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HYDROGENATION AND OZONOLYSIS OF SUBMICROGRAM QUANTITIES OF UNSATURATED ORGANIC COMPOUNDS ELUTED FROM GAS CHROMATOGRAPHIC COLUMNS

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SUMMARY

Procedures are described whereby small amounts of organic compounds are trapped from gas chromatographic columns on short lengths of cooled glass porous layer open tubular capillaries containing either an inert support like Celite or an adsorbent such as alumina. Compounds collected on traps coated with palladium catalyst may be cleanly hydrogenated and the products analysed by simply breaking the traps in the heated inlet of a gas chromatograph. In a similar manner, ozonolysis may be performed on compounds in uncoated traps, and the resulting ozonides directly cleaved by heat to the carbonyl compounds. The cleavage may be carried out in the presence of an added reagent either to form derivatives suitable for gas chromatography, or to produce a characteristic colour reaction. The methods outlined are capable of providing structural information on trapped compounds at the 0.05-μg level, and in favourable cases, as low as 0.01 μg.

INTRODUCTION

In the analysis of complex mixtures of organic compounds such as those derived from natural products, mass spectrometry (MS) when combined with high-efficiency gas chromatography (GC) is the most effective (and indeed the only spectrometric) method capable at the present time of yielding structural information on components eluted from GC columns in submicrogram quantities. However, it is often difficult even with this powerful combination to identify an unknown compound if a reference spectrum is not available, or if the compound is not completely separated from other components, and a mixed mass spectrum is produced. Valuable additional information regarding the elements present in a compound may be obtained with the aid of various specific and highly sensitive GC detectors which have been developed in recent years, such as the flame photometric (FPD), thermionic (TID), and electron capture (ECD) detectors. The recently described microwave plasma detector[1], which

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may be tuned not only to detect different elements, but also to determine empirical formulae, is potentially the most useful and versatile of all such detectors.

Another approach capable of yielding valuable structural information on minute amounts of compounds involves the application of microchemical methods in conjunction with GC. The development of 'reaction GC', including such techniques as pre-column abstractors to remove specific functional groups, as well as methods for hydrogenation, hydrogenolysis (carbon-skeleton chromatography), and ozonolysis, owes much to the work of Beroza and his co-workers. Reviews of these and related techniques for structure determination appear in two recent texts[2,3].

While some of the most valuable structural information may be obtained by scaling down such techniques as hydrogenation, hydrogenolysis and ozonolysis and examining either by GC or by GC–MS the products or fragments produced, the majority of work reported to date has been concerned with pure compounds or simple mixtures. The analysis of complex mixtures, such as concentrates of volatile compounds isolated from foodstuffs in aroma research, is best performed on either small-diameter wall coated or porous layer open tubular (PLOT) capillary columns. The application of the above techniques in an on-line mode for such systems is really only feasible for straight hydrogenation of double bonds. In flavour research, Issenberg *et al.*[4] have used hydrogenation to confirm identification of *cis*- and *trans*-4-hexen-1-ol in hydrolysed banana essence by placing a hydrogenation reactor between the column and the inlet in a GC–MS system, thereby obtaining spectra of the hydrogenated products at the same retention times as the original compounds. As a general method, this procedure suffers from the disadvantage that all unsaturated compounds encountered in a complex mixture may not be hydrogenated at the same rate, thereby producing superimposed spectra of hydrogenated and unreacted compounds; these may be difficult to interpret. Also, with high-efficiency capillary columns the size and geometry of the reactor would have to be carefully determined so that mixing, accompanied by loss of resolution of already separated compounds, did not occur.

In our view an off-line system, whereby submicrogram quantities of compounds eluted as discrete peaks from high-efficiency columns are trapped and then subjected to any of the above treatments (or indeed to other chemical modification) with the minimum of manipulative difficulties, represents a much more flexible approach. An effective system, capable of achieving maximum sensitivity throughout, should incorporate the following features:

(a) Peaks to be examined should be trapped with minimal loss on elution from the column.

(b) It should be possible to store the trapped components until required for examination.

(c) The system should permit the desired reactions to be carried out on the trapped compounds *in situ*, *e.g.*, in ozonolysis it would be desirable to form the ozonides and then to decompose them, either alone to produce the normal carbonyl fragments, or, if necessary, in the presence of specific reagents to form characteristic derivatives for fragments normally difficult to detect such as formaldehyde and glyoxal.

(d) Finally, it should be possible to re-inject the products into a GC column to produce sharp peaks for identification by retention times with or without MS.

None of the procedures so far reported seem capable of fulfilling all of the above requirements. Stanley and Murray[5] have developed a useful technique for hydrogenation and hydrogenolysis of samples as small as 0.1 μg, in which a short stainless-steel tube packed with GC packing and catalyst forms a combined trap-reactor. Samples may be trapped, stored until required, reacted with hydrogen at elevated temperatures and then re-injected into a GC column.

