hydrolases, such as horse serum (Armour and Co.) or acetyl-cholinesterase (Winthrop Labs.) or penicillinase (Baltimore Biological Laboratory) do not hydrolyze dibutyrylfluorescein and do not interfere. Acylase (Armour and Co.) and chymotrypsin (Calbiochem) will produce fluorescein and interfere.

Fluorescein exhibits a maximum fluorescence at pH 8 to 10, and the rate of hydrolysis of substrate reaches a maximum at about pH 7.0. For greatest accuracy, all determinations were made in tris buffer, pH 8.0. A concentration of dibutyrylfluorescein of $5 \times 10^{-5}M$ was optimum for all lipase determinations, and all runs were made at a constant temperature of 25° ± 1° C. Over the range of enzyme concentrations tested, 0.00213 to 0.0425 units per ml., there is a linear relationship between the concentration of lipase and the rate of hydrolysis, expressed as $\Delta F/\Delta t$.

The method described is considerably faster than other previously reported methods for the determination of lipase activity (*1*, *3*). Only lipase, acylase, and chymotrypsin of all the hydrolases tested catalyzed the hydrolysis of the substrate, and the method is sensitive and accurate. More information on the possible analytical applications of this procedure will be described in a future publication. In addition, a study is being made on a comprehensive substrate series of fluorescein esters to increase further the specificity and sensitivity of the method described herein.

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Analysis of Peroxyacyl Nitrates by Gas Chromatography with Electron Capture Detection

Sir: The peroxyacyl nitrates [$RCO-ONO_2$, PAN] are important constituents of photochemical air pollution (Los Angeles-type smog), causing both plant damage and eye irritation (*7*, *9*). Plant damage attributable to PAN has been observed not only in California but also in 19 other states of the nation, thus indicating the widespread occurrence of this specific photochemical product of air pollution (*4*).

PAN was first detected in the atmosphere, and partially characterized, with long-path infrared spectrometry (*5*, *8*, *10*). The first two members of the series, peroxyacetyl nitrate (PAN), and peroxypropionyl nitrate (PPN), were produced in high yields in the laboratory, purified with gas chromatography, and further characterized with the combined use of infrared spectrometry and time-of-flight mass spectrometry (*6*, *7*). Long-path infrared spectrometry is presently the only means available for measuring PAN at concentrations approaching those found in polluted atmospheres, and even this method has limitations because of expense of instruments, the large volume of sample required, and a threshold of detectability of about 5 parts per hundred million (p.p.h.m.), which is somewhat above that required to induce plant damage.

Characteristic damage to vegetation is a good indicator of the presence of PAN but is not a measure of concentration. Taylor (*11*) has shown that exposure to 1 to 2 p.p.h.m. of PAN for 6 hours will cause moderate damage to plants; further, PPN is about 5 times more toxic than PAN. Concentrations for these experiments were calculated by dilution from 500- to 600-p.p.m. sources.

Gas chromatography with flame ionization detection seemed to offer a potential method to determine PAN at atmospheric concentrations. However, atmospheric samples can be expected to contain numerous hydrocarbons, aldehydes, ketones, etc., which could not be readily distinguished from PAN in a chromatogram. Furthermore, PAN has a relatively high molecular weight for its carbon number compared to compounds of these other classes. Since the flame ionization detector responds according to the number of unoxidized carbon atoms, it is relatively insensitive to PAN.

Electron capture detectors (*2*, *3*), on the other hand, are much less sensitive to hydrocarbons and simple oxygenates but were reported to be very sensitive to nitrates, so it was logical to investigate their response to PAN. Such a detector offers an extremely sensitive method of measuring peroxyacyl nitrates in the parts per hundred million range using untreated air samples of less than 5 ml.

