Beta Oxidation of Chlorophenoxybutyric Acid Herbicides in Guinea Pigs

by CARLOS H. VAN PETEGHEM and AUBIN M. HEYNDRICKX

Department of Toxicology
State University of Ghent
Ghent, Belgium

Chlorophenoxy acids find extensive use as systemic herbicides in weed control of broadleaf plants. They are mainly divided into acetic acid, 4-butyric acid and 2-propionic acid derivatives. While propionic acid compounds are not metabolized (WAIN 1955), butyric acids are only active against plants with an effective beta oxidation capacity, that degrades them to the corresponding acetic acid homologs (WEBLEY et al.1957)
Various legumes, lacking that beta-oxidative system, are therefore not appreciably harmed. Linscott et al. (1968) reported that Alfalfa (Medicago sativa L.) converts 4-(2,4-dichlorophenoxy)butyric acid (2,4-DB) to homologs. The corresponding 2,4-dichlorophenoxycaproic and 2,4-dichlorophenoxydecanoic acids as well as limited amounts of 2,4-dichlorophenoxycrotonic acid and 2,4-dichlorophenoxyacetic acid were found.
They explain the resistance of Alfalfa to 2,4-DB by suggesting the synthesis of herbicidally inactive compounds, having longer side chains than the parent herbicide.
On the other hand 2,4-dichlorophenoxycrotonic acid has been shown to yield 2,4-D in soil, so that it was considered as the first intermediate in the beta oxidation of 2,4-DB (GUTENMANN and LISK 1964). A similar phenomenon has been ascertained in the leafs of soybean (Glycine max L.) and cocklebur (Xanthium sp.) plants (WATHANA and CORBIN 1972)

Bulletin of Environmental Contamination & Toxicology,
Vol. 14, No. 5 © 1975 by Springer-Verlag New York Inc.

METHODS AND MATERIALS

A guinea pig, weighing 400 g, was administered orally 40 mg 4-(2,4-dichlorophenoxy)butyric acid methyl ester (2,4-DB-CH_3), dissolved in 3 ml soybean salad oil. The herbicide was purchased from Polyscience Corporation, Evanston, Il. 60201, purity degree 99 %. The oral LD_{50} in rat of 2,4-DB in the form of free acid varies between 700 and 1500 mg/kg (WEGLER and EUE 1970)

A second guinea pig, weighing 350 g, was administered orally a suspension of 40 mg 4-(2-methyl-4-chlorophenoxy)butyric acid (MCPB) in 3 ml soybean salad oil. The herbicide was synthesized according to SYNERHOLM and ZIMMERMAN (1945) and was found chromatographically pure. The oral LD_{50} in rats of MCPB is estimated at 700 mg/kg (WEGLER and EUE 1970)

A third guinea pig, weighing 650 g, was administered orally a suspension of 50 mg 4-(2,4,5-trichlorophenoxy) butyric acid in 3 ml soybean salad oil. This compound was also synthesized according to SYNERHOLM and ZIMMERMAN (1945) and found chromatographically pure.

Urine volumes were kept separate from the faeces and collected for 24 hours after administration of the pesticide. They were stored in a freezer until analysis. All animals survived and were still alive 4 weeks after the start of the experiments.

ANALYTICAL PROCEDURES

Reagents

- All reagents were analytical grade; solvents were redistilled in glass.
- Diazomethane (CH_2N_2) was generated from N,N'-dimethyl-N,N'-dinitrosoterephtalamide in alkaline medium.

An ethereal solution was stored in a freezer for several weeks.

Extraction

In small reagent tubes 1 ml urine portions of each sample were acidified with 10 % H_2SO_4 and extracted with 1,5 and 1 ml diethylether. The combined organic phases were treated with diazomethane solution until a yellow colour persisted in the reaction vessel. Ether phases were then passed onto small clean-up columns (8 cm x 6 mm), plugged with glass-wool and fitted with 150 mg Al_2O_3 (activity degree II-III) and 150 mg anhydrous Na_2SO_4 (upper layer). Columns were washed with two 1 ml portions of ether and the organic layer subsequently evaporated to dry in a dry nitrogen stream. Excess diazomethane was removed by passing through the column. Finally the residue was taken up in isooctane.

