

CHROM. 13,025

## QUANTITATIVE STRUCTURE-RETENTION RELATIONSHIPS IN CATION-EXCHANGE CHROMATOGRAPHY

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(First received March 24th, 1980; revised manuscript received June 5th, 1980)

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### SUMMARY

A study of quantitative structure-retention relationships has been made in the cation-exchange chromatography of primary monoamines, such as aliphatic amines,  $\beta$ -phenylethylamine and amino alcohols. Retention data for twelve amines on three columns in the  $K^+$ ,  $Na^+$  and  $Li^+$  forms were analysed. The relationship between the Stokes radius of the counter ion and retention was elucidated by comparing the selectivity coefficients of amines for the three different counter ions. The structure parameters,  $\tau$ , of methyl, phenyl, hydroxyl and ionized amino groups were obtained as the increments in the selectivity coefficient of the amine,  $\Delta \ln K_M^{A_{\text{amine}}}$ , on replacing a hydrogen atom with these groups.

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### INTRODUCTION

Liquid chromatography has contributed greatly to the separation and analysis of various compounds. However, optimization of the elution conditions for new problems still require much effort and time. Many studies have been made of the relationship between retention and chromatographic conditions.

In a previous paper<sup>1</sup>, the chromatographic behaviour of primary amines in cation-exchange chromatography was investigated. The correlation of the distribution coefficient with the composition of the mobile phase was elucidated, and the influence of the degree of dissociation of the amines on retention was expressed by an experimental equation.

This paper describes the effect of the molecular structure of amines on retention in ion-exchange chromatography. The contribution of hydrophobic interactions to the selectivity coefficient is discussed. Hydrophobic properties of amine molecules with respect to the resin matrix are measured by applying the method of quantitative structure-activity relationships (QSAR). Recently, a study of QSAR, developed by Hansch<sup>2</sup>, has been applied to the evaluation of the contribution of substituents to chromatographic retention<sup>3-6</sup>.

## EXPERIMENTAL

Methylamine, ethylamine, *n*-propylamine, *n*-butylamine, isobutylamine, *n*-amylamine, isoamylamine, *n*-hexylamine, ethanolamine, 2-aminobutanol, 5-aminopentanol, 6-aminohexanol and  $\beta$ -phenylethylamine were purchased from Nakarai Chemicals (Kyoto, Japan) or Tokyo Chemicals (Tokyo, Japan). Other chemicals were obtained from Nakarai Chemicals or Wako (Osaka, Japan).

A Hitachi Model KLA-3B amino acid analyser (Hitachi, Tokyo, Japan) was employed throughout this work. The chromatography was carried out on a  $3 \times 0.6$  cm column of Aminex A-4 sulphonated polystyrene cation-exchange resin (Bio-Rad Labs., Richmond, CA, U.S.A.) with a flow-rate of 30 ml/h at 50°C. Potassium borate, sodium borate and lithium borate (pH 8.0) buffers were used as eluents. A 0.1- $\mu$ mole amount of each amine was applied to the column and detected with the ninhydrin system at 440, 570 and 640 nm.

## RESULTS AND DISCUSSION

The retention of the amines was expressed as the logarithm of the selectivity coefficient, which was proportional to the free energy change associated with the distribution process. In the buffer solution of pH 8.0 most of the amine molecules are dissociated, as the dissociation constants of these amines are well below  $10^{-8}$ . The selectivity coefficient can be expressed by the following equation:

$$\ln K_M^{\text{Amine}} = \ln(D_v[M^+]/[\overline{M^+}]) \quad (1)$$

where  $K_M^{\text{Amine}}$  is the selectivity coefficient of the amine for the counter ion, M, and  $D_v$  denotes volume distribution coefficients.  $[M^+]$  and  $[\overline{M^+}]$  are the concentrations of univalent counter ions in the mobile phase and in the stationary phase, respectively. The  $[M^+]$  value was that of the cations in the buffer salts. The  $[\overline{M^+}]$  value was approximated as the concentration of the fixed ionic groups in the resin, as the amounts of the amine molecules and hydrogen ions should be negligible in the stationary phase. The concentration of the fixed ionic groups depends on the ionic form of the resin and can be calculated by correcting the weight capacity for the density and solvent content of the resin<sup>7</sup>. The concentration of the fixed ionic group in the  $K^+$  form of the resin was found to be 2.73, that in the  $Na^+$  form of the resin 2.69 and that in the  $Li^+$  form of the resin 2.57 mequiv/ml of the resin bed. The  $D_v$  value was obtained from the retention volume,  $V_R$ , according to the usual method;  $D_v = (V_R - V_0)/V_M$ , where  $V_0$  is the hold-up volume and  $V_M$  the column bed volume. The selectivity coefficients of amines were calculated by eqn. 1. Each  $\ln K_M^{\text{Amine}}$  value used here was the mean value of several determinations, using eluents with different buffer salt concentrations.

