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Correlation between n-octanol/water partition coefficient and liquid chromatographic retention for caffeine and its metabolites, and some structure-pharmacokinetic considerations

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A method to establish the correlation between the reverse phase high performance liquid chromatographic retention of caffeine and its metabolites and their n-octanol/water partition coefficients is described. The log (P) values were always below zero, ranging from −0·02 to −2·03. The capacity factor quadratically back-extrapolated to 100% water eluent (k'_w) was used as the index of lipophilicity. The compounds examined gave a good correlation ($r > 0·99$) with $\log(k'_w)$ if considered in separate series, depending on the substituent position. For a structure–pharmacokinetic relationship study, correlations were found between the partition coefficient and some pharmacokinetic parameters, suggesting that for drugs that are widely metabolized, any predictions of their disposition from physicochemical characteristics are hazardous.

To evaluate the lipophilicity of a variety of compounds several authors have used reverse phase high performance liquid chromatography (HPLC) as a convenient, rapid and accurate method (Henry et al 1976; Unger & Chiang 1981; Brent et al 1983; Caron & Shroot 1984; Carney & Graham 1985). McCall (1975) discussed the relationship

$$\log(P) = \log(k') + \log(K) \qquad (1)$$

where P is the partition coefficient, K is a constant for the chromatographic system and k′ is the capacity factor for the compound in that chromatographic system.

Solute–eluent interactions play a role in the mechanism of retention. The effect on retention behaviour of an organic modifier such as methanol or acetonitrile has been described by linear relationships between the logarithm of the capacity factor, log(k′), and the per cent in volume of the organic modifier, x:

$$\log(k') = \mathbf{a}.x + \log(k'_w) \qquad (2)$$

where **a** is a constant for a given organic modifier and a given solute and $\log(k'_w)$ is the logarithm of the extrapolated capacity factor in 100% water eluent (Snyder et al 1979; Braumann & Grimme 1981; Grushka et al 1982; Hammers et al 1982; Harnish et al 1983; Dufek 1983). Jandera et al (1979) showed that the effect of organic modifiers was not strictly linear and Schoenmakers et al (1979) suggested that equation 2 was generally not valid and should be replaced by the quadratic regression

$$\log(k') = \mathbf{a}.x^2 + \mathbf{b}.x + \log(k'_w) \qquad (3)$$

In that study the $\log(k'_w)$ values were used as a lipophilicity index of the compounds for correlation with log (P).

Although many investigations have been made on the physiological and behavioural effects of caffeine (see Dews 1984), no information is available about any correlation between its physicochemical characteristics and kinetic profile and those of its metabolites.

Therefore we have investigated the behaviour of methylxanthines to assess the relationship between capacity factor, mobile phase properties (acetonitrile percentage) and solute properties (lipophilicity) and to establish the role of lipophilicity for caffeine and its metabolites in their pharmacokinetic profile in the rat (Bonati et al 1983, 1984b, 1985; Bonati & Garattini 1984; Latini et al 1984; Bortolotti et al 1985). As previously described for other compounds (Craig & Welling 1977, Jusko & Gretch 1976; Seydel et al 1980; Hinderling et al 1984), the relation between elimination rate constant, elimination half-life, total, renal, non-renal and intrinsic clearance, volume of distribution and the lipophilic/hydrophilic properties of caffeine and its metabolites is now reported.

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MATERIALS AND METHODS

Materials

All the compounds were used as supplied. Caffeine (1,3,7-TMX) and its metabolites theophylline (1,3-DMX), theobromine (3,7-DMX), paraxanthine (1,7-DMX), 1-, 3- and 7-methylxanthine (1-MX, 3-MX, 7-MX), 1,3,7-trimethyluric acid (1,3,7-TMU), 1,3-, 1,7- and 3,7-dimethyluric acid (1,3-DMU, 1,7-DMU, 3,7-DMU), 1-, 3- and 7-methyluric acid (1-MU, 3-MU, 7-MU), 6-amino-5-(*N*-methylformylamino)-1,3-dimethyluracil (1,3,7-TAU), 6-amino-5-(*N*-methylformylamino)-3-methyluracil (1,7-DAU) and 6-amino-5-(*N*-formylamino)-1,3-dimethyluracil (1,3-DAU) were generously supplied by ILSI (International Life Sciences Institute, Washington, DC, USA); 6-amino-5-(*N*-methylformylamino)-1-methyluracil (3,7-DAU) was a gift from Drs Arnaud and Philippossian (Nestlé, La Tour De Peilz, Switzerland). For ease of reference, in this work the position of the methyl substituent in uracils is numbered as in the 'parent' xanthines.

Buffer solution (pH 3·0) was prepared by adjusting the pH of 0·05 M KH_2PO_4 with phosphoric acid. HPLC grade acetonitrile was obtained from Omnia Res (Milan, Italy), n-octanol from Merck (Darmstadt, GFR) and analytical grade acetic acid from Farmitalia Carlo Erba (Milan, Italy).

