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# GAS CHROMATOGRAPHIC BEHAVIOR AND CHEMICAL STRUCTURE OF METHYL PARATHION AND METHYL PARAOXON IN RELATION TO THEIR HOMOLOGS

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## SUMMARY

Gas chromatography of methyl parathion and methyl paraoxon homologs on nonpolar phases shows that oxons elute before thions while the trend is reversed on polar phases. The pattern of elution of homologs shows linear relationship to chemical and thermodynamic properties as obtained from plots of retention value *versus* number of carbon atoms or number of methyl groups, and a plot of reciprocal of temperature *versus* log of retention time on polar and nonpolar phases shows straight relationships.

## INTRODUCTION

Due to increased use of organophosphorus pesticides to replace the persistent organochlorine compounds, methods for their reliable determination are needed. The parent compounds are usually thionates which are essentially noninhibitory to cholinesterase but which are metabolized to toxic "oxons" which are invariably potent cholinesterase inhibitors; methods for their determination are therefore also of particular interest. Several reports of their separation by GLC are available. The general consensus is that "oxons" elute before "thions" on nonpolar liquid stationary phases, while the elution pattern is reversed on polar phases[1]; the opposite behavior has been also reported, however[2].

In order to clarify these conflicting data as well as to develop a method for the simultaneous separation of both types of compounds, a detailed study of the behavior of methyl parathion and methyl paraoxon homologs was undertaken; this study also permitted us to relate their chemical structures to their retention behavior.

## EXPERIMENTAL

Methyl parathion (O,O'-dimethyl *p*-nitrophenyl phosphorothioate) and methyl paraoxon (dimethyl *p*-nitrophenyl phosphate) were prepared in the usual manner[3]. Parathion (O,O'-diethyl *p*-nitrophenyl phosphorothioate), *n*-propyl parathion (O,O'-dipropyl *p*-nitrophenyl phosphorothioate), and paraoxon (diethyl *p*-nitro-

phenyl phosphate) were received as analytical samples from the American Cyanamid Co., while *n*-butyl parathion (O,O'-dibutyl *p*-nitrophenyl phosphorothioate) and *n*-butyl paraoxon (*n*-dibutyl-*p*-nitrophenylphosphate) were obtained from Dr. T. R. FUKUTO of this department.

Gas chromatographic analyses were done as previously described[3] using Apiezon L and DEGS as liquid phases with a Hewlett-Packard Model 402 high-efficiency gas chromatograph equipped with hydrogen flame detector. The detector was modified for the thermionic detection of phosphorus by mounting a KCl pellet on the burner jet.

## RESULTS AND DISCUSSION

Results of gas chromatography of a limited number of homologs of methyl parathion and methyl paraoxon on Apiezon L and DEGS columns are given in Table I.

TABLE I

RELATIVE AND OBSERVED RETENTION TIMES OF INDIVIDUAL INJECTIONS OF METHYL PARATHION, METHYL PAI OXON AND THEIR HOMOLOGS

Temperatures of flash heater and detector: 210°; flow rates of nitrogen, hydrogen, and air: 40, 21, and 300 r min, respectively.

| *Column temp. (°C)* | *Relative and observed[a] retention times* | | | | | | |
|---|---|---|---|---|---|---|---|
| | *Methyl paraoxon* | *Methyl parathion* | *Paraoxon* | *Parathion* | *n-Propyl parathion* | *n-Butyl paraoxon* | *n-Butyl parathion* |
| *Column: 5% Apiezon L on Gas-Chrom Q 80/100, 2 ft. × 4 mm I.D.* | | | | | | | |
| 170 | 0.77 (1.85) | 1.0 (2.40) | 1.0 (2.45) | 1.62 (3.90) | 3.41 (8.2) | 5.4 (13.0) | 8.0 (19.3) |
| 190 | 0.63 (0.85) | 1.0 (1.35) | 1.0 (1.35) | 1.47 (2.00) | 2.81 (3.80) | 4.08 (5.50) | 5.85 (7.9) |
| 210 | 0.60 (0.40) | 1.0 (0.65) | 1.0 (0.65) | 1.39 (0.90) | 2.70 (1.75) | 3.70 (2.45) | 5.70 (3.70) |
| *Column: 1.4% DEGS on Gas-Chrom Q 80/100, 2 ft. × 4 mm I.D.* | | | | | | | |
| 170 | 1.25 (2.65) | 1.0 (2.10) | 1.15 (2.40) | 0.95 (1.95) | 1.21 (2.55) | 2.43 (5.1) | 1.81 (3.80) |
| 190 | 1.25 (1.25) | 1.0 (1.00) | 1.15 (1.15) | 0.95 (0.95) | 1.10 (1.10) | 2.30 (2.30) | 1.70 (1.70) |
| 210 | 1.20 (0.56) | 1.0 (0.47) | 1.12 (0.53) | 0.95 (0.44) | 1.10 (0.52) | 2.02 (0.95) | 1.59 (0.75) |

[a] Figures in parentheses denote the observed retention time in minutes.

