 4.

The results of the concentration study are presented in Table III. These data reveal some variation in percent degradation with changes in concentration, but the variation was small over short concentration increments.

p-Values for all compounds and their degradation products are listed in Table IV.

TABLE III

PER CENT UNDEGRADED PARENT COMPOUND REMAINING AFTER U.V. IRRADIATION

Compound	*U.V. irradiation time (min)*	*Initial concentration in solution (p.p.m.)*				
		0.2	*1.0*	*2.5*	*5.0*	*10.0*
Dieldrin	60	35.5	35.6	35.6	36.4	44.8
Aldrin	60	29.6	33.0	27.4	23.6	21.1
Heptachlor	60	61.4	63.0	61.5	53.7	60.3
Heptachlor epoxide	60	36.5	40.5	42.2	45.6	56.6
DDT	15	[a]	16.9	14.7	22.4	36.4
DDD	60	[a]	22.6	17.8	11.3	9.5
DDE	6	[a]	7.5	8.0	9.5	11.3

[a] Not determined.

TABLE IV

p-VALUES OF PARENT INSECTICIDES AND THEIR DEGRADATION PRODUCTS

Compound	*p-Values*[a,b]						
	Parent	*1*	*2*	*3*	*4*	*5*	*6*
Dieldrin	0.335	0.210					
Aldrin	0.511	0.710					
Heptachlor	0.753	0.446	0.386	0.287	0.124	0.163	0.463
Heptachlor epoxide	0.290	[c]	0.118				
DDT	0.346	0.201	[c]	0.263	0.210		
DDD	0.145	[c]	[c]				
DDE	0.602	0.279	0.523	0.592			

[a] p-Value = concentration in acetonitrile/concentration in hexane.
[b] Average of two trials.
[c] Completely partitioned into acetonitrile.

At present, the identities of the degradation products of the compounds involved in this study are unknown. The degradation product of dieldrin noted in this study may be the pentachloro derivative obtained through U.V. irradiation by HENDERSON AND CROSBY[5] since the GLC relative retention time on similar columns is in agreement. Other photochemical products have been obtained from dieldrin[4,7]. The products of the U.V. degradation of DDT have been reported[2,3,10]. However, for

the purpose of identification of the parent compound by this method, the identity of the degradation products need not be known.

Using the GLC parameters described herein, DDT, DDD and DDE can be identified in any combination. Dieldrin can be analyzed in combination with DDT and DDD or with heptachlor and heptachlor epoxide. Aldrin and dieldrin can be analyzed in combination. The above combinations are frequently encountered in biological samples. Although some degradation peaks were obscured, sufficient resolution was obtained for the analysis of all the CIs included in this study when they were combined and analyzed. Obviously, the GLC columns and operating conditions can be found that will provide better resolution for various combinations of CIs and their degradation products. It is clear at this point, however, that the sample must be prepared in a manner that excludes nearly all interfering non-insecticide compounds from the final hexane solution. The standard Florisil cleanup procedure[12,13] has proven adequate for lipid or lipid extracts analyzed thus far.

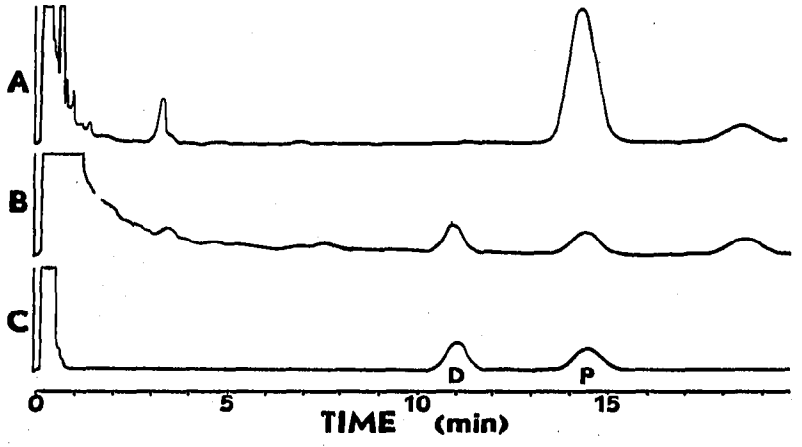

Fig. 2. Identification of dieldrin at a level of 10 p.p.b. in hamburger. Chromatogram A: hexane solution of the sample preparation. Chromatogram B: hexane solution of the sample preparation following U.V. irradiation for 45 min. Chromatogram C: hexane solution of a similar concentration of authentic dieldrin following U.V. irradiation for 45 min. P = parent peak (dieldrin). D = degradation product.

