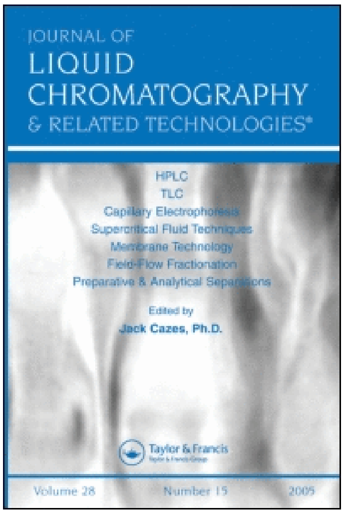

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# Effect of Substituents on the Retention in HPLC Chromatography of Strychnine Derivatives

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# EFFECT OF SUBSTITUENTS ON THE RETENTION IN HPLC CHROMATOGRAPHY OF STRYCHNINE DERIVATIVES.

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## ABSTRACT

The capacity factor k, relative retentions $\alpha_{SP}$ and log $\alpha_{SP}$ values measured on $\mu$Porasil columns for 33 strychnine derivatives using $CHCl_3$:MeOH (containing ca 2% $NH_4OH$) (93:7) as eluent in normal-phase chromatography. The results allow for the estimation of the effect of various substituents on the retention of these alkaloids.

## INTRODUCTION

The rapidly advancing technique of high pressure liquid chromatography made possible the microanalytical identification, as well as the preparative separation of synthetic and naturally occuring complex organic molecules which were, otherwise, insolable by the traditional t.l.c. and column chromatography techniques.

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Many examples can be cited where both analysis and isolation of plant active substances which may have some physiological significance, was successfully achieved e.g. steroidal (1), tropane (2), morphine (3) and strychnos alkaloids (4).

The main aim of the present work is to give a qualitative correlation of the effects of substituents on the retention of compounds, which are strychnine alkaloid derivatives modified in the aromatic and non-aromatic part of the molecule. The results shown can lead to a "prediction"of the behavior of similar molecules when treated under the same conditions of HPLC.

## EXPERIMENTAL

The present analyses were carried out on a high pressure liquid chromatograph consisting of the following parts: A Waters 6000 pump, a Waters U 6K Universal injector and a Varian Variscan 635 u.v. detector. Chromatograms were obtained at 254 nm wavelength. All measurments were performed on 8 $\mu$m Porasil column (300 x 4.5mm i.d).

The solvent system used was $CHCl_3$ and MeOH (containing 2% $NH_4OH$) (93:7). This was isocratically eluted at a flow rate of 3ml/min. at <u>ca</u> 2 500 psi, and at ambient temperature. Stock solutions of the solutes were made in the eluent and about 0.5-10 $\mu$g of each individual sample was injected. The elution time of an unsorbed solute, $t_M$, was measured as described earlier (5). The retention times of the solutes, $t_R$, was evaluated at the peak maxima of the symmetrical peaks. The capacity factors, $k=(t_R-t_M)/t_M$, and the relative retentions $\alpha_{SP}$ were also evaluated as described (5).

## RESULTS AND DISCUSSION

The chromatograms in fig. 1 illustrate the speed and effeciency of HPLC for the analysis of the systems studied. The use of Porasil column at ambient temperature and the relatively high flow rates, together, afford ease of manipulation and efficiency.

