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Note

Ionization constants and lipophilicity of 1-arylpiperazines investigated by reversed-phase high-performance liquid chromatography

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The lipophilicity of drugs is usually evaluated by using partition ratios, in particular the octanol–water partition coefficient, P[1–4]. When comparing the lipophilicity of a series of homologous compounds, chromatographic methods may be useful in determining quantitative structure–activity relationships. Reversed-phase high-performance liquid chromatography (HPLC) has been used for this purpose; it partitions and separates drugs partly on the basis of their polarity, yielding retention data (capacity factors, k) closely correlated with log P[5–8]. The capacity factor of ionogenic substances in an HPLC column containing a non-polar stationary phase is greatly affected by the eluent pH[9], so the disturbance of ionization in the measurement of lipophilicity is commonly avoided by inhibiting ionization by raising the eluent pH for bases or lowering it for acids. However, the operating range of a C_{18} column is limited to the range pH 2–8; thus for acids with $pK_a < 2$ or bases with $pK_a > 8$ the ionization is still present.

Whether log k of highly ionizable substances can still be used as a suitable estimate of the lipophilic character, and how to achieve the best correlation between log k and log P in such cases, is of interest. In HPLC on μBondapak C_{18} the present study we investigated as an approach to quantitating the relative lipophilicity of 1-arylpiperazines, which are ionogenic bases of biological interest[10,11] and whose P values have recently been reported[12]. The k values were determined at different eluent pH values and the values for the protonated, k_i, and neutral, k_0, forms of arylpiperazines were estimated using a non-linear regression fitting program. The correlation between log k and log P was then examined.

METHODS

HPLC was carried out on a Waters system (Waters Assoc., Milford, MA, U.S.A.) equipped with a Model U6K universal liquid injector, a Model 6000 A solvent-delivery system and a reversed-phase column (μBondapak C_{18}, 30 cm × 3.9

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mm I.D.), at room temperature. The mobile phase was 0.01 *M* dipotassium hydrogenphosphate–acetonitrile (70:30, v/v) adjusted to different pH values (5–7.7) by adding phosphoric acid. Pure reference standards of the twelve 1-arylpiperazines listed in Table I, supplied by their manufacturers[10], were dissolved in methanol and injected sequentially into the chromatograph. The flow-rate was 1.5 ml/min and the injection volume 20 μl. compounds eluted from the column were detected with a Model 440 UV monitor set at 229 nm.

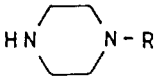
The capacity factor, k was calculated as follows:

$$k = (T_x - T_0)/T_0$$

Methanol (considered a non-retained compound) was used to define T_0, and T_x is the retention time of the test compound.

TABLE I

CHEMICAL STRUCTURE OF 1-ARYLPIPERAZINES



Compound	*R*
1-(2-Pyrimidinyl)piperazine (PmP)	
1-(2-Thiazolyl)piperazine (TzP)	
1-(2-Pyridyl)piperazine (PdP)	
1-(2-Quinolyl)piperazine (QuP)	
1-Phenylpiperazine (PP)	
1-(*o*-Methoxphenyl)piperazine (*o*-OCH_3PP)	
1-(*o*-Tolyl)piperazine (*o*-CH_3PP)	
1-(*p*-Fluorophenyl)piperazine (*p*-FPP)	
1-(*o*-Chlorophenyl)piperazine (*o*-ClPP)	
1-(*m*-Chlorophenyl)piperazine (*m*-ClPP)	
1-(*p*-Chlorophenyl)piperazine (*p*-ClPP)	
1-(*m*-Trifluoromethylphenyl)piperazine (*m*-CF_3PP)	

CALCULATIONS

For basic substances the pH dependence of the capacity factor, k, on a reversed-phase HPLC column containing a non-polar stationary phase has been described by Horváth *et al.*[9].

$$k = (k_0 + k_i\, 10^{(pK_{am} - pH)})/(1 + 10^{(pK_{am} - pH)}) \quad (1)$$

where k_0 and k_i are the capacity factors of the neutral and the protonated base, and pK_{am} is the log of the acid dissociation constant of the protonated base in the mobile phase. Eqn. 1 was derived on the assumption that solute retention occurs because of a reversible association between the dissociated and undissociated base and the hydrocarbonaceous ligand (L) of the stationary phase. The equilibrium constants of these processes, K_{LBH^+} and K_{LB} for the protonated and neutral base respectively, define the capacity factors

$$k_0 = \varphi\, [L]_s\, K_{LB};\; k_i = \varphi\, [L]_s\, K_{LBH^+} \quad (2)$$

where $[L]_s$ is the concentration of the stationary phase ligand and φ is the ratio of the volume of stationary phase to that of the mobile phase. Values of pK_{am}, k_0 and k_i were calculated according to eqn. 1 by use of a non-linear fitting program on a HP85 microcomputer[12].

