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LIPOPHILICITY MEASUREMENTS OF PROTONATED BASIC COMPOUNDS BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

II. PROCEDURE FOR THE DETERMINATION OF A LIPOPHILIC INDEX MEASURED BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

The lipophilic properties of a series of basic neuropharmacological compounds were determined by reversed-phase high-performance liquid chromatography. Apparent capacity factors ($\log k_x$, pH = 7.5) were measured at different compositions of the methanol–water eluent and extrapolated to 100% water as the eluent. While parabolic extrapolation was feasible only for a limited number of compounds, linear extrapolation to $\log k_w$ values was possible for all 25 drugs investigated. Correction for solute ionization yielded $\log k_w^0$ values which correlate well with published water–octanol partition coefficient ($\log P_{oct}$) values. The best procedure to obtain $\log k_w$ values is discussed.

INTRODUCTION

The lipophilic properties of drugs play an important rôle in drug design. The distribution of solutes between water and *n*-octanol, as expressed by the partition coefficient ($\log P_{oct}$), is often used as an index of lipophilicity. Extensive compilations of experimental partition coefficients are available[1]. Hydrophobic substituent and fragmental constants have been developed for the calculation of $\log P$ values[2–6]. Many studies of quantitative structure–activity relationships (QSARs) indicate that experimental partition coefficients should be used whenever possible.

The traditional shake-flask method used for the measurement of $\log P$ values has a number of practical disadvantages such as, (a) slowness, (b) large errors caused by small impurities with strong chromophores and (c) limitation to $\log P$ values above -2 and below 4. Partition chromatography is being explored as an alternative means of measuring lipophilicity. In reversed-phase thin-layer chromatography (RP-TLC) using a support impregnated with an organic phase, *e.g.*, octanol, silicone oil, a number of R_M values are linearly related to $\log P_{oct}$[7–10]. This method thus

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provides a rapid and reproducible technique for obtaining an hydrophobic index for many drugs[11]. However, problems with standardization of plates in order to obtain daily reproducibility and some limits of detection make this procedure of limited practical use.

High-performance liquid chromatography is considerably more promising. The availability of alkyl-bonded phases (RP-HPLC) provides a simple, accurate and reproducible method to determine the lipophilic character of a wide variety of compounds. As eluent, aqueous solvent mixtures have been used, consisting of water and organic modifiers such as methanol, tetrahydrofuran or acetonitrile. A number of studies[12–14] indicate that a monolayer of methanol molecules is adsorbed on the alkyl-bonded stationary phase from methanol–water mobile phases. Therefore, it can be suggested that the stationary phase has a partly polar character and thus resembles the *n*-octanol phase in *n*-octanol–water systems, implying that there is no need to use an octanol-coated column technique[15,16]. In addition, the transfer equilibrium of solute molecules between the mobile and stationary phases (expressed by the capacity factor, k) takes place in a dynamic manner as in biomembranes. However, the presence of free silanol sites on the stationary phase influences the mechanism of retention, and the observed retention behaviour becomes the result of at least two processes, namely partition and adsorption. These adsorption interactions can be suppressed either by using a deactivated column[17] or by adding to the eluent a lipophilic basic compound such as *n*-decylamine or N,N-dimethyloctylamine[18–20] which competes with the solute for the silanol sites.

In the preceding paper[21], the retention behaviour of a series of basic compounds was investigated. A minimum was observed in the relationship between log k and the methanol content (x) in the eluent. The methanol concentration at which the minimum in the capacity factor was found depends on the proportion of protonated species. At high pH, the proportion of protonated species decreases, and as consequence the magnitude of the ionic interaction between the solute and solvent molecules will decrease. The minimum is thus displaced to higher methanol concentrations or even disappears for compounds in their un-ionized forms.

This paper describes and discusses an improved RP-HPLC procedure to determine a lipophilic index for basic compounds. Capacity factors were measured at a pH of 7.5 which is close to the maximum value (pH = 8) permitted by the present HPLC technique and allows a fair proportion of neutral solute molecules to be present. The transformation of apparent log k_w values (pH = 7.5) to log k_w^0 values (neutral species) requires the knowledge of pK_a values which were measured when not available.

EXPERIMENTAL

Materials

All compounds were of the best available purity and were obtained from different pharmaceutical companies. Analytical grade methanol and 3-morpholinopropanesulphonic acid were purchased from Merck (Darmstadt, F.R.G.).

