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High-performance liquid chromatographic study of some biologically active analogues of arabinosylcytosine

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ABSTRACT

The effects of methanol concentration, pH and flow-rate of the mobile phase and column temperature on the retention of two cytidine deaminase-resistant analogues (cyclocytidine hydrochloride and 5′-chloro-5′-deoxyarabinosylcytosine) of the antineoplastic agent arabinosylcytosine (araC) and their metabolites in a reversed-phase system were examined. Cyclocytidine hydrochloride (pK_a 6.60) changed its retention significantly in the pH range 6–7 and when the methanol concentration increased from 3 to 5% comparing to other compounds. The greatest changes in the capacity factors were observed for 5′-chloro-araC (pK_a = 3.84) in mobile phases with low methanol concentrations (up to 5%, v/v). The effect of temperature was most significant in the case of 5′-chloro-araC. Its retention decreased the most when the temperature was changed from ambient to 30°C. The relationship between capacity factors and hydrophobicity (logarithm of partition coefficients) of the studied compounds was also studied.

INTRODUCTION

Arabinosylcytosine (araC) is a cell cycle (S-phase) specific antitumour agent widely used in the treatment of leukaemias and lymphomas [1]. Its clinical utility is severely limited by its rapid deamination to a biologically inactive compound, arabinosyluracil (araU) [2].

Drug research has been focused on araC analogues resistant to deamination in order to improve the stability of araC. For example, O^2,2′-cyclocytidine hydrochloride, an anhydride derivative of araC, has been found to be biologically active against a variety of tumours [1,3] and it is not deaminated by cytidine deaminase. It is more effective and less toxic than araC. It is hydrolysed to araC and therefore it can serve as an araC pro-drug.

Studies of structure–activity relationships for nucleoside analogues [4] indicate that 5′-chloro-5′-deoxyderivatives of nucleosides, especially of araC, are a potentially interesting new group of biologically active compounds. The results show that 5′-haloderivatives of araC are superior in their transport rate and metabolic stability to the clinically used compound araC [5,6]. The results have stimulated further investigation of the mechanism of action and biological activity of the 5′-chloro derivative of araC. As high-performance liquid chromatography (HPLC) has often been used for the determination of araC nucleosides and nucleotides in physiological fluids, we report in this paper an HPLC study of the effects of mobile phase methanol concentration, pH, column temperature and flow-rate on the retention of 5′-chloro-araC, cyclocytidine, araC and their possible metabolites and related compounds 2′,5′-anhydro-araC, 2′,5′-anhydro-araU, araU, cyclouridine and the natural nucleosides cytidine and uridine in reversed-phase systems.

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We have also investigated the relationship between lipophilicity expressed as the logarithm of partition coefficients (log P) and capacity factors of the compounds in reversed-phase LC; although no correlation was found between lipophilicity and transport rate but there was a good correlation between lipophilicity and metabolic transformation [5].

