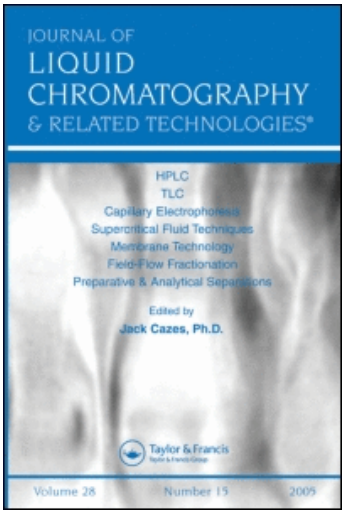

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High Performance Liquid Chromatography of Isoxazolyl-Naphthoquinones: A Comparison Between Experimental and Theoretical Lipophilicity

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF ISOXAZOLYL-NAPHTHOQUINONES: A COMPARISON BETWEEN EXPERIMENTAL AND THEORETICAL LIPOPHILICITY

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ABSTRACT

An RP-HPLC procedure was developed for determining the lipophilicity of a series of isoxazolyl-naphthoquinones which possess antibacterial, trypanosidal and antineoplasic activity. The experimental results were compared with theoretical log P values, and it was found that there was a good relationship between the two methods, except for very lipophilic compounds.

INTRODUCTION

In search of bioactive compounds, we prepared a series of naphthoquinones bearing different isoxazole substituents.[1-3] Extensive studies carried out with some of these compounds have revealed antibacterial,[4,5] trypanosidal[6] and antineoplasic[7] activity.

The lipophilicity of drugs has been shown repeatedly,[8-10] to be of great importance in determining the body distribution, as well as the relative potency of drugs that are members of an analogous series. A useful descriptor of global lipophilicity has been the octanol-water partition coefficient (log P_{oct}), traditionally obtained by the shake-flask method.

Because this method has a number of disadvantages, other procedures have been developed, for example chromatographic, such as reverse phase high performance liquid chromatography (RP-HPLC).[11-12] This method assumes a linear relationship between the logarithm of capacity factor (log k′) and log P, by a Collander-type equation.[13]

The reason for log P being accurately determined by RP-HPLC is that the dominant mode of retention in the stationary phase is that of partitioning, not absorption.[14]

In addition to the experimental methods, theoretical procedures for the calculation of log P values have been developed.[8,15]

The aim of this study was to determine the lipophilicity of a series of isoxazolyl-naphthoquinones, because between their members are biologically relevant molecules, and the knowledge of this parameter is important in view of their possible clinical use. We selected the following compounds:

1a-2-(3,4-dimethyl-5-isoxazolylamine)-N-(3,4-dimethyl-5-isoxazolyl)-1,4-naphthoquinone- 4-imine.

1b- 2-(4-methyl-5-isoxazolylamine)-N-(4-methyl-5-isoxazolyl)-1,4-naphthoquinone-4-imine.

1c- 2-(5-methyl-3-isoxazolylamine)-N-(5-methyl-3-isoxazolyl)-1,4-naphthoquinone-4-imine.

2a- 4-N-(3,4-dimethyl-5-isoxazolyl)-1,2-naphthoquinone.

2b- 4-N-(4-methyl-5-isoxazolyl)-1,2-naphthoquinone.

2c- 4-N-(5-methyl-3-isoxazolyl)-1,2-naphthoquinone.

3a- 2-hydroxy-N-(3,4-dimethyl-5-isoxazoyl)-1,4-naphthoquinone-4-imine.

3b- 2-hydroxy-N-(4-methyl-5-isoxazolyl)-1,4-naphthoquinone-4-imine.

3c- 2-hydroxy-N-(5-methyl-3-isoxazolyl)-1,4-naphthoquinone-4-imine.

