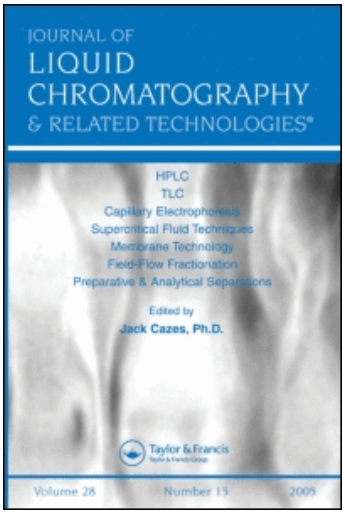

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Relationships Between Biological Activity of N-Phenylamides of Benzoylacetic Acid and Their Capacity Ratios in Reversed-Phase Systems

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RELATIONSHIPS BETWEEN BIOLOGICAL ACTIVITY OF N-PHENYLAMIDES OF BENZOYLACETIC ACID AND THEIR CAPACITY RATIOS IN REVERSED-PHASE SYSTEMS

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ABSTRACT

The relationships between lipophilicity of N-phenylamides of benzylacetic acid derivatives and their biological activity were investigated. Experimental R_M and k' values for systems of the type water + methanol-octadecyl silica were determined by HPTLC and HPLC, respectively. The equations describing the activity relationships confirm the importance of hydrophobic character of the compounds in determining their pharmacological properties.

INTRODUCTION

Quantitative relationships between the chemical structure and the biological acitivty of drugs /QSAR/ are frequently evaluated by Hansch's analysis, where the hydrophobicity of compounds is characterized by the logarithm of partition coefficient /log P/ in the reference system n-octanol + water /1-4/. The relationships between

the partition coefficient P and analogous chromatographic parameters of hydrophobicity such as the R_M value can be expressed by Collander's equation

$$\log P + b R_M + a$$

where a, b are constants /5-9/.

Recently it has been demonstrated that HPLC can also be employed for the determination of hydrophobic properties of organic compounds. The advantages of HPLC over TLC are due to the better accuracy and reproducibility; reversed-phase adsorbents now available are practically totally inert /10-13/. The capacity ratio k' of a compound determined by HPLC is related to the retention volume by

$$k' = (V_R - V_o) / V_o$$

and

$$\log k' = R_M = \log (V_R - V_o)/V_o$$

where V_R and V_o-the retention volumes of a retained and unretained compound, respectively.

It has been shown that the R_M value defined by Bate-Smith and Westall /14/ is for liquid-liquid systems linearly related to log P and that ΔR_M is therefore analogous to the π values - introduced by Hansch as a measure of hydrophobicity of substituents /1/.

For ionogenic compounds, if necessary association of molecules in the mobile phase can be neglected, a correction for the ionization can be expressed by equation:

$$R_M = R'_M + \log \frac{K_A + [H^+]}{[H^+]}$$

where R_M is the corrected value, R'_M if the experimental value, K_A is the ionization constant of the solute and $[H^+]$ is the hydrogen ion concentration in the aqueous phase.

The present study attempts to elucidate how the relationships between R_M values /HPTLC/ or log k' values /HPLC/ and biological activity of the investigated compounds are modified by the presence of methanol and acetic acid in the mobile phase and whether the reversed phase chromatographic systems used differ considerably from the Hansch reference partition system n-octanol - water.

EXPERIMENTAL

High-performance thin layer chromatography was carried out using 10 x 10 cm precoated HPTLC plates with octadecyl silica RP-18 /E.Merck, Darmstadt, F.R.G./.20 µl samples /1 mg/1 ml of methanol/ were spotted 1 cm from the edge and eluted in conventional saturated tanks over a distance of 8.5 cm. As eluent a 7:3 mixture of methanol and 3% v/v aqueous solution of acetic acid was used.

