Identification of a Mirex Metabolite[1,2]

by PIO ANDRADE, JR. and W. B. WHEELER
University of Florida
Pesticide Research Laboratory
Gainesville, Fla. 32611
and
DAVID A. CARLSON
U.S.D.A.-A.R.S.
Insects Affecting Man Laboratory
Gainesville, Fla. 32604

Several investigators have been unsuccessful in detecting metabolites of Mirex [dodecachlorooctahydro-1,3,4-metheno-2H cyclobuta (cd) pentalene] in biological systems. GIBSON et al. (1972) reported that rats did not metabolize Mirex or its major photoproduct. MEHENDALE et al. (1972) found no Mirex metabolites after Mirex was incubated with rat, mouse, and rabbit liver preparations and with bean and pea root preparations. Investigations into the ability of several aerobic soils, anaerobic lake sediments, and pure cultures of soil microorganisms to degrade Mirex have also yielded negative results (JONES and HODGES, 1974). Recently, however, ANDRADE and WHEELER (1974) reported the biodegradation of Mirex by sewage sludge microorganisms under anaerobic conditions. This paper presents data concerning the isolation and identification of a Mirex metabolite.

Experimental

Samples and Chemicals

Anaerobic sludge was taken from the anaerobic digester of the City of Gainesville's (Florida) sewage treatment plant.

Uniformly labeled ^{14}C-Mirex (6.34 mCi/mM) was obtained from Mallinckrodt Chemicals, Inc., St. Louis, Missouri. Nonradioactive Mirex was supplied by the USDA Insects Affecting Man Laboratory, Gainesville, Florida.

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[2]This paper reflects the results of research only. Mention of a pesticide or commercial or proprietary product in this paper does not constitute a recommendation or an endorsement of this product by the U. S. Department of Agriculture or by the University of Florida.

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Four authentic Mirex photoproducts were supplied by Drs. Earl G. Alley and Bobby R. Layton of the Mississippi State Chemical Laboratory, Mississippi State, Mississippi. These products were the 10-monohydro, the 8-monohydro, the 2 or 3,8-dihydro, and the 5,10-dihydro. Preparation and purification of these compounds has been described (ALLEY et al., 1973).

Incubation

Fifty mg of unlabeled Mirex plus 1.2 uCi ^{14}C-Mirex were added to 250 ml of anaerobic sludge in a 500 ml Erlenmeyer flask. The flask was fitted with a rubber stopper which was equipped with a vent that could be opened periodically to release the pressure which developed within the flask. Incubation was done in a dark environmental room maintained at 30° ± 1° C for two months.

Extraction

After two months incubation, the sludge was transferred to two 250 ml centrifuge tubes with the aid of 200 ml acetone. The tubes were centrifuged at 2000 RPM for 30 minutes, after which the supernatants were transferred to a 1 liter volumetric flask, and the extracted incubation residues were washed with two 25 ml portions of acetone. The washings were added to the supernatant, followed by 20 ml n-hexane and the mixture was shaken for 30 minutes with a Burrell wrist-action shaker at 200 cycles per minute. Two percent aqueous Na_2SO_4 was added to bring the n-hexane layer into the stem of the flask. It was then set aside overnight to allow complete separation of the phases.

Extract Purification

The n-hexane layer was pipetted into a 50 ml centrifuge tube, concentrated to 2 ml under a stream of nitrogen, and then transferred to a 10 x 300 mm chromatographic column containing 3 g of Florisil overlaid with approximately 2 cm of anhydrous Na_2SO_4; the column was eluted with 40 ml of n-hexane. The eluate was concentrated and then streaked on a 0.100 mm thick silica gel-MH (Analtech) thin layer plate for further purification.

The plate was developed twice in n-heptane (solvent was allowed to dry between developments) to a height of 17 cm. X-ray film (Kodak No-Screen) was exposed to the plate for several days and the autoradiogram was developed. The radioactive band corresponding to the major metabolite was scraped and then eluted with 30 ml n-hexane. The eluate was concentrated

and for further purification was applied to a 0.250 mm thick silica gel thin layer plate. The plate was developed and the metabolite was recovered as previously described.

Instrumentation

A Research Specialties gas chromatograph equipped with an electron capture detector was operated isothermally. The column, detector and injection port temperatures were 200°, 205° and 230° C respectively. The glass column was 1.8 m x 4 mm i.d. Column packings used were: 1) 10% OV-1 on 80/100 Supelcoport; 2) 3% SE-30 on HP Chromsorb W 80/100; and 3) 1.5% OV-17, 1.95% QF-1 on 80/100 Gas Chrom Q. The nitrogen carrier gas flow rate was 80 ml/min.

The mass spectra of the authentic compounds and the metabolite were determined with a Model 1015 Finnigan computerized chemical ionization mass spectrometer interfaced with a Model 1400 Varian gas chromatograph. The gas chromatograph was equipped with a 150 cm x 2 mm i.d. stainless steel column containing 3% SE-30 on Varaport 30. Methane was used as carrier gas and as ionizing gas at 0.7 torr pressure in the ion source. The authentic standards and the metabolite were injected into the gas chromatograph unit and the mass spectra of the peaks corresponding to the compounds were obtained.