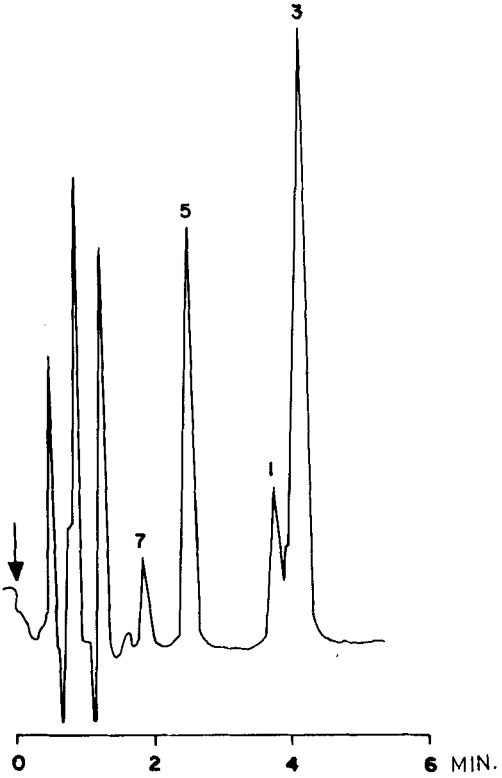
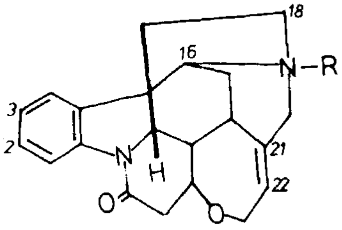


FIGURE 1

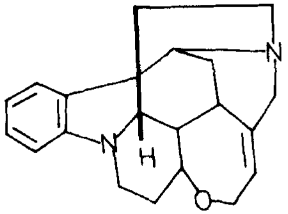
Chromatogram of some derivatives. The conditions and symbols are given in text.

In the present study, the use of the slightly basic solvent system allowed for the separation of the relatively polar substrates, which otherwise may need less strongly retentive columns.

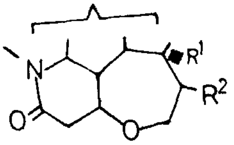
The capacity factor k, the relative retention $\alpha_{SP}$ together with the corresponding $\log \alpha_{SP}$ values are given in tables 1,2 and 3 for 33 compounds of type I, II and III. The data in general offer an overview on the



I



II



III

effect of the various substituents on the retention of such compounds. The results generally show the solute-solvent interaction wherein an increase in the energy of interaction between the solute and the solvent reduces binding. The interaction can be optimised to Van der Waals interaction, which is primarily dependent on the molecular size of the solute and the solvent, and the electrostatic interactions as is evident for the 2-carbamoyl strychnines (fig. 2). In this series, the increse in size of the "non-polar" alkyl fragment of the amide side chain, increases retention. In either case the plot

TABLE 1. Relative retentions and capacity factors of 2-carbamoylstrychnines.

| Compound (I) | Name | $\alpha_{SP}$ | $\log\alpha_{SP}$ | k |
|---|---|---|---|---|
| 2 | Amino | 1.0 | 0.0 | 0.96 |
| 3 | Acetamido | 4.4 | 0.64 | 7.63 |
| 4 | *n*-propionamido | 2.8 | 0.45 | 4.49 |
| 5 | *n*-butyramido | 2.0 | 0.30 | 3.12 |
| 6 | *iso*-butyramido | 2.0 | 0.30 | 2.96 |
| 7 | *n*-valeramido | 1.7 | 0.23 | 2.33 |
| 8 | *iso*-valeramido | 1.8 | 0.25 | 2.53 |
| 9 | benzamido | 1.4 | 0.15 | 1.74 |
| 10 | pivalamido | 1.4 | 0.15 | 1.74 |
| 1 | strychnine | 1.0 | 0.0 | 5.86 |

TABLE 2. Relative retentions and capacity factors of 2- and 3-substituted strychnines.

| Compound (I) | Name | $\alpha_{SP}$ | $\log\alpha_{SP}$ | k |
|---|---|---|---|---|
| 3 | 2-acetamido | 4.4 | 0.64 | 7.63 |
| 2 | 2-amino | 1.0 | 0.0 | 0.96 |
| 11 | 2-hydroxy | 0.69 | -0.16 | 3.71 |
| 12 | 2-methanesulfonamido | 0.60 | -0.22 | 3.12 |
| 9 | 2-benzamido | 0.40 | -0.40 | 1.74 |
| 13 | 2-*p*-toluenesulfonamido | 0.34 | -0.47 | 1.35 |

(continued)

TABLE 2/ conti.