Chromatography and determination of k' values

The HPLC analyses of caffeine and its metabolites have been described by Bonati et al (1980, 1982). In summary a Perkin-Elmer (Norwalk, CT, USA) Series 2/2 liquid chromatograph equipped with an LC 15 spectrophotometer operating at 254 nm was used. The column (25 cm × 4 mm i.d.) was LiChrosorb RP-18, 7 μm (Merck, Darmstadt, GFR). The mobile phases consisted of acetonitrile at various concentrations and acetic acid 0·5% (v/v) in freshly glass-distilled water. The pH of the mobile phases was adjusted to 3·0, if necessary, by addition of a few drops of acetic acid. All solutions were deaerated before use by vacuum filtration through a 0·4 μm pore size filter (Nucleopore, Pleasanton, CA, USA). Flow rate was 1·0 mL min^{-1}.

Caffeine and its metabolites were studied individually as aqueous solutions (10 μg mL^{-1}), 50 μL of each being injected for analysis. Retention times were measured at room temperature (22 °C) and the column dead time, t_0, was determined using methanol as the non-retained compound. The capacity factor, k', is defined as

$$k' = (t_r - t_0)/t_0 \quad (4)$$

where t_r is the retention time of the solute.

Each compound was run six times at different acetonitrile concentrations (5, 7·5, 10, 12·5, 15 and 20%) and the results were used to back-extrapolate $\log(k')$ to 100% water eluent, $\log(k'_w)$.

Partition coefficients

Partition coefficients were determined according to a routine laboratory shake-flask method (Bonati et al 1984a). A known amount of solute was dissolved in n-octanol-saturated buffer and partitioned with an equal volume of buffer-saturated n-octanol. The two phases were shaken for 3 h and then left to equilibrate at room temperature for 2 h. The phases were separated by centrifugation at 1500 rev min^{-1} for 15 min and the concentration in the aqueous phase was determined by HPLC. All determinations were run in triplicate. The partition coefficients, (P), of the compounds were calculated from the relationship:

$$P = V_w/V_o \times [(C_{w,o} - C_w)/C_w] \quad (5)$$

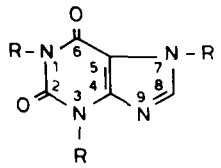
where V_W and V_o are the volumes of the aqueous and organic phases, respectively, and $C_{w,o}$ and C_w are the respective concentrations of the compound in the aqueous phase before and after shaking.

The apparent partition coefficients at pH 7·4 were calculated using the following equation:

$$P_{7.4} = P/(1 + 10^{7.4 - pK_a}) \quad (6)$$

Kinetic data

During recent years our laboratory has described various aspects of the disposition of caffeine and some of its metabolites in different mammalian species (Bonati et al 1983, 1984b, 1985; Bonati & Garattini 1984; Latini et al 1984; Bortolotti et al 1985). Data obtained in the rat at dosages showing linear kinetics have been used for the present study.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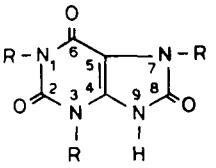
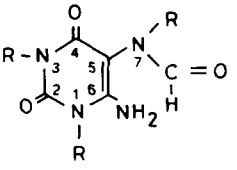

Uric acids


Uracils

Details of parameter estimates are reported in the papers cited (λ_z, elimination rate constant; $t_{\frac{1}{2}}$, elimination half-life; V, apparent volume of distribution; CL total body clearance; CL_R, renal clearance; CL_H non-renal clearance; CLu_{int}, intrinsic clearance; fraction of unbound drug, f_u).

According to the disposition profile of xanthines, their elimination from the body is retricted to hepatic metabolism and renal excretion. Although the apparent volume of distribution and clearance are the most important kinetic parameters describing the disposition profile of a drug (Rowland & Tozer 1980), in view of the number of values available and their wide utilization, the secondary parameters λ_z and $t_{\frac{1}{2}}$ were also considered.

Data analysis

Data were fitted by linear and non-linear regression iterative programs and a two-sided *t*-test was used to evaluate the significance of correlations; levels of $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Partition coefficients and capacity factors

The values of log(P) and $\log(k'_w)$ (Table 1), vary widely, these compounds having a poor lipophilicity, with log(P) values always below zero. Because caffeine and its metabolites are weak acids, pH 3·0 was chosen to determine log(P) and $\log(k'_w)$ so no corrections for ionization were needed.

Table 1. n-Octanol/water partition coefficients, log(P), and lipophilicity indices, log (k'_w) of of caffeine and its metabolities.

