

ISOTOPIC EFFECTS IN GAS-LIQUID CHROMATOGRAPHY

I. GAS CHROMATOGRAPHIC BEHAVIOUR OF BENZENE
AND PERDEUTEROBENZENE

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The possibility of using gas chromatography for the separation of natural isotopes has been investigated so far only for hydrogen¹⁻⁴ and neon⁵. The introduction of high efficiency columns, however, opens new possibilities for the determination of isotopic molecules. A partial separation of deuterated and tritiated cyclohexane has been obtained by WILZBACH AND RIESZ⁶, but more recently FALCONER AND CVETANOVIC⁷ have shown that a number of hydrocarbons can be effectively separated from their partially and fully deuterated isomers on capillary columns.

The purpose of this investigation was to evaluate the factors which affect the separation and the determination of two isotopically substituted molecules by gas-liquid chromatography with the aim of obtaining a number of fundamental data, which may then be extended to the analysis of any system. This work deals with the gas chromatographic investigation of benzene and perdeuterobenzene where the mass difference is about 7.7 %.

EXPERIMENTAL

A home-made gas chromatograph was used with a hydrogen flame ionization detector; the main advantage is, in comparison to commercially available instruments, that it can be thermostated by a circulating liquid so that an accuracy of about 0.1°C can easily be obtained in the temperature range 0-50°.

Perdeuterobenzene was obtained from Fluka (Buchs); its purity was higher than 99 %.

Experiments were performed to estimate the response of the hydrogen flame detector towards benzene and perdeuterobenzene. It was found that the ratio of the peak areas is the same as the ratio of the molar concentrations in the range of the experimental error (1-2 %).

Glass capillary columns, 0.3 mm internal diameter were used, and prepared as described by DESTY *et al.*⁸. The experiments were carried out on columns coated, as described elsewhere⁹, with the following liquid phases: dinonyl phthalate (A), squalane (B) and silicone oil 702 (C). The number of theoretical plates and the length of these columns, (taking *n*-heptane as internal standard), were: A 40,000 (40 m long); B 40,000 (40 m long); C 160,000 (80 m long).

Retention volumes have been corrected for the dead volume according to PETERSON AND HIRSH¹⁰.

The gas chromatograms of benzene and perdeuterobenzene, performed on these columns under the same experimental conditions, are shown in Fig. 1. The ratio of the retention volumes is 1.0095 on A, 1.0357 on B and 1.0198 on C.

The nature of the stationary phase plays an important role in the separation of these substances. From Fig. 1 it appears that on a less polar phase *e.g.* squalane, better results are obtained, only a barely appreciable separation being observed on the dinonyl phthalate column.

