

Note

Correlation of pK_{BH^+} of acetanilides with liquid chromatographic behaviour*

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The effect of molecular structure on chromatographic behaviour has been extensively investigated¹. However, with the exception of thin-layer chromatographic (TLC)² and high-performance liquid chromatographic (HPLC)³ studies, the correlation of basicity with retention time has received limited attention. The liquid-liquid partition chromatography of methyl derivatives of aniline in acidic systems indicated that their chromatographic behaviour is determined by the basicity of the amino group in addition to steric effects and their molecular volumes². HPLC investigations³ of weakly basic aromatic amines using slightly acidic silica gel adsorbent columns revealed an empirical and linear correlation amongst the *o*-, *m*- and *p*-isomers of the amines, but such a correlation between amines containing different substituents was not clearly established.

The direct correlation of basicity constants (pK_{BH^+}) of acetanilides with HPLC retention times has now been established and is discussed in this paper. Substituted acetanilides are very weak bases, much weaker than the corresponding anilines. Spectroscopic or potentiometric determinations of their pK_{BH^+} values are difficult, as they lack adequate spectral differences in their acid and base forms and have insufficient solubility. Therefore, we have attempted to calculate the pK_{BH^+} values of acetanilides by methods based on a linear free energy relationship and by extrapolation of the available data.

EXPERIMENTAL

Preparation of monoacetyl derivatives

A mixture of the aniline (1 mole), pyridine (1 mole) and acetic anhydride (1.1 mole) was stirred and heated in a boiling water-bath for 2 h. The monoacetyl derivatives were isolated by precipitation on pouring in water, filtered, washed well with water and purified by recrystallization.

HPLC procedure

A Waters Model ALC/GPC-202R401 liquid chromatograph with a UV detector (280 nm) and a dual pen recorder was used. A Corasil-II silica gel column (61 cm

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TABLE I

RELATIVE BASICITIES OF SUBSTITUTED ACETANILIDES AND HAMMETT σ -VALUES OF SUBSTITUENTS

Substituent	$-pK_{BH^+}$	σ^*	Ref.
4-OH	1.02	-0.38	4
4-OCH ₃	1.21	-0.28	4
3-OCH ₃	1.66	0.11	5
2-OCH ₃	1.45	0.00	5
4-OC ₂ H ₅	1.18	-0.24	4
4-CH ₃	1.27	-0.14	4
3-CH ₃	1.67	-0.06	5
2-CH ₃	1.63	0.10	5
4-H	1.59	0.00	4
4-F	1.70	0.06	4
4-Cl	1.88	0.24	4
3-Cl	2.05	0.37	4
4-Br	1.91	0.22	4
4-I	2.07	0.21	4
3-NH ₂	1.68	0.00	5
4-COOH	2.12	0.44	4
4-NO ₂	2.59	0.78	4

* Ref. 6.

$\times 2$ mm I.D.) was used with chloroform as the mobile phase at a flow-rate of 2.0 ml/min at ambient temperature and a column pressure of 650 p.s.i. Spectral-grade chloroform was filtered prior to use as the mobile phase. The sample concentrations were adjusted to 1.0 mg/ml in the mobile phase (chloroform) and injections of 25 μ l were made. The capacity factor (k') for each compound was calculated from the equation $k' = t_R - t_0/t_0$, where t_R is the sample retention time and t_0 is the hold-up time, which was recorded by injecting pure carbon tetrachloride in the mobile phase. The retention time and hold-up time were means of at least ten consecutive injections.

RESULTS AND DISCUSSION

Correlation with Hammett σ -values of substituents

The pK_{BH^+} values of acetanilides and the σ -values of their substituents are summarized in Table I. A linear correlation was found (Fig. 1), and by regression analysis the equation

$$pK_{BH^+} = -1.549 - 1.394 \Sigma\sigma$$

was derived. The pK_{BH^+} values of disubstituted acetanilides utilized in the study of HPLC behaviour were obtained using this regression equation.

Correlation with HPLC retention time

The pK_{BH^+} values of substituted acetanilides with their HPLC retention data are summarized in Table II. As a measure of the reproducibility, the relative standard deviations of the mean k' values (means of ten determinations) from the actual values