In Fig. 1 the values of  $\ln K_K^{\text{Amine}}$  and  $\ln K_{Li}^{\text{Amine}}$  are plotted against the value of  $\ln K_{Na}^{\text{Amine}}$ . A linear relationship was found, with a slope of unity. The correlation equations are as follows:

$$\ln K_{Li}^{\text{Amine}} = 0.38 + \ln K_{Na}^{\text{Amine}}$$

$$\ln K_K^{\text{Amine}} = -0.36 + \ln K_{Na}^{\text{Amine}}$$

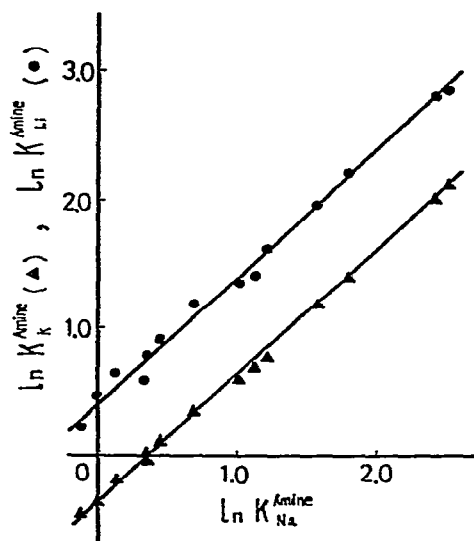


Fig. 1. Correlation between  $\ln K_M^{Amine}$  values of primary monoamine measured with columns with different counter ions.

The intercepts, 0.38 and  $-0.36$ , indicate the values of  $\ln K_{Li}^{Na}$  and  $\ln K_K^{Na}$ , respectively. The values of the selectivity coefficients for the exchange systems Na/Li and Na/K were obtained as  $K_{Li}^{Na} = 1.46$  and  $K_K^{Na} = 0.70$ , which compare reasonably well with values reported in the literature<sup>8</sup>. Additionally, the values of  $K_{Li}^{Na}$  and  $K_K^{Na}$  obtained here could be related to the ratio of the Stokes radii (effective hydrated radii) of the ions:  $r_{Li}/r_{Na} = 1.30$ ,  $r_K/r_{Na} = 0.68$ , which were calculated from Kiso's data<sup>9</sup> and 1.30 and 0.69 from the Landolt-Brönstein's Tabellen<sup>10</sup>. This shows that in ion-exchange chromatography the selectivity coefficient can be expressed as a function of the ratio of the Stokes radii of ions.

In the above discussion only ionic interactions are being considered. In fact, the selectivity coefficient of the dissociated amine depends on two different interactions; one is due to ionic interactions with the fixed ionic group of the resin and the other to hydrophobic interactions with the resin matrix. The overall selectivity coefficient can be expressed as follows:

$$\ln K_M^{Amine} = \ln(K_M^{Amine})_{ionic} + \ln(K_M^{Amine})_{hydrophobic} \quad (2)$$

The retention characteristic of each amine is dominated by the second term in eqn. 2, as the first term for compounds having the same ionized group is constant.

The effects of hydrophobic interactions on the selectivity coefficient are shown in Figs. 2 and 3. In Fig. 2 the logarithm of the selectivity coefficients of aliphatic monoamines and  $\beta$ -phenylethylamine for various counter ions is plotted against the carbon number of the amine,  $n$ . The values for  $n = 0$  were obtained from the logarithm of the selectivity coefficients of the ammonium ion for the various counter ions. The  $\ln K_M^{Amine}$  value increased with increasing carbon number. However, isobutylamine and isoamylamine were eluted faster than  $n$ -butylamine and  $n$ -amylamine, respectively.

