also supported our view. The contamination level was about 17 pg/sample with the standard deviation of 1.5 pg of selenium. The working detection limit was, therefore, calculated to be 3 pg/sample, taking twice the standard deviation of blank value as the detection limit.

Table II summarizes the detection limits for selenium by various methods. The present method gave the lowest detection limit compared with other values reported. It should be also pointed out that a fairly large amount of the extract can be injected into the HPLC system without loss of selectivity. This is one of the advantages of HPLC over other extraction–detection systems that use TLC or GC as the separation step (*9–11*, *14*). The injection volume can be increased to 80 μL without loss of selectivity. Furthermore, the volume of cyclohexane can be reduced considerably with little loss of extraction efficiency. For example, the recovery was 94% when 5 mL of the reaction mixture containing 100 pg of selenium was extracted with 1 mL of cyclohexane and 86% when the same solution was extracted with 100 μL of cyclohexane. Thus we should obtain a potentially lower detection limit (0.19 pg of Se/sample) by extracting with a 100-μL volume and injecting 80 μL of the extract, provided that the blank peak could be completely removed. Our system has now reached a level where the working detection limit seems to be determined by the contamination level of selenium(IV) in the reagents.

Registry No. H_2O, 7732-18-5; Se, 7782-49-2; NSD, 269-20-5; DAN, 771-97-1.

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Determination of Oxirane Ring Position in Epoxides at the Nanogram Level by Reaction Gas Chromatography

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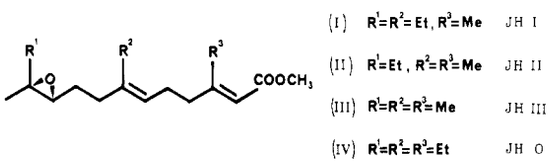
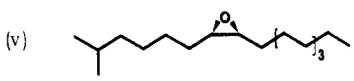
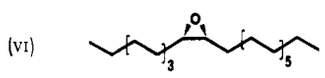
Epoxide groups are present in many biologically important natural products. The juvenile hormones (I–IV) are one group of important epoxides encountered in insects. Disparlure (V), (*Z,Z*)-*cis*-9,10-epoxyheneicosa-3,6-diene, and *cis*-9,10-epoxytricosane (VI) found in the gypsy moth (*1*), the saltmarsh caterpillar moth (*2*), and the house fly (*3*), respectively, are some examples of epoxide sex pheromones. Therefore the techniques to locate the epoxide position are of great importance to the natural products chemist. When mass spectrometry (MS) facilities are available, EI-MS (*4*) and particularly CI-MS (*5*) are useful to locate the position of the oxirane ring. As an alternative to MS, simple microchemical methods are often employed to determine the epoxide positions. Bierl et al. (*6*) performed this by the cleavage of 1–100 μg samples with periodic acid in a chlorinated solvent and subsequent examination of the carbonyl products by GC. A column of periodic acid on calcium sulfate has been used by Schwartz et al. (*7*) to cleave micromole amounts of epoxides to aldehydes. Similarly, Mizuno et al. (*8*) used HIO_4 in anhydrous ether and subsequently analyzed the carbonyl products formed by GC.

We have recently described some reaction gas chromatography methods, without solvent, for the identification of nanogram quantities of natural products (*9*). In the present study, a simple reaction gas chromatographic technique was developed to locate the oxirane position in nanogram quantities of unknown epoxides by cleavage of the epoxide to corresponding carbonyl compounds with a periodic acid precolumn.







EXPERIMENTAL SECTION

Apparatus. A Pye-Unicam PU 4500 gas chromatograph with a flame ionization detector (FID) was used for GLC, using one of the following columns: (A) 2.75 m × 4 mm (i.d.) glass column packed with 10% PEG 20M on Chromosorb W, 100–120 mesh; and (B) 1.5 m × 4 mm (i.d.), glass column packed with Porapak Q (Waters Associates, Milford, MA), 100–120 mesh. Nitrogen was used as the carrier gas at a flow rate of 50 mL/min.