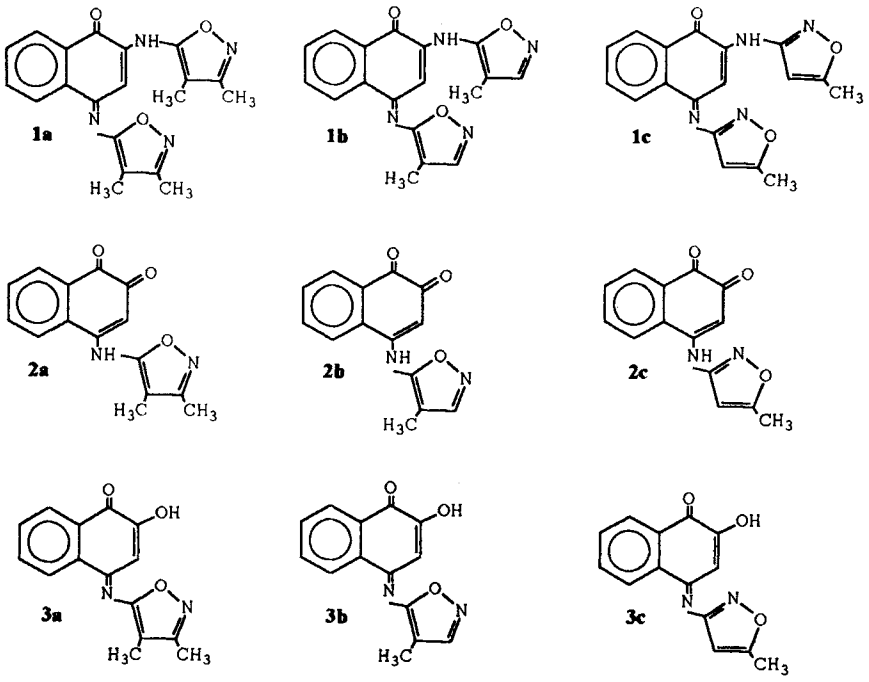

Figure 1. Chemical structures of compounds studied.

We chose the RP-HPLC technique due to the low water solubility of these compounds. The reliability of this methodology is checked by comparison of the experimental data with the calculated log P values.

MATERIALS AND METHODS

Materials

The isoxazolyl-naphthoquinone derivatives (1-3) were obtained as in previously reported procedures.[1-3] All other chemicals and solvents were of *analytical* reagent grade and were used without further purification. Reagent grade water was generated by a Millipore Milli-Q Water purification system.

Chromatography

HPLC chromatography was performed with a KONIK model 500G, with a UV-V-KNK-029-757 absorbance detector with the wavelength set at 245 nm, a Rheodyne 7125 injector, a Spectra Physics 4600 Data Jet integrator, and a 250 x 4.6 mm Supelcosil LC-18 5-μm HPLC column (Supelco). The mobile phase composition ranged from 60 to 90% (v/v) methanol with water. The flow rate was 1.0 mL/min.

Analytes were dissolved in methanol and then they were injected separately from each other. The experiments were repeated three times and the mean value of the retention time for each compound was determined.

Retention times (t_r) can be transformed into a capacity factor as $k' = (t_r - t_0)/t_0$ where t_r and t_0 are the retention times of the analytes and the methanol, respectively. Capacity factors (log k′) were determined at six to seven different concentrations of methanol in water (90%, 85%, 80%, 75%, 70%, 65%, and 60%). Experiments with lower percentage of methanol than 60%, afforded retention times too long to be measured.

The average log k′was graphed against the percent of methanol, and the value of log k_w (where k_w represents the capacity factor in absence of organic solvent) was obtained by extrapolating to 100% water, according to the following equation: log $k' = ax + \log k_w$. The extrapolated log k_w values are used in order to suppress the effect of the organic modifier and to obtain lipophilicity values independent of the eluent conditions. The system was calibrated by determining log k_w for a set of compounds, which included the following ones: pyridine (log P = 0.64), aniline (log P = 1.08), acetanilide (log P = 1.42), 1,4-naphthoquinone (log P = 1.71), p-nitroacetanilide (log P = 2.34), 1-naphthol (log P = 2.98), and phenanthrene (log P = 4.46).