	Compound	log(P)	$\log(k'_w)$ Quadratic	$\log(k'_w)$ Linear
1	1,3,7-TMX	−0·07[a]	2·105	1·811
2	3,7-DMX	−0·78[a]	1·448	1·122
3	1,3,7-TMU	−0·37	1·718	1·471
4	3,7-DMU	−1·33	0·964	0·694
5	1,3,7-TAU	−1·32	1·083	0·672
6	3,7-DAU	−2·03	0·308	−0·226
7	1,7-DMX	−0·22	1·604	1·307
8	7-MX	−0·89	0·935	0·609
9	1,7-DMU	−0·22	1·429	1·077
10	7-MU	−1·18	0·625	0·205
11	1,7-DAU	−1·30	0·523	0·110
12	1,3-DMX	−0·02[a]	1·629	1·353
13	3-MX	−0·50	0·966	0·634
14	1,3-DMU	−0·52	0·935	0·702
15	3-MU	−1·08	0·338	−0·299
16	1,3-DAU	−0·64	0·859	0·522
17	1-MX	−0·27	1·105	0·753
18	1-MU	−0·57	0·684	0·010

[a] This work and from Leo et al (1971).

The quadratically obtained $\log(k'_w)$ values were higher than those obtained by linear extrapolation and a good correlation was observed between $\log(k'_w)$ values of linear and quadratic origin:

$$\log(k'_w)_{(quadr.)} = 0{\cdot}835\ (\pm\ 0{\cdot}071) \times \log(k'_w)_{(lin.)} + 0{\cdot}489\ (\pm 0{\cdot}064) \quad (7)$$
$$n = 18,\quad r = 0{\cdot}987,\quad P < 0{\cdot}001$$

Numbers in parentheses are 95% confidence limits; n is the number of compounds; r is the correlation coefficient. Despite the fact that only eluents with low acetonitrile concentration (≤20%) were considered, and partly parabolic plots of acetonitrile percentage vs log(k') were obtained, quadratic regressions gave better correlations than linear regressions for each compound. El Tayar et al (1985) found that quadratic extrapolations to $\log(k'_w)$ were readily made for quite polar compounds, and Schoenmakers et al (1979) and Toon & Rowland (1981) found that the values of k' and log(k') did not always vary linearly with mobile phase composition, and (Hanai & Hubert 1982) that curvature could occur at high or low acetonitrile levels. Garst (1984) found that curvature could be column-dependent, either concave or convex, and that it could depend on the organic modifier and on the compound.

Instead of capacity factor k', Toon et al (1984) used R_q values, where $R_q = \log[(t_r - t_o)/t_r]$, because they found R_q varied linearly with mobile phase composition even when marked non-linearity had been shown for log(k'); we found this only for the less polar compounds. For these reasons $\log(k'_w)$ extrapolated by quadratic regression was used. The relationship between log(P), the n-octanol/water partition coefficient measured by the shake-flask method, and the $\log(k'_w)$ values determined in the present study gave a poor correlation, although statistically significant, as shown by:

$$\log(P) = 0{\cdot}821\ (\pm 0{\cdot}379) \times \log(k'_w) - 1{\cdot}618\ (\pm 0{\cdot}445) \quad (8)$$
$$n = 18,\quad r = 0{\cdot}754,\quad P < 0{\cdot}001$$

This might be because of the low log(P) values for caffeine and its metabolites and confirms previous general statements (El Tayar et al 1985; Thus & Kraak 1985) that deviations from the straight line are larger for less lipophilic compounds. Unger et al (1978), noting that only small unhindered compounds deviated from regression between log(P) and log(k'), attributed these findings to probable binding to residual silinol sites.

Garst (1984) found a log(P) value of 0·63 for caffeine, far removed from the value of Anderson (−0·07) (cited by Leo et al 1971) and suggested inconsistencies in the xanthine series because theophylline, log(P) = −0·02, and theobromine, log(P) = −0·78, two dimethylated xanthines, should have similar values. Mirrlees et al (1976) found a value of −0·07 for caffeine with a Kieselguhr-packed column.

Yalkowsky et al (1983) measured the partition coefficients by the shake-flask technique and obtained values for caffeine, log(P) = −0·20, and theophylline, log(P) = −0·09, lower than those reported (Leo et al 1971); in this case also, caffeine had a lower log(P) value than theophylline.

Leo et al (1971) explored the possibility of deriving an additive group contribution to the partition coefficient; an n-octanol/water system was chosen as the standard. The group contributions or 'π values' are defined by equation 9:

$$\log(P_R) - \log(P_H) = \pi_R \qquad (9)$$

where P_R and P_H are the n-octanol/water partition coefficients for the compound containing the substituent R and the 'parent' compound, respectively. Those authors found the substituent constant mean for a methyl group was 0·56.

Table 2 lists the π values for caffeine and its metabolites. The data suggest that π values are not always additive and vary depending on the substituent position; this might be for steric reasons or electron and hydrogen bonding effects. Xanthine, uric acid and uracil derivatives are nitrogen heterocycles and substituents on the ring may undergo perturbations due to the intramolecular associations with the ring nitrogens. It is reasonable therefore to assume that π values for the substituents would differ considerably from the reported values. Moreover the nitrogens at positions 1, 3 and 7 are not equivalent: N_1 is near two carbonyl groups, N_3 is near a carbonyl group and a double bond, N_7 is in the imidazole ring near two double bonds in the xanthine derivatives and near a double bond and a carbonyl group in uric acids and uracils. Ionization constants of these compounds (Gaspari et al 1983) confirm that nitrogen atoms are quite different (hydrogen at N_1 is always less acidic than that at N_3).