Reagents. Periodic acid supplied as H_5IO_6 by Fluka AG (Buchs, Switzerland) was ground to a fine powder and dried to a constant weight in an evacuated drying pistol at 100 °C. *m*-Chloroperbenzoic acid was purchased from Aldrich Chemical Co. (Milwaukee, WI).

Test Compounds. (7*S*,8*R*)-*cis*-7,8-Epoxy-2-methyloctadecane (disparlure) was a gift of B. A. Bierl-Leonhardt (USDA). The juvenile hormones were purchased from Sigma Chemical Co. (St. Louis, MO). The noncommercially available epoxides were synthesized from the corresponding alkenes by reacting with *m*-chloroperbenzoic acid (*10*).

Precolumn Preparation. The precolumn packing (10%, w/w) was prepared by evaporating a solution of anhydrous periodic acid (100 mg) in absolute ethanol in contact with 5% OV-101 on

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100–120 mesh Chromosorb W (1 g) in a rotating evaporator. The dry and free-flowing powder was packed into a glass tube, between two silanized glass wool plugs and further dried by passing nitrogen (50 mL/min) through it at 200 °C for 2 h. Sufficient material was packed between silanized glass wool plugs, in the injection end of a PEG 20M or Porapak Q column to make a 7 cm long precolumn. The column was conditioned by injecting hexane (5 × 5 μL) onto it at 200 °C.

Cleavage of Epoxides. The epoxides (200 ng) were trapped into glass capillaries (*9*) from the gas chromatographic effluent and the ends were sealed. The tubes were crushed (*11*) and the material vaporized onto the 10% Carbowax column which contained a precolumn of 10% periodic acid. An appropriate isothermal oven temperature between 150 and 220 °C was used according to the sample. Alternatively the low molecular weight epoxides (200–300 ng) were injected as solutions in carbon tetrachloride on the Porapak Q column containing a periodic acid precolumn. The oven temperature was 200 °C. The alkylidene-group analysis of terpenes was performed by injecting a sample (1 μL) of the reaction mixture of terpene (5 μg) and *m*-chloroperbenzoic acid (50 μg) in carbon tetrachloride (20 μL).

RESULTS AND DISCUSSION

A method has been described to cleave epoxides trapped in glass capillaries by coinjecting with periodic acid (*9*). The epoxide and periodic acid were sealed in a glass tube and heated in the injection port before crushing. In the present modification the efficiency of the process has been improved by using a precolumn of periodic acid. This modification allows not only trapped epoxides but also solutions and crude reaction mixtures to be examined.

The commercially available periodic acid (H_5IO_6) contained water and was dried in a drying pistol. A number of preliminary trials were made to determine the best formulation. A 10% (w/w) loading of HIO_4 upon 5% OV-101 on Chromosorb W, 100–120 mesh gave the best results. A higher loading of HIO_4 increased byproduct formation. After the precolumn was conditioned, preinjection of a solvent such as hexane helped to reduce adsorption of the products. The precolumn could cleave epoxides quantitatively at any oven temperature between 150 and 220 °C but, preferably, it was operated at the highest temperature suitable to observe the expected product peaks. The precolumn material did not show any loss of activity on storage for months but always overnight conditioning was essential before the use of the precolumn. The precolumn material needed to be changed when the activity decreased but usually more than 100 injections of 1-μg samples could be made before any decrease of activity was seen. It is recommended to keep the periodic acid loaded column separately for the specific analysis of epoxides.

The HIO_4 precolumn on top of either a Porapak Q or a PEG 20 M column was used to determine the epoxide position of a number of known compounds. The compounds were either trapped in glass capillaries and solid injected or injected as solutions. Figure 1 shows the results obtained from the injection of three low-mass epoxides on a Porapak Q column. 1,2-Epoxypropane (propylene oxide) and 2,3-epoxy-2,3-dimethylbutane yielded ethanal and propanone, respectively, whereas 2,3-epoxy-2-methylbutane gave both ethanal and propanone. The reactions were quantitative. Many solvents were tried and carbon tetrachloride was found to be useful to examine low mass products up to C_6 on a Porapak Q column. Table I summarizes the results on the Porapak Q column of some other compounds which produce smaller fragments. When ethanal was a product, trace amounts (<5%) of ethanoic (acetic) acid were always observed due to further oxidation. A peak corresponding to methanal was not observed perhaps due to the poor response factor of methanal toward the FID. The technique clearly demonstrated by the production of butanone, in samples of 200 ng, the presence of an ethyl and a methyl group attached to the epoxide ring of juvenile hormones I (I) and II (II). In the case of juvenile hormone III (III), propanone was produced, indicating the presence of two methyl groups attached to the epoxide ring.

