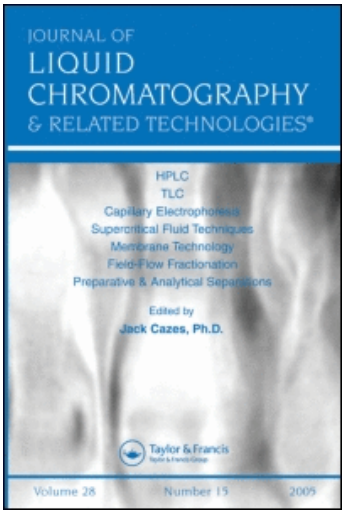

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Investigation of the Lipophilicity of Antiphlogistic Pyrazole Derivatives: Relationships Between Log Kw and Log P Values of 5-Arylamino and Arylhydrazono-3-Methyl-4-Nitro-1-Phenylpyrazoles

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INVESTIGATION OF THE LIPOPHILICITY OF ANTIPHLOGISTIC PYRAZOLE DERIVATIVES: RELATIONSHIPS BETWEEN LOG Kw AND LOG P VALUES OF 5-ARYLAMINO AND ARYLHYDRAZONO-3-METHYL-4-NITRO-1-PHENYLPYRAZOLES

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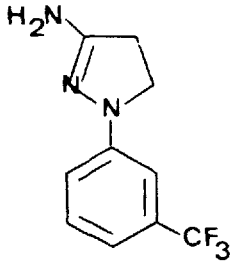
ABSTRACT

The log kw values of a series of 3-methyl-4-nitro-1-phenylpyrazoles substituted at C-5 with arylamino and arylhydrazono moieties were obtained through extrapolation to 100% water from capacity factors data from reversed-phase HPLC. The partition coefficients for some key compounds were obtained through the "shake-flask" method and used to calculate, with aid of fragmental constants, the values expected for the series. The log P data showed a definite correlation with log Kw, confirming the feasibility of using the latter as hydrophobicity descriptors. This study indicates also the need for reassignment of the fragmental constant value for the hydrazono (**-NH-N=CH-**) group.

INTRODUCTION

The pyrazole nucleus is present in several molecules showing biological activity at the arachidonic acid cascade, for example, the phenylbutazone antiinflammatory agents and the more recently described (1) dual cycloxygenase-lipoxygenase inhibitor BW755C (I). In order to further explore these leads, a series of 3-methyl-4-nitro-1-phenyl-pyrazole derivatives were prepared presenting, at C-5, arylamino (II) or arylhydrazono (III) groups with varied aromatic substitution patterns. These compounds were evaluated in the carrageenin-induced edema and the acetic acid-induced writhing response in rats showing an antiphlogistic activity comparable to standard NSAI drugs (2). As the lipophilic character of several antiinflammatory agents has been well correlated with their biological activities (3,4), the need for hydrophobic descriptors in the search for QSAR in this class of drugs was envisaged. The purpose of the present paper is to study the lipophilicity of the pyrazole derivatives by means of a reversed-phase HPLC technique. The reliability of this methodology is checked by correlation of the log Kw data with the more classical parameter log P, obtained through calculation via fragmental constants from the "shake-flask" values for parent compounds.



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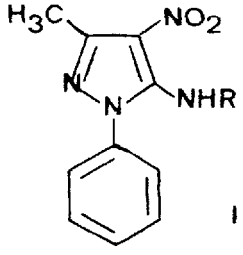
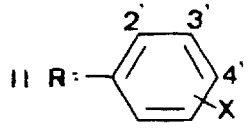
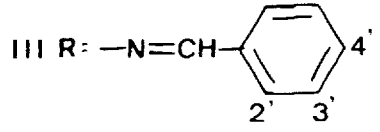






MATERIALS AND METHODS

Chemicals. The pyrazole derivatives II were obtained from 5-chloro-3-methyl-4-nitro-1-phenylpyrazole (5) by reaction with substituted anilines (2). Treatment of the same chlorinated precursor with hydrazine followed by condensation with substituted benzaldehydes led to the corresponding hydrazones III (2). All compounds were characterized from spectral data (IR, ^{1}H NMR and MS) and shown to be more than 99% pure by HPLC. All other chemicals and solvents were of analytical reagent or HPLC grade. In the following text, II will be referred as anilinopyrazoles and III as hydrazonopyrazoles.

Octanol-Water Partition Coefficients Measurements. The partition coefficients were determined by a conventional methodology (6). Samples in a weight range of 5-10 mg were partitioned between 5 ml of n-octanol satured with potassium phosphate buffer and 100-200 ml of the buffer satured with n-octanol. Only the n-octanol phase was analyzed to obtain the partition coefficients, using recently prepared calibration curves. The quoted values represent the mean from at least five measurements. Phosphate buffer (0,1M, pH 8.5) was used as the water phase, ensuring that all compounds were more than 99% unionized. The log P values for parent compounds IIa and IIIa were used as the basis for the calculation of data for the other molecules through summing the corresponding fragmental constants (7-10).

