Confirmation of Pesticide Residue Identity, Part III. Derivative Formation in Solid Matrix for the Confirmation of Endrin by Gas Chromatograph

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As discussed in Part II of this series (1), derivation of pesticide residue in solid matrix offers certain advantages which are lacking in the conventional "wet" method of derivation. Especially noted is the simplicity of the procedure, the economy of glassware used, and the minimum mechanical loss involved. In addition, because of the simplicity of the procedure, an analyst can handle a few dozen chemical confirmatory tests simultaneously. Unlike some of the derivation procedures carried out in solution, this approach of using a solid matrix for derivation for subsequent GLC analyses appears to be easier to carry out even by a person with minimum knowledge in pesticide chemistry and little experience in chemical derivation techniques. Since the approach of using solid matrix for the confirmation of DDT-type compounds and chlordanes has been proved successful in routine analysis in the Water Quality laboratories in Ottawa, this approach is being extended to other pesticides. This paper reports results on the derivation of endrin in solid matrix for confirmation of its identity.

It is well known that endrin is isomerized thermally (2), photochemically (3) and also under acidic media (4,5) to a hexachloro-pentacyclic ketone (1). Up to the time of writing this communication, there appears to be only two types of methods reported for the confirmation of endrin by chemical derivation-gas chromatographic technique. One type of method is based on the acid-catalyzed intramolecular isomerization of endrin (4) as investigated independently by Chau et al (5,6), and by Wiencke and Burke (7). Another method (8) resulted from the original finding of Chau (6,9,10,11) is based on the consecutive acid-catalyzed isomerization and reductive dechlorination isomerization in acidic chromous chloride solution. These two approaches involve reaction of endrin or sample extract suspected to contain endrin with the appropriate reagent followed by the appropriate work-up of the reaction mixture involving some or all of the following steps: extraction, neutralization, washing, drying and concentration of reaction extract, and subsequent examination of extract by GLC. In this paper, the author wishes to report an alternative procedure for the acid-catalyzed isomerization of endrin in solid matrix for confirmatory purpose particularly for water samples. The present approach of using solid matrix and the previous reported method for acid-catalyzed isomerization in solution (5) are also discussed and compared.

Bulletin of Environmental Contamination & Toxicology,
Vol. 8, No. 3, 1972, © by Springer-Verlag New York Inc.

Materials and Methods

Reagents

(Unless specified, use reagents without prior purification; however, test all chemicals and solvents for interferences before use.)

a) Petroleum ether, ethyl acetate and benzene - commercially available Pesticide Grade Solvents.

b) Concentrated Sulphuric acid (H_2SO_4), analytical grade.

c) Technical endrin standard (99% purity) - City Chemical Corporation, U.S.A. Purify endrin by column chromatography as described previously (6). Omit purification if pure standard is available.

d) Endrin hexachloro-pentacyclic ketone (I) - Prepared as reported previously (6).

e) Alumina, acidic, activity grade one, Woelm - Purify alumina as described in Part II of this series.

f) Alumina/sulfuric acid (Al_2O_3/H_2SO_4) solid matrix - Dilute 10 ml of concentrated sulfuric acid with 5 ml water. Cool in ice bath for 20-30 minutes. In a pestle and mortar grind rapidly to mix thoroughly 100 gram of ice-cold acidic alumina with the dilute sulfuric acid. Transfer the mixture to a glass-stoppered flask and tumble for two hours at room temperature. Keep solid matrix in a tightly closed glass container in a dessicator.

Instruments

Gas chromatography - Dual channels Varian gas chromatograph Model 2800 was equipped with tritium electron capture detector and 6" x 1/4" o.d. coiled glass column packed with 3% OV-101 on 80-100 mesh chromosorb W, HP. Operating parameters were: temperature, (^{o}C) - column 215^{o}C, detector 220^{o}C, injector block 220^{o}C; nitrogen carrier gas, 70 ml/min - 75 ml/min.

