# Anomalous retention behaviour of some synthetic nucleosides on aluminium oxide layers

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## ABSTRACT

The retention of 21 nucleoside derivatives with various alkyl substituents at the 5'-position was determined on unimpregnated and paraffin oil-impregnated alumina layers using dichloroethane–methanol, water-methanol, water-ethanol and water-2-propanol eluent systems. The nucleosides showed anomalous retention behavior in each system: their retention first decreased with increasing concentration of the stronger component in the eluent, reached a maximum, and then increased with further increase in the concentration of the stronger component in the eluent. The data indicate a mixed (adsorption and reversed-phase) retention mechanism independently of the impregnation of the alumina surface. A quadratic equation described well the dependence of the $R_M$ value on the concentration of the stronger component in the eluent; the parameters of the quadratic equation showed a high intercorrelation. The chain length of the alkyl substituent and the number of triple bonds in the alkyl chain governed the retention of nucleoside derivatives in each eluent system.

## INTRODUCTION

In recent years quantitative structure-activity relationship (QSAR) studies have often promoted the design of novel bioactive compounds [1]. QSAR methods may also help in the elucidation of the role of hydrophobic interactions in various biochemical [2] and biophysical processes [3]. In the search for the best correlation between chemical structure and biological activity, a wide range of molecular parameters have been applied [4] and many of these parameters can readily be determined by various chromatographic techniques [5]. Chromatographic methods have several advantages: they are rapid and relatively simple, very small amounts of the substances are required and the compounds used need not be very pure. Lipophilicity as the molecular parameter used most frequently in QSAR studies can also be determined by reversed-phase high-performance liquid chromatography [6], reversed-phase thin-layer chromatography (RPTLC) [7] and gas chromatography [8].

The $R_M$ value (related to the molecular lipophilicity), determined through the use of RPTLC, generally depends on the concentration of the organic component in the eluent [9]. In most instances the correlation is linear. The $R_M$ value extrapolated to zero organic phase concentration ($R_{M0}$) was regarded as the most accurate estimate

of lipophilicity. However, marked deviations from linearity have been observed with quaternary amino steroids [10], crown ether derivatives [11] and peptides [12]. The $R_M$ value decreased with increasing organic component concentration in the lower concentration range, reached a maximum and then increased with further increase in the proportion of organic component. This phenomenon was tentatively explained in terms of a silanophilic effect: at higher organic component concentrations, the solute molecules have an enhanced probability of access to the silanol groups uncovered by the impregnating agent. The interaction with the free silanol groups results in an increased retention and an increased apparent lipophilicity [13,14]. It was further established that the relative polarity of the mobile and stationary phases is the main distinguishing attribute for the classification of different chromatographic systems; in other words, in RPTLC, the stationary phase has to be less polar than the mobile phase [14]. This means that the impregnation or chemical bonding of hydrophobic substituents to the support is not a prerequisite in RPTLC. The validity of the hypothesis outlined above was proved to be correct for unimpregnated cellulose [15]. For example, the application of water-alcohol mixtures on unimpregnated layers may result in an adsorption or reversed-phase retention mode depending on the relative polarity of the stationary and mobile phases.

It has additionally been stated that not only the $R_M$ value extrapolated to zero organic phase concentration but also the slope (b) value of the linear correlation is characteristic of molecular lipophilicity [15]. With a homologous series of compounds the slope and the $R_{M0}$ value exhibited a significant intercorrelation [16], but for a non-homologous series both parameters were needed to describe the lipophilicity accurately [17]. The slope has been regarded as a characteristic of the hydrophobic surface of the compounds [18].

The various molecular parameters determined by means of adsorption chromatography (relative energy of adsorption, specific adsorption surface) have found only limited application in QSAR. A good correlation was found between the tumour-inhibiting activity and the relative energy of adsorption for a series of benzofluorene derivatives [19].

Synthetic nucleosides have many biological effects [20]; they can be incorporated into DNA [21], resulting in the modification of some enzymatic processes [22]. The dependence of their biological activity on certain chromatographic parameters has recently been demonstrated [23]. The adsorption capacity, the specific adsorptive surface, the lipophilicity and the specific hydrophobic surface of some nucleosides have recently been determined using a silica support [24].

Nucleosides may be partially ionized in an aqueous environment, with the result that the pH and the ionic strength of the eluent may influence their retention. As the nature of the buffer [25] and the buffer mobility [26] also influence the retention, we assumed that the inclusion of so many variables (pH, ionic strength, buffer type and buffer mobility) would overcomplicate the experimental design and the calculation. Accordingly, we used unbuffered, ion-free eluent systems. Our choice was also guided by the fact that the adsorptive, non-aqueous systems were also unbuffered and ion free, and hence the comparison of the systems is facilitated. The objectives of our investigations were to determine some hydrophilic and hydrophobic parameters of nucleosides using alumina as a support, to select the molecular substructures that significantly influence the parameters and to find correlations between the various parameters.