| Compound (I) | Name | $\alpha_{SP}$ | $\log\alpha_{SP}$ | k |
|---|---|---|---|---|
| 14 | 2-methoxy | 0.29 | -0.53 | 0.96 |
| 15 | 2-bromo | 0.20 | -0.70 | 0.37 |
| 16 | 2-nitro | 0.17 | -0.77 | 0.18 |
| 17 | 3-acetamido | 0.57 | -0.24 | 2.92 |
| 18 | 3-amino | 0.54 | -0.27 | 2.73 |
| 19 | 3-hydroxy | 0.54 | -0.27 | 2.73 |
| 20 | 3-methanesulfonamido | 0.46 | -0.34 | 2.14 |
| 21 | 3-methoxy | 0.29 | -0.53 | 0.96 |
| 22 | 3-nitro | 0.26 | -0.59 | 0.76 |
| 23 | 3-bromo | 0.20 | -0.70 | 0.37 |
| 24 | 2,3-dimethoxy | 1.20 | 0.08 | 7.56 |

TABLE 3. Relative retentions of other strychnine and strychnidine derivatives.

| Compound | Name | $\alpha_{SP}$ | $\log\alpha_{SP}$ | k |
|---|---|---|---|---|
| (I) 25 | 16-hydroxystrychnine | 0.29 | -0.53 | 0.96 |
| (I) 26 | 16-methoxystrychnine | 0.17 | -0.77 | 0.18 |
| (I) 27 | 16-ethoxystrychnine | 0.17 | -0.77 | 0.18 |
| (I) 28 | 16-isopropoxystrychnine | 0.17 | -0.77 | 0.18 |
| (III; $R^1=R^2=H$) | 21,22-dihydrostrychnine | 0.28 | -0.56 | 0.90 |
| (III; $R^1=R^2=OH$) | 21,22-dihydroxystrychnine | 0.62 | -0.21 | 3.30 |

(continued)

TABLE 3/ conti.

| Compound | Name | $\alpha_{SP}$ | $\log \alpha_{SP}$ | k |
|---|---|---|---|---|
| (I; R=O) | strychnine -N Oxide | 1.06 | 0.025 | 6.25 |
| (I; R=Me Cl$^-$) | N-methylstrychnine chloride | 1.26 | 0.10 | 7.60 |
| (II) | 21,22-dihydrostrychnidine | 0.74 | -0.13 | 4.10 |

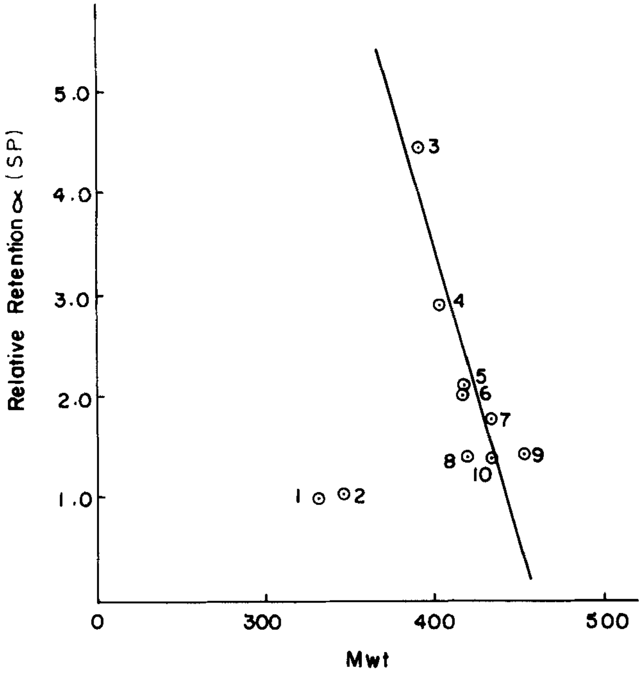


FIGURE 2

Relative retention vs. Molecular weight of 2-Carbamoylstrychnines.

