Studies on a systematic analysis of terpenic hydrocarbons by gas–liquid chromatography

KLOUWEN AND TER HEIDE[1] have treated gas chromatographic data of over 22 isomeric terpenic hydrocarbons in connection with differences in polarisability. They concluded that for "non-polar" columns such as Apiezon-L or Silicone Oil a direct proportionality exists between the logarithm of retention volume and boiling point. Such columns were characterised as "non-selective" in contrast to the "selectivity" shown by polar chromatographic columns. The comparison of the logarithms of the retention volumes for one stationary phase with those in another stationary phase, first suggested by JAMES et al.[2], and used successfully in the identification and characterisation of members of homologous series, was found inapplicable to terpenic isomeric hydrocarbons. To obtain reasonable results, classification of terpenes into groups, based on rather vague structure similarities, was found necessary[1]. From other methods of coordinating gas chromatographic data, the method of WEHRLI AND KOVATS[3], where comparisons of "retention indices" are used, is applicable in homologous series but not always in the case of isomers.

Considering possibilities of developing graphical presentations for the identification of terpenic hydrocarbons from retention data given in the literature, we found that relative retention times, or volumes, or the logarithmic values, for one column, when plotted against those in another column of the "same selectivity", gave linear graphs. Since the term "same selectivity" is scarcely satisfied for columns of different stationary phases, relationships of data taken under different conditions but in columns of the same stationary phase were worked out. This linear relationship is still valid when flow rate, column dimensions, nature of carrier gas or temperature are varied (Figs. 1, 2 and 3). The results obtained can be correlated with those of JAMES et al.[2] in their relative retention volume logarithmic plots for two different stationary
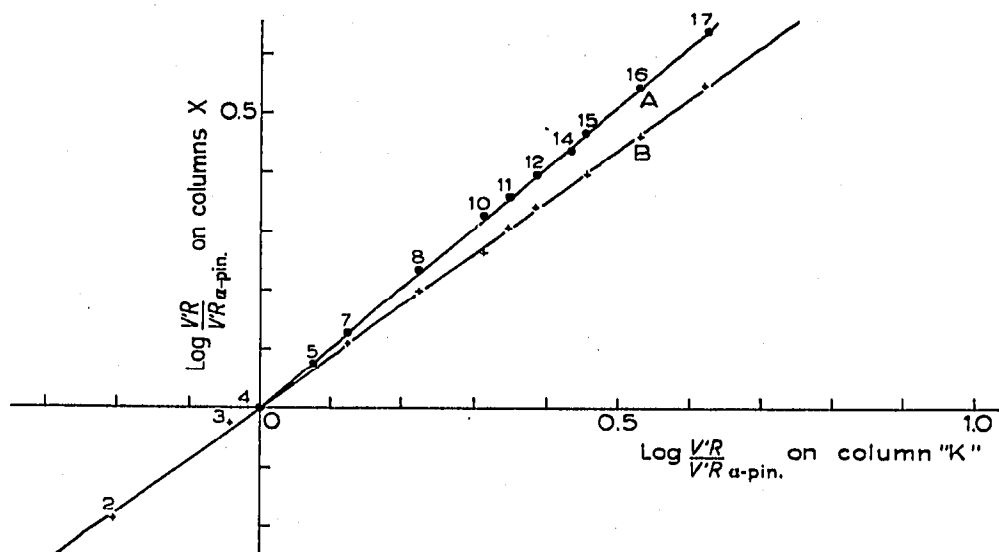