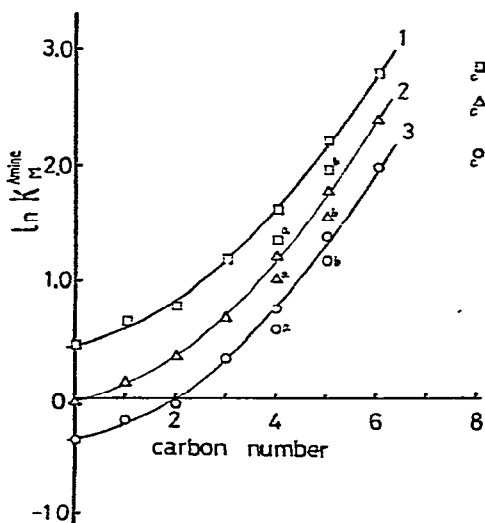


Fig. 2. Correlation between carbon number (chain length) of *n*-alkylamines and  $\ln K_M^{\text{Amine}}$ . Column: 1 =  $\text{K}^+$  form; 2 =  $\text{Na}^+$  form; 3 =  $\text{Li}^+$  form. (a) Isobutylamine; (b) isoamylamine; (c)  $\beta$ -phenylethylamine.

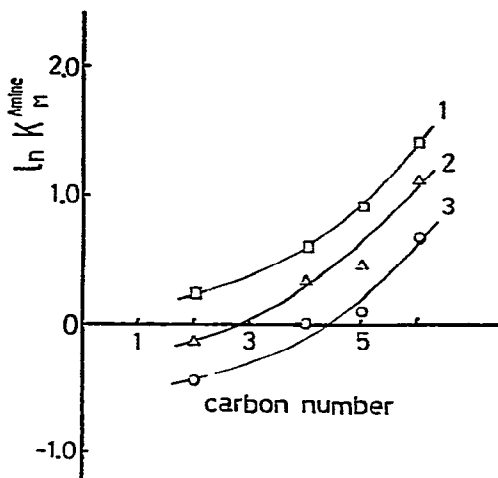


Fig. 3. Correlation between carbon number (chain length) of amines and  $\ln K_M^{\text{Amino alcohol}}$ . Column: 1 =  $\text{K}^+$  form; 2 =  $\text{Na}^+$  form; 3 =  $\text{Li}^+$  form.

The selectivity coefficient of these two amines corresponded to those of *n*-alkylamines with carbon numbers of 3.7 and 4.7, respectively. The selectivity coefficient of  $\beta$  phenylethylamine ( $n = 8$ ) corresponded to that of an *n*-alkylamine with a carbon-number of 6.2.

Fig. 3 shows the plots of the logarithm of selectivity coefficients of amino alcohols against the carbon number of the main alkyl chain. Comparing the results in Fig. 3 with those in Fig. 2, it can be seen that the introduction of a hydroxyl group into the aliphatic amine molecule causes a decrease in adsorption, corresponding to a decrease in the carbon number of about 2. The results suggest that hydrophobic interactions depend on the molecular shape of the amines.

The QSAR technique developed by Hansch was applied to measurements of the hydrophobic properties of the groups constituting the amine. The increments in the  $\ln K_M^{\text{Amine}}$  values on replacing a hydrogen atom by a group such as methyl, hydroxyl or phenyl were calculated from the retention data of amines. In Table I the  $\Delta \ln K_M^{\text{Amine}}$  values are shown as the structure parameter,  $\tau$ . As retention is subjected to a linear free energy relationship,  $\tau$  represents the effect of the substituted group on the free energy change of hydrophobic adsorption. A larger  $\tau$  value implies that the substituted group requires a greater hydrophobic adsorption energy to interact with the resin matrix. In Table I the simple numbers before the substituents in the first column shows the position of the carbon atoms on the alkyl chain; the numbers with primes show the position of the carbon atom on the branched chain. The  $\tau$  values for the same substituent obtained on the three different ionic forms of the columns did not vary considerably, and the  $\tau$  values depended on the position of the substituent. The following sequence of  $\tau$  values for the substituents was found:  $1\text{-CH}_3 < 2\text{-CH}_3 < 3\text{-CH}_3 < 4\text{-CH}_3 < 5\text{-CH}_3 \approx 6\text{-CH}_3$ .