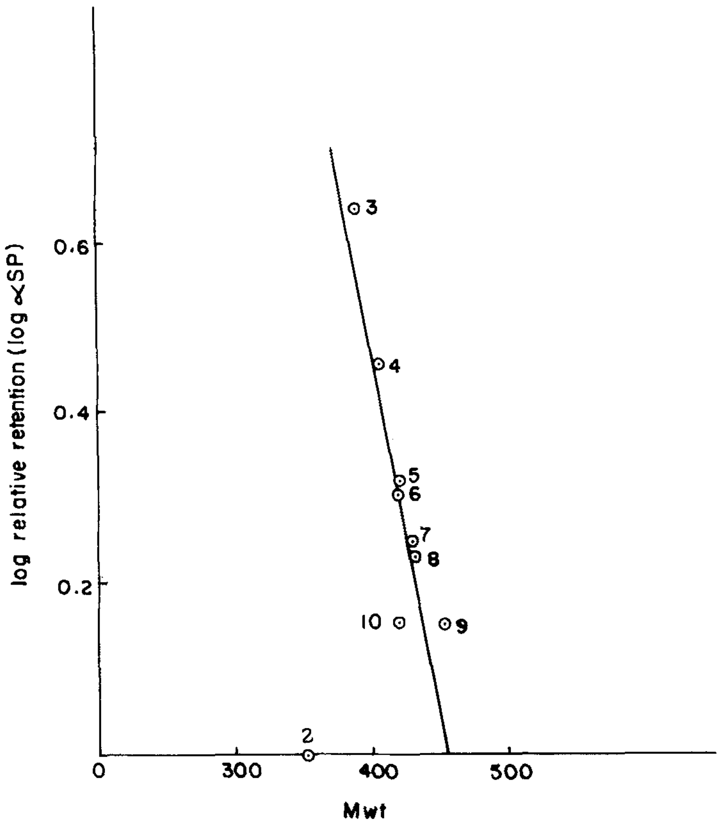


FIGURE 3

Relative retention vs. Molecular weight of 2- Carbamoylstrychnines.

of the relative retention $\alpha_{SP}$ or $\log\alpha_{SP}$ versus molecular weight evidently show a linear relationship (figs. 2 and 3). In other words, the effect of the methylene group increment on the aliphatic side chain is surprisingly consistent. This is, however, unmatched by the benzamido group even though the replacement of a methyl group by an aromatic ring significantly increases

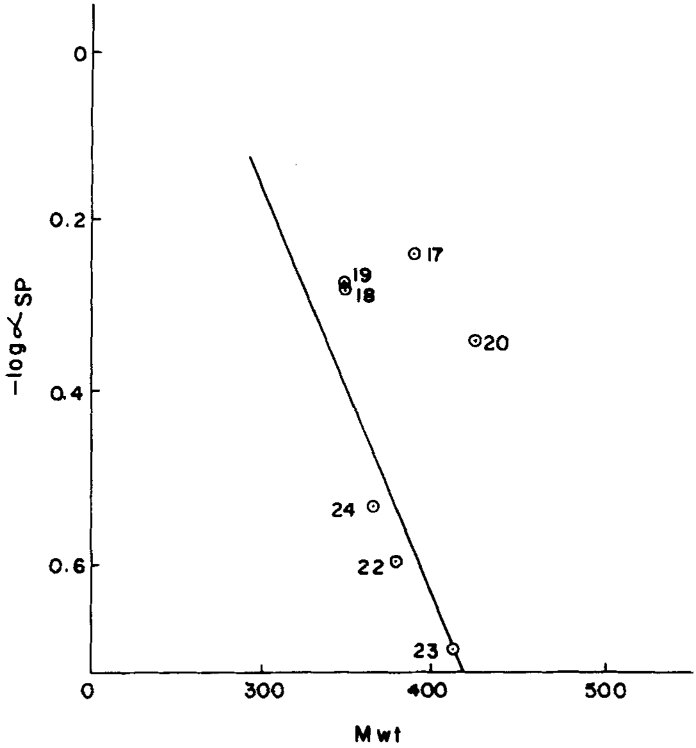


FIGURE 4
-Log Relative retention vs. Molecular weight of 3-Substituted strychnines.