Isothermal separation of these compounds on the Apiezon L column is shown in Fig. 1. On the Apiezon L column "oxons" elute before "thions" in all instances, while on the DEGS column this trend is reversed. Although *n*-propyl paraoxon was not available, from the behavior of the other compounds it would be expected to give a separate peak. These data, therefore, lend support to the observed gas chromatographic behavior of methyl paraoxon reported earlier[3].

However, under the conditions employed, methyl parathion and paraoxon could not be resolved when injected simultaneously on the Apiezon L column, and most of the compounds could not be resolved when injected simultaneously on the DEGS column. Although methyl parathion and paraoxon may be separable on other columns or by low-temperature GLC, their separation was not investigated further because they can be distinguished relatively easily by using a flame photometric detector in both phosphorus and sulfur modes.

Although the *n*-propyl and *n*-butyl homologs are not commercial insecticides,

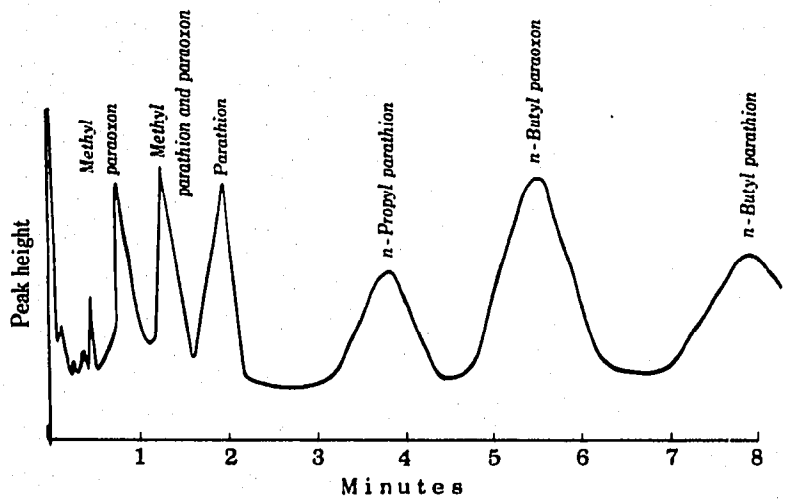



Fig. 1. Isothermal separation of methyl parathion and methyl paraoxon homologs. Column: 5% Apiezon L on Gas-Chrom Q 80/100, 2 ft. × 4 mm I.D.; temperatures of column, flash heater, and detector: 190, 210, and 200°, respectively; flow rates of nitrogen, hydrogen, and air: 40, 21, and 300 ml/min, respectively.

this system of separation could be extremely useful for studies in which the relationship of molecular dimensions, chemical structure, and biological activity are to be compared.

Although the resolution of this series of compounds was inferior on the DEGS column, the pattern of "oxons" eluting later than "thions" was as expected. However, the data present a very puzzling situation since parathion elutes before methyl parathion (also previously observed by BOWMAN AND BEROZA[4]) and paraoxon elutes before methyl paraoxon. HRIVNAK AND PASTOREK[5] also observed a shift of compounds containing a methyl group toward higher retention values on liquid phases of increasing polarity. Although no explanation can be advanced for this anomalous behavior on the basis of our present knowledge of the GLC behavior of organophosphorus compounds, the observation is significant since it points out that an attempt to correlate the behavior of organophosphorus compounds directly with that for saturated hydrocarbons in the literature could be very deceptive.

## IDENTIFICATION AND BEHAVIOR OF COMPOUNDS

If the GLC column used for separation is sufficiently specific and has enough theoretical plates, each sample component can be theoretically separated from the others. If these compounds are not the same, there will usually be at least slight differences in retention volumes, indicated by the occurrence of a shoulder on the augmented peak or by rounding of the peak top accompanied by an increase in peak width.