We have described the production of narrow-bore glass PLOT columns in which a relatively thick layer of diatomaceous earth support is fused to the inner wall of the tube[6]. Short traps of such tubing have been shown to be highly effective for collecting very small amounts of compounds eluted from GC columns[7]. In addition, lengths of PLOT tubing, coated with various reagents in the manner of normal liquid phases, have been shown to be useful in functional group subtraction chromatography[8], and the trapping of small amounts of organic compounds in PLOT tubes containing activated alumina has provided a very sensitive method for identifying compounds by means of colour reactions[9]. The present paper describes the logical extension of the utility of PLOT capillaries into the area of hydrogenation and ozonolysis and shows how these techniques may be applied to peaks eluted from GC columns in amounts less than 0.1 μg, using procedures which largely satisfy the four criteria stated above.

EXPERIMENTAL

Method

The basis of the method involves the trapping of eluted compounds in short straight lengths of PLOT capillaries cooled by powdered dry-ice. For hydrogenation, tubing pre-coated with activated palladium catalyst and hydrogen carrier gas is used. After trapping, the tubes are immediately sealed off in a microburner flame and then stored at low temperatures until required for analysis. Reacted samples are readily analysed by crushing the PLOT trap in a simple, low-volume, glass injection device, fitted with a plunger to release the reaction products on to the GC column. Various capillary crushing devices, usually made of metal components, have been described for such applications as examining volatiles evolved from polymers[10] and from natural products[11], when the latter are heated in sealed glass capillaries. In a very recent note[12], the technique has been described for hydrogenation and ozonolysis of samples collected from GC columns in glass capillary tubes, which are then filled with reactive gases such as hydrogen or ozone and sealed.

In the present work, ozonolysis is readily performed *in situ* by passing ozone through the PLOT trap at low temperature (generally —70°), followed by nitrogen to remove excess of ozone from the trap. Traps may then be sealed and direct pyrolytic cleavage of the ozonide to the carbonyl fragments carried out in the heated injection block before crushing the trap to release the fragments onto the GC column. Alternatively, a small amount of a specific reagent, *e.g.*, *o*-phenylenediamine, to form the quinoxaline derivatives of glyoxal fragments (which themselves do not respond to the FID) may be applied to the trap from a hypodermic syringe fitted with a long needle, before the trap is sealed and subjected to analysis. Colour reactions where appropriate may also be used for purposes of identification, *e.g.*, compounds containing a terminal methylene group may be trapped out very sharply on a PLOT trap containing activated

alumina, subjected to ozonolysis and the resulting formaldehyde fragment detected by decomposing the ozonide in the presence of the very sensitive and specific chromotropic acid reagent.

Apparatus and materials

Except for the GC–MS work, which was carried out on a Philips Model PV4000 research chromatograph linked by means of a jet separator to an Edwards (E60) low-resolution fast-scanning mass spectrometer[13], a Pye Series 104 temperature-programmed flame ionisation gas chromatograph was used throughout. It was equipped with a heated fraction collecting port mounted on the side of the oven, which was used to heat a stainless-steel capillary line in the transfer of column effluent to PLOT traps. For peak trapping the effluent was split by means of a three-way (1.55 mm O.D.) Simplifix stainless-steel coupling (Simplifix, Maidenhead, Great Britain) between the FID and a 12-cm length of stainless-steel capillary line (1.55 mm O.D. × 0.65 mm I.D.) which led through the wall of the oven. The PLOT traps were connected to the exit line by means of a short length of high-temperature silicone rubber tubing (bore 1 mm) and cooled in a small (25 ml) plastic beaker filled with finely powdered dry-ice as shown in Fig. 1.

