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GROUP CONTRIBUTIONS TO HYDROPHOBICITY AND ELUTION BEHAVIOUR OF PYRIDINE DERIVATIVES IN REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

FEDERICO GAGO
Department of Pharmacology, University of Alcalá de Henares, Madrid (Spain)
JULIO ALVAREZ-BUILLA*
Department of Organic Chemistry, University of Alcalá de Henares, Madrid (Spain)
and
JOSÉ ELGUERO
Instituto de Química Médica, C.S.I.C., Madrid (Spain)
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SUMMARY

The mean hydrophobic contributions, π^*_m, of substituents in a sample of 34 monosubstituted pyridines has been determined by using reversed-phase high-performance liquid chromatography. The values are compared with those obtained for benzene.

INTRODUCTION

The logarithm of the octanol–water partition coefficient of a compound, log $P_{o/w}$, is a parameter frequently used as a measure of hydrophobicity in the study of quantitative structure–activity relationships (QSARs) in drug design and pharmacology. It has been classically determined by the "shake-flask" method[1], but lately many attempts have been made to simplify this determination, especially by means of chromatographic techniques[2,3]. Although some novel methods such as droplet counter-current chromatography[4] and micellar chromatography[5] have been reported, reversed-phase high-performance liquid chromatography (RP-HPLC) with hydro-organic mobile phases continues to be the most widely used and the best studied alternative so far.

Two different methods exist for the calculation of approximate log $P_{o/w}$ values, namely the π approach and the fragment approach. π was initially proposed[6] as a substituent parameter for the replacement of an hydrogen on an aromatic carbon atom and was later extended to aliphatic carbon systems. The summation of these hydrophobic contributions from each constitutive group will afford estimates of log $P_{o/w}$, but deviations are often observed that need be accounted for by interaction terms[7,8]. *De novo* calculation of these log $P_{o/w}$ values may be attempted by making use of the fragment method of Rekker[9] and Hansch and Leo[10].

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Work directed to the determination of hydrophobicity by indirect means has focused on the benzene ring, taking into account its relative simplicity. Heteroaromatics, on the other hand, have received comparatively much less attention due in part to their greater complexity but also to the fact that many of their log $P_{o/w}$ values are lacking. For these reasons, few papers have dealt with the problem in detail[11,12].

Before the determination of the hydrophobic contributions from substituents in this sort of structures is undertaken, it should be borne in mind that a monosubstituted ring system possessing one or several heteroatoms behaves as a di- or polysubstituted benzene, since the heteroatoms exert π-donor or π-acceptor effects[13]. Therefore, the substituent electronic and/or steric effects on each position of the ring are different and, as has already been discussed for benzenoid compounds[7,8], hydrophobic contributions are also expected to vary. This question has also been raised for heterocycles, but to date information is scarce[11]. These discrepancies may generate errors capable of vitiating any attempt at QSAR analysis in which π values obtained from the benzene ring are incautiously used to study substituent effects on heterocyclic systems.

In this paper we present a simple and rapid RP-HPLC method of interest for the estimation of log $P_{o/w}$ values of pyridine derivatives. By means of multiple regression analysis (MRA) it has been possible to derive π^*_m values for several substituents chosen for their frequent use in QSAR studies: F, Cl, Br, CH_3O, CHO, $COCH_3$, $CONH_2$, CO_2CH_3, CN, CH_3, NH_2, phenyl and *tert.*-butyl, in a similar fashion to that described for substituents on the benzene ring[14].

EXPERIMENTAL

Materials

The chemicals were of analytical grade (Merck and Aldrich) and were collected from laboratory stock and from colleagues. Methyl carboxylates were synthesized from the corresponding acids, and amides (commercial nicotinamide excepted) were obtained from the respective carbonitriles by standard procedures. 4-Methoxypyridine was synthesized by treating the corresponding commercial N-oxide with phosphorus tribromide[15]. The identity of these compounds was verified by IR and NMR spectroscopy, and the purity by HPLC.

Apparatus

All experiments were performed on a Series 10 Perkin-Elmer liquid chromatograph equipped with a fixed-wavelength detector at 254 nm. A 10-μm C_{18} Perkin-Elmer and a 5-μm LC-18 Supelco column, both 25 cm × 4.6 mm I.D., were used throughout. Retention times were computed by an Hewlett-Packard 3390A integrator. Isocratic elution with three different mixtures of HPLC-grade methanol and 0.015 *M* triethylamine, *i.e.*, 65:35, 55:45 and 45:55 was maintained at a constant flow-rate of 1.0 ml min^{-1} at room temperature.

