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GAS–LIQUID CHROMATOGRAPHY OF TRIAZINE HERBICIDES AS HEPTAFLUOROBUTYRYL DERIVATIVES AND SOME APPLICATIONS TO ANALYSIS IN FOODS

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SUMMARY

The heptafluorobutyryl (HFB) derivatives of ten triazine herbicides were prepared by reacting the pesticides with heptafluorobutyric anhydride in benzene, in the presence of trimethylamine or pyridine as catalyst. The reactions produced mainly the mono-HFB products while some of the herbicides had small quantities of the di-HFB derivatives present. The derivatives were 300 fold to several thousand fold more sensitive to electron-capture detection than the underivatized triazines. They also were 5–10 fold more sensitive than the parents by electrolytic conductivity detection in the halogen mode while they were of similar sensitivity with the same detector in the nitrogen mode. The derivatives eluted in the same general order as the parent triazines on stationary phases of OV-1, OV-101, OV-101/QF-1, and OV-210. This method was successfully applied to the analysis of potatoes, peas and tomatoes spiked with various triazines at levels of 0.13–0.86 ppm.

INTRODUCTION

Triazine herbicides are one of the most abundantly used classes of pesticidal compounds. As a result many methods have been developed for their analysis and, have been reported in reviews[1–3] and comparison studies[4–9]. The detector found most useful for triazines has been the nitrogen-selective electrolytic conductivity detector. The high weight-percentage of nitrogen in these compounds often permits detection at sub-nanogram levels, whereas with the electron-capture detector, the sensitivity can vary from sub-nanogram to microgram levels depending upon the triazine and its substituents. Chloro-*s*-triazines are the most sensitive to electron-capture detection while the methoxy-*s*-triazines are the least. While most pesticide-

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residue laboratories are equipped with electron-capture detectors many may not have nitrogen-selective electrolytic conductivity or alkali-flame ionization detectors. Thus, it was decided to investigate the formation of electron-capture sensitive derivatives. Heptafluorobutyric anhydride (HFBA) is well known to react under various conditions with pesticides which contain -NH or -OH groups to produce highly sensitive electron-capture derivatives often detectable in low picogram quantities[10–13]. This report describes the results of our investigation of the reactions and detector responses of the heptafluorobutyryl (HFB) derivatives of several triazine herbicides with application to residue determinations in selected foods.

EXPERIMENTAL

Apparatus

Gas chromatography was carried out on a Tracor Microtek MT220 instrument (Austin, Texas, U.S.A.) equipped with a Coulson conductivity detector (in halogen or nitrogen mode) and a Varian 1200 or a Varian 1400 gas chromatograph (Palo Alto, Calif., U.S.A.) equipped with tritium foil electron-capture detectors. The chromatographic systems employed were: (a) 2.1 m × 4 mm I.D. column packed with 3% OV-1 on 100–120 mesh Gas-Chrom Q; helium flow-rate, 35 ml/min; oven temperature programmed; initial temperature 190°; initial hold, 3 min; rate, 6°/min; final temperature 240°; final hold 8 min; cooling 2 min; (b) 0.76 m × 4 mm I.D. column packed with 3% OV-101 on 100–120 mesh Chromosorb 750; nitrogen flow-rate, 26 ml/min; temperature 182°; (c) 2.4 m × 4 mm I.D. column packed with 1.5% OV-101/2.25% QF-1 on 80–100 mesh Gas Chrom Q; nitrogen flow-rate, 67 ml/min; temperature 200°; (d) 2.4 m × 4 mm I.D. column packed with 3% OV-210 on 80–100 mesh Chromosorb W HP; nitrogen flow-rate 45 ml/min, temperature 186°.

The Coulson detector parameters for both halogen and nitrogen modes were: hydrogen, 35 ml/min; helium purge 35 ml/min; furnace 800°.

Mass spectra were obtained on a Varian MAT (Model 311A) high-resolution mass spectrometer equipped with an electron-impact ionization source.

