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# Relationship between retention parameters in reversed-phase high-performance liquid chromatography and antitumour activity of some pyrimidine bases and nucleosides

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## ABSTRACT

The relationship between retention parameters on octadecyl stationary phases of some pyrimidine bases and nucleosides and their antitumour activity was studied. The differences between the free energy of sorption and hydrophobic contact area of the natural compounds and their synthetic analogues can be used as sensitive parameters correlated with the potential activity of the compounds.

## INTRODUCTION

The relationship between the hydrophobic properties of a substance and its biological activity has long been established. The correlations between the activity and the distribution coefficients of a substance in an octanol–water system are widely used [1–4]. An octanol–water system can be regarded as a model of a biomembrane–water system. However, this model is only a coarse approximation. The isotropic liquid octanol differs appreciable from an anisotropic biomembrane. The stationary phase in reversed-phase high-performance liquid chromatography (RP-HPLC) also has a strongly anisotropic character and there is a great similarity between the mobile phase–stationary phase surface and the membrane–water surface [2]. Hence a chromatographic system consisting of water and a hydrocarbon layer bonded to a hydrophilic matrix may serve as a more suitable model of a membrane than an octanol–water system. Moreover, the parameters of interaction with the surface of an RP sorbent can produce additional information on the physico-chemical properties of substances that are difficult to study in an octanol–water system. These include many pyrimidine bases and nucleosides, which are highly polar compounds containing ionogenic groups of acidic and basic character. The aim of this work was to compare the parameters of the interactions of some compounds with an octadecyl sorbent surface and their antitumour activity.

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## EXPERIMENTAL

### *Chromatographic conditions*

The experiments were performed on an LKB (Bromma, Sweden) liquid chromatographic system consisting of a Model 2151 variable-wavelength monitor, two Model 2150 HPLC pumps, a Model 2152 LC controller, a Model 2154 injector and a Model 2220 recording integrator. The columns used were a Separon SIX $C_{18}$ (5 $\mu$m) glass column (150 × 3.3 mm I.D.) (Laboratorní Přistroje, Prague, Czechoslovakia), a Silasorb $C_{18}$ (10 $\mu$m) column (100 × 1.0 mm I.D.) (LaChema, Brno, Czechoslovakia) (these sorbents with a silica gel matrix) and an octadecyl polyol Si 100 (5 $\mu$m) stainless-steel column (250 × 4.6 mm I.D.) (Serva, Heidelberg, Germany) with a propylglycerol matrix. The mobile phases were 0–60% water–methanol mixtures for compounds **II–IX** (Fig. 1) or 0.1 *M* phosphate buffer–0.1–1.0 *M* ammonium sulphate for the other compounds at a flow-rate 0.01 ml/min (column of 1 mm I.D.) or 0.5 ml/min (other columns).

### *Materials*

Compound names are abbreviated as in Fig. 1. 6-AzaCyd and its derivatives were obtained from the Institute of Molecular Biology and Genetics of the Ukrainian SSR Academy of Sciences, 5-AzaCyd, Ara-C and Ara-U from Chemical Dynamic (Sourth Plainfield, NY, USA) and other bases and nucleosides from Reakhim, (Moscow, USSR). Orthophosphoric acid, methanol and ammonium sulphate were obtained commercially (analytical-reagent grade) and were used without further purification. Water was doubly distilled and filtered for HPLC use.

## RESULTS AND DISCUSSION

We studied 16 synthesized analogues of pyrimidine bases and nucleosides: N-4- and O′-substituted 6-azacytidine (Fig. 1), 6-azauridine (6-AzaUrd), 6-azauracil (6-AzaUra), 6-azathymine (6-AzaThy), 6-azacytosine (6-AzaCyt), 5-azacytidine (5-

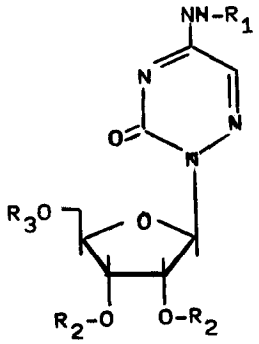


Fig. 1. 6-AzaCyd and its derivatives. **I**, $R_1 = R_2 = R_3 = H$(6-AzaCyd); **II**, $R_1 = C_6H_5COOH$, $R_2 = R_3 = H$; **III**, $R_1 = C_6H_5COOCH_3$, $R_2 = R_3 = H$; **IV**, $R_1 = C_6H_5$, $R_2 = R_3 = H$; **V**, $R_1 = CH_2C_6H_5$, $R_2 = R_3 = H$; **VI**, $R_1 = R_2 = H$, $R_3 = COC_6H_5$; **VII**, $R_1 = H$, $R_2 = R_3 = COC_6H_5$; **VIII**, $R_1 = CH_2CONH_2$, $R_2 = H$, $R_3 = COC_6H_5$; **IX**, $R_1 = (CH_2)OH$, $R_2 = R_3 = COC_6H_5$; **X**, $R_1 = CH_2CONH_2$, $R_2 = R_3 = H$ (Gl).

