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GAS CHROMATOGRAPHY OF SUBSTITUTED PHENYLUREAS BY FLASH-HEATER METHYLATION WITH TRIMETHYLANILINIUM HYDROXIDE*

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SUMMARY

A simple method for the methylation of substituted phenylureas with trimethylanilinium hydroxide (methelute) is described. The methylation reaction product was the 3-N-methyl analog of the substituted phenylurea. Seven phenylureas with a variety of structures were analyzed using six different chromatographic columns. The gas chromatographic response was linear with increased concentration of the 3-N-methyl analogs of monuron and diuron for both synthetic standards and flash-heater reaction products. Maximum methylation was obtained when the ratio of methylating reagent to phenylurea was approximately 2.5:1. This methylation technique provides for rapid qualitative and quantitative chromatography of substituted phenylureas without side-chain decomposition.

INTRODUCTION

Gas–liquid chromatography (GLC) provides a useful technique for detecting small quantities of substituted phenylurea compounds[1,2]. Owing to the thermal instability of urea herbicides, however, only the aromatic portion of the molecule is normally detected[3]. Usually the side-chain is identified by means of thin-layer chromatography[4,5]. In view of the instability of substituted phenylureas, Fishbein and Zielinski[6] investigated the GLC analysis of their trimethylsilyl derivatives. They found that silylation did cause a general improvement in peak appearance due to a decrease in polarity of the compounds. The elution times of the trimethylsilyl derivatives, however, were very close to those of the parent compounds. McKone and Hance[7], on the other hand, investigated the conditions under which gas chromatography of the unchanged substituted ureas could be achieved. By means of electron capture detection, several of the compounds were detected at the 1-ng level. However, many of the compounds that contained identical aromatic moieties with different side-chain substitution could not be resolved under the conditions utilized. Katz and Strusz[8] also indicated that substituted phenylureas could be gas chromatographed

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without change. By implementation of a very short XE-60 column with an oven temperature program, material could be trapped from the column effluent which appeared to be identical with the parent phenylurea by chromatographic and spectroscopic analysis.

Flash-heater methylation of drugs containing =NH groups has been shown to stabilize and improve the GLC characteristics of these compounds[9–11]. This derivatization technique has proved very useful as a rapid GLC method for drug screening in serum and urine. Since substituted phenylureas also have a reasonably reactive =NH group, attempts have been made to apply this methylation technique to the GLC analysis of these compounds. We have found that derivatization by flash-heater methylation provides a rapid means for identifying and estimating phenylureas by GLC under isothermal conditions. Furthermore, the phenylureas can be chromatographed without side-chain decomposition.

EXPERIMENTAL

Materials

Standard solutions of fluometuron (1.0 μg/μl), fenuron (1.0 μg/μl), monuron (2.5 μg/μl), linuron (2.5 μg/μl), diuron (2.5 μg/μl), metobromuron (3.5 μg/μl), and neburon (4.0 μg/μl) were prepared by dissolving samples of greater than 99% purity in a small quantity of acetone (nanograde) and diluting to volume with hexane (nanograde). A mixture containing all seven compounds at the concentrations specified above was prepared in acetone.

The reagent for flash-heater methylation was trimethylanilinium hydroxide (methelute). The methelute was purchased from Pierce, Rockford, Ill., U.S.A., as a 0.2 *M* solution in methanol.

Synthesis of methylated monuron and diuron

Into a 100-ml 3-necked flask fitted with mechanical stirrer, reflux condenser with drying tube, and addition funnel was placed 0.5 g (12 mmol) of sodium hydride (58% dispersion in mineral oil). Then 15 ml of dimethylformamide (DMF) and 15 ml of tetrahydrofuran (THF) were added to the sodium hydride. The mixture was heated to 60° and stirred for about 30 min at that temperature. After the sodium hydride was dissolved, the solution was cooled in an ice-bath to about 0°. A solution of 2.0 g (10 mmol) of monuron and 1.25 ml (20 mmol, 2.84 g) of methyl iodide dissolved in a mixture of 10 ml of DMF and 10 ml of THF was added with stirring to the sodium hydride. The flask was then heated in an oil-bath to approximately 48°, and the reaction mixture stirred for 1 h. After completion of reaction, the mixture was poured on to crushed ice and extracted with diethyl ether. The product, 3-(4-chlorophenyl)-1,1,3-trimethylurea, was purified by vacuum distillation. Boiling point at 1 torr was 120°; nuclear magnetic resonance (NMR), ($CDCl_3$, 37°, Me_4Si internal reference) 2.70 [6 H, singlet, $-CO-N(C\underline{H}_3)_2$], 3.15 [3 H, singlet, phenyl–$N(C\underline{H}_3)$–CO–], 7.10 and 7.40 ppm (4 H, 2 doublets of the *para* substituted phenyl ring, J=9 Hz, 4-chlorophenyl group); mass spectrum, *m/e*, 212 (molecular ion).

