

Observations on the gas-liquid chromatography of *m*-monoalkenes with reference to the systematic identification of esters of unsaturated fatty acids through separation factors and log retention time plots

Introduction

Separation factors between any two materials in gas-liquid chromatography may be defined as the greater retention time divided by the lesser retention time. In the case of the methyl esters of unsaturated fatty acids on polyester substrates it has been possible to show that systematic relationships exist by separation factors among various acids of any one fixed chain length dependent on the relative positions of single double bonds or of normal methylene-interrupted multiple double bond systems^{1,2}. These separation factors, designated type I and type III, are virtually independent of chain length and number of double bonds provided the differences in the number of double bonds are the same. It has been further suggested that these separation factors would also apply to related unsaturated materials, including hydrocarbons such as the *n*-alkenes.

In the type I and type III separation factors established for the methyl esters of unsaturated fatty acids it was possible, through varying the number of double bonds, to fix either the carboxyl end chain (the inclusive number of carbon atoms from the carboxyl group to the center of the first double bond) or the end carbon chain (the inclusive number of carbon atoms between the center of the last double bond and the terminal methyl group). In the type III separation factors the same end concepts are applied to chains of the same length containing the same number of double bonds in isomeric positions. The type I and type III relationships cannot be shown with the available hydrocarbon data, but the type III system can be explored, using the terms "long end carbon chain" and "short end carbon chain" to refer to the respective chain portions on either side of the center of the double bond in the *n*-monoalkenes. The effects of the different positions of the double bond systems for type I and III separation factors in the case of the longer chain fatty acids were ascribed to relative changes in volatility due to the three-carbon changes in the lengths of the carboxyl end chains and the end carbon chains. This was based on the observation that as the double bond in octadecenoic fatty acids approached either the carboxyl group³, or particularly the terminal methyl group^{2,4}, of the fatty acid chain the retention time increased drastically. A similar observation may be made in part with octadecadienoic esters⁵, while in the case of saturated esters of common chain length the same rule of increasing retention time is observed as either the acid or alcohol moiety of the chain is reduced⁶. In the latter example this may be compared with known boiling points of similar esters of shorter chain lengths⁷, suggesting that the increased retention time is associated with a higher boiling point.

The separation factors to be examined are therefore of the type III system, with one double bond in various positions. The particular relationships are set up on the basis of the ratios of the "short end carbon chains" and are limited to pairs of

(Text continued p. 274)

TABLE I
A. COMPARISONS OF SOME SEPARATION FACTORS FOR *n*-ALKENES BASED ON 2/3, 3/1 AND 2/1 SHORT END CARBON CHAIN RATIOS

Substrate	Column I		Column II		Column III		Hexadecane (with P/S and DmS) ^b				
	1	II	1	II	1	II	2/3	2/1			
Operating temperature											
	24°				25°						
Hydrocarbon	B.P. °C	Long end carbon chain	Short end carbon chain	Rel. ret. time ^c	2/3	3/1	2/1	Rel. ret. time ^c	2/3	3/1	2/1
<i>cis</i> -2-Heptene	98.41	5	2	3.19	—	—	—	—	—	—	—
<i>cis</i> -3-Heptene	95.75	4	3	2.84	1.12	—	—	—	—	—	—
<i>i</i> -Heptene	93.64	6	1	2.50	—	1.14	1.28	—	—	—	—
<i>cis</i> -2-Hexene	68.89	4	2	1.16	—	—	—	1.42	—	—	—
<i>cis</i> -3-Hexene	66.45	3	3	1.03	1.12	—	—	1.26	1.12	—	—
<i>i</i> -Hexene	63.49	5	1	0.89	—	1.16	1.30	1.10	—	1.14	1.29
<i>cis</i> -2-Pentene	36.94	3	2	0.41	—	—	—	0.51	—	—	—
<i>i</i> -Pentene	29.97	4	1	0.31	—	—	1.32	0.39	—	—	1.31
<i>trans</i> -2-Heptene	97.95	5	2	2.95	—	—	—	—	—	—	—
<i>trans</i> -3-Heptene	95.67	4	3	2.76	1.07	—	—	—	—	—	—
<i>i</i> -Heptene	93.64	6	1	2.50	—	1.10	1.18	—	—	—	—
<i>trans</i> -2-Hexene	67.88	4	2	1.05	—	—	—	1.31	—	—	—
<i>trans</i> -3-Hexene	67.09	3	3	1.03	1.02	—	—	1.21	1.08	—	—
<i>i</i> -Hexene	63.49	5	1	0.89	—	1.16	1.18	1.10	—	1.10	1.19
<i>trans</i> -2-Pentene	36.35	3	2	0.38	—	—	—	0.48	—	—	—
<i>i</i> -Pentene	29.97	4	1	0.31	—	—	1.22	0.39	—	—	1.23

alkenes with short end carbon chains of 1, 2 and 3 giving ratios of 2/3, 3/1 and 2/1. The appropriate separation factors will therefore be found under these headings in Table I.