Chemicals and reagents

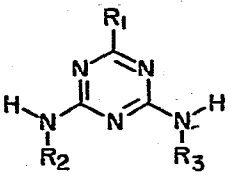
The triazines studied are shown in Table I and were obtained from suppliers as analytical standards. All organic solvents were distilled-in-glass grade. HFBA was obtained from PCR (Gainesville, Fla., U.S.A.) while anhydrous trimethylamine (TMA) was obtained in ampoules from Eastman-Kodak (Rochester, N.Y., U.S.A.). The TMA solution was prepared by adding the cooled (0°) contents of the ampoules to cool tared benzene to produce a 0.5 *M* solution. This solution was refrigerated in a volumetric flask with tight-fitting stopper when not in use. It was stable for 1–2 months. Pyridine was obtained from BDH (Toronto, Canada).

Derivatization

A 15-μl volume of HFBA was added to a 15-ml screw-capped culture tube containing the dry triazine residue (1–25 μg). Then 1.0 ml of 0.5 *M* TMA solution (or 1 ml benzene plus six drops of pyridine) were added and the tube capped and shaken gently for 30 sec. The tube was then left to stand at room temperature for

TABLE I

STRUCTURE OF TRIAZINES



Triazine	*Substituent groups*		
	R_1	R_2	R_3
Atrazine	Cl	C_2H_5	$CH(CH_3)_2$
Atratone	OCH_3	C_2H_5	$CH(CH_3)_2$
Ametryne	SCH_3	C_2H_5	$CH(CH_3)_2$
Simazine	Cl	C_2H_5	C_2H_5
Simetone	OCH_3	C_2H_5	C_2H_5
Propazine	Cl	$CH(CH_3)_2$	$CH(CH_3)_2$
Prometone	OCH_3	$CH(CH_3)_2$	$CH(CH_3)_2$
Prometryn	SCH_3	$CH(CH_3)_2$	$CH(CH_3)_2$
Terbuthylazine	Cl	C_2H_5	$C(CH_3)_3$
Terbutryn	SCH_3	C_2H_5	$C(CH_3)_3$

30 min. After this time, 4.0 ml hexane were added and the contents were shaken gently by hand for 1 min. Following this 10 ml of water were added and the contents were shaken vigorously for 1 min. After the phases had separated, a suitable aliquot of the hexane layer was injected into the gas chromatograph.

Sample analysis

Extraction and clean-up of the samples were carried out by a multi-residue method as described earlier[14,15]. Briefly, the sample is blended with acetone; the filtrate partitioned between water and dichloromethane–hexane (1:1); the organic phase reduced to a small volume and cleaned up on a 3% deactivated Florisil column. The triazines were eluted from the column with 15% acetone in hexane. An aliquot of this fraction was reduced just to dryness for derivatization. The crops examined were potatoes, peas and tomatoes.

RESULTS AND DISCUSSION

The use of pyridine as a catalyst produced the mono-substituted HFB products only for the triazines studied. The derivatization occurred at one of the N–H moieties on the molecules. The mono-products were confirmed by combined gas chromatography–mass spectrometry (GC–MS). Molecular ions were found in every case corresponding to the addition of one HFB-moiety. Thus for the symmetrically substituted triazines such as simazine, propazine and their corresponding methoxy- and methylthio-analogues, only one product peak was obtained. However for atratone, atrazine and ametryne, two mono-HFB products were found. This was expected since two mono-products were possible. Terbuthylazine and terbutryne

produced only one mono-HFB product each although two were possible. Probably, the *t*-butyl-amino substituent was too sterically hindered to react with HFBA, thus only the ethylamino-HFB product was observed. All mono-HFB products eluted earlier than the parent triazines on the columns studied.

The TMA-catalyzed reactions produced single peaks with the chloro-*s*-triazines. However, the derivatives in these cases eluted later than the parent compounds compared to earlier for the confirmed mono-derivatives from the pyridine-catalysed reaction. It was first thought that these might be the di-substituted HFB products, however GC–MS indicated differently. The molecular ions were in all cases eight mass units higher than expected for the di-HFB products. Also the characteristic chlorine lines were absent from the spectra of all of these products while they were present for the derivatives obtained from the pyridine-catalysed reaction. It appears that a substitution reaction occurred at the chlorine position in addition to the HFB reaction at the two amino substituents. Further identification of these products was not attempted.