EXPERIMENTAL

DC-Fertigplatten Aluminiumoxid 60 F$_{254}$ plates (Merck, Darmstadt, Germany) were used for adsorptive TLC without any pretreatment. For RPTLC the plates were impregnated with paraffin oil as described earlier [27]. The nucleosides were the 5'-alkylated derivatives of deoxyuridine (1), *i.e.,* methyl (2), ethyl- (3), *n*-propyl- (4), butyl- (5), hexyl- (6), heptyl- (7), octyl- (8), vinyl- (9), pentenyl- (10), hexenyl- (11), heptenyl- (12), octenyl- (13), propynyl- (14), butynyl- (15), pentynyl- (16), hexynyl (17), heptynyl- (18), octynyl- (19), isopropyl- (20) and bromovinyldeoxyuridine (21). They were synthesized by the research group of Dr. J. Sági at the Central Research Institute for Chemistry of the Hungarian Academy of Sciences (Budapest, Hungary).

The nucleoside derivatives were dissolved separately in methanol to give a concentration of 5 mg/ml, and 2 $\mu$l of solution were spotted on the plates with a Camag (Muttenz, Switzerland) applicator. The developments were carried out in sandwich chambers, the running distance being about 16 cm. Dichloroethane-methanol (1:0, 3:1, 1:1, 1:3 and 0:1, v/v) and water-methanol, water-ethanol and water-2-propanol mixtures (15–90%, v/v, alcohol in steps of 5%) were applied as eluents for adsorption TLC. The application of the uncommon water-alcohol mixtures as adsorption-mode eluents was motivated by the considerations mentioned in the Introduction about the distinguishing features of adsorption and reversed-phase separation modes. For RPTLC, ethanol was applied as the organic mobile phase in the concentration range 0–90% (v/v) in steps of 10%.

After development the plates were dried at 105°C and the nucleoside spots were detected under a CAMAG UV lamp at 254 nm. Each determination was run in quadruplicate. The $R_M$ values were calculated for both TLC and RPTLC systems.

When in a given chromatographic system the nucleoside spot remained at the start or was very near to the front (deformed spot shape) or when the relative standard deviation of parallel determinations was higher than 8%, the data were omitted from the calculations. As the linear correlation between the $R_M$ value of the nucleosides and the alcohol concentration in the eluent did not fit the experimental data well, quadratic correlations were calculated for each nucleoside-organic modifier pair:

$$R_M = R_{M0} + b_1 C + b_2 C^2 \tag{1}$$

where $R_M$ = actual $R_M$ value of a compound at an alcohol concentration C in the eluent, $R_{M0}$ = $R_M$ value extrapolated to zero alcohol concentration, C = alcohol concentration in the eluent (%, v/v), $b_1$ = change in $R_M$ value caused by a unit change in alcohol concentration and $b_2$ = change in $R_M$ value caused by a unit change in the alcohol concentration squared.

To assess the contributions of the nucleoside lipophilicity and the nature of the organic component to the anomalous retention behaviour, the organic compound concentration corresponding to minimum retention ($C_M$) was calculated with the parameters of eqn. 1. Mathematically, $C_M$ corresponds to the point where the first derivative of eqn. 1 is equal to zero.

To elucidate the similarities and dissimilarities between the retention behaviour of the nucleosides and the parameters of eqn. 1, principal component analysis (PCA)

was applied [28]. The nucleosides were taken as observations, and the parameters ($R_{M0}$, $b_1$, $b_2$ and $C_M$ values separately for methanol, ethanol and 2-propanol on unimpregnated alumina; a total of twelve variables) served as variables. Two-dimensional non-linear mapping of the PC loadings and variables was also carried out [29].

To find the molecular substructures accounting for the retention behaviour, stepwise regression analysis was applied [30]. The calculation was carried out sixteen times; the $R_{M0}$, $b_1$, $b_2$ and $C_M$ values of eqn. 1. determined in methanol, ethanol and 2-propanol as organic mobile phases on unimpregnated alumina and in ethanol on impregnated alumina were the dependent variables. The length of the alkyl chain, the number of double and triple bonds in the chain and the number of branching and bromo substitutions were the independent variables in each instance. The acceptance limit for the individual independent variables was set to the 95% significance level.

## RESULTS AND DISCUSSION

Solvent demixing was not observed in the eluent systems; in each instance the first and second eluent fronts were very near to each other and the nucleoside spots were always under the second eluent front.

The nucleosides showed anomalous retention behaviour even under strictly adsorption conditions (unimpregnated alumina, dichloroethane-methanol mixtures as eluents). They remained at the start in pure dichloroethane and pure methanol and showed low mobility in eluents of 1:3 and 3:1 (v/v). The lowest retention was observed in the 1:1 (v/v) eluent. This phenomenon is similar in character to the silanophilic effect. It has been customary to use this term for the attraction (retention) of solutes by SiOH groups. However, in this instance the support was unimpregnated alumina, which does not contain any silanol groups. We are well aware that the application of the term "silanophilic effect" to describe the anomalous retention behaviour of nucleosides is somewhat misleading. As the phenomenon is similar we subsequently use the term "silanophilic-like effect", bearing in mind the differences between the two retention mechanisms discussed above.

It was expected that the mobility of the nucleosides would increase with increasing concentration of methanol. The data contradict this supposition. We have no valid hypothesis to explain this anomalous retention behaviour. The $R_M$ value decreased with increasing length of the alkyl chain (Fig. 1), which indicates a normal-phase separation mode.

The relationship between the chain length and the $R_M$ value was not linear. The presence of a double bond in the alkyl chain increased the retention slightly and a triple bond increased it strongly; the difference between the effects of double and triple bonds was higher than expected from their physico-chemical characteristics.