Column high performance liquid chromatography was carried out using a liquid chromatograph/Institute of Physical Chemistry of the Polish Academy of Sciences, Warsaw/ with a syringe 200 cm^3 pump and a UV 254 nm detector. Stainless steel column, 150 x 4 mm I.D., was packed with 10 µm LiChrosorb RP-18 /E.Merck, Darmstadt, F.R.G./. The samples were dissolved in the eluent /1 ml/1 cm^3/ and introduced into the column with a 5 µl switching valve. The flow rate was 1.2 cm^3 min^{-1}. The column dead volume was determined using an aqueous solution of potassium bichromate as the nonretained compound. The temperature was kept at 20 $\pm$ 1°. The results are averages of three measurements. The compounds studied were synthesized in the Department of Pharmaceutical Chemistry of the Medical Academy of Cracow and their biological activities were determined in the Department of Pharmacology of the Medical Academy of Cracow. The coefficients in the regression equations were calculated from the experimental data by the multiple regression analysis, using the least squares method on a Hewlett-Pacard 65 programmable calculator START 1-22 A.

RESULTS AND DISCUSSION

Reversed-phase high performance column /HPLC/ and thin-layer /HPTLC/ chromatography with chemically bonded octadecylsilica sta-

tionary phase were used to determine R_M and k' values of N-phenyl-amides of benzoylacetic acid derivatives as an indication of their hydrophobic character assuming some analogy between the sorption of biologically active compounds in suitably chosen chromatographic system and their transport through lipid membranes in vivo; thus, a correlation between their biological activities and R_M or log k' values could be expected /Table 1 and Fig.1/. It follows from the basic Hansch equation than when compounds with a broad range of log P values are studied then the relative activity depends parabolically on log P. The concept of an optimum log P value has been mathematically justified by consideration of a simple kinetic model to describe the movement of the molecule through a series of aqueous compartments separated by liquid barriers. In most simple tests in vitro a quadratic term in π or log P is not necessary; log P is then a measure of the energy of hydrophobic binding between the compound and reception site /also binding to protein/. In the case when compounds within a limited range of log P values /all higher or all lower than the optimum/ are studied and electronic and steric effects could be neglected, approximately linear relationships between the biological activity and log P can be expected and a simple Collander type equation can be applicable:

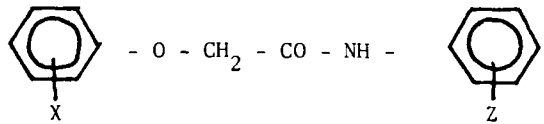
$$\log \frac{1}{C} = b\, R_M + a$$

where a and b are constants and C is the molar concentration of substance producing an equivalent biological effect.

The investigated compounds which have complicated structures with respect to hydrophobic and hydrophilic substituents should provide a good estimate of the validity of chromatographic behaviour for determining physico-chemical parameters important to QSAR. To increase the accuracy, the k' values were determined using several concentrations of the modifier /methanol/ in the eluent /3% v/v concentration of acetic acid was used to suppress the ionization of the solutes/.

TABLE 1

The Chromatographic and Pharmacological Data of Investigated Compounds:



No	X	Z	Inhibition of synthetase of prostagle. ID_{50}/log μm/	Strength of binding to albumin ID_{50} /log μm/	HPLC log k' values Eluent				
					40% MeOH	Pure Water	70% Me in 3% acetic acid	3% acetic acid	70% MeOH in acetic acic
1	4-Me	2-COOH	1.58	2.30	0.44	1.74	0.61	2.60	0.60
2	4-Me	3-COOH	2.70	3.67	0.11	1.04	0.24	2.28	0.70
3	-	2-COOH	2.04	2.78	0.18	1.61	0.41	2.31	0.41
4	2-Me	2-COOH	2.70	2.65	0.41	2.10	0.65	2.72	0.37
5	2-Me	3-COOH	2.70	3.56	0.25	1.72	0.30	2.06	0.50
6	3-Me	2-COOH	2.70	2.40	0.42	1.91	0.61	2.31	-
7	4-Me	2-COOH, 4C1	0.28	1.40	0.70	2.02	0.95	3.03	0.87
8	4-Me	2-COOH, 6C1	2.12	2.75	0.37	1.94	0.37	2.21	0.18
9	4-Me	2-COOH, 4Br	0.00	1.34	0.80	2.64	1.03	3.07	0.79
10	4-Me	2-COOH, 4Me	0.94	2.00	0.58	2.17	0.78	2.53	0.72
11	4-ME	2-COOH, 6Me	2.70	2.80	0.44	2.06	0.35	2.80	0.09
12	4-C1	2-COOH, 4C1	0.04	1.40	0.85	3.40	0.97	3.54	0.41