Log P Calculations

For the calculation of log P of all studied isoxazolyl-naphthoquinones we used the Leo-Hansch fragmental method.[8]

RESULTS AND DISCUSSION

The chemical formulae of the tested isoxazolyl-naphthoquinones are given in Figure 1.

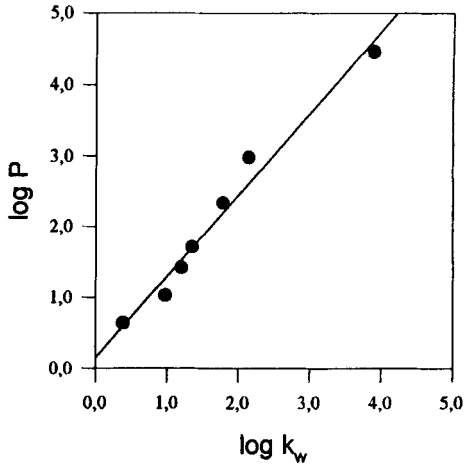

Figure 2. Relationships between log P and log k_w for selected standards.

Determination of Partition Coefficients by HPLC

The application of an HPLC system for the determination of partition coefficients by correlation, requires previous calibration of the system using standards for which classical shake-flask partition coefficients are known.[16] In our case, we selected seven compounds, which exhibited intense UV absorption at 245 nm, and the set of standards chosen covered a log P range from 0.64 (pyridine) to 4.46 (phenanthrene), where most of the log P values for the naphthoquinone derivatives could be included.

As shown in Figure 2, excellent correlations were obtained for all standards assayed, and the relationship between log k_w and log P for the set of standards was fitted into the following linear equation:

$$\log P = 1.15\ (\pm 0.08) \log kw + 0.15\ (\pm 0.16) \qquad (1)$$

with $n = 7$, $r^2 = 0.992$, and $S = 0.051$, where n is the number of data used, r^2 the correlation coefficient, S the estimated standard error, and the 95% confidence limits on the regression coefficients are given in parenthesis. This correlation can be considered as very satisfactory.

To estimate reproducibility of retention times and, consequently, of log k′ parameters, the above standards were tested. As depicted in Table 1, the results showed excellent reproducibility, which, allowed us to perform the whole HPLC analysis with three independent injection runs for every solute.

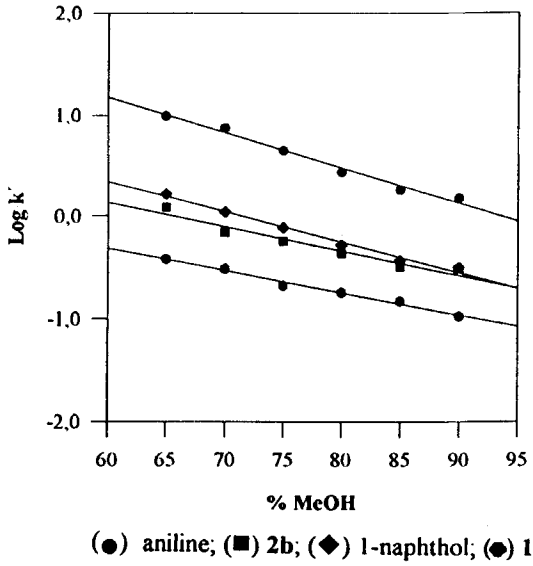

Figure 3. Typical graph of log k´at different methanol concentrations.

