Journal of Chromatography, 396 (1987) 399–403
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 19 473

Note

Preparation, identification, separation, gas–liquid and thin-layer chromatography of the 2,4-dinitrophenylhydrazone derivatives of acetone and acrylaldehyde

KEVIN J. MELKSHAM*
Agricultural Chemistry Branch, Queensland Department of Primary Industries, Meiers Road, Indooroopilly, Queensland 4068 (Australia)
and
SIMON AXELSEN
Agriculture Branch, Queensland Department of Primary Industries, c/o Operations Division, Queensland Water Resources Commission, P.O. Box 2454, Brisbane 4001 (Australia)

(First received December 24th, 1986; revised manuscript received February 2nd, 1987)

Acrolein (containing 92–94% acrylaldehyde) is a biocide used in the U.S.A. and Australia to control submerged weeds in irrigation channels[1,2]. In Australia, the use of acrolein is generally restricted to State Government irrigation authorities. The present acrolein use system in Australia involves injecting acrolein into channels at concentrations up to 15 mg/l. The irrigation system must be shut down and the treated water must be withheld from use for 48 h, causing considerable inconvenience to irrigators and irrigation authorities.

To avoid these problems it would be desirable to use a system similar to that used in the U.S.A. where acrolein is injected into channels without shutting down and water is used immediately for irrigation. To investigate the effects of irrigating crops with water containing acrolein, a trial was to be conducted in which tomatoes would be irrigated with water containing various concentrations of acrolein.

To allow detection of acrylaldehyde residues in the fruit, a sensitive and reliable method of detection was required. Methods which had been used included 2,4-dinitrophenylhydrazone colorimetry[1–3], gas–liquid chromatography (GLC) of acrylaldehyde itself, ultraviolet spectroscopy[3], *m*-aminophenol fluorescence[3] and differential pulse polarography[3]. These methods suffer from a range of problems (*e.g.* lack of sensitivity, presence of interferences) with the result that when the same sample is analysed by these different methods, different results are obtained[3].

Acrylaldehyde is highly reactive[5], flammable[6] and highly volatile[6] (boiling point 52°C). It is toxic at high concentrations and is intolerably irritating to the skin and eyes at concentrations of 0.1 to 1.0 mg/kg of air. To avoid working with such unpleasant material and to overcome losses caused by volatility, conversion to a derivative during initial extraction appeared appropriate. The 2,4-dinitrophenylhydrazone derivative appealed because of the apparent simplicity of formation and ease and sensitivity of detection. However, the colorimetric procedure[1–3] suffered from the disadvantage that carbonyl-containing compounds such as acetone (routinely used

0021-9673/87/$03.50

for cleaning glassware in residue laboratories and possibly present in plant material[7]) would interfere.

Despite some unpublished reports that acrylaldehyde 2,4-dinitrophenyhydrazone could not be chromatographed successfully, there is a published report[7] on the GLC behaviour of this compound. These authors employed a non-polar column (2% SE-30) and electron-capture detection (ECD). This seemed ideal because of the sensitivity of ECD, but linearity was limited, and the acrylaldehyde derivative still could not be separated from the potentially interfering acetone derivative. Therefore, alternative GLC columns were investigated with the intention of finding one which would separate the acrylaldehyde and acetone derivatives.

EXPERIMENTAL AND RESULTS

Two published procedures[7,8] were available for preparing appropriate derivatives, one of which[8] appeared suitable for preparation of bulk quantities and the other[7] more appropriate for preparing small quantities. Initially, small quantities of acrylaldehyde and acetone derivatives were prepared by the method of Kallio *et al.*[7] using the 2,4-dinitrophenylhydrazine reagent of Furniss *et al.*[9].

Several available GLC columns and detectors were tried. A 6 m × 4 mm I.D. 5% OV-1 on Supelcoport glass column at 220°C with carrier gas (nitrogen) flow-rate 30 ml/min would not separate the derivatives (retention time for both, 7.00 min). A 3/BP1-0.5 capillary column (12 m × 0.33 mm I.D.) at 200°C with a 30:1 split ratio and carrier gas (helium) flow rate of 2 ml/min (connected to a nitrogen–phosphorus detector) could not separate them (retention time 4 min 45 s). A DB5 (J and W bonded-phase SE-54) capillary column (30 m × 0.25 mm I.D., 0.25-μm film) (initial column temperature 120°C; after 2 min the temperature increased at 20°C/min to a final temperature of 260°C) gave minimal separation. Retention times were 11.00 min (acetone derivative) and 11 min 9 s (acrylaldehyde derivative).