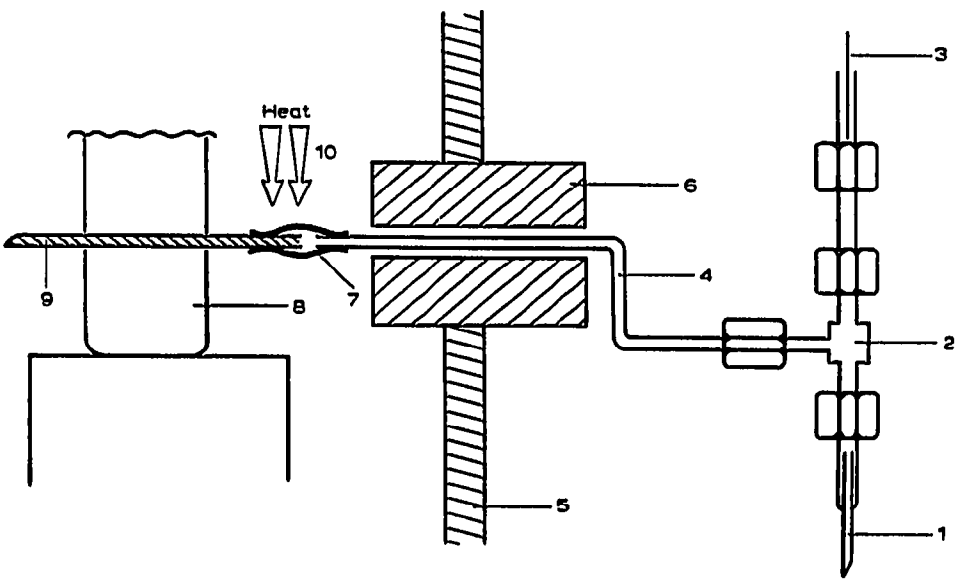


Fig. 1. Schematic representation of a peak trapping system. 1 = Hypodermic connection to column; 2 = 1.55 mm O.D. Simplifix three-way connector; 3 = restricting wire to FID; 4 = stainless-steel capillary transfer line; 5 = oven wall; 6 = outlet heater; 7 = silicone rubber sleeve (bore 1 mm); 8 = 25-ml plastic beaker with dry-ice coolant; 9 = PLOT trap; 10 = heat from hair dryer.

The device for crushing and re-injecting the contents of the sealed PLOT traps after reaction is shown in Fig. 2 and was constructed as follows: An 8-cm length of Pyrex capillary tubing (7.0 mm O.D. × 2.5 mm I.D.) was joined in a gas–oxygen flame to a 2-cm length of narrower tubing (6.0 mm O.D. × 1.00 mm I.D.). A plug of glass wool and a short length (4–5 mm) of glass rod to act as an anvil for crushing

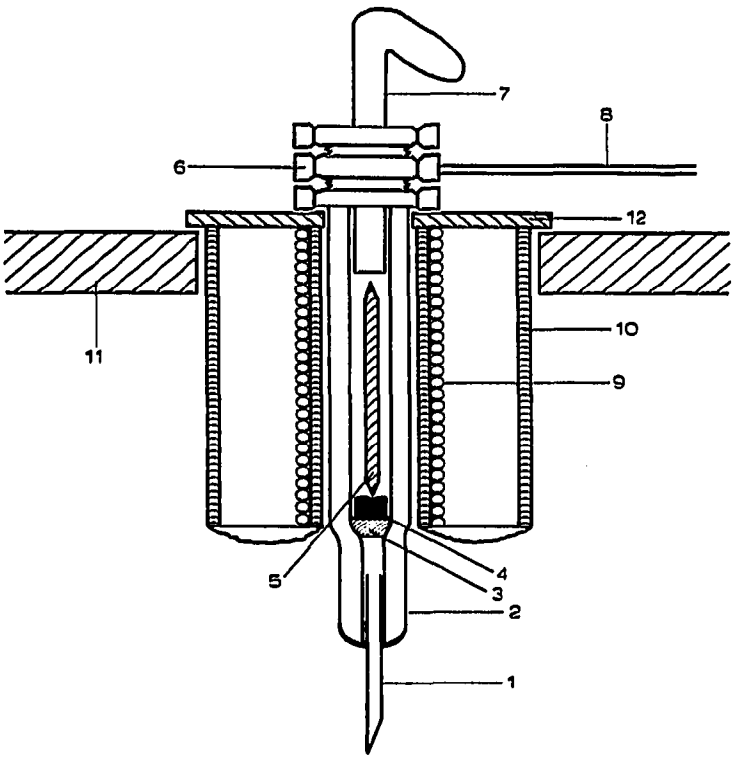


Fig. 2. Heated capillary crushing and re-injection system for PLOT traps. 1 = Hypodermic connection to column; 2 = 2-cm glass capillary tube (6 mm O.D. × 1 mm I.D.) jointed to 8-cm tube (7 mm O.D. × 2.5 mm I.D.); 3 = glass wool plug; 4 = glass rod anvil; 5 = PLOT trap; 6 = Drallim (6.25 mm) metal compression coupling; 7 = glass plunger; 8 = carrier gas inlet line; 9 = heater winding; 10 = ceramic outer casing; 11 = top of chromatograph oven; 12 = asbestos retaining ring.

the PLOT traps was inserted in the larger capillary. A 21-gauge hypodermic needle to provide the connection to the PLOT analytical column via a piece of thin-wall polytetrafluorethylene (PTFE) tubing was then cemented into the narrow capillary and sealed using epoxy resin in a manner previously described[8]. The outside diameter of the larger tube was ground down over a short distance from the end in order to fit tightly into a 6.25-mm Drallim compression coupling (Drallim, Bexhill-on-Sea, Great Britain) which was connected to the carrier gas supply. Crushing of the trap was performed with a length of glass rod of slightly smaller diameter than the larger capillary, which could be moved up and down through gas-tight seals, comprising two overlapping silicone rubber septa set into the Drallim nut. A metal rod of suitable diameter may also be used here if desired.