The methylated diuron, 3-(3,4-dichlorophenyl)-1,1,3-trimethylurea, was prepared by the same procedure as described above using 2.3 g (10 mmol) of diuron. The 3-N-methylated product was purified by recrystallization from a solution in

benzene and hexane. Melting point (uncorrected) was 80°; NMR, ($CDCl_3$) 2.76 [6 H, singlet, $-CO-N(C\underline{H}_3)_2$], 3.23 ppm [3 H, singlet, phenyl$-N(C\underline{H}_3)-CO-$]; mass spectrum, *m/e*, 246 (molecular ion).

Gas–liquid chromatography

A Barber-Coleman 5000 chromatograph equipped with flame ionization detector and glass columns (6 ft. × 3.5 mm I.D.) was used. Stationary phases were deposited on the solid support (Anakrom ABS 80–90 mesh) by the slurry method. All columns were preconditioned by temperature programming at a rate of 1° per min until a final temperature of 235° was obtained, and the columns were then held at this temperature for 18 h. For all analyses the detector temperature (310°), inlet temperature (220°), hydrogen flow-rate (50 ml/min), air flow-rate (450 ml/min), and the nitrogen carrier flow-rate (85 ml/min) were held constant.

Flash-heater methylation

Two methods were used to introduce the sample plus methylating reagent into the gas chromatograph. Using the method described by Barrett[9], the methelute was mixed in a small test tube with the material to be analyzed before the mixture was drawn into the syringe for injection. In the second method, about 2 μl of methelute was drawn into the syringe followed by 1–10 μg of the substituted phenylurea dissolved in approximately 5 μl of solvent. For these on-column injections, flash-heater methylation did not occur if the methelute was drawn into the syringe after the phenylurea solution. The first described method gave good reproducible results and was the better method for quantitative determination.

Identification of flash-heater methylation products

The products derived from reaction of methelute with monuron and diuron were trapped from the column effluent of the gas chromatograph in capillary tubes. The trapped materials were compared with the synthesized 3-(4-chlorophenyl)-1,1,3-trimethylurea and 3-(3,4-dichlorophenyl)-1,1,3-trimethylurea. Both unknown compounds were identical with their respective synthetic 3-N-methyl analogs upon direct comparison by co-chromatography (GLC) and infrared spectrometry. Furthermore, the mass spectra of these materials gave molecular ions (*m/e*, 212 and 246) and fragmentation patterns identical with the synthesized 3-N-methyl analogs of monuron and diuron. These results confirmed the identity of the two methylated phenylureas.

Requirement of methelute for maximum methylation

To prepare a sample with a 1:1 molar ratio of methelute to monuron, 7.92 mg (0.2 mmol) of monuron was dissolved in 1 ml of methelute (0.2 mmol). For a 1:1 molar ratio of methelute to diuron, 9.28 mg (0.2 mmol) of diuron was dissolved in 1 ml methelute. The molar ratios were increased until a methelute to phenylurea ratio of 10:1 was obtained. For analysis of these samples, 5 μl of solution (0.2 μmol of phenylurea) was injected into the gas chromatograph. The extent of methylation was estimated by comparison of the peak heights of the flash-heater methylation products with those on a standard curve prepared from the synthesized 3-(4-chlorophenyl)-1,1,3-trimethylurea and 3-(3,4-dichlorophenyl)-1,1,3-trimethylurea. The samples were analyzed on a column of 8% SE-30 and 2% DC-LSX-3-0295.