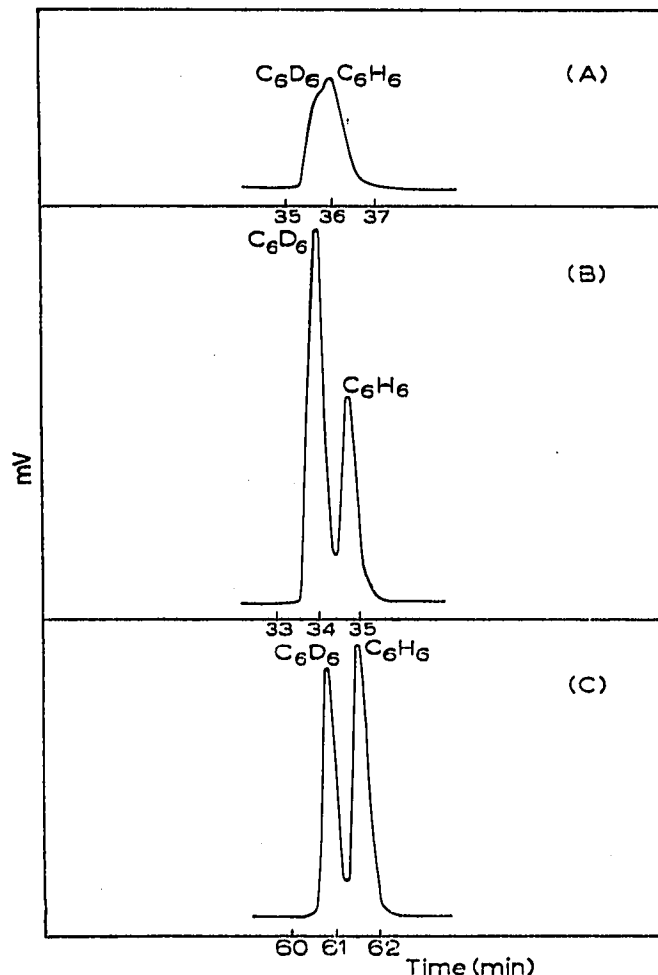


Fig. 1. Gas chromatograms of benzene and perdeuterobenzene on capillary columns coated with dinonyl phthalate (A: 40 m), squalane (B: 40 m) and silicone oil (C: 80 m). Temperature: 20°; carrier gas: nitrogen; flow rate: 0.5 ml/min.

In order to make a quantitative appraisal of this behaviour, the thermodynamics of the chromatographic process were studied. The results were calculated not in terms of the absolute values but as the differences between the changes of the thermodynamic functions. It should be pointed out that absolute retention volumes can be measured only with an accuracy of about 3%. Calculations on the ratio of relative retention volumes obtained by running a combined sample afford a degree of accuracy about one hundred times higher. Gas chromatograms of benzene (Bz) and perdeuterobenzene (dBz) were run at different temperatures on the squalane and silicone oil columns and the ratio of the corrected retention volumes: $(V^{\circ}_R \text{ Bz}/V^{\circ}_R \text{ dBz})$, is

TABLE I

T	Squalane				Silicone oil 702				
	$\frac{P_{dBz}}{P_{Hz}}$	$\log \frac{P_{dBz}}{P_{Hz}} \times 10^3$	$\frac{V_R^\circ Bz}{V_R^\circ dBz}$	$\log \frac{V_R^\circ Bz}{V_R^\circ dBz} \times 10^3$	$\frac{\gamma_{dBz}}{\gamma_{Hz}}$	$\log \frac{\gamma_{dBz}}{\gamma_{Hz}} \times 10^3$	$\log \frac{V_R^\circ Bz}{V_R^\circ dBz} \times 10^3$	$\frac{\gamma_{dBz}}{\gamma_{Hz}}$	$\log \frac{\gamma_{dBz}}{\gamma_{Hz}} \times 10^3$
273.2	1.0340	14.520	1.0421	17.909	1.0073	3.1157	—	—	—
283.2	1.0332	14.142	1.0395	16.824	1.0061	2.6411	1.0205	0.9880	-5.24
293.2	1.0315	13.469	1.0357	15.234	1.0041	1.7770	1.0198	0.9887	-4.94
303.2	1.0304	13.006	1.0336	14.352	1.0031	1.3442	1.0191	0.9890	-4.80
313.2	1.0295	12.626	—	—	—	—	1.0186	0.9894	-4.63
323.2	1.0286	12.240	—	—	—	—	1.0178	0.9895	-4.58

compared in Table I with the corresponding ratio of the vapour pressures, (P_{dBz}/P_{Bz}) measured by INGOLD *et al.*¹¹. With the squalane column the former ratio is higher than the latter. The converse is observed with the silicone oil column. The interaction of the two molecules is therefore quite different with the two liquid phases.

