Gas Chromatographic Separation of Several Urea Herbicides and Their Metabolites

A gas chromatographic procedure for the separation of several substituted urea herbicides, their anilines, and other known or suspected metabolites was developed using a XE-60 as the liquid phase and the programmed mode of operation from 75° to 230° C. The separations are useful in following transformations of some of these compounds under various environmental conditions.

Although there are several gas chromatographic procedures for the determination of substituted urea herbicides, all are dependent upon the classical alkaline hydrolysis of the urea herbicide to the corresponding aniline and the determination of the aniline either directly (Kirkland, 1962; Webley and McKone, 1964), or as a halogenated derivative (Gutenmann and Lisk, 1964, 1966). Henkel (1966) reported a direct gas chromatographic procedure which converted the urea herbicide to the aniline after hydrolysis by tetraethylammonium hydroxide and determination by a flame ionization detector. The sensitivity of all these procedures varied from 0.02 to 0.20 p.p.m., quite reasonable figures for residue analysis.

Recently, Baunok and Geissbuhler (1968) reported an aniline-derivative gas chromatographic procedure based upon the classical alkaline digestion procedure followed by formation of an iodinated derivative, through the Sandmeyer reaction, followed by detection and determination by an electron-capture detector. The procedure was quite sensitive, capable of determining residues in the order of 0.01 to 0.05 p.p.m. of parent herbicide. The results were equivalent in sensitivity to the classical alkaline hydrolysis procedure (Bleidner *et al.*, 1954a,b; Dalton and Pease, 1962; Pease 1962), or the acid hydrolysis procedure (Katz, 1967) all of which depend upon the colorimetric Bratton-Marshall (1939) determination of the aromatic amine.

Onley *et al.* (1968) used gas chromatographic procedures for studying the metabolism of diuron in corn seedlings. Gas chromatographic determinations were used to measure metabolic products only after very extensive clean-up and separation by column chromotography.

For many studies, such as metabolic transformations in soils, water, rumen fluid, ensilage as well as photolysis reactions, the previously described methods did not answer the need for a separative system that permitted separation and eventually identification of the parent herbicide as well as the metabolites. The gas chromatographic separations presented in this report allow for the separation of most, if not all, the known and/or suspected metabolites of many substituted urea herbicides directly with no modification of the molecule.

EXPERIMENTAL

Apparatus and Reagents. The MT-220 gas chromatograph (Micro-Tek Instruments, Inc., Baton Rouge, La.) was equipped with a flame ionization detector. The recorder was a Sargent SR equipped with a Disc Integrator and operated at 1 mv.

The gas chromatographic column was 1.5% General Electric XE-60 silicone gum on 80 to 100-mesh Gas Chrom Q (Applied Science Laboratories, Inc., State College, Pa.) 16-inch or 4-foot glass, $^1/_4$-inch o.d., $^3/_{16}$-inch i.d.

Standard reference materials of metobromuron, chlorobromuron, fluometuron, and chloroxuron were supplied by Ciba Agrochemical, Vero Beach, Fla. The standard reference materials of linuron, diuron, fenuron, monuron, and neburon were supplied by DuPont, Wilmington, Del.

Gas Chromatographic Parameters and Conditions. The parameters of operation of the gas chromatograph are as follows: injector temperature 220° C.; initial column temperature, 75° C.; final column temperature, 230° C.; detector temperature, 250° C.; program rate 5° C. per minute; carrier gas (nitrogen) flow, 50 cc. per minute; hydrogen flow, 40 cc. per minute; air flow, 1.2 c.f.h. The column is aged at 240° C. for 48 hours with a nitrogen flow of 60 cc. per minute.

The column is conditioned after aging by injecting several 5-μl. quantities of a mixture of compounds under study and programming after each injection over the range of 75° to 235° C.

Calibration curves of the various compounds under study can be obtained by plotting concentration in nanograms *vs.* peak area as measured by the Disc Integrator.

RESULTS AND DISCUSSION

The separations presented here have many potentials for use in metabolic studies, transformation by microorganisms, photolytic conversions, and for qualitative and quantitative residue analysis.