Table I. Reaction Gas Chromatography by a HIO_4 Precolumn on a Packed Column of Porapak Q[a]

compound injected[b]	products and comments
1,2-epoxypropane	ethanal + ethanoic acid (trace, <5%) + *c*
1,2-epoxybutane	propanal + *c*
2,3-epoxy-2-methylbutane	ethanal + propanone + ethanoic acid (trace)
2,3-epoxy-2,3-dimethylbutane	propanone
1,2-epoxypentane	butanal + *c*
cis-2,3-epoxypentane	ethanal + propanal + ethanoic acid (trace)
cis-2,3-epoxyhexane	ethanal + butanal + ethanoic acid (trace)
3,4-epoxy-4-methyl-2-pentanone	propanone + *d*
juvenile hormone I [I][e]	butanone + *d*
juvenile hormone II [II][e]	butanone + *d*
juvenile hormone III [III][e]	propanone + *d*
ethanol[f]	ethanal (low yield, 20%)
2-propanol[f]	propanone (low yield, 30%)
1,2-propandiol	ethanal + ethanoic acid (trace)
2-hydroxypropanoic acid	ethanal + ethanoic acid (trace)
ethyl 2-oxopropanoate	ethanal (trace)
2-hydroxy-1,4-butanedioic acid[f]	no ethanal
2-hydroxy-1,2,3-propantricarboxylic acid (citric acid)	no ethanal
ethyl 3-oxobutanoate	ethanal (poor yield, <5%)
3,7-dimethyloct-6-enal[g] (citronellal)	propanone + *d*
(*Z*,*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-ol[g] (farnesol)	propanone + *d*
1-methyl-4-(1-methylethenyl)-cyclohexene[g] (limonene)	no propanone + *d*
3,7-dimethyloct-2,6-dienal[g] (citral)	propanone + *d*
5-methyl-2-(1-methylethylidene)-cyclohexanone[g] (pulegone)	propanone + *d*

[a] A 1.5 m × 4 mm packed column of 100–150 mesh Porapak Q, with a 70 mm × 4 mm precolumn of 5% OV-101 silicone on 100–120 mesh Chromosorb W loaded with 10% w/w HIO_4. Oven temperature 200 °C, isothermal. [b] Unless otherwise stated, liquid injection of 200–300 ng samples in carbon tetrachloride. [c] Methanal not observed due to its poor flame response. [d] Sample not analyzed for other products. [e] Solid injection of 200-ng samples trapped in glass capillaries. [f] Liquid injection of 300-ng samples as aqueous solutions. [g] The reaction mixture (1 μL) of terpene (5 μL) and *m*-chloroperbenzoic acid (50 μL) in carbon tetrachloride (20 μL).

The method was not limited to epoxides. It can be extended to study other substances capable of being oxidized by a HIO_4 precolumn. For example, 1,2-propandiol (propylene glycol) and 2-hydroxypropanoic acid (lactic acid) were both cleaved to yield ethanal (Table I). One of the methods currently employed to determine lactic acid in blood and other biological tissues is to convert lactic acid to ethanal by HIO_4 oxidation and determine the ethanal by GC (*12*). The HIO_4 precolumn has the potential to determine lactic acid in a more efficient and convenient manner. Similarly, the method can be extended for the estimation of propylene glycol and other vicinal diols.

A HIO_4 precolumn placed before a Carbowax 20 M column was used to study compounds that produce large fragments on cleavage. Table II summarizes the compounds studied.