Peak identifications from comparisons of retention values on a single column are, however, limited because the results are only characteristic but not specific. Specificity can be increased and further confirmation can be obtained by determining relative retentions on two columns having widely different polarities, or it can be buttressed by the use of selective detectors which are responsive only to certain atomic groupings[6]. Ancillary systems including chemical tests, TLC, cholinesterase inhibition,

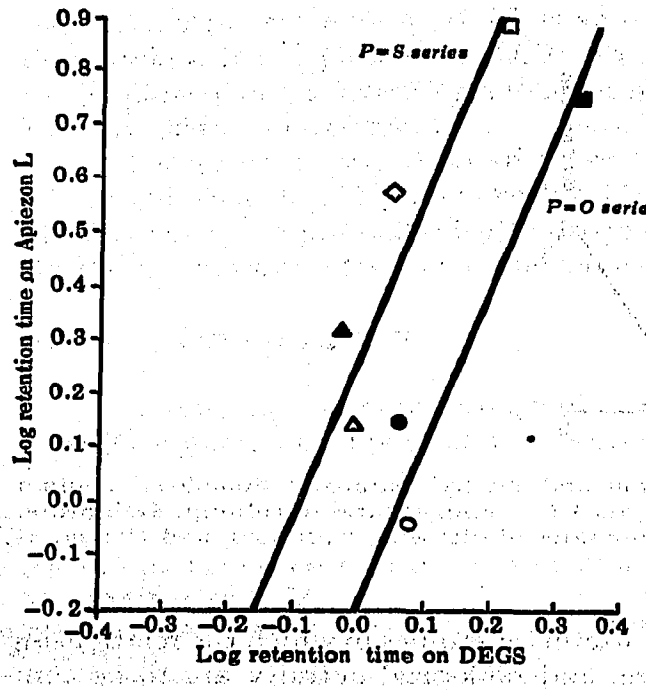



Fig. 2. Relationship between log retention time on DEGS and Apiezon L columns (data from Table I at 190°, legends as in Fig. 3).

mass spectrometry, and IR spectrometry can also be used for confirmation of identifications.

The uncertainties and ambiguities which arise in the use of coincidence methods by the use of a single column are perhaps greatest when working with compounds which have not been rigorously studied previously by GLC. The confusion can be further enhanced if some unexpected or unconsidered reaction or if some adsorption or chemical change occurs in the column.

The determination of retention data on columns of different polarities is very valuable for qualitative identification. This was first described by JAMES[7], who observed that when the retention volumes of aliphatic amines were plotted on two different columns, the respective points of each homologous series (primary, secondary, and tertiary) always gave a straight line. However, it was later demonstrated that it is more useful to plot the logarithm of the retention values than the actual values because in the latter case the graph becomes crowded in the region close to the origin[8–10]. Slopes of such graphs are characteristic of the chemical structure of the individual homologous series.

Using a log/log scale, the resulting plots are almost parallel to each other and now the intercepts are characteristic of the individual homologous series. Such a relationship for log retention time on Apiezon L *versus* DEGS, for example, is depicted in Fig. 2 for the dialkyl-substituted *p*-nitrophenyl phosphates and phosphorothioates.

The retention values of a homologous series can usually be related to various physical properties such as boiling points or to structural properties such as number of carbon atoms. The logarithms of retention times, relative retentions, or retention volumes *versus* such characteristics show a linear relationship. A plot of retention time

