Gas Chromatographic Method for the Analysis of Metaldehyde in Crop Tissue

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A simple and sensitive gas chromatographic method is described for the analysis of residues of the molluscicide metaldehyde in crop tissue. After extraction with benzene and cleanup on Florisil, metaldehyde was converted to acetaldehyde, which was determined as its 2,4-dinitrophenylhydrazone derivative using alkali-flame ionization gas chromatography. Recoveries of 94 to 102% were obtained for artichoke and strawberry samples fortified at 0.1, 1.0, and 10 ppm. Acetaldehyde as well as other aldehydes and ketones may be determined separately by the same derivatization procedure through analysis of the sodium bisulfite extract of the benzene solution.

Since its accidental discovery as a snail and slug attractant and poison (Hadden, 1936), metaldehyde (2,4,6,8-tetramethyl-1,3,5,7-tetroxocane) has been widely used in spray and bait formulations for control of these pests in several vegetable and fruit crops. The molluscicide currently has a tolerance of 0 on food crops. Its use is thus restricted to applications which result in no residue in edible fruit or foliage. An efficient and sensitive method is required to support continued use and allow study of the degradation and metabolism of metaldehyde in the environment.

Reported methods for quantitative analysis of metaldehyde residues are based on acid hydrolysis to form acetaldehyde which is steam distilled and determined photometrically after reaction with *p*-phenylphenol. Giang and Smith (1956) reported determination of fortifications as low as 1.8 ppm using this colorimetric method. The procedure was modified by Bowman *et al.* (1961) to allow extension to analysis of acetals, vinyl ethers, and other compounds which liberate acetaldehyde upon vigorous acid hydrolysis. Kimura and Miller (1964) included a Florisil column cleanup step to minimize interference. Even with this modification, background from control samples ranged from 0.1 to 0.7 ppm of metaldehyde equivalent in corn, bean foliage, and strawberry fruits. These high control levels, probably generated to a considerable extent during the hydrolysis step, make applications to residues less than 1 ppm subject to considerable variability.

The following method provides quantitative determination of metaldehyde at the 0.1-ppm level with little interference. It is based on conversion of metaldehyde to acetaldehyde at room temperature, formation of the 2,4-dinitrophenylhydrazone (DNPH) and subsequent determination of the derivative by gas chromatography using the nitrogen-selective alkali-flame ionization detector. Acetaldehyde, occurring naturally in the crops and from possible decomposition of metaldehyde, is first removed from the benzene extract by washing with sodium bisulfite; it may then be determined by the same derivatization procedure if desired.

EXPERIMENTAL SECTION

Reagent grade tetrahydrofuran, sodium metabisulfite, 2,4-dinitrophenylhydrazine (Matheson, Coleman and Bell), acetaldehyde, Nanograde benzene (Mallinckrodt), and Florisil (PR grade, 60/100 mesh, Floridin Co., moisture content 6.7% by weight) were used as received. The 2,4-dinitrophenylhydrazine reagent (DNPH reagent) was prepared by dissolving 0.25 g of 2,4-dinitrophenylhydrazine in a solution of 50 ml of water and 50 ml of concentrated hydrochloric acid by warming on a water bath. The reagent was kept at room temperature for use as needed. A standard of acetaldehyde DNPH was prepared by adding two drops of acetaldehyde solution to 3 ml of the DNPH reagent. The filtered precipitate was recrystallized from methanol to give an orange product, mp 168°.

Nmr spectra were obtained in deuterochloroform (TMS internal standard) using an Hitachi Perkin-Elmer Model R-20 high-resolution spectrometer. A Finnigan Model 3000 peak identifier containing a 3-ft 3% OV-17 on 100/120 mesh, A/W, DMCS-treated Chromosorb W column was used for gas chromatography–mass spectrometry.