EXPERIMENTAL

Apparatus and Procedure. A Wilkens Instrument and Research, Inc., Aerograph A 600, HY-FI chromatograph fitted with an electron capture detector head was used for this work. A 3-foot × 3-mm. o.d. (1.5-mm. i.d.) glass column, packed with 5% Carbowax 400 on 100- to 120-mesh Chromosorb W was operated at 35° C. with a nitrogen carrier gas flow of 25 standard ml. per minute at 10 p.s.i.g. Electrical potential to the electron capture cell was set at 40 volts which was near the inflection point on the current-voltage curve. This gave a standing current of 5.5×10^{-9} amp. One- to 3-ml. samples were injected directly into a glass-lined injector port from a gas-tight syringe fitted with a 4-inch steel needle. Preliminary experiments indicated that it was not feasible to use the gas sample valve, external sample loop, or a metal column, as PAN was decomposed by, or adsorbed on, the metal surface.

PAN and PPN were prepared and purified as described previously (*6*, *7*). Gas-phase samples in nitrogen were made up in 35-liter stainless steel low-pressure cylinders at calculated concentrations of 500 to 1000 p.p.m. at 5 atm. and actual concentration was determined on a Perkin-Elmer 221-G infrared spectrophotometer using a 10-cm. cell at atmospheric pressure. By dilution from these cylinders, final samples in the parts per hundred million range were made either in glass flasks at atmospheric pressure or in low-pressure cylinders at 5 atm. Syringe samples were taken directly from the flasks or cylinders for injection into the chromatograph.

RESULTS

Samples containing approximately 5 p.p.h.m. of PAN or of PPN were injected separately into the chromatograph to establish emergence times. Resolution was quite good; with a

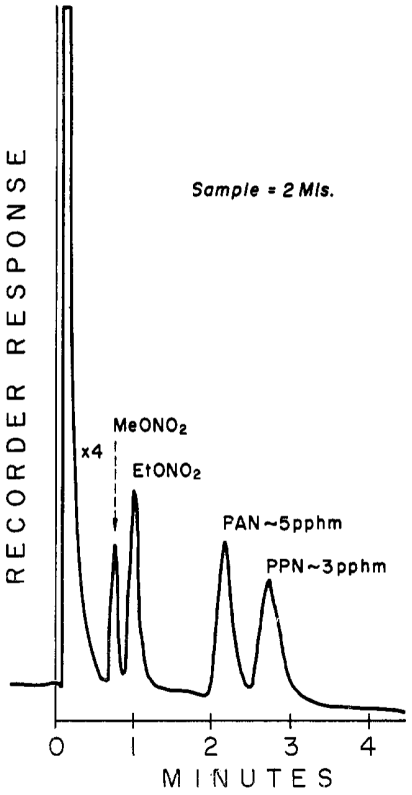

Figure 1. Chromatogram of mixture of peroxyacyl nitrates and alkyl nitrates

25 ml. per minute carrier gas flow, PAN and PPN emerged at 2 minutes 10 seconds and 2 minutes 45 seconds, respectively. Both of these compounds decompose if allowed to stand at elevated temperature (40° to 60°C.) (*7*). Methyl and ethyl nitrate are the main decomposition products and these compounds are also coproducts of the same reactions which produce the peroxyacyl nitrates. The chromatogram resulting from the injection of a 2-ml. sample containing all four compounds is shown in Figure 1. Identification of the PAN and PPN was confirmed by warming the sample flasks whereupon the PAN and PPN peaks decreased while the methyl and ethyl nitrate peaks increased. At maximum sensitivity, peak heights for 2-ml. sample injections were about 0.17 mv. per p.p.h.m. for both PAN and PPN. The present noise level of the instrument (about 0.01 mv. or 3.27×10^{-12} μa.) would permit measuring either component at 0.3 to 0.5 p.p.h.m.

Atmospheric samples taken on an afternoon of heavy (for Riverside) air pollution showed peaks indicating about 5 p.p.h.m. of PAN and 0.6 p.p.h.m. of PPN (Figure 2). The difference in peak heights for equivalent amounts of PAN in Figures 1 and 2 is due to a lower sensitivity of the detector in the latter case. Samples taken simultaneously in a greenhouse equipped with activated carbon filters for the removal of oxidant type air pollutants (*1*) revealed less than 0.3 p.p.h.m. of PAN and no PPN. An outdoor sample taken the next morning shortly after sunrise showed less than 0.2 p.p.h.m. PAN and no PPN. These results constitute the first detection of PPN in polluted atmospheres.