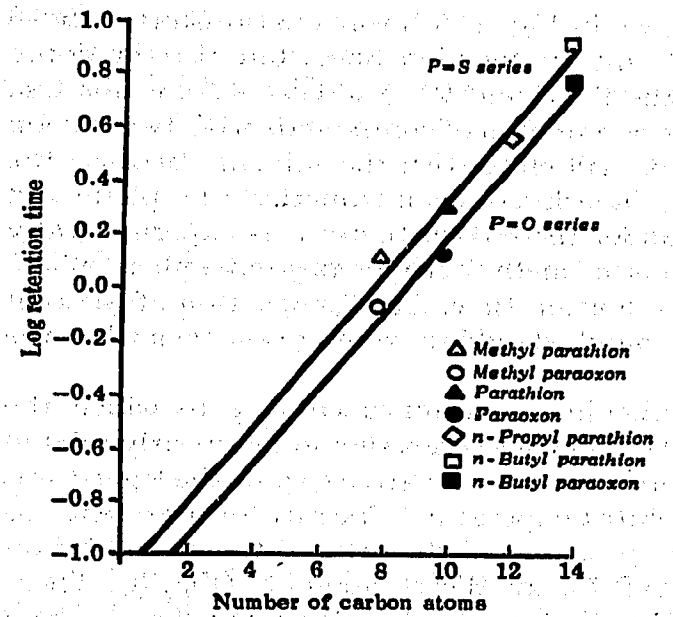
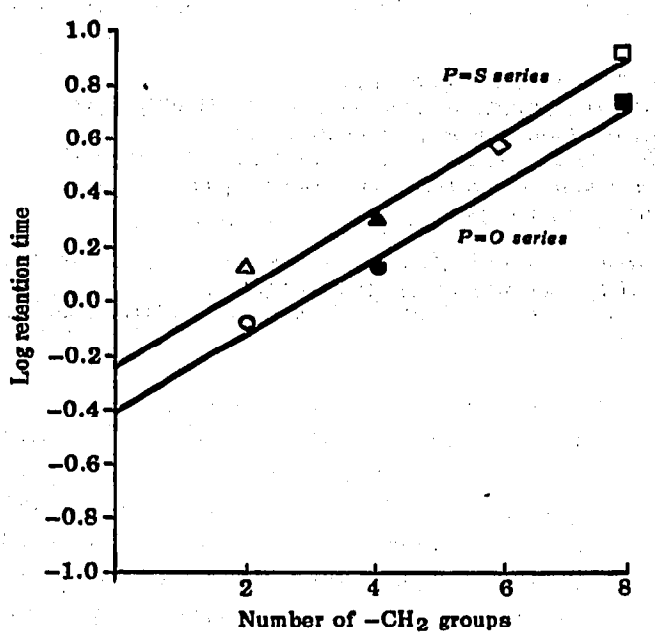



Fig. 3. Relationship between log retention time *versus* number of carbon atoms in the compound (data from Table I at 190° on Apiezon L).



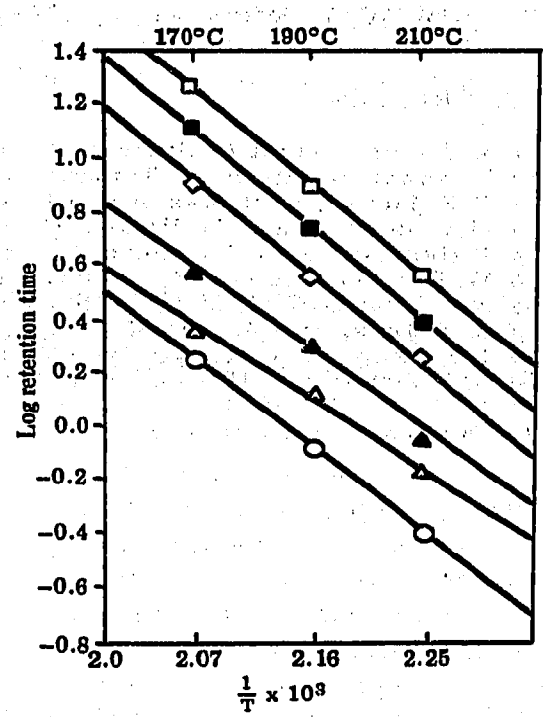




Fig. 4. Relationship between log retention time and number of substituted methylene groups in *p*-nitrophenylphosphorus esters (data and legend as in Fig. 3).

Fig. 5. Relationship between log retention time *versus* reciprocal of absolute temperature (data from Table I on Apiezon L, legends as in Fig. 3).

*versus* total number of carbon atoms is shown in Fig. 3. A linear relationship for both the P=S and P=O series is observed. Not only are the plots linear but also the slopes are parallel. The fact that the curves for the P=O and P=S series cross the abscissa at about two carbon atoms indicates that the retention of compounds with two carbon atoms will be zero, that is, the compounds will elute with the solvent. Because the solvent peak also has a measurable width, dimethyl or even trimethyl phosphate and phosphorothioates will not be resolved under these conditions, as is experimentally observed. Fig. 4 shows a plot of the number of methyl groups in *p*-nitrophenylphosphorus esters *versus* the logarithm of the retention time. The intersection of the plot with the *y*-axis indicates that all such compounds should show retention even when the number of methyl groups is zero.

Because of the validity of the Arrhenius equation, according to which the equilibrium constants of compounds in a homologous series should be linearly related to the reciprocal of the absolute temperature, it was informative to plot the logarithms of the retention times *versus* reciprocal absolute temperature. The ratio of the retentions of two solutes has been shown to be directly related to the ratio of their equilibrium constants. A linear relationship is observed for all the compounds (Fig. 5). These demonstrated relationships give considerable credence to the validity of the observed elution of methyl paraoxon before methyl parathion on the Apiezon L column and question the results of NAKATSUGAWA *et al.*[2], who reported the opposite behavior.

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