TABLE I  
 $\tau$  VALUES

Substituent	$\Delta \ln K_M^{\text{Amine}}$	$\tau$		
		$M = \text{Li}^+$	$M = \text{Na}^+$	$M = \text{K}^+$
1-CH <sub>3</sub>	$\ln K_M^{\text{Methylamine}} - \ln K_M^{\text{Ammonia}}$	0.19	0.17	0.17
2-CH <sub>3</sub>	$\ln K_M^{\text{Ethylamine}} - \ln K_M^{\text{Methylamine}}$	0.14	0.22	0.13
2'-CH <sub>3</sub>	$\ln K_M^{\text{Isobutylamine}} - \ln K_M^{\text{n-Propylamine}}$	0.17	0.33	0.24
3-CH <sub>3</sub>	$\ln K_M^{\text{n-Propylamine}} - \ln K_M^{\text{Ethylamine}}$	0.40	0.33	0.40
3'-CH <sub>3</sub>	$\ln K_M^{\text{Isopropylamine}} - \ln K_M^{\text{n-Butylamine}}$	0.34	0.36	0.42
4-CH <sub>3</sub>	$\ln K_M^{\text{n-Butylamine}} - \ln K_M^{\text{n-Propylamine}}$	0.44	0.53	0.42
5-CH <sub>3</sub>	$\ln K_M^{\text{n-Amylamine}} - \ln K_M^{\text{n-Butylamine}}$	0.59	0.58	0.64
6-CH <sub>3</sub>	$\ln K_M^{\text{n-Hexylamine}} - \ln K_M^{\text{n-Amylamine}}$	0.59	0.62	0.60
2-C <sub>6</sub> H <sub>5</sub>	$\ln K_M^{\text{n-Phenylethylamine}} - \ln K_M^{\text{Ethylamine}}$	2.07	2.16	2.17
2-OH	$\ln K_M^{\text{Ethanolamine}} - \ln K_M^{\text{Ethylamine}}$	-0.56	-0.48	-0.39
5-OH	$\ln K_M^{\text{n-Aminopentanol}} - \ln K_M^{\text{n-Amylamine}}$	-1.31	-1.35	-1.30
6-OH	$\ln K_M^{\text{n-Aminohexanol}} - \ln K_M^{\text{n-Hexylamine}}$	-1.39	-1.29	-1.33

Negative  $\tau$  values for a substituted hydroxyl group indicate that the alcoholic hydroxyl group weakens the hydrophobic interaction to the resin matrix. The sequence of  $\tau$  values for substituted hydroxyl groups was as follows: 2-OH > 5-OH  $\approx$  6-OH.

In this method the structure parameter of the ionized amino group was not available. However, an approximate value for the ionized amino group could be estimated as follows. As discussed above, the contribution of ionic interactions to the selectivity coefficient can be expressed as the ratio of the hydrated radii of the ionized groups. For the exchange between the amine and counter ion,  $\text{Na}^+$ , the relationship was as follows:

$$\ln (K_{\text{Na}}^{\text{Amine}})_{\text{ionic}} = \ln r_{\text{Na}}/r_{\text{A}}$$

where,  $r_{\text{Na}}$  is the hydrated radius of the sodium ion and  $r_{\text{A}}$  that of the ionized amino group. The hydrated radius of the ammonium ion was used as an approximate value of  $r_{\text{A}}$ . The  $\ln r_{\text{Na}}/r_{\text{A}}$  was 0.38 from the values of the hydrated radii in the literature<sup>9,10</sup>. On the other hand, the overall selectivity coefficient,  $\ln K_{\text{Na}}^{\text{Amine}(n=0)}$ , was -0.04, as shown in Fig. 2. From eqn. 2, the  $\ln [K_{\text{Na}}^{\text{Amine}(n=0)}]_{\text{hydrophobic}}$  value of -0.42 was obtained as the remainder on subtracting the  $\ln r_{\text{Na}}/r_{\text{A}}$  value from the  $\ln K_{\text{Na}}^{\text{Amine}(n=0)}$  value. It was estimated that the value of -0.42 was the approximate structure parameter of the ionized amino group.

In ion-exchange chromatography, ionized compounds are drawn towards the ion-exchange resin through ionic interactions between the ionized group in the molecule and the fixed ion of the resin, and this makes the hydrophobic groups in the molecule adsorb on the resin matrix. The logarithm of the selectivity coefficient could be expressed as the sum of the logarithms of the contributions of both ionic and hydrophobic interactions. The contribution of ionic interactions to the selectivity coefficient was related to the ratio of the hydrated radii of ionized amino groups and counter ions in this work. If ionized amino groups were present in the molecule the contribution of hydrophobic interactions was represented as the structure parameter,

$\tau$ , which was calculated for each substituent. The  $\tau$  values showed the hydrophobic property of the methyl substituent and hydrophilic properties of hydroxyl and ionized amino substituents with respect to the resin matrix. Methyl substitution in different positions on the *n*-alkyl chain caused differences in the  $\tau$  values. The  $\tau$  value for the hydroxyl group was also affected by its position in the *n*-alkyl amino alcohols. The sequences of  $\tau$ -value observed for methyl and hydroxyl substituents suggest an effect of the ionized amino group on the interaction between the substituents and the resin matrix.

#### ACKNOWLEDGEMENTS

The author expresses her thanks to Prof. H. Hatano and Dr. S. Rokushika for helpful discussions and suggestions.

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