Pestic. Sci. 1994, **40**, 31–35

Some Comments on a Recently Proposed Method of Determining Chlormequat Residues by Derivatization With Pentafluorothiophenol

Richard D. Mortimer* & Dorcas F. Weber

Food Research Division, Food Directorate, Bureau of Chemical Safety, Health Protection Branch, Health and Welfare Canada, Ottawa, Ontario, Canada, K1A 0L2

(Revised manuscript received 5 May 1993; accepted 19 August 1993)

Abstract: A recent publication concerning the analysis of residues of the herbicide chlormequat in cotton seed by gas chromatography contains a significant error. A derivative prepared by reacting pentafluorothiophenol with chlormequat is actually produced by the reaction of the initial product of demethylation of chlormequat with a second molecule of pentafluorothiophenol. As the derivative is not derived from the chlormequat backbone, the method is not specific to chlormequat and could be misleading to an analyst using it or adapting it to other produce.

1 INTRODUCTION

Chlormequat (**1**) is a herbicide which, although not heavily used in Canada, is registered for use on wheat and is used in a number of provinces. A recent publication by W. J. Allender[1] which proposed to determine chlormequat residues in cotton seed by derivatization with pentafluorothiophenol (**2**) was, therefore, of interest to us in regard to its further application to cereal grains. The first item that caught our attention in that publication was the improbable mechanism suggested by the author to explain his results. The author had observed a peak in the GC–MS analysis of the reaction mixture with a molecular ion of *m/z* 394 and had proposed a chemical structure to fit this fact with seemingly little regard for expected chemical reactivities. In addition, the mass spectrum had both a strong $[M + 2]^+$ ion, suggesting at least two sulphur atoms, and an intense $[M - 15]^+$ fragment ion, characteristic of the loss of a methyl group. The fragmentation pattern was not consistent with the author's proposed structure.

* To whom correspondence should be addressed.

We would like to provide an alternative explanation of Allender's observations and to point out the implications for using this reaction to determine chlormequat residues.

2 MATERIALS AND METHODS

2.1 Reagents

Solvents were all distilled-in-glass grade from Caledon Labs, Inc. (Georgetown, Ontario). Pentafluorothiophenol (PFTP) and 2-dimethylaminoethylchloride hydrochloride were purchased from Aldrich Chemical Co. (Milwaukee, Wisconsin) and used as received. Tetramethylammonium bromide was purchased from Eastman Kodak (Rochester, New York). Chlormequat and parathion-methyl were manufacturer's analytical standards of $\geqslant 95\%$ purity. Stock solutions were prepared by dissolving chlormequat, 2-dimethylaminoethylchloride·HCl (DMAEC) and tetramethylammonium bromide individually in methanol (5 mg ml^{-1}) and pentafluorothiophenol (20 mg ml^{-1}) and parathion-methyl (5 mg ml^{-1}) in acetone. Methyl

Pestic. Sci. 0231-613X/94/$09.00

iodide was purchased from BDH Chemicals (Poole, England) and stored over copper wire. A methanolic sodium hydroxide solution was prepared by dissolving sodium hydroxide pellets (0·49 g) in methanol (10 ml).

2.2 Derivatization

2.2.1 Heterogeneous Conditions

To a 4-ml Reactival was added a magnetic stirring bar, anhydrous potassium carbonate (5 mg) and acetone (2 ml). The vial was fitted with a serum cap and the headspace purged with nitrogen for 2 min. While stirring, an aliquot of chlormequat stock solution (10 μl) was added, followed by an aliquot of PFTP stock solution (0·1 ml). The serum cap was replaced with a Teflon-lined cap while the nitrogen purge was continued. The sealed vial was placed in an aluminum block on a stirring hot-plate at 95°C for 45 min. A control experiment was done in the same way except that no chlormequat was added, only methanol (10 μl). After cooling, an aliquot of the reaction mixture (2 μl) was transferred to a vial containing hexane (1·0 ml) and shaken with sodium hydroxide solution (1 M; 1 ml). A sample of the hexane (1 μl) was analyzed by GC.

The derivatizations of parathion-methyl, methyl iodide and tetramethyl ammonium bromide were done in the same way. When 2-dimethylaminoethylchloride hydrochloride was derivatized, the potassium carbonate was increased to 12 mg.

2.2.2 Homogeneous Conditions

Derivatizations with sodium hydroxide were done by replacing the potassium carbonate with an aliquot of sodium hydroxide in methanol (10 μl).