Residue analytical procedures for the substituted urea herbicides suffer from the fact that the procedures necessary for qualitative identification and for quantitative measurement are long and tedious following the alkaline hydrolysis. Use of the programmed technique with the aniline of the substituted ureas allowed for a good separation. Figure 1 shows the separation of these anilines using a 4-foot × $^1/_4$-inch XE-60 column. One hundred nanograms is represented by each peak with the limits of detectability in the order of 5 ng. Quantitation can be accomplished with a minimum of 15 to 25 ng. depending on the aniline. Preliminary studies indicate that this separation can be the basis for a residue procedure (Katz *et al.*, 1968a).

Similarly separations of many of the parent compounds can be accomplished by this technique. The separation of the parent herbicides is seen in Table I. Metobromuron and fluometuron cannot be separated since their retention times are almost identical. However, fenuron, linuron, chlorobromuron, and monuron are easily separated while diuron is barely detectable. Neither neburon nor chloroxuron can be determined using this programmed technique. No satisfactory reason can be advanced concerning the inability of this procedure to affect separation.

Separation of some of the parent substituted urea herbicides can also be accomplished using a 5% SE-30 liquid phase on Chromosorb W 60 to 80-mesh solid support under isothermal conditions at 130° C. The column was 5-foot × $^1/_8$-inch S.S. operated under the same flows as mentioned previously with the injector temperature of 175° C. Metobromuron and fluometuron were easily separated with this column. Chloroxuron which could not be chromatographed on the XE-60

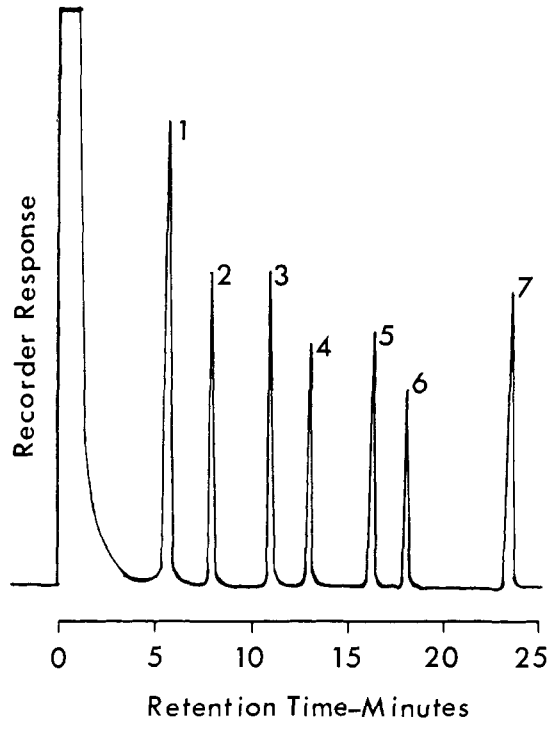

Figure 1. Separation of anilines of substituted urea herbicides on 4-foot XE-60 column

1. Aniline (fenuron)
2. *m*-Trifluoromethylaniline (fluormeturon)
3. *p*-Chloroaniline (monuron)
4. *p*-Bromoaniline (metobromuron)
5. 3,4-Dichloroaniline (diuron, linuron, neburon)
6. 3-Chloro,4-bromoaniline (chlorobromuron)
7. *p*-Chlorophenoxyphenylaniline (chloroxuron)

column was now easily distinguishable. Monuron, diuron, and neburon all had poor response and were essentially inseparable using SE-30. Some success was obtained in the separation of monuron and diuron using 5% OV-17 on Gas Chrom Q 80 to 100-mesh under the same conditions. Programming techniques with this column from 75° to 200° C did not improve the elution characteristics of these three herbicides. No relationship between molecular weight, polarity, methyl, phenyl, or nitrile modification of the silicone liquid phase could be developed to explain the separations or lack of separation of these substituted area herbicides.

