Confusion of Identification of o,p′-Kelthane as Heptachlor in Orange Rind Extractives

by W. E. WESTLAKE, R. T. MURPHY, and F. A. GUNTHER
Department of Entomology,
University of California Citrus Research Center
and Agricultural Experiment Station, Riverside, California

Gas and thin-layer chromatograms of Valencia and Navel orange rind stripping solutions from mature fruits treated commercially on the trees early in the season with the acaricide 1,1-bis (p-chlorophenyl)-2,2,2-trichloroethanol (Kelthane) had revealed the presence of an unknown compound not present in control samples. It had a tlc R_f value and a glc retention time practically identical with those of heptachlor and was reported as apparent heptachlor in a "market" survey by an independent laboratory using published methods, yet today heptachlor is not used in citriculture. The data herein presented prove conclusively that this compound was not heptachlor and show that it has retention and other characteristics of o,p'-Kelthane, a compound known to be present in small amounts in technical grade Kelthane. Microcoulometric glc analysis of the stripping solution further showed the isolate contained organochlorine. Soil samples from under the treated trees contained both isomers of Kelthane but again no detectable heptachlor.

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Method

Principles

Samples of Valencia and Navel orange rinds were equilibrated with mixed hexanes. Soil samples were equilibrated with 2:1 hexane-isopropyl alcohol, the alcohol was washed out, and the hexane stripping solutions were dried over anhydrous sodium sulfate. A residue was partitioned into acetonitrile and then back into hexane by addition of water to the acetonitrile phase. After drying, the hexane was concentrated and chromatographed through a Florisil column. Aliquots of the eluate fractions were analyzed by electron-capture gas chromatography. All 3 types of column packings used to separate the organochlorine residues gave different retention times relative to aldrin, allowing partial characterization by reference to purified standards. Portions of the eluates were also fractionated by glc and by tlc for further characterization by R_f values, spectral absorption, and polarography.

Determinations

Extraction and Partition -- Five-hundred grams of finely chopped orange peel were equilibrated with 1000 ml. of mixed hexanes by end-over-end tumbling for 1 hour. After filtration,

200 ml. of the stripping solution was extracted with two 50-ml. portions of acetonitrile. Combined acetonitrile extracts, diluted with 300 ml. of water, were extracted with three 50-ml. portions of hexane. Combined hexane extracts were dried over anhydrous sodium sulfate and concentrated to 10 ml. through Snyder columns. Soil samples of 1000 g. each were equilibrated with 2:1 hexane-isopropyl alcohol by tumbling for 1 hour. The alcohol was washed out with distilled water, the hexane solutions were dried over anhydrous sodium sulfate, then concentrated to about 100 ml. through Snyder columns.

Column Chromatography -- Ten grams of Florisil was added to a Shell-type glass column (2) and washed with 100 ml. of hexane. A sample was washed onto the Florisil layer with two 10-ml. portions of hexane followed by an additional 50 ml. The eluate was collected as a single fraction beginning with the addition of the sample; heptachlor added to equivalent portions of control orange rind stripping solutions was eluted in this fraction.

Then 100 ml. of 6% diethyl ether in hexane was passed through the column and collected. Both o,p'- and p,p'-Kelthane were eluted in this fraction. Further elution with 25% ether in hexane did not remove more of these compounds from the Florisil.

Gas chromatography -- Gas chromatographic analyses of aliquots of these column fractions were with an electron-capture detector (Aerograph Pestilyzer Model 680). Most evaluations involved a 2-foot borosilicate, 1/8-inch O.D. column packed with 100/200 mesh acid-washed HMDS, Chromosorb-W support coated with 5% SF-96, at 150° C. Verifications were on a 29-cm. Teflon, 0.034-inch I.D. column packed with 60/80 mesh Teflon coated with 1.0% Apiezon L, also at 150° C. Nitrogen at 20 ml./min. was maintained through each column. Further aliquots were put through an Aerograph Autoprep 705 glc unit with electron-capture detector. A 2-foot x 1/8-inch stainless steel column packed with 3.4% QF-1 and 6.2% DC-200 on Gas Chrom Q was used, at 180° C.; nitrogen flow was 75 ml./min.

Thin-layer chromatography -- Aliquots were concentrated to near dryness and spotted on fluorescent silica gel thin-layer plates. After development with 5:1 ether-benzene, R_f values were determined. Appropriate areas were scraped off, the compounds were extracted, and the resulting solutions were gas chromatographed.

Spectrophotometry -- Infrared spectra were recorded with a Perkin-Elmer 21 instrument equipped with sodium chloride optics and 0.3-ml. cavity cells of 5-mm. light path. Ultraviolet spectra were recorded on a Beckman DK-2 instrument and silica cells of 1-cm. light path.

Polarography -- Polarograms were determined at 25° C. oscillographically with a Davis Southern Analytical Differential Cathode Ray Polarotrace, Type A.1660A equipped with an amalgamated silver wire reference electrode and Gajan micropolarographic cells. Reference solutions contained 5 to 10 μg. of compound/ml. of equal volumes of 95% ethanol and 0.2 M tetramethylammonium bromide solution.

Results and Discussion

Breakdown of purified p,p'-Kelthane to p,p'-dichlorobenzophenone in 15 to 85% yields in gas chromatographic systems has been reported by Gunther et al. (1). Both p,p'- and o,p'-Kelthane were converted in good yields to their corresponding dichlorobenzophenones in the 3 gas chromatographic systems used in this study.

Technical grade Kelthane could not be gas chromatographed reproducibly until it had been passed through the Florisil column cleanup procedure. The fraction eluted by 6% ether in hexane contained compounds with the same glc retention times as those from purified o,p'- and p,p'-Kelthane.