2.3 Methylation of PFTP

A larger-scale methylation of PFTP was done in the same way as the derivatizations except that 150 mg of potassium carbonate was used and PFTP (100 μl) and methyl iodide (20 μl, 0·43 equiv.) were added neat sequentially. As the methyl iodide was added dropwise, the yellow coloration of the thiophenolate anion disappeared and reappeared. After 45 min heating, the reaction mixture was cooled and poured into sodium hydroxide solution (1 M; 20 ml) and extracted with methylene chloride (20 ml). The organic layer, which had a slightly milky appearance, was dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure at 40°C to yield a pleasant-smelling, white powder (130 mg). The GC chromatogram (see Section 2.4) of the diluted reaction product (1 mg ml^{-1} in acetone, then 1 μl of the acetone solution in 1 ml hexane) showed three principal volatiles eluting at 5·4 min (104°C), 15·5 min. (205°C) and 28·5 min (3·5 min after reaching 300°C).

2.4 Gas Chromatography-Electron Capture Detector

Gas chromatographic analyses were done on a HP 5890 Series II instrument with an automatic sampler using a DB-5 capillary column (J&W Scientific, Folsom, Calif.), 30 m × 0·25 mm i.d., 0·25 μm film thickness, with a 1-m retention gap and on-column injection. The helium carrier gas flow was set at 35 cm sec^{-1} and the electron capture (^{63}Ni) detector temperature was set at 325°C. Initial oven temperature of 70°C was held for 2 min after injection, programmed to 300°C at 10°C min^{-1}, then held at that temperature for 6 min. The oven was then raised rapidly to 325°C and held for 10 min to clean off late eluters.

2.5 Gas Chromatography–Mass Spectroscopy

A VG Analytical Model 7070EQ hybrid mass spectrometer (with configuration EBQQ) coupled to a Varian VISTA 6000 GC equipped with an on-column injector and an identical DB-5 capillary column was used. The column was held initially at 60°C for 2 min, then ramped to 300°C at 10°C min^{-1} and held for 20 min. Only the conventional section (configuration EB) of the mass spectrometer was used. MS conditions: resolution 1 K for low resolution and 10 K for accurate mass measurement; electron energy, 70 eV; trap current, 200 μA; source, re-entrants and GS transfer line temperatures, 200°C. The mass range was *m/z* 50 to 650, scanned exponential down at a scanning rate of 0·6 s decade^{-1} and an interscan delay of 0·2 s.

3 RESULTS AND DISCUSSION

In attempting to derivative chlormequat with PFTP, Allender expected the reaction to proceed initially as outined in eqns (1) and (2) of Fig. 1 by analogy with earlier work with thiophenol and acetylcholine and chlorocholine.[1] Demethylation of the quaternary ammonium group and subsequent substitution of the chlorine atom in **4** was expected to give product **5**. It would appear, however, that Allender found no evidence for **5**, but did find a compound with a molecular ion of *m/z* 394 whose mass spectrum was included in his publication. Unfortunately, he misinterpreted the mass spectrum and overlooked the possibility of the perfluorinated aromatic ring undergoing a nucleophilic substitution.[2,3] It occurred to us that the initially expected pentafluorophenylmethyl sulphide (**3**) might have reacted with a second molecule of pentafluorothiophenol, which was in large excess, to generate product **6** (eqn (3), Fig. 1). The latter compound was expected to have mass

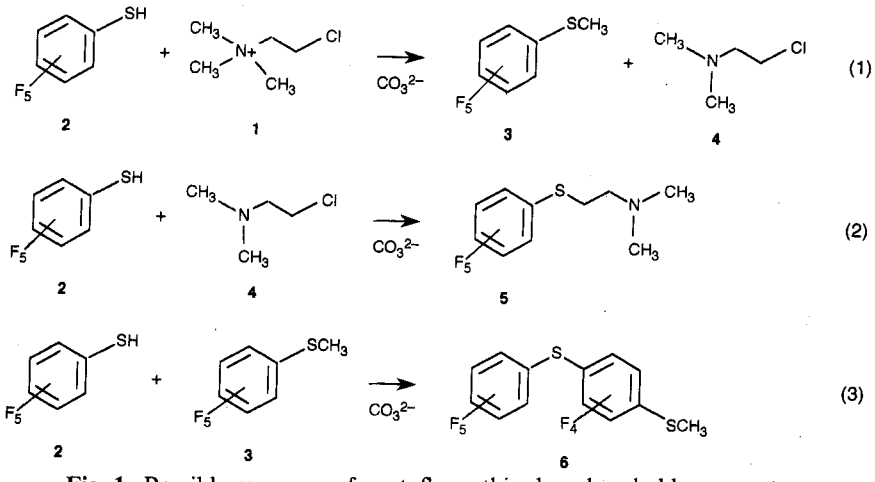

Fig. 1. Possible reacuons of pentafluorothiophenol and chlormequat.