The separations possible through the use of the XE-60 column with the programming mode were most apparent in considering the degradation of some of the urea herbicides in the environment. Geissbuhler *et al.* (1963) showed that the substituted urea herbicide, chloroxuron, was metabolized in plants and in soil through successive demethylation to the urea followed by hydrolysis to the aniline. Separation and quantitation of some of the transformations of this family of herbicides can be followed easily. Chlorobromuron, metobromuron, linuron, and fluometuron and many of the known and/or suspected metabolites were separated using the 16-inch XE-60 column (Figure 2) illustrating the ease of following the conversions. Quantitative estimation of the transformations is possible because of the linearity of the response curves. The limit of detectability of the compounds shown in Figure 2 ranges from 5 ng. for the anilines of the herbicides to 50 ng. for the ureas of the herbicides. A summary of the retention time of the various compounds illustrated is given in Table I.

The peaks separated under these conditions represent, as far as can be determined, the unchanged compounds. Trapping of the column effluent by means of a stream splitter and

Table I. Retention Times of Substituted Urea Herbicides and Related Compounds

Retention Time of Anilines on 4-Foot XE-60 Column

Compound	Retention Time, Min.
Aniline	5.50
3-Trifluromethylaniline	7.90
p-Chloroaniline	11.05
p-Bromoaniline	12.80
3,4-Dichloroaniline	16.15
3-Chloro,4-bromoaniline	17.90
p-Chlorophenoxyphenylaniline	23.45

Retention Times of Substituted Urea Herbicides

Compound	Retention Time 16-Inch XE-60	Retention Time SE-30
Fenuron	12.60	5.90
Metobromuron	13.95	15.15
Fluometuron	13.95	7.30
Linuron	16.15	23.65
Chlorobromuron	16.95	26.40
Monuron	17.75	1.00
Diuron	20.30	2.35
Neburon	...	1.55
Chloroxuron	...	20.90

Retention Times of Metobromuron and Related Compounds on 16-Inch XE-60 Column

Compound	Retention Time, Min.
N-Hydroxy-1-(*p*-bromophenyl)-urea	2.15
p-Bromoaniline	5.40
p-Bromophenol	7.30
p-Bromophenylcarbamic acid methyl ester	10.95
Metobromuron	13.85
3-(*p*-Bromophenyl)-1-methoxy-urea	16.95
p-Bromophenylurea	22.10

Retention Times of Fluometuron and Related Compounds on 16-Inch XE-60 Column

Compound	Retention Time, Min.
3-Trifluoromethylaniline	1.75
3-Trifluoromethylphenol	3.35
2-Hydroxy,3-trifluoromethyl-phenylurea	9.45
Fluometuron	13.30
3-(3-Trifluoromethylphenyl)-1-methylurea	16.95
3-Trifluoromethylphenylurea	17.95

Retention Times of Linuron and Related Compounds on 26-Inch XE-60 Column

Compound	Retention Time, Min.
3,4-Dichloroaniline	8.55
Linuron	17.15
3-(3,4-Dichlorophenyl)-1-methoxyurea	20.30
3-(3,4-Dichlorophenyl)-1-methylurea	25.00

Retention Times of Chlorobromuron and Related Compounds on 16-Inch XE-60 Column

Compound	Retention Time, Min.
3-Chloro,4-bromoaniline	9.65
Chlorobromuron	18.10
3-(3-Chloro,4-bromophenyl)-1-methoxyurea	21.10
3-(3-Chloro,4-bromophenyl)-1-methylurea	25.65

