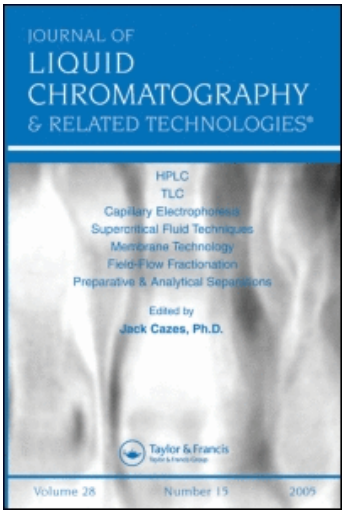

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DETERMINATION OF PARTITION COEFFICIENTS FOR SEVERAL PROPRANOLOL ANALOGUES BY REVERSED PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

The partition coefficients, log P_{oct}^{app}, for a series of propranolol analogues have been obtained by reversed phase-high performance liquid chromatography (RP-HPLC). A Collander type equation was set for 22 different standards that have the same trend as their H-bond capability. Fluorinated propranolol analogues are known to possess enhanced pharmacological properties and thus they were also investigated according to their capability of forming H-bonding.

It was found that these fluorinated analogues do behave differently from their H-substituted counterparts. The results suggest that this behaviour may not be related to the number of fluorine atoms in the side chain, but rather to the strong polarisation of the molecules.

INTRODUCTION

Propranolol is considered the model parent drug for non-selective β-adrenergic blockers and been used for the treatment of illnesses such as hypertension, angina pectoris, supraventricular, and ventricular arrhythmias and reducing the frequency and intensity of migraine headaches.[1] However, it has various side effects such as mental depression, nausea, vomiting, light-headedness, and visual disturbances.[2,3] Recently, increasing interest has been devoted to the synthesis of fluorinated medicinals since fluorine leads to strong polarization of the molecules and increases their biological activity and enhances their pharmacological properties.[4,5]

In search for more potent and less toxic β-adrenoceptors antagonists, several propranolol analogs were synthesized as a racemic mixture which included three fluorinated analogs.[6] The chemical structures of these analogs are shown in (Table 1).

The synthesis and pharmacological activity of these analogs were recently presented.[6,7] Aboul-Enein et al.[8,9] also studied the enantiomeric separation of these analogs on several polysaccharide-type chiral stationary phases.

The quantitative relationship between the structure of solutes and their chromatographic retention (QSRR) may be of interest due to the possibility of explaining the mechanisms of chromatographic separations and the characterisation of the solute physicochemical properties which is important for reactivity and bioactivity.[10]

Our on-going study on QSRR[11-13] has enabled us to carry out the current research in order to investigate the effect of various substitution patterns for several propranolol analogues as compared with the parent drug propranolol which is used as a reference compound.

The aim of this paper is to address the effect of modifying the α-substitution at naphthalene to β-substitution; the side chain (fluorinated versus nonfluorinated) and also the role of naphthalene versus phenyl groups. To undertake this, we have used RP-HPLC measurements as a mean of obtaining partition coefficients for all studied compounds.

Table 1

Chemical Structures and Values of Log k_w^{app} and Log P_{app} for Propranolol and Analogues Used in this Study