Discussion

In the case of the gas-liquid chromatography of certain *n*-alkenes on a polyester substrate⁸ (Table I-B, Column V) the 2/1 separation factors for the *cis*-hexenes and *cis*-pentenes are not the same, nor are the *trans* examples. Moreover the *cis* and *trans* forms of the same alkenes do not separate, indicating that on this polar substrate, although the retention times are in order of boiling point for the isomeric alkenes of the same chain length, there must be a further overriding influence, that of inductive forces from the respective end carbon chains, which would change the polarity of the double bonds in their interaction with the polyester. If the 2/1 separation factors for the butenes are also considered (1.43 at 50°, 1.33 at 100°), an alternating order of magnitude is apparent. The reversal of the relative magnitude of the separation factors for the pentenes with temperature increase further indicates that at moderate temperatures complex interactions govern the retention times of isomeric alkenes on polar substrates and hence the type III separation factors. However, as the temperature increases these same data indicate that the differences in the 2/1 separation factors for various chain lengths become less. Certainly the polarity of the 1-hexenes and 1-pentenes is notably altered, since they no longer separate from the corresponding alkanes at 100°. In the gas-liquid chromatography of the longer-chain unsaturated fatty acid esters with more centrally located unsaturation, inductive effects should play only a minor role in positional isomer separation at normal operating temperatures (180°–220°), although this would not apply to double bond systems very near the carboxyl group⁹. For example in the type II separation factors² it would be unreasonable to expect the inductive effects from the 3, 6 or 9 carbon atom end carbon chains to act universally on double bond systems containing from one to six double bonds.

A potential variable which has not been explored in alkene separations at moderate temperatures on polyester substrates is the physical state of the substrate. It has recently been shown¹⁰ that the performance of inert solid and liquid hydrocarbon substrates in the gas-liquid chromatography of alkanes may differ drastically. Dependent on temperature, a polyester substrate might be solid, solid with a film of amorphous low molecular weight or degraded polyester, or a homogeneous liquid, with consequent variation in polarity and behaviour.

In contrast, examination of retention times at 24° for both *cis* and *trans* 2- and 3-alkenes in relation to 1-alkenes, on SF-96, an inert substrate¹¹ (Table I-A, Column I), not only gives very good correlation of retention times and boiling points, but also good agreement in the separation factors for the *cis*-heptenes and *cis*-hexenes. The *cis*-pentene data also agrees reasonably well, but the retention times for the shorter chain materials are not necessarily as satisfactory as for the longer chain materials¹². Thus there is reason to believe (see also log plot discussion below) that the retention time for 1-pentene may be slightly low, and it will be noted that a relative retention time of 0.32 in lieu of 0.31 for this material would markedly improve the 2/1 separation factor correlation. This does not necessarily reflect on the accuracy of the experimental data, but rather on the number of significant figures in the particular relative retention time.

These observations suggest that in the case of the *cis* hydrocarbons on an "inert" substrate it may not be necessary to have the same "long end carbon chains" in order to have the same values for separation factors based on the ratios of the "short end carbon chains" only, provided that the number of carbon atoms in the "long end carbon chains" exceeds four or possibly three. This is in agreement with the type III separation factors obtained with polyunsaturated fatty acid esters on polyesters², where in those pairs of acids of common end carbon chain ratio the position of the unsaturation in relation to the carboxyl group is of little significance, provided the number of double bonds involved is the same in the various pairs of acids (e.g. 11,14- and 8,11-eicosadienoates and 9,12- and 6,9-octadecadienoates, end carbon chain ratios both 6/9, have respective type III separation factors of 1.05 and 1.04). Inductive forces are therefore unlikely to affect these separations. The particular acids in each set of pairs are those which may be respectively related by a linear log plot^{13,14}.

Unfortunately the necessary data for longer chain hydrocarbons to support this view are not available, but some comparison may be made of data from different workers using the same shorter chain *cis* hydrocarbons on other "inert" substrates. At nearly the same temperature, data for a hexadecane column (coupled to a short section of column packed with *n*-propyl sulfone and 2,4-dimethylsulfolane)¹⁵ agrees very well in the case of the *cis*-hexenes and *cis*-pentenes (Table I-A, Column II). At a somewhat higher temperature, 50°, silicone grease⁸ also gives reasonably similar values, although these do not correlate at 100° (Table I-B, Column III). At the same elevated temperatures D.C. 200 silicone oil⁸ (Table I-B, Column IV) gives very poor correlation. It will be noted that with the silicone grease both the *cis* and *trans* separation factors increase with increasing temperature, whereas with the silicone oil they decrease, suggesting that one of these last two substrates may not be truly non-polar (D.C. 200 silicone oil has been reported to be more polar than squalane¹⁶).