RESULTS AND DISCUSSION

The capacity factors, k of arylpiperazines determined at different eluent pH values were fit well by eqn. 1, yielding sigmoid curves (see Fig. 1).

Arylpiperazines are bases with $pK_a > 8$ (ref. 12). Since the maximum operating pH of a C_{18} column is pH 8, in this experiment the capacity factor of the neutral form of the arylpiperazines, k_0 cannot be determined directly. However, the k_0 values as well as the capacity factors of the protonated bases, k_i, and the dissociation constants, pK_{am}, of these arylpiperazines in the HPLC eluent containing 30% acetonitrile can be calculated from eqn. 1.

The pK_{am} values were lower than the corresponding pK_a values determined at the same temperature (20°C) in aqueous buffer. These differences may be due to the presence of acetonitrile, a weak basic solvent that inhibits the ionization of the basic solutes[13,14]. The pK_{am} values for the arylpiperazines calculated from eqn. 1 showed a linear decrease with increasing acetonitrile content in the range tested (see Fig. 2).

In this study we tried to correlate the estimated partition coefficient of the un-ionized substance, log P_t, with log k measured at different eluent pH values. Despite the change in ionization of the test compound, a correlation was found between log P_t and log k at all the tested pH values (see Table II), showing that the disturbance due to slightly different ionization was negligible, at least for this series of compounds. The lines resulting from linear regression have nearly equal slopes (see Fig. 3) and the estimated intercepts correlate very well with pH (see Fig. 4).

This suggested a multiple regression between log P_t, log k and pH, and the following equation was obtained:

$$\log P_t = 0.029 \log k - 2.003\ \text{pH} + 14.923 \quad (R^2 = 0.95)$$

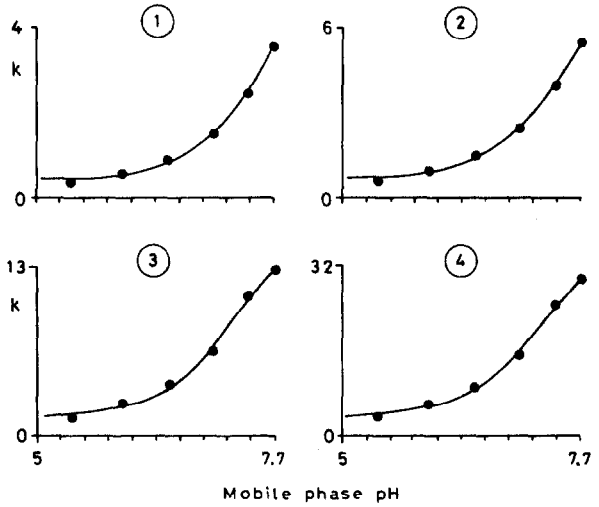


Fig. 1. HPLC capacity factor, k, *vs.* pH: ●, experimental points: —, calculated curve using eqn. 1. 1, PdP; 2, PP; 3, mClPP; 4, mCF_3PP (see Table I for key to compounds).

A comparison of observed and calculated log P_t values showed that this relationship adequately predicted the lipophilicity of this group of arylpiperazines, providing k and the eluent pH were known.

Unlike the octanol–buffer system in which the partition into octanol of the ionized form can be assumed to be nearly zero, the HPLC C_{18} column can still retain the ionized arylpiperazines to a certain extent. In this experiment the capacity factor of the protonated bases ranged from 0.25 to 2.55, and linear regression analysis showed that both log k_0 and log k_i were closely correlated with log P_t (see Table II). Since k_i values can easily be determined by adjusting the eluent pH to two units lower than the pK_a, this parameter can conveniently be used as a lipophilicity index for these highly ionizable arylpiperazines and possibly for other groups of bases in HPLC systems.

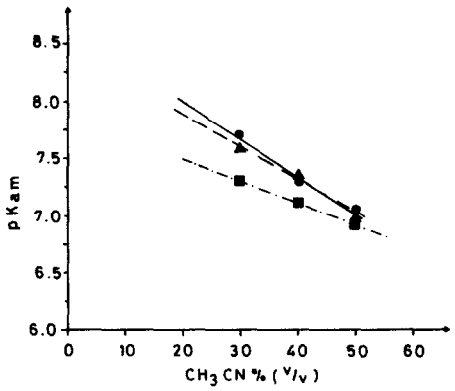


Fig. 2. pK_{am} values estimated from eqn. 1 *vs.* acetonitrile content (see text). ●—●, *o*-CH_3PP; ▲—▲, *o*-ClPP; ■—■, *m*-CF_3PP (see Table I for key to compounds).