Terbutryn and prometryn also produced single products with the TMA reaction. These corresponded to the mono-HFB products found with the pyridine-catalyzed reaction. Ametryne produced two peaks, both corresponding to the mono-HFB products. The other triazines produced various amounts of mono- and di-substituted products when using TMA as catalyst. Thus, atratone produced a total of three peaks (one di-, two mono-) and simetone and prometone each produced two (one di-, one mono-). The di-substituted products eluted earlier than the mono-HFB compounds and were normally in lower quantities, often barely detectable.

Attempts at forcing the reaction to the di-substituted products were unsuccessful with both pyridine and TMA-catalysed systems, either by increasing reaction time, temperature or catalyst concentration. No reaction was observed for the triazines in the absence of a base catalyst. Attempts at silylation of triazines also produced mixtures of products[8]. Table II lists the relative retention times of the triazines and HFB products on four different stationary phases.

Table III lists the sensitivities of the products and parent triazines with the three detection systems investigated. The derivatives were found to be about an order of magnitude more sensitive than the parents with the Coulson detector in the halogen mode due to the large number of fluorine atoms in the derivative. The responses of simetone, prometone and atratone parents to this detector appear to be anomalous since these compounds contain no halogens or sulfur. It is possible that some reduction to ammonia occurred causing the responses. The Coulson nitrogen mode results showed parent and products to be of similar sensitivity. This was expected since both parent and products retain the same number of nitrogen atoms. The greatest increase in sensitivity was found with electron-capture detection. The derivatives were at least several hundred to several thousand fold more sensitive than the parents. All derivatives eluted as narrower peaks than the parent compounds, on the columns studied.

The quantitative and practical aspects of this reaction were studied for application to residue analysis in foods. Samples of 2, 5 and 10 μg of atrazine were reacted in triplicate and then analysed by GC (on the 3% OV-101 system, electron-capture detection). The relative responses per ng were as follows: 2-μg reaction, 13.4, 13.2, 13.2; 5-μg reaction, 15.5, 14.6, 16.1; 10-μg reaction, 14.6, 14.6, 15.0. This

TABLE II

RELATIVE RETENTION TIMES OF TRIAZINES AND HFB-PRODUCTS

Compound	*Relative retention time (atrazine = 1.0)*			
	OV-1	*OV-101*	*OV-101/QF-1*	*OV-210*
Simetone	0.94	—	0.90	0.92
Simetone-HFB	0.67	0.62	0.72	—
(m)	0.86	0.86	0.86	0.82
Atratone	0.96	—	0.90	0.89
Atratone HFB (d)	0.70	0.66	0.77	—
(m)	0.85	0.84	0.85	0.89
(m)	0.89	0.93	0.90	0.91
Prometone	0.98	—	0.93	0.88
Prometone HFB (d)	0.75	0.71	0.80	—
(m)	0.89	0.90	0.89	0.91
Simazine	0.98	0.97	0.98	1.00
Simazine HFB (m)	0.72	—	—	—
(o)	1.01	1.10	1.23	1.50
Atrazine	1.00	1.00	1.00	1.00
Atrazine HFB (m)	0.61	—	—	—
(m)	0.72	—	—	—
(o)	1.06	1.16	1.26	1.55
Propazine	1.02	1.04	1.02	1.01
Propazine HFB (m)	0.70	—	—	—
(o)	1.12	1.32	1.34	1.60
Terbuthylazine	1.09	1.13	1.13	1.11
Terbutylazine HFB (m)	0.74	—	—	—
(o)	1.13	1.26	1.39	1.70
Ametryne	1.60	—	1.64	1.50
Ametryne HFB (m)	1.35	1.52	1.41	1.40
(m)	1.43	1.66	1.51	1.48
Prometryn	1.64	—	1.65	1.47
Prometryn HFB (m)	1.39	1.58	1.46	1.41
Terbutryn	1.80	—	1.84	1.64
Terbutryn HFB (m)	1.47	1.74	1.54	1.51

m = mono-HFB derivative; d = di-HFB derivative; o = unknown derivative obtained for the chloro-*s*-triazines with TMA as catalyst.

repeatability indicates a good potential for quantitating triazine residues in samples. Generally, the stability of the derivatives in the hexane solution after the water partition when refrigerated at about 4° was such that less than 25% was lost over a two-week period. Drying the solutions with Na_2SO_4 and storing in a freezer probably would improve the stability of the derivatives.