In Fig. 2 the logarithm of the ratio of the retention volumes on squalane column

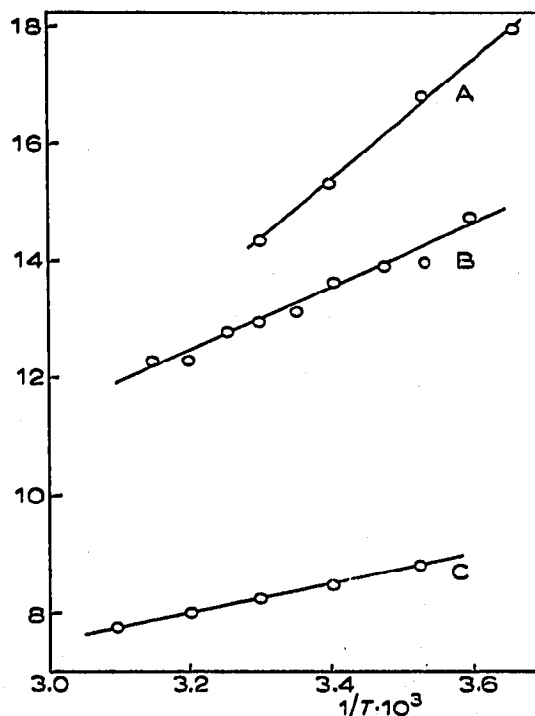


Fig. 2. Logarithm $\times 10^3$ of the ratio of the retention volumes (benzene/perdeuterobenzene) on squalane (A) and silicone oil (C) and of the vapour pressures (perdeuterobenzene/benzene) versus $1/T$ (B).

(A) and on silicone oil column (C) and the logarithm of the ratio of the vapour pressures (B)⁶ is plotted against the reciprocal of the absolute temperature. There is a linear relationship between these values, which holds in the range of temperature investigated. It can be expressed by the following equation:

$$\log \frac{V_{RBz}^0}{V_{RdBz}^0} = - \frac{(\Delta H_{Bz} - \Delta H_{dBz})_{chr.}}{2.3 R} \cdot \frac{1}{T} + C \quad (1)$$

From the slopes of A and C on the graph the difference of enthalpy related to the chromatographic process can be calculated; the results are:

$$\text{on squalane: } (\Delta H_{Bz} - \Delta H_{dBz})_{chr.} = -46.2 \text{ cal.}$$

$$\text{on silicone oil: } (\Delta H_{Bz} - \Delta H_{dBz})_{chr.} = -11.4 \text{ cal.}$$

By extrapolation to $1/T = 0$, the value of the constant C is determined and from it the difference of the entropy change calculated by the following equation:

$$\Delta S_{Bz} - \Delta S_{dBz} = 2.3 RC \quad (2)$$

The difference of entropy can also be calculated from the following relationship:

$$\Delta G_{Bz} - \Delta G_{dBz} = \Delta H_{Bz} - \Delta H_{dBz} - T(\Delta S_{Bz} - \Delta S_{dBz})_{chr.}$$

The differences of free energy changes are obtained from the retention volumes:

$$\Delta G_{Bz} - \Delta G_{dBz} = -2.3 RT \log \frac{V^{\circ}_{R Bz}}{V^{\circ}_{R dBz}}$$

Both values are given in Table II.

TABLE II

T	$\frac{(\Delta S_{Bz} - \Delta S_{dBz})_c}{\times 10^3}$	Squalane			Silicone oil 702		
		$\frac{\Delta G_{Bz} - \Delta G_{dBz}}{\Delta G_{dBz}}$	$\frac{(\Delta S_{Bz} - \Delta S_{dBz})_{chr.}}{\times 10^3}$	$\frac{(\Delta S_{Bz} - \Delta S_{dBz})_m}{\times 10^3}$	$\frac{\Delta G_{Bz} - \Delta G_{dBz}}{\Delta G_{dBz}}$	$\frac{(\Delta S_{Bz} - \Delta S_{dBz})_{chr.}}{\times 10^3}$	$\frac{(\Delta S_{Bz} - \Delta S_{dBz})_m}{\times 10^3}$
273	-7.69	-22.31	-8.75	-1.06	-	-	-
283	-7.42	-21.73	-8.65	-1.23	-11.38	0	7.42
293	-7.17	-20.37	-8.82	-1.65	-11.38	0	7.17
303	-6.93	-19.84	-8.70	-1.77	-11.36	0	6.93
313	-6.71	-	-	-	-11.43	0	6.71
323	-6.50	-	-	-	-11.29	0	6.50