Compound	Ar-O-CH_2-CHOH-CH_2-NHR_1 R1	Ar	Log $P_{HPLC\ (app)}$(C8)	Log k_w^{app}
A. 1-Napthyloxyaminopropan-2-ol				
1 (Propranolol) Hydrochloride)	$CH(CH_3)_2$	1-naphthyl	-0.413*	-0.163
2-H_2	$CH_2CH_2CH_2CH_3$	1-naphthyl	-0.332	-0.093
3-H_3	$CH_2CH_2CH_3$	1-naphthyl	-0.234	-0.011
4-H_4	CH_2CH_3	1-naphthyl	-0.151	-0.060
5-H-tB	$C(CH_3)_3$	1-naphthyl	-0.302	-0.068
6-F_2	$CH_2CF_2CF_2CF_3$	1-naphthyl	-0.547	-0.275
7-F_3	$CH_2CF_2CF_3$	1-naphthyl	-0.326	-0.089
8-F_4	CH_2CF_3	1-naphthyl	-0.456	-0.198
B. 2-Naphthyloxyaminopropan-2-ol				
9	CH_2CH_3	2-naphthyl	-0.399	-0.150
10	$CH_2CH_2CH_3$	2-naphthyl	-0.286	-0.055
11	$CH_2CH_2CH_2CH_3$	2-naphthyl	-0.404	-0.154
12	$C(CH_3)_3$	2-naphthyl	-0.256	-0.029
C. Phenyloxyaminopropan-2-ol				
13	CH_2CH_3	phenyl	-0.377	-0.132
14	$CH_2CH_2CH_3$	phenyl	-0.334	-0.095
15	$CH_2CH_2CH_2CH_3$	phenyl	-0.328	-0.090
16	$C(CH_3)_3$	phenyl	-0.412	-0.161

* Literature value: -0.43[23].

This approach was used because log $P_{RP\text{-}HPLC}$ may give some good insight into the hydrogen bonding capabilities of solutes and also allow their values to be used in QSRR and quantitative structure-activity relationships (QSAR) studies.[10-13]

EXPERIMENTAL

The RP-HPLC experiments were recorded on a Shimadzu instrument equipped with two LC-10AD pumps, UV detector SPD-6AV set at 254 nm and LC-R6A Chromapac recorder. The stationary phase was 5 μM C_8 NUCLEOSIL

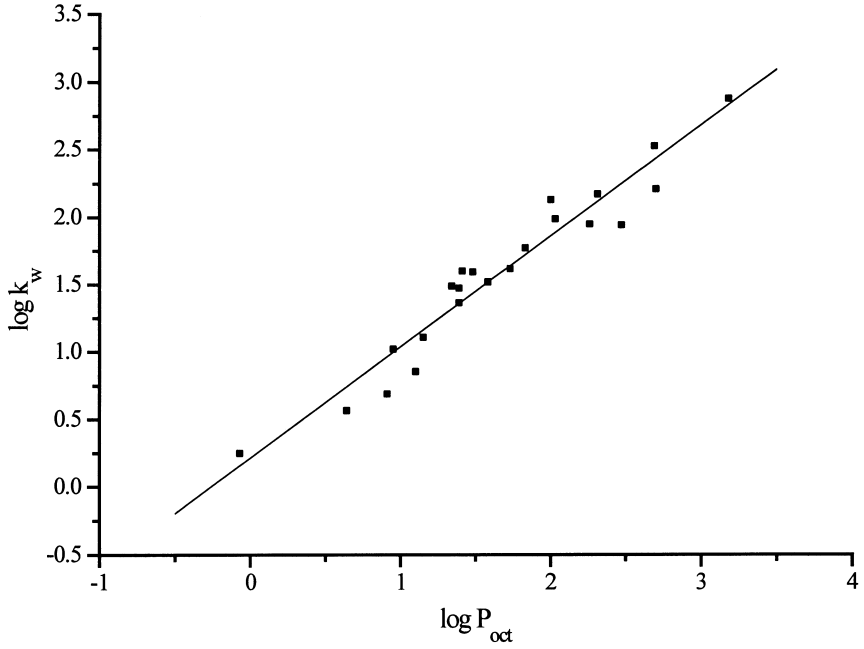

Figure 1. Correlation between log k_w and log P for the standards as depicted from Figure 2.

column (250 x 4.6 mm) obtained from Sigma-Aldrich, St. Louis, MO. The mobile phase consisted of a buffer of 5 x 10^{-3} M of phosphoric and 5 x 10^{-3} M glacial acetic acids adjusted to pH 4.6 by the addition of 1 M sodium hydroxide and methanol as organic modifier. The pH was measured before the addition of methanol since we are using a polycratic measurement.

The methanol content in the mobile phase composition (φ_{MeOH}) was in the range of 25/75 (% v/v). The flow rate of 1 mL/min was used throughout all the experiments. The retention time of sodium nitrate, detected at 214 nm, was used as the column dead-time (T_O) which is equal to 2.774 min.