In water-alcohol eluents on unimpregnated alumina the nucleosides showed typical silanophilic-like retention behaviour; the $R_M$ value decreased or did not change in the lower concentration range, then increased with increasing alcohol concentration (Fig. 2). Methanol had the smallest and 2-propanol the greatest impact on the retention at higher alcohol concentrations. This order corresponds to the order of reversed-phase eluent strengths; however, higher concentrations of water with respect to alcohol caused a decrease in $R_M$, which would be compatible with a normal-phase mechanism. These observations indicate a mixed mode of retention that is not well
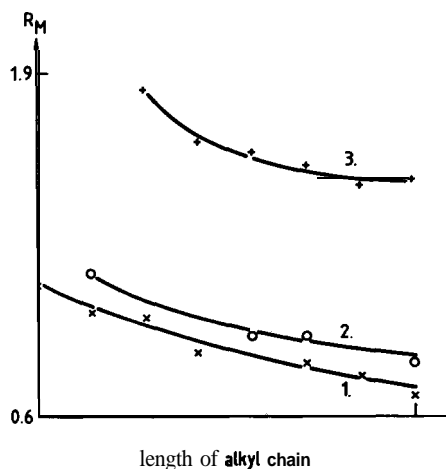
Fig. 1. Effect of the length of the alkyl chain on the $R_M$ values of some synthetic nucleosides. Eluent: dichloroethane-methanol (1:1, v/v). 1 = Saturated alkyl chain; 2 = double bond in the alkyl chain; 3 = triple bond in the alkyl chain.

understood. The water-alcohol eluents at higher alcohol concentrations behaved similarly to dichloroethane-methanol (1: 1, v/v) eluent; the nucleosides with the longest alkyl chain showed the highest mobility and the difference between the retentions of nucleosides with saturated and unsaturated chains was the same (Fig. 3). This finding suggests that the retention is governed by the adsorption separation mechanism; however, the eluent is of reversed-phase character.

The retention order of nucleosides changed continuously with changing alcohol concentration and it showed a curious picture at the lowest alcohol concentration (Fig. 4). The $R_M$ value decreased with increasing length of the alkyl chain for the shorter chains, which indicates an adsorption-type separation. It increased again with
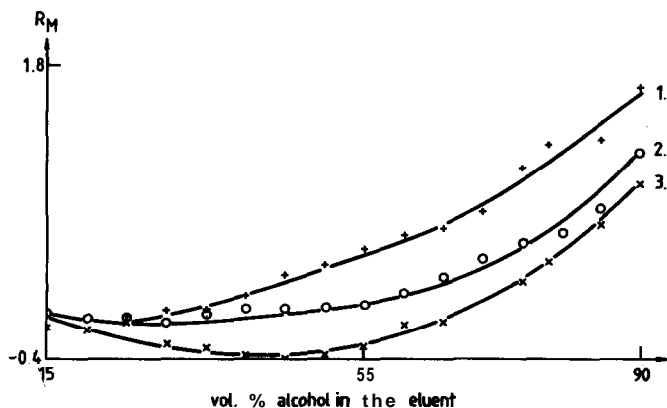


Fig. 2. Effect of the alcohol concentration in the eluent on the $R_M$ value of 5′-hexynyldeoxyuridine. Unimpregnated alumina, water-alcohol eluents. 1 = Methanol; 2 = ethanol; 3 = 2-propanol.
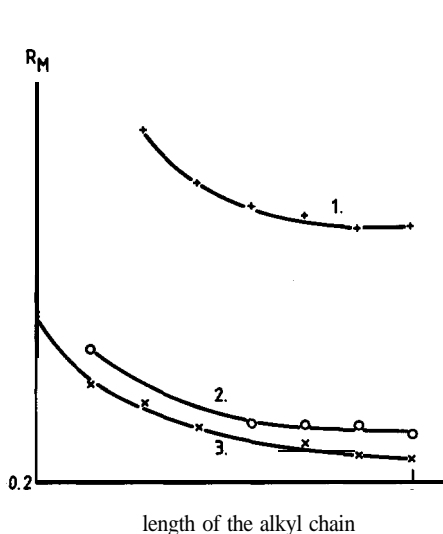
Fig. 3. Effect of the length of the alkyl chain on the $R_M$ values of some synthetic nucleosides. Eluent, water-2-propanol  (1:9, v/v). 1 = Saturated alkyl chain; 2 = double bond in the alkyl chain; 3 = triple bond in the alkyl chain.
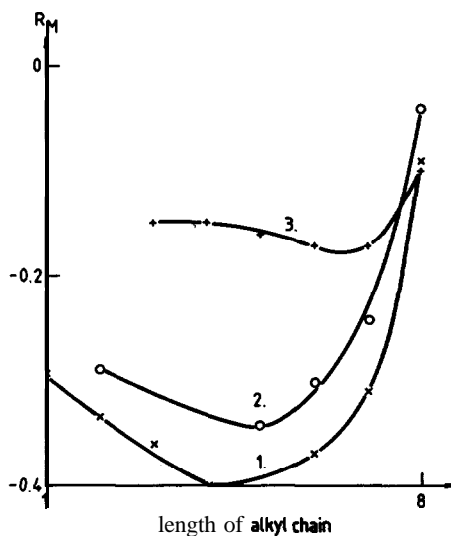
Fig. 4. Effect of the length of the alkyl chain on the $R_M$ values of some synthetic nucleosides. Eluent, water–2-propanol (85:15, v/v). 1 = Saturated alkyl chain; 2 = double bond in the alkyl chain; 3 = triple bond in the alkyl chain.

increasing length of the alkyl chain at longer chain lengths, which indicates a re-versed-phase separation mechanism. This result can be explained by the assumption that the retention mechanism in the same chromatographic system may depend heav-ily on the type of solute because its partitioning between the stationary and mobile phases may depend on the length and lipophilicity of the alkyl chain substituents.