Fig. 1. Log (relative retention volumes) for "K" Perkin-Elmer column (Carbowax 1500 in Chromosorb W, length 4 m, helium flow 84 ml/min, temp. 100°) against those on Carbowax co umns under different conditions. A[1] = 20 % Carbowax 4000 on EMBACEL, length 3 m, hydrogen flow 75 ml/min, temp. 100°). B[3] = 28.5 % Carbowax 4000 on Chromosorb C-44857, length 1.82 m, helium flow 45 ml/min, temp. 130°).
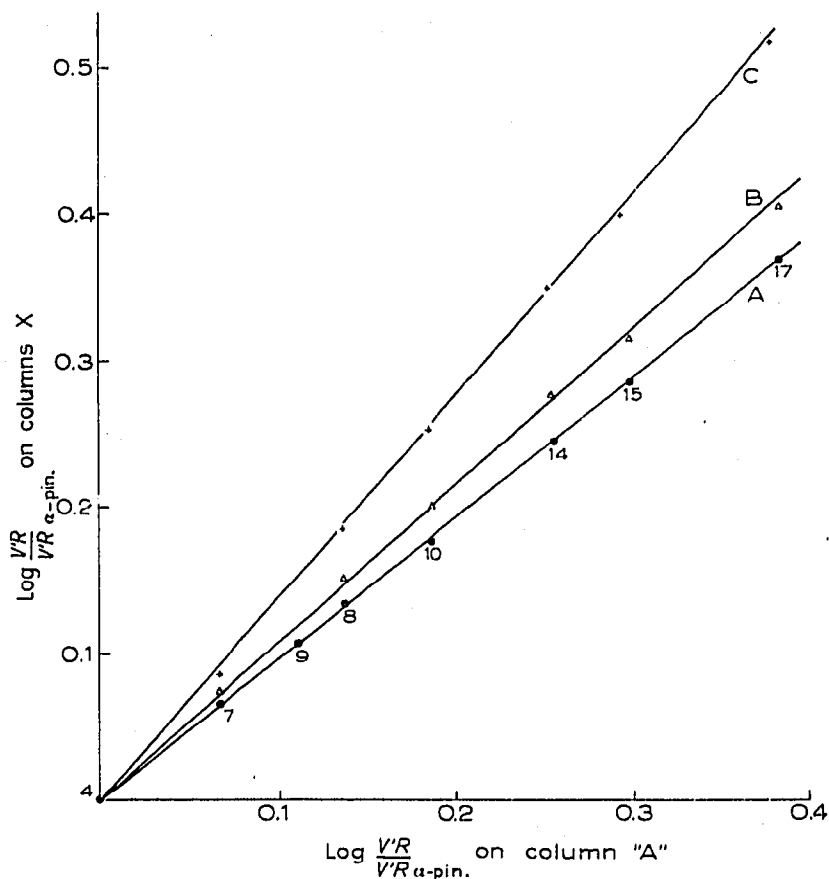
Fig. 2. Log (relative retention volumes) for "A" Perkin-Elmer column (diisodecyl phthalate, length 4 m, helium flow 86 ml/min, temp. 161°) against those on diisodecyl phthalate columns under different conditions. A = 34 % diisodecyl phthalate on Celite, length 4.5 m, helium flow 45 ml/min, temp. 150°. B = Perkin-Elmer "A" column, length 2 m, helium flow 50 ml/min, temp. 161°. C = Perkin-Elmer "A" column, length 2 m, helium flow 80 ml/min, temp. 99.5°.
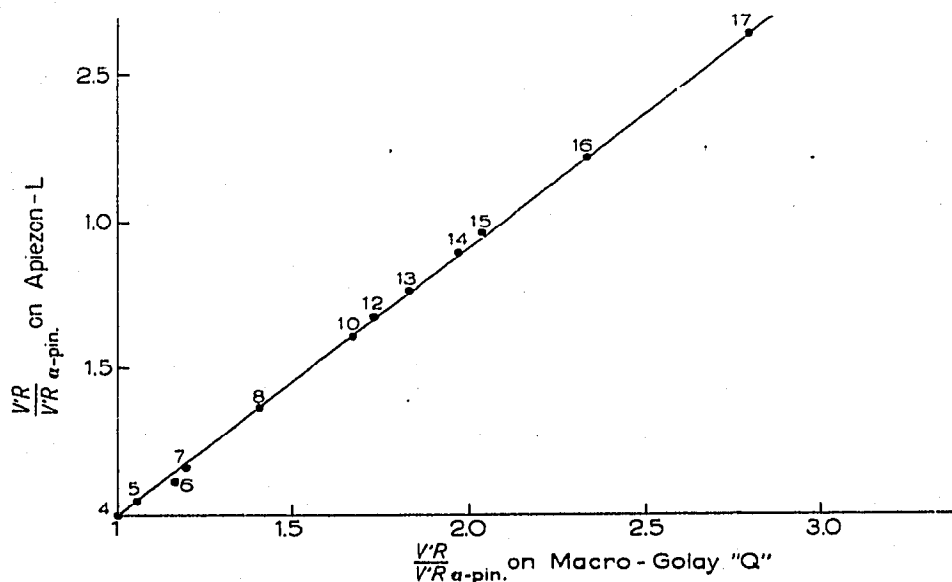

Fig. 3. Relative retention values for "Q" Perkin-Elmer Macro-Golay column (Apiezon L, length 100 m, helium flow 5 ml/min, temp. 133°) against those on a conventional column of the same stationary phase[1] (20 % Apiezon L, length 3 m, hydrogen flow 75 ml/min, temp. 120°).

phases, where the variation in column "selectivity" is replaced here by variation in structure.

No exceptions were observed in applying this graphical presentation. It was found, however, that small peak areas, when overlapping large peak areas, appear somewhat earlier. This phenomenon is related to the established fact that big size samples and highly concentrated sample components overload parts of the column, thus disturbing flow rate and column efficiency[4].

