Influence of ionization and ion-pair formation on lipophilicity of some 4-hydroxycoumarin derivatives in the octanol–water system

Willem F. van der Giesen and Lambert H.M. Janssen

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Utrecht, Catharijnesingel 60, 3511 GH Utrecht (The Netherlands)

Summary

The pH-dependent distribution of weak acids in the octanol–water system was investigated. For a series of six 4-hydroxycoumarins, substituted at position 3, the apparent partition coefficient or the distribution coefficient, P_{app}, was measured in the pH range 1–11. The log P_{app}–pH profiles obtained are in complete agreement with the theory. At high pH the value of P_{app} is independent of pH, but dependent on the concentration of counterions (Na^+ in this study) in the aqueous phase; in other words ion-pair extraction occurs. The pNa ($-\log [Na^+]$)-dependent distribution is analogous to the pH-dependent distribution, except in the case of 4-hydroxycoumarin. Evidence was found that the sodium ion-pair of 4-hydroxycoumarin dissociates considerably in the octanol phase. A large discrepancy was noted between the experimentally obtained log P values and the theoretical values calculated using the hydrophobic fragmental constant system. Log P_{app} values obtained under conditions where the compounds are ionized in the aqueous phase depend on both the partition coefficient and the dissociation constant of the acid or salt. Partition coefficients of the sodium ion-pairs obtained by extrapolation are just slightly lower than the partition coefficients of the undissociated acids.

Introduction

Hansch and co-workers (Hansch et al., 1963, 1973) were the first to use the linear free-energy approach in studies concerning the quantitative structure–activity rela-

tionships (QSAR) of biologically active compounds. It has become increasingly clear since then that the lipophilicity is often an important factor in determining the extent of the biological activity of compounds. The partition coefficient is always used as a quantitative measure of lipophilicity, and is generally measured in the octanol–water system (Smith et al., 1975).

Many biologically active compounds are weak acids or bases and occur as ionized species at the pH of interest. It seems likely that the activities associated with ionized and unionized forms will differ. This difference may be due to the different lipophilicity of the two forms. To obtain more insight into this problem one needs to study the influence of ionization on the lipophilicity in more detail by exploring the effect of pH on the distribution process. In the relevant literature there are a number of studies (Ezumi and Kubota, 1980; Moser et al., 1975; Murthy and Zografi, 1970; Le Petit, 1977, 1980; Schaper, 1979, Tsuji et al., 1977; Unger and Feuerman, 1979; Vezin and Florence, 1979; Wang and Lien, 1980; Yalkowsky and Morozowick, 1980) dealing with the pH-dependent partitioning of protolytes, some of which are more detailed than others. In this literature one frequently encounters the assumption that the charged species of a molecule are not found in the organic phase. This assumption, however, should be viewed critically (Dearden and George, 1978). In this connection the possible occurrence of ion-pairs in the organic phase should be taken into consideration (Colaizzi and Klink, 1969; Dearden and George, 1978; Ezumi and Kubota, 1980; Irwin et al., 1969; Irwin and Li Wan Po, 1979; Murthy and Zografi, 1970; Terada et al., 1981).

For this paper we decided to investigate the pH-dependent partitioning of some derivatives of 4-hydroxycoumarin in the octanol–water system. It has been established in our laboratories that the binding of the 4-hydroxycoumarin anticoagulants to serum albumin is pH-dependent (Wilting et al., 1980, 1981) and it is known that hydrophobic forces often play an important role in protein binding. To obtain good insight into the pH-dependent albumin binding we need a full understanding of the pH-dependent lipophilicity of these compounds. Special attention is given to ion-pair formation.

Theory

For the acid HA the dissociation can be represented by:

$$HA \rightleftharpoons H^+ + A^- \tag{1}$$

and the corresponding dissociation constant as

$$K_a = \frac{[H^+]_w [A^-]_w}{[HA]_w} \tag{2}$$

The subscript w indicates the water phase. The partition coefficient P of the undissociated acid HA is defined according to:

$$P_{HA} = \frac{[HA]_0}{[HA]_w} \tag{3}$$

The subscript o indicates the organic phase. Symbols or concentrations without the subscript w or o are assumed to refer to the aqueous phase. Molar concentrations are used.