Methods

General procedures. Samples were dissolved in methanol at a concentration that permitted their UV detection with injections of up to 10 μl (typically 5 μl) and yielded similar peak areas. The column dead time, t_0, was determined by injection of 10 μl of

pure methanol. From the solute retention time, t_R, the logarithm of the capacity factor, k', was calculated:

$$\log k' = \log [(t_R - t_0)/t_0]$$

All measurements were made at least in triplicate. The average reproducibility of each run was better than 3%.

Evaluation of the chromatographic system. In RP-HPLC, addition to the eluent of amines that "mask" the unmodified surface silanols present on the stationary phase can serve to "deactivate" the column. One of the favourite amines used for this purpose is triethylamine (TEA), of relatively small size and soluble enough in the usual mobile phases[16,17]. It has been shown to compete for silanols with solutes having a basic nitrogen in their molecule[17].

Pyridine ($pK_a = 5.20$) is particularly sensitive to free silanols, and for this reason it has been used as solute to test the capacity of various modifiers to reduce peak tailing[18]. The presence of TEA ($pK_a = 11.01$) produces more rapid elution and improves peak shape at relatively low concentrations. Because of these properties, TEA was chosen as the base modifier in this work, at a concentration of 0.015 *M* in water, mixed in different proportions with methanol. In spite of the high pH of the resulting solutions, no column deterioration was detected for as long as the experiment lasted.

A preliminary study using the conventional C_{18} Perkin-Elmer column showed that the quality of the correlations between the reported log $P_{o/w}$ values[10,11] and the retention times (expressed as log k') was very good (Table I). The compound that deviated most was 4-aminopyridine (32) which showed an irregular behaviour, and for that reason was excluded from the regression analysis. This deviation was dependent upon the concentration of organic modifier in the eluent and was least for an eluent composition of methanol–0.015 *M* TEA in water (45:55).

The latter mobile phase composition was chosen to investigate the elution behaviour of the whole set of 28 derivatives for which log $P_{o/w}$ was reported, including pyridine itself (Table II), in a persilylated column packed with a C_{18} alkylated adsorbent of smaller particle size (5 μm). Under these new conditions (Fig. 1), 4-aminopyridine deviated even less, and the quality of the regression line for log $P_{o/w}$ *vs.* log k' was very good indeed (Fig. 1), as is shown by the squared correlation

TABLE I

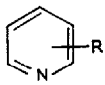
CORRELATION BETWEEN LOG $P_{o/w}$ AND LOG k' FOR PYRIDINE DERIVATIVES IN THREE MOBILE PHASES OF DIFFERENT COMPOSITIONS

$\log P_{o/w} = a + b \log k'$

	a	*b*	*r*	*n*	*s*	*F*
65:35	1.751	2.407	0.970	16	0.0102	444
55:45	1.371	2.083	0.982	22	0.085	1069
45:55	1.005	1.754	0.982	22	0.085	1079

TABLE II

PHYSICO-CHEMICAL DATA FOR THE PYRIDINE DERIVATIVES



Hydrophobicity values (log P) described in the literature[10,11]; retention data (log k') on a Supelco LC-18 column with methanol–0.015 M TEA (45:55); experimentally determined log P^* values; difference between literature log P and log P^* values (Δlog P) and pK_a values[19].