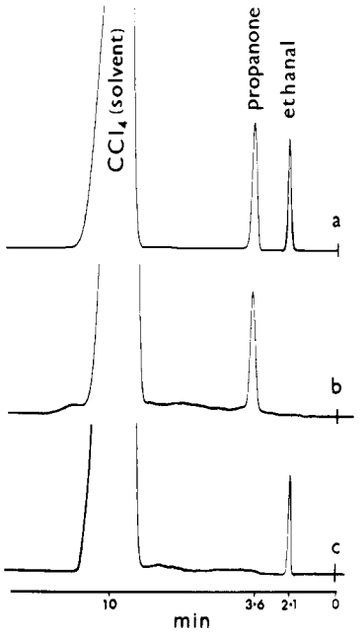

Figure 1. Cleavage of epoxides by a periodic acid precolumn, chromatograms on a Porapak Q column with a precolumn of periodic acid. Oven temperature was 205 °C. Approximately 200 ng each of (a) 2,3-epoxy-2-methylbutane (b) 2,3-epoxy-2,3-dimethylbutane, and (c) 1,2-epoxypropane was injected as solutions in carbon tetrachloride.

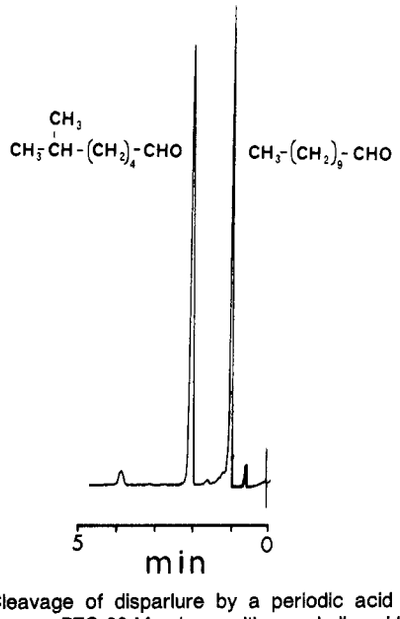

Figure 2. Cleavage of disparlure by a periodic acid precolumn, chromatogram on a PEG 20 M column with a periodic acid precolumn. Disparlue (200 ng) sealed in a glass capillary was introduced at 180 °C by a solid injection technique.

The HIO_4 precolumn cleaved *cis*-7,8-epoxy-2-methyloctadecane [disparlure (V)], quantitatively into 6-methylheptanal and undecanal (Figure 2). Epoxyethylbenzene (styrene oxide) produced phenylethanal as a minor product, along with the expected major product benzaldehyde. The precolumn was able to oxidize 3-octanol to 3-octanone to demonstrate its nonspecificity, but the yield was poor (<30%, for 200-ng samples). The yield improved to 60% when sample size was made smaller (50 ng). Although isolated double bonds are known not to be cleaved by periodic acid in solution, surprisingly, the precolumn was capable of cleaving alkenes directly though the yields were poor. Presumably this was caused by the high temperature or possibly some catalytic effect of the silica support. 8-Heptadecene and 9-nonadecene (in nanogram quantities) were completely removed by the precolumn but the corresponding aldehyde peaks represented less than 40% of theoretical yield and no other products were observed.

Table II. Reaction Gas Chromatography by a HIO_4 Precolumn on a Packed Column of Carbowax 20M[a]

compound injected[b]	products and comments
cis-8,9-epoxyheptadecane	octanal + nonanal
trans-8,9-epoxyheptadecane	octanal + nonanal
cis-9,10-epoxynonadecane	nonanal + decanal
trans-9,10-epoxynonadecane	nonanal + decanal
cis-9,10-epoxytricosane	nonanal + decanal
methyl *cis*-9,10-epoxyoctadecanoate	nonanal + aldehyde ester[c]
1,2-(epoxyethyl)benzene	benzaldehyde + phenylethanal (minor product)
trans-2,3-epoxy-3-phenylpropanal	benzaldehyde + few other products
trans-2,3-epoxy-3-phenylpropanoic acid	benzaldehyde
cis-1,2-epoxy-1,2-diphenylethane	benzaldehyde
1,2-epoxy-2-phenylpropane	acetophenone + 5% starting material
1,2-epoxy-1,1,2-triphenylethane	benzophenone + benzaldehyde
2,3-epoxy-3-phenylpropanoic acid	benzaldehyde
cis-7,8-epoxy-2-methyloctadecane (disparlure)	6-methylheptanal + undecanal
8-heptadecene	octanal + nonanal (low yield, <40%)
9-nonadecene	nonanal + decanal (low yield, <40%)
3-octanol	3-octanone (low yield, <30%)