AzaCyd), 5-fluorouracil (F-Ura), cytosine arabinoside (Ara-C) and uracil arabinoside (Ara-U).

The synthetic analogues studied can be classified in terms of their antitumour activity in three groups: a highly active group (Ara-C, F-Ura and 5-AzaCyd [5–8]), a moderately active group (6-AzaCyd, 6-AzaUrd, Ara-U [9–11] and Gl) and an inactive group (**II–IX**). The following parameters were used: the hydrophobic contact area, the change in free sorption energy and ionization constants. Previously we studied the parameters of some compounds on a Silasorb $C_{18}$ [12,13] and on octadecyl polyol Si 100 [14] sorbents.

All the compounds used are ampholytes. The values of the constants that characterize their acid–base properties are given in Table I (taken from ref. 12).

The changes in the acid–base properties of the compounds considerably affect their retention by a hydrophobic sorbent. The sharp decrease in the capacity factor on transition of the compounds from a molecular to an ionized form is observed for 5- and 6-aza derivatives at a much lower pH than for the unmodified compounds (Fig. 1 in ref. 12).

To compare the interactions of bases and nucleosides with a hydrophobic surface, we determined the values of the change in the area of contact with a hydrophobic surface $\Delta(\Delta\Phi)$ and the change in the free energy of sorption on this surface $\Delta(\Delta G)$ on going from synthetic analogues to natural bases and nucleosides.

The values of $\Delta(\Delta G)$ were determined as $-RT\ln\alpha$ [separation factor $\alpha = k'_1/k'_2$, where $k'_1$ and $k'_2$ are the capacity factors for compounds 1 and 2, respectively (Table I), $R$ is the molar gas constant and $T$ is the temperature] in an eluent containing no methanol.

It is useful to introduce two values of $\Delta(\Delta G)$: $\Delta(\Delta G)_1$ for molecular forms and $\Delta(\Delta G)_2$ for physiological pH (7.0–7.5). To determine $\Delta(\Delta G)_1$, we measured $k'$ for pH values of MP that correspond to deriving compounds in the molecular form (pH 7 for

TABLE I

PARAMETERS OF THE COMPOUND–SORBENT SURFACE CONTACT

| Compound | | $\Delta(\Delta\Phi)$ | | $-\Delta(\Delta G)_1$ (kJ/mol) | $-\Delta(\Delta G)_2$ (kJ/mol) |
|---|---|---|---|---|---|
| 1 | 2 | Å$^2$ | % | | |
| Ura | 6-AzaUra | 13 | 40 | 0.1 | 3.3 |
| Cyt | 6-AzaCyt | 12 | 52 | 0.5 | |
| Thy | 6-AzaThy | 6 | 8 | 1.1 | 2.3 |
| Ura | F-Ura | − 6 | 8 | −0.35 | |
| Cyd | 6-AzaCyd | 16 | 21 | 2.1 | |
| Urd | 6-AzaUrd | 23 | 27 | 2.2 | 4.2 |
| Cyd | 5-AzaCyd | 8 | 10 | 0.7 | |
| Cyd | Ara-C | − 5 | 7 | −0.9 | |
| Urd | Ara-U | − 10 | 12 | −1.5 | |
| Cyd | Gl | − 15 | 19 | −1.2 | |
| Cyd | **II** | − 80 | 103 | −3.4 | |
| Cyd | **III–IX**[a] | >100 | >130[a] | −4 to −15[a] | |

[a] The compounds having the $\Delta(\Delta\Phi)a$ values exceeding 100% compared with Cyd (**III–IX**) are combined in one group.

Cyt, Cyd and their derivatives; pH 3 for Ura, Urd and their derivatives). The value of $\Delta(\Delta\Phi) = \Delta\Phi_1 - \Delta\Phi_2$ for compounds 1 and 2 can easily be calculated by using the dependence of $\ln \alpha$ on the surface tension of MP. For uncharged compounds in eluents that contain no organic solvents, solvophobic theory gives the following relationship [15,16]:

$$\ln \alpha = \frac{N\Delta(\Delta\Phi)\gamma}{RT} + \text{constant} \tag{1}$$

where $N$ is Avogadro's number and $\gamma$ is the surface tension. The surface tension of an inorganic salt solution in water is, to a good approximation, a linear function of the salt concentration and can be expressed by $\gamma = \gamma_0 + rm$, where $m$ is the molal salt concentration, $r$ is a coefficient that depends of the nature of the salt [$r = 2.17$ for $(NH_4)_2SO_4$] and $\gamma_0$ is the surface tension of pure water ($\gamma_0 = 72.0$ dyn/cm). By finding the dependence $\ln \alpha = f(m)$ we obtained the values of $\Delta(\Delta\Phi)$ for pairs of natural compounds, their unsubstituted derivatives and Gl (**X**) (Table I).