Determination of log K'and log Kw values by reversed-phase HPLC. Chromatography was performed with a Waters 600E System Controller apparatus using a Lichro CMT column (125x4 mm I.D.) packed with LiChrosphere 100RP-18 (particle size 5 um). A UV detector (Waters Model 490E) set at 360 nm for the anilinopyrazoles and 390 nm for the hydrazonopyrazoles was used. The compounds were dissolved in acetone and applied to the column in 5 ul volumes, employing Hamilton 802 chromatographic syringes (25ul). Separation was run at pH 7.5 using methanol-potassium phosphate buffer mixtures as the mobile phase at a flow rate of 1.5 ml/min. The methanol content ranged from 50 to 70%. The experiments were performed at room temperature (20-25 ºC). The column dead-time of the system, t_0, was measured as the time from injection to the first

TABLE 1

Lipophilicity Parameters for 5-Substituted-3-Methyl-4-Nitro-1-Phenylpyrazoles

Chemical Class		Log P		Log Kw			
Nº	Anilinopyrazoles	Exp.	Calc.a	Exp.	Eq.1	Eq.2	Eq.3
IIa	H	2.98	2.97	3.84	3.90	3.94	3.90
b	2'-Methyl	3.80	3.44	4.79	4.30	4.35	4.45
c	3'-Methyl	3.25	3.44	4.35	4.30	4.35	4.31
d	4'-Methyl	3.43	3.44	4.43	4.30	4.35	4.31
e	2'-Nitro	----	2.71	3.91	3.68	3.71	3.86
f	3'-Nitro	----	2.71	3.64	3.68	3.71	3.67
g	4'-Nitro	2.78	2.71	3.83	3.68	3.71	3.67
h	2'-Methoxy	----	3.02	3.91	3.94	3.98	4.15
i	3'-Methoxy	----	3.02	3.87	3.94	3.98	3.94
j	4'-Methoxy	----	3.02	3.84	3.94	3.98	3.94
k	3'-Chloro	----	3.68	4.42	4.51	4.56	4.52
l	4'-Chloro	----	3.68	4.42	4.51	4.56	4.52
m	4'-Bromo	----	3.83	4.53	4.64	4.69	4.65
n	4'-Trifluormethyl	3.89	3.85	4.70	4.66	4.70	4.66

Nº	Hydrazonopyrazoles (aryl ring)						
IIIa	Phenyl	3.92	----	4.63	----	----	----
b	3'-Nitrophenyl	3.95	3.66 [b]	4.51	4.49	4.54	4.50
c	4'-Methoxyphenyl	----	3.97 [b]	4.94	4.76	4.81	4.77
d	4'-Dimethylamino-phenyl	----	4.54 [b]	5.27	5.25	5.31	5.26
e	3',4'-Dimethoxy-phenyl	----	4.00 [b]	4.60	4.79	4.83	4.79
f	3',4'-Methylene-dioxyphenyl	----	3.87 [b]	4.80	4.67	4.72	4.68
g	2'-Bromo-3',4'-Methyl-enedioxiphenyl	----	4.73 [b]	5.63	5.41	5.47	5.66
h	2'-Nitro-3',4'-Methyl-enedioxiphenyl	3.39	3.61 [b]	3.61	4.45	----	----

a: Calculed from Rekker's fragmental constants (7,8).

b: Calculed from Rekker's f (7,8) values and the experimental value for IIIa.

distortion of the baseline and the log Kw value for each compound was obtained by regression analysis of log K' data, expressed from the retention times, t_x, through the formula:

$$K' = (t_x - t_0)/t_0 ,$$

and extrapolation to 0% methanol content (Table 1).

Correlation/Regression Analysis. Statistical evaluation of data and the correlation/regression analysis were carried out with the programs STATGRAPHICS and QSAR - PC:PAR (Biosoft/Elsevier) using a PC/XT computer.

RESULTS

Determination of partition coefficients. The log P data for the anilino- (IIb-n) and the hydrazonopyrazoles (IIIb-h) were obtained through calculations using fragmental constants (7-10) and the experimental values for parent compounds IIa and IIIa, respectively. For 6 substances (IIa, IIIa and IIIh were excluded due to the lack of all fragmental contributions of the substituents), Norrington's pi (o,m,p) (10) afforded the best equivalency between measured and calculated values. Using Hansch's pi (7) and Rekker's f (7,8) also afforded statistically good correlations. The latter descriptor was chosen as the general one due to the grater number of compounds which could be included in the analysis and the close similarity between the calculated (2.97) and the experimental datum (2.98) for compound IIa. On the other side, hydrazonopyrazole IIIa showed a predicted value of 0.31 (using -2.75 as the f value for the HN-N=CH moiety (7)) which is clearly discrepant with the experimental one (3.92). Use of this latter value afforded predicted data for derivatives IIIb and IIIh closer to experimental ones; it is therefore proposed to assign 0.94 as a better value for the fragmental constant of a NH-N=CH group flanked by two aromatic rings.