The methyl group is a substituent which can donate electrons to the xanthine nucleus because of its inductive effect, and this could be related to alteration of hydrogen bonding, ring dipoles, or any combination of these and other parameters. Thus a

Table 2. π Values for caffeine and its metabolites.
(a) Substitution of a methyl group for the hydrogen atom.

Compound	'Parent' compound	Substituted position 1	3	7
1-MX	X[i]	0·72	—	—
3-MX	X[i]	—	0·49	—
7-MX	X[i]	—	—	0·10
1,3-DMX	1-MX	—	0·25	—
1,3-DMX	3-MX	0·48	—	—
3,7-DMX	3-MX	—	—	−0·28
3,7-DMX	7-MX	—	0·11	—
1,7-DMX	1-MX	—	—	0·05
1,7-DMX	7-MX	0·67	—	—
1,3,7-TMX	1,3-DMX	—	—	−0·05
1,3,7-TMX	3,7-DMX	0·71	—	—
1,3,7-TMX	1,7-DMX	—	0·15	—
1-MU	U[i]	0·69	—	—
3-MU	U[i]	—	0·18	—
7-MU	U[i]	—	—	0·08
1,3-DMU	1-MU	—	0·05	—
1,3-DMU	3-MU	0·56	—	—
3,7-DMU	3-MU	—	—	−0·25
3,7-DMU	7-MU	—	−0·15	—
1,7-DMU	1-MU	—	—	0·35
1,7-DMU	7-MU	0·96	—	—
1,3,7-TMU	1,3-DMU	—	—	0·15
1,3,7-TMU	3,7-DMU	0·96	—	—
1,3,7-TMU	1,7-DMU	—	−0·15	—
1,3,7-TAU	1,3-DAU	—	—	−0·68
1,3,7-TAU	3,7-DAU	0·71	—	—
1,3,7-TAU	1,7-DAU	—	−0·02	—
	Mean	0·72	0·10	−0·06
	± s.d.	±0·16	±0·20	±0·30

[i] Log(P) value for xanthine (X) and uric acid (U) from Leo et al (1971).

(b) Substitution of OH for the hydrogen atom.

Compound	'Parent' compound	π Value
U[i]	X[i]	−0·27
1-MU	1-MX	−0·30
3-MU	3-MX	−0·58
7-MU	7-MX	−0·29
1,3-DMU	1,3-DMX	−0·50
3,7-DMU	3,7-DMX	−0·55
1,7-DMU	1,7-DMX	0
1,3,7-TMU	1,3,7-TMX	−0·30
	Mean	−0·35
	± s.d.	±0·19

[i] Log(P) values for xanthine (X) and uric acid (U) from Leo et al (1971).

(c) Ring opening (uric acids → uracils).

Compound	'Parent' compound	π value
1,3-DAU	1,3-DMU	−0·12
3,7-DAU	3,7-DMU	−0·70
1,7-DAU	1,7-DMU	−1·08
1,3,7-TAU	1,3,7-TMU	−0·95
	Mean	−0·71
	±s.d.	±0·43

different effect on the overall lipophilicity could be expected when substitutions occur at different positions of the pyrimidine or imidazole ring, taking into account that the electron density of the two moieties should be different (as in the purine molecule).

Steric effects could also help to explain the variability of the π values of substituents. The steric hindrance of the methyl group could prevent hydrogen bonding with the carbonyl oxygen and water hydrogen; moreover these compounds in solution are capable of lactim–lactam tautomerization and the lactim tautomer can give hydrogen bonding with water oxygen. When hydrogen is replaced by a methyl group, lactim tautomer formation is prevented. Again, the non-equivalence of nitrogens should play an important role in the variance of the π value because of the difference in steric effect.

Table 2 also indicates that substitution of a methyl group for the N_1 hydrogen significantly increases lipophilicity and the mean π value observed is higher than the reported value (Leo et al 1971). Changes at N_3 (except for 3-MX) and N_7 have little effect; the π values for substitution at N_3 and N_7 are near zero and somewhat erratic (especially N_7). At C_8 the substitution of OH for H (methylxanthines $\rightarrow$ uric acids) slightly reduces the lipophilicity ($-0{\cdot}35 \pm 0{\cdot}19$); Leo et al (1971) reported a value of $-1{\cdot}16$ and the difference might be due to the existence of these compounds in solution as lactim–lactam tautomers.