Chromatography

A Siemens S101 chromatograph equipped with an Orlita pump Type DMP-

AE 10.4 was used. The detector was a Uvikon 740 LC model (Kontron) operating at 254 nm; the column (25 cm × 4 mm I.D.) was prepacked with LiChrosorb RP-18, particle size 10 μm (Knauer). A Hewlett-Packard 3390A integrator was used for peak registration and calculation of retention times. *n*-Decylamine (0.2%, v/v) was used as masking agent to eliminate silanophilic (adsorption) interactions. The pH was adjusted to 7.5 in the aqueous solution by use of hydrochloric acid. The buffering agent and preparation of the mobile phase were as described previously[21].

Measurement of dissociation constants by potentiometry

Dissociation constants (pK_a) were calculated from data obtained by titrating solutions of the salts of basic compounds of various concentrations with standard sodium hydroxide. Titration curves were recorded using the following Metrohm equipment: Dosimat E535, potentiograph E536 combined with a glass electrode EA125, temperature probe EA 911-Pt-100. The temperature was kept at 25.0 ± 0.1°C using a Heto 01T623 thermostat. All the water used was CO_2-deprived. Some basic compounds were available as hydrochloric acid salts. The salt was dissolved (*ca.* $7.5 \cdot 10^{-4} M$) and titrated with 0.01 *M* sodium hydroxide; potassium chloride was added to obtain an ionic strength of 0.1. Compounds supplied as the free base were dissolved in the stoichiometric amount of hydrochloric acid before making up to volume with CO_2-free water. Other compounds were supplied as a salt of a weak acid such as maleate and phosphate. Since the weak acid affected the titration curve, it was necessary to convert the compound into a salt of a strong acid.

The pK_a values were calculated using a non-logarithmic linearization of the titration curve proposed by Benet and Goyan[22] and modified by Leeson and Brown[23] to overcome the problem of dilution during titration. For the back titration of a base as a salt of a strong acid, eqn. 1 applies

$$Z' = A^0 - (1/K_a)\, Z'[H^+] \tag{1}$$

where A^0 = number of moles of salt present at the beginning of the titration, $Z' = M - H^+ + OH^-$ and M, H^+ and OH^- are the number of moles of strong base titrant, hydrogen ion and hydroxyl ion, respectively; K_a is the stoichiometric dissociation constant. For each titration curve, 10–30 points were calculated and the slope ($1/K_a$) and intercept (A^0) in eqn. 1 obtained by linear regression. Titration curves were determined in triplicate for each compound. All calculations were performed with an Apple III microcomputer using a program written in BASIC.

RESULTS

The experimental results which form the basis of the present study are capacity factors (log k_x values) at a fixed pH value of 7.5 with eluents having the broadest possible range of methanol–water ratios (Table I). Such capacity factors however are too condition-dependent to be useful as lipophilicity indices (see Discussion), and were therefore (a) extrapolated to 100% water as eluent (log k_w values), and (b) corrected for solute ionization (extrapolation to 100% neutral species, $\log k_w^0$).

The plots of methanol content *versus* log *k* (pH 7.5) (not shown) either display a minimum and appear as parabolic (hydrophilic compounds) or are linear (lipophilic

TABLE I

RP-HPLC CAPACITY FACTORS OF NEUROLEPTIC COMPOUNDS AT pH 7.5

Underlined log k values are observed minima; a broken line indicates that the minimum was not reached.

*Compound**	$\log k_{80}$	$\log k_{70}$	$\log k_{60}$	$\log k_{50}$	$\log k_{40}$	$\log k_{30}$	$\log k_{20}$	$\log k_{10}$
Tiapride	−0.115	−0.331	−0.463	−0.611	−0.663	−0.661	−0.623	−0.580
Prosulpride	−0.096	−0.258	−0.348	−0.372	−0.321	−0.301	0.048	0.331
Tigan	0.049	0.121	0.360	0.467	0.667	0.840	1.015	1.172
Amisulpride	0.039	−0.048	−0.154	−0.153	−0.036	0.165	0.416	0.813
Clozapine	0.486	0.782	1.091	1.432	–	–	–	–
Tropapride	−0.005	0.110	0.277	0.529	0.877	1.208	–	–
YM 09151-2	0.405	0.709	1.052	1.520	–	–	–	–
Domperidone	0.108	0.395	0.774	1.300	–	–	–	–
Halopemide	0.237	0.548	1.095	1.570	–	–	–	–
trans-Flupenthixol	0.789	1.215	1.676	–	–	–	–	–
Fluphenazine	0.574	0.990	1.512	–	–	–	–	–
cis-Flupenthixol	0.765	1.206	1.686	–	–	–	–	–
Chlorpromazine	0.782	1.133	1.423	–	–	–	–	–
Thioridazine	0.817	1.230	1.584	–	–	–	–	–
Pimozide	0.371	0.873	1.540	–	–	–	–	–

* The corresponding values for sulpiride, sulmepride, alizapride, clebopride, metoclopramide, flubepride, pipamperone, haloperidol and spiperone can be found in Table IV of the preceding paper[21] and are not repeated here.

compounds). Some intermediate cases exist. However, for all 25 compounds investigated, a linear extrapolation to log k_w can be performed using either log k_x values measured at low methanol contents for the hydrophilic compounds ($r > 0.99$, $n \geqslant 3$), or all log k_x values measured (lipophilic compounds, $r > 0.99$). The resulting log k_w values are collected in Table II.