TABLE I

RETENTION VOLUMES OF TERPENE HYDROCARBONS RELATIVE TO THAT OF α-PINENE

	Hydrocarbon	"K"[a]	"A"[b]	"Q"[c]	"R"[d]
1	Bornylene	—	—	0.6	0.69
2	Cyclofenchene	0.623	0.685	0.77	0.8
3	Tricyclene	0.932	—	0.94	0.985
4	α-Pinene	1.00	1.00	1.00	1.00
5	β-Fenchene	1.18	1.12	1.05	1.12
6	α-Fenchene	1.30	—	1.12	1.23
7	Camphene	1.32	1.155	1.17	1.24
8	β-Pinene	1.67	1.365	1.37	1.5
9	Myrcene	2.04	1.285	—	—
10	3-Carene	2.05	1.535	1.61	1.81
11	α-Phellandrene	2.22	1.6	—	—
12	α-Terpinene	2.42	1.67	1.67	1.98
13	p-Cymene	—	—	1.76	2.61
14	Dipentene (limonene)	2.67	1.795	1.89	2.18
15	β-Phellandrene	2.86	1.95	1.96	—
16	γ-Terpinene	3.4	2.08	2.22	2.4
17	Terpinolene	4.16	2.465	2.64	3.14

[a] "K" = Perkin-Elmer column (Carbowax 1500 on Chromosorb W), length 4 m, helium flow 84 ml/min, temp. 100°±0.5°.
[b] "A" = Perkin-Elmer column (diisodecyl phthalate on Chromosorb W), helium flow 86 ml/min, length 4 m, temp. 162°±0.5°.
[c] "Q" = Perkin Elmer Macro-Golay column (Apiezon L), length 100 m, helium flow 5 ml/min, temp. 133°±0.5°.
[d] "R" = Perkin-Elmer Macro-Golay column (polypropylene glycol UCON LB-550-X), helium flow 7 ml/min, length 100 m, temp. 132°±0.5°.

The graphical presentation described is even more useful for identification purposes. Chemical reactions can lead to products whose nature is characteristic of the particular system. In examining the interaction of α-pinene with fatty acids we obtained in the chromatogram of the isomerisation products some 12 terpenic hydrocarbons for two of which we had no samples for comparison. By plotting our data successively against those of KLOUWEN AND TER HEIDE[1] and ZUBYK AND CONNER[5] we established that the unknown compounds coincide with β-fenchene and cyclofenchene. Later we succeeded in preparing pure samples of these two compounds and this result was further confirmed.

We have employed the Macro-Golay columns Apiezon-L and Polypropylene glycol (Perkin–Elmer Q and R columns) in the analysis or over 15 terpenic hydrocarbons with very satisfactory results[6]. These columns were found to cause some isomerisation during analysis, especially above 130°. The retention data obtained can be correlated with those obtained using conventional chromatographic columns (Fig. 3). Because of

the ideal equilibration conditions during analysis, these data are most reliable and could be suggested as standards.

Our results suggest that in order to compare gas chromatographic retention data, it is not necessary to reproduce working conditions. A chromatogram obtained under a definite set of conditions can be compared with one obtained under different conditions, providing equilibrium of flow rate and temperature is attained and columns of the same stationary phase are used. The validity of this statement has been proved for the terpenic hydrocarbons; we do not see why this should not apply to other systems.

Laboratories of Organic Chemical Technology and
Pharmaceutical Chemistry, Swiss Federal Institute of
Technology, Zurich (Switzerland)

G. VALKANAS
N. ICONOMOU

[1] M. H. KLOUWEN AND R. TER HEIDE, *J. Chromatog.*, 7 (1962) 297.
[2] A. T. JAMES, A. J. P. MARTIN AND G. H. SMITH, *Biochem. J.*, 52 (1952) 238.
[3] A. WEHRLI AND E. KOVATS, *Helv. Chim. Acta*, 42 (1959) 2728.
[4] S. DAL NOGARE AND R. S. JUVET, JR., *Gas-Liquid Chromatography*, Interscience, New York–London, 1962, p. 16.
[5] W. J. ZUBYK AND A. Z. CONNER, *Anal. Chem.*, 32 (1960) 912.
[6] G. VALKANAS AND N. ICONOMOU, *Pharm. Acta Helv.*, in the press.

Gas chromatographic analysis of polar–non polar mixtures

In the study of thermodynamic properties of multicomponent mixtures, such as vapor–liquid equilibrium compositions, the gas chromatographic technique has been used extensively. The methods commonly used in the determination of concentrations from a chromatograph assume that either the peak height or the peak area is proportional to the concentrations of the components present in the mixture. Recently, WAGNER AND WEBER[1] studied polar–non polar mixtures, such as ethanol–benzene–heptane, and proposed a modified method in which the mole fraction ratio to the peak area ratio is considered constant, namely:

$$\frac{(x_i/x_j) \text{ mole fraction}}{(x_i/x_j) \text{ peak area}} = K_{ij} \tag{1}$$

These constants and the condition that the sum of the mole fractions must be equal to unity were used to calculate the compositions.

We have determined the composition of ethanol–benzene–cyclohexane mixtures in this laboratory, using a Perkin-Elmer Model 154C Vapor Fractometer coupled with a Leeds and Northrup series 6000 Recorder and a Perkin-Elmer Model 194 Integrator. In the Vapor Fractometer, two 2-m type R columns were employed in series to separate the three components. A curve was obtained on a (x_i/x_j)mole fraction *vs.* (x_i/x_j)peak area