The gas chromatographic process might be taken as consisting of two reactions, one due to condensation (c) of the eluted component and the other due to mixing (m) with the partition liquid. The calculated enthalpy and entropy changes are therefore the sum of the contributions due to each reaction:

$$\begin{aligned} (\Delta H_{Bz} - \Delta H_{dBz})_{chr.} &= (\Delta H_{cBz} + \Delta H_{mBz}) - (\Delta H_{cdBz} + \Delta H_{mdBz}) \\ (\Delta S_{Bz} - \Delta S_{dBz})_{chr.} &= (\Delta S_{cBz} + \Delta S_{mBz}) - (\Delta S_{cdBz} + \Delta S_{mdBz}) \end{aligned} \tag{3}$$

By using the difference between the latent heat of these molecules determined by INGOLD *et al.* ($\Delta H_{cBz} - \Delta H_{cdBz} = -20.96$ cal)¹¹, the difference of the changes of heat of mixing in excess is calculated as:

and:

$$\begin{aligned} \Delta H_{mBz} - \Delta H_{mdBz} &= -25.2 \text{ cal for squalane} \\ \Delta H_{mBz} - \Delta H_{mdBz} &= 9.6 \text{ cal for silicone oil.} \end{aligned}$$

The values of entropy change of the process of mixing are calculated according to:

$$(\Delta S_{Bz} - \Delta S_{dBz})_m = (\Delta S_{Bz} - \Delta S_{dBz})_{chr.} - (\Delta S_{Bz} - \Delta S_{dBz})_c$$

By considering the Clausius-Clapeyron equation for the vaporization of benzene and perdeuterobenzene

$$\log \frac{P^{\circ}_{dBz}}{P^{\circ}_{Bz}} = -\frac{\Delta H_{vdBz} - \Delta H_{vBz}}{2.3 R} \cdot \frac{1}{T} + C' \tag{4}$$

and for the chromatographic process of these compounds (eqn. 1) and by introducing

the relationship¹²

$$\log \frac{V^{\circ}_{RBz}}{V^{\circ}_{RdBz}} = \log \frac{(\gamma P^{\circ})_{dBz}}{(\gamma P^{\circ})_{Bz}} \quad (5)$$

where γ is the activity coefficient, and by combining eqns. (1), (4) and (5) and accounting for eqn. (3), the following equation is obtained, being $\Delta H_v = -\Delta H_c$:

$$\log \frac{\gamma_{dBz}}{\gamma_{Bz}} = -\frac{\Delta H_{mBz} - \Delta H_{m dBz}}{2.3 R} \frac{1}{T} + (C' - C) \quad (6)$$

From the plot of the logarithm of the ratio of the activity coefficients, calculated according to eqn. (5), *versus* $1/T$ (Fig. 3), the difference between the integration constants

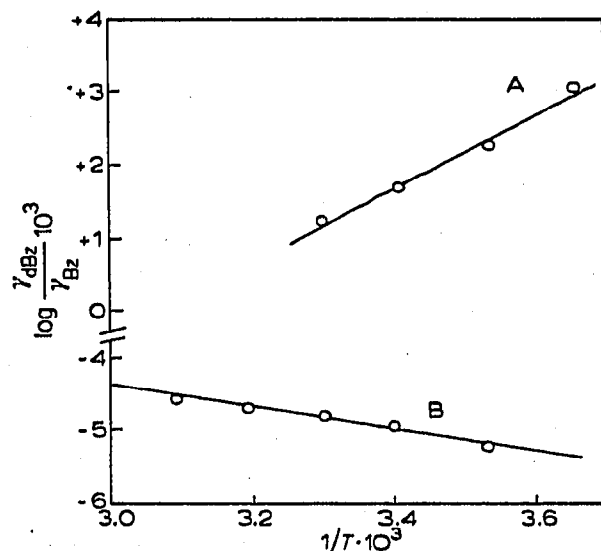


Fig. 3. Logarithm of the ratio of the activity coefficients (perdeuterobenzene/benzene) *versus* $1/T$.