RESULTS AND DISCUSSION

The set of compounds studied included 11 propranolol analogues (α and β substitutions to the naphthalene ring), and 4 analogues having phenyl ring, previously synthesised,[6] the structure of these compounds are shown in Table 1. Log k and log k_w have been determined for such compounds through the RP-HPLC measurements as described in the experimental section. The use of a

single C-8 column has proven to be very reproducible for such protonated compounds in deriving log P measurements (see below). The comparison between log P allows validation of these complex structures and their charge effects as they are fairly solved by RP-HPLC chromatographic whose values might be useful in QSAR studies.

The calibration of C-8 column has been done through a set of standards. Equation 1 shows the relationship between log k_w and log P_{oct} for the used standards as measured for methanol/water content and literature values, respectively.[14] The correlation between log k_w and log P_{oct} is shown in Figure 1.

Model for the Standards

$$\log k_w = 0.844(\pm 0.11)\log P_{oct} + 0.187(\pm 0.20) \quad (1)$$

$$(n = 22,\ r = 0.963,\ s = 0.185,\ F = 254{,}25\ \ r^2_{cv} = 0.910)$$

Equation 1 can be regarded as a good Collander type equation[15] and then C-8 column can be used for the measurement of log P_{oct}. Log k_w can, thereof, also be known as a good descriptor for log P_{oct}. This is also due to the homo-energetic interactions which are essentially the same and occur in these two-phase systems.[16] As the partitioning is guaranteed through Equation 1, this homo-energetic interaction is of fundamental importance since a pure retention mechanism is taking place.

It has been suggested that the slope obtained for log k versus φ_{MeOH} (the methanol content of mobile phase), S, is a measure of lipophilicity;[17-19] straight lines in the composite diagram found in Figure 2, for all standards, are nearly parallel, which supports the same mechanism of retention and the capability of S being responsible for the hydrogen bond activity.[19]

The chromatographic retention mechanism is dependent, basically, on two components: the solute's size (measured by its volume or surface area) and its capacity of establishing hydrogen bond. It has been shown that the slope, S, versus log k_w can display differences in the capacity of forming hydrogen bonding for a set of compounds.[20]

Figure 3 (the straight lines are fictitious and non-numerical and serve only to show the general behaviour of S dependence upon log k_w). S values measure the hydrogen bond characteristics of all studied compounds. Since only one straight line for all compounds was obtained (Figure 3), we may suggest that the hydrogen bond formation for all compounds studied behaves in the same manner.

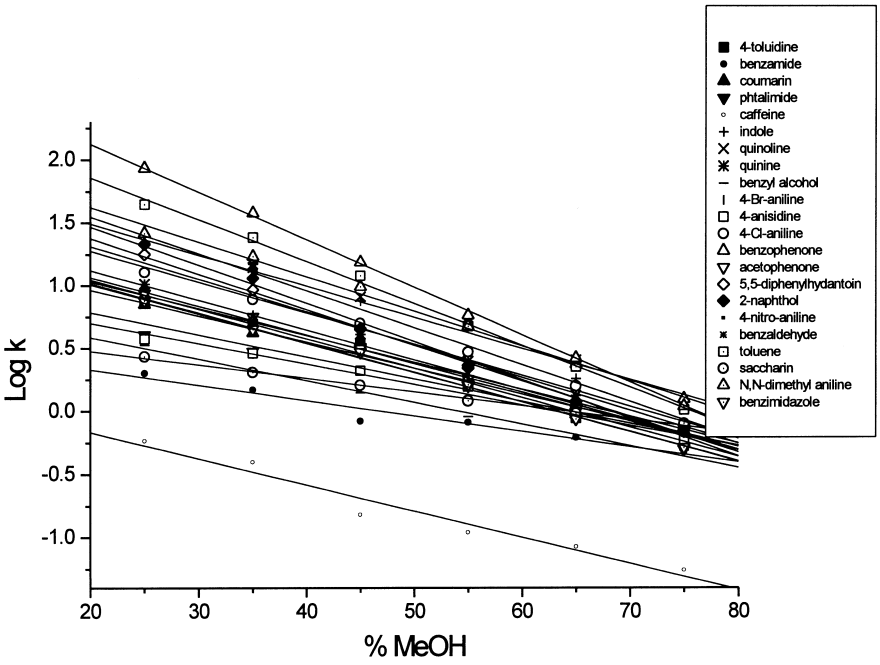

Figure 2. Composite diagram for the standards.