A larger decrease in lipophilicity ($-0{\cdot}71 \pm 0{\cdot}43$) is observed for imidazole ring opening to obtain uracilic compounds (uric acids $\rightarrow$ uracils). The small decrease in lipophilicity for 1,3-DAU could be due to the lack of the methyl group at position 7: the low steric hindrance of hydrogen permits free rotation of the bond C_5 –N_7. Thus intramolecular hydrogen bonding could occur with a consequent decrease in affinity for the aqueous phase. Leo et al (1971) reported a log (P) = $-1{\cdot}07$ for uracil and a log (P) = $-1{\cdot}20$ for 3-methyluracil (i.e. 1-methyluracil). Substitution of a methyl group for H slightly reduced the lipophilicity; the π value observed ($-0{\cdot}13$) is close to our finding ($\pi = -0{\cdot}02$) for uracilic metabolites of caffeine (1,3,7-TAU–1,7-DAU).

The data in Table 2 also indicate that the π values of substituents are influenced by the presence of other substituents on the molecule. The π value of a methyl group at N_1 (π-Me_1) seems to be the least affected; only the presence of another methyl group at N_3 reduces the π-Me_1 value (mean 0·52) while in uric acids when there is a methyl group at N_7 the π-Me_1 value is higher (0·96).

The effects of other substituents on the π value of a methyl group at N_3 (π-Me_3) vary. The concomitant presence of a methyl group at N_7 produces a slight increase in lipophilicity (mean 0·13) in xanthines but a slight decrease (mean $-0{\cdot}15$) in uric acids; uracil seems to be unaffected (π-Me_3: $-0{\cdot}02$). When there are no other methyl substituents the increase in lipophilicity due to a methyl group at N_3 is small though greater than when there is a methyl group at N_1.

It is noteworthy that in uric acids the contribution of a methyl group at N_3 to the overall lipophilicity is reduced by an almost constant value ($\Delta\pi$: $0{\cdot}27 \pm 0{\cdot}05$).

A methyl group at N_7 (π-Me_7), i.e. on the imidazole ring, takes small but opposite π values when methyl groups are at N_1 and N_3 in the xanthine or in uric acid. When another methyl group is at N_1, the π-Me_7 value is positive in both xanthine and uric acid, but becomes negative if the second methyl group is at N_3. When there are no substituents the π-Me_7 value is positive but very low.

The replacement of H by OH at C_8 gives a mean value different from that expected, as stated before, and for uric acids bearing a methyl group at N_3 the π value is more negative (mean $-0{\cdot}54$) than for the other uric acids (about $-0{\cdot}29$).

The π value observed for 1,7-DMU suggests that the concomitant presence of methyl groups at N_1 and N_7 matches the effect on lipophilicity of the OH group at C_8, and it is noteworthy that the difference in π value (0·30) with 1,3,7-TMU, which bears a methyl group at N_3, is similar to that observed for the other uric acids (mean 0·25).

These findings suggest that new sets of physicochemical parameters must be determined for each of the three (considered) nitrogen positions of the xanthine nucleus (and its derivatives).

On the basis of the above considerations, a different approach was tried to correlate log (P) with log (k'_w) for caffeine and its metabolites and good correlations were found in the series of compounds substituted at specified positions.

A similar approach was taken by Thus & Kraak (1985) studying n-octanol/water partition coefficients of some aromatic compounds: they found a much better correlation between log (P) and log (k') if they correlated separately only polychlorinated biphenyls and polyaromatics or only simple aromatics rather than all the compounds together.

Haky & Young (1984) also improved the correlation by splitting their data into two sets, i.e. phenolic

compounds and all compounds except phenols. Fig. 1 shows the regression lines obtained considering:

1,3,7- and 3,7-substituted compounds:

$$\log(P) = 1{\cdot}134\ (\pm\ 0{\cdot}170) \times \log(k'_w) - 2.425\ (\pm\ 0{\cdot}237) \quad (10)$$

$$n = 6,\quad r = 0{\cdot}994,\quad P < 0{\cdot}001$$

1,7- and 7-substituted compounds:

$$\log(P) = 1{\cdot}069\ (\pm 0{\cdot}266) \times \log(k'_w) - 1{\cdot}855\ (\pm\ 0{\cdot}294) \quad (11)$$

$$n = 5,\quad r = 0{\cdot}991,\quad P < 0{\cdot}002$$

1,3- and 3-substituted compounds:

$$\log(P) = 0{\cdot}821\ (\pm\ 0{\cdot}139) \times \log(k'_w) - 1{\cdot}328\ (\pm 0{\cdot}143) \quad (12)$$

$$n = 5,\quad r = 0{\cdot}996,\quad P < 0{\cdot}001.$$

The compounds substituted only at N_1 (1-MU, 1-MX), can be included in regression with compounds substituted at position 1,3- and 3-, even though these two metabolites seem to belong to another series. Adding these two compounds, regression of 1,3- and 3- and 1-substituted compounds yields:

$$\log(P) = 0{\cdot}797\ (\pm 0{\cdot}261) \times \log(k'_w) - 1{\cdot}257\ (\pm 0{\cdot}262) \quad (13)$$

$$n = 7,\quad r = 0{\cdot}962,\quad P < 0{\cdot}001.$$

The resulting equations have much better correlation coefficients than the equation of the overall data set and the other regression parameters differ significantly. Of particular significance is the large difference in the intercept of correlation lines (>0·52 log (P) units) suggesting different types of partitioning mechanism for each set of compounds, as observed by Haky & Young (1984).