Gas–Liquid Chromatography. A Varian Aerograph Model 1700 gas chromatograph equipped with a rubidium sulfate alkali-flame ionization detector was used with a 4-ft 2% OV-17 on 100/120 mesh, A/W, DMCS-treated Chromosorb W column. The column was 3 mm i.d. glass. Nitrogen carrier gas, hydrogen, and air flow rates were 20, 25, and 200 ml/min, respectively. Column, injector, and detector temperatures were 270, 300, and 215°, respectively. The column was conditioned each day of use by injecting 1-μg quantities of acetaldehyde DNPH until a constant response was obtained. The glass injector insert was changed frequently when crop samples were being analyzed.

Conversion of Metaldehyde to Acetaldehyde. The nmr spectra of a saturated solution of metaldehyde in deuterochloroform were recorded before and after shaking in a capped nmr tube with an equal volume of 6 *N* hydrochloric acid for 2 min. The spectrum of metaldehyde (d at 1.4, q at 4.9 ppm) almost completely disappeared and was replaced by the characteristic spectrum of acetaldehyde (d at 2.2, s at 8.7 ppm). No other peaks were observed from intermediates or by-products.

Microscale Derivatization Procedure. The DNPH reagent (15 ml) was added to 15 ml of a benzene solution of metaldehyde in a round-bottomed flask. The two phases were mixed with a magnetic stirrer at room temperature for 20 min; the aqueous phase was extracted with 10 ml of fresh benzene and the combined benzene phases were concentrated to an appropriate volume (usually 1 ml) for measurement of the derivative. Derivatization efficiency was determined by carrying 10, 100, and 1000 μg of metaldehyde through this procedure. The amount of acetaldehyde DNPH formed was calculated by comparing peak heights with a standard curve constructed from gas chromatographic analysis of known quantities of the authentic standard. The amount of metaldehyde recovered, from which percent conversion was derived, was calculated from the formula:

$$\mu\text{g metaldehyde} = \mu\text{g acetaldehyde DNPH from standard curve} \times 44/224 \times \frac{\text{final volume of benzene (ml)}}{\text{amount injected (ml)}}$$

To check conversion as a function of time, aliquots of the benzene phase from a reaction solution containing 40 μg of metaldehyde were removed at intervals and transferred to a test tube containing a small amount of aqueous sodium carbonate to neutralize the acid present. Analysis of the benzene aliquots and calculation of percent conversion were carried out as outlined above. The reaction was

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repeated using a DNPH reagent prepared in 1 *N* hydrochloric acid.

Analysis of Metaldehyde in Crop Samples. An homogenized crop sample (100 g) was shaken for 30 min on a rotating shaker with 200 ml of benzene in a 1-l. Erlenmeyer flask. The benzene phase was decanted to a 250-ml separatory funnel. Extraction of the remaining crop tissue was repeated with two 100-ml portions of fresh benzene. The combined benzene extracts were filtered through glass wool if necessary to remove suspended solid material. The benzene was then extracted in a 500-ml separatory funnel with two portions, 10 ml each, of freshly prepared 2% sodium metabisulfite solution. The bisulfite extracts were saved for acetaldehyde analysis and the benzene layer was washed with 10 ml of water and then filtered through sodium sulfate into a 500-ml round-bottomed flask. The solution was concentrated to *ca.* 5 ml on a rotary evaporator (bath temperature 50°) and transferred quantitatively by means of a Pasteur pipette to a 10 × 2 cm chromatographic column containing 13 g of Florisil previously rinsed with 50 ml of benzene. The column was eluted with 125 ml of 5% tetrahydrofuran in benzene, and the eluate was concentrated to *ca.* 15 ml on a rotary evaporator (bath temperature 50°). The concentrated solution was derivatized and analyzed as in the preceding section.

Two other extraction techniques were examined. One involved blending 100 g of crop sample with 100 ml of benzene for 3 min in a Waring Blendor, centrifugation of the resulting mixture at 2000 rpm, and removal of an aliquot of the clear benzene layer for bisulfite extraction and cleanup as outlined above. The other involved extraction of 50 g of crop sample with 500 ml of benzene in a Soxhlet extractor for 6 hr. The benzene extract was processed as above.

