

Prulaurasin (D- und L-Mandelsäurenitril- β -D-glucosid) gar nicht, Isoamygdalin (D- und L-Mandelsäurenitril- β -D-gentiobiosid) nur andeutungsweise getrennt (Fig. 1).

An OV-1 konnten beide Diastereomeren-Gemische deutlich getrennt werden (Fig. 2). Gegenüber OV-1 zeigt OV-17 ab 250° eine deutlich höhere Flüchtigkeit (Fig. 3). Die Trennung der Amygdalin-Isomeren ist an OV-17 schlechter als an OV-1.

Additionsanalyse ergab, dass die Peaks mit kürzeren Retentionszeiten der L-, diejenigen mit längeren R_t -Werten der D-Form zuzuordnen sind. Die Einstellung eines Gleichgewichts bei der Isomerisierung verläuft bei beiden Glycosidgemischen zugunsten der L-Form. Dies wird für Amygdalin in der Literatur angegeben⁸. Amygdalin isomerisiert bereits in wässrigem Pyridin⁸. Eine ähnliche Beobachtung wurde von uns für Prunasin gemacht. Dies ist bei der praeparativen Papier- und Dünnschichtchromatographie mit dem häufig verwendeten Fließmittel Butanol-Pyridin-Wasser (6 : 4 : 3) zu beachten.

Eine Kontrolle der Einheitlichkeit der Glycoside ist mit der angegebenen Methode leicht möglich.

Pharmakognostisches Institut
der Universität Freiburg,
78 Freiburg i. Br. (B.R.D.)

A. NAHRSTEDT

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Effect of liquid phase loading and temperature on the relative retentions in the gas chromatography of methyl parathion and parathion

Differences in equilibrium constants of various solutes on a particular liquid phase are directly related to the separation achieved. The determination of equilibrium constants has limited value in the practical utilization of gas chromatography in comparison to theoretical studies, because their calculation requires precise knowledge of values such as amount of liquid phase, its density at operating temperatures, and its surface area, all of which are usually unknown and cumbersome to determine. A practical, simple operational substitute is the determination of relative retention time or volume in which retention of one solute is measured relative to that of a

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reference solute on the same column and under the same operating conditions.

However, in the GLC of methyl parathion and metabolites relative retentions of $P = O$ analogs were observed to be higher on low-loaded columns than on high-loaded columns¹. KEULEMANS² explained that adsorption by the support of tube wall affects retention values particularly if polar solutes are analyzed on a column with a non-polar stationary phase, and such effects may be observed with other phases as well, and that absence of these effects can be verified when retention values are identical on two or more columns containing increasing amounts of stationary phases³.

GIUFFRIDA *et al.*⁴ evaluated the effect of liquid phase loading (20, 10, and 5% DC-200) on the relative retentions of various pesticides, including DDT, which is nonpolar, and observed a most significant effect due to loading. They evaluated these effects at different temperatures, but when they transposed these data they rightly concluded that liquid phase loading has no effect and the most significant differences are due to temperature, a fact well in agreement with theory⁵.

In this communication are examined the effects of liquid phase loading and temperature on the relative retentions of methyl parathion and parathion, both nonpolar solutes, on Apiezon L liquid phase, which is classified as a nonpolar phase⁶, in order to substantiate whether this general theory as developed for saturated hydrocarbons applies to these and related compounds. Although these compounds are termed nonpolar, they also have oxygen functions which may interact with the support or with the wall of the column.

Experimental

The GLC analyses were carried out with a Hewlett-Packard, Model 402, high-efficiency gas chromatograph equipped with two 2 ft. \times 4 mm I.D. U-shaped glass columns with liquid phase loading of 3% and 12% Apiezon L on Gas-Chrom Q 80/100. The columns were conditioned at 240° for one week. Nitrogen, hydrogen, and air flow were identical and precisely controlled through flow controllers (Brooks Instrument Corp.). The hydrogen flame detectors were modified for the thermionic detection of phosphorus through the mounting of KCl pellets on the burner jets.

Standard solutions of purified methyl parathion and parathion (American Cyanamid Co.) in acetone were prepared. Two or three μ l of 5 ng/ μ l of each were injected separately as well as in mixture. A given temperature was equilibrated for at least 1 h.

Results and discussion

Limited changes in retention time normally occur for various reasons². In addition to adsorption by solid support and tube wall, variation can result from over-loading of the column due to large sample size, from changes in the chemical composition of the liquid phase, or some unresolved impurity in the solute peak.

Another factor can be an on-column reaction. The eluted peak of the product formed in such a reaction is 6–10 sec delayed in comparison to that when the product itself is injected. This phenomenon is discussed in detail by JAGLAN *et al.*⁷.

The relative retentions of methyl parathion and of parathion are given in Table I. At a given temperature, the differences in the relative retention are small in comparison to the differences between temperatures. According to theory⁵ the

TABLE I

COMPARISON OF RETENTION TIMES OF METHYL PARATHION AND PARATHION WITH DIFFERENT LIQUID PHASE LOADINGS AND TEMPERATURES

Column, Apiezon L on Gas-Chrom Q 80/100, 2 ft. \times 4 mm I.D.; temperature of flash heater and detector, 210°; flow rates of nitrogen, hydrogen, and air, 40, 21, and 300 ml/min, respectively.

Temperature (°C)	3% Apiezon L			12% Apiezon L		
	Methyl parathion	Parathion	Ratio of relative retentions	Methyl parathion	Parathion	Ratio of relative retentions
185	1.45	2.20	1.517	7.0	10.50	1.500
195	1.05	1.55	1.476	5.1	7.5	1.471
205	0.8	1.15	1.437	3.7	5.25	1.419

liquid phase loading has no effect on relative retention when nonpolar solutes are analyzed on nonpolar phases. This is true for highly nonpolar compounds alone. Because the relative retention is higher on low liquid phase loading, probably adsorption with support does take place with these compounds. Therefore, it is concluded that it is fallacious to assume that these compounds are nonpolar in the classical sense, since these compounds are hundreds of times more soluble in this liquid phase than are the saturated hydrocarbons.

The data also verify that relative retention increases with decreasing temperature. The temperature dependence of the relative retention has been expressed as $\log \alpha = (a/T) + b$, where α is the relative retention, T is the absolute temperature, and a and b are constants⁸. Very often $a > 0$ and the relative retention increases with decreasing temperature. When a is approximately equal to zero the relative retention remains practically the same when the temperature is changed, and this is noticed with a number of compounds⁹. Infrequently a/T becomes smaller than b when the temperature is changed; thus the order of peaks can be reversed.

The variation of relative retention of the solutes with temperature is similar to changes in their equilibrium constants or their retention volumes. Therefore, linear plots of log retention time *versus* reciprocal absolute temperature can be obtained as reported previously¹⁰.

Department of Entomology, University of California,
Riverside, Calif. 92502 (U.S.A.)

P. S. JAGLAN
F. A. GUNTHER

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