THE PARTITION OF POLAR AND NON-POLAR LIPIDS IN A REVERSED-PHASE CHROMATOGRAPHIC SYSTEM

A. G. VERESHCHAGIN
K. A. Timiryazev Institute of Plant Physiology, Academy of Sciences of the U.S.S.R., Moscow (U.S.S.R.)

(Received July 25th, 1963)

The additive nature of the chromatographic partition process was postulated by MARTIN already in 1950[1]. He formulated the equation:

$$\ln\left(\frac{\alpha_B}{\alpha_A}\right) = \frac{\Delta\mu_X}{RT} \qquad (1)$$

where A, B are members of a homologous series, differing by the functional group X, α is the partition coefficient and $\Delta\mu_X$ is the difference in chemical potential of the group X in polar and non-polar phases of the chromatographic system.

It follows, that each group in the solute molecule contributes more or less independently to the difference in standard free energy of the solute between the two different phases. Thus, in general, there is a linear relationship between $\ln\alpha$ or $\log_{10}\alpha$ and the number of functional groups in a homologous series. This relationship has been widely used for the chromatographic identification of flavonoids[2], 2,4-dinitrophenylhydrazones of aliphatic aldehydes and methyl-ketones[3], aliphatic and aromatic hydrocarbons[4], and other substances.

In a reversed-phase partition chromatography of lipids[5,6] several attempts were made to derive some information as regards identity from the behaviour of these substances during separation. The R_F value[7] and the logarithm of the retention volume[8] were reported to be linear functions of the number of carbon atoms in an aliphatic chain. In other studies no such linearity was found[9].

It seemed desirable to find the relation between properties of lipid molecules and the chromatographic partition pattern. The following reversed-phase systems were used: for the fatty acids—natural rubber + aliphatic hydrocarbons, b.p. 220–260°/90 % acetic acid; for triglycerides — aliphatic hydrocarbons, b.p. 260–310°/a mixture of acetone and acetic acid (85:15 v/v). The experimental conditions used for the separation and localization of lipids have been described previously[10,11].

The molecular properties of lipids were characterized by the polarity constant:

$$K_{1,2} = 100 - m + 2e \qquad (2)$$

where m is the number of carbon atoms and e the number of double bonds in a lipid molecule.

In a reversed-phase system the absolute R_F values of fatty acids are not readily reproducible owing to slight fluctuations of temperature during the chromatographic

procedure; therefore, the stable relative value R_1 was used instead of R_F for the calculation of the partition coefficient α_1. The R_1 value is computed from the equation:

$$R_1 = \frac{\text{distance travelled by a given fatty acid zone}}{\text{distance travelled by ricinoleic acid zone}} \quad (3)$$

Table I gives the K_1 and R_1 values of higher fatty acids (the abbreviated designations of the acids are given in parentheses).

TABLE I

POLARITY CONSTANTS AND R_1 VALUES OF HIGHER FATTY ACIDS

Fatty acid	*Stearic (S)*	*Palmitic (P)*	*Oleic (O)*	*Linoleic (L)*	*Myristic (M)*	*Linolenic (Le)*	*Lauric (La)*
K_1	82	84	84	86	86	88	88
R_1	0.10	0.18	0.18	0.28	0.34	0.39	0.52

The partition coefficient was calculated according to MARTIN AND SYNGE[12]. For the reversed-phase system at a constant temperature the effect of the water content of the solid support was negligible; therefore, it was assumed that:

$$\frac{q_{\text{polar}}}{q_{\text{non-polar}}} = 1 \quad (4)$$

and:

$$\alpha_{1,2} = \frac{1}{R_{1,2}} - 1 \quad (5)$$

There is a linear relationship between $\log_{10}\alpha_1$ and the polarity constant of saturated fatty acids (Fig. 1). The unsaturated fatty acid with carbon number m and the saturated acid with $m - 2e$ carbon atoms have equal polarity constants, but the $\log_{10}\alpha_1$ values of these pairs differ considerably from each other, with the exception of the pair oleic–palmitic acid. The $\log_{10}\alpha_1$ value is a linear function of the polarity constant of unsaturated fatty acids. Using the curves of Fig. 1, it is possible to determine the values of m and e from the experimental R_1 value of the unknown fatty acid.

The chromatographic behaviour of triglycerides obtained from linseed, poppyseed, and cottonseed oils was characterized by the value R_2 (ratio of R_F of triglyceride to the R_F of butyl hexabromostearate). The triglycerides, together with their polarity constants, K_2, and R_2 values are listed in Table II, columns 1–3. There was a linear relationship between K_2 and the partition coefficient of triglycerides α_2 (Fig. 2).