Table 1

Dispersion Analysis for Retention Times (R_t) and log k' Values of Seven Calibration Standards. Mobile Phase: Methanol-Water 75:25 (v/v)

Standards	n	Rt (min) ± SD	log k'
Pyridine	7	3.01 ± 0.02	-0.755
Aniline	7	3.10 ± 0.03	-0.676
Acetanilide	7	3.28 ± 0.05	-0.551
1 Naphthoquinone	7	3.38 ± 0.03	-0.494
p-Nitroacetanilide	8	4.30 ± 0.05	-0.168
1-Naphthol	8	4.54 ± 0.04	-0.112
Phenanthrene	7	5.88 ± 0.06	0.113

Table 2

Experimental and Calculated Lipophilicity Values

Compound	log kw	log P (RP-HPLC)	log P (CLOGP)
1a	3/93	4.68	4.50
1b	3.34	4.00	4.12
1c	3.87	4.61	4.26
2a	2.36	2.87	3.01
2b	1.74	2.15	2.37
2c	2.96	3.56	3.33
3a	2.79	3.37	3.51
3b	2.72	3.28	3.44
3c	6.32	7.44	5.86

The above HPLC analytical treatment was then applied to compounds 1-3. Thus, respective log k′ values were obtained from analysis of the retention behaviour, using the same methanol volume fractions as in standards. The log k′ of the compounds and standards, decreased linearly with increasing methanol percentage of mobile phase (Figure 3).

The log k_w values for each naphthoquinone analogue, were obtained by regression analysis of log k′ data. Then, extrapolation of respective log k_w values in equation 1, permitted calculation of the corresponding partition coefficients of the derivatives assayed. These results are depicted in Table 2.

Calculated Log P

The log P values for the nine naphthoquinones were calculated by means of the fragmental method (CLOGP) of Leo and Hansch, which is based on the additivity of fragmental contributions. These results are shown in Table 2. When it was neccesary, appropriate correction factors were applied.[15]

Correlation Between Lipophilic Indexes

The lipophilicity values of the naphthoquinones determined by HPLC, have been compared to calculate log P values (Figure 4). A close relationship has been found to exist between these pairs of values, according to the equation ($n = 9$, $r^2 = 0.962$, $S = 0.084$):

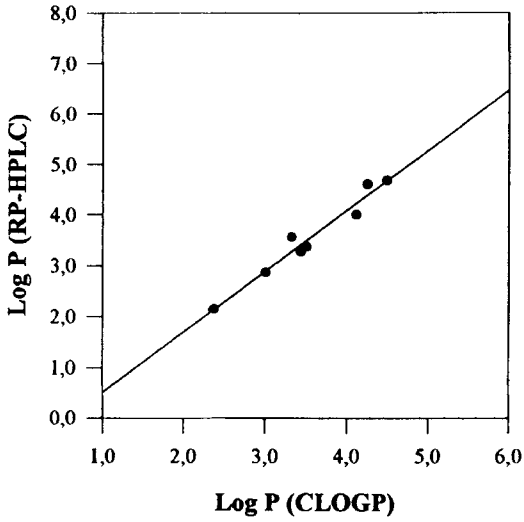

Figure 4. Correlation between calculated and experimental log P values.

$$\log P\ (\text{RP-HPLC}) = 1.19\ (\pm 0.11)\ \log P\ (\text{CLOGP}) - 0.68\ (\pm 0.44) \quad (2)$$

The plot indicates a linear relationship between the experimental and calculated values, with a slope closer to unity, that allows us to postulate that lipophilicity of new isoxazolyl-naphthoquinone analogues could be predicted from their retention in HPLC using Eq. 2. However, in Fig. 4 we can see that the data point of 3c appreciably deviated from linearity and exhibited a lipophilicity much higher than other analogues.

Using the RP-HPLC technique, we observed a log P value of 7.44 for 3c, which lies above the upper limit of accuracy (log P = 4.60) for most experimental methods for measuring log P. For this reason, the log P values of very lipophilic molecules are calculated, rather than measured.[17] This latter fact obviously indicates the limit of the applicability of Eq. 2 for very lipophilic compounds.

On the other hand, it may be interesting to compare the log P values of the keto/enol tautomers of the three series: a, b, and c. In all cases, it was observed that the enol forms, as was established in other works for related compounds,[15] are always more lipophilic and, in addition, 3c has the highest lipophilicity in the group of compounds studied.