Analysis of Acetaldehyde in Crop Samples. To the sodium metabisulfite extract from above was added 20 ml of DNPH reagent. The mixture was left for 2 hr and then extracted with two 10-ml portions of benzene. The benzene was concentrated and analyzed as before by gas chromatography. The amount of free acetaldehyde was calculated from the same formula used for metaldehyde quantitation. The efficiency of the method was tested by extracting benzene fortified with 100 μg of acetaldehyde with sodium metabisulfite solution; the addition of DNPH reagent and subsequent determination of the derivative were carried out as before. The recovery was 95%.

RESULTS AND DISCUSSION

The hydrolysis conditions employed by Giang and Smith (1956), Bowman *et al.* (1961), and Kimura and Miller (1964) necessarily involved prolonged boiling in aqueous acid, since acetaldehyde was isolated by steam distillation. This procedure unfortunately tends to increase interferences. In fact, conversion of metaldehyde to acetaldehyde has been reported to occur in aqueous acid solution at room temperature (Craven *et al.*, 1962), although the rapidity of the reaction was not specified.

The overall conversion of metaldehyde to acetaldehyde DNPH occurs in two steps: conversion of metaldehyde to acetaldehyde in the presence of acid, and formation of the DNPH derivative from the liberated aldehyde.

$$C_8H_{16}O_4 \xrightarrow{H^+} 4\ CH_3CHO \quad (1)$$

$$CH_3CHO \xrightarrow{\text{DNPH reagent, } H^+} CH_3CH{=}N{-}NH{-}C_6H_3(NO_2)_2 \quad (2)$$

The formation of the DNPH derivative from acetaldehyde is almost instantaneous. The rate of conversion of metaldehyde to acetaldehyde was found to be slower and dependent on the concentration of acid. When a solution of metaldehyde in deuterochloroform was shaken for 2 min with 1 *N* hydrochloric acid at room temperature, essentially no acetaldehyde was found to be formed by nmr analysis of the deuterochloroform phase; with 6 *N* hydrochloric acid, nearly complete conversion of metaldehyde to acetaldehyde was observed under the same conditions. The overall conversion of metaldehyde to acetaldehyde DNPH was found to be similarly dependent on the acid concentration (Figure 1); conversion was essentially complete within 7 min using 6 *N* hydrochloric acid, while 30 min was required to complete the reaction using 1 *N* hydrochloric acid. The higher acid concentration and 20 min reaction time were employed for routine analysis to ensure complete conversion in the presence of crop extracts. The high conversion efficiency allows quantitation through reference to weighed standards of the DNPH derivative, and thus obviates the need for preparing derivatives from standard metaldehyde each time the analysis is carried out.

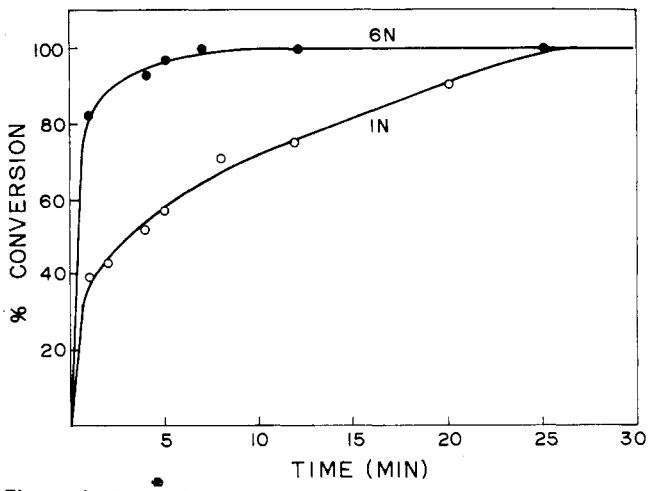

Figure 1. Rate of conversion of metaldehyde to acetaldehyde DNPH using two different concentrations of hydrochloric acid.