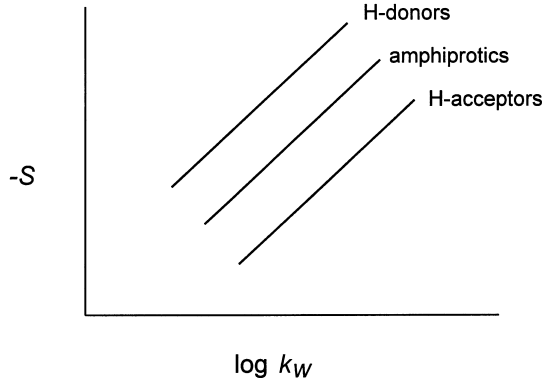

Figure 3. Generalised composite diagram showing the H-bonding capability for fictitious data.

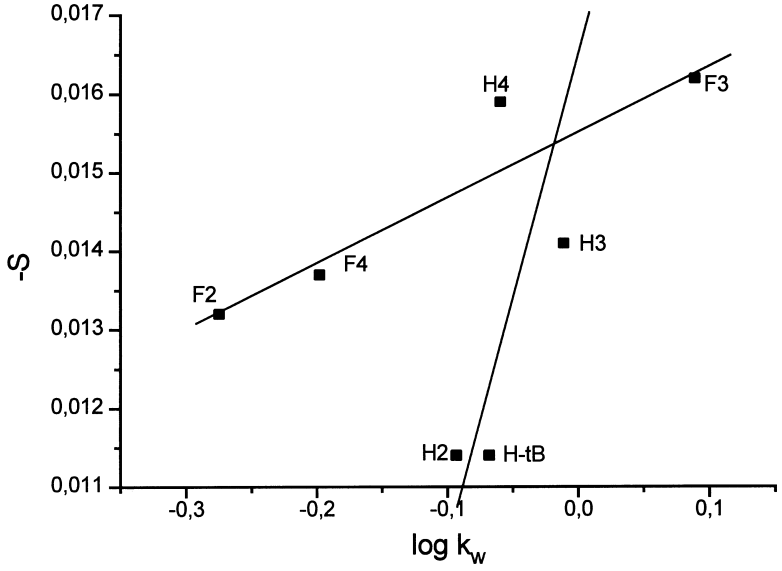

Figure 4. Hydrogen bonding behaviour for compounds belonging to 1-naphthyloxyaminopropan-2-ol (compounds 1-8 in Table 1). The two lines are not fitted to data points, but rather show the different trend capacity of forming hydrogen bonds.

We have carried out this study for the propranolol analogues and have found quite interesting results for 1-naphthyloxyaminopropan-2-ol with two sets of hydrogen and fluorine isoteric analogues (compounds 1-8), Figure 4. It is of interest to mention that for both of them there is a separate straight line, i.e. they can be classified to two different classes of hydrogen-bonding compounds.[14] These results are in agreement with chemical structures for such propranolol compounds: the first one belonging to the aliphatic hydrocarbon series and the last to the fluorinated ones.

The test set used in this work covers a narrow range of lipophilicity from log P_{app} = -0.547 to -0.151, Table 1, and all of them have a hydrophilic behaviour towards the C-8 column. The observed high correlation of log P_{oct} and log k_w confirms the results and conclusions presented by Minick.[19,21] For this range of logs P_{app} values there is no need for adding any OH-suppresser upon C-8 column.