Our findings confirm that the substituent position plays an important role in determining the lipophilic properties and the chromatographic behaviour of these compounds, and that capacity factor values extrapolated to 100% water eluent, $\log(k'_w)$, can be linearly related to n-octanol/water partition coefficients, but only if caffeine and its metabolites are considered as separate groups of molecules, depending on the position of substituents.

The regression line obtained correlating the n-octanol/water partition coefficients determined by the shake-flask method, log (P), and $\log(P)_{HPLC}$ (reverse phase method) values calculated from equations 10, 11, 12 and 13, as listed in Table 3, is described by:

$$\log(P) = 1{\cdot}008\ (\pm 0{\cdot}068) \times \log(P)_{HPLC} + 0{\cdot}020\ (\pm 0{\cdot}063) \quad (14)$$

$$n = 18,\quad r = 0{\cdot}992,\quad P < 0{\cdot}001.$$

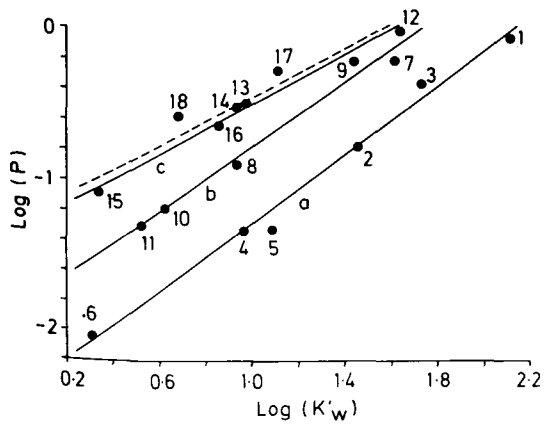


FIG. 1. Log–log relationships between log (P) and $\log(k'_w)$ of: 1,3,7- and 3,7-substituted compounds (line a); 1,7- and 7-substituted compounds (line b); 1,3- and 3-substituted compounds (line c), and 1,3-, 3- and 1-substituted compounds (dashed line). Compounds: 1 = 1,3,7-TMX; 2 = 3,7-DMX; 3 = 1,3,7-TMU; 4 = 3,7-DMU; 5 = 1,3,7-TAU; 6 = 3,7-DAU; 7 = 1,7-DMX; 8 = 7-MX; 9 = 1,7-DMU; 10 = 7-MU; 11 = 1,7-DAU; 12 = 1,3-DMX; 13 = 3-MX; 14 = 1,3-DMU; 15 = 3-MU; 16 = 1,3-DAU; 17 = 1-MX; 18 = 1-MU.

Table 3. Partition coefficients measured (shake-flask method) and calculated (by equations 10, 11, 12 and 13) and their differences, Δ.

Compound	log (P) (shake-flask)	$\log(P)_{HPLC}$	Δ
1 1,3,7-TMX	−0·07	−0·04	+0·03
2 3,7-DMX	−0·78	−0·78	0
3 1,3,7-TMU	−0·37	−0·48	−0·11
4 3,7-DMU	−1·33	−1·33	0
5 1,3,7-TAU	−1·32	−1·20	+0·12
6 3,7-DAU	−2·03	−2·08	−0·05
7 1,7-DMX	−0·22	−0·14	+0·08
8 7-MX	−0·89	−0·86	+0·03
9 1,7-DMU	−0·22	−0·33	−0·11
10 7-MU	−1·18	−1·19	−0·01
11 1,7-DAU	−1·30	−1·30	0
12 1,3-DMX	−0·02	0·01	+0·03
13 3-MX	−0·50	−0·53	−0·03
14 1,3-DMU	−0·52	−0·56	−0·04
15 3-MU	−1·08	−1·05	+0·03
16 1,3-DAU	−0·64	−0·62	+0·02
17 1-MX	−0·27	−0·38	−0·11
18 1-MU	−0·57	−0·71	−0·14

The close correlation, the slope indistinguishable from unity and the close to zero intercept indicate that the two approaches gave equivalent results, confirming the reliability of the proposed HPLC method.

Structure–pharmacokinetic relationships

In line with the requisite that data on physicochemical and biological properties exist for a sufficient number of compounds belonging to the same class (Hinderling et al 1984) and because the n-octanol/water partition coefficient is a useful parameter in structure–activity relationship studies, an attempt was made to determine a possible structure–pharmacokinetic relationship in rats for caffeine and its metabolites with calculated log ($P_{7.4}$) values. Table 4 lists log ($P_{7.4}$), pK_a values and available pharmacokinetic parameters in the rat for caffeine and ten of its metabolites.

Linear and parabolic correlations were attempted between the pharmacokinetic parameters and log ($P_{7.4}$), and five discontinuous variables, I-1, I-3, I-7, OX and RO were used to investigate the steric influences of substituents. These dummy parameters take the value of 0 for the model compound, the xanthine; I-1, I-3 and I-7 take the value of 1 when the methyl substituent is in position 1, 3, 7, respectively; OX is equal to 1 when at position 8 the hydrogen atom is replaced by OH-yielding uric acids and RO takes the value of 1 when imidazole ring opening occurs with formation of uracilic derivatives.