Apparatus

1) Kontes evaporative concentrator (K-569350) or equivalent

2) Kontes concentrator tube (K-570050)

Sample Extraction and Clean-Up

See Part I of this series (12)

TABLE I

Effects of Water and Sulfuric Acid Contnent on the Derivation of Endrin to Ketone I by Solid Matrix*

No.	Solid Mat. $Al_2O_3/H_2SO_4/H_2O$	Conc. of Endrin Tested	Reaction Time at Room Temp.	Yield of I
1	50gm/10ml/0ml	1 ug	1½ hr. or overnight	< 5%
2	50gm/5ml/0ml	1 ug	1½ hr. or overnight	< 5%
3	50gm/5ml/2½ml	1 ug	1½ hr. or overnight	70-75%
4	50gm/2½ml/2½ml	1 ug	1½ hr. or overnight	65-70%
5	50gm/5ml/5ml	1 ug	1½ hr. or overnight	65-70%
6	50gm/5ml/10ml	1 ug	1½ hr. or overnight	< 50%
7	50gm/10ml/10ml	1 ug	1½ hr.	< 20%
8	50gm/5ml/20ml	1 ug	1½ hr.	< 5%
	Silicic acid (28-200mesh) H_2SO_4/H_2O	--	--	--
9	50gm/5ml/5ml	1 ug	1½ hr. overnight	< 10% < 20%

* All derivations were carried out in two inch of solid matrix and after the specified time, eluted with benzene until 5 ml of eluant was collected.

Acid-Catalyzed Isomerization of Endrin in Solid Matrix

Pack micro-columns by plugging cleaned disposable pipets with a piece of pre-cleaned glass wool. Add 1/2" of anhydrous sodium sulfate and fill column with 2" of solid support. Tap column to settle solid support and arrange set-up similar to that described in Part II (1). By means of a micro-syringe with a long needle, apply an aliquot of standard solution or concentrated clean-up extract onto the solid support. Avoid touching the glass surface inside the column with the solution. Up to 300-320 ul of standard solution or sample extract can be applied to the column. After one and one-half hours or overnight at room temperature, elute column with 5 ml of benzene and collect eluate in a 10 ml Kontes concentrator tube. Concentrate eluate in an evaporative concentrator to 0.5 ml.

Results and Discussion

The water content in the solid matrix greatly affects the yield of ketone 1. As illustrated in Table I, when the water content increases in the solid matrix, the yield of ketone I decreases. On the other hand, when no water was added in the solid matrix, the amount of ketone eluted by benzene was practically zero. In the first case, the reason may be attributed to the dilution of sulfuric acid with increase of water content in the solid matrix and hence, only sufficient to isomerize endrin partially to the ketone I. In the second case, the presence of concentrated sulfuric acid alone in the alumina increases the activity to such an extent that 5 ml benzene could not elute out ketone I which is a polar compound. The fact that substitution of a polar solvent such as ethyl acetate eluted over 65% of ketone I appears to substantiate the above postulation. Replacement of silicic acid for alumina also gave a low yield of ketone I. The best yield of ketone I was obtained from derivation of endrin in solid support no. 3 (Table I, Fig. 1). As indicated in Table I, a reaction time of 1 1/2 hours or overnight gave similar yield of ketone I; therefore, either conditions can be chosen. Reaction overnight offers an advantage in that after the reaction is set up, it can be left unattended overnight until ready for elution and analysis by GLC next morning. Figure I illustrates endrin before and after derivation in solid matrix no. 3 at room temperature for 1 1/2 hours, at which time endrin was completely reacted (based on disappearance of parent peak) at the range 50 ng-2 ug studied. For endrin concentration below 500 ng, a reaction time of 1 hour at room temperature was found to be sufficient; however, in order to ensure complete conversion at higher range, a minimum reaction time of 1 1/2 hours was chosen.