The two-phase reaction medium is advantageous in several ways. Equilibria between acetaldehyde, metaldehyde, and other condensation products of acetaldehyde are shifted to favor acetaldehyde, since this product immediately reacts with the DNPH reagent. Loss of acetaldehyde through vaporization is minimized for the same reason. Furthermore, a large excess of the DNPH reagent may be used since unreacted reagent remains for the most part in the aqueous phase, and thus does not interfere in subsequent gas chromatography.

The gas chromatography of the DNPH derivatives of different aldehydes has been previously reported (Kallio *et al.*, 1972, and references therein) utilizing flame ionization and electron-capture detection. The alkali-flame ionization detector was chosen for the residue method because of the consistently lower background obtained from derivatized crop extracts. The standard curve for acetaldehyde DNPH indicated a detectibility limit of approximately 50 ng using this detector. Additional analytical specificity is derived from the high temperature required to elute the relatively nonvolatile derivative.

The use of chloroform as the extracting solvent in the method of Giang and Smith (1956) led to high background from solvent-derived interferences. Substitution of benzene eliminated this problem. Furthermore, while metaldehyde is soluble in both solvents, the less polar benzene leads to a cleaner primary extract. Florisil column chromatography was included for further cleanup. Complete elution of metaldehyde in the first 125 ml of eluting solvent (5% tetrahydrofuran-in-benzene) was conveniently checked using the thin-layer chromatographic method of Mays *et al.* (1968).

Table I. Recovery of Metaldehyde from Artichokes and Strawberry Fruit

Crop	Metaldehyde added, ppm	% recovery	Avg % recovery
Artichokes	0.1	103	102
		99	
		105	
	1	104	101
		102	
		96	
	10	98	96
		96	
		93	
Strawberries	0.1	109	98
		103	
		83	
	1	95	94
		88	
		98	
	10	90	94
		100	
		91	

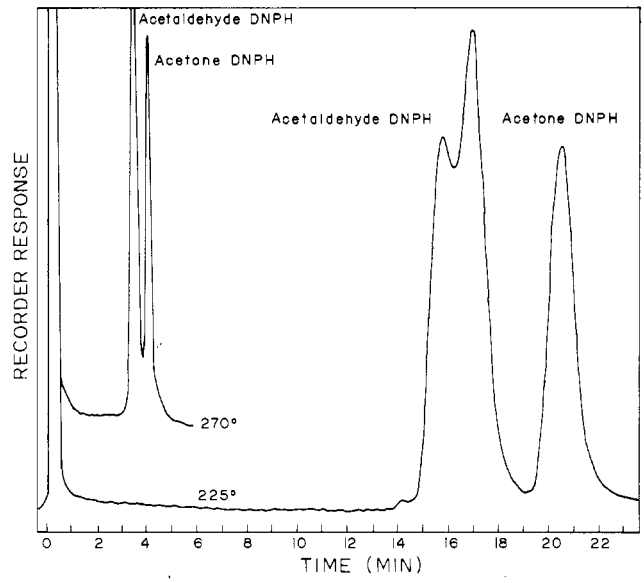

Figure 3. Gas chromatograms of acetaldehyde DNPH and acetone DNPH at two column temperatures.

Recovery of metaldehyde from fortified artichoke and strawberry samples (Table I) was excellent. The control level expressed as metaldehyde was less than 0.02 ppm for both crops. While several peaks were noted in chromatograms of samples fortified at 0.1 ppm (Figure 2), none interfered seriously with that of the DNPH derivative of acetaldehyde. Extension of the analysis to tomato and bean control samples revealed no significant interference at the 0.1-ppm level in these crops as well. Although the DNPH derivative of acetone has a retention time slightly greater than that of acetaldehyde (Figure 3), some overlap can occur; precaution must be taken to remove this potential interference from glassware, reagents, and solvents.