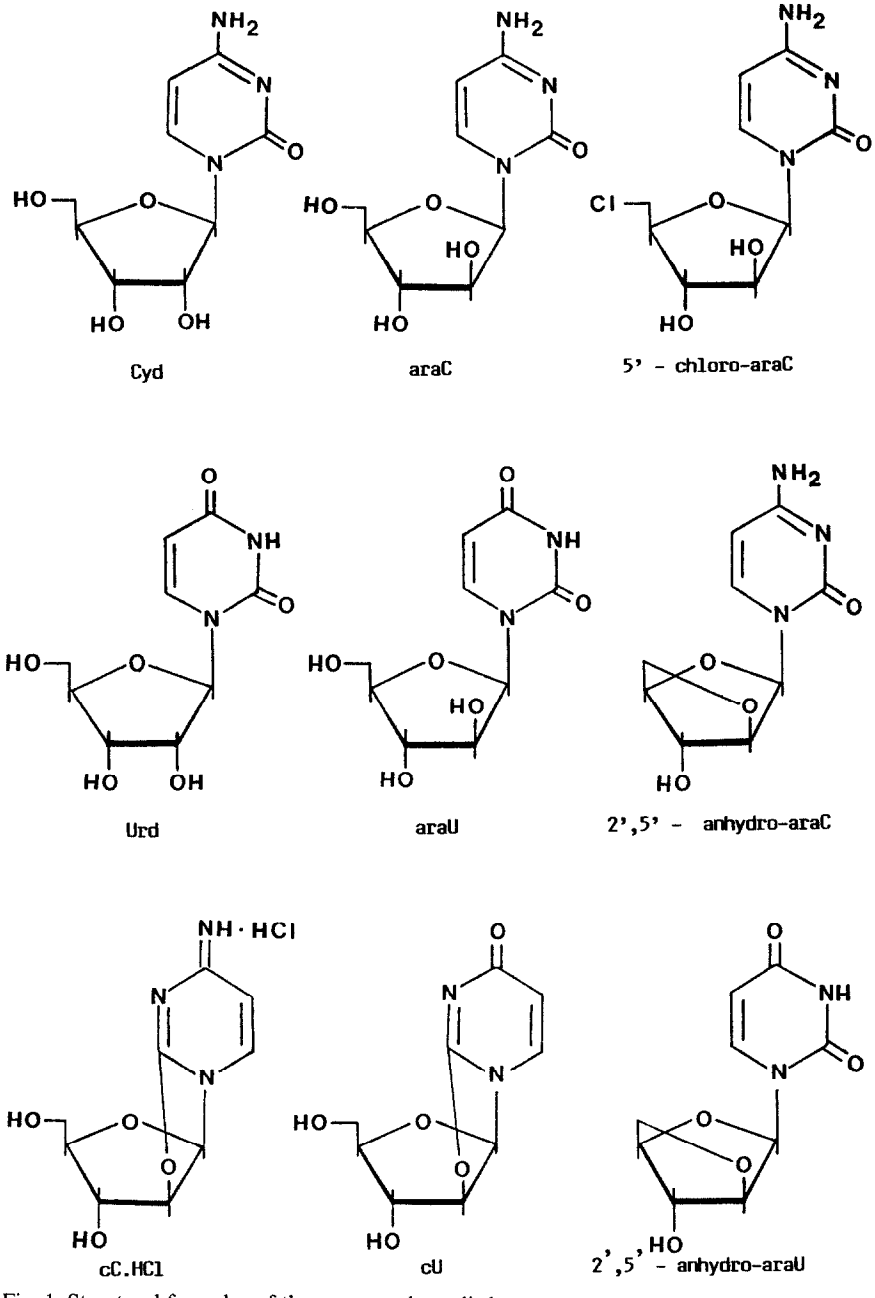

Fig. 1. Structural formulae of the compounds studied.

EXPERIMENTAL

Materials

The studied compounds (Fig. 1) were synthesized according to the references indicated in Table I.

Cytidine and uridine were obtained from Pharma-Waldhof (Mannheim, Germany), potassium dihydrogenphosphate from Merck (Darmstadt, Germany) and methanol and potassium hydroxide of the highest purity available from Lachema (Brno, Czechoslovakia). Doubly-distilled, deionized water was purified using an Elgastat Spectrum SC 20 system (Elga, High Wycombe, UK).

Single-compound stock solutions of nucleosides were prepared to give concentrations of about 0.4 m*M* (Urd, cC · HCl, 5′-Cl-araC, 2′,5′-An-araC and 2′,5′-An-araU), 0.27 m*M* (Cyd and araC) or 0.5 m*M* (araU and cU). Working standard solutions were prepared by diluting the stock solutions tenfold with doubly distilled, deionized water. The working standard solutions were diluted to a final volume of 50 ml containing 7 ml of cU and 5 ml of other nucleosides. All stock and working standard solutions were stored at −4°C.

Chromatography

The HPLC equipment consisted of a Pye Unicam PU 4003 gradient elution high-performance liquid chromatograph equipped with a Rheodyne Model 7125 injector (20-μl sample loop), PU 4030 controller, PU 4031 column oven and PU 4020 variable-wavelength UV detector (Philips Scientific, Cambridge, UK). Peak recording was achieved using a dual-channel SP 4200 computing integrator (Spectra-Physics, Darmstadt, Germany).

Separon SGX C_{18} (150 × 3 mm I.D.; 7 μm) analytical columns were obtained from Tessek (Prague, Czechoslovakia).

The isocratic mobile phases consisted of 0.01 *M* potassium dihydrogenphosphate solutions containing various concentrations of methanol by volume. The pH was adjusted with a few drops of either potassium hydroxide or orthophosphoric acid. The eluents were filtered through 0.45-μm nylon 66 membranes (Supelco, Bellefonte, PA, USA) and vacuum degassed prior to the analysis. The flow-rate was 0.45 ml/min. UV detection was performed at 275 nm (1.28 a.u.f.s.).