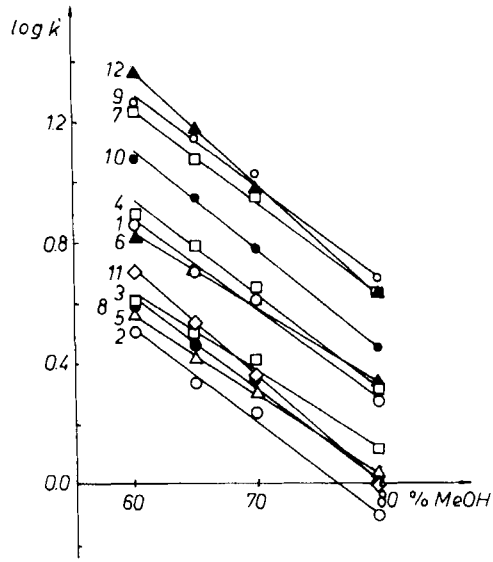


FIGURE 1: Correlation between - log C and log k' values
a) inhibition of synthetase of prostaglandins;
b) binding to albumina.

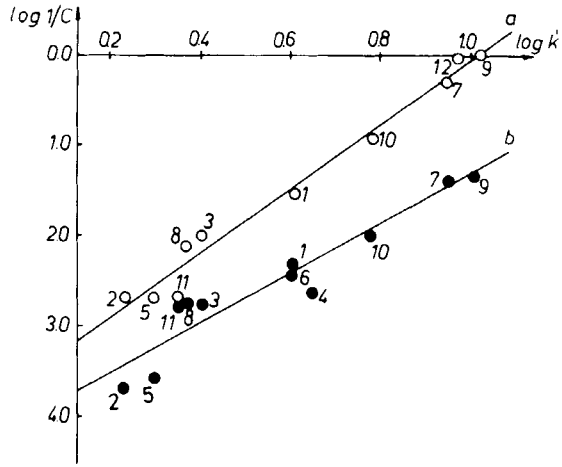


FIGURE 2: log k' values plotted against % concentration of methanol in 3% aqueous acetic acid. Adsorbent: octadecylsilane silica /RP-18/. For notation of solutes see Table 1

Fig.2 demonstrates the plots of log k' against per cent concentration of methanol in 3% aqueous solution of acetic acid. In all cases linear relationships were obtained /18,19/. The lines sometimes cross /change of sequence of k' values/. The structural effects are generally in agreement with those observed for liquid-liquid chromatography /20,21/. Thus, highest k' values were obtained for solutes with ortho carboxylic group; their hydrophilic properties were decreased owing to interaction /intramolecular H-bond/ between vicinal carboxylic and amide groups. Increases of selectivity for lower concentrations of the modifier in the eluent is caused by increasing contribution of hydrophobic interactions, especially in the case of less polar compounds /17/.

Higher k' values were also obtained for solutes with additional halogen atom in the para position. One or two halogen atoms introduced into the molecule, especially in the para position /Fig.1/, caused significant increase of hydrophobic properties. A halogen atom in the ortho position had a smaller effect on the chromatographic behaviour owing to steric shielding of the second hydrophobic amide group H-bonded with the ortho carboxylic group. Equations of the straight lines of relationships between the log k' values and per cent concentration of methanol in the aqueous eluent /log k = bc + a; a and b - constants, c-methanol concentration, v/v/, derived for each compound by regression analysis permitted to estimate by extrapolation the high capacity ratios in systems with pure water or 3% aqueous solution of acetic acid.