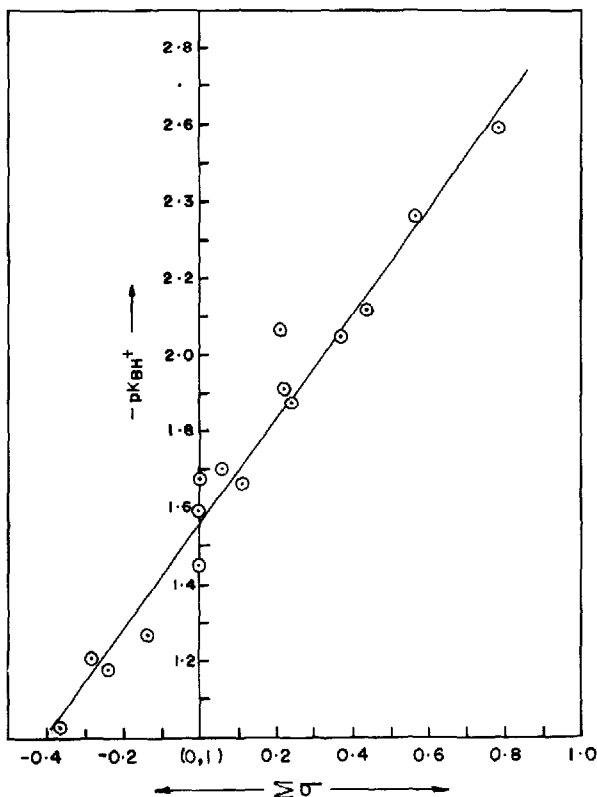


Fig. 1. Variation of pK_{BH^+} of acetanilides with Hammett σ -values of substituents.

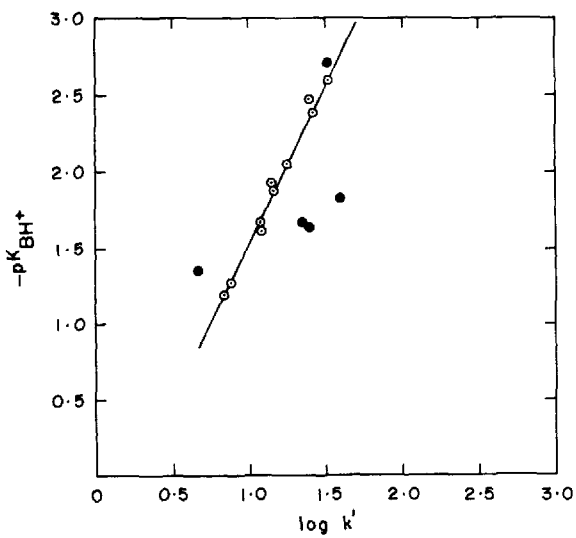


Fig. 2. Variation of basicity constants of acetanilides with capacity factor. \circ , *meta*- and *para*-substituted acetanilides; \bullet , *ortho*-substituted acetanilides.

TABLE II

COMPARISON OF BASICITY CONSTANTS (pK_{BH^+}) OF SUBSTITUTED ACETANILIDES AND CAPACITY FACTORS (k')

Substituent	$-pK_{BH^+}$	k'	Log k'^*	Relative standard deviation of k' (%)
—	1.59	11.9	1.0755	0.6
4-OCH ₃	1.21	6.7	0.8261	0.3
4-CH ₃	1.27	7.5	0.8751	0.4
3-CH ₃	1.67	11.8	1.0750	0.4
2-CH ₃	1.63	24.7	1.3927	1.2
4-Cl	1.88	14.1	1.1500	0.2
3-Cl	2.05	17.7	1.2500	1.0
2-Cl	1.35	4.6	0.6628	0.2
4-Br	1.91	14.1	1.1500	0.5
4-NO ₂	2.59	33.1	1.5185	1.5
3-NO ₂	2.46	25.1	1.4000	2.0
4-CH ₃ , 3-NO ₂	2.38	26.0	1.4150	1.9
2-CH ₃ , 5-NO ₂	2.72	32.5	1.5118	2.0
2,6-Di-CH ₃	1.83	36.2	1.5587	1.8
2,6-Di-C ₂ H ₅	1.69	22.5	1.3521	1.2

* In Fig. 2, log k' values rounded up to the second decimal place were utilized for plotting.

for each of the fifteen monoacetyl derivatives were estimated and were found to be in the range 0.2–2.0%. The graph of pK_{BH^+} against logarithm of the capacity factor (Fig. 2) is linear for the *meta*- and *para*-substituted acetanilides, but the *ortho*-substituted acetanilides show some deviations. This behaviour suggests that basicity is a predominant factor in the adsorption process. The deviations shown by *ortho*-substituted acetanilides may be attributed to the additional field and steric effects exhibited by *ortho*-substituents in addition to the inductive and resonance contributions^{7–10}.

Apart from their contribution towards a better understanding of the liquid chromatographic behaviour of very weak bases, such as acetanilides, the significance of such linear correlations lies in the fact that they can be used to predict the values of basicity constants of compounds whose experimental determination is difficult for the reasons mentioned above.

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