RESULTS AND DISCUSSION

Effect of pH and methanol concentration

It is known that the solute retention in a reversed-phase system can be decreased by the addition of an organic modifier (such as methanol) to an aqueous mobile phase [14,15]. We studied the effect of various methanol concentrations in 0.01 *M* KH_2PO_4 mobile phase at pH 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0. Similarly to the results for purine nucleosides, inosine and guanosine compounds [15], we also observed that the retention times of all the compounds studied decreased with increasing percentage of methanol in the mobile phase.

Except for cC · HCl and 5′-chloro-araC, the ca-

TABLE I

COMPOUNDS STUDIED AND THEIR ABBREVIATIONS

Compounds are listed according to increasing log *P* values

Compound	Abbreviation	Ref.	pK_a [ref.]
Cyclocytidine	cC · HCl	7	6.60[11]
Cyclouridine	cU	8	
Cytidine	Cyd	–	4.22[12]
Arabinosylcytosine	araC	9	4.15[11]
Uridine	Urd	–	9.17[12]
Arabinosyluracil	araU	9	9.20[13]
2′,5′-Anhydroarabinosylcytosine	2′,5′-An-araC	10	
2′,5′-Anhydroarabinosyluracil	2′,5′-An-araU	10	
5′-Chloroarabinosylcytosine	5′-Cl-araC	10	3.84[a]

[a] Unpublished result.

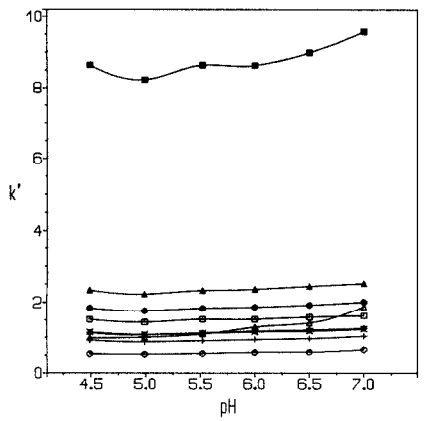

Fig. 2. Effect of pH of the mobile phase on capacity factors (k') in the isocratic reversed-phase LC separation of (○) cU, (△) cC · HCl, (+) Cyd, (×) araC, (◇) Urd, (□) araU, (●) 2′,5′-An-araC, (▲) 2′,5′-An-araU and (■) 5′-Cl-araC. Column, Separon SGX C_{18} (150 × 3 mm I.D.; 7 μm); mobile phase, 0.01 *M* KH_2PO_4 containing 8% of methanol; flow-rate, 0.45 ml/min; detection, 275 nm, 1.28 a.u.f.s.; temperature, 23°C.

pacity factors (k') of the compounds did not change significantly when the pH of the mobile phase changed from 4.5 to 7.0 (Fig. 2). At all methanol concentrations (0–10%), the k' of cC · HCl increased with increasing pH of the mobile phase (Fig. 3). The k' of 5′-chloro-araC increased noticeably with increasing pH of the mobile phase only at less than 3% of methanol (Fig. 4). cC · HCl was the only solute studied that showed a reversed elution

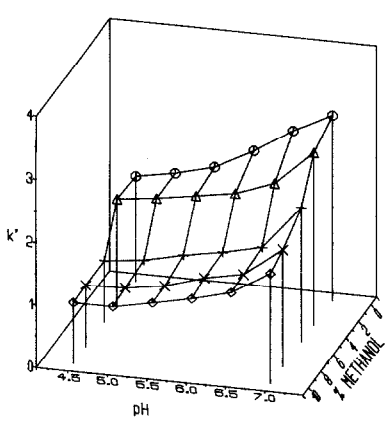

Fig. 3. Effect of pH of the mobile phase on capacity factor (k') of cC · HCl in a mobile phase containing (◇) 10%, (×) 8%, (+) 5%, (△) 3%, and (○) no methanol. Other chromatographic conditions as in Fig. 2.

