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Note

Microchemical method for determining formaldehyde, lower carbonyl compounds and alkylidene end groups in the nanogram range using the Keele micro-reactor

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There is a demand for methods of increasing sensitivity for the determination of many classes of organic compounds. This may arise from decreasing limits of tolerance in environmental sampling, increasing use of ultra-high purity substances, or as in our case, the need to detect and quantify very small amounts of biologically active substances such as pheromones. Gas chromatography (GC) is still an extremely versatile, sensitive and accurate method for many substances, but very small molecules, the lower carbonyl compounds among them, present difficulties because they can be lost in the solvent peak. The chemist may wish to separate and quantify these carbonyl compounds directly, or as frequently happens, he may wish to determine them as fragments from ozonolysis of larger compounds.

Many natural terpenoid substances contain either vinyl or isopropylidene end groups. Among the ants, *Myrmicaria natalensis* contains important quantities of monoterpene hydrocarbons such as myrcene, β-pinene, limonene, camphene, sabinene[1]. Four farnesenes have been identified in the trail pheromone of the ant *Solenopsis invicta*[2]. We have found four compounds of farnesene structure from C_{15}–C_{18} in many species of *Myrmica* ants, with vinyl, isopropylidene and isobutylidene end groups[3]. The structure of these end groups cannot be determined from mass spectrometry (MS) alone. For example in the case of the pheromone from the wing gland of *Eldana saccharina*, after GC–MS, micro-ozolysis at the microgram level did not distinguish between an isopropylidene or propylidene end group and resort was made to microgram NMR analysis (268 glands) to demonstrate the isopropylidene group[4]. A micro-chemical method for such alkylidene groups would be very useful.

A variety of techniques have been applied to determining the position of double bonds on a micro-chemical scale (summarized in ref. 5). Micro-ozonolysis has been a particularly useful technique, but its main drawback has been the difficulty in identifying the small molecules formed in the presence of the solvent[6]. We have recently extended the sensitivity of micro-ozonolysis down to the nanogram range[3], and, by avoiding the use of solvents, made it possible to determine acetaldehyde, acetone and higher carbonyl compounds directly from ozonides[3]. Formaldehyde still presents a difficulty because of its near-zero response factor in the flame ionization detector.

Moore and Brown[7] described a method for derivatizing formaldehyde with cyclohexane-1,3-dione to give a pyran derivative. Combined with ozonolysis, the

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method was useful for samples down to 10 μg. Others have more recently recommended formation of pentafluorophenylhydrazones[8], O-benzyloximes[9,10] O-(*p*-nitrobenzyl)oximes[10] and O-pentafluorobenzyl oximes[11], all in combination with GC, and 2,4-dinitrophenylhydrazones in combination with high-performance liquid chromatographic detection[12,13].

We describe here a method for determining low-molecular-weight carbonyl compounds, including formaldehyde, as benzyloximes with GC detection, making use of the very small working volume of the Keele micro-reactor[14]. The method is applicable down to 10^{-8} g of formaldehyde determined directly or when produced by ozonolysis, for acetone it is somewhat less sensitive.

EXPERIMENTAL

Apparatus

A Pye 104 Series gas chromatograph with a flame ionisation detector was used for GC with a 2.75 m × 4 mm (I.D.) glass column packed with 10% PEG 20M on 100–120 mesh Chromosorb W. Nitrogen was used as the carrier gas at a flow-rate of 40 ml/min^{-1} with the oven temperature at 155°C.

Preparation of reagent

The reagent O-benzylhydroxylamine hydrochloride (0.1109 g) was dissolved in water (5 ml) and acidified with 2 *M* hydrochloric acids. After extraction with hexane (3 × 5 ml) the aqueous layer was evaporated to dryness. The residue was dissolved in water (150 ml) and adjusted to pH 4.5 with 2 *M* sodium hydroxide.

Formation of derivatives

The reagent solution (15 μl) was added to standard aqueous solutions (2 μl) of acetaldehyde, acetone or formaldehyde (16 ng/μl) in a Keele micro-reactor (Wheaton Scientific, Millville, NJ, U.S.A.). After 1 h the mixture was acidified and hexane (5 μl) was added. The two phases were contacted by drawing the mixture up into a 100-μl syringe and by pumping the plunger up and down a few times[14]. Subsequently, it was transferred to the top of the vial and air was withdrawn from the lower chamber until the hexane was in the neck. A portion of the hexane layer (0.5 μl) was removed with a microlitre syringe and chromatographed.

Ozonolysis

Ozone was generated by passing oxygen through an aqueous solution of hydroxylamine hydrochloride buffered to pH 4.5, drying it through molecular sieves and then passing it through an ozone micro-generator[6]. A fine stream of ozonized oxygen was passed for 10 s over a solution of pure alkene (200 ng) in cyclohexane (40 ng/μl, 5 μl taken) in a Keele micro-reactor. A crystal of triphenylphosphine was added to cleave the ozonide, the aqueous reagent (15 μl) was added and the mixture was left for 1 h. After acidification with 2 *M* hydrochloric acid, 0.5 μl of the cyclohexane layer was chromatographed.