A different situation is observed for compounds **II–IX**. To elute these substances, it is necessary to use methanol-containing eluents. Then, according to [15,16], we have

$$\ln \alpha = \frac{N\Delta(\Delta\Phi)\gamma}{RT} + \Delta(\Delta G)_{\text{e.s.}} + \Delta(\Delta G)_{\text{vdW}} + \text{constant} \tag{2}$$

where $\Delta(\Delta G)_{\text{e.s.}}$ and $\Delta(\Delta G)_{\text{vdW}}$ are the changes in the free energy of electrostatic and Van der Waals interaction with MP, respectively. As shown by Horváth *et al.* [15,16], the electrostatic interactions of uncharged molecules with MP, with the methanol content in MP changing slightly, are virtually constant. For the compounds studied (**II–IX**), a linear $\ln k' = f(\gamma)$ dependence is observed [14]. This enables us to conclude that for the compounds studied the term $\Delta(\Delta G)_{\text{vdW}}$ either changes slightly with changing methanol content in the mobile phase, or varies linearly with the surface tension of the mobile phase. In the latter instance we have

$$\ln \alpha = \frac{N\Delta(\Delta\Phi)\gamma a}{RT} + \text{constant} \tag{3}$$

where $a$ is a factor allowing for the change in the term $\Delta(\Delta G)_{\text{vdW}}$. Thus, in this instance also, the value of $\Delta(\Delta\Phi)a$ can be used to find correlations. The values of $\Delta(\Delta\Phi)$ and $\Delta(\Delta G)$ are given in Table I. It can be seen that for the compounds studied there is some correlation between the values of $\Delta(\Delta\Phi)$ and $\Delta(\Delta G)$, indicating a considerable contribution from the cavity term to the selectivity of separation of the compounds studied.

From the results, the compounds can be divided into three groups. The first group includes F-Ura, 5-AzaCyd, Ara-C, Ara-U and 6-AzaThy, for which the differences in the area of hydrophobic contact of their molecules with a surface are minimal in comparison with the natural analogues. The differences for these compounds are not more than 10% compared with the natural analogues.

The second group is characterized by moderate deviation (20–25%). The third group, which is the largest, includes compounds that exhibit a 40–50% higher deviation from the value of hydrophobic contact area for the natural analogues. It should be noted that the compounds displaying a large deviation in the values of $\Delta(\Delta\Phi)$ do not show antitumour activity. On the other hand, all compounds showing high antitumuour activity belong to the first group, characterized by low values of $\Delta(\Delta\Phi)$. Moderately active compounds belong to the second group. If we consider the relative changes in the free energy of sorption, $\Delta(\Delta G)$, we can see that this value as a rule does not exceed $\pm 1.0$ kJ/mol for highly active nucleosides; it is $\pm(1.5–2.2)$ kJ/mol for moderately active compounds and more than $\pm 3.0$ kJ/mol for inactive compounds.

For bases, the values of $\Delta(\Delta G)_i$ are small for the molecular forms of 6-AzaThy and 6-AzaUra (pH 2–5) and they rise sharply as a result of their ionization at pH 6.5–7.5, reaching values of 2.1–3.3 kJ/mol. Thus, as distinct from their natural analogues, 6-AzaThy, 6-AzaUra and 6-AzaUrd are characterized by a high degree ionization under physiological pH, so that it is energetically disadvantageous for them to penetrate into a weakly polar medium. Highly active F-Ura has minimal values for both $\Delta(\Delta G)$ and $\Delta(\Delta\Phi)$.

We can therefore conclude that the differences between the hydrophobic contact areas of a natural pyrimidine compound and its synthetic analogues $\Delta(\Delta\Phi)$ is a sensitive parameter correlated with the potential activity of the compounds. A useful parameter for this purpose is also $\Delta(\Delta G)$. We consider it most promising to search for active compounds among analogues of natural substances that have simultaneously the following characteristics; (1) the difference in the areas of contact with a hydrophobic surface do not exceed 20%; (2) the change in the free energy of sorption on transition from the natural compound to a potential inhibitor or an antagonist does not exceed $\pm 1.5–2.5$ kJ/mol in water at pH 7.0–7.5; and (3) the p$K$ is such that under physiological pH the ionization state of the synthetic analogues is not different from that of the natural compound.

It should be noted that parameters such as $\Delta(\Delta\Phi)$, $\Delta(\Delta G)$ and p$K$ are relatively easy to determine.

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