Depending on the experimental circumstances the acid HA may be found as several molecular species in both the aqueous and the organic phase. The apparent partition coefficient or distribution coefficient, P_{app}, is defined as the sum of the concentrations of all species of the acid in the organic phase, divided by the sum of the concentrations of all species in the aqueous phase.

When the partitioning process is represented by Scheme a in Fig. 1, P_{app} is given by:

$$P_{app} = \frac{[HA]_0}{[HA]_w + [A^-]_w} \tag{4}$$

Combining Eqns. 2, 3 and 4 and transferring to log gives:

$$\log P_{app} = \log P_{HA} - pH - \log([H^+] + K_a) \tag{5}$$

or, when α is the degree of dissociation of HA

$$\log P_{app} = \log P_{HA} + \log(1 - \alpha) \tag{6}$$

It is clear that when $pH = pK_a$, $\log P_{app} = \log P_{HA} - 0.301$. For certain pH regions Eqn. 5 can be replaced by a simpler equation. In the pH region where $pH \ll pK_a$, or $[H^+] \gg K_a$, Eqn. 5 becomes:

$$\log P_{app} = \log P_{HA} \tag{7}$$

Under these conditions $\log P_{app}$ is independent of pH, and plotting $\log P_{app}$ against pH will yield a straight line with zero slope. In the pH region where $pH \gg pK_a$, or
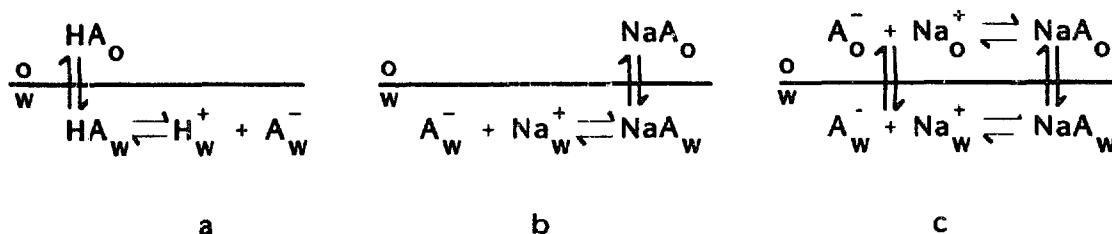

Fig. 1. Possible partition mechanisms of a weak acid, HA, between an aqueous phase (w) and an organic phase (o). a: pH not too far above pK_a; b: pH far above pK_a; c: pH far above pK_a and including dissociation of the ion-pair in the organic phase.

$[H^+] \ll K_a$, Eqn. 5 becomes:

$$\log P_{app} = \log P_{HA} - pH + pK_a \tag{8}$$

Plotting $\log P_{app}$ against the pH results in a straight line with a slope value of -1.

The straight lines given by Eqns. 7 and 8 intersect at $pH = pK_a$. This means that if one is able to measure the partitioning of a substance in the pH region where Eqn. 7 is valid, the $\log P_{HA}$ can be found directly. If, in addition, the partitioning is known in the pH region where Eqn. 8 is valid, then one can find the pK_a value or, if the pK_a value is already known from another source, the partition pH profile can be checked. If only the distribution has been studied in the pH region where Eqn. 8 is valid, then the $\log P_{HA}$ can be estimated by extrapolating, provided the pK_a value is known.

The use of Eqn. 7 instead of the full expression as given in Eqn. 5 or Eqn. 6 is allowed when $pH < pK_a - 1.5$, because then the value of $\log (1 - \alpha)$ is less than 0.013. The accuracy of $\log P_{app}$ values obtained experimentally is certainly not better than 0.01, so it reasonable to use Eqn. 7 in the pH region, as stated above. On similar grounds it can be demonstrated that Eqn. 8 can be used at $pH > pK_a + 1.5$.