A heater designed to provide uniform heat to the injection block over its length was constructed by winding a length (8 cm) of Pyrex tubing (10 mm O.D. × 8 mm I.D.) with Nichrome heating wire (resistance 17 Ω/m); the latter was then cemented to the glass with a paste made from refractory cement mixed with waterglass. After hardening overnight at 100°, a thermocouple was cemented to the outer wall of the tube about half way along its length and the whole then cemented into a cylindrical

ceramic case having approximately the same outer diameter (3 cm) as the standard Pye injection block heater. The space between the wound tube and the wall of the outer tube was packed with asbestos wool to provide good thermal insulation. The block was positioned in the oven as shown in Fig. 2, and held in place by an overlapping asbestos ring fixed with epoxy resin to the top of the block. A variable voltage regulator in series with a stepdown transformer provided the temperature control.

GC columns and PLOT traps. Two glass PLOT columns (diameter 0.5 mm) prepared from 120–150 mesh Celite 545 mixed with powdered B-37 Pyrex glass as a binder, as previously described[6], were used as analytical columns. Column A, 35 m, was coated with 0.8% (w/v) solution of Carbowax 20M in methylene chloride. Column B, 21.5 m, was coated with a 0.5% (w/v) solution of silicone rubber (SE-30) in chloroform. Column A was used for all the trapping experiments and also for examining a large proportion of the reaction products. Carrier gas flow-rates (hydrogen was used exclusively for the hydrogenation work and nitrogen for the ozonolysis) were usually in the range 3–5 ml/min for both trapping and re-running of fractions. Temperature parameters varied widely and depended on the compounds under examination. Column C (3.2 m × 1.3 mm I.D.), packed with preconditioned (240° for 8 h under nitrogen) 80–100 mesh Porapak Q and operated isothermally at 150° with a flow-rate of 6 ml/min, was used solely for the analysis of acetaldehyde and acetone formed by ozonolysis.

The traps were made from straight lengths of the above PLOT capillaries. Lengths (about 2 m) were washed by filling successively under suction with concentrated hydrochloric acid, distilled water until completely neutral, and finally with AnalaR acetone. The latter was removed by purging with nitrogen gas for 30 min. The traps were then broken into sections about 10 cm in length and sealed at both ends until required for use in the ozonolysis work.

Catalyst-coated traps. Traps containing 'neutral' palladium catalyst[14] were prepared as follows: palladium chloride (32 mg) was dissolved by stirring with heated 5% aqueous acetic acid solution (2 ml), and potassium carbonate (24 mg) was added. A 2-m length of PLOT capillary was bent over a microflame into a series of narrow U tubes each about 20 cm in length and parallel to one another. The purpose of this was to permit the entire length of tube to fit in the GC oven for subsequent activation. The tubing was then filled with the catalyst solution using a hypodermic syringe. Excess of solution was expelled by connecting the tubing to a nitrogen supply and the purging continued for about 30 min until the tube was dry. The catalyst was then reduced and conditioned under a hydrogen flow of 5 ml/min by heating for consecutive 10-min periods at 100 and 150°. The activated tubing was stored in sealed 10-cm lengths.

PLOT capillaries containing activated alumina for examination of formaldehyde fragments were prepared as recently described[9].

Ozonisation apparatus. A simple laboratory ozoniser, using a Tesla coil (Edwards High-Vacuum Model T1 tester) as the electrical discharge supply, was constructed according to the design of Beroza and Bierl[15]. The flow of oxygen from the cylinder was accurately set to the low value required (4–6 ml/min) by means of a fine control needle valve and the ozonised oxygen was dried by passing through a tube of anhydrous calcium chloride. A short length of stainless-steel capillary provided a connection from the latter to the cooled PLOT trap via a piece of silicone rubber tubing.

Test materials and reagents. Test materials for hydrogenation were commercially available samples. Redistilled ether, pentane and ethyl acetate were used as solvents and were shown to be chromatographically pure.

o-Phenylenediamine was recrystallized successively from distilled water and chloroform to yield yellow plates, m.p. 100–101°.

The chromotropic acid reagent was prepared by dissolving 10 mg of the sodium salt, after recrystallisation from anhydrous methanol, in distilled water (0.3 ml) and then adding slowly and with stirring concentrated sulphuric acid (0.7 ml). Freshly prepared reagent should be used at all times.