[a] A 2.75 m × 4 mm packed column of 10% Carbowax 20M with a 70 mm × 4 mm precolumn of 5% OV-101 silicone on 100–120 mesh Chromosorb W loaded with 10% w/w HIO_4. An appropriate isothermal oven temperature between 150 and 220 °C was used according to the sample. [b] 200–300 ng samples sealed in glass capillaries were vaporized on-column. [c] No synthetic sample was available for comparison, but R_t of the peak corresponds to the expected product.

Alkenes can be better examined by this method by converting them first to epoxides by using *m*-chloroperbenzoic acid (*10*). The crude reaction mixtures in carbon tetrachloride, dichloromethane, or hexane can be directly injected onto the HIO_4 precolumn. However, the decomposition of the reagent mixture gives some impurity peaks; therefore a blank GC run of reagent mixture without the alkene was found to be useful to identify the product peaks. This method was employed to examine the alkylidene terminals (the part of a molecule between its end and the first double bond) of some terpenes (Table I). The production of propanone from citronellal, farnesol, citral, and pulegone readily demonstrated the presence of an isopropylidene group in their structures. Limonene did not produce propanone hence demonstrated the absence of an isopropylidene group in its structure.

Honda et al. (*13*) have described the cleavage of partially protected sugars at a vicinal diol group with periodic acid and identified the products by gas chromatography. The present method should be applicable to the identification of sugars in the same way, but more conveniently and with small quantities, though we have not attempted such experiments.

ACKNOWLEDGMENT

The authors thank B. A. Bierl-Leonhardt for a sample of disparlure.

Registry No. I, 13804-51-8; II, 34218-61-6; III, 22963-93-5; V, 29804-22-6; VI, 66640-79-7; 1,2-epoxypropane, 75-56-9; 1,2-ep-

oxybutane, 106-88-7; 2,3-epoxy-2-methylbutane, 5076-19-7; 2,3-epoxy-2,3-dimethylbutane, 5076-20-0; 1,2-epoxypentane, 1003-14-1; *cis*-2,3-epoxypentane, 3203-99-4; *cis*-2,3-epoxyhexane, 6124-90-9; 3,4-epoxy-4-methyl-2-pentanone, 4478-63-1; ethanol, 64-17-5; 2-propanol, 67-63-0; 1,2-propanediol, 57-55-6; 2-hydroxypropanoic acid, 50-21-5; ethyl 2-oxopropanoate, 617-35-6; 2-hydroxy-1,4-butanedioic acid, 6915-15-7; citric acid, 77-92-9; ethyl 3-oxobutanoate, 141-97-9; citronellal, 106-23-0; farnesol, 4602-84-0; limonene, 138-86-3; citral, 5392-40-5; pulegone, 89-82-7; *cis*-8,9-epoxyheptadecane, 85267-93-2; *trans*-8,9-epoxyheptadecane, 85267-94-3; *cis*-9,10-epoxynonadecane, 85267-95-4; *trans*-9,10-epoxynonadecane, 85267-96-5; methyl *cis*-9,10-epoxyoctadecanoate, 2566-91-8; 1,2-(epoxyethyl)benzene, 96-09-3; *trans*-2,3-epoxy-3-phenylpropanal, 71403-94-6; *trans*-2,3-epoxy-3-phenylpropanoic acid, 1566-68-3; *cis*-1,2-epoxy-1,2-diphenylethane, 1689-71-0; 1,2-epoxy-2-phenylpropane, 2085-88-3; 1,2-epoxy-1,1,2-triphenylethane, 4479-98-5; 2,3-epoxy-3-phenylpropanoic acid, 5694-02-0; 8-heptadecene, 2579-04-6; 9-nonadecene, 31035-07-1; 3-octanol, 589-98-0.