Separation was achieved using a 1 m × 2 mm I.D. 10% OV-225 on 80–100 mesh Chromosorb W HP glass column (glass wool and walls treated with dichlorodimethylsilane). With an injector temperature 240°C, detector temperature (ECD) 350°C, oven temperature 220°C, relatively high carrier gas flow-rate (50 ml/min) and a nitrogen input purge of 15 ml/min, the acetone derivative had a retention time of 5 min 34 s while the acrylaldehyde derivative eluted with a retention time of 7 min 5 s. Typical chromatograms are shown in Fig. 1. The column had an efficiency of 620 theoretical plates for acrylaldehyde (symmetry factor 1.0). Linearity (peak area) was excellent over a wide range (200 pg to at least 150 ng). Assuming a relative pure standard of the acrylaldehyde derivative, a detection limit of <100 pg of acrylaldehyde per injection would be possible in the absence of interferences.

To check on the purity of the derivative and because a relatively large quantity of the derivative would be required for method development and future analytical work, an attempt was made to prepare a bulk supply of the acrylaldehyde derivative by the alternative method of Allen[8]. This gave, for acrylaldehyde (prepared from technical material) a reddish orange product melting in the range 161–164°C (literature value[8] 165°C). When a standard prepared from this material was compared to the material produced by the method of Kallio *et al.*[7] a relatively small peak ($<1\%$ of that expected) was obtained with the same retention time as that of the Kallio *et*

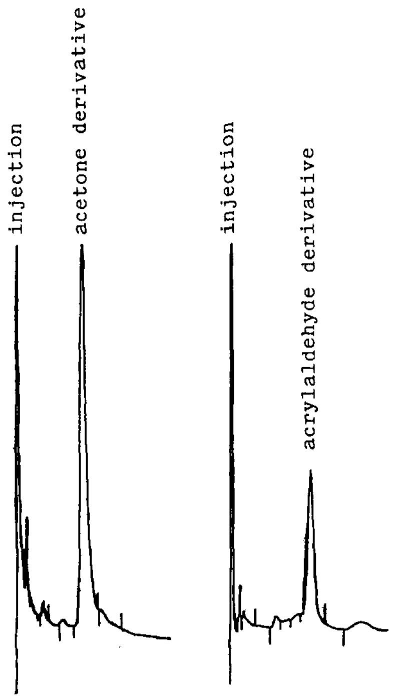

Fig. 1. Typical chromatograms for acetone 2,4-dinitrophenylhydrazone and acrylaldehyde 2,4-dinitrophenylhydrazone (the latter equivalent to *ca.* 25 ng of acrylaldehyde).

al.[7] material. This, together with the melting point range, suggested that the bulk product was impure.

Attempts to recrystallise the reddish orange material from ethanol[8] resulted in the loss of the major part of the product and gave a small amount of product melting in the range 151–154°C. When problems were experienced with the prepa-

TABLE I

MOST INTENSE *m/e* VALUES OF ACRYLALDEHYDE AND ACETONE DERIVATIVES AT 70 eV

Derivative of acrylaldehyde (mol.wt. 236)		*Derivative of acetone (mol.wt. 238)*	
m/e	*Relative intensity*	*m/e*	*Relative intensity*
236	71	238	49
189	40	152	33
159	45	79	68
116	41	78	53
89	54	59	100
69	58	56	77
63	78	51	38
39	100	41	76

ration of a standard by this method, the procedure of Kallio *et al.*[7] was scaled up to give enough acrylaldehyde derivative for a melting point determination. The product was orange (not reddish orange) and melted sharply at 149.9 ± 0.3°C as determined by a Mettler FP 61 melting point apparatus.

The derivatives prepared by the method of Kallio *et al.*[7] were checked by thin-layer chromatography (TLC) using a 0.25 mm silica gel G plate with toluene solvent. Both showed only one spot each. The acetone 2,4-dinitrophenylhydrazone had an R_F value of 0.30 while the (presumed) acrylaldehyde 2,4-dinitrophenylhydrazone had an R_F value of 0.41. Detection was visual by the yellow-orange colour of the compounds. The limit of detection was approximately 0.1 μg on the TLC plate.