No.	*R*	*log P*	*log k'*	*log P**	*Δlog P*	*pK_a*
1	H	0.65	−0.167	0.80	−0.150	5.20
2	2-F	–	−0.153	0.82	–	−0.44
3	3-F	–	−0.079	0.92	–	2.97
4	2-Cl	1.27	0.067	1.12	0.152	0.72
5	3-Cl	1.33	0.248	1.37	−0.036	2.81
6	4-Cl	1.28	0.255	1.37	−0.096	3.83
7	2-Br	1.38	0.151	1.23	0.147	0.79
8	3-Br	1.58	0.343	1.50	0.084	2.84
9	4-Br	1.54	0.342	1.49	0.045	3.78
10	2-OCH_3	1.34	0.228	1.34	0.002	3.28
11	4-OCH_3	1.00	0.014	1.04	−0.040	6.62
12	2-CHO	–	−0.373	0.51	–	3.80
13	3-CHO	–	−0.538	0.29	–	3.80
14	4-CHO	–	−0.578	0.23	–	4.77
15	2-$COCH_3$	0.83	−0.081	0.91	−0.086	–
16	3-$COCH_3$	0.43	−0.369	0.52	−0.091	–
17	4-$COCH_3$	0.54	−0.320	0.59	−0.048	–
18	2-CONH	0.29	−0.554	0.27	0.022	–
19	3-CONH	−0.37	−1.061	−0.43	0.056	–
20	4-CONH	−0.28	−1.061	−0.43	0.146	–
21	2-$COOCH_3$	0.27	−0.549	0.27	−0.005	–
22	3-$COOCH_3$	0.81	−0.043	0.97	−0.160	–
23	4-$COOCH_3$	0.87	0.002	1.03	−0.159	–
24	2-CN	0.50	−0.376	0.51	−0.012	–
25	3-CN	0.36	−0.437	0.43	−0.068	–
26	4-CN	0.46	−0.412	0.46	−0.002	–
27	2-CH_3	1.11	0.064	1.11	−0.004	5.97
28	3-CH_3	1.20	0.150	1.23	−0.032	5.60
29	4-CH_3	1.22	0.151	1.23	−0.013	6.03
30	2-NH	0.49	−0.369	0.52	−0.031	6.86
31	3-NH	0.11	−0.709	0.05	−0.054	5.98
32	4-NH	0.26	−0.317	0.59	−0.330	0.17
33	4-Phenyl	2.45	0.986	2.37	0.074	5.55
34	4-*tert.*-Butyl	–	1.016	2.42	–	5.99
35	2,4,6-$(CH)_3$	2.01	0.690	1.97	0.039	–

coefficient (r^2 = 0.983), standard error (s = 0.087) and F test (F = 1466), for 28 examples (compound 32 was excluded). The resulting equation was:

$$\log P_{o/w} = 1.019\ (\pm\ 0.017) + (1.367 \pm 0.036) \log k' \qquad (1)$$

This points to the fact that hydrophobicity is the major factor affecting the retention of pyridine in RP-HPLC under these conditions. This chromatographic

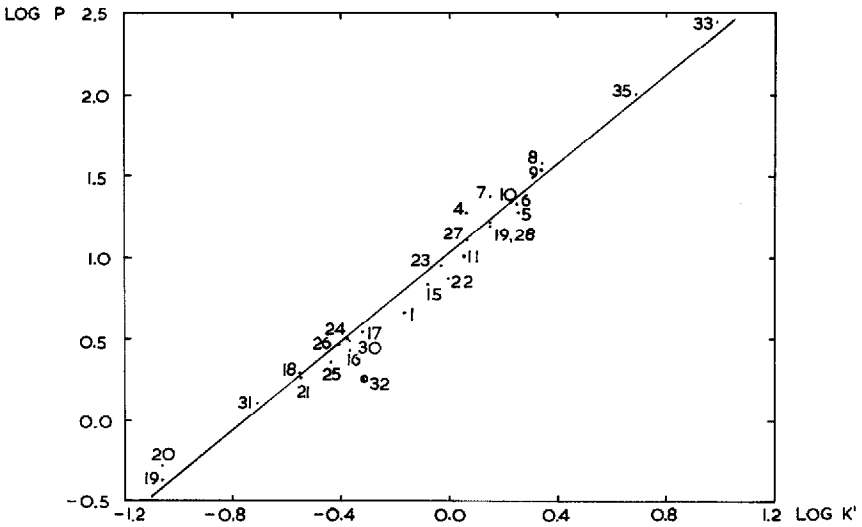


Fig. 1. Correlation, as expressed in eqn. 1, between log $P_{o/w}$ for 28 pyridines (Table II) and their log k' values in methanol–0.015 M TEA (45:55).

system provides a longer lifetime of the stationary phase and a more stable baseline during experiments than when octanol-coated silica is used[11,12]. In addition, its greater simplicity and the fact that no special techniques are required to prepare the column make this method a useful alternative for obtaining hydrophobicity data for pyridines.