The substantial role of triple bonds in determining retention prevails for the shorter alkyl chains and disappears with longer alkyl chains.

The results indicate that the retention of nucleosides is highly irregular on unimpregnated alumina; at least two retention mechanism (adsorption and reversed-phase) are involved, their relative importance depending on the type and composition of the eluent and on the character of the alkyl substituent. The data do not exclude the possibility that the change in intermolecular forces (caused by the changing die-lectric constant of the eluents) may also influence the retention, as was observed with silica-based supports [31].

Similar anomalies were observed on impregnated alumina (Fig. 5). The $R_M$ values decreased with growing ethanol concentration in water in the lower concentra-tion range, reached a minimum, then increased again.

The importance of triple bonds is manifested only at higher ethanol concentra-tions (adsorption-like separation mechanism). In this instance the anomaly can be explained by the silanophilic-like effect when we assume that for the appearance of the silanophilic-like effect a support with uncovered adsorption centres is sufficient and the adsorption centres need not to be silanol groups. However, this explanation is
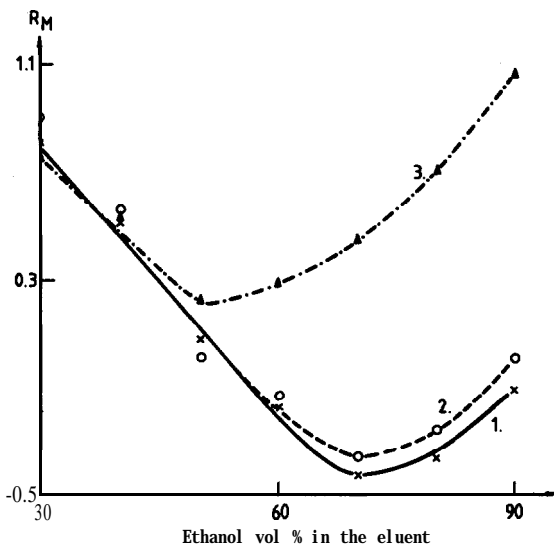
Fig. 5. Effect of ethanol concentration in the water-ethanol eluent on the $R_M$ value of (1) hexyl-, (2) hexenyl- and (3) hexynyldeoxyuridine. Impregnated alumina.

not valid for the unimpregnated alumina, showing similar retention behaviour. We suggest two possible mechanisms to account for the irregularities:

(a) The water or the alcohol is adsorbed on the alumina depending on their relative concentrations in the eluent. The quasi-stationary phase of water or alcohol behaves as an adsorption or reversed-phase support, respectively, depending on the equilibrium of the various nucleoside derivatives between the stationary and mobile phases.

(b) The extent of dissociation of the nucleosides depends strongly on the dielectric constant of the eluent, which decreases with increasing proportion of organic component in the mobile phase. The dissociated and undissociated nucleosides may be retained in the same eluent by an adsorption or reversed-phase retention mechanism, the retention being influenced by the changing intramolecular forces.

We assume that both processes have a considerable impact on the retention and the retention order is the result of the interplay of the processes outlined above.

The parameters of eqn. 1 are compiled in Tables I-IV. Blanks in Table I indicate that the linear regression coefficient did not differ significantly from zero. The quadratic function fits the experimental data well, the significance level in each instance being higher than 99.9%, confirming the applicability of eqn. 1 (see $F$ values). The equation accounted for about 92-99% of the total variance (see $r^2$ values). The normalized slope values ($b'_1$ and $b'_2$ values) clearly show that the contribution of the linear and quadratic parameters to the retention is commensurable. The numerical values of the slope ($b_1$) increased in the order methanol < ethanol < 2-propanol on unimpregnated alumina. As this order corresponds to the reversed-phase elution strength of the alcohols, this phenomenon indicates a reversed-phase separation mechanism. For deoxyuridine derivatives with a saturated alkyl chain and with a double bond in the alkyl chain the intercept ($R_{M0}$) values generally increased with

TABLE I

PARAMETERS OF THE QUADRATIC RELATIONSHIP BETWEEN THE $R_M$ VALUES OF NU-
CLEOSIDES AND THE METHANOL CONCENTRATION ($C$) IN THE ELUENT USING UN-
IMPREGNATED ALUMINA