The data of Table II show that glycerides with different fatty acid compositions may still have the same polarity constants. Triglycerides of the same polarity always form a separate chromatographic zone. Further separation of such mixtures into individual triglycerides was achieved by quantitative bromination of the double bonds by the KAUFMANN reagent[13]. The polarity constant of brominated triglycerides can be expressed by the equation:

$$K_3 = m - s \quad (6)$$

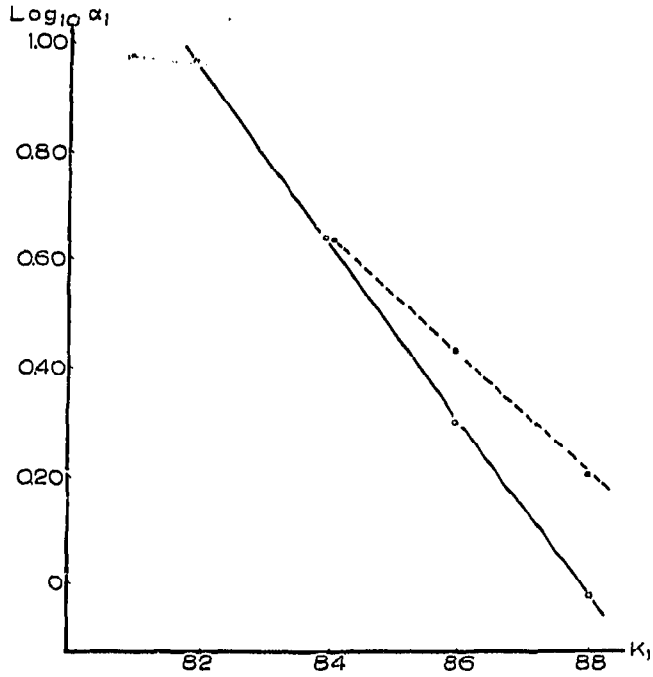

Fig. 1. Dependence of the logarithm of the partition coefficient of fatty acids on the polarity constant K_1. O——O saturated fatty acids; ●- - - - -● unsaturated fatty acids.

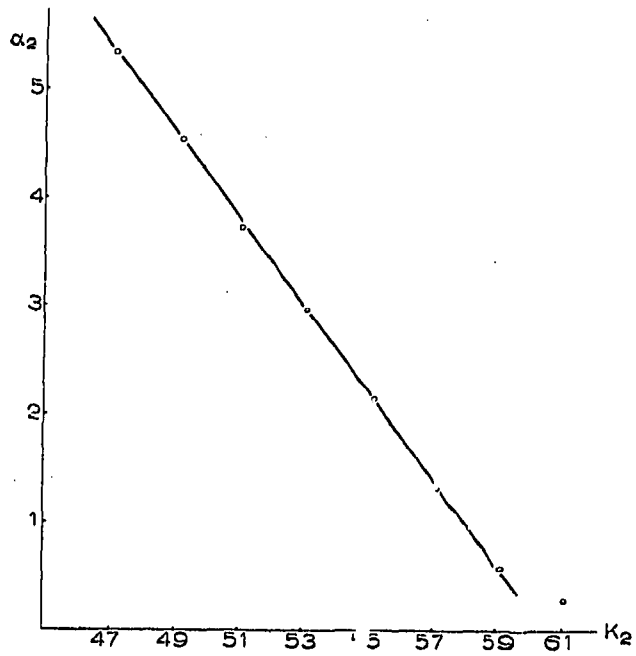

Fig. 2. Dependence of the partition coefficient of higher fatty acid triglycerides on the polarity constant K_2.

TABLE II

POLARITY CONSTANTS AND R_2 VALUES OF TRIGLYCERIDES BEFORE AND AFTER BROMINATION

Triglycerides	K_2	R_2	K_3	R_2'
SPO	47	0.16	—	—
PPO	49	0.18	51	0.21
SPL			53	0.25
POO			54	0.27
SOL			56	0.30
OOO			57	0.3-r
PPL	51	0.21	51	0.29
POL			54	0.36
SLL			56	0.42
OOL			57	0.47
PLL	53	0.25	54	0.47
OLL			57	0.57
LLL	55	0.32	57	0.69
PLeLe	57	0.42	—	—
OLeLe				
LLeLe	59	0.63	—	—
LeLeLe	61	0.78	—	—

* For abbreviations, see Table I.

where s is the number of saturated fatty acid acyls in a triglyceride molecule. The chromatographic separation was carried out as described for non-brominated triglycerides.

Table II (columns 4 and 5) shows that each polarity constant value K_3 corresponds to a different R_2' value, making possible the isolation and subsequent identification of all component triglycerides. As in the case of non-brominated glycerides, the partition coefficient of the bromides α_2' is a linear function of the polarity constant K_3 (Fig. 3).