Ozonolysis of the cyclohexane solvent

Ozonised oxygen from the ozone micro-generator was passed over pure cy-

clohexane (5 μl) in a Keele micro-reactor, for 10 s and a crystal of triphenylphosphine was added. The reagent (15 μl) was added and after 1 h the mixture was acidified; 0.5 μl of the hexane layer were chromatograped at the same attenuation as used for the derivatives. This served as a blank determination.

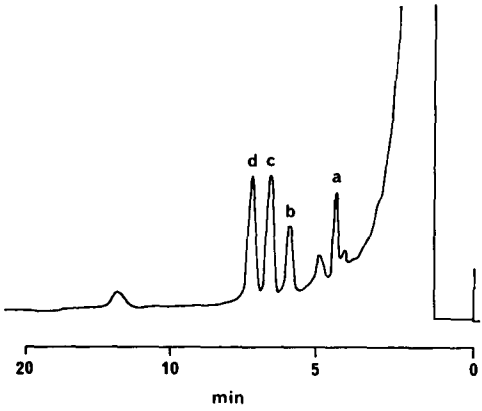
Ozonolysis of the alkenes trapped from the gas chromatograph

The GC effluent was passed through a splitter of the design of Baker *et al.*[15] to give a 95:5 (outlet: flame ionisation detector) split ratio. The trapping was carried out using the method of Attygalle and Morgan[16]. Cyclohexane (5 μl) was injected into the glass capillary containing the trapped alkene, using a 5-μl syringe fitted with a 75 mm × 0.23 mm I.D. steel needle. The capillary was then rocked back and forth to dissolve all the alkene in the cyclohexane. The cyclohexane solution was drawn back up into the syringe and placed in the Keele micro-reactor. Ozone was then passed over the solution and a crystal of triphenylphosphine was added. The aqueous reagent (15 μl) was added and the mixture left for 1 hr. After this time the volume was increased to 5 μl with cyclohexane and the mixture acidified with 2 *M* hydrochloric acid, 0.5 μl of the cyclohexane layer was chromatographed.

RESULTS AND DISCUSSION

Essentially a method was sought by which nanogram samples of formaldehyde could be detected by GC. Because of its near-zero flame response, formaldehyde had to be converted to a derivative, suitable for GC,with a normal flame response. But also, since the formaldehyde we wished to determine was usually formed in micro-ozonolysis, and other small carbonyl fragments might be produced at the same time, a single method, applicable to quantifying at least formaldehyde, acetaldehyde and acetone in a single sample was required. Since none of the methods presently available was applicable to nanogram quantities, adaptation was required. A method was sought which required minimum manipulation, and one which could take advantage of the very small working volume of the Keele micro-reactor[14], in which the reaction can be performed, quenched, and the product extracted into as little as 5 or 10 μl of solvent for GC analysis. Excessive dilution of the sample or evaporation of the excess solvent (which can lead to losses by entrainment) can thereby be avoided.

Of the derivatives available, O-substituted oximes seem most suitable for GC. Both O-benzyloximes and O-pentafluorobenzyloximes were examined. The reaction times for quantitative formation of O-pentafluorobenzyloximes were about 2–3 h whereas, once the correct pH conditions were found, benzyloxyamine reacted quickly and quantitatively with all the lower carbonyl compounds. With formaldehyde reaction was almost instantaneous, acetone reacted more slowly, 1 h was sufficient to ensure complete reaction. Heating to 70°C actually reduced yields, presumably through by-product formation. The derivatives could be gas chromatographed at moderate temperatures and were well separated from the solvent peak for quantification. Polar stationary phases, such as polyethylene glycol, gave better peak shape than less polar phases. The derivatives of several lower carbonyls are shown in Fig. 1. The method was found to be quantitative and reproducable for as little as 40 ng of formaldehyde. Below that there was too much ambient carbonyl compounds in the atmosphere. Greater sensitivity was limited by the size of derivative peaks in



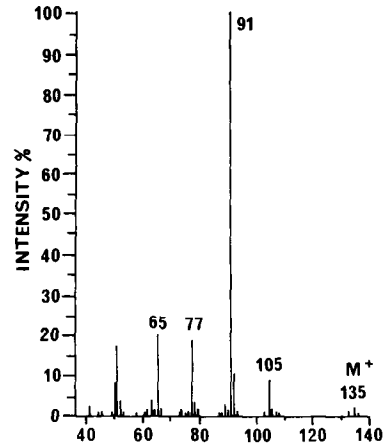


Fig. 1. Gas chromatogram of the separated O-benzyloximes of the lower carbonyl compounds at 155°C on a 10% PEG 20M column, using 32 ng of each of the parent compounds. The peaks correspond to the derivatives formed from (a) formaldehyde, (b) acetaldehyde, (c) acetone, (d) propionaldehyde.