Determination of log Kw values. The reversed-phase HPLC at pH 7.5 of the compounds in Table 1 was accomplished with buffer/methanol mixtures as mobile phases in the range of 50-70% methanol, since smaller concentrations of this component led to unreliabling high retention times. However, for all compounds,

linear relationships ($r > 0.99$) were proved to exist between the log K' values and methanol concentrations, allowing the calculation of log Kw through extrapolation. The slopes for the equations were mostly constant with a mean value of -0.057 (±0.005).

Following N. El Tayart et al (11) and assuming a maximum basic pKa of 5.5 for the studied compounds, a correction factor for ionization smaller than 0.004 was obtained at pH 7.5, which showed that all substances were mostly un-ionized at the experimental conditions.

One point that deserves comment is the absence of a lipophilic amine as a "silanol-masking" co-eluent in our experiments (12). The compounds hereby described present high lipophilicity ($4.73 < \log P < 2.71$) and, consequently, interact rather strongly with the stationary reversed-phase ($5.63 < \log Kw < 3.61$). In instances similar to these, as it has been previously pointed out (13), the supression of silanophilic interactions is incomplete and negatively dependent on methanol content, leading, after all, to deviations from the expected linearity between log K'and organic modifier concentration. Thus, it seemed more reasonable to consider these silanol-solute interactions as part of the retention mechanism in a kind of "mixed model" (13). In the present work, the dimethylamino derivative IIId must be the only compound affected more profoundly by this effect, which explains the log Kw value higher than that predicted on grounds of its lipophilicity (log P) (see infra).

Correlation between lipophilic indexes. The log Kw values were correlated with log P, affording equation 1 for the experimental data listed in Table 1.

$$\log Kw = 0.859\ (\pm 0.100)\ \log P + 1.349\ (\pm 0.357) \qquad (1)$$

$$(n = 21;\ r = 0.891;\ s = 0.253;\ F = 73.99;\ p < 0.005)$$

Compound IIIa was not included as its "all-predicted" value was very discrepant from the experimental one (vide supra).

Examination of predicted and observed log Kw data (Table 1) shows a clear deviation for compound IIIh, even after consideration of eletronic parameters in the correlation (vide infra). Maybe, resonance interaction between the nitro group and one of the

oxygens of the methylenedioxy moiety imparts a more polar character to this substance than that expected on grounds of individual lipophilic/lipophobic contributions. Removal of this compound from the regression leads to a better correlation, v.g., eq.2:

$$\log Kw = 0.872\ (\pm 0.064)\ \log P + 1.346\ (\pm 0.229) \quad (2)$$

$$(n = 20;\ r = 0.954;\ s = 0.163;\ F = 183.23;\ p < 0.005)$$

As the interaction of the compounds with the mobile phase (and most probably, with the stationary phase, too) involves eletrostatic factors which may modify their chromatographic behaviour, multiple correlations were investigated, with the addition of the so-called electronic and steric parameters (7). The most statistically reliable correlation is described by eq.3:

$$\log Kw = 0.869\ (\pm 0.057)\ \log P + 0.030\ (\pm 0.012)\ MRo + 1.259\ (\pm 0.207) \quad (3)$$

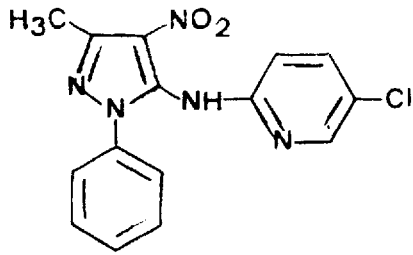
$$(n = 20;\ r = 0.966;\ s = 0.144;\ F = 119.08;\ p < 0.005)$$

In eq.3, MRo describes the sum of the molar refractivity data for the substituents at the positions adjacent to the amino and the hydrazono moieties in the phenyl rings. Addition of a dummy variable to account for the amino (I = 0) and hydrazono (I = 1) groups linking the pyrazolo and the phenyl nucleus also improves the correlation, although with less statistical significance.

DISCUSSION AND CONCLUSIONS

The present work shows that good correlations exist between HPLC data and the log P values calculated from fragmental constants and the experimental partition coefficients of parent, unsubstituted compounds. Equations 1,2 and 3 account for 79%, 91% and 93% of the total variance in the data (r^2). The lipophilic behaviour in Table 1 from the log Kw values, can be explained through the probable retention mechanism of all these substances in reversed-phase HPLC. For the anilinopyrazoles, the affinity to the mobile aqueous phase, which is central to the retention process (12), is dependent on the chemical environment at and near the amino

group. Ortho-substituents clearly disturb this interaction, leading to retention times greater than those obtained for meta- and para- derivatives. In the hydrazonopyrazoles, the influence of the ortho group is expected to be lessened by the greater distance to the analogous NH moiety, although there are few data to support this. The deviation for IIIh may result, at least in part, from this effect.
The reliability of these correlations could be confirmed by comparison of the log Kw value predicted from the "shake-flask" log P (3.01) of derivative IIo, i.e., 3.94 with the experimental one, i.e., 3.74.



IIo

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