To check whether the acrylaldehyde material produced by the method of Kallio *et al.*[7] was truly the 2,4-dinitrophenylhydrazone, both this material and the acetone derivative were analysed by GLC–mass spectrometry (Table I).

DISCUSSION

As can be seen from Table I, the acrylaldehyde derivative prepared by the method of Kallio *et al.*[7] has a parent ion of 236 which is what would be expected for acrylaldehyde 2,4-dinitrophenylhydrazone. Similarly, as would be expected, the acetone derivative has a parent ion of 238 corresponding to that of acetone 2,4-dinitrophenylhydrazone. All of the evidence presented here suggests that the product prepared from acrylaldehyde by the method of Kallio *et al.* is acrylaldehyde 2,4-dinitrophenylhydrazone of high purity and that this product melts at 149.9 ± 0.3°C. This is markedly different from the literature value[8] of 165°C which appears to be based on a product of suspect purity. The problem with the formation of acrylaldehyde 2,4-dinitrophenylhydrazone which this work has highlighted could account for many of the problems which have been experienced previously with both the colorimetric and GLC detection of acrylaldehyde 2,4-dinitrophenyl-hydrazone.

This work has established that the GLC detection of acrylaldehyde 2,4-dinitrophenylhydrazone is possible (either using ECD, nitrogen–phosphorus detection, or by monitoring by specific ion (*e.g.* 236) by GLC–mass spectrometry. A TLC procedure suitable for confirmation of these results has also been described. Previously unreported mass spectra have also been described.

These procedures should provide the basis for a relatively simple, sensitive procedure for detecting and confirming acrylaldehyde residues in a variety of matrices. Such an analytical procedure will be less susceptible to interferences from carbonyl-containing compounds (in particular acetone) than current procedures.

Following the preparation of the initial draft of this paper, a very recent alternative method[10] for acrolein determination was encountered. That method employed a similar principle to that used here, *i.e.* derivatization followed by ECD. Limits of detection for both procedures appear to be comparable and at least one order of magnitude lower than those previously reported.

The procedure described here has the advantage of being a simple, one-step derivitization with readily available reagents. Confirmation is just as important as detection in the development of residue methods and relatively simple, sensitive confirmatory procedures have been described here. The separation of acetone and acrylaldehyde derivatives described here is very important for obtaining meaningful results. This separation may, or may not, be a problem with the procedure of Nishikawa *et al.*[10], since ketones can also form oximes, but it has not been mentioned by them.

ACKNOWLEDGEMENT

We wish to thank Dr Chris Moore of Biochemistry Branch, Animal Research Institute, Yeerongpilly for providing mass spectra on these compounds.

REFERENCES

1 K. H. Bowmer and M. L. Higgins, *Arch. Environ. Contam. Toxicol.*, 5 (1966) 87.
2 K. H. Bowmer, A. R. G. Lag, M. L. Higgins, A. R. Pillary and Y. T. Tchan, *Weed Res.*, 14 (1974) 325.
3 C. L. Kissel, J. L. Brady, A. M. Guerra, M. J. Meshishnek, B. A. Rockie and F. F. Caserio, Jr., in J. L. Johnson, J. R. Stanford, C. C. Wright and A. G. Ostroff (Editors), *Waters for Subsurface Injection, ASTM.STP 735*, American Society for Testing and Materials, Philadelphia, PA, 1981, p. 102.
4 F. A. Dreisch and T. O. Munson, *J. Chromatogr. Sci.*, 21 (1983) 111.
5 E. Y. Spencer, *Guide to the Chemicals Used in Crop Protection*, Ministry of Supply and Services, London, Canada, 1982, p. 2.
6 Weed Science Society of America, *Herbicide Handbook*, 4th ed., 1979, p. 4.
7 H. Kallio, R. R. Linko and J. Kaitaranta, *J. Chromatogr.*, 65 (1972) 355.
8 C. F. H. Allen, *J. Am. Chem. Soc.*, 52 (1930) 2955.
9 B. A. Furniss, A. J. Hannaford, V. Rogers, P. W. G. Smith and A. R. Tatchell (Editors), *Vogel's Textbook of Practical Organic Chemistry*, Longman, London, 4th ed., 1978, p. 1071.
10 N. Nishikawa, T. Hayakawa and T. Sakai, *J. Chromatogr.*, 370 (1986) 327.