In the region $pK_a - 1.5 < pH < pK_a + 1.5$ none of the terms in Eqn. 5 can be ignored, so the full equation must be used. This equation can be written as

$$P_{app}^{-1} = P_{HA}^{-1} + K_a \cdot P_{HA}^{-1} \cdot [H^+]^{-1} \tag{9}$$

Plotting P_{app}^{-1} against $[H^+]^{-1}$ results in a straight line with intercept P_{HA}^{-1} and slope $K_a \cdot P_{HA}^{-1}$. At first glance Eqn. 9 seems to be very suitable for determining K_a and P_{HA} values simultaneously (Ezumi and Kubota, 1980). However, one should realize that this method can only be used if the pH is in region of $pK_a - 1.5 < pH < pK_a + 1.5$.

At relatively high pH values $[HA]_w$ becomes very small compared to $[A^-]_w$ and partitioning of the anion should be taken into account as well. The partitioning process can now be represented by Scheme b in Fig. 1. Due to the fact that electroneutrality in each phase must be maintained, ions can pass to the other phase only if they are accompanied by an equivalent amount of ions of opposite charge. In other words ion-pair extraction occurs. This model for the partitioning of entirely ionized compounds is widely accepted in the theory of ion-pair extraction and in ion-pair liquid–liquid chromatography (Schill, 1978).

At sufficiently high pH and when only H^+ and Na^+ are present as cations, P_{app} is given by:

$$P_{app} = \frac{[NaA]_0}{[A^-]_w} \tag{10}$$

This implies that the $\log P_{app}$–pH profile reaches a constant level at high pH, provided the counterion concentration is kept constant. Of course there will be a gradual change from the situation described by Eqn. 8 to the situation described by

Eqn. 10. This means that Scheme a and Scheme b in Fig. 1 represent two extreme situations.

The model represented by Scheme b in Fig. 1 can be described in a similar way as the model of Scheme a if we introduce the term P_{NaA} as the partition coefficient of the sodium ion-pair and the term pK_{NaA} defined by

$$pK_{NaA} = -\log \frac{[Na^+]_w [A^-]_w}{[NaA]_w} \tag{11}$$

In principle a $\log P_{app}$–pNa profile can be obtained by analogy with a $\log P_{app}$–pH profile. It is very unlikely that NaA will be found in the aqueous phase because the high concentration of Na^+ needed cannot be reached. This means that only the equivalent of Eqn. 8, viz.

$$\log P_{app} = \log P_{NaA} - pNa + pK_{NaA} \tag{12}$$

can be found. Plotting $\log P_{app}$ against the pNa results in a straight line with a slope of -1.

Materials and methods

The chemical formulae of the 4-hydroxycoumarin derivatives and their sources are summarized in Table 1. The purity of the compounds was checked by means of high-performance liquid chromatography (see below) and by means of thin-layer

TABLE 1

CHEMICAL FORMULAE AND SOURCES OF THE 4-HYDROXYCOUMARIN DERIVATIVES
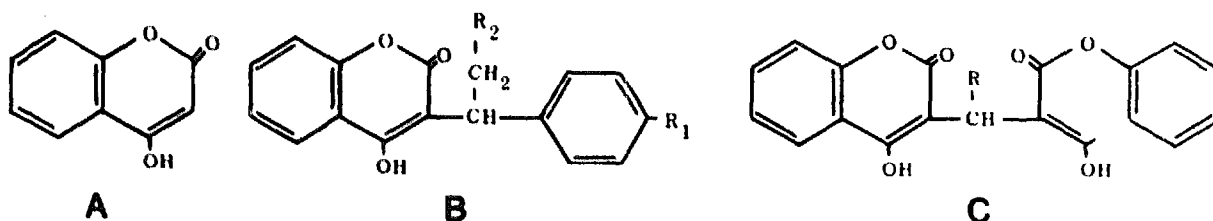
No.	Compound	Formula	Source
1	4-hydroxycoumarin	A	M
2	warfarin	B R_1 = H R_2 = -CO-CH$_3$	ACF
3	acenocoumarin	B R_1 = NO$_2$ R_2 = -CO-CH$_3$	CG
4	phenprocoumon	B R_1 = -H R_2 = -CH$_3$	HR
5	ethylbiscoumacetate	C R = -CO-OC$_2$H$_5$	CG
6	coumetarol	C R = -CH$_2$-OCH$_3$	ACF