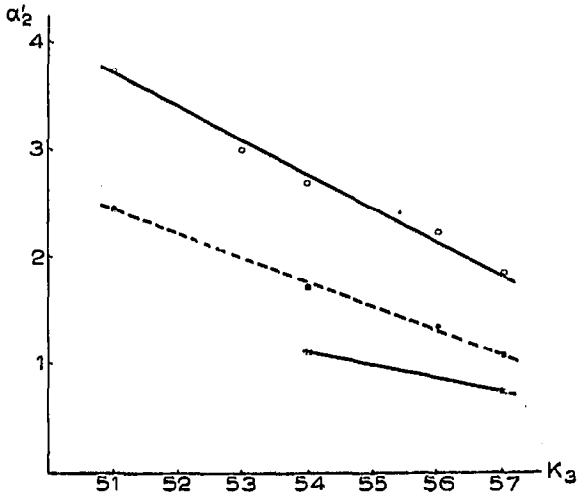

Fig. 3. Dependence of the partition coefficient of brominated triglycerides on the polarity constant K_3. O——O brominated triglycerides, $K_2 = 49$; ●- - - -● $K_2 = 51$; ×——× $K_2 = 53$.

The chromatographic partition of brominated glycerides is of an additive nature. The following relationship exists between the partition coefficients of non-brominated and brominated triglycerides:

$$\alpha_2 = k \cdot x_2' \cdot e \tag{7}$$

where $k = 0.75 \pm 0.10$ is a constant, determined by the nature of the halogen and the parameters of the reversed-phase system. The value of e is given by the expression

$$e = \alpha_2 / k \cdot \alpha_2' \tag{8}$$

Thus, for the identification of a triglyceride it is necessary to carry out its chromatographic separation before and after bromination. The polarity constant may be determined by reference to Fig. 2 and the number of double bonds is calculated from eqn. (8). Identification is accomplished by comparison of the data obtained with the theoretically possible composition of the triglycerides of the given polarity[11].

It is clear from the results that polar lipids (fatty acids) as well as non-polar lipids (triglycerides) follow MARTIN's rule for their partition in a reversed-phase system. There are, however, certain differences in the chromatographic behaviour of these lipids. The fact that the polarity constants of saturated and unsaturated fatty acids are equal does not necessarily imply equality of their partition coefficients, whereas the triglycerides strictly follow this rule. There is a linear relationship between polarity constant and logarithm of partition coefficient in the homologous series of fatty acids, but in the case of triglycerides it is the value of the partition coefficient itself that is a linear function of the polarity constant.

The experimental results seem to disagree with the data cited for other reversed-phase systems[7-9]. Further investigation is necessary to explain the reasons for this apparent difference.

SUMMARY

Reversed-phase chromatography studies have shown that the logarithm of the partition coefficient of saturated and unsaturated higher fatty acids and the partition coefficient of triglycerides are linear functions of the polarity constants of these lipids. By quantitative bromination of double bonds the polarity of triglycerides is increased. The reversed-phase chromatography of brominated glycerides makes possible the separation of otherwise inseparable triglyceride mixtures. The methods of chromatographic identification of lipids are discussed.

REFERENCES

1 A. J. P. MARTIN, *Biochem. Soc. Symposia, (Cambridge, Engl.)* 3 (1950) 4.
2 E. C. BATE-SMITH AND R. G. WESTALL, *Biochim. Biophys. Acta*, 4 (1950) 427.
3 H. VAN DUIN, *Biochim. Biophys. Acta*, 9 (1952) 580; 10 (1953) 198; 12 (1953) 489, 490.
4 G. J. PIEROTTI, C. H. DEAL, S. L. DELL AND P. E. PORTER, *J. Am. Chem. Soc.*, 78 (1956) 2989.
5 J. BOLDINGH, *Rec. Trav. Chim.*, 69 (1950) 247.
6 G. A. HOWARD AND A. J. P. MARTIN, *Biochem. J.*, 46 (1950) 532.
7 V. KOBRLE AND R. ZAHRADNIK, *Chem. Listy*, 48 (1954) 1189.
8 J. HIRSCH, *J. Lipid Res.*, 4 (1963) 1.
9 H. P. KAUFMANN *et al.*, *Fette, Seifen, Anstrichmittel*, 62 (1960) 153; 63 (1961) 125, 1021.
10 A. G. VERESHCHAGIN, *Biokhimiya*, 23 (1958) 721.
11 A. G. VERESHCHAGIN, *Biokhimiya*, 27 (1962) 866.
12 A. J. P. MARTIN AND R. L. M. SYNGE, *Biochem. J.*, 35 (1941) 1358.
13 A. G. VERESHCHAGIN, S. V. SKVORTSOVA AND N. I. ISKHAKOV, *Biokhimiya*, 28 (1963) 868.