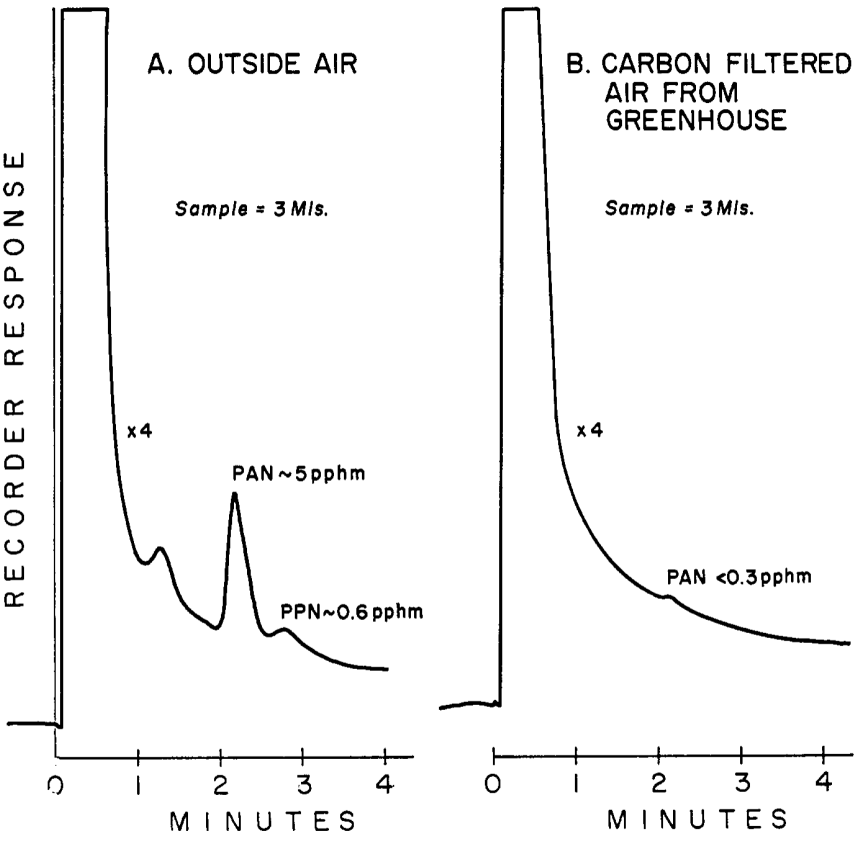

Figure 2. Chromatograms of air samples taken on a day of heavy air pollution in Riverside

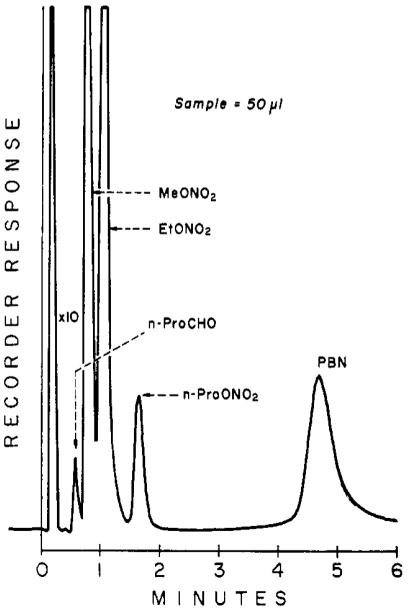

Figure 3. Chromatogram of vapors from liquid products of the reaction of ozone, nitrogen dioxide, and butyraldehyde in the dark

The four-carbon homolog, peroxybutyryl nitrate (PBN), is produced by the gas phase reaction of ozone, nitrogen dioxide, and butyraldehyde (*12*) in the dark. This reaction was carried out in the same reactor that was used for the preparation of PAN and PPN and the products were frozen out in a dry ice–acetone bath. Vapors from above this liquid were injected to give the chromatogram shown in Figure 3. In addition to the various alkyl nitrates and unreacted butyraldehyde, there is a large peak at 4 minutes 20 seconds which is probably PBN.