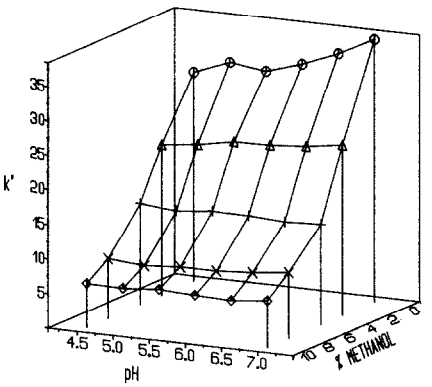

Fig. 4. Effect of pH of the mobile phase on capacity factor (k') of 5′-chloro-araC in a mobile phase containing (◇) 10% (×) 8%, (+) 5%, (△) 3% and (○) no methanol. Other conditions as in Fig. 2.

order in mobile phases containing more than 5% of methanol at pH 6.0–7.0 (data not shown). We assume that cC · HCl (pK_a 6.60) can undergo ionization at pH 6.0–7.0 and its ionized form can participate in non-hydrophobic interactions that influence the retention of cC · HCl in the reversed-phase LC system. 5′-Chloro-araC, the least polar analogue of araC, is the most retained of studied nucle-

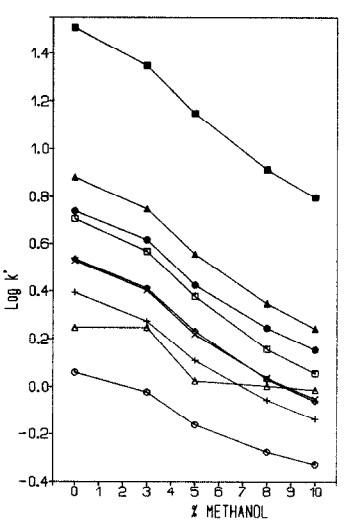

Fig. 5. Effect of the concentration of methanol in the mobile phase (pH 5.5) on logarithm of capacity factor (log k') in reversed-phase systems for (○) cU, (△) cC · HCl, (+) Cyd, (×) araC, (◇) Urd, (□) araU, (●) 2′,5′-An-araC, (▲) 2′,5′-An-araU and (■) 5′-chloro-araC. Other conditions as in Fig. 2.

osides on the hydrophobic ligand. This can provide a possible explanation for the variations of the retention times of 5′-chloro-araC.

A remarkable change in the retention of cC · HCl was observed when the methanol concentration in the mobile phase was changed from 3 to 5% (v/v), as can be seen from the log k' *vs.* methanol concentration plot in Fig. 5. Unlike purine nucleosides, for which the plot of log k' vs. methanol concentration was linear at pH 5.5 and non-linear at pH 6.5 [15], the plots for the present compounds were non-linear at all pH values studied.

According to theory, if the solvophobic mechanism is operative, the plots of log k' *vs.* methanol concentration at different pH values should be linear [15]. However, it was observed that log k' *vs.* methanol concentration plot was non-linear when k' was measured over a wide range of organic modifier (methanol) concentrations. Log–log plots of k' *vs.* 1/(concentration of organic modifier in aqueous mobile phase) have been attempted to describe the non-linearity of the log k' *vs.* methanol plot concentration [16].

The requirement of four premises was reported for the linearity of the log k' *vs.* 1/[methanol] plot based on the displacement model [17]. First, an intermediate concentration of organic modifier (methanol) of about 30–80% [18] or 45–80% [19] was reported for the hydrophobic (solvophobic) retention mechanism on an ODS ligand. However, for nucleosides the amount of methanol in the mobile phase is usually less than 10% [20]. This also interferes with the second premise that the solvation and the conformation of the ligand should be unchanged. At lower methanol concentrations the amount of methanol adsorbed on the ligand will be decreased and the ligand conformation will be changed as, for example, alkyl ligands can contact each other. As for the third premise, that non-hydrophobic interactions can be negligible, at relatively low methanol concentrations a hydrophilic solute may suffer from non-hydrophobic interactions with the stationary phase, such as silanophilic interactions with residual silanols [21] and from the interaction with the organic modifier adsorbed on the ligand [22]. The solute–solvent interaction, called the mobile phase effect, involved in the fourth premise should be negligible. However, hydrogen bonding between the polar functional groups of a

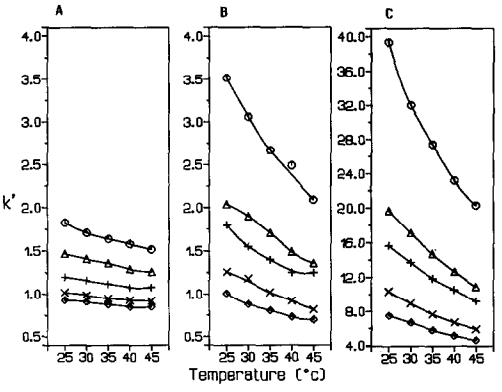

Fig. 6. Effect of temperature on capacity factors (k') of (A) cC · HCl, (B) araC and (C) 5′-chloro-araC in mobile phase of pH 5.5 containing (◇) 10%, (×) 8%, (+) 5%, (△) 3% and (○) no methanol. Other conditions as in Fig. 2.

solute and the mobile phase solvent (especially water) leads to a negative contribution to the hydrophobic interaction with the stationary ligand [19].