Results and Discussion

Table 1 shows the retention times relative to Mirex of the metabolite and the four photoproducts standards as chromatographed on the three columns. For all the columns, the metabolite and the 10-monohydro derivative have identical retention times. This indicates that the metabolite is not the 8-monohydro, the 2 or 3,8-dihydro, or the 5,10-dihydro derivatives and may be identical to the 10-monohydro derivative.

The metabolite and the 10-monohydro derivative also have the same R_f values when chromatographed on silica gel thin layer plates using two different solvent systems (Table 2).

TABLE 1

Retention time relative to Mirex of metabolite and Mirex derivatives in several gas chromatography columns

Compound	10% OV-1 Supelcoport	3% SE-30 H.P. Chrom W	1.5% OV-17 Gas Chrom Q
Metabolite	.74	.72	.80
10-monohydro	.74	.72	.80
8-monohydro	.65	.62	.68
5,10-dihydro	.55	.51	.64
(2 or 3),8-dihydro	.41	.38	.41

TABLE 2

R_f value of Mirex metabolite, 8-monohydro and 10-monohydro derivatives in two solvent systems on silica gel thin layer plates

Compound	Developing Solvents	
	n-heptane*	acetone:n-hexane (1:9 v/v)
Metabolite	0.69	0.65
10-monohydro	0.69	0.65
8-monohydro	0.76	0.74

*double development

The methane chemical ionization mass spectra of the metabolite, the 10-monohydro and the 8-monohydro derivatives show very similar fragmentation patterns. The spectra of the metabolite and the 10-monohydro derivative from 200 m/e to 560 m/e are shown in Fig. 1. Both have base peaks at 475 m/e and major ion clusters at 470-480 m/e and at 235-242 m/e corresponding to $C_{10}Cl_{10}^+$ and $C_5Cl_5^+$ (one half of the pentacyclodecane nucleus) fragments, respectively. The latter peak is characteristic of Mirex and Mirex derivatives which fragment by loss of chlorine atoms and cleavage into halves of the pentacyclodecane nucleus (DILLING and DILLING, 1967). This mass spectral evidence indicates that the metabolite is a monohydro derivative.