GLC analysis

Gas liquid chromatography was performed on a Varian 1840 gas chromatograph, fitted with a 8 mc ^{63}Ni detector. A glass column 1/8 inch o.d. and 6 feet in length, was packed with 3 % DC-11 on Varaport 100-120 mesh (AW-HMDS-treated). Nitrogen was used as the carrier gas at a rate of 25-30 ml/min. Operating temperatures were kept at 180° C, 200° C and 250° C for column, injector and detector respectively.

GLC-MS analysis

The instrument used was a Varian 1400 gas chromatograph coupled by a Gohlke Jet Separator to a Finnigan 3000 Quadrupole Mass Spectrometer. A glass column, 1/4 inch o.d. and 5 feet in length was packed with 2 %-DC 11 on Varaport 100-120 mesh (AW-HMDS-treated)

Helium was the carrier gas at a rate of 25-30 ml/min. Temperatures of the column, injector and interface (separator) were 210° C, 230° C and 210° C respectively.
The electron energy in the ion source was 70 eV, temperature of the manifold was 130° C, the pressure 5×10^{-6} Torr, filament current 450 µA and electron multiplier voltage 2.0 kV.
Mass spectra were normalised after background subtraction by means of a Finnigan 6000 Interactive Data System and plotted by a Zeta Plotter (Zeta Research Inc.)

RESULTS AND DISCUSSION

After appropriate dilution with isooctane, 1 µl of the urine extract of the guinea pig that was fed 2,4-DB-CH_3, was injected in the chromatograph fitted with EC detection. No peak, corresponding to the unchanged 2,4-DB could be detected (Figure 1)
On the other hand, a peak with the same retention time as 2,4-D-CH_3 (1'25") appeared.
After concentration of the extract to 100 µl in a nitrogen stream, 1 µl was injected in the GLC-MS combination and a mass spectrum recorded of the peak with the same retention time as 2,4-D-CH_3 (Figure 2)

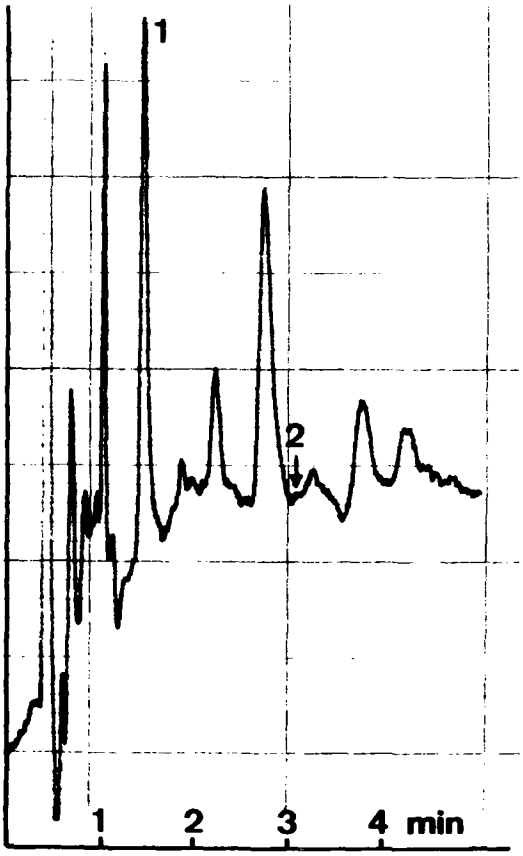

Figure 1

Gas chromatogram of the urine extract of the guinea pig that was fed 2,4-DB-CH_3. Retention time of the original compound is indicated by the arrow, marked 2. The peak, marked 1, has the same retention time as 2,4-D-CH_3 (1'25")