Procedures for collecting and handling trapped fractions

Solutions of the compounds to be examined were prepared in ether or pentane so that a 0.5-μl injection contained either 0.01, 0.05, 0.1, 0.5 etc., μg of the compound as desired. With a PLOT trap immersed in the coolant, one end was snapped off and the trap connected as shown in Fig. 1. Under these conditions the entire column effluent passed to the FID. When the peak of interest was about to be eluted, the sealed end of the trap was snapped off. The restriction to the detector was arranged so that 95% of the effluent then went to the trap and the remainder to the FID. A hair dryer positioned next to the GC exit by the side of the beaker eliminated any condensation in the external transfer lines. It was sometimes useful, especially if two peaks eluting rapidly after one another were to be trapped separately, to dislodge the packing from the first 2 cm or so of the trap and to tap it free. This facilitated an even more rapid transfer of the sample into the cooled portion of the trap, and is also advantageous when dealing with high-boiling polar materials. After collecting the peak, the trap, still in the coolant, was disconnected from the silicone sleeve and immediately sealed at both ends by drawing out close to the wall of the beaker, giving a sealed trap 5–6 cm in length. Traps were stored at low temperatures and out of contact with light until required for re-running.

The above procedure was always adopted for the hydrogenation experiments using the palladium-coated PLOT tubes. Before re-running, the outsides of the traps were carefully cleaned with a tissue dipped in ether, dried, and then placed in the injection system as shown in Fig. 2. With the plunger placed in position the carrier gas was switched on to flush air from the block, the GC column connected and after the flow had stabilised, the GC oven was raised to the appropriate operating temperature. The injection block was then raised rapidly by means of the voltage regulator to the desired hydrogenation temperature (usually 150–200°) for a predetermined time, at which the trap was broken with one sharp downward movement of the plunger, and the contents swept on to the column.

Ozonolysis was performed either on compounds which had been trapped, sealed and stored, or directly after their elution from the column. Ozone was passed through the cooled (−70°) traps at a flow-rate of about 4–6 ml/min for up to 1 min depending on the amount of compound being ozonised. Excess of ozone was usually indicated after 20–30 sec by a blue coloration imparted to starch/iodide paper dipped in 1 *N* sulphuric acid and held against the open end of the PLOT capillary. After ozonolysis the excess of ozone was removed from the trap by purging with nitrogen at 4 ml/min for 1–2 min. Depending on the nature of the fragments to be studied, one of the following procedures was applied to the trapped ozonides.

(1) For the examination of simple monofunctional carbonyl fragments (formaldehyde excepted), the traps were sealed immediately after the nitrogen purge and the ozonides subjected to pyrolytic cleavage (usually at around 100°) in the heated injection head followed by analysis of the fragments on columns A, B, or C, under operating conditions designed to give the best separations for the expected products in each case.

(2) Ozonides yielding glyoxal or its homologues were treated as follows: After the nitrogen purge and with the traps still cooled, 0.25 μl of a 10% solution of *o*-phenylenediamine in ethyl acetate was injected just in front of the cooled portion of the trap by inserting the needle of a 1-μl hypodermic syringe through the end of the PLOT capillary from which the packing had been tapped free. The reagent solution was then blown into the trap by re-connecting the latter to the nitrogen supply for a few seconds. The trap was sealed and heated in boiling water for a few minutes to ensure complete formation of the quinoxaline(s). The products were chromatographed isothermally at 180° on column A, the injection block being first raised to 200° before breaking the trap.

(3) Compounds containing a terminal methylene group which were trapped out sharply at the start of PLOT capillaries containing activated alumina were always ozonised immediately after trapping. After removal of excess of ozone as described, one drop of the chromotropic acid reagent was applied to the tip of the trap using a narrow capillary glass rod. The tubes were developed by heating at 100° for 2 min. A purple coloration indicated a positive test.

The identities of the carbonyl fragments and derivatives formed in the ozonolysis experiments as well as the saturated compounds produced in the hydrogenation work, were established by checking their retention data with those of standard compounds. The latter were trapped out on PLOT capillaries containing only Celite and then re-run under exactly the same conditions as the compounds under study. Suitable *n*-alkanes in appropriate amounts were trapped out along with the compounds being examined, and also with the standards, and were used as references both for the determination of relative retention times, as well as for measuring the efficiencies of conversion of the reactants to the expected products.

Confirmation of the identities of the products in most cases was obtained by combined GC-MS using trapped samples in the range 0.5–1 μg, which yielded large GC peaks and strong mass spectra. The samples were injected as described above, by mounting the capillary crushing device on the Philips PV4000 chromatograph.

RESULTS AND DISCUSSION

It has been shown that PLOT traps in the form of U tubes cooled in liquid nitrogen provide a highly effective means of quantitatively trapping small amounts of organic compounds eluted from narrow-bore columns[7]. When it was decided to apply the capillary crushing method of re-injection in the present work, it was necessary to first establish whether trapping of compounds on straight lengths of PLOT capillary cooled by crushed dry-ice was similarly effective. It was found that for compounds containing a range of functional groups, all but the most volatile solvents were trapped with efficiencies of greater than 90% (ref. 16).