CONCLUSIONS

We conclude, that with some few exceptions (very lipophilic compounds), the log P values of the isoxazolyl-naphthoquinone derivatives in n-octanol/water can be determined using RP-HPLC, and that the log P of all these compounds can be calculated from the theoretical method of Leo and Hansch.

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REFERENCES

1. A. Fernández, M. M. de Bertorello, R. y Manzo, "Síntesis y Propiedades Espectroscópicas de 1,2-naftoquinona-4-aminoisoxazoles", Anal. Asoc. Quím. Argentina, **70**, 49 (1982).

2. C. Ortiz, M. Longhi, M. M. de Bertorello, M. Briñón , "Synthesis of bis-Isoxazolylnaphthoquinones", Org. Prep. Proc. Int., **23**, 181 (1991).

3. M. Longhi, M. M. de Bertorello, "Isoxazoles VI. Aspects of the Chemical Stability of a New Naphthoquinone-Amine in Acidic Aqueous Solution", J. Pharm Sci., **79**, 754 (1990).

4. P. Bogdanov, I. Albesa, N. Sperandeo, M. M. de Bertorello, "Actividad Antibacteriana on nitro de Isoxazolilnaftoquinonas, Rev. Arg. Microbiol., **25**, 119-128 (1993).

5. I. Albesa , P. Bogdanov, A. Eraso, N. Sperandeo, M. M. de Bertorello "Antibiotic Activity of Isoxazolylnaphthoquinone Imines on Mice Infected with Staphylococcus aureus", J. Applied Bacteriol., **78**, 373-377 (1995).

6. M. Schwarcz, S. Goijman, M. Molina, A. Stoppani, "Effects of Isoxazolyl-naphthoquinoneimines on Growth and Oxygen Radical Production in Trypanosoma cruzi and Crithidia fasciculata", Experientia, **46**, 502-505 (1990).

7. V. Narayanan, Ph.D. (National Cancer Institute, USA), personal communication.

8. A. Leo, C. Hansch, D. Elkins, "Partition Coefficients and their Uses", Chem. Rev., **71**, 525 (1971).

9. R. Silverman, **The Organic Chemistry of Drug Design and Action**, Academic Press, San Diego, 1992.

10. H. Kubinyi, **QSAR: Hansch Analysis and Related Approaches**, vol. 1, VCH Publishers, New York, 1993.

11. F. Camps, O. Colomina, A. Messeguer, F. Sánchez, "Evaluation of Liquid-Liquid Partition Coefficients of Precocenes and Related Analogues by HPLC",J. Liq. Chromatog., **91**, 23 (1986).

12. C. Yamagami, M. Yokota, N. Takao, "Hydrophobicity Parameters Determined by RP-HPLC", Chem. Pharm. Bull., **42**, 907 (1994).

13. J. Thomas, O. Adetchessi, C. Jarry, "High Performance Liquid Chromatography of New 5-Aryloxy-Methyl-2-Oxazolines: A Comparative Study of their Lipophilicity", J. Liq. Chromatogr., **18**, 1429 (1995).

14. D. Schmit, J. Votaw, R. Kessler, T. De Pauli, "Aromatic and Amine Substituent Effects on the Apparent Lipophilicities of N-[(2-Pyrrolidinyl)-methyl]-Substituted Benzamides", J. Pharm. Sci., **83**, 305 (1994).

15. A. Leo, "Clculating log P_{oct} from Structures" Chem. Rev., **93**, 1282 (1993).

16. A. Kibara, H. Hohda, N. Hirata, M. Hirose, T. Nakagawa, "Evaluation of Solute Hydrophobicity by RP-HPLC Using Aqueous Binary Mobile Phases", Chromatographia, **29**, 275-288 (1990).

17. C. Lipinski, E. Fiese, R. Korst, "pKa, log P and MedChem CLOG P Fragment Values of Acidic Heterocyclic Potential Bioisosteres", Quant. Struct.-Act. Relat., **10**, 109-117 (1991).

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