As the plots of log k' *vs.* methanol concentration for cC · HCl for all the mobile phases used differed substantially from the non-linear plots for the other compounds (Fig. 5), we assume that the retention mechanism of cC · HCl is the most affected by processes other than solvophobic.

Effect of temperature

We also investigated the effect of temperature from 25 to 45°C on the retention of araC and its polar derivative cC · HCl and the less polar derivative 5′-chloro-araC (Fig. 6). Although the values decreased with increasing temperature, the differences among capacity factors (at different temperatures) were found to be negligible for cC · HCl. The greatest changes in the k' values were observed for 5′-chloro-araC in mobile phases with low methanol concentrations (up to 5%) and especially when the temperature was changed from ambient to 30°C. Relative retention (α) values for cC · HCl and araC of nearly 1.00 (when the peaks merged) were observed at higher methanol concentrations (from 8 to 10%) and at higher temperatures (Table II).

From a temperature study for purine compounds [15], the conclusion was made that if faster separations are necessary, it is preferable to increase the flow-rate rather than temperature, because temperature affects not only the mass transfer terms, both

TABLE II

CAPACITY FACTORS (k') AND RELATIVE RETENTIONS (α) OF CYCLOCYTIDINE (cC · HCl) AND ARABINOSYLCYTOSINE (araC)

Sample, 20 μl of a standard solution; column, Separon SGX C_{18} (150 × 3 mm I.D.; 7 μm); mobile phase, 0.01 M KH_2PO_4; pH, 5.5; flow-rate, 0.45 ml/min; detection, 275 nm, 1.28 a.u.f.s.

Methanol concentration (%)	25°C			30°C			35°C			40°C			45°C		
	k'		α	k'		α	k'		α	k'		α	k'		α
	cC·HCl	araC		cC·HCl	araC		cC·HCl	araC		cC·HCl	araC		cC·HCl	araC	
0	1.83	3.52	1.92	1.71	3.06	1.79	1.64	2.67	1.63	1.58	2.38	1.51	1.52	2.09	1.38
3	1.47	2.04	1.39	1.41	1.90	1.35	1.36	1.71	1.26	1.29	1.49	1.16	1.26	1.36	1.08
5	1.20	1.80	1.50	1.16	1.55	1.34	1.12	1.40	1.25	1.08	1.25	1.15	1.08	1.25	1.16
8	1.02	1.26	1.24	0.99	1.18	1.19	0.95	1.02	1.07	0.93	0.92	0.99	0.92	0.82	0.89
10	0.93	1.01	1.09	0.91	0.88	0.97	0.88	0.81	0.92	0.85	0.73	0.86	0.85	0.70	0.82

in the mobile and stationary phases, but also solute–solvent and solute–stationary phase interactions.

In our experiments we confirmed the widely accepted fact that capacity factors are independent of flow-rate of the mobile phase (Fig. 7). Therefore, we assume that an increase in temperature could have a more significant effect than an increase in flow-rate on accelerating the separation of the present compounds.

The optimized separation of all the nucleosides studied is shown in Fig. 8. The chromatographic

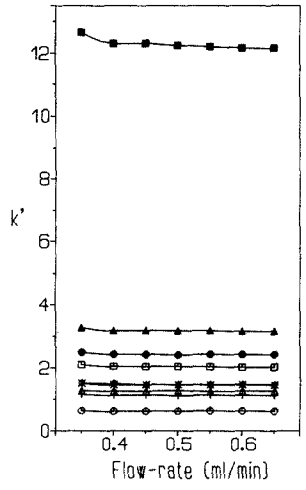

Fig. 7. Effect of flow-rate on capacity factors (k') of (○) cU, (△) cC · HCl, (+) Cyd, (×) araC, (◇) Urd, (□) araU, (●) 2′,5′-An-araC, (▲) 2′,5′-An-araU and (■) 5′-chloro-araC. Mobile phase, 0.01 M KH_2PO_4 containing 8% of methanol (pH 5.5); other conditions as in Fig. 2.