Hydrogenation of unsaturated compounds

The techniques described above provided a convenient and flexible method for the collection and hydrogenation of small quantities of organic compounds. Hydrogenation was found to occur rapidly and efficiently for a range of compounds containing different functional groups.

The efficiencies of conversion of a number of substances at around the 1-μg level were compared by trapping out the compound with an *n*-alkane, first on a palladium-coated trap and then on an uncoated trap. Both traps were then re-run under identical conditions, *i.e.*, by raising the temperature of the injection block rapidly to 150° over a period of 1.5 min before breaking. The ratios of the areas of unsaturated and saturated compounds (assuming equivalent response factors) to those of the alkane were then measured. Some typical conversion efficiencies were as follows: 2-nonenal (97%), 3-hexen-1-ol, 5-hexen-1-ol, and 3,5-hexadien-1-ol (70%), 2-hexenal and 2,4-hexadienal (83%), 5-hexen-2-one (90%), 4-nonene (90%), and 1-nonene (97%). With the exception of the alcohols the temperature/time of heating parameters were found to be not critical with respect to the yields of products obtained. For example, when 5-hexen-1-ol was heated in a palladium trap at 190° for 5 min before releasing, the peak area of hexanol was only about 15% of the theoretical value, while under the same conditions the yield of 2-hexanone from 5-hexen-2-one was scarcely affected. Hydrogenolysis rather than dehydration appeared to be occurring in the case of the alcohol, since neither hexanol nor 5-hexen-1-ol were affected by similar heat treatment in an uncoated trap. In general, the best procedure was to heat the traps for the minimum time to a temperature sufficient to yield sharp peaks for the hydrogenated compounds.

The minimum amounts of material which could be easily trapped and hydrogenated by this technique were determined for a few compounds. These quantities were: 1-nonene (0.01 μg), 2,4-hexadienal (0.015 μg), and 5-hexen-1-ol (0.02 μg). These limits were based on peak heights for the hydrogenated compounds at least three times that of the background noise and seem capable of being improved upon by careful optimisation of the chromatograph performance.

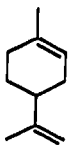
Ozonolysis

A number of unsaturated compounds chosen to yield a range of common ozonolysis fragments were examined by the procedures outlined above. The results are summarised in Table I.

The most effective systematic procedures described in the literature for the ozonolysis of small amounts of organic compounds have generally involved bubbling ozone through solutions of the substances in solvents like carbon disulphide and ethyl acetate, followed by reductive cleavage of the ozonides to carbonyl compounds, usually in the presence of triphenylphosphine. Limits of detection as low as 1 μg have been achieved for carbonyl fragments using such procedures[15], while Moore and Brown[17] have recently developed methods for forming derivatives of low-molecular-weight ozonolysis fragments in solution, which were amenable to GC. These included quinoxalines for glyoxal, azines and hydrazones for aldehydes and ketones, and a pyran derivative for formaldehyde. Detection limits as low as 10 μg were achieved. The principal factor which limits the ready extension of the above methods to submicrogram amounts is the presence of a relatively large excess of solvent, only a small

TABLE I

GAS CHROMATOGRAPHIC ANALYSIS OF OZONOLYSIS PRODUCTS

Compound	*Products identified**
$CH_3(CH_2)_2CH=CH(CH_2)_3CH_3$ 4-Nonene (0.01 μg)**	$CH_3(CH_2)_2CHO$ + $CH_3(CH_2)_3CHO$ *n*-butanal *n*-pentanal
$CH_2=CH(CH_2)_6CH_3$ 1-Nonene (0.05 μg)**	$CH_3(CH_2)_6CHO$ + HCHO *n*-octanal
 limonene (0.05 μg)**	—§ + HCHO
$Ph\text{-}CH_2C(CH_3)=CH_2$ 2-Methylallylbenzene	$Ph\text{-}CH_2COCH_3$ + —§ 1-phenylpropan-2-one
$Ph\text{-}CH_2CH=CHCH_3$ 1-Phenyl-2-butene	$Ph\text{-}CH_2CHO$ + CH_3CHO phenylacetaldehyde
$CH_2=CH(CH_2)_3CH_2OH$ 5-Hexen-1-ol (0.05 μg)**	—§ + HCHO
$CH_3CH=CH\text{-}CH=CHCHO$ 2,4-Hexadienal (0.05 μg)**	CH_3CHO + glyoxal as quinoxaline
$CH_3(CH_2)_5CH=CHCHO$ 2-Nonenal	$CH_3(CH_2)_5CHO$ + quinoxaline not formed
$Ph\text{-}CH=CHCHO$ Cinnamaldehyde	$Ph\text{-}CHO$ + quinoxaline not formed Benzaldehyde
$(CH_3)_2C=CHCOCH_3$ Mesityl oxide	$(CH_3)_2C=O$ + methylglyoxal as methylquinoxaline
$CH_3CO(CH_2)_2CH=C(CH_3)_2$ 6-Methyl-5-hepten-2-one(0.05 μg)**	$CH_3COCH_2CH_2CHO$ + $(CH_3)_2C=O$ laevulinaldehyde
$CH_3COCH_2CH_2CH=CH_2$ 5-Hexen-2-one (0.05 μg)**	$CH_3COCH_2CH_2CHO$ + HCHO laevulinaldehyde
$CH_3COCH_2CH_2\text{-}C(CH_3)=CH_2$ 5-Methyl-5-hexen-2-one (0.05 μg)**	$CH_3COCH_2CH_2COCH_3$ + —§ 2,5-hexanedione