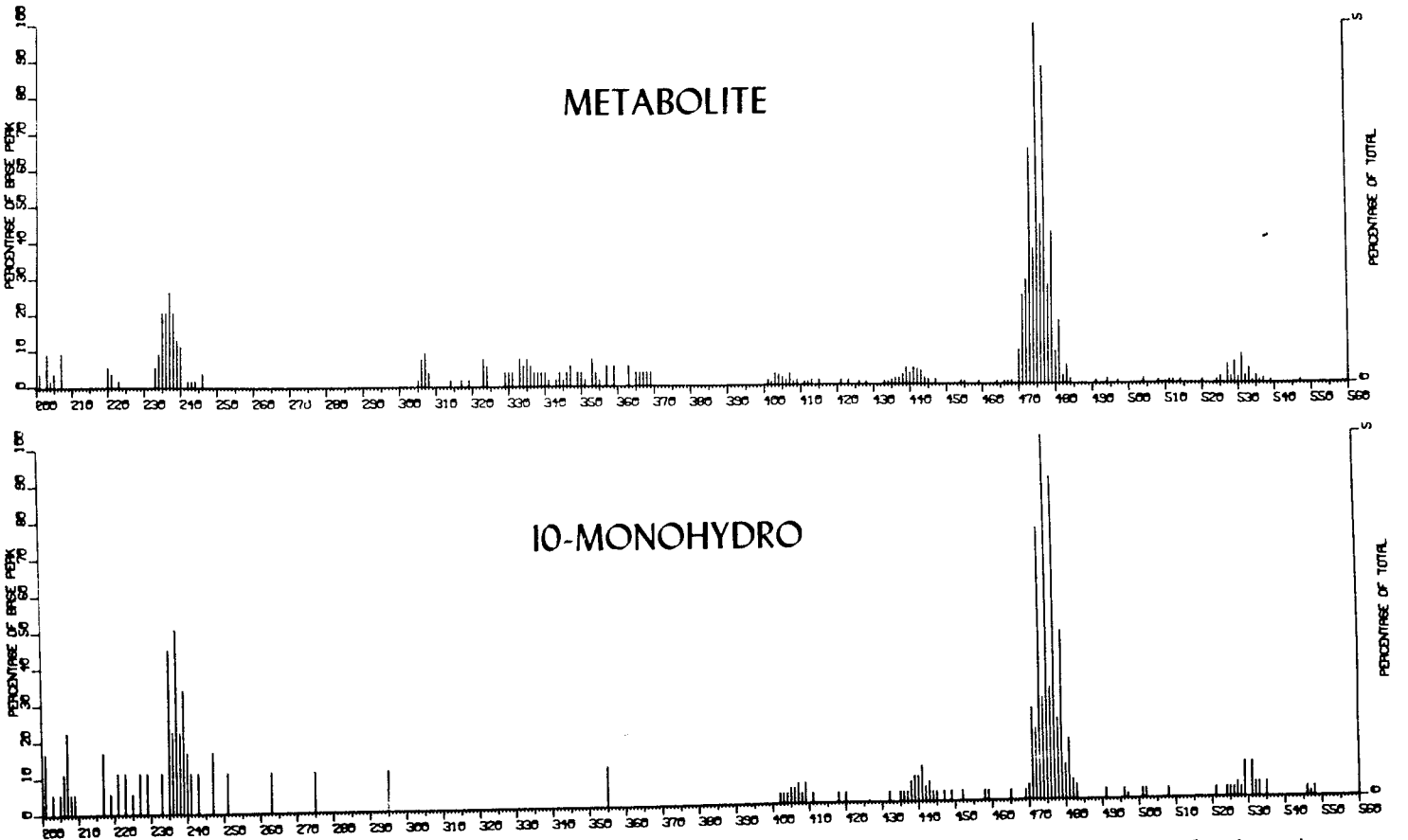
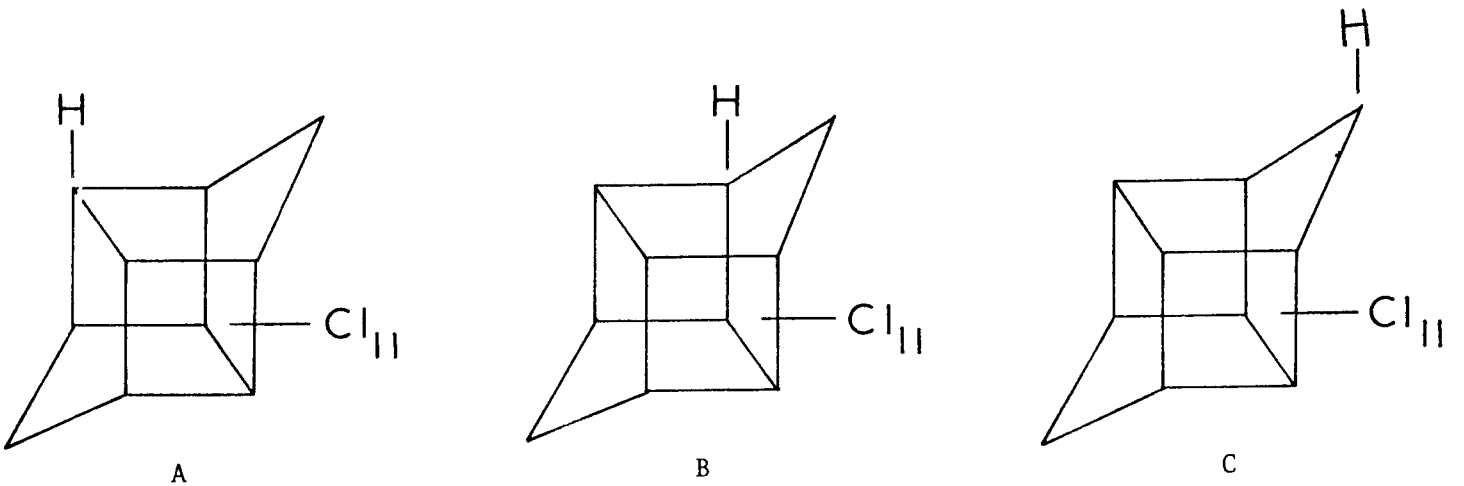


Fig. 1. Chemical ionization mass spectra of metabolite and 10-monohydro derivative



A
8-MONOHYDRO

B
9-MONOHYDRO

C
10-MONOHYDRO

Fig. 2. The three monohydro derivatives of Mirex

The three possible monohydro derivatives are shown in Fig. 2. A-(1,2,3,4,5,5,6,7,9,10,10-undecachloropentacyclo {5.3.0.$0^{2,6}$.$0^{3,9}$.$0^{4,8}$} decane), B-(1,2,3,4,5,5,6,7,8,10,10-undecachloropentacyclo {5.3.0.$0^{2,6}$.$0^{3,9}$.$0^{4,8}$} decane), and C-(1,2,3,4,5,5,6,7,8,9,10-undecachloropentacyclo {5.3.0.$0^{2,6}$.$0^{3,9}$.$0^{4,8}$} decane). For simplicity these compounds are referred to as 8-monohydro, 9-monohydro, and 10-monohydro respectively. The 8-monohydro derivative is the photoproduct of Mirex which was first reported by GIBSON et al. (1972) and whose structure was unequivocally assigned by ALLEY et al. (1974). The 10-monohydro derivative was synthesized unambiguously from Kepone by DILLING et al. (1967). The 9-monohydro derivative has not yet been reported.

The combined mass spectral, TLC and GLC data for the metabolite and the Mirex derivative standards obtained in this investigation suggest that the Mirex metabolite formed by sewage sludge organisms is the 10-monohydro derivative. Since there was no authentic 9-monohydro for comparison, the possibility that the metabolite is the 9-monohydro derivative has not been eliminated.

Acknowledgement

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