$R_M = R_{M0} + b_1C + b_2C^2.$

| Parameter | No. of nucleoside | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| $R_{M0}$ | 50.4 | $-1.38$ | 11.1 | 6.49 | $-5.27$ | 9.81 | 39.9 |
| $b_1$ | $-4.02$ | $-1.55$ | -2.12 | $-2.08$ | $-1.65$ | $-2.54$ | $-3.52$ |
| $b_2 \cdot 10^2$ | 4.96 | 2.79 | 2.99 | 3.03 | 2.57 | 3.48 | 4.23 |
| $F$ | 104.9 | 136.1 | 12.3 | 67.8 | 100.4 | 77.5 | 73.6 |
| $r^2$ | 0.9589 | 0.9612 | 0.9393 | 0.9250 | 0.9481 | 0.9337 | 0.9305 |
| $b_1'(\%)$ | 41.01 | 32.28 | 37.88 | 37.13 | 35.50 | 38.56 | 41.69 |
| $b_2'(\%)$ | 58.99 | 61.12 | 62.12 | 62.87 | 64.50 | 61.44 | 58.31 |
| | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
| $R_{M0}$ | 51.1 | -45.5 | 56.1 | 32.0 | 38.3 | 19.6 | -24.3 |
| $b_1$ | -3.83 | — | — | -3.21 | -3.60 | -4.65 | — |
| $b_2 \cdot 10^2$ | 4.45 | 1.50 | 1.56 | 4.23 | 4.12 | 5.26 | 2.14 |
| $F$ | 102.2 | 190.5 | 162.0 | 182.1 | 173.7 | 180.4 | 709.6 |
| $r^2$ | 0.9489 | 0.9407 | 0.9310 | 0.9707 | 0.9693 | 0.9704 | 0.9834 |
| $b_1'(\%)$ | 42.53 | — | — | 39.46 | 39.58 | 43.15 | — |
| $b_2'(\%)$ | 51.41 | — | — | 60.54 | 60.42 | 56.85 | — |
| | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
| $R_{M0}$ | $-25.9$ | $-24.1$ | $-22.9$ | $-23.4$ | -21.5 | $-58.1$ | $-52.6$ |
| $b_1$ | — | — | — | — | — | — | — |
| $b_2 \cdot 10^2$ | 2.10 | 2.02 | 2.15 | 1.92 | 1.89 | 1.21 | 1.86 |
| $F$ | 756.8 | 463.2 | 616.6 | 323.5 | 292.6 | 155.9 | 291.6 |
| $r^2$ | 0.9844 | 0.9748 | 0.9809 | 0.9672 | 0.9606 | 0.9285 | 0.9605 |
| $b_1'(\%)$ | — | — | — | | — | — | — |
| $b_2'(\%)$ | — | — | | — | — | — | — |

increasing length of the alkyl substituent, which also supports the reversed-phase
retention mechanism. However, the derivatives with triple bonds did not show a
similar retention behaviour, that is, for these the reversed-phase retention mechanism
is not valid. The unsubstituted deoxyuridine deviates considerably from the retention
order. It shows a higher retention than would be expected under reversed-phase
conditions. We assume that the free hydrogen can form hydrogen bonds with the free
adsorption centres of alumina, resulting in increased retention.

Only one background variable explains the majority of the variance in the data
matrix for PCA (Table V). It means that may exists only one chromatographic pa-
rameter that accounts for about 80% of variance. We must stress that the PCA did
not state that such a parameter really exists, it only indicates the mathematical possi-
bility of it. Each parameter has a high loading in the first component, which demon-

TABLE II

PARAMETERS OF THE QUADRATIC RELATIONSHIP BETWEEN THE $R_M$ VALUES OF NUCLEOSIDES AND THE ETHANOL CONCENTRATION ($C$) IN THE ELUENT USING UNIMPREGNATED ALUMINA

$R_M = R_{M0} + b_1 C + b_2 C^2$.

| Parameter | No. of nucleoside | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 1 |
| $R_{M0}$ | 183.1 | 2.96 | 30.8 | 47.2 | 5.50 | 64.3 | 100.6 |
| $b_1$ | −8.71 | −1.16 | −3.02 | -3.81 | −2.63 | −5.08 | −6.45 |
| $b_2 \cdot 10^2$ | 1.53 | 2.36 | 3.12 | 3.78 | 2.17 | 4.78 | 5.89 |
| $F$ | 81.6 | 97.9 | 15.4 | 108.2 | 188.8 | 79.7 | 140.1 |
| $r^2$ | 0.9411 | 0.9311 | 0.9263 | 0.9516 | 0.9667 | 0.9300 | 0.9589 |
| $b_1'(\%)$ | 41.89 | 41.11 | 46.45 | 46.41 | 47.00 | 48.76 | 49.53 |
| $b_2'(\%)$ | 52.11 | 58.89 | 53.55 | 53.59 | 53.00 | 51.24 | 50.47 |
| | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
| $R_{M0}$ | 106.7 | 14.4 | 41.1 | 51.3 | 58.2 | 102.1 | 16.8 |
| $b_1$ | −6.73 | −2.29 | -4.06 | -4.36 | -4.75 | -6.50 | −1.52 |
| $b_2 \cdot 10^2$ | 6.10 | 2.74 | 4.09 | 4.23 | 4.67 | 6.07 | 2.19 |
| $F$ | 76.9 | 110.2 | 89.4 | 105.1 | 77.8 | 95.7 | 254.1 |
| $r^2$ | 0.9276 | 0.9443 | 0.9322 | 0.9417 | 0.9229 | 0.9671 | 0.9150 |
| $b_1'(\%)$ | 49.12 | 43.85 | 48.12 | 49.10 | 48.73 | 50.02 | 33.16 |
| $b_2'(\%)$ | 50.28 | 56.15 | 51.88 | 50.90 | 51.21 | 49.98 | 66.24 |
| | 15 | 16 | 11 | 18 | 19 | 20 | 21 |
| $R_{M0}$ | 16.8 | 11.3 | 24.6 | 25.1 | 34.7 | 5.19 | 6.66 |
| $b_1$ | −1.52 | −1.94 | −2.22 | −2.39 | −2.70 | −2.48 | −2.38 |
| $b_2 \cdot 10^2$ | 2.19 | 3.11 | 3.40 | 3.48 | 3.62 | 2.57 | 2.89 |
| $F$ | 254.1 | 138.4 | 216.0 | 208.8 | 145.3 | 149.2 | 99.1 |
| $r^2$ | 0.915 1 | 0.9551 | 0.9108 | 0.9698 | 0.9572 | 0.9582 | 0.9388 |
| $b_1'(\%)$ | 33.76 | 36.81 | 37.88 | 39.16 | 41.11 | 41.46 | 43.55 |
| $b_2'(\%)$ | 66.24 | 63.19 | 62.12 | 60.24 | 58.89 | 52.54 | 56.45 |