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Open Split Interface for Capillary Gas Chromatography/Mass Spectrometry

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The successful application of capillary gas chromatography/mass spectrometry (GC/MS) relies heavily upon the interface system. Two alternatives exist at this time. First, the capillary GC column can be directly connected to the mass spectrometer source (*1*, *2*). This method has many advantages; however, it also has several disadvantages, including: (1) all of the sample, including solvent, elutes directly into the mass spectrometer source, and (2) changing columns is laborious and time consuming, as the mass spectrometer vacuum system is vented with each column change. Direct connection has its greatest applicability for routine analysis, when capillary columns are changed infrequently. The second alternative for connecting GC columns to the mass spectrometer source is an open split interface (*3*). Some advantages of an open split interface include the following: (1) Capillary columns can be changed without an isolation valve or venting the mass spectrometer vacuum system. (2) The capillary column chromatography, in some modes of operation, is unaffected by the vacuum system. (3) The dynamic range of sample component concentrations can be greatly increased by purging portions of the highly concentrated sample, such as solvent components. (4) Chemical inertness of all surfaces, contacted by the sample prior to transfer to the mass spectrometer, is equivalent to that of the capillary GC column.

Our laboratory analyzes a diverse array of complex samples, requiring a wide variety of capillary columns. Because capillary columns must be changed frequently, an open split interface was the best choice for our capillary GC/MS use. Although our interface design is functionally similar to commercially available or previously reported units (*3*–*5*), it has some unique characteristics. Some of these are as follows: (1) The interface body is completely inside the GC oven, improving accessibility, compared to interfaces housed in the heated interface oven. (2) The use of Pyrex glass allows precise visual adjustment of fused silica (FS) capillary columns and the interface tubing [FS or vitreous silica (VS), Superox-4 deactivated] (*6*, *7*). (3) The interface tubing (0.15 mm i.d.) can easily be inserted inside the FS capillary column (0.3 mm i.d.) for maximum chromatographic efficiency, without distortion by the vacuum system ("the ideal mode", discussed later). (4) Component parts are relatively inexpensive and assembly is simple and rapid.

The platinum/iridium capillary tubing SGE isolation valve interface that came with our Hewlett-Packard 5985B GC/MS system was unsatisfactory, due to a high dead volume and to catalytic and adsorptive activities of the platinum/iridium and the glass-lined stainless steel (SS) tubing. Commercially available interfaces and those described in the literature are more complicated in design, are more expensive, and are composed of metal and/or glass-linked stainless steel (SS) tubing, making visual adjustment of the capillary column and interface tubing impossible. The design and construction of our interface and the modification of the mass spectrometer for capillary GC/MS applications are discussed. The application of the interface is illustrated by analyses of a variety of complex mixtures.

EXPERIMENTAL SECTION

Materials. Vitreous silica (VS) capillary tubing (0.15 mm i.d.) was obtained from Scientific Glass Engineering (Austin, TX), Superox-4 from Alltech (Deerfield, IL), 0–60 psi pressure regulator from Supelco (Bellefonte, PA), Swagelok $^1/_8$ in. × $^1/_{16}$ in. SS reducing unions, Swagelok $^1/_{16}$ in. × $^1/_4$ in. SS reducing unions, Nupro "J" series miniature forged body shut-off valves, and Nupro "MG" series fine metering valve from Georgia Valve and Fitting Co. (Atlanta, GA), graphite and graphite–vespel ferrules from Scientific Glass Engineering (Austin, TX), and $^1/_8$ in. SS tubing ($^1/_8$ in. o.d. × 0.085 in. i.d.) and $^1/_{16}$ in. SS tubing ($^1/_6$ in. o.d. × 0.03 in. i.d.) from Analabs (North Haven, CT). The Pyrex glass