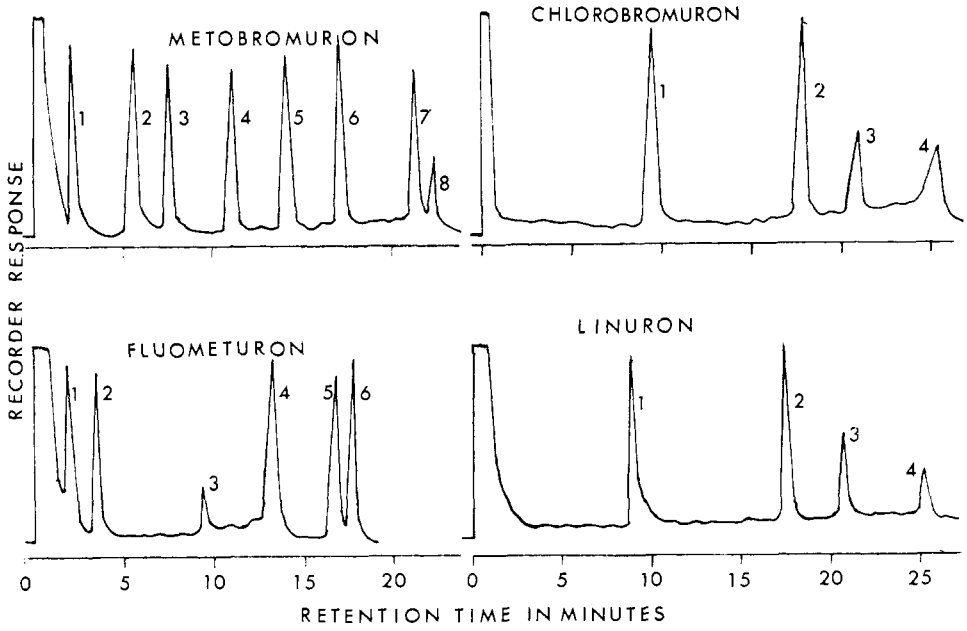

Figure 2. Separation of some urea herbicides and metabolities

Metobromuron
1. *N*-Hydroxy-1-(*p*-bromophenyl)urea
2. *p*-Bromoaniline
3. *p*-Bromophenol
4. *p*-Bromophenylcarbamic acid methyl ester
5. Metobromuron, 3 - (*p* - bromophenyl)-1-methoxy-1-methylurea
6. 3-(*p*-Bromophenyl)-1-methoxyurea
7. 3-(*p*-Bromophenyl)-1-methylurea
8. *p*-Bromophenylurea

Chlorobromuron
1. 3-Chloro,4-bromoaniline
2. Chlorobromuron, 3(3 - chloro,4 - bromophenyl)-1-methoxy-1-methylurea
3. 3 - (3 - Chloro,4 - 4 - bromophenyl)-1-methoxyurea
4. 3 - (3 - Chloro,4 - bromophenyl) - 1-methylurea

Fluometuron
1. 3-Trifluoromethylaniline
2. 3-Trifluoromethylphenol
3. 3 - Hydroxy,3 - trifluoromethylphenylurea
4. Fluometuron, 3 - (3 - trifluoromethylphenyl)-1,1-dimethylurea
5. 3 - (3 - Trifluoromethylphenyl) - 1 - methylurea
6. 3-Trifluoromethylphenylurea

Linuron
1. 3,4-Dichloroaniline
2. Linuron, 3(3,4 - dichlorophenyl) - 1-methoxy-1-methylurea
3. 3 - (3,4 - Dichlorophenyl) - 1 - methoxyurea
4. 3 - (3,4-Dichlorophenyl) - 1 - methylurea

cooled trap yielded infrared spectra identical to standards of unchromatographed compounds. Similarly, thin-layer chromatograms yielded spots identical to standards co-chromatographed with the trapped compounds. The XE-60 column life expectancy, of course, is variable. Consistent values were obtained with the 16-inch XE-60 and 75 programming cycles.

Application of these separations has been made by Rosen *et al.* (1968a) in studies on the photochemical degradation of metobromuron and in his study of the photochemical conversion of linuron (1968b). These separations have been useful in the studies of the transformations of representatives of the substituted ureas in an artificial rumen and in ensilage (Katz *et al.* 1968b).

The separations presented are for relatively known systems. No positive identification can be made based on retention time. Confirmatory thin-layer chromatography would give added support to any presumptive conclusion, but the characterization of any compound must be performed by spectrochemical means. These separations are useful starting points for the development of analytical procedures to solve specific problems.

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