TABLE II

SLOPE, A, INTERCEPT, B, AND CORRELATION INDEX, R^2, ESTIMATED BY LINEAR REGRESSION BETWEEN LOG P AND LOG k AT DIFFERENT pH VALUES

Regression equations: $\log P = 2.307 \log k_0 - 2.169$ ($R^2 = 0.94$); $\log P = 2.075 \log k_i - 3.749$ ($R^2 = 0.94$).

pH	*A*	*B*	R^2
5.39	2.073	3.941	0.95
5.97	1.010	2.991	0.96
6.50	2.030	2.071	0.95
7.00	2.089	0.923	0.97
7.40	2.086	−0.098	0.97
7.70	2.196	−0.916	0.97

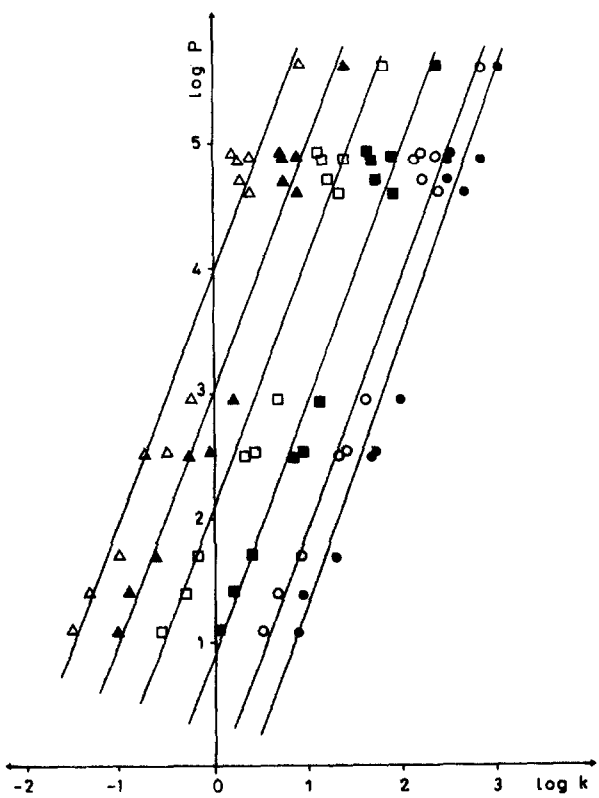


Fig. 3. log P_t *vs.* log k: regression lines and experimental points at different pH values: 7.7 (●); 7.4 (○); 7 (■); 6.5 (□); 5.47 (▲); 5.39 (△).

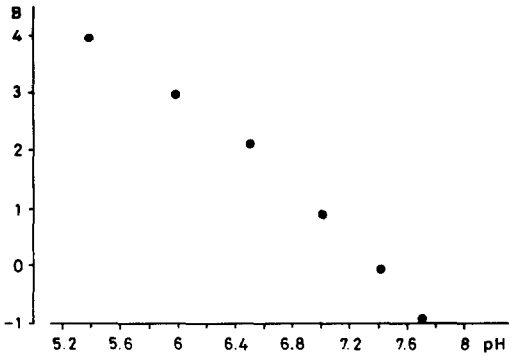


Fig. 4. Intercept (*B* in Table I) *vs.* pH.

In conclusion, the HPLC retention factors of arylpiperazine bases were greatly affected by eluent pH and were fit well to the equation previously described by Horváth *et al.*[9]. Moreover, the correlation found between log P_t, log k and pH suggests that the ratio of the partition equilibrium constants of the neutral and ionized forms with the stationary phase ligands is constant for all the compounds tested. From eqn. 2

$$k_0^j/k_i^j = K_{LB}^j/K_{LBH^+}^j$$

and assuming that

$$\log K_0^j = A_0 + B_0 \log P^j$$

$$\log K_i^j = A_i + B_j \log P^j$$

$$B_0 = B_i$$

where the superscript j refers to compound j, then:

$$K_{LB}^j / K_{LBH^+}^j = \exp (A_0 - A_j)$$

Results have recently been presented[12] which show that 1-arylpiperazines, centrally active metabolites of a number of psychotropic drugs bearing an arylpiperazine side-chain[10,11], can be classified according to their lipophilic character and that this may be related to the extent to which these metabolites enter the brain. The present report indicates that HPLC retention could be used to quantify the lipophilicity of these compounds, and comparison of *in vitro* HPLC retention with *in vivo* 1-arylpiperazine brain uptake indicates that HPLC retention is at least as reliable an index of brain concentration as the partition ratio.

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