Linearity of response to 3-N-methyl monuron and diuron

Eight standard solutions were prepared from the synthesized 3-N-methyl analogs of monuron and diuron ranging in concentration, by 1-μg increments, from 1 μg/5 μl to 8 μg/5 μl. To estimate linearity of response by the flash-heater reaction, methanolic solutions of monuron and diuron were prepared containing methelute at a methelute to phenylurea molar ratio of 10:1. Monuron solutions of 2.0, 4.0, and 7.0 μg/5 μl were prepared with methelute. The concentrations of the diuron solutions with methelute were 2.3, 4.6, and 9.3 μg/5 μl. All samples for these studies were prepared in triplicate. For each determination, 5 μl of solution were introduced into the gas chromatograph. Chromatographic separation was performed on a column of 8% SE-30 and 2% DC-LSX-3-0295.

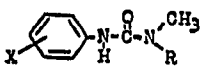
Samples were also introduced into the gas chromatograph by the second method, where the substituted phenylurea and methelute were not premixed before injection. For these experiments, the molar ratio of methelute to phenylurea was about 15:1. The concentrations of the monuron and diuron solutions in this study were identical with those prepared for the premixed samples of methelute and phenylurea.

RESULTS AND DISCUSSION

A wide variety of substituted phenylurea herbicides are currently available for use. In order to examine a number of compounds with different aromatic substitutions and different side-chains, the compounds in Table I were utilized so that a correlation between structure and retention time might be drawn.

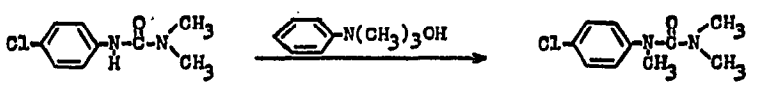
TABLE I

CHEMICAL STRUCTURES OF SUBSTITUTED PHENYLUREA COMPOUNDS



Common name	*X*	*R*	*Chemical name*
Fluometuron	3-CF_3	CH_3	3-(3-Trifluoromethylphenyl)-1,1-dimethylurea
Fenuron	--	CH_3	3-(Phenyl)-1,1-dimethylurea
Monuron	4-Cl	CH_3	3-(4-Chlorophenyl)-1,1-dimethylurea
Metobromuron	4-Br	OCH_3	3-(4-Bromophenyl)-1-methoxy-1-methylurea
Linuron	3,4-Cl,Cl	OCH_3	3-(3,4-Dichlorophenyl)-1-methoxy-1-methylurea
Diuron	3,4-Cl,Cl	CH_3	3-(3,4-Dichlorophenyl)-1,1-dimethylurea
Neburon	3,4-Cl,Cl	$(CH_2)_3CH_3$	3-(3,4-Dichlorophenyl)-1-butyl-1-methylurea

In the GLC analysis of substituted phenylurea compounds, the parent material can decompose during chromatography. Flash-heater methylation with methelute provides a means for the stabilization of phenylureas for GLC analysis. The following reaction illustrates the flash-heater methylation of monuron with methelute.



Methylated monuron and diuron were synthesized by reaction with sodium hydride and methyl iodide. The position of methylation was assigned to the 3-N position (3.15 ppm) of urea based on analysis by NMR spectrometry. Monuron and diuron were methylated by flash-heater reaction, trapped from the gas chromatograph, and compared with the synthesized materials. The trapped products were identical with the synthetic materials in all respects upon examination by GLC co-chromatography, infrared and mass spectroscopy.

Retention times (R_t) and retention values with respect to monuron (R_{mon}) for the separation of the seven phenylureas with six different chromatographic columns are given in Table II. Other columns that gave unsatisfactory results were: 10% OV-17; 6% DC-LSX-3-0295 and 4% OV-17; 10% XE-60; and 3% SE-30. These columns gave numerous extraneous peaks, and the observed peaks were not separated adequately. These extraneous peaks were apparently caused by the phenylureas decomposing during chromatography. Flash-heater methylation of the monomethyl phenylurea also yields more than one peak. The multiple peaks in this case appear to be caused by incomplete reaction of the methelute with the monomethyl phenylurea. Therefore, this methylation technique appears useful only for ureas that have alkyl or aryl substitutions on three of the four urea nitrogen positions. Chloroxuron, 3-[*p*-(*p'*-chlorophenoxy)phenyl]-1,1-dimethylurea, was successfully derivatized by this technique, but at the column temperature used for the elution of the other phenylureas, the retention time of chloroxuron was too long to be included in this study.