In the *trans* alkenes the situation is less clear, owing to the anomalous boiling point and retention time for the *trans*-3-hexene. The separation factors on hexadecane are interesting since the *trans*-hexene separation factors are virtually the same as those obtained for the *trans*-heptenes on SF-96. Unfortunately the former cannot be considered entirely reliable owing to the possible slight influence of the short length of mildly polar substrate preceding the hexadecane, although this does not seem to have an appreciable effect on the *cis* values, or on the *trans* 2/1 short end carbon chain ratio separation factor. In the case of the *trans* alkenes the silicone grease and D.C. 200 silicone oil separation factors are both in poor agreement with the SF-96 data.

In lieu of the common practice of plotting log retention time against boiling point, the log retention time data for the SF-96 column were plotted against number of carbon atoms^{13,14}. The C₄ points only partly fit the other data since the errors in relative retention time may be relatively large, but some significant correlations may be found. The *cis*-2-, *cis*-3- and *trans*-2-alkene lines are all parallel, and if the point for 1-pentene is slightly in error (see above), the 1-alkene line is also parallel to these. The *trans*-3-alkene line is abnormally inclined to the others owing to the anomalous *trans*-3-hexene point, and none are parallel to the *n*-alkane line. The latter is, however, based on data¹¹ at 25°. The results are therefore broadly similar to those suggested for positional isomers of methyl esters of certain polyunsaturated fatty acids on polyester substrates¹³.

Conclusions

On limited evidence it appears that as operating temperatures increase, inductive effects in *n*-alkenes governing the polarity of the double bonds decrease, suggesting that other influences must govern the gas-liquid chromatographic separation of isomerically unsaturated materials on polar substrates at elevated temperatures.

The examination of hydrocarbon retention times on various substrates from the point of view of separation factors and in one case of the linear log plot system supports the viewpoint that these systematic relationships in the gas-liquid chromatography of unsaturated fatty acid esters on polyester substrates may be largely based on similarities in volatility due to contributions from similar structural elements.

The application of systematic separation factors to the analysis of hydrocarbon mixtures on strictly inert substrates may be useful if such mixtures are essentially straight-chain alkenes and if the relationships proposed can be shown to hold for longer chain materials where reference compounds of known structure may not be readily available.

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- ¹ R. G. ACKMAN, *J. Am. Oil Chemists' Soc.*, submitted for publication.
- ² R. G. ACKMAN AND R. D. BURGHER, *J. Chromatog.*, 11 (1963) 185.
- ³ A. T. JAMES, *J. Chromatog.*, 2 (1959) 552.
- ⁴ C. R. SCHOLFIELD, personal communication.
- ⁵ R. A. LANDOWNE AND S. R. LIPSKY, *Biochim. Biophys. Acta*, 46 (1961) 1.
- ⁶ D. LEFORT, C. PAQUOT AND A. POURCHEZ, *Oleagineux*, 16 (1961) 253.
- ⁷ K. S. MARKLEY, in *Fatty Acids, Their Chemistry and Physical Properties*, 1st. Ed., Interscience, New York, 1947, p. 169.
- ⁸ P. R. SCHOLLY AND N. BRENNER, in H. J. NOEBELS, R. F. WALL AND N. BRENNER (Editors), *Gas Chromatography*, Academic Press, New York, 1961, p. 263.
- ⁹ J. JANÁK, M. DOBIAŠOVÁ AND K. VEREŠ, *Collection Czech. Chem. Commun.*, 25 (1960) 1566.
- ¹⁰ T. O. TIERNAN AND J. H. FUTRELL, *Anal. Chem.*, 34 (1962) 1838.
- ¹¹ A. G. POLGÁR, J. J. HOLST AND S. GROENNINGS, *Anal. Chem.*, 34 (1962) 1226.
- ¹² J. F. SMITH, *Nature*, 193 (1962) 679.
- ¹³ R. G. ACKMAN, *Nature*, 194 (1962) 970.
- ¹⁴ R. G. ACKMAN, *Nature*, 195 (1962) 1198.
- ¹⁵ M. G. BLOCH, in H. J. NOEBELS, R. F. WALL AND N. BRENNER (Editors), *Gas Chromatography*, Academic Press, New York, 1961, p. 133.
- ¹⁶ H. J. MAIER AND O. C. KARPATHY, *J. Chromatog.*, 8 (1962) 308.

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