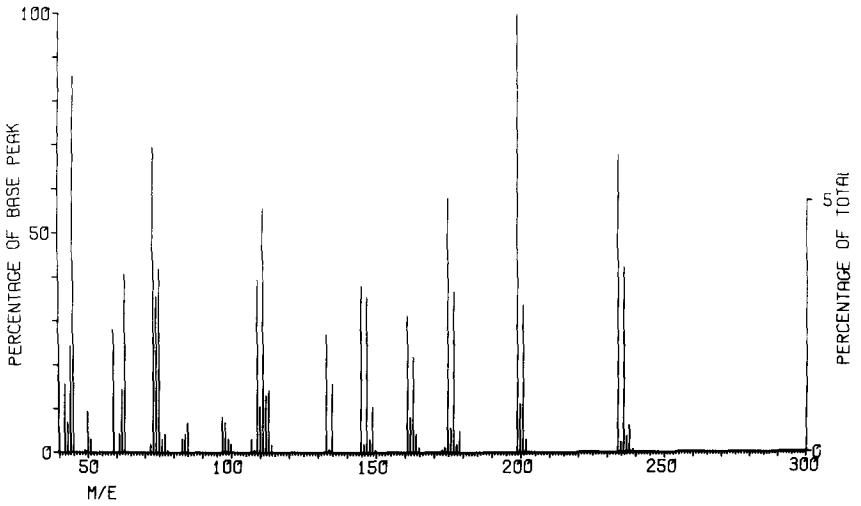

Figure 2

Mass spectrum of the compound with the same retention time as 2,4-D-CH_3 from the urine extract of the guinea pig that was administered 2,4-DB-CH_3.

A molecular ion at m/e 234 suggested the identity of 2,4-dichlorophenoxyacetic acid methyl ester.
This evidence was corroborated by the presence of 2 chlorine atoms (P/P+2/P+4 isotopic abundance ratios : 9/6/1) and the fragmentation pattern : m/e 199 (loss of 1 chlorine atom), m/e 175 (loss of $COOCH_3$), m/e 161 (loss of CH_2COOCH_3) and m/e 145 (loss of OCH_2COO-CH_3)
Injection of a standard-solution of 2,4-D-methyl ester yielded a completely identical spectrum.

In the same way MCPA was found to be a guinea pig formed metabolite of MCPB (Figure 3). The compound showed a molecular ion at m/e 214 bearing 1 chlorine atom (P/P+2 isotopic abundance ratio 3/1) and important fragment ions at m/e 155 (loss of $COOCH_3$), m/e 141 (loss of CH_2COOCH_3) and 125 (loss of OCH_2COOCH_3).

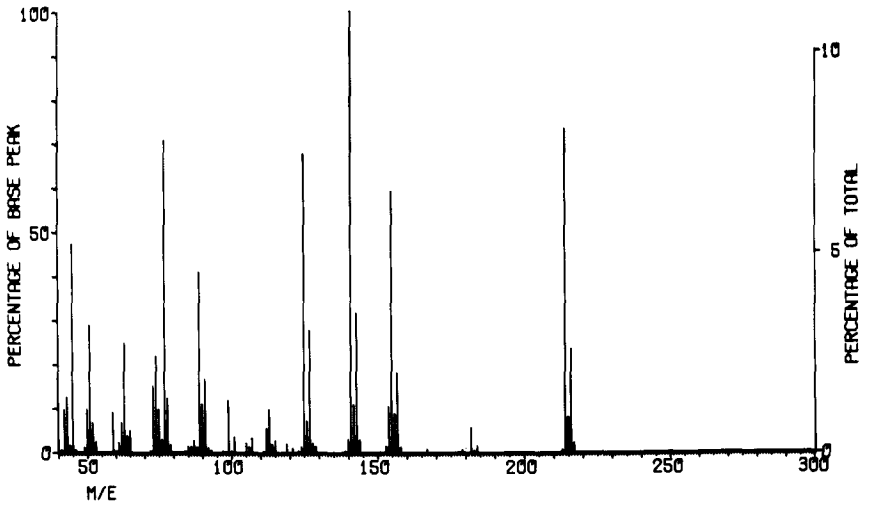

Figure 3

Mass spectrum of the compound with the same retention time as MCPA-CH_3 from the urine extract of the guinea pig, that was administered MCPB.