strates again the strong intercorrelation between them. The linear regression coefficients form a distinct cluster on the two-dimensional non-linear map of PC loadings, whereas the other parameters form another cluster (Fig. 6). This finding suggests that the intercept value, the quadratic regression coefficient and the critical alcohol concentration contain similar information. As the slope value is generally related to the lipophilicity, we assume that these three parameters are related to the lipophilicity of the nucleoside derivatives. The nucleosides form three separate clusters on the two-dimensional non-linear map of PC variables (Fig. 7). The nucleosides with triple bonds in the alkyl chain (cluster C) form the most compact group, indicating the important role of triple bonds in the retention of nucleosides on unimpregnated alumina. The nucleosides with a saturated alkyl chain form a separate group (cluster A), whereas the nucleosides with double bonds and with branching or bromo sub-

TABLE III

PARAMETERS OF THE QUADRATIC RELATIONSHIP BETWEEN THE $R_M$ VALUES OF NUCLEOSIDES AND THE 2-PROPANOL CONCENTRATION C IN THE ELUENT USING UNIMPREGNATED ALUMINA

$R_M = R_{M0} + b_1 C + b_2 C^2$.

| Parameter | No. of nucleoside | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| $R_{M0}$ | 269.3 | 16.8 | 25.2 | 45.2 | 53.0 | 96.1 | 136.0 |
| $b_1$ | −14.4 | −2.58 | −3.41 | -4.66 | −5.53 | -8.15 | -9.99 |
| $b_2 \cdot 10^2$ | 12.7 | 3.05 | 3.58 | 4.52 | 5.30 | 7.66 | 9.25 |
| $F$ | 96.9 | 197.4 | 135.6 | 88.4 | 115.4 | 173.7 | 132.8 |
| $r^2$ | 0.9556 | 0.9681 | 0.9542 | 0.9315 | 0.9467 | 0.9639 | 0.9533 |
| $b_1'(\%)$ | 48.73 | 44.18 | 41.58 | 49.03 | 49.38 | 49.87 | 50.24 |
| $b_2'(\%)$ | 51.27 | 55.82 | 52.42 | 50.97 | 50.62 | 50.13 | 49.76 |
| | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
| $R_{M0}$ | 154.3 | 11.7 | 96.4 | 124.8 | 153.1 | 165.7 | 7.85 |
| $b_1$ | −10.6 | −3.04 | -1.64 | -9.19 | −10.9 | -11.3 | −1.95 |
| $b_2 \cdot 10^2$ | 9.67 | 3.29 | 1.25 | 8.64 | 10.3 | 10.4 | 3.44 |
| $F$ | 93.7 | 110.5 | 95.8 | 120.0 | 83.7 | 104.6 | 110.5 |
| $r^2$ | 0.9351 | 0.9444 | 0.9365 | 0.9486 | 0.9279 | 0.9415 | 0.9526 |
| $b_1'(\%)$ | 50.61 | 46.38 | 49.60 | 49.85 | 49.81 | 50.22 | 34.38 |
| $b_2'(\%)$ | 49.39 | 53.62 | 50.40 | 50.15 | 50.13 | 49.78 | 65.62 |
| | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
| $R_{M0}$ | 8.95 | 18.5 | 31.0 | 45.8 | 61.7 | 57.0 | 15.3 |
| $b_1$ | −2.06 | -2.71 | −3.57 | -4.36 | −5.29 | -5.10 | −5.80 |
| $b_2 \cdot 10^2$ | 3.37 | 3.78 | 4.67 | 5.15 | 5.93 | 4.61 | 5.48 |
| $F$ | 126.4 | 83.2 | 80.9 | 186.6 | 144.9 | 144.3 | 108.9 |
| $r^2$ | 0.9583 | 0.9380 | 0.9363 | 0.9664 | 0.9571 | 0.9569 | 0.9437 |
| $b_1'(\%)$ | 36.26 | 39.83 | 41.38 | 44.20 | 45.41 | 50.83 | 49.73 |
| $b_2'(\%)$ | 63.14 | 60.17 | 58.62 | 55.80 | 54.53 | 49.17 | 50.27 |

stitution in the alkyl chain form the third cluster (cluster B). This means that branching and bromo substitution of the alkyl chain have a similar effect to the double bond on the retention of nucleosides.