the molecular size. On the other hand, with the solutes studied of approximately similar dimensions and with a fixed eluent, the magnitude of the relative retention $\alpha_{SP}$, is primarily affected by the polarity of the respective substituents (tables 2,3) and (figs. 4,5). Compounds with a "zwitterionic moiety" as in strychnine N-oxide (I; R = O) and quaternary strychnine salts (I; R = Me Cl$^-$) show a markedly reduced biophobicity and hence maximum retention values. Similar findings were reported

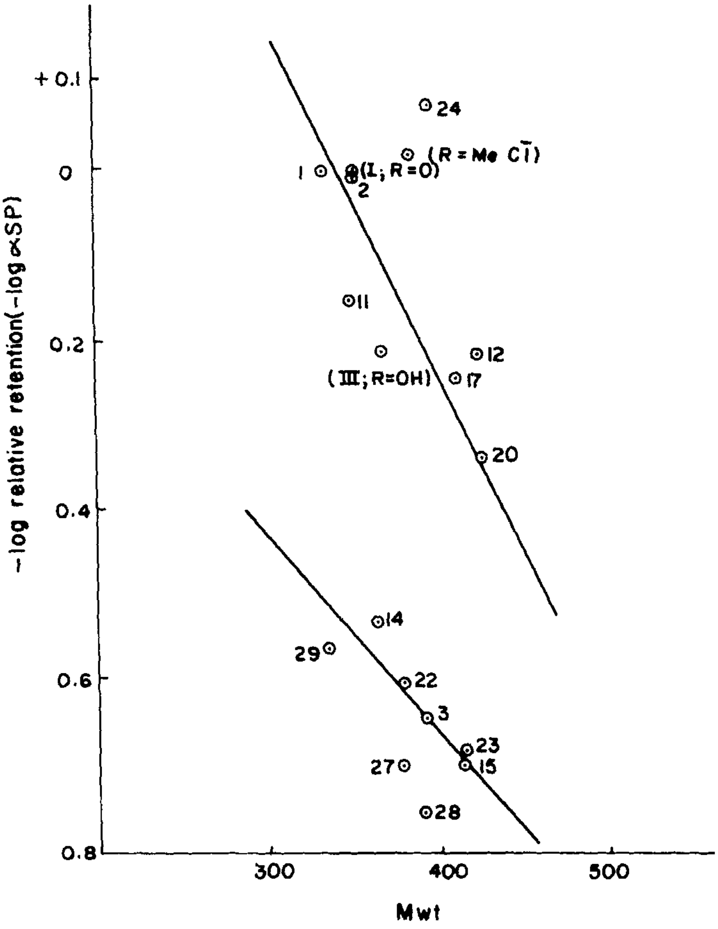


FIGURE 5

−Log Relative retention vs. Molecular weight of Strychnine and Strychnine derivatives.

for the effects of substituents on the retention values of catecholamines (6). This is expected in view of the behavior of monopoles, dipoles and zwitterions (7).

It is apparent that the substituents on C-2, C-3 and on the nitrogen atoms provide most drastic changes on retention (tables 2,3). The replacement of a C-2 and/or C-3 hydrogen atom(s) on the aromatic ring reduces retention even though the molecular properties, e.g. dipolemoments, are markedly changed (*cf* figs. 4,5). The 2-acetamido and 2,3-dimethoxystrychnines are surprisingly anamolous. These positions are, however, notably found to be phytochemically most effective (8). The sample population here is not large enough to establish a statistically valid quantitative structure-retention relation; nevertheless, the quantitative aspect of replacing a hydrogen at C-2, C-3 and C-16 on retention of these alkaloids is clearly illustrated.

To summarise, the lengthening of the hydrocarbon chain of a given substituent is associated with an equivalent increment on the relative retention and capacity factors in this chromatographic system. In contrast, polar functional substituents, cause a change in the net dipolemoment of the substrate molecule and thus rigorously change the $\alpha_{SP}$ and k factors, which in many instances are unequalled by the relative increase in the molecular size.

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