PARTITION CHROMATOGRAPHY OF HOMOLOGOUS SERIES II

by

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In a previous communication the derivation was described of the linear relationship between the true retention volumes R_t of terms of homologous series and the order of these terms; this relationship was valid in partition chromatography for ideal behaviour of solutions and column. The true retention volume R_t was defined as the retention volume minus the volume of the mobile phase in the column itself[1]. These considerations are extended in the following treatment:

Assuming that to a first approximation the free energy required to transport a given group, *e.g.* CH_2, from one solvent to another is independent of the rest of the molecule, MARTIN derived in 1949 the expression: $\ln \alpha_A - \ln \alpha_B = \frac{\Delta\mu_x}{RT}$ in which α_A and α_B represent the partition coefficients of two substances A and B which differ by a group X, $\Delta\mu_x$ the difference in chemical potential of this group in the phases of the partition-system and R the Gas Constant[2]. By means of this equation we found[1] that also for the true retention volumes Rt: $\ln R_{t_A} - \ln R_{t_B} = \frac{\Delta\mu_x}{RT}$.

If now a series of compounds is formed by adding the same groups to consecutive terms, then a linear relationship must exist between both $\ln \alpha$ and $\ln R_t$ and $\log \alpha$ and $\log R_t$, resp., and the order n of the series. Series of this kind are obtained by addition of a group X or by interchanging groups X and Y either in a nucleus or a chain. The graph of $\log R_t$ or $\log \alpha$ against n may be called the partition graph and the curves which indicate the relationship between the two, partition curves.

In the partition graph of a given partition system under ideal conditions the partition curves should be linear; moreover in series whose terms differ by the same group or groups these partition curves should be parallel. Theoretically, this should be true for all forms of partition between two phases, namely both for partition chromatography and for counter-current methods. The question is to what extent is ideal behaviour approached in reality.

For liquid-liquid chromatography with columns, the linear and parallel course of the partition curves has been confirmed for a number of extended series of aliphatic homologues ($X = CH_2$) in the system light petroleum/nitromethane, namely for 2,4-dinitrophenylhydrazones of aldehydes, methylketones and esters of keto-acids (occurring in isomeric forms), and for esters of 4-dialkylamino-3,5-dinitrobenzoic acid. By this means it has been found possible to separate, identify and determine spectrophotometrically aliphatic aldehydes, ketones and alcohols in the range C_1–C_{18}[3].

The linear and parallel course of the partition curves has also been demonstrated for liquid-gas chromatography, which has been worked out by JAMES AND MARTIN, although the (here small) correction term for the volume of the mobile phase in the column itself was not included[4].

In 1950 BATE-SMITH AND WESTALL derived a corresponding linear relationship for paper chromatography between $\log (1/R_F - 1)$ and the number of substituents in a given nucleus[5]. They confirmed this expression experimentally, while their data indicate a more or less parallel course. Homologous series were not investigated. However, MEIGH later found linearity for a short series of 2,4-dinitrophenylhydrazones[6] and 3,5-dinitrobenzoates[7].

If we compare partition chromatography with adsorption chromatography on polar adsorbents, both applied to aliphatic homologues (2,4-dinitrophenylhydrazones), then we see that in the former theoretically higher terms should be just as readily separated as lower terms, while in the other method the effect decreases so that the derivatives are difficult to separate when the chain length is greater than 6 C-atoms[8].

The relationships thus found for similar series in the same partition system can in principle be used for identification purposes. Thus, the terms of an aliphatic series should be identifiable by means of their retention volumes, provided the slope of the partition curve for aliphatics is known for the system used and also the behaviour of one term of the series (preferable C_3 or higher). Practical application in this way presupposes the existence of more or less universally usable systems. The system light-petroleum/nitromethane (on silicagel) was also applicable to the separation of fatty acids up to and including C_{10}, although deviations soon appeared at higher concentrations owing to association.

Finally, the connection between $\log R_t$, $\log \alpha$, or $\log (1/R_F - 1)$ and the order of the series may be of use in investigating the behaviour of the support of the immobile phase (silicagel, starch, paper, etc.). By comparison of the partition curves found on partition with and without the support,

the effect of the latter can be determined. If the linearity of the curves is maintained when the support shows an influence, then the latter and the immobile phase appear to form together a new immobile phase.

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BOOK REVIEWS

The Chemistry and the Physiology of the nucleus. (Proceedings of the Symposium held August, 1951 by the Biology Department, Brookhaven National Laboratory). Edited by V. T. BOWEN. Experimental Cell Research, supplement 2 (Academic Press Inc., New York), 402 pp., $ 7.00.

L'ouvrage qui vient d'être édité, sous la forme d'un supplément par la revue *Experimental Cell Research*, constitue une excellente mise au point de nos connaissances actuelles sur la biochimie et le rôle du noyau cellulaire; il est d'ailleurs le fruit d'un Symposium où se sont rencontrées de nombreuses autorités en la matière, en grande majorité d'outre-Atlantique.

Le livre débute par une mise au point générale due à K. G. STERN; elle est suivie d'un article particulièrement intéressant de F. HAUROWITZ ET C. F. CRAMPTON qui développent leurs idées sur le rôle des acides nucléiques, qui agiraient à la façon d'un modèle, dans la synthèse des protéines. A. W. POLLISTER, puis M. J. MOSES discutent ensuite les résultats et les techniques cytochimiques. Après un exposé de J. G. GALL sur les chromosomes plumeux de *Triturus viridescens*, A. L. DOUNCE examine les résultats fournis par l'étude biochimique des noyaux isolés; A. B. NOVIKOFF expose ensuite la délicate question de la détection cytochimique des phosphatases dans le noyau. On lira avec un intérêt tout particulier le travail de F. BINKLEY, qui conclut de ses expériences que la cystéinylglycinase serait un acide ribonucléique et qui émet des hypothèses intéressantes sur le rôle de ces acides dans la synthèse des protéines.

Après un exposé de D. F. POULSON ET V. T. BOWEN sur les constituants inorganiques du noyau, A. BENDICH aborde le métabolisme des acides nucléiques, dont G. R. WYATT discute la composition et la spécificité. Les effets des radiations sur les acides nucléiques (G. SCHOLES ET J. WEISS) et sur le noyau cellulaire (A. H. SPARROW, M. J. MOSES ET R. J. DUBOW) sont examinés à leur tour; le métabolisme des chromosomes, suivi par autoradiographie, est discuté alors par S. R. PELC ET A. HOWARD.

J. BIESELE et ses collaborateurs examinent ensuite les effets des purines et de leurs analogues sur la mitose, tandis que S. INOUÉ analyse l'action de la colchicine sur le fuseau. Après une mise au point de E. D. DE LAMATER, M. E. HUNTER ET S. MUDD sur le noyau bactérien, F. W. PUTNAM, puis L. M. KOZLOFF, discutent le problème du bactériophage, tel qu'il résulte d'une analyse au moyen des radio-isotopes. Enfin, R. D. HOTCHKISS termine par une excellente étude sur la nature biologique des facteurs de transformation chez les bactéries.

Ce bref énoncé des chapitres suffit à montrer tout l'intérêt de l'ouvrage; chaque article est d'ailleurs suivi d'une discussion, souvent fort intéressante. Ce livre, qui devrait être lu par tous les biochimistes et biologistes qui se préoccupent du rôle du noyau dans la vie cellulaire, est présenté de façon impeccable. Tout au plus peut-on exprimer le regret qu'un an se soit écoulé entre sa publication et le Symposium qu'il résume: il en résulte nécessairement quelques lacunes, d'ailleurs d'importance secondaire.

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