This approach was employed only in correlating λ_z or $t_{\frac{1}{2}}$ because of the small number of observations for the other parameters, and stepwise regression analysis was performed to explain the observed variations in λ_z and $t_{\frac{1}{2}}$.

The most significant equations are listed in Table 5. No significant correlations were found between pharmacokinetic parameters and pK_a, and multiple regressions on log (P) and pK_a were equivalent to single regressions of the parameters on log ($P_{7.4}$).

Only regressions of CLu_{int} and CL_H vs log ($P_{7.4}$) were statistically significant suggesting that the lipophilicity of the compound is important in the variance of the parameter, accounting for 88% (r^2 = 0·880) and 79% (r^2 = 0·792), respectively, and indicating that both parameters increase with increasing lipophilicity.

For CL_H the findings agree with the observation that for caffeine and its metabolites lipophilicity seems to be important in determining the metabolic pattern because compounds with a log ($P_{7.4}$) lower than −1·0 are excreted virtually 100% unchanged (Dews 1984) and an increase in log ($P_{7.4}$) lowers this percentage. This was confirmed by the linear correlation (r = 0·861) found between log (R) (where R is the ratio between non-renal and renal excretion), and log ($P_{7.4}$).

For λ_z and $t_{\frac{1}{2}}$, significant (though poor) correlations with log ($P_{7.4}$) were found, and lipophilicity accounted for only 37% of the variance of the parameters. In stepwise regressions only steric parameter I-3 reached significance and its inclusion in the equations was statistically justified. For $t_{\frac{1}{2}}$ and λ_z the variance of the parameter was 66% (r^2 = 0·661) and 75% (r^2 = 0·753), respectively, in terms of both lipophilicity and the presence of a methyl substituent at N_3 (steric effect). The signs of coefficients of log($P_{7.4}$) and I-3 indicate that $t_{\frac{1}{2}}$ and λ_z, respectively increase and decrease with increasing lipophilicity and with the presence of a substituent at position 3. Again, the steric effect of the methyl groups differs depending on the substituent position.

Correlations taking only 1,3,7- and 3,7-substituted compounds did not improve the significance of regressions, but these results may be due to the small number of compounds.

For these parameters other authors found relationships with log ($P_{7.4}$), suggesting that the kind of correlation depends on the class of compounds considered. Seydel et al (1980) studying antibacterial sulphonamides, observed linear relationships for λ_z

Table 4. Physicochemical and pharmacokinetic parameters of caffeine and some of its metabolites.[a]

Compound	pK_a	log $P_{7.4}$	V ($L\,kg^{-1}$)	CL ($L\,h^{-1}\,kg$)	LCu_{int}[b] ($L\,h^{-1}\,kg$)	CL_H ($L\,h^{-1}\,kg$)	CL_R ($L\,h^{-1}\,kg$)	λ_z (h^{-1})	$t_{\frac{1}{2}}$[c] (h)	f_u
1,3,7-TMX	14·00[d]	−0·07[e]	0·78	0·380	0·441	0·361	0·019	0·46	1·51	0·90
1,3-DMX	8·77	−0·04	0·88	0·200	0·338	0·130	0·070	0·22	3·15	0·40
3,7-DMX	10·05	−0·78	0·94	0·230	0·136	0·115	0·115	0·24	2·89	0·88
1,7-DMX	8·81	−0·24	0·70	0·390	0·231	0·187	0·203	0·55	1·26	0·85
3-MX	8·32	−0·55	—	—	—	—	—	0·50	1·39	—
7-MX	8·33	−0·94	—	—	—	—	—	0·79	0·88	—
1,3,7-TMU	6·00	−1·79	5·08	1.080	—	—	—	0·24	2·89	—
3,7-DMU	5·50	−3·24	—	—	—	—	—	0·82	0·85	—
7-MU	5·60	−2·99	—	—	—	—	—	1·70	0·41	—
1,3,7-TAU	12·58	−1·32	0·94	0·400	0·004	0·004	0·386	0·42	1·65	1·00
3,7-DAU	10·09	−2·03	—	—	—	—	—	0·82	0·85	—

[a] For pharmacokinetic data sources see text.
[b] Calculated according to Bonati et al (1985).
[c] Harmonic mean.
[d] pK_a values taken from Gaspari et al (1983).
[e] From log (P) reported in Table 1, corrected using eqn 6.

Table 5. Regression of the pharmacokinetic parameters on the apparent n-octanol/water (pH 7·4) partition coefficient.