A comparison was made between the quantitation of the triazines spiked in food samples either directly or as their HFB derivatives. The Coulson halogen mode was used as a detector. Table IV shows the results for the compounds examined. In general, the derivatization technique appears at least as good as the direct method. However, while the method worked well for potatoes and tomatoes, a large off-

TABLE III

SENSITIVITIES OF HFB-TRIAZINES

m = mono-HFB derivative; o = unknown derivative.

Compound	*Detector*		
	Coulson (halogen)	*Coulson (nitrogen)*	*Electron capture*
Simetone	21.8	8	N**
Simetone HFB (m)	1.2	6	0.08
Atratone	13.4	8	N
Atratone HFB (m)	3.2	5	0.17
(m)	3.3	5	0.17
Prometone	10.9	7	N
Prometone HFB (m)	1.7	6	0.08
Simazine	10.8	8	36
Simazine HFB (o)	0.8	8	0.06
(m)	—	6	—
Atrazine	7.4	5	36
Atrazine HFB (o)	1.2	5	0.07
(m)	—	6	—
(m)	—	6	—
Propazine	9.7	7	28
Propazine HFB (o)	1.3	6	0.07
(m)	—	5	—
Terbutylazine	11.5	11	23
Terbutylazine HFB (o)	1.6	17	0.07
(m)	—	8	—
Ametryne	27.9	8	1500
Ametryne HFB (m)	5.1	5	0.18
(m)	7.6	10	0.26
Prometryn	26.0	16	1500
Prometryn HFB (m)	3.4	14	0.12
Terbutryn	25.6	16	1500
Terbutryn HFB (m)	4.0	16	0.13

* Sensitivities reported as nanograms for 50% full-scale deflection.

** N = not detectable at 1.0 microgram. Dashes indicate that no analyses were performed.

scale peak appeared after the triazines, in the derivatized pea samples which was absent from the samples before the HFB reaction. Although this peak did not interfere with the quantitation, it was necessary to wait about 30 min (or to reprogram with no injection) before another injection could be made. This problem was not observed with the potatoes or tomatoes. Thus for peas, an additional clean-up step would be required to remove the cause of the interfering peak if analysis on a routine basis is desired.

CONCLUSION

The HFB derivatives of triazine herbicides provide a great increase in sen-

TABLE IV

RECOVERIES FROM FOODS*

Compound	*Food product*	*Spike level (ppm)*	*Recovery (%)*	
			Parent	*Derivative*
Simazine	peas	0.13	175.5	93.9
	potatoes	0.46	76.9	77.4
Atrazine	peas	0.57	106.9	78.0
	potatoes	0.57	87.3	99.2
Propazine	potatoes	0.46	92.8	81.9
Terbuthylazine	tomatoes	0.86	100.9	101.9
	potatoes	0.29	89.4	99.5
Ametryn	potatoes	0.65	89.8	91.9
Prometryn	potatoes	0.59	89.0	92.4
Terbutryn	tomatoes	0.57	89.7	89.1
		0.57	98.0	99.4
	potatoes	0.57	102.0	93.3
Atratone	potatoes	0.46	—	91.0
Prometone	potatoes	0.46	—	93.4

* Detection made with the Coulson, halogen mode.

sitivity by electron-capture detection. This is a definite aid to those who have only an electron capture detector and who wish to carry out triazine analysis. The derivatization reaction is reproducible enough to permit quantitative measurement. Where nitrogen- or halogen-specific detectors are available, the technique can serve either for quantitation or as a confirmatory test.

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