A plot of S versus log k_w, for the standard compounds can be found in Figure 5, a straight line with a correlation coefficient equal to 0.976, if caffeine is excluded and 0.906 for the whole set.

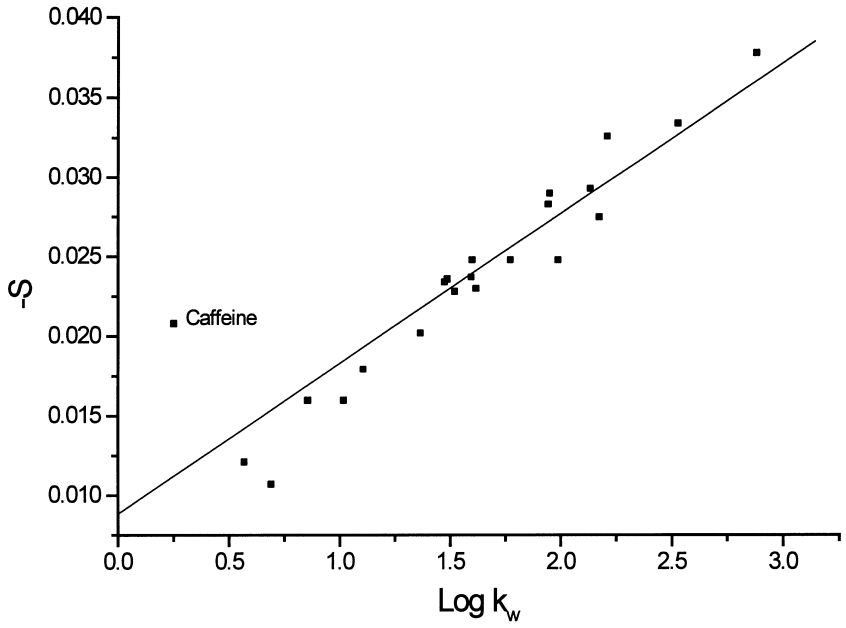

Figure 5. Linear dependence of *S* over log k_w for the standards.

It means that all of them behave similarly and can, therefore, be evaluated in the same way. There is no clear separation between their capacity of forming hydrogen bond. Thus, it seems obvious that for this descriptor they do have the same sort of retention to the chromatographic column used in this study.

Finally, a monoprotic substance partitions in two forms: neutral and ion-pair, often with a log P difference of 3-4 units,[22] which should be seen in the chromatographic measurements. However, this is not the case so far in our studies since we have only one peak, i.e. propranolol and its analogues have been eluted as a hydrochloride, as suppressed by the buffered mobile phase. Thus, it is important to point out the log P_{HPLC} measurement carried out for the reference compound, is propranolol hydrochloride, and has a quite similar magnitude value for the one that determined octanol log P, at pH 2.0, which is –0.45.[23]

Propranolol base has a log P_{oct} in the range of 0.73-3.54 at pH of 7-7.5, which are not for ion-corrected values.[23] The lipophilicity profile log D versus pH for propranolol[22] shows that in the pH range of 3(or less) till ca. 7 (zone of zero-slope) there is no log D (distribution coefficient: "apparent" partition, log P_{app}) variation through pH change, and this fixes the log Ps.

Our results thus show the RP-HPLC single peaks as a good and straightforward way of getting log P for such compounds. Overall, propranolol analogues have a log $P_{RP\text{-}HPLC\ app}$ in the range of (–0.547) – (-0.151), i.e. all of them have a hydrophilic character when measured via a C-8 column, that resembles quite well an octanol/water system. It seems that there is no ion-pair effect since all peaks are single and sharp. The pH of 4.6 also seems to be a good choice as depicted by the distribution profiles and, thus, no correction for ionisation is needed and, therefore, the log Ps are fixed. There is a clear difference for fluorinated and non-fluorinated propranolol analogues, and their H-bonding capabilities can be envisaged from these partitioning studies. It is of interest to mention that this difference was not related to the number of fluorine atoms in the side chain, but rather to the strong polarization of the molecules due to the strong inductive effect induced by the strong electronegative fluorine atoms.

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