Whenever feasible, quadratic extrapolations to log k_w were performed using the parabolic equation of Schoenmakers *et al.*[24] (Table II). A good correlation between log k_w values of linear and quadratic origin is observed for those compounds which could be studied over a wide range of eluent composition ($10 \leqslant x \leqslant 80$), *i.e.*, the most polar compounds. This correlation is expressed by eqn. 2:

$$\log k_w(\text{quadr}) = 1.196\ (\pm\ 0.134)\ \log k_w(\text{lin}) + 0.009\ (\pm\ 0.171) \qquad (2)$$

$$r = 0.991,\ s = 0.145,\ n = 10,\ F = 423$$

In contrast, the quadratically obtained log k_w values of less polar compounds are much higher than those obtained by linear extrapolation. This is due to the fact that only a part of the parabola is within experimental reach. Moreover because lipophilic compounds cannot be eluted at low methanol concentrations ($x < 50$) due to excessive retention, the calculated parabola has little statistical significance and yields misleading intercepts.

It thus appears that despite parabolic or partly parabolic plots of methanol content *versus* log k, quadratic extrapolation to log k_w values can be readily performed only for quite polar compounds (10 out of 25 compounds in the present broad series). In contrast, linear extrapolation was feasible for all compounds.

In order to compare the log k_w values with published log P_{oct} values and thus

TABLE II

IONIZATION CONSTANTS AND LIPOPHILICITY VALUES OF A SERIES OF NEUROLEPTIC COMPOUNDS

Compound	pK_a*	*log* k_w**		*log* k_w^{0}*** *neutral*	*log* P§
		linear	*quadratic*		
Tiapride	9.14	−0.540 (±0.003)	−0.450 (±0.026)	1.110	0.66
Sulmepride	8.73	0.504 (±0.043)	0.646 (±0.030)	1.759	–
Prosulpride	8.99	0.658 (±0.041)	0.742 (±0.071)	2.162	–
Sulpiride	9.12	0.613 (±0.021)	0.728 (±0.019)	2.243	0.58/−0.50
Tigan	8.78	1.354 (±0.021)	1.472 (±0.046)	2.656	2.29
Sultopride	9.40	0.765 (±0.008)	0.926 (±0.043)	2.670	1.20
Alizapride	7.48	2.524 (±0.263)	4.167 (±0.189)	2.815	–
Metoclopramide	9.27	1.052 (±0.009)	1.202 (±0.038)	2.829	2.74/2.32
Amisulpride	9.37	1.113 (±0.091)	1.249 (±0.035)	2.989	–
Pipamperone	8.28	2.378 (±0.065)	2.982 (±0.110)	3.224	2.40
Clozapine	7.50	2.994 (±0.048)	3.454 (±0.088)	3.295	3.93
Flubepride	8.25	2.693 (±0.090)	3.400 (±0.119)	3.514	2.08
Tropapride	8.91	2.136 (±0.069)	2.734 (±0.099)	3.563	–
Clebopride	8.19	2.860 (±0.190)	4.771 (±0.167)	3.631	3.70/<3.46
YM 09151-2	7.82	3.527 (±0.219)	4.999 (±0.398)	4.017	4.07
Domperidone	7.90§§	3.538 (±0.256)	5.664 (±0.255)	4.084	3.90
Haloperidol	8.66§§	3.106 (±0.178)	4.415 (±0.108)	4.295	3.36/4.31/4.18
Halopemide	7.82§§	4.139 (±0.126)	5.498 (±1.426)	4.629	4.02
trans-Flupenthixol	7.80§§	4.331 (±0.071)	–	4.807	4.51/4.25
Fluphenazine	7.90§§	4.308 (±0.261)	–	4.854	4.36/4.52/3.49
Spiperone	9.09§§	3.284 (±0.197)	4.685 (±0.109)	4.885	4.04/3.03
cis-Flupenthixol	7.80§§	4.442 (±0.079)	–	4.918	4.51/4.25
Chloropromazine	9.36§§	3.363 (±0.124)	–	5.229	5.05/5.35/4.04
Thioridazine	9.50§§	3.896 (±0.120)	–	5.900	5.65/5.53/5.90
Pimozide	8.63§§	5.018 (±0.336)	–	6.179	6.23/4.88

* Measured in our laboratory with a standard deviation < 0.03 except for YM 09151-2 (±0.23).