Fig. 2. Mass spectrum of the O-benzyloxime of formaldehyde showing the very prominent tropylium ion, suitable for single-ion monitoring. The spectra of the other lower carbonyl derivatives are similar.

blank determinations. Indeed it was found that the work could not be carried out in a laboratory where other organic chemists occasionaly used acetone for rinsing apparatus and the use of acetone for washing the micro-reactor or any other glassware had to be scrupulously avoided. The glassware was always washed with chromic acid and then used immediately as it was found even if the apparatus was washed with chromic acid and then left for a week strong acetone peaks were detected in blank runs.

Mass spectra

The mass spectra of the O-benzyloximes are very characteristic, with weak molecular ions and very prominent tropylium ions at *m/z* 91 (Fig. 2). Sensitivity could be increased by single-ion monitoring at *m/z* 91 if required. The principle ions of the mass spectra are listed in Table I.

TABLE I

RETENTION TIMES AND MASS SPECTRAL DATA FOR THE O-BENZYLOXIMES OF THE LOWER CARBONYL COMPOUNDS

Chromatography was on a 10% PEG 20M column at 155°C. Further details are given in the Experimental section.

Compound	*Retention time (min)*	M^+ *(m/z)*	*Relative intensity (%)*			
			M^+	*M − 30*	*91*	*77*
Formaldehyde	3.36	135	3.4	13	100	24
Acetaldehyde	4.58	149	1.9	10	100	12
Acetone	5.18	163	18.3	35	100	22

TABLE II

EFFICIENCY OF DETECTION OF CARBONYL COMPOUNDS PRODUCED BY OZONOLYSIS OF VARIOUS ALKENES

Compounds were either ozonized directly, and products derivatized or else alkenes were trapped from GC, ozonized and then converted to derivatives. In each case 200 ng alkene was used.

Compound ozonized	*Product obtained*	*Amount found by direct determination*		*Amount found via trapping*	
		ng	*% recovery*	*ng*	*% recovery*
Limonene	Formaldehyde	38	85	65	74
Nerolidol	Formaldehyde + acetone	21, 47	78, 90	41, 94	76, 90
Eugenol	Formaldehyde	24	67	44	61
Safrole	Formaldehyde	36	90	65	87
Citronellal	Acetone	48	86	82	75
8,10-Dodecadienyl acetate	Acetaldehyde	31	73	–	
Methyl linolenate	Propionaldehyde	29	72	56	70
Ethyl crotonate	Acetaldehyde	–		62	78

Ozonolysis

Ozonolysis is a powerful method for locating double bonds in natural products. We have recently discussed micro-ozonolysis and alternative methods for locating double bonds[5]. However, we lacked the method now provided for determining formaldehyde, and any other small carbonyl fragments from ozonolysis. Table II lists a number of substances which were used as models for ozonolysis and determination of the small carbonyl fragments.

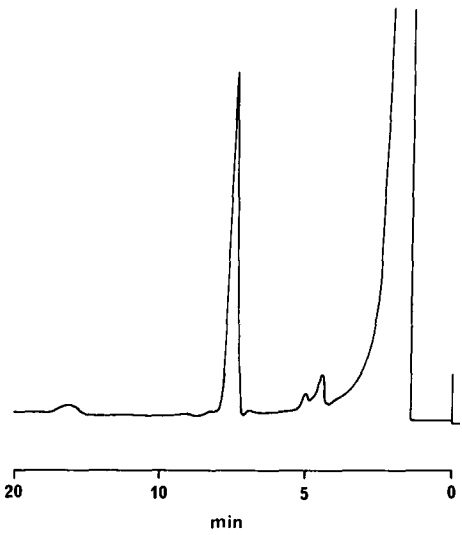


Fig. 3. Gas chromatogram of acetone O-benzyloxime produced by ozonolysis of 200 ng of citronellal in the Keele micro-reactor followed by derivative formation.

The ozonolysis worked well and in the model compounds each gave the expected lower carbonyl compounds. However, methyl linolenate gave a strong acetone peak each time as well as the propionaldehyde product as expected; this is presumed to be due to the presence of an appreciable amount of isolinolenic acid in the linolenic acid sample used. Fig. 3 shows the derivative peak obtained when 200 ng of citronellal were ozonized and the mixture of carbonyl compounds produced was converted to the O-benzyloximes. There is very little evidence of other products at this sensitivity. At lower amounts of alkene, peaks for acetone derivatives become significant, when none should be seen, due to the ambient acetone in the laboratory.

Solvent

It was difficult to find a solvent which did not give traces of ozonolysis products which obscured the quantitation. Using 5 μl of solvent, if 0.01% decomposition occurred during ozonolysis it would give rise to *ca.* 400 ng products. Even highly purified hexane was not satisfactory, neither were carbon disulphide, carbon tetrachloride nor chloroform. However, cyclohexane proved to be sufficiently stable and 10 μl gave virtually no carbonyl derivatives after treatment with ozone.

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