The similarity of the retention times of heptachlor and o,p'-Kelthane was shown by the formation of a distinct doublet peak when a mixture of the two compounds was chromatographed on the SF-96 or Apiezon L columns. The mixed QF-1-DC-200 column

completely resolved the two peaks despite their close relative retention times (0.77 and 0.94, respectively, when aldrin = 1.00).

The 6% ether fraction of control orange rind samples, processed as described, produced no peaks in the area of interest. This same fraction from subject field-treated oranges, however, showed the glc presence of two compounds with retention times identical to those of o,p'- and p,p'-Kelthane. When o,p'- and p,p'-Kelthanes were added to this fraction each corresponding peak height was increased with no indication of new doublet formation. When heptachlor was added, however, a new doublet was formed with the SF-96 and Apiezon L columns and distinct heptachlor and o,p'-Kelthane peaks resulted when the QF-1-DC-200 column was used. When both heptachlor and o,p'-dichlorobenzophenone were added to the subject orange rind extractives, using the combination column a distinct heptachlor peak and an o,p'-Kelthane (o,p'-dichlorobenzophenone) peak of correspondingly increased height resulted.

As stated earlier, heptachlor would have been eluted from the Florisil column in the hexane fraction. Gas chromatography as above of this fraction from the subject orange rind samples did not yield a peak with a retention time even remotely similar to that of ehptachlor.

Table I gives the retention times, relative to aldrin, for the 3 gas chromatographic columns used. The most notable feature

is the efficiency of the QF-1-DC-200 column in separating heptachlor and o,p'-Kelthane (o,p'-dichlorobenzophenone) despite the very small difference in retention times. This column, however, would not separate aldrin and o,p'-dichlorobenzophenone, and an analyst might easily be led to believe that aldrin was present in a routine examination of a Kelthane-treated sample. This again emphasizes the necessity for confirming gas chromatographic analyses by a more specific method.

TABLE I

Effect of column packing on retention times relative to aldrin

Compound	Time relative to aldrin		
	SF-96	Apiezon L	QF-1 + DC-200
Heptachlor	0.83	0.73	0.77
o,p'-Kelthane	0.89	1.10	0.94
o,p'-Dichlorobenzophenone	0.89	1.10	0.94
Aldrin	1.00	1.00	1.00
p,p'-Kelthane	1.20	1.80	1.16
p,p'-Dichlorobenzophenone	1.20	1.80	1.16

Attempts to confirm the identity of the unknown compound in the subject oranges by infrared spectrophotometry failed because less than 10 μg. of pooled material was available, and both glc

and tlc fractionation let some interfering substances through. Comparative ultraviolet spectrophotometry of these same fractions indicated the probable presence of o,p'-dichlorobenzophenone. Similarly, micropolarography was inconclusive because of overlap of reduction potentials (half-wave potentials were -0.66 v and -0.85 to -0.89 v for the two peaks of p,p'-Kelthane, -1.25 to -1.29 v for p,p'-dichlorobenzophenone, and -1.29 to -1.31 v for the o,p'-ketone).

The remainder of the pooled glc fractions, analyzed in a Dohrmann microcoulometric gas chromatograph, contained chlorine in the amount estimated from the previous analyses using the electron-capture detector.

Soil samples from the Kelthane-treated plots, analyzed by glc in the same manner as the rind, contained compounds that produced peaks identical with those for o,p'- and p,p'-Kelthane but no peak corresponding to heptachlor. Microcoulometric glc analyses again showed that organochlorides were present. Thin-layer chromatography of the soil extracts failed to separate the unknown from p,p'-Kelthane with the solvent system used. Thus, chromatograms developed on silica gel plates with 5:1 ether-benzene showed an elongated area with 3 distinct zones which were carefully removed, eluted, and gas chromatographed. Zone 1, with an R_f range of 0.66 to 0.73, contained the major part of a compound having a retention time identical with that of o,p'-Kelthane

Zone 2, with an R_f range of 0.54 to 0.66, contained a small amount of the unknown plus p,p'-Kelthane. Zone 3, with an R_f range of 0.47 to 0.54, gave no peaks in the area of interest. The R_f range for purified p,p'-Kelthane was 0.64 to 0.71 which agrees with the above observed distribution of this compound between zones 1 and 2. The R_f range for o,p'-Kelthane was 0.65 to 0.72. A 1:1 mixture of the 2 isomers afforded R_f 0.62 to 0.72. The R_f range for heptachlor in this system was the same as that of this mixture, making it impossible to distinguish between it and technical grade Kelthane (a mixture containing up to several % of the o,p'-isomer).

Conclusions

Both the glc and the tlc data prove that the unknown compound in the rind of Kelthane-treated oranges was not heptachlor. While the characterization is not absolutely conclusive, there can be no reasonable doubt that it is o,p'-Kelthane, known to be present in technical grade Kelthane.

Other workers, using glc, reported p,p'- and o,p'-Kelthane in the ratio of 100:1 in 21 market fruit samples from different areas of Southern California. By analogy with DDT, if technical grade Kelthane is assumed to contain up to 20% of the o,p'-isomer this ratio found on fruits treated months previously means that on and in oranges the o,p'-isomer has much the longer half-life

(RL_{50}) of the two isomers. On the other hand, approximately equal half-lives are indicated for the two isomers in soil.

This brief study emphasizes the need for checking on gas chromatographic data by a more specific method and clearly demonstrates again that improper interpretation of data may be avoided by using at least two columns possessing different retention characteristics for the compounds of interest.

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