** Values in parentheses are the standard deviation of the intercept.

*** Linearly obtained log k values corrected for ionization.

§ Taken from the literature[25,35–38]; in the case of many log P values, only the first value was used in the correlation analysis (eqn. 4).

§§ Taken from the literature[8,33,34].

to assess their interest as a lipophilicity index, they were corrected for solute ionization using eqn. 3 and the pK_a values shown in Table II:

$$\log k_w^0 = \log k\ (\mathrm{pH} = 7.5) + \log (1 + 10^{pK_a - 7.5}) \quad (3)$$

The resulting log k_w^0 values are collected in Table II. For 20 compounds in Table II, log P_{oct} values are reported in the literature. The relationship between these two sets of values is expressed by eqn. 4

$$\log P_{oct} = 1.142\ (\pm\ 0.212) \log k_w^0 - 0.998\ (\pm\ 0.876) \quad (4)$$

$$r = 0.937,\ s = 0.562,\ n = 20,\ F = 129$$

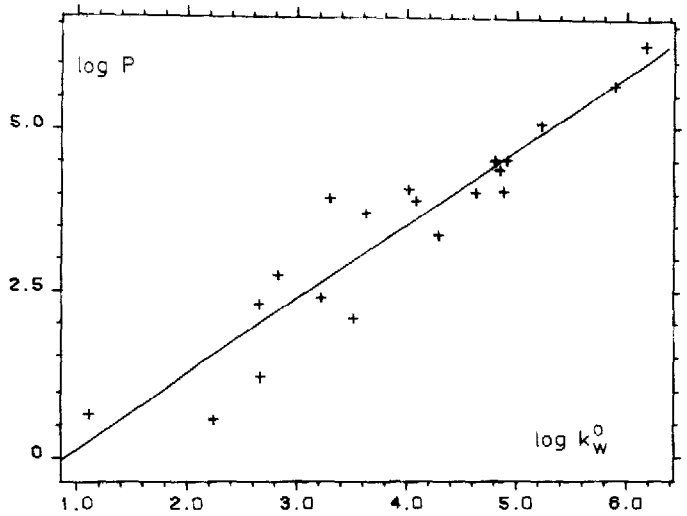

Fig. 1. Relationship between published log P_{oct} values and log k_w^0 values determined in the present study for 20 neuroleptics.

where the uncertainties are 95% confidence limits. This equation shows a good correlation despite the uncertainties in log P_{oct} values taken from various sources. The same relationship is shown in Fig. 1, which indicates that the observations are well distributed over the range of variables, but that deviations are larger for the less lipophilic compounds.

DISCUSSION

Capacity factors (log k) obtained from RP-HPLC measurements can be taken as a lipophilic index in three different ways. First, isocratic capacity factors, which are the capacity factors measured at a certain eluent composition x (log k_x), are used to calculate octanol–water partition coefficients (log P_{oct}) using a Collander-type equation

$$\log P_{oct} = a \log k_x + b \tag{5}$$

based upon the observed similarity in the hydrophobic partitioning processes occurring in both systems. The estimated log P values have been used in QSAR studies[25–28].

However, different classes of compounds were described by separate lines on a log k_x *versus* log P_{oct} plot when measured in acetonitrile–water (50:50, v/v) eluent using an octadecyl-bonded stationary phase[29]. Overestimation and/or underestimation of log P values often occurs. This may be related to the fact that single isocratic capacity factors cannot determine the hydrophobic properties of all kinds of compounds, *i.e.*, polar and non-polar. For example, the hydrophobic expulsion is relatively attenuated for polar compounds at high concentration of the organic modifier.

The second approach has been to relate isocratic capacity factors measured at a given pH directly to biological activity[30–32]. However, their extrapolation to log k values of the neutral species ("true" log k values) meets with difficulties, since pK_a values under eluent conditions must be known.

A third possibility is to use capacity factors extrapolated to 0% of the organic modifier (log k_w) in correlation with biological activity. However, the extrapolation of capacity factors to 100% water as the eluent is not straightforward, as shown in the present study. Reasons for the differences between plots of log k *versus* methanol content for various compounds have been discussed previously[21]. As a result of these studies, we suggest that the most adequate technique to determine a RP-HPLC lipophilicity index is by linear extrapolation of log k_x values to log k_w values:

(a) For neutral and/or non-polar compounds, in the range $10 \leqslant x \leqslant 80$

(b) For ionogenic polar compounds, in water-rich ranges of eluent composition

The log k_w values thus obtained can be corrected for solute ionization using pK_a values measured in water, yielding log k_w^0 values which were shown to yield a good correlation with literature log P_{oct} values over a very broad range of lipophilicity.

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