To check the efficiency of the benzene slurry extraction for recovering metaldehyde from field-weathered samples, separate portions of composited strawberries harvested on the day of spraying (zero-day sample) and 3 days following spraying were extracted by this method, by blending, and by Soxhlet extraction. The residues found for the zero-day sample were 2.4, 1.9, and 2.1 ppm, respectively, and for the 3-day sample were 0.3, 0.3, and 0.4, respectively. The pesticide was applied as a 50% wettable powder at the rate of 3 lb of active ingredient per acre. Furthermore, the recoveries and background obtained from strawberry samples fortified with 0.1 ppm of metaldehyde were in substantial agreement when extraction was carried out by either the slurry or blending techniques.

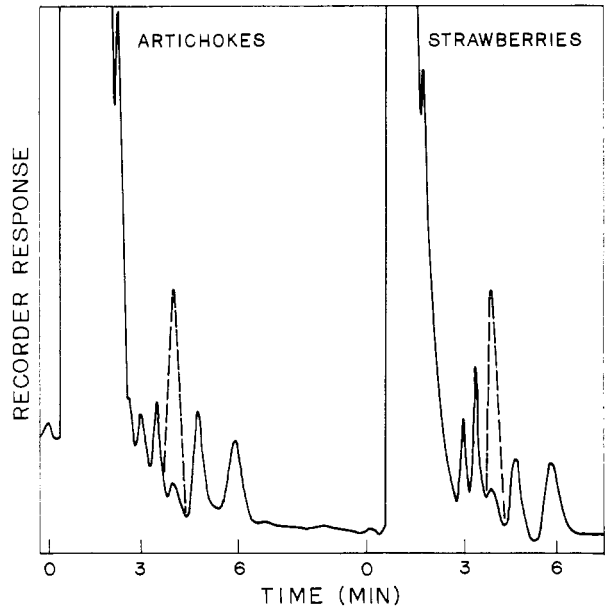

Figure 2. Gas chromatograms from artichoke and strawberry crop blanks, with peak corresponding to 0.1 ppm of metaldehyde superimposed.

Analysis of the bisulfite extract of an unfortified strawberry sample showed that the amount of free acetaldehyde was small, 0.03 ppm. Little significance can be attached to this result since no special precautions were taken to ensure freshness of the sample. The method could be of use in this way for determination of lower molecular weight aldehydes and ketones, important flavor constituents in a variety of products, as well as for analysis of acetaldehyde from possible breakdown of metaldehyde. Some confirmation of peak identification can result from the partial resolution of the syn and anti forms of the DNPH derivatives of aldehydes on gas chromatography (Kallio *et al.*, 1972; Karabatsos *et al.*, 1963). Observation of two peaks, the smaller of which has the shorter retention time, is best made by lowering the column temperature over that normally used in the analysis (Figure 3). Aldehydes, exemplified by acetaldehyde, may easily be differentiated from symmetrical ketones, exemplified by acetone, using this simple expedient. The two peaks of several aldehyde DNPH derivatives were completely separated on OV-17 and analyzed by coupled mass spectrometry; each peak within pairs had identical parent and fragmentation patterns, confirming their representation as the syn and anti isomers as proposed by Kallio *et al.* (1972). This criterion was used to differentiate naturally occurring acetaldehyde from background interference in crop extracts.

The chief advantage of this method over the modified procedure of Kimura and Miller (1964) is a lower detectibility limit of at least tenfold for metaldehyde. The higher sensitivity is apparently due to a number of factors: the use of benzene results in a cleaner primary extract; the conversion of metaldehyde to acetaldehyde at room temperature in the presence of the derivatizing reagent generates fewer interferences and minimizes loss of the highly volatile acetaldehyde; the low volatility of the derivative places the peak of interest in a region relatively free of interference; and the use of a selective detector further minimizes background from interfering compounds. The method appears adaptable to the quantitative analysis of

free aldehydes and ketones in plant tissue, though this application was not pursued for compounds other than acetaldehyde.