The utility of this method as an aid in the identification of chlorinated insecticide residues occurring in biological samples is demonstrated in Fig. 2. A 10 g sample of hamburger was extracted with hexane–petroleum ether (1:1) and the extract passed through a Florisil column[12,13] to remove the bulk of the lipid material. The eluate was analyzed by GLC after suitable concentration, and the chromatogram revealed a peak having the appropriate retention time for dieldrin. Based on the assumption that the component was dieldrin, its concentration was determined from a standard curve prepared from the injection of known amounts of authentic dieldrin. The eluate was then concentrated to 2.5 ml and irradiated for 45 min, rather than 60 min, due to the low initial concentration of the compound suspected to be dieldrin. GLC analysis of the irradiated eluate disclosed a degradation peak whose retention time agreed with that of the degradation peak resulting from the irradiation of a similar concentration of authentic dieldrin. The ratio of the area of the degradation peak to the area of the remaining parent peak was 0.859 for the sample and 0.932 for authentic dieldrin. The p-values obtained for the degradation and parent peak in the

sample were 0.355 and 0.161, respectively; for authentic dieldrin, the p-values obtained were 0.335 and 0.210, respectively. The concentration of dieldrin in the original hamburger sample was approximately 10 p.p.b. However, the data yield a reasonably conclusive identification of dieldrin in the hamburger sample at even this low concentration. Needless to say, concentrations of CIs of this magnitude are nearly impossible to identify by other classical means. For concentrations of insecticides that occur in the usual range of interest (around 1 p.p.m.) the method yields data in closer agreement between unknown and authentic compounds.

The following method is proposed for the identification of U.V. degradable chlorinated insecticides or similar compounds:

(1) Prepare sample for electron capture GLC analysis with final solution in hexane.

(2) Examine by GLC and estimate the concentration of each suspected chlorinated insecticide present.

(3) Concurrently irradiate the sample solution and solutions of comparable concentrations of authentic chlorinated insecticides. If suspected compounds have different OITs, it will be necessary to split the sample or run duplicate samples to obtain the correct U.V. exposure of each compound.

(4) Following irradiation, determine the p-values of the parent and degradation compounds from aliquots of the sample solution and the authentic compound solutions.

(5) Analyze each irradiated solution by GLC.

(6) Compare GLC patterns for retention time of degradation peaks, ratio of the size of degradation peaks to the size of the undegraded parent peak, and percent destruction of parent peak.

(7) Compare p-values of the components of the irradiated sample solution and the irradiated solutions of authentic compounds.

The concurrent irradiation of authentic compounds with each analysis could be eliminated after sufficient experience has been acquired. It would be advisable, however, to include known compounds periodically to compensate for the diminishing radiation intensity that occurs with the repeated use of most U.V. sources. The use of dissimilar GLC columns for the analysis of the parent compounds and degradation products and the determination of p-values in additional two-phase systems[1] would add to the conclusiveness of the identification.

Further work is in progress to extend this method to the analysis of other pesticide residues.

REFERENCES

1 M. BEROZA AND M. C. BOWMAN, *Anal. Chem.*, 37 (1965) 291.
2 ELMER E. FLECK, *Ind. Eng. Chem.*, 40 (1948) 706.
3 ELMER E. FLECK, *J. Am. Chem. Soc.*, 71 (1949) 1034.
4 R. B. HARRISON, D. C. HOLMES, J. ROBURN AND J. O'G. TATTON, *J. Sci. Food Agr.*, 18 (1967) 10.
5 G. L. HENDERSON AND D. G. CROSBY, *J. Agr. Food Chem.*, 15 (1967) 888.
6 C. F. LI AND R. L. BRADLEY, Jr., *J. Dairy Sci.*, 50 (1967) 944.
7 J. MITCHELL, *J. Assoc. Offic. Agr. Chemists*, 44 (1961) 643.
8 J. ROBINSON, A. RICHARDSON AND B. BUSH, *Bull. Environ. Contam. Toxicol.*, 1 (1966) 127.
9 J. D. ROSEN, D. J. SUTHERLAND AND G. R. LIPTON, *Bull. Environ. Contam. Toxicol.*, 1 (1966) 133.
10 H. F. WICHMANN, W. I. PATTERSON, P. A. CLIFFORD, A. K. KEIN AND H. V. CLABORN, *J. Assoc. Offic. Agr. Chemists*, 29 (1946) 218.
11 K. K. CARROLL, *Nature*, 191 (1961) 377.
12 W. A. MOATS, *J. Assoc. Offic. Agr. Chemists*, 46 (1963) 172.
13 B. E. LANGLOIS, A. R. STEMP AND B. J. LISKA, *J. Agr. Food Chem.*, 12 (1964) 243.