The significant correlations between the chromatographic parameters and structural characteristics of the nucleoside derivatives are collected in Table VI. As the PCA proved that the chromatographic parameters did not show large differences and are strongly intercorrelated, it was reasonable to assume that the structure–chromatographic parameter correlations will be very similar. The calculation entirely supported this assumption; the equations did not differ considerably from each other. Therefore, only the equations derived from the 2-propanol-unimpregnated alumina system are presented. Each chromatographic parameter was related to the structural characteristics of the nucleoside derivatives and the significance level was over 99.9%

TABLE IV

PARAMETERS OF THE QUADRATIC RELATIONSHIP BETWEEN THE $R_M$ VALUES OF NU-
CLEOSIDES AND THE ETHANOL CONCENTRATION $(C)$ IN THE ELUENT USING IMPREG-
NATED ALUMINA

$R_M = R_{M0} + b_1 C + b_2 C^2.$

| Parameter | No. of nucleoside | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| $R_{M0}$ | 974.1 | 47.5 | 83.4 | 125.4 | 167.2 | 322.9 | 441.5 |
| $b_1$ | − 24.9 | -3.11 | -4.40 | − 5.80 | − 6.26 | − 9.78 | − 12.9 |
| $b_2 \cdot 10^2$ | 15.6 | 3.47 | 4.16 | 5.12 | 4.89 | 6.68 | 8.61 |
| $F$ | 483.8 | 79.8 | 59.6 | 47.4 | 131.1 | 63.2 | 168.9 |
| $r^2$ | 0.9979 | 0.9580 | 0.9445 | 0.9405 | 0.9740 | 0.9693 | 0.9883 |
| $b_1'(\%)$ | 55.18 | 48.97 | 53.09 | 55.04 | 57.81 | 54.71 | 55.19 |
| $b_2'(\%)$ | 44.82 | 51.03 | 46.91 | 44.96 | 42.19 | 45.29 | 44.81 |
| | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
| $R_{M0}$ | 526.9 | 60.2 | 231.6 | 346.5 | 467.2 | 536.6 | 63.8 |
| $b_1$ | − 14.9 | − 3.08 | − 7.48 | − 10.5 | − 13.7 | − 15.2 | − 2.66 |
| $b_2 \cdot 10^2$ | 9.83 | 3.00 | 5.47 | 4.34 | 9.37 | 10.2 | 3.71 |
| $F$ | 182.0 | 116.1 | 159.0 | 77.2 | 185.0 | 153.3 | 41.8 |
| $r^2$ | 0.9747 | 0.9707 | 0.9815 | 0.9747 | 0.9979 | 0.9871 | 0.9227 |
| $b_1'(\%)$ | 55.56 | 52.41 | 57.16 | 54.10 | 54.59 | 55.18 | 43.45 |
| $b_2'(\%)$ | 44.44 | 47.59 | 42.84 | 45.90 | 45.41 | 44.82 | 56.55 |
| | 15 | 16 | 17 | 18 | 19 | **20** | 21 |
| $R_{M0}$ | 123.2 | 180.9 | 313.7 | 303.2 | 421.9 | 129.9 | 168.1 |
| $b_1$ | -4.70 | − 6.67 | − 9.85 | -9.37 | − 12.5 | − 6.69 | − 7.27 |
| $b_2 \cdot 10^2$ | 5.14 | 6.60 | 8.46 | 7.82 | 9.80 | 5.94 | 6.54 |
| $F$ | 98.4 | 75.2 | 38.1 | 69.0 | 38.5 | 40.9 | 81.8 |
| $r^2$ | 0.9657 | 0.9619 | 0.9384 | 0.9787 | 0.9506 | 0.9424 | 0.9703 |
| $b_1'(\%)$ | 49.48 | 52.18 | 51.01 | 49.65 | 51.20 | 54.61 | 54.31 |
| $b_2'(\%)$ | 50.52 | 47.82 | 48.99 | 50.35 | 48.80 | 45.39 | 45.69 |

in each instance (see *F* values). The substructures accounted for about 81-94% of the
total variance (see $r^2$ values). The normalized slope values (b') indicate that the length
of the alkyl chain and the number of triple bonds in the chain have the greatest impact
on the retention. The role of the triple bond is higher than would be expected. We do
not have any valid explanation for the phenomenon. It is probable that the triple
bond specially binds to the adsorption site on the alumina surface, but the character
of the binding is not known.

TABLE V

RESULTS OF PRINCIPAL COMPONENT ANALYSIS USING UNIMPREGNATED ALUMINA

| No. of PC | Eigenvalue | Explained variance (%) |
|---|---|---|
| 1 | 9.57 | 79.76 |
| 2 | 1.06 | 8.86 |
| 3 | 0.92 | 7.68 |

Principal component loadings

| No. of variable" | No. of principal component | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| 1 | 0.82 | 0.48 | 0.22 |
| 2 | -0.93 | -0.11 | -0.33 |
| 3 | 0.92 | 0.24 | 0.24 |
| 4 | 0.84 | 0.05 | 0.52 |
| 5 | 0.85 | 0.28 | -0.35 |
| 6 | − 0.91 | 0.01 | 0.16 |
| 7 | 0.94 | 0.14 | − 0.27 |
| 8 | 0.86 | − 0.43 | 0.15 |
| 9 | 0.93 | − 0.03 | -8.32 |
| 10 | − 0.95 | 0.17 | 0.17 |
| 11 | 0.94 | − 0.07 | -0.22 |
| 12 | 0.12 | − 0.66 | 0.12 |