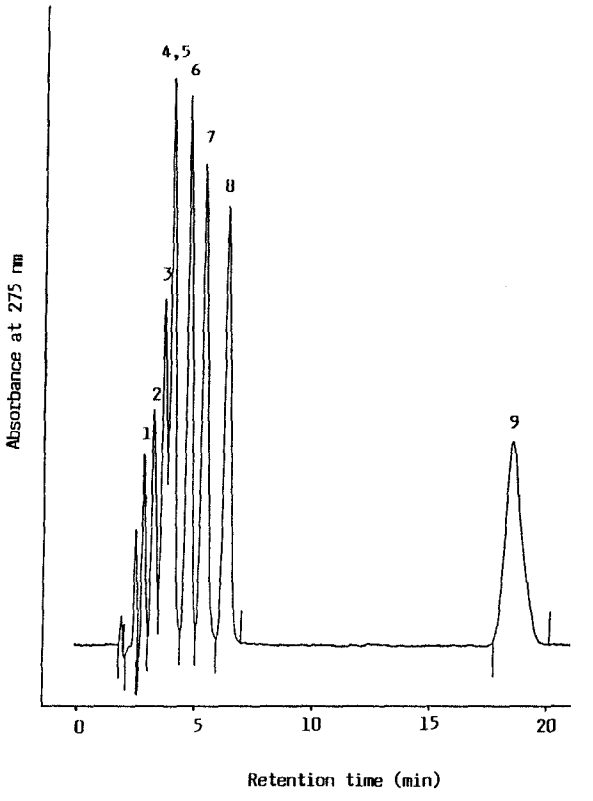

Fig. 8. Reversed-phase isocratic HPLC separation of the nucleosides (1) cU, (2) Cyd, (3) cC · HCl, (4) araC, (5) Urd, (6) araU, (7) 2′,5′-An-araC (8) 2′,5′-An-araU and (9) 5′-chloro-araC. Sample, 20 μl of a standard solution; column, Separon SGX C_{18} (150 × 3 mm I.D.; 7 μm); mobile phase, 0.01 M KH_2PO_4 containing 8% of methanol; pH, 5.5; flow-rate, 0.45 ml/min; detection, 275 nm, 1.28 a.u.f.s.; temperature, 23°C.

conditions were as follows: eluent, 0.01 *M* potassium dihydrogenphosphate solution containing 8% of methanol; pH, 4.5–5.5; column, Separon SGX C_{18} (150 × 3 mm I.D.; 7 μm); flow-rate, 0.45 ml/min; and detection at 275 nm (1.28 a.u.f.s.).

Correlation between hydrophobicity and retention in reversed-phase LC

The ability of a chemotherapeutic agent to be effective depends on the drug hydrophobicity. Hydrophobicity, one of the most important physicochemical properties that affect a drug's biological activity, is commonly expressed as the logarithm of the partition coefficients (log *P*) of the compound between *n*-octanol and an aqueous phase.

Cheung and Kenney [20] reported a linear correlation between log *P* and log *k'* values of diverse nucleoside analogues. They used monocratic LC to obtain log *k'* values, although the major weakness of the method is that the Collander relationship between log *k'* and log *P* [23]:

$$\log k' = a \log P + b$$

has been shown to be valid for congeners only [24,25]. From their data, it is evident that the hydrophobicities of nucleoside analogues can be calculated from their log *k'* values. However, the LC system should closely resemble the octanol–aqueous partition system and the analytes should be kept in the neutral form.

TABLE III

PARTITION COEFFICIENTS AND CAPACITY FACTORS OF SOME NUCLEOSIDE ANALOGUES

Log *P* values were determined by the traditional shake-flask method and *k'* values by HPLC. Sample, 20 μl of a standard solution; column, Separon SGX C_{18} (150 × 3 mm I.D.; 7 μm); mobile phase, 0.01 *M* KH_2PO_4 containing 3% of methanol; pH, 7.0; flow-rate, 0.45 ml/min; detection, 275 nm, 1.28 a.u.f.s.