Columns 2 and 5 gave the best separation of the seven phenylureas (Table II). The mixed column of 2% DC-LSX-3-0295 and 8% SE-30 gave the best separation of linuron from diuron. The separation achieved by the mixed column is illustrated in Fig. 1. All of the columns gave satisfactory separation of the remaining five substituted phenylureas. Linuron and diuron were the most difficult phenylureas to separate. Therefore, if either linuron of diuron was not present in the mixture, any of the columns in Table II would give good separation.

Examination of the retention values for the substituted phenylureas shows that some simple conclusions may be drawn with respect to structure and retention time. The trifluoromethyl group appears to enhance the volatility of fluometuron over that of fenuron. Substitution of a methoxyl group (linuron) for one of the methyl groups of diuron reduces the retention time slightly. Substitution of a longer alkyl chain (neburon) for a methyl group of diuron substantially increases the retention time. Substitution of chlorine in the aromatic ring increases the retention time with each additional substitution, but an increase in molecular weight does not necessarily mean that a longer retention time should be expected.

In order to determine the quantity of methelute required to obtain maximum methylation, different molar ratios of methelute to phenylurea were examined with monuron and diuron as representative compounds. Starting with molar ratios of 1:1, the quantity of methelute was increased for each analysis until a molar ratio of 10:1 was obtained. The percent methylation with respect to each given molar ratio is shown in Table III. From these data, it appears that a molar ratio of methelute to phenylurea of approximately 2.5:1 will induce maximum flash-heater methylation.

The linearity of response of the gas chromatograph to the 3-N-methyl analogs of monuron and diuron was determined. Standard solutions were prepared from

TABLE II

RETENTION VALUES OF THE FLASH-HEATER METHYLATION PRODUCTS OF SOME SUBSTITUTED PHENYLUREAS

Column No.	*T (column), °C*	*Stationary phase*
1	175	10% DC-200 (viscosity 12,500 cs, methyl)
2	190	10% SE-30 (methyl)
3	180	15% SE-30
4	195	1% DC-LSX-3-0295 (trifluoropropyl, vinyl, methyl) and 4% OV-17
5	165	2% DC-LSX-3-0295 and 8% SE-30
6	180	3% OV-17 (phenyl, methyl)

R_t = Retention time, min.
R_{mon} = Retention, relative to monuron.

Compound	*Column 1*		*Column 2*		*Column 3*		*Column 4*		*Column 5*		*Column 6*	
	R_t	R_{mon}	R_t	R_{mon}	R_t	R_{mon}	R_t	R_{mon}	R_t	R_{mon}	R_t	R_{mon}
Fluometuron	3.6	0.44	2.4	0.44	3.9	0.45	2.1	0.36	2.8	0.46	1.8	0.32
Fenuron	4.3	0.51	3.0	0.56	4 5	0.52	2.9	0.49	3.2	0.51	2.7	0.49
Monuron	8.3	1.00	5.5	1.00	8.8	1.00	5.9	1.00	6.2	1.00	5.6	1.00
Metobromuron	12.0	1.44	7.3	1.33	12.1	1.38	8.6	1.46	7.9	1.27	8.4	1.51
Linuron	15.7	1.89	9.1	1.67	15.6	1.78	10.5	1.79	10.1	1.63	10.5	1.89
Diuron	17.0	2.04	10.0	1.83	16.2	1.85	11.6	1.97	11.8	1.90	11.6	2.09
Neburon	35.8	4.03	21.5	3.94	36.1	4.10	22.4	3.82	23.2	3.73	23.4	4.22

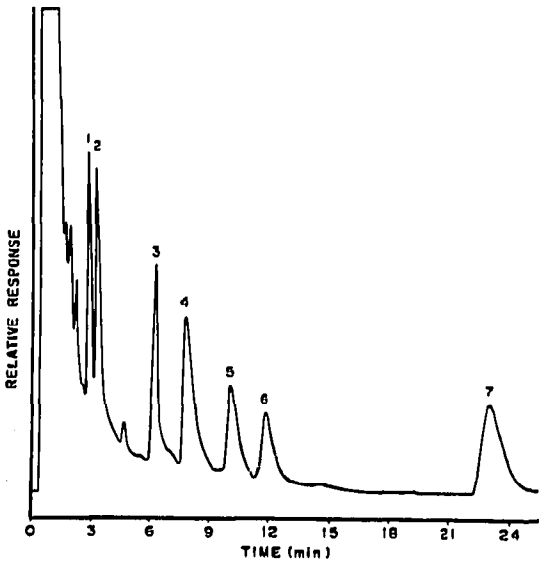


Fig. 1. The separation of a mixture of substituted phenylureas by flash-heater methylation on a 2% DC-LSX-3-0295 and 8% SE-30 column. 1, Fluometuron; 2, fenuron; 3, monuron; 4, metobromuron; 5, linuron; 6, diuron; and 7, neburon.