Linear regressions:

$$\log(V) = -0{\cdot}352\,(\pm 0{\cdot}378) \times \log(P_{7{\cdot}4}) - 0{\cdot}193\,(\pm 0{\cdot}366)$$
$$n = 6,\ r = -0{\cdot}792,\ P < 0{\cdot}1$$

$$CL = -0{\cdot}334\,(\pm 0{\cdot}409) \times \log(P_{7{\cdot}4}) + 0{\cdot}211\,(\pm 0{\cdot}395)$$
$$n = 6,\ r = -0{\cdot}750,\ P < 0{\cdot}1$$

$$CLu_{int} = 0.290\,(\pm 0{\cdot}197) \times \log(P_{7{\cdot}4}) + 0{\cdot}372\,(\pm 0{\cdot}137)$$
$$n = 5,\ r = 0{\cdot}938,\ P < 0{\cdot}02^*$$

$$\log(CL_H) = 1{\cdot}232\,(\pm 1{\cdot}158) \times \log(P_{7{\cdot}4}) - 0{\cdot}475\,(\pm 0{\cdot}805)$$
$$n = 5,\ r = 0{\cdot}890,\ P < 0{\cdot}05^*$$

$$CL_R = -0{\cdot}217\,(\pm 0{\cdot}267) \times \log(P_{7{\cdot}4}) + 0{\cdot}052\,(\pm 0{\cdot}186)$$
$$n = 5,\ r = -0{\cdot}830,\ P < 0{\cdot}1$$

$$\log(t_{\frac{1}{2}}) = 0{\cdot}148\,(\pm 0{\cdot}147) \times \log(P_{7{\cdot}4}) + 0{\cdot}321\,(\pm 0{\cdot}244)$$
$$n = 11,\ r = 0{\cdot}607,\ P < 0{\cdot}05^*$$

$$\log(\lambda_z) = -0{\cdot}148\,(\pm 0{\cdot}147) \times \log(P_{7{\cdot}4}) - 0{\cdot}481\,(\pm 0{\cdot}244)$$
$$n = 11,\ r = -0{\cdot}605,\ P < 0{\cdot}05^*$$

Stepwise regressions:

$$\log(t_{\frac{1}{2}}) = 0{\cdot}138\,(\pm 0{\cdot}116) \times \log(P_{7{\cdot}4}) + 0{\cdot}319\,(\pm 0{\cdot}274) \times (I\text{-}3) + 0{\cdot}078\,(\pm 0{\cdot}282)$$
$$n = 11,\ r = 0{\cdot}813,\ P < 0{\cdot}05^*$$

$$\lambda_z = -0{\cdot}238\,(\pm 0{\cdot}151) \times \log(P_{7{\cdot}4}) - 0{\cdot}510\,(\pm 0{\cdot}362) \times (I\text{-}3) + 0{\cdot}683\,(\pm 0{\cdot}373)$$
$$n = 11,\quad r = 0{\cdot}868,\quad P < 0{\cdot}005^*$$

* Levels of $P < 0{\cdot}05$ were considered significant.

and CL, the value decreasing with increasing lipophilicity and ionization. Hinderling et al (1984) found parabolic or linear dependence on lipophilicity of many primary and secondary pharmacokinetic parameters for β-adrenoceptor blocking agents. Watanabe & Kozaki (1978a, b) studied the relationship between partition coefficient and apparent volume of distribution for basic drugs. They found a good correlation in the region of medium and high $\log(P_{7{\cdot}4})$, i.e. $\log(P_{7{\cdot}4}) > 0$, where V values rise with increasing $\log(P_{7{\cdot}4})$, while for $\log(P_{7{\cdot}4}) < 0$ they found no significant correlation because V values appeared almost constant in the low $\log(P_{7{\cdot}4})$ region. The model developed and the proposed equation were in good agreement with their experimental data and with the general statement that drugs with larger partition coefficients have larger V values.

It is noteworthy that excluding 1,3,7-TMU, considered to have an aberrant V value, caffeine and its metabolites have almost constant V values (mean $0{\cdot}85 \pm 0{\cdot}11\,L\,kg^{-1}$). This seems to validate the observations by Watanabe & Kozaki (1978a, b) for drugs with low $\log(P_{7{\cdot}4})$. Using this approach the average error between found and predicted volumes of distribution is only 11% (range 4–18%).

Taking into account the piecemeal relationships found, it must be underlined that previously reported correlations between physicochemical characteristics and pharmacokinetic parameters were established employing homologous agents, and not closely related compounds (parent drug and its metabolites), as reported here.

In conclusion, a good relationship was described between n-octanol/water partition coefficient and the lipophilicity index $\log(k'_w)$ for caffeine and its metabolites considered in separate series, depending on the substituent position, and only a few correlations were found between $\log(P_{7{\cdot}4})$ and pharmacokinetic parameters considered.

These results suggest it is unwise to estimate pharmacokinetic parameters from physicochemical values, at least for drugs that are largely metabolized. It must also be borne in mind that whereas physicochemical values are constant, kinetic parameters are (or may be) related to physiological functions (Rowland & Tozer 1980). Thus, they can differ widely not only inter- and intrasubject, but interspecies too. This has in fact been described for caffeine (Bonati et al 1985).

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