The compounds were supplied by Ciba-Geigy (CG; Arnhem, The Netherlands), Hoffmann-La Roche (HR; Mijdrecht, The Netherlands), A.C.F. Chemiefarma (ACF; Maarssen, The Netherlands) or purchased from Merck (M; Darmstadt, G.F.R.).

chromatography (van der Giesen and Janssen, 1982). In all cases only one compound proved to be present. The melting points of the 4-hydroxy compounds used were: compound 1: 205–207°C (lit. 206°C, Handbook of Tables for Organic Compound Identification, 1967); compound 3: 199–200°C (lit. 196–199°C, Merck Index, 1976); compound 4: 178–179°C (lit. 179–180°C, Merck Index, 1976); compound 5: 177–179°C (lit. 177–182°C, Merck Index, 1976) and compound 6: 161°C (lit. 156–157°C, Merck Index, 1976). Compound 2 was of British Pharmacopoeia quality. Octanol (Merck, Darmstadt, G.F.R.) was purified by successive washings with an equal volume of 4 M NaOH, 2 M H_2SO_4 and 1 M Na_2CO_3. Finally the octanol was washed repeatedly with water until the water phase reacted neutral. The buffers used for the determination of the pH-dependent partitioning had an ionic strength, I, of about 0.1 and were composed as follows: pH 1.1–3.0 HCl–KCl (Biochemists' Handbook, 1961); pH 3.0–4.0 citric acid–sodium citrate (Biochemists' Handbook, 1961); pH 4.0–5.8 HAc–NaAc (Biochemists' Handbook, 1961); pH 5.8–8.0 NaH_2PO_4–NaOH; pH 8.2–9.2 H_3BO_3–NaOH; pH 9.0–11.0 $NaHCO_3$–Na_2CO_3 (Biochemists' Handbook, 1961) or glycine–NaOH (Biochemists' Handbook, 1961). The buffers used for the determination of the Na^+-dependent partitioning were 0.025 M, to which NaCl was added to achieve one of the desired Na^+ concentrations. The octanol and buffer solutions were mutually saturated. The samples were dissolved in the phase in which they were most soluble. The distribution coefficients were determined by gently shaking 5 ml octanol with 10 ml buffer solution for 2 h. Equilibration was generally reached within half an hour. A shake-waterbath (Precision Scientific, Chicago, U.S.A.) provided with a thermostat (25°C) was used. After letting the mixture stand for a few minutes the pH was measured in the water phase and the phases were centrifuged for 10 min at 5000 rpm. For the pNa profiles the pNa was measured too. The pH was measured using a digital pH meter, model PHM 62 with a combined glass-reference pH electrode, type GK 2401C (Radiometer, Copenhagen, Denmark). The pNa was measured using the same pH meter equipped with a sodium-sensitive glass electrode, type G 502 Na and a calomel reference electrode, type K 401 (Radiometer).

The concentrations of the 4-hydroxycoumarin derivatives were determined in the water phase when log $P_{app} > 1$, in the octanol phase when log $P_{app} < -1$, in both phases when $-1 < $ log $P_{app} < 1$. When the concentration in only one phase was determined experimentally the concentration in the other phase was obtained by calculation. The concentrations were determined using a high-performance liquid chromatograph consisting of an autosampler, model WISP, a high-pressure pump, model 6000 and a UV detector type 440 with a 313 nm filter (Waters Associates, Etten-Leur, The Netherlands) combined with a flat-bed recorder, model BD 8 (Kipp, Delft, The Netherlands). A prepacked reversed-phase column Lichrosorb 10 RP 18, 25 cm × 4.6 mm (Chrompack, Middelburg, The Netherlands) was used and 1% acetic acid in methanol–water mixtures as mobile phase (1.5 ml/min). Methanol concentrations ranged from 30 to 70% (v/v) and were chosen to achieve log k' values of about 0.5. Depending on the concentration, 25–100 μl of the water phase was injected. Always 5 μl of the octanol phase was injected because larger volumes influenced the retention times and could therefore make quantitative determinations