Compound	Log *P*	*k'*	Log *k'*
5'-Cl-araC	−0.71	24.56	1.390
2',5'-An-araC	−1.64	4.45	0.648
araU	−1.71	3.82	0.582
Urd	−1.98	2.65	0.423
araC	−2.05	2.63	0.420
cU	−2.85	1.05	0.021
cC · HCl[a]	−4.03	2.74	0.438

[a] Data for cC · HCl were not included in regression analysis.

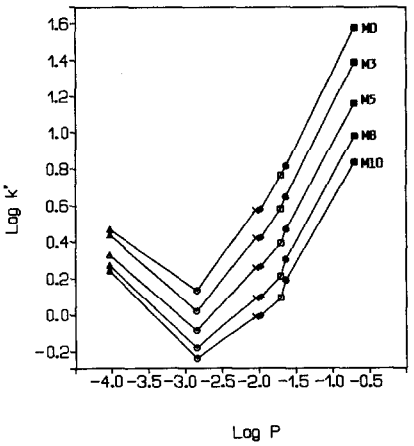

Fig. 9. Relationship between log *k'* and log *P* for (△) cC · HCl, (○) cU, (×) araC, (◇) Urd, (□) araU, (●) 2',5'-An-araC and (■) 5'-chloro-araC in mobile phases of 0.01 *M* KH_2PO_4 (pH 7.0) containing (M10) 10%, (M8) 8%, (M5) 5%, (M3) 3% and (MO) no methanol. Other conditions as in Fig. 2.

We tried to find a correlation between log *P* determined by the traditional shake-flask method in octanol–water [26] and log *k'* obtained from reversed-phase LC at different mobile phase compositions for seven nucleosides. Plots of log *k' vs.* log *P* in mobile phases of pH 7.0 with different methanol concentrations are shown in Fig. 9. For simplicity, we tried to analyse the relationship between log *k'* and log *P* (Table III) excluding cC · HCl. Although a correction for the degree of ionization in octanol–water was made and cC · HCl should have been eluted as the first of the studied compounds according to its log *P*, its retention in a reversed-phase system differed considerably from that expected. We do not suppose that it is the effect of the already mentioned possible ionization of cC · HCl in a mobile phase of pH 7.0, because the plots of log *k' vs.* log *P* for cC · HCl in mobile phases of pH 4.5–7.0 (data not shown) were very similar.

We assume that this can possibly be explained by the fact that naturally the log *P* value is affected by the non-hydrophobic nature of a solute, especially by solute–solvent complex formation, owing to hydrogen bonding [17]. This effect is obviously different from the effect on the hydrophobic retention of a solute in an aqueous–methanolic mobile phase. The difference in relative solvation effects through the hydrogen bonding between the stationary

TABLE IV

LINEAR REGRESSION ANALYSIS OF LOG k' *VS.* LOG P

Log k' = Slog P + I; S = slope, I = intercept, S.D. = standard deviation, r = correlation coefficient, n = number of compounds studied

Compounds[a]	$S \pm$ S.D.	$I \pm$ S.D.	r	n
Group I	0.737 ± 0.045	1.900 ± 0.070	0.997	3
Group II	0.485 ± 0.028	1.399 ± 0.062	0.999	3
Group I + II	0.641 ± 0.071	1.750 ± 0.138	0.985	6

[a] See text.

phase–mobile phase and octanol–water systems is expected to be increased with decrease in the methanol concentration in the eluent [27].

Linear plots and linear regression analyses between log P and log k' values in a mobile phase containing 3% of methanol (pH 7.0) were performed for group I (araC derivatives including 5'-chloro-araC, its metabolite 2',5'-anhydro-araC and araC) and group II (uracil nucleosides, araU, cU and Urd). The results are summarized in Table IV and the linear plots are presented in Fig. 10. The linear correlation coefficient for group I, except for cC · HCl, was r = 0.997. An even better correlation coefficient r = 0.999 was obtained for group II.

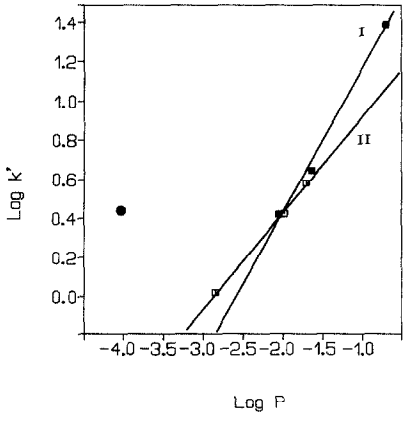

Fig. 10. Linear plot of log k' *vs.* log P for the araC analogues. Group I (■) fits the equation log k' = 0.737log P + 1.900 and group II(□) log k' = 0.485log P + 1.399. cC · HCl (●) was not included in the regression analysis. Mobile phase, 0.01 M KH_2PO_4 containing 3% of methanol (pH 7.0); other conditions as in Fig. 2.