Reaction at elevated temperature did not improve the yield; in fact, at elevated temperature (e.g. 100°C) the yield decreases. In earlier experiments, various solvents were tried. Hexane only eluted ketone I partially and polar solvents were

TABLE II

Yield of endrin ketone 1 from derivation of endrin in 2" Al_2O_3/H_2SO_4 solid matrix

NO.	Conc. of endrin used	Vol. of Benzene Eluates	% Yield of endrin Ketone(I)
1	50 ng	5 ml*	75%
	"	2 ml●	70%
2	100 ng	5 ml	75%
	"	2 ml	65%
3	400 ng	5 ml	74%
	"	2 ml	57%
4	500 ng	5 ml	78%
	"	2 ml	60%
5	800 ng	5 ml	75%
	"	2 ml	62%
6	1 ng	5 ml	77%
	"	2 ml	66%
7	5 ng	5 ml	75%
8	10 ng	5 ml	65%

* The results for 5 ml benzene eluates were average of 4 trials
● The results for 2 ml benzene eluates were averaged of 2 trials

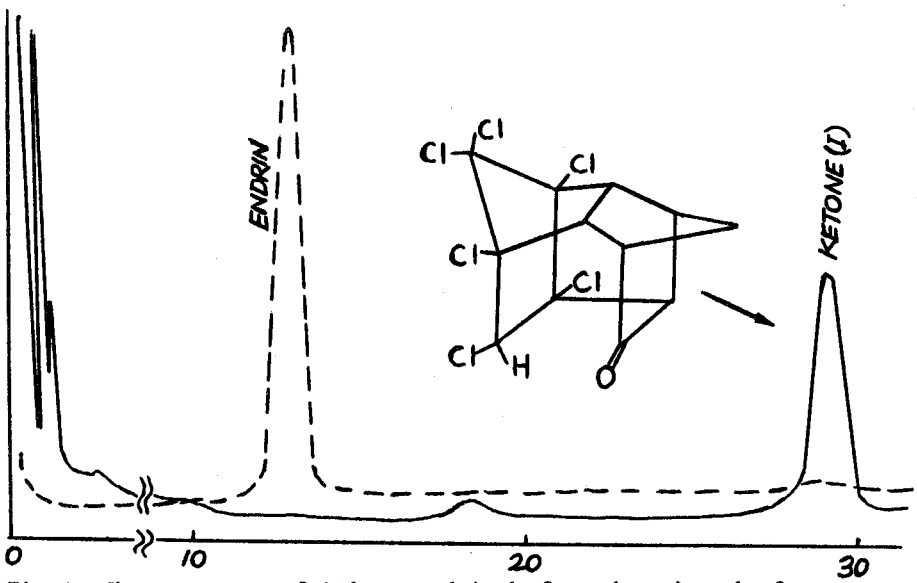

Fig.1 Chromatogram of 110 pg endrin before (----) and after (———) derivation in 2" Al_2O_3/H_2SO_4 solid matrix.