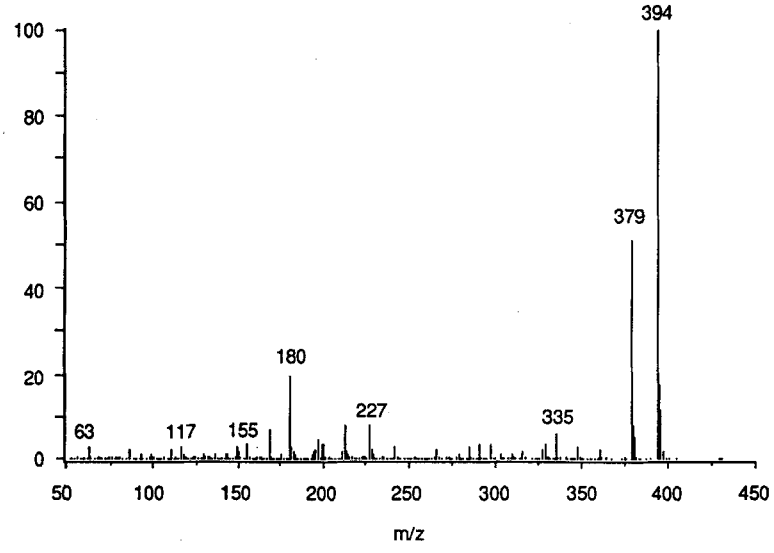

Fig. 2. Mass spectrum of the volatile product **6** from the reaction of pentafluorothiophenol with methyl iodide or with chlormequat.

spectroscopic features consistent with the mass spectrum observed by Allender.

In order to favour the formation of compound **6**, two equivalents of thiophenol were reacted with one equivalent of methyl iodide on a larger scale, but using conditions otherwise similar to those of the derivatization. The GC chromatogram of the reaction mixture showed three principal peaks on the electron capture detector. Using an identical column in the GC–MS, the first peak was identified as compound **3** by comparison of its mass spectrum with the NBS library spectrum of pentafluorophenylmethyl sulphide. The second peak produced a mass spectrum essentially identical with that published by Allender (Fig. 2). Precise mass determination of this component gave the best fit to a molecular formula of $C_{13}H_3S_2F_9$. The measured mass was 393·9548 while the expected mass for structure **6** is 393·9532 and for Allender's structure is 393·9880. Although the exact structure of this peak has not been established (*para*-substitution is assumed at this point), it is reasonably assigned structure **6**. The third peak had a parent ion of m/z 574, a strong $[M + 2]^+$ ion (20%) and a strong $[M - 15]^+$ fragment (20%). These data are consistent with a trimeric species for the third peak as a result of the further reaction of product **6** with pentafluorothiophenol. Presumably, even higher-molecular-weight products could be formed and this may account for the late eluting peaks and the insoluble material observed on workup.

When chlormequat was derivatized by Allender's procedure, additional GC peaks (Fig. 3) were found in the reaction mixture that were not found in the control reaction. The retention times and mass spectra of three of these peaks were identical to those of the three principal volatile products from the methyl iodide reaction above (mainly **6**).

Although the product **6** does derives from chlormequat, the reaction is not necessarily specific to this herbicide; other methylating species could be expected to produce compound **6** under the same conditions. In the USA,

