Identification of a Mirex Metabolite from Monkeys

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Studies by ANDRADE and WHEELER (1975) have produced evidence that Mirex [dodecachloro-octahydro-1,3,4-metheno-2H cyclobuta (CD) pentalene] is metabolized by sewage sludge micro-organisms under anaerobic conditions and have identified the metabolite as one of the monohydro derivatives of Mirex. Gas chromatography, thin layer chromatography and mass spectrometry were utilized in identifying the metabolite. Stein et al. (1976), utilizing high pressure liquid chromatography, have characterized a ^{14}C-labelled metabolite of Mirex from monkey feces.

The present communication reports the identification, as a monohydro derivative, of a Mirex metabolite found in extracts of feces of rhesus monkeys (Macaca mulatta) given daily doses of Mirex.

Materials and Methods

Chemicals: Mirex (obtained from Allied Chemical Co.) was checked for purity by thin-layer (TLC), gas-liquid (GLC), and high pressure liquid chromatography (HPLC), as reported previously (STEIN et al., 1976). Fractions collected on HPLC were analyzed by GLC to determine the location of Mirex and other organochlorine compounds.

The Mirex photodegradation products used as standards were the same as those used previously, and solutions were prepared in the same manner (STEIN et al., 1976). The purification and preparation of these compounds has been described (ALLEY et al., 1973).

Sample Collection: Fecal samples from adult rhesus monkeys receiving 1.0 mg Mirex/kg/day, p.o., were collected separately from the urine for a period of 24 hours on two separate occasions. The samples were stored immediately in a freezer until they were analyzed.

Extraction and Purification of Metabolites: Fecal samples of approximately 2-6 grams were homogenized in 10 ml of acetonitrile using a Super Dispax Model SDT 182 Tissuemizer (Tekmar Company). The mixture was centrifuged and the acetonitrile layer drawn off using a pasteur pipette. This procedure was repeated three times with 5 ml of acetonitrile, and the extracts were combined in a 250-ml round bottom flask and evaporated to near dryness using a rotary evaporator. The residue was transferred to a large extraction tube, 7.5 ml of 2% sodium sulfate added and the acetonitrile extract partitioned into hexane with one 10-ml and four 5-ml portions of hexane. Preliminary purification of these extracts was carried out by a procedure described earlier (STEIN et al., 1976). A total of approximately 100 grams of feces was extracted. The suspected Mirex metabolite in the cleaned-up fecal extracts was separated

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from Mirex and other impurities by HPLC. Replicate samples in acetonitrile were injected into the liquid chromatograph and only those fractions containing the metabolite were collected. These fractions were combined and extracted with hexane. The hexane extracts were evaporated to dryness using a rotary evaporator and transferred to small glass vials, which were wrapped with aluminum foil to reduce the possibility of u.v. degradation.

Characterization of the Mirex Metabolite: Samples of the purified suspected Mirex metabolite were characterized by GLC, alone and by co-chromatography with the 8- and 10-monohydro derivatives of Mirex, and by HPLC, alone and by co-chromatography with the 8- and 10-monohydro derivatives of Mirex and with samples containing the ^{14}C-Mirex metabolite previously described (STEIN et al., 1976), except that characterization by GLC was carried out at an oven temperature of 220°C. Samples from HPLC were analyzed by GLC to determine the elution volume of the metabolite formed from the unlabelled Mirex and also counted by liquid scintillation spectrometry to determine the elution volume of the metabolite formed from the ^{14}C-Mirex.

The mass spectra of the isolated metabolite and standards of monohydro and dihydro Mirex derivatives were determined using a Hewlett-Packard Model 5982 computerized chemical ionization mass spectrometer interfaced with a Hewlett-Packard Model 5711 gas chromatograph and a Hewlett-Packard Model 5933 data system. The gas chromatograph contained a 6' x 2 mm I.D. glass column filled with 3% OV-1 on Chromsorb Q, 100/120 mesh, maintained at an oven temperature of 220°C. A carrier gas of methane was used at a flow rate of 10 ml/minute. The standards and isolated metabolite were injected into the gas chromatograph (100 µl) and mass spectra of the relevant peaks were obtained.

Results and Discussion

Thin-layer, gas and high pressure liquid chromatographic analysis of the unlabelled Mirex failed to show the presence of detectable quantities of chemical impurities.

Co-chromatography of the 8-monohydro derivative of Mirex with the suspected metabolite by GLC and HPLC resulted in separation of the two compounds, which eliminates the possibility that the metabolite is the 8-monohydro derivative. Co-chromatography by GLC of the 10-monohydro derivative of Mirex with the suspected metabolite did not result in separation of the two compounds. The 10-monohydro derivative of Mirex could not be co-chromatographed with the suspected metabolite since there was an insufficient amount of the suspected metabolite remaining after mass spectral analysis.

The co-chromatography, on HPLC, of the ^{14}C-Mirex metabolite and the suspected metabolite from unlabelled Mirex clearly showed that they are identical.

Mass spectra of the metabolite, the 8-monohydro and 10-monohydro derivatives of Mirex show very similar fragmentation patterns. Major ion clusters are found between 470-480m/e with a base peak at 475m/e corresponding to the $C_{10}HCl_{10}^+$ fragment. These mass spectral data are similar to that of ANDRADE and WHEELER (1975), and confirm that the metabolite is a monohydro derivative of Mirex.

The GLC, HPLC and mass spectral data clearly confirms that the metabolite is either the 10-monohydro or 9-monohydro derivative of Mirex. The 9-monohydro derivative of Mirex has not been synthesized and therefore was not available for comparison. Since our previous work (STEIN et al., 1976) resulted in some separation of the 10-monohydro derivative of Mirex from the ^{14}C-Mirex metabolite, the metabolite may be the 9-monohydro derivative of Mirex. The possibility that the separation may be due to an isotope effect (KLEIN 1966), rather than structural differences, should not be overlooked.

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