TABLE III

ESTIMATION OF METHELUTE REQUIREMENT FOR MAXIMUM METHYLATION OF MONURON AND DIURON

Column for separation was 2% DC-LSX-3-0295 and 8% SE-30.

Molar ratios methelute:phenylurea	*Monuron* % methylated*	*Diuron** % methylated*
1:1	79	77
1.25:1	91	91
2:1	97	98
2.5:1	98	98
5:1	99	98
10:1	99	98

* Quantity injected: 39.5 μg (0.2 μmol) in 5 μl.
** Quantity injected: 46.4 μg (0.2 μmol) in 5 μl.

the synthesized 3-N-methyl analogs of monuron and diuron, and a concentration range of 1 to 8 μg was introduced into the gas chromatograph. The GLC response was linear with increased concentration as shown in Fig. 2. Next, experiments were performed to determine if the flash-heater methylation products would give a linear response with increased concentration. For this study, premixed samples of monuron and diuron were prepared containing methelute at a methelute to phenylurea ratio of 10:1. A constant volume (5 μl) of the premixed sample for each concentration was injected, and the results are shown as the solid spots in Fig. 2. These samples also

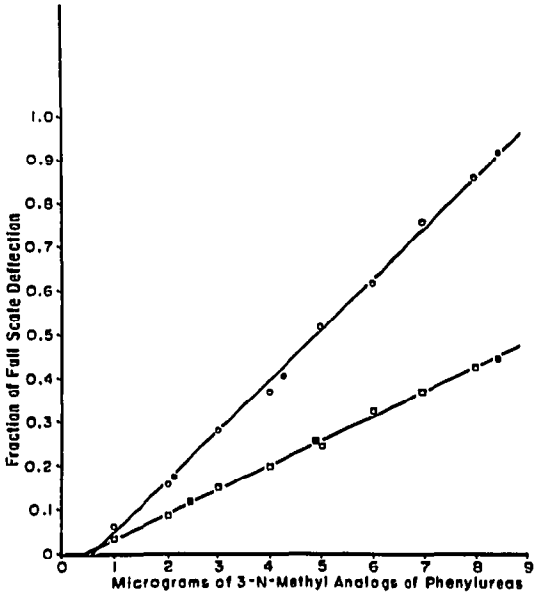


Fig. 2. Linearity of response of the gas chromatograph with increased concentration of the 3-N-methyl analogs of monuron and diuron. ○, 3-N-Methyl monuron; □, 3-N-methyl diuron; ●, monuron + methelute (1 : 10); ■, diuron + methelute (1 : 10).

gave a linear response with increased concentration. Thus, flash-heater methylation appears to give a valid estimation of substituted phenylureas.

Samples were also introduced into the gas chromatograph by the second method where the substituted phenylurea and methelute were not mixed prior to injection. By this technique the results for diuron were satisfactory with a relatively consistent 95–100% response to the introduced material. On the other hand, wide deviations were observed in the detection of monuron, and the results showed an inconsistent 75–100% response to the injected material. Therefore, the second method of sample introduction appears useful only for rapid qualitative and semiquantitative analysis.

The sensitivity of detection using the flash-heater reaction was not as high as is normally desired for low-level residue analysis. In our studies, approximately 1 μg of material must be injected in order to obtain a reliable response. As observed in Fig. 2, the lines of this graph do not intersect through the origin. Therefore, at low levels some of the injected material is apparently unavailable for detection. Our sensitivity of response is, however, the same as that reported by Barrett[9,10] for the detection of drugs by flash-heater methylation. Attempts were made to improve the sensitivity of detection by implementation of a ^{63}Ni electron capture detector. For some unknown reason this detector responded with broad peaks and a significant amount of tailing even though the column parameters were identical with those used with the flame ionization detector. Therefore, the use of the electron capture detector in our studies was unsatisfactory. Perhaps the use of a capillary column might enhance sensitivity or make electron capture detection feasible.

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