* Identities confirmed by MS, except formaldehyde, which was identified by the chromotropic acid test only.

** Signifies the minimum amount which gave an identifiable fragment or derivative.

§ — indicates that the second product was not examined.

portion of which may be injected onto the column for subsequent analysis of the products at high sensitivity settings.

For the analysis of the simple carbonyl fragments produced from the range of compounds in Table I, the present techniques have the advantage that the ozonides may be readily formed *in situ* without solvent, and then cleanly cleaved to the desired products simply by heating the sealed traps. In preliminary work 0.25-μl injections of a 5% solution of triphenylphosphine in carbon disulphide were added to the ozonides before the traps were sealed. The purpose of the reagent was to avoid peroxide formation in the cleavage of the ozonides, and to thereby minimise side reactions. However, the yields of the carbonyl fragments varied little whether the reagent was used

or not, and it was found more convenient to dispense with it in subsequent work. A recent paper[18] has shown that the yields of carbonyl compounds obtained by heating in solution the ozonides of substituted vulpinic acids are the same in the presence or absence of added reducing agent. Although the decomposition mechanism was uncertain, it was suggested that when only small quantities are subjected to ozonolysis, traces of reducing agents present as impurities in the system sufficed to cleave the ozonides. A similar explanation may apply in the case of the PLOT traps. A crucial step in the present work was to ensure the complete removal of excess of ozone from the traps after forming the ozonides. Failure to do this often resulted in spurious peaks, and markedly reduced yields of the carbonyl compounds on re-running the traps.

Peaks due to unreacted starting materials were sometimes observed in the chromatograms of the carbonyl fragments indicating incomplete ozonolysis. The amount of unreacted compound depended on the nature of the compound and on the amount being ozonised, the efficiency of conversion being better for smaller amounts of substance. For example, with 2-nonenal and 5-hexen-2-one (2–4 μg) from 80–90% of each compound was converted to the ozonide, while for the same amounts of cinnamaldehyde and 6-methyl-5-hepten-2-one ozonolysis occurred only to the extent of 50–60%. The behaviour of the latter compound was studied in some detail. An increase in the ozonolysis time at −70° from 1–5 min did not increase the yield of the ozonide. However, as the amount of the compound was decreased, the efficiency gradually improved, complete ozonolysis occurring at the 0.05-μg level. No unreacted starting material was recovered when the ozonolysis temperature was raised to −10° for the larger quantities, but yields of the carbonyl fragments were no greater than at −70°. While these results were somewhat inconclusive, they do indicate that incomplete ozonolysis did not limit the sensitivity of the method for very small quantities.

Satisfactorily low limits of detection for simple carbonyl fragments were obtained throughout. These ranged from the detection of *n*-butanal and *n*-pentanal from 0.01 μg of 4-nonene to about 0.05 μg for fragments from such compounds as 5-methyl-5-hexen-2-one and 6-methyl-5-hepten-2-one. The presence of acetaldehyde and acetone was always confirmed using the relatively 'slow' Porapak Q column, where, under the conditions given above, the compounds had retention times of 2.15 and 5.25 min, respectively.

When the ozonolysis products from 2-methylallylbenzene and 1-phenyl-2-butene were first examined from fractions which had been trapped with the stainless-steel capillary transfer line from the column at 180–200°, benzaldehyde was found to be the principal product in both cases. This suggested that re-arrangement of the double bond to the more stable conjugated position had occurred due to excessive heating in contact with a metal surface. By lowering the temperature of the transfer line to about 100° during trapping, the expected carbonyl fragments were identified as the major ozonolysis products. These results highlighted the well-known harmful catalytic effects of even very short lengths of heated metal tube on labile compounds, and indicated the desirability of using not only glass columns and injection systems, but also either glass or glass-lined stainless-steel capillary lines during peak trapping experiments.