TABLE 2

Equations 1-5 and 1a-5a show correlations between chromatographic data and pharmacological activity expressed by two effects - inhibition of synthetase of prostaglandins /i/ and binding to albumins /b/; C denotes the micromolar concentration of the investigated solutes corresponding to ID_{50}, n-number of compounds in the set, n-regression coefficient.

The best correlations were obtained for eluent composed of methanol and 3% aqueous acetic acid /Egs. 3, 3a/. System with acetic

TABLE 2

Relationships Between log k'Values /HPLC/ or R_M Values /HPTLC/ and Biological Activity /i-inhibition, b-binding/

Mobile Phase : water /extrapolated/

$$\log \frac{1}{C_i} = 1.33/\pm 0.41/\log k' - 4.22/\pm 0.86/ \quad n\text{-}10 \; r\text{-}0.927 \quad /1/$$

$$\log \frac{1}{C_b} = 1.05/\pm 0.27/\log k' - 4.55/\pm 0.58/ \quad n\text{-}12 \; r\text{-}0.748 \quad /1a/$$

Mobile phase : 40% methanol

$$\log \frac{1}{C_i} = 3.39/\pm 0.63/\log k' - 3.38/\pm 0.34/ \quad n\text{-}10 \; r\text{-}0.911 \quad /2/$$

$$\log \frac{1}{C_b} = 3.15/\pm 0.37/\log k' - 3.88/\pm 0.18/ \quad n\text{-}12 \; r\text{-}0.938 \quad /2a/$$

Mobile phase : 70% methanol in 3% aqueous solution of acetic acid

$$\log \frac{1}{C_i} = 3.59/\pm 0.18/\log k' - 3.67/\pm 0.12/ \quad n\text{-}10 \; r\text{-}0.990 \quad /3/$$

$$\log \frac{1}{C_b} = 2.69/\pm 0.25/\log k' - 4.05/\pm 0.17/ \quad n\text{-}12 \; r\text{-}0.959 \quad /3a/$$

Mobile phase : 3% aqueous solution of acetic acid /extrapolated/

$$\log \frac{1}{C_i} = 1.93/\pm 0.50/\log k' - 6.61/\pm 1.35/ \quad n\text{-}10 \; r\text{-}0.806 \quad /4/$$

$$\log \frac{1}{C_b} = 1.46/\pm 0.34/\log k' - 6.25/\pm 0.89/ \quad n\text{-}12 \; r\text{-}0.812 \quad /4a/$$

HPTLC-Mobile phase : 70% methanol in 3% aqueous solution of acetic acid

$$\log \frac{1}{C_i} = 2.98/\pm 0.93/ \; R_M - 2.98/\pm 0.49/ \quad n\text{-}11 \; r\text{-}0.731 \quad /5/$$

$$\log \frac{1}{C_b} = 2.10/\pm 0.72/ \; R_M - 3.38/\pm 0.38/ \quad n\text{-}11 \; r\text{-}0.693 \quad /5a/$$

acid /to suppress ionization/ was found to be very suitable for the study of hydrophobic properties of compounds expressed by log k' or R_M and their activity data.

Introduction of extrapolated log k' values for pure water /egs.1, 1a/ and for 3% aqueous solution of acetic acid /egs.4, 4a/ did not improve the correlation coefficients but a slope 1.05 and 1.33 for pure water, are not far from unity, what could mean that mechanisms of separation of the investigated compounds is closely related to the penetration of drugs to their sites of action through the liquid phases and various membranes which like octadecylsilica bonded stationary phase do not behave as a bulky liquid /8, 22/.

The relatively low correlation coefficients of egs. 5 and 5a seem to be due to the lower accuracy of data obtained on the thin layers in comparison to the HPLC data. The positive slope of relationship of log 1/C vs log k' or R_M indicate the importance of the lipophilic character of the compounds in determining both the inhibition of synthetase of prostaglandins and the strength of binding to albumin.

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