The urine extract from the guinea pig, that was given 2,4,5-TB, contained a compound with the same retention time as 2,4,5-T-CH_3. After comparison of its mass spectrum (Figure 4) with that of 2,4,5-T-CH_3 it was shown to be quite identical. It contained a molecular ion at m/e 268, with an isotopic cluster indicating 3 chlorine atoms, and fragmentations at m/e 233 (loss of 1 chlorine atom), m/e 209 (loss of $COOCH_3$),

m/e 195 (loss of CH_2COOCH_3) and m/e 179 (loss of OCH_2COOCH_3)

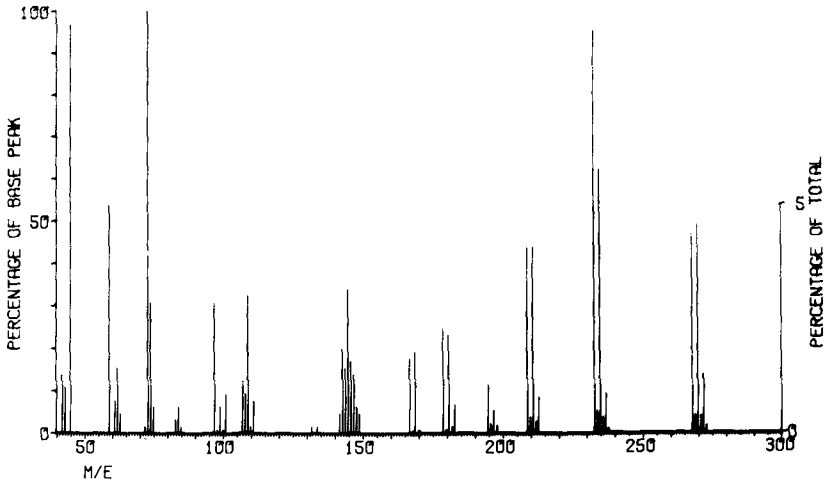

Figure 4

Mass spectrum of the compound with the same retention time as 2,4,5-T-CH_3 from the urine extract of the guinea pig that was administered 2,4,5-T.

In all studies, no original butyric acid derivative could be detected. Free acids (MCPB and 2,4,5-T) as well as methyl esters (2,4-DB-CH_3) had completely disappeared.

In further experiments, we would like to investigate if the beta oxidation of the alkyl side chain is a biochemical process, comparable to the beta oxidation of fatty acids. The elucidation of the identity of some other constituents of the extract, as revealed on the chromatogram (Figure 1) will certainly be extremely helpful to this aim.

REFERENCES

GUTENMANN, W.H., and LISK, D.J. : J. Agr. Food Chem. 12, 322 (1964)

LINSCOTT, D.L., HAGIN, R.D., and DAWSON, J.E. : J. Agr. Food Chem. 16, 844 (1964)

SYNERHOLM, M.E., and ZIMMERMAN, P.W. : Contrib. Boyce Thompson Inst. 14, 91 (1945)

WAIN, R.L. : J. Agr. Food Chem. 3, 128 (1955)

WATHANÀ, S., and CORBIN, F.T. : J. Agr. Food Chem. 20, 23 (1972)

WEBLEY, O.M., DUFF, R.B., and FARMER, V.C. : Nature 179, 113 (1957)

WEGLER, R., and EUE, L. : Chemie der Pflanzenschutz- und Schädlingsbekämpfungsmittel, Band 2. Springer-Verlag Berlin-Heidelberg-New York 1970, p. 280-281