RESULTS

Group contributions to hydrophobicity

By interpolating the experimentally obtained log k' value for each compound in eqn. 1, a new series of 35 log P values were calculated and termed log P^* (Table II). The difference, if any, between the experimental log P^* and the reported log $P_{o/w}$ was indicated as log P. Furthermore, Scherrer and Howard[19] proposed to correct the log P values by taking into account the pK_a of the substrate. In order to check whether the goodness of fit of eqn. 1 was improved by adding a second term in pK_a, we carried out a multiple regression[20] with the sixteen pyridines of Table II whose pK_a values were known[21]. The resulting equation (eqn. 2) is very similar to eqn. 1:

$$\log P_{o/w} = 1.179\ (\pm 0.029) + (1.398 \pm 0.033) \log k' - (0.035 \pm 0.005)\ pK_a \qquad (2)$$

$$r^2 = 0.979$$

The coefficient of the basicity term is small but its sign shows that when the basicity increases this somewhat corrects the retention term. However, 4-amino-pyridine (32) is still the compound that deviates most (from eqn. 1, log $P_{o/w} = 0.59$; from eqn. 2, log $P_{o/w} = 0.41$; experimental value from Table II, 0.26).

The retarding effect of basic compounds, and especially 32, is presumably due to their binding to the unmasked residual silanol sites at the surface of the C_{18} stationary phase[16].

An examination of the log P^* values of Table II shows that, excluding 4-aminopyridine (32), these values are quite similar for positions 3 and 4 (mean difference, 0.03; maximum difference, 0.07 log P^* units, for $COCH_3$) but quite different for *ortho*-substituted pyridines (mean difference, 0.33; maximum difference, 0.73 log P^* units, for $COOCH_3$).

Thus, it is justifiable to consider the log P^* value for a substituent at a position 3 or 4 (or the average value when both are available) as representative of the contribution of this group to the hydrophobicity of substituted pyridines: F (0.92), Cl (1.37), Br (1.50), OCH_3 (1.04), CHO (0.26), $COCH_3$ (0.55), $CONH_2$ (−0.43), $COOCH_3$ (1.00), CN (0.44), CH_3 (1.23), NH_2 (0.05), phenyl (2.37) and *tert.*-butyl (2.42).

In Table III are reported the π^*_m values for pyridines corresponding to

$$\log P^*_{3(4)} - \log P^*_H = \log P^*_{3(4)} - 0.80$$

together with π^*_m values for benzenes taken from one of our earlier studies[14], and to Hansch's π values[10].

The substituent contributions from halogen and methoxy groups to pyridine hydrophobicity in 3- and 4-positions were quantitatively similar to those obtained from the benzene system by RP-HPLC[14]. In the case of formyl, acetyl, carboxamide, methyl carboxylate and cyano, however, they were significantly higher, *i.e.*, their character was less hydrophilic. The methyl, phenyl and *tert.*-butyl groups were the only ones to show lower hydrophobicity, although this result might be partly due to the

TABLE III

MEAN HYDROPHOBIC CONTRIBUTIONS OF SUBSTITUENTS ON THE PYRIDINE RING, π^*_m

Calculated from subset of 3- and 4-isomers, compared with the corresponding π^*_m[14] and π[10] values found for benzene ring derivatives.

Substituent	π^*_m *Pyridine*	π^*_m *Benzene*	π *Benzene*
F	0.12	0.08	0.14
Cl	0.57	0.58	0.71
Br	0.70	0.67	0.86
OCH_3	0.24	0.21	−0.02
CHO	−0.54	−0.64	−0.65
$COCH_3$	−0.25	−0.43	−0.55
$CONH_2$	−1.23	(−1.71)	−1.49
CO_2CH_3	0.20	0.12	−0.01
CN	−0.36	−0.60	−0.57
CH_3	0.43	0.54	0.56
NH_2	−0.75	–	−1.23
Phenyl	1.57	–	1.96
tert.-Butyl	1.62	–	1.98

excessive relative retention of pyridine ($\pi = -0.150$), which would lead to an underestimate of the effect of the substituent on the pyridine ring.

We failed to explain the deviation of the group contribution at the 2-position from this mean value in terms of electronic or steric parameters, but it is suggested that the substituent at this position might affect the hydration state of the annular nitrogen, resulting in a variation both in its partitioning between *n*-octanol and water and in its retention in the chromatographic system. A similar hypothesis has been advanced to explain the effect of *ortho* substitution in disubstituted benzenes[8].

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