is calculated by graphic extrapolation. From this the difference between the heats of mixing in excess is obtained. The values -20.50 cal for squalane and $+6.4$ cal for silicone oil are in fair agreement with the ones previously reported.

DISCUSSION

The data obtained from the gas chromatographic behaviour of benzene and perdeuterobenzene, given in Table I, show that the ratio of the activity coefficients in squalane is greater than one and in silicone oil less than one. This means that in squalane the difference between the heats of solution acts in the same direction as that of the heat of mixing, while the converse holds for silicone oil. Both ratios tend to unity on raising the temperature.

As a tentative explanation for this behaviour, the fact that in the liquid state there is a stronger attraction among the benzene molecules than is the case in perdeuterobenzene might be taken into account. These attractions, due to the interaction of π electrons in the two molecules, are responsible for the different vapour pressures of the two molecules. The dispersion strengths, due to the dipoles of a polar

phase, exert a strong competitive action against the association strengths with the result that on a polar stationary phase the two substances behave almost in the same way and therefore a lower separation is achieved.

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SUMMARY

The separation of benzene and perdeuterobenzene by gas-liquid chromatography has been investigated on glass capillary columns. The nature of the liquid phase plays a very important role in the separation of isotopically substituted molecules, the less polar (squalane) being more effective than silicone oil. With the former, the separation is better than expected on account of the differences of vapour pressure, the reverse is found with the latter phase.

The thermodynamics of the chromatographic process have been studied, and the difference of ΔG , ΔH and ΔS for both substances together with the ratio of the activity coefficients on squalane and silicone oil have been calculated.

REFERENCES

- ¹ W. R. MOORE AND W. R. WARD, *J. Am. Chem. Soc.*, 80 (1958) 2909.
- ² C. O. THOMAS AND H. A. SMITH, *J. Phys. Chem.*, 63 (1959) 427.
- ³ S. OHKOSHI, Y. FUGITA AND T. KWAN, *Bull. Chem. Soc. Japan*, 31 (1958) 770.
- ⁴ M. MOHNKE AND W. SAFFERT, in M. VAN SWAAY (Editor), *Gas Chromatography 1962*, Butterworths, London, 1962.
- ⁵ E. GLUECKAUF, K. H. BARKER AND G. P. KITT, *Discussions Faraday Soc.*, 7 (1949) 199.
- ⁶ K. E. WILZBACH AND P. RIESZ, *Science*, 126 (1957) 748.
- ⁷ W. E. FALCONER AND R. J. CVETANOVIC, *Anal. Chem.*, 34 (1962) 1064.
- ⁸ D. H. DESTY, J. N. HASEMAPE AND B. H. F. WHYMAN, *Anal. Chem.*, 32 (1960) 302.
- ⁹ F. BRUNER, G. CARTONI AND A. LIBERTI, *Chim. Ind. (Milan)*, 44 (1962) 999.
- ¹⁰ D. L. PETERSON AND J. HIRSH, *J. Lipid Res.*, 1 (1959) 32.
- ¹¹ C. K. INGOLD, G. G. RAISIN, C. L. WILSON AND C. R. BAYLEY, *J. Chem. Soc.*, (1936) 915.
- ¹² A. I. M. KEULEMANS, *Gas Chromatography*, Reinhold Publishing Corp., New York, p. 16.

J. Chromatog., 12 (1963) 8-14