Further work on the calibration factors for the various homolog remains to be done with studies of the transportation of samples to the laboratory. The results to date demonstrate that the important peroxyacyl nitrates can be measured in polluted atmospheres at less than 1 p.p.h.m. with an electron capture detector using very small untreated samples.

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Gas Chromatography of the Antihistamines

SIR: The antihistamines are a group of basic compounds which are relatively nontoxic. However, many of them produce drowsiness as a side effect, and others are used as mild sedatives. These facts, plus the possibility of synergistic effects with alcohol, give the antihistamines forensic importance.

On a column utilizing Carbowax 20M as the liquid phase they generally have retention values which lie between those of the sympathomimetic amines (*2*) and the phenothiazines. The structures of the antihistamines are given by Vecerkova, Sulcova, and Kacl (*3*) with a paper chromatographic method for their analysis.

The Hy-Fi gas chromatograph (Wilkens Instrument and Research Co., Walnut Creek, Calif.) with hydrogen flame ionization detector, Aerograph Model 600, and the Leeds and Northrup Speedomax H, 0- to 1-mv. recorder, Model S, were employed in the study reported here. The chromatographic column was a stainless steel tube $^1/_8$-inch o.d., 0.093-inch i.d., 5 feet in length. It was packed with 60- to 80-mesh Chromosorb W, acid-washed, treated with potassium hydroxide 10% (w./w.) and coated with Carbowax 20M 2% (w./w.). The column was preconditioned at 200° C. for 10 hours.

The stationary phase was prepared in two steps. First a slurry was made of the weighed Chromosorb W and a volume of methanolic potassium hydroxide sufficient to cover the solid support, and containing the specific weight percentage of base. The methanol was evaporated at once on a steam bath with the air over the slurry being aspirated by vacuum, and with occasional stirring. Then the Carbowax was dissolved in chloroform and coated on the previously-KOH-treated support in a similar manner.

The operating conditions employed were: injector temperature 230° C., oven temperature 190° C., flow rate of carrier gas (nitrogen) 79 cc. per minute, an oxygen flow of 90 cc. per minute being used to maintain the flame.

Table I. Retention Values for Some Antihistamines Relative to Methapyrilene HCl

Antihistamine	Relative retention time	Antihistimine	Relative retention time
Diphenhydramine HCl	0.460	Parabromdylamine maleate	1.55
Doxylamine succinate	0.579	Bromophenhydramine HCl	1.82
Antazoline HCl	0.603	Chlorothen citrate	1.84
Tripelennamine base	0.857	Triprolidine HCl	3.02, 5.18[b]
Chlorpheniramine maleate	0.984	Pyrilamine maleate	3.33
Methapyrilene HCl	1.00[a]	Methdilazine HCl	8.00
Carbinoxamine maleate	1.53	Clemizole HCl	No peak observed

[a] 6.3 minutes.
[b] Larger of the two peaks, and asymmetrical.

Responses were obtained from samples of 1 to 10 μg. of the compounds dissolved in 95% ethyl alcohol.

Table I lists relative retention values for the antihistamines chromatographed.

All of the above antihistamines were also chromatographed in the free base form. Their relative retention values corresponded with those of their respective salts within the limits of experimental error. It would appear that the injector temperature was sufficient to dissociate the salts, and that the observed peaks were actually those of the free bases.

In a preliminary presentation of this study (*1*) the use of a similar column was reported—one which had been prepared in a slightly different manner. During use the retention characteristics of that column changed continuously. The retention times for the antihistamines became increasingly shorter, in some instances as much as 14% in a week.

The column used for the study reported in this communication did not show this undesirable characteristic, exhibiting no drift in its three days of use. The reason for the shortening of retention times with use is not understood, but the following factors may contribute to this effect: slow bleeding of the liquid phase, uneven deposition of the liquid phase, and disruption of particle integrity both in coating the liquid phase on the solid support, and in packing the column. These three factors would permit active sites in the solid support to become exposed.

The efficiency of this column, or any similar one, could probably be improved by rescreening the solid support after its preparation, to remove crushed particles. The KOH treatment of the solid support makes the particles very liable to disruption, and the support must be handled with care.

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