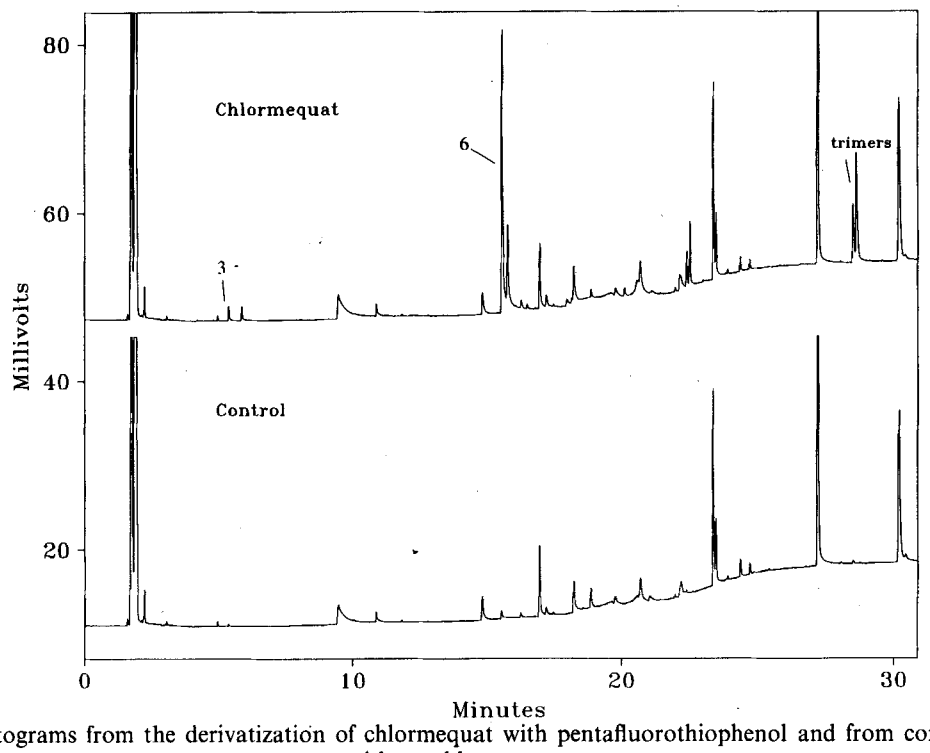

Fig. 3. Gas chromatograms from the derivatization of chlormequat with pentafluorothiophenol and from control derivatization without chlormequat.

parathion-methyl is the insecticide most heavily used on cotton. When the derivatization reaction was done with parathion-methyl in place of chlormequat, two major peaks corresponding to **3** and **6** were observed. In Canada, chlormequat is used on wheat; so is difenzoquat. The latter herbicide is a methyl pyrazolium compound which is readily demethylated by nucleophiles. As a result, difenzoquat residues could also be expected to generate **6**. Adapting Allender's procedure to the analysis of chlormequat in wheat could not differentiate one from the other. This experiment was not attempted because difenzoquat is produced commercially as the methylsulfate salt and reaction with this material to produce **6** would not differentiate between the methylsulfate ion and the methylpyrazolium ion as the source of the methyl group.

In order to understand the fate of the chlormequat molecule (only the methyl group had been accounted for) and to understand why parathion-methyl gave strong peaks for both **3** and **6** while chlormequat gave principally **6**, a number of experiments were done in a qualitative manner using additional methylating species and comparing heterogeneous conditions with homogeneous conditions. The temperature, solvent and concentration conditions of Allender's derivatization were maintained. The results are summarized in Table 1.

It is evident from these results that the derivatization reaction is complex and a complete understanding of it is beyond the scope of this paper. In general, heterogeneous conditions with an ionic methylating species gave mainly the dimer **6** (parathion-methyl produced a charged species after a loss of the first methyl group). On other hand, homogeneous conditions or heterogeneous conditions with a neutral methylating species (parathion-methyl is initially neutral) gave the monomer **3**. These results suggest that formation of **6** may be due to a reaction occurring on the carbonate surface. A peak corresponding to a trimeric product is observed under heterogeneous conditions with ionic methylating agents but not under homogeneous conditions. In fact, the homogeneous conditions produce only **3**($<1\%$ of **6**, and no trimer) and may have some use for analytical purposes for general screening of methylating species.

Derivatization of 2-dimethylaminoethyl chloride under heterogeneous and homogeneous conditions did not yield any appreciable amount of ECD-detectable products that corresponded to products found in the chlormequat reactions. The fate of the chlormequat backbone during derivatization remains a puzzle.

TABLE 1
Principal Products Formed during Derivatization

Compound	*Heterogenous* (K_2CO_3)	*Homogeneous* (NaOH)
Chlormequat	**6**	**3**
Parathion-methyl	**3** and **6**	**3**
Methyl iodide	**3**	**3**
$Me_4N^+Br^-$ [a]	**6**	**3**

[a] Appears to be less reactive than others, judging by smaller peaks.

4 CONCLUSION

There is an error in Allender's interpretation of the results from his attempt to derivatize chlormequat with pentafluorothiophenol which could be misleading to potential users. Although pentafluorothiophenol is a potent nucleophile that gives reasonably volatile derivatives which are very sensitive to electron-capture detectors, its products may undergo further nucleophilic substitution of the fluorinated aromatic ring particularly under heterogeneous conditions. The derivative resulting from reaction of PFTP with chlormequat is not specific to this herbicide because it is produced by demethylation of the quaternary ammonium centre. It is a reaction product that other pesticides with alkylating potential also generate.

REFERENCES

1. Allender, W. J., *Pestic. Sci.* **35** (1992) 265–9.
2. Burdon, J., Hollyhead, W. B. & Tatlow, J. C., *J. Chem. Soc.*, (1965) 5152–6.
3. Chou, T-Z., Voros, P., David, M., Saha, M. & Giese, R. W., *Biomed. Environ. Mass Spectrom.*, **13** (1986) 23–7.