Glyoxal poses a problem in that although it is a product of conjugated double bond systems, it does not give a response to the flame detector. Although it can be de-

tected with an ECD, which gives a sensitive and selective response, such detectors are not always available and an alternative method based on the FID is desirable. The formation of the quinoxaline from the ozonide as described provided such a method. Quinoxalines produced from the ozonolysis of 2,4-hexadienal and mesityl oxide were detectable above background noise from 0.05 μg of starting material. A typical analysis from 1 μg of 2,4-hexadienal is shown in Fig. 3. Simple α,β-unsaturated aldehydes (see Table I) failed to give the quinoxaline after ozonolysis, either on the PLOT capillaries or in ethyl acetate solution, in the presence or absence of triphenylphosphine. A possible explanation is that formation of the ozonide is accompanied by oxidation of the aldehyde group already present to the corresponding acid. The product after cleavage would then be glyoxylic acid rather than glyoxal, and the quinoxaline would not be formed.

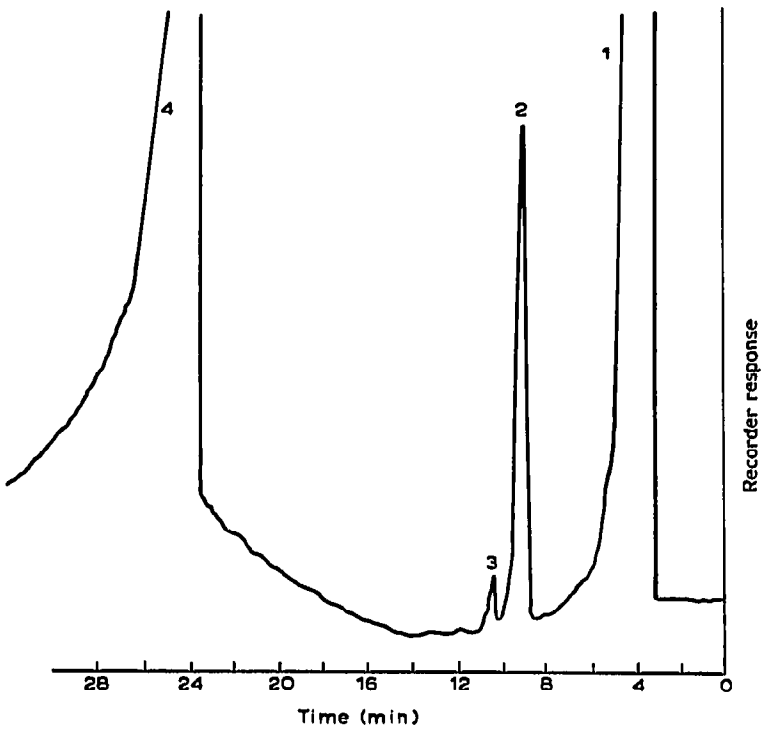


Fig. 3. Chromatogram showing quinoxaline from the ozonolysis products of 1 μg of 2,4-hexadienal by reaction with *o*-phenylenediamine in a sealed PLOT capillary. Column, 35 m × 0.5 mm I.D. PLOT, coated with 0.8% (w/v) Carbowax 20M; nitrogen flow-rate, 4 ml/min; temperature, 180°; Attenuation, × 50. Peak identity: 1 = Ethyl acetate; 2 = quinoxaline; 3 = unknown; 4 = excess of *o*-phenylenediamine.

Formaldehyde is an important fragment from compounds having a terminal methylene group and presents analytical problems on account of its volatility and lack of response to the FID. In the present work, the application of the sensitive and specific chromotropic acid colour test to the ozonides of compounds trapped as sharp bands at the tip of cooled alumina-coated PLOT traps provided a very rapid and simple method of testing for the presence of formaldehyde. Detection limits of

about 0.05 μg were obtained for a number of compounds (see Table I). While it was always important to run blanks throughout this work, especially when operating near the limits of detection, it was particularly so for the chromotropic acid test, *i.e.*, the reagent was applied to a clean PLOT capillary which was subjected to the same treatment with ozone and nitrogen purging as the sample being examined. It was also essential to use only freshly prepared chromotropic acid reagent, since slightly aged reagent sometimes gave blanks with a purple colour similar to that produced by formaldehyde itself. Other compounds such as acetone and acetaldehyde did not interfere, even when present in amounts in excess of 1 μg. Acetaldehyde gave an immediate purple colour which changed rapidly to a weak yellow, while the purple colour produced by formaldehyde was stable for several hours.

CONCLUSIONS

The procedures outlined in this paper represent a considerable extension of the utility of reaction GC to yield structural information on peaks eluted from GC columns in amounts not normally amenable to further examination by most other currently available techniques. While the methods have been demonstrated only for hydrogenation and ozonolysis of a relatively small range of compounds, a much wider applicability seems certain. For example, the flexible handling procedures should permit optimum conditions for hydrogenolysis of submicrogram amounts of compounds to be readily established. Since PLOT traps may either be precoated with various reagents, or a reagent applied to a compound already trapped to form a suitable derivative, the study of many processes such as oxidation, reduction, alkylation, acylation, etc., applied to very small amounts of trapped compounds is envisaged.

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