When all six nucleosides were considered as a whole, the correlation coefficient between log P and log k' was only r = 0.985.

In conclusion, this work has shown that the polar derivative cC · HCl of araC, changed its behaviour in a reversed-phase system depending on the pH and the concentration of methanol in the mobile phase. On the other hand, the retention of the less polar araC derivative 5'-chloro-araC was the most influenced by the concentration of methanol in the mobile phase and the temperature.

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REFERENCES

1 B. A. Chabner, *Pharmacologic Principles of Cancer Treatment*, Saunders, Philadelphia, 1982, p. 387.
2 R. K. Y. Zee-Cheng and C. C. Cheng, *Methods Findings Exp. Clin. Pharmacol.*, 11 (1989) 439.
3 D. H. W. Ho, V. Rodriques, T. L. Loo, G. P. Bodey and E. J. Freireich, *Clin. Pharmcol. Ther.*, 17 (1975) 66.
4 J. Beránek, *Drugs Exp. Clin. Res.*, 12 (1986) 355.
5 L. Novotný, H. Farghali, M. Ryba, I. Janků and J. Beránek, *Chemother. Pharmacol.*, 13 (1984) 195.
6 G. I. Birnbaum, M. Buděšinský and J. Beránek, *Can. J. Chem.*, 66 (1988) 1203.
7 J. Beránek, J. Brokeš and H. Hřebabecký, *Czech. Pat.*, 1501 (1977).
8 J. P. H. Verheyden, D. Wagner and J. G. Moffatt, *J. Org. Chem.*, 36 (1971) 250.
9 J. Brokeš and J. Beránek, *Collect. Czech. Chem. Commun.*, 39 (1974) 3100.
10 H. Hřebabecký, J. Brokeš and J. Beránek, *Collect. Czech. Chem. Commun.*, 45 (1980) 599.
11 L. Novotný, V. Reichelová, E. Balážová and V. Ujházy, *Neoplasma*, 37 (1990) 1.
12 *Properties of the Nucleic Acid Derivatives*, California Corporation for Biochemical Research, Los Angeles, 4th revision 1961.
13 M. Tuncel, R. E. Notari and L. Malpeis, *J. Liq. Chromatogr.*, 4 (1981) 887.
14 A. M. Krstulovic and P. R. Brown, *Reversed-Phase High Performance Liquid Chromatography, the Theory, Practice and Biomedical Applications*, Wiley, New York, 1982, p. 9.
15 Ch. Yi, J. L. Fasching and P. R. Brown, *J. Chromatogr.*, 339 (1985) 75.
16 F. Muramaki, *J. Chromatogr.*, 178 (1979) 393.
17 A. Kaibara, C. Hohda, N. Hirahata, M. Hirose and T. Nakagawa, *Chromatographia*, 29 (1990) 275.
18 E. H. Slaats, W. Markovski, J. Fekete and H. Poppe, *J. Chromatogr.*, 207 (1981) 299.

19 A. Kaibara, M. Hirose and T. Nakagawa, *Chromatographia*, 29 (1990) 551.
20 A. P. Cheung and D. Kenney, *J. Chromatogr.*, 506 (1990) 119.
21 K. E. Bij, Cs. Horvath, W. R. Melander and A. Nahum, *J. Chromatogr.*, 203 (1981) 65.
22 W. E. Hammers, G. J. Meurs and C. L. de Ligny, *J. Chromatogr.*, 246 (1982) 169.
23 R. Collander, *Acta Chem. Scand.*, 5 (1951) 774.
24 K. Miyake, F. Kitawa, N. Mizuno and H. Terada, *Chem. Pharm. Bull.*, 35 (1987) 377.
25 V. D. Biasi and W. J. Lough, *J. Chromatogr.*, 353 (1986) 279.
26 H. Farghali, L. Novotný, M. Ryba, J. Beránek and I. Janků *Biochem. Pharmacol.*, 33 (1984) 655.
27 C. Yamagami, T. Ogura and N. Takao, *J. Chromatogr.*, 514 (1990) 123.