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Inorganic and Organic Bromide Residues in Foodstuffs Fumigated with Methyl Bromide and Ethylene Dibromide at Low Temperatures

Theodore Dumas

Fruit that was fumigated with methyl bromide over a range of temperatures had residues that diminished as the temperatures were lowered. When ethylene dibromide was used at a lower temperature (13°), the fumigant was highly adsorbed and retained for many days when stored at this temperature. Also, the inorganic bromide determination by ashing and bromate titration did not account for all the bromide resulting from ethylene dibromide present. Study of the distribution showed bromide in fruits was highest in the seed and skin and lowest in the pulp.

For the eradication of insects in harvested fruits, fumigants such as methyl bromide and ethylene dibromide are often used. The fixed residues formed as a result of chemical reaction between the fumigant and the tissue of the fruit are particularly important because they may tend to reduce quality of fruit or present a health hazard to the consumer. To avoid hazard to the consumer, residue limits are set by health authorities. When foodstuffs are treated with methyl bromide, some of the fumigant may react with the products to form inorganic bromide residue and the remaining fumigant is readily desorbed on aeration. However, with ethylene dibromide desorption is slow, especially at lower temperatures, and unreacted fumigant may remain for long periods after treatment (Heuser and Freeman, 1955). For this reason it is necessary to know the residual amount and the rate of desorption. Ethylene dibromide is very toxic and residues have been shown to affect the fertility of bulls (Amir and Volcani, 1965) and to cause biochemical changes in organs of chicks and rats that may affect growth, sexual development, and fertility (Alumot *et al.*, 1968; Nachtomi *et al.*, 1968). Also, it will affect the formation or release of hormones in hens (Alumot and Mandel, 1969).

Residue levels will vary with the temperature at which fumigation treatments are carried out. Many successful fumigations of various commodities have been conducted at temperatures down to 4° (30°F). With a decrease in temperature, the dosage of fumigant has to be increased to kill the insects and hence sorption of the gas by the commodity is increased. The question now arises as to whether the dosage of fumigant applied to foodstuffs at lower temperatures will lead to an increase of residues. This paper reports the levels of residues occurring in certain fruits and walnuts fumigated with methyl bromide and ethylene dibromide over a range of temperatures. Since early results showed that the "total bromide" method of determination ashing by Neufeld (1936), oxidation, and titration by Kolthoff and Yutzy (1937) failed to account for all of the bromide resulting from ethylene dibromide, the quantitative aspect of its conversion to inorganic bromide had to be investigated. The accuracy of the method used for the inorganic bromide determination was of ±10%.

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MATERIALS AND METHODS

The kinds of fruit used in this investigation were peaches from the United States and Ontario, cherries (Bing and Schmidt varieties) from the United States, apples (Delicious variety from British Columbia, MacIntosh from Ontario), and plums and walnuts from the United States. They were treated with methyl bromide and ethylene dibromide at temperatures ranging from −4 to 25° (25° to 77°F) in 525-l. chambers described by Monro and Buckland (1956). The required dosage of methyl bromide was introduced as a gas from a pressure cylinder into the evacuated dispensers of the above mentioned chambers and then released into the main component of the chambers. Ethylene dibromide as liquid was placed in a shallow glass dish and evaporated by heat from a small hot plate in the chamber. A fan was employed to ensure uniform distribution of the fumigant. Dosage of fumigant and duration of the fumigation were selected according to the product treated.

Preparation of Fruits for Analysis. The fruits were purchased in local markets during the time that each was at its peak in the marketing season. As far as possible, boxes of fruit were left packed in normal shipping containers and placed in the chamber to simulate commercial conditions. The load for fruits was 40–50 lb and for walnuts was 5 lb per treatment. Following fumigation, indi-