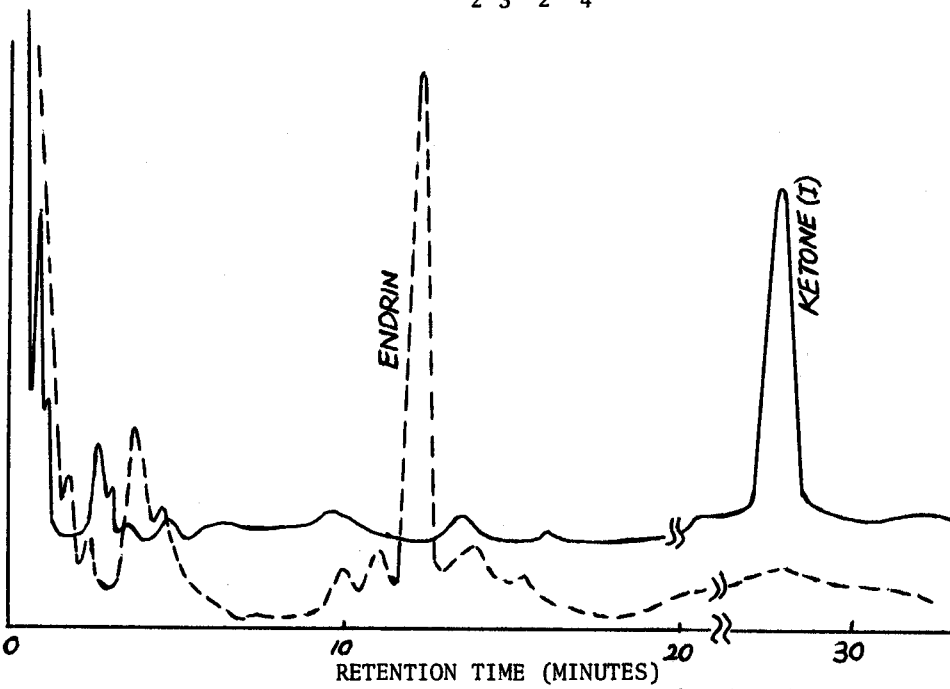

Fig.2 Chromatogram of clean-up fish extract (15% ether in petroleum ether fraction from Florisil column) containing 150 pg endrin before (----) and after (———) solid matrix derivation. Note the disappearance of the unidentified peaks and the narrowing of solvent peak.

found undesirable because the eluates could not be injected directly into the GLC systems due to "poinsoning" of ECD detectors. Furthermore, polar solvents elute more by products and other materials from the reaction column; thus, gave less clean chromatograms as when benzene was used. At 1 ug level the use of 2 ml benzene for a 2" column elute approximately 60% of the ketone I whereas 4 ml benzene elute over 90-95%. 5 ml benzene elute all the ketone I (Table II). Therefore 5 ml benzene was used in the procedure although 2 ml can be satisfactory if endrin concentration in a sample extract is high. Analogous to the situation of derivation in Al_2O_3/t-BuOK solid matrix as discussed in Part II of this series (1), the amount of solid support does not affect the yield; therfore, either a 1" or 2" column can be used for derivation. In the present described procedure a 2" column was used in order to accommodate more sample extract. On the other hand, a 1" column requires half of the volume of benzene as eluant thus saving time in evaporation of the eluate. This step is necessary due to low concentration of the parent pesticide (and hence its derivative) in the sample extract. Therefore, the use of 1" or 2" solid support for derivation is more or less a matter of personal choice. Fig. 2 illustrates a fish extract containing endrin before and after derivation in 2" solid matrix.

The yield of ketone I by the solid matrix derivation procedure is very similar to that obtained form "wet" chemistry at the upper scale of the range. At lower range, the yield is higher than from "wet"method, probably due to mechanical loss in using "wet" method which requires extractions, transferring and washing of extracts rather than the chemistry itself. In the solid matrix method, all such steps are eliminated.

As already discussed (5), other pesticide residues such as lindane, heptachlor, its epoxide, aldrin, DDT-type compounds, endosulfans and chlordanes do not interfere with the acid-catalyzed isomerization of endrin in solution or in solid matrix. Dieldrin, however, forms a compound which has close retention time to that of endrin ketone I. This compound which was previously thought to be a ketone is now proven to be a dimeric compound probably an ether based on elemental analysis, mass spectormetry and nuclear magnetic resonance spectroscopy. This and related studies will be published elsewhere with Drs. J. ApSimon and J. Buchini of Carleton University.

In the solid matrix approach, the presence of 1 ug dieldrin will yield a derivative peak less than 10% the peak height of the endrin ketone I obtained after reaction of 1 ug endrin. Thus, if the endrin to dieldrin ratio is large, the solid matrix acid - catalyzed isomerization procedure can still be used if they are present together in the sample extract. If dieldrin is present in large quantity compared with endrin, the chromous chloride conversion of endrin to a pentachloro-ketone as reported earlier (8,9) is recommended.

Conclusion:

Application of the present method to water, fish and mud extract have been used routinely in this laboratory. As little as 1 ng of endrin in a clean-up sample extract can be confirmed by this procedure.

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