[a]1–4 = Water-methanol eluents: 1 = intercept (a) value; 2 = linear regression coefficient (b,); 3 = quadratic regression coefficient ($b_2$); 4 = critical alcohol concentration ($C_M$). 5-8 = Water-ethanol eluents: 5 = intercept (a) value; 6 = linear regression coefficient ($b_1$); 7 = quadratic regression coefficient (b,); 8 = critical alcohol concentration ($C_M$). 9–12 = Water-2-propanol eluents: 9 = intercept (a) value; 10 = linear regression coefficient ($b_1$); 11 = quadratic regression coefficient ($b_2$); 12 = critical alcohol concentration ($C_M$).
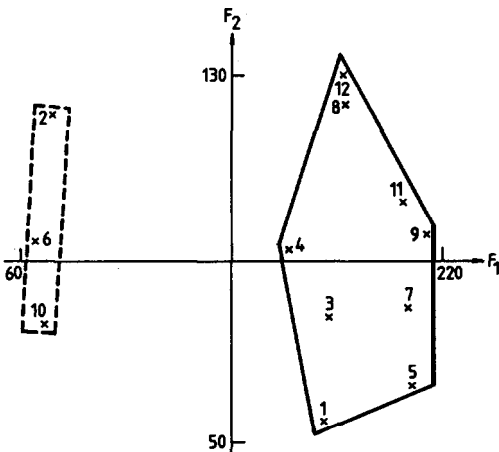


Fig. 6. Two-dimensional non-linear map of PC loadings. Number of iterations, 116 Maximum error, 1.83 · $10^{-3}$. 1–4 = Water-methanol eluents: 1 = intercept (a) value; 2 = linear regression coefficient ($b_1$); 3 = quadratic regression coefficient ($b_2$); 4 = critical alcohol concentration ($C_M$). 5-8 = Water-ethanol eluents: 5 = intercept (a) value; 6 = linear regression coefficient (b,); 7 = quadratic regression coefficient (b,); 8 = critical alcohol concentration ($C_M$). 9-12 = Water-2-propanol eluents: 9 = intercept (a) value; 10 = linear regression coefficient (b,); 11 = quadratic regression coefficient (b,); 12 = critical alcohol concentration ($C_M$).
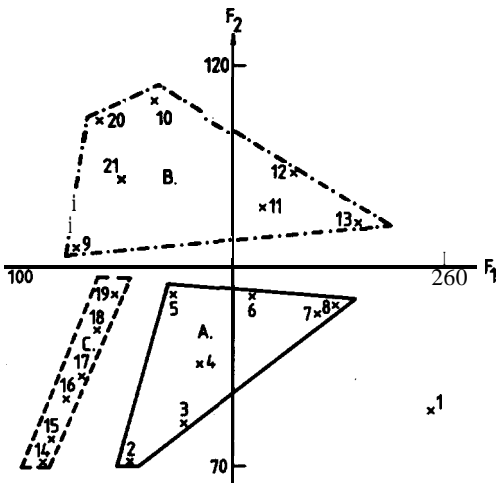
Fig. 7. Two-dimensional non-linear map of PC variables. Number of iterations, 73. Maximum error, 9.59 . $10^{-3}$. Numbers refer to nucleosides given in Experimental.

TABLE VI

PARAMETERS OF SIGNIFICANT CORRELATIONS BETWEEN THE CHROMATOGRAPHIC PARAMETERS AND STRUCTURAL CHARACTERISTICS OF SOME NUCLEOSIDE DERIVATIVES

Results of stepwise regression analysis. Unimpregnated alumina; eluent, water–2-propanol mixtures.
(I) $R_{M0} = a + b_1 x_1 + b_2 x_2 + b_3 x_3 + b_4 x_4$.
(II) Regression coefficient (linear) = $a + b_1 x_1 + b_3 x_3 + b_4 x_4$
(III) Regression coefficient (quadratic) $= a + b_1 x_1 + b_3 x_3 + b_4 x_4$.
(IV) $C_M = a + b_1 x_1 + b_2 x_2 + b_3 x_3$.
$x_1$ = length of the alkyl chain; $x_2$ = number of double bonds in the alkyl chain; $x_3$ = number of triple bonds in the alkyl chain; $x_4$ = number of bromo substituents in the alkyl chain.

| Parameter | Equation No. | | | |
|---|---|---|---|---|
| | I | II | III | IV |
| $a$ | − 8.37 | − 1.10 | 0.017 | 44.2 |
| $b_1$ | 19.13 | − 1.21 | 0.010 | 1.86 |
| $b_2$ | 16.43 | — | — | -4.61 |
| $b_3$ | − 66.88 | 4.49 | − 0.030 | − 17.15 |
| $b_4$ | 45.39 | − 2.26 | 0.017 | — |
| $F$ | 64.7 | 79.4 | 67.4 | 25.4 |
| $r^2$ | 0.9418 | 0.9334 | 0.9225 | 0.8175 |
| $b'_1(\%)$ | 47.11 | 51.71 | 56.96 | 28.86 |
| $b'_2(\%)$ | 8.30 | — | — | 14.67 |
| $b'_3(\%)$ | 33.78 | 39.03 | 33.96 | 56.47 |
| $b'_4(\%)$ | 10.81 | 9.26 | 9.08 | — |

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