inaccurate. The concentrations were calculated from the chromatograms from peak height measurements. Compounds 5 and 6 were found to decompose in the aqueous phase. The decomposition products are unknown. Compound 5 and 6 formed one and two decomposition products, respectively. Samples from both the aqueous and the organic phase which were analyzed as quickly as possible after shaking produced only minor extra peaks in the chromatograms, which means that compounds 5 and 6 hardly decompose during shaking and that reasonably accurate partition coefficients can be obtained. However, the concentrations of the decomposition products in the aqueous phase increased with time and since it was impossible to analyze all the samples immediately after shaking we decided to sum the heights of the peaks in the chromatograms of the aqueous phase.

When the analysis is carried out within 24 h after shaking—and it always was in our case—the relative error in the concentration of compounds 5 and 6 in the aqueous phase is smaller than 5%.

The pK_a values were determined by potentiometric titration or by spectrophotometry, as proposed by Albert and Serjeant (1971). An ionic strength of about 0.1 was achieved by the addition of potassium chloride.

Results and discussion

pH profiles

The distribution coefficients, P_{app}, were determined using the method described above. In Fig. 2 log P_{app} is plotted against pH for compounds 1–4. At relatively low pH straight lines with zero slopes have been drawn, according to Eqn. 7. As an example the calculated slope value for warfarin from the experimental points is -0.02 (± 0.01)[1]. There is no doubt that at low pH, log P_{app} is independent of pH. Also at relatively high pH log P_{app} is independent of pH in accordance with theory. At intermediate pH values, straight lines with a slope value of -1 have been drawn, according to Eqn. 8. In Table 2 the values of the slopes are given as calculated from the experimental points of the straight lines obtained when log P_{app} is plotted against pH. It is clear from this table that in the pH region mentioned Eqn. 8 is valid. When the lines with slope values of -1 are extrapolated, points of intersection with the extrapolated zero slope lines are obtained. The pH values of the intersections at low pH are in good agreement with the pK_a values of the compounds determined independently (see Table 2).

In a given solvent system the height of the plateau of the graphs at low pH is only dependent on the lipophilicity of the compounds and is equal to log P_{HA} and is therefore characteristic for the compound under study. The log P_{HA} values (the average of log P_{app} values at pH $<$ pK_a $-$ 1.5) are also given in Table 2. However, the height of the plateau at high pH is dependent on the kind of counterion, on the counterion concentration and on the lipophilicity of the ion-pair formed (see later).

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[1] The standard deviation is given in parentheses.

TABLE 2

CHARACTERISTICS OF THE pH PROFILES OF THE PARTITIONING OF 4-HYDROXYCOUMARIN DERIVATIVES

No.	Compound	log P_{HA} [a]	n [h]	Slope [c]	n [h]	pH region [d]	pK_a [e]	pK_a
1	4-hydroxycoumarin	2.37	3	−0.983 (±0.009)	6	4.77– 7.11	4.13	4.10[f]
2	warfarin	3.28	5	−0.995 (±0.007)	8	6.02– 8.05	5.02	5.00[f]
3	acenocoumarin	3.24	3	−0.973 (±0.020)	6	5.68– 7.39	4.98	5.05[f]
4	phenprocoumon	4.56	3	−1.021 (±0.011)	6	5.68– 7.39	4.02	4.20[f]
5	ethylbiscoumacetate	4.93[b]		−1.037 (±0.049)	4	2.16– 3.61		1.25[g]
				−0.972 (±0.074)	6	8.54– 9.98	7.50	7.50[f]
6	coumetarol	4.55[b]		−0.74	5	1.21– 3.59		0.65[g]
				−0.85	4	9.68–10.87		9.00[f]

[a] Average of the log P_{app} values at pH<pK_a −1.5.
[b] Obtained by extrapolating as indicated in the text.
[c] Slope of straight lines obtained when log P_{app} is plotted against pH, the standard deviation is given in parentheses.
[d] pH region in which the slope was calculated.
[e] From points of intersection of the drawn lines in pH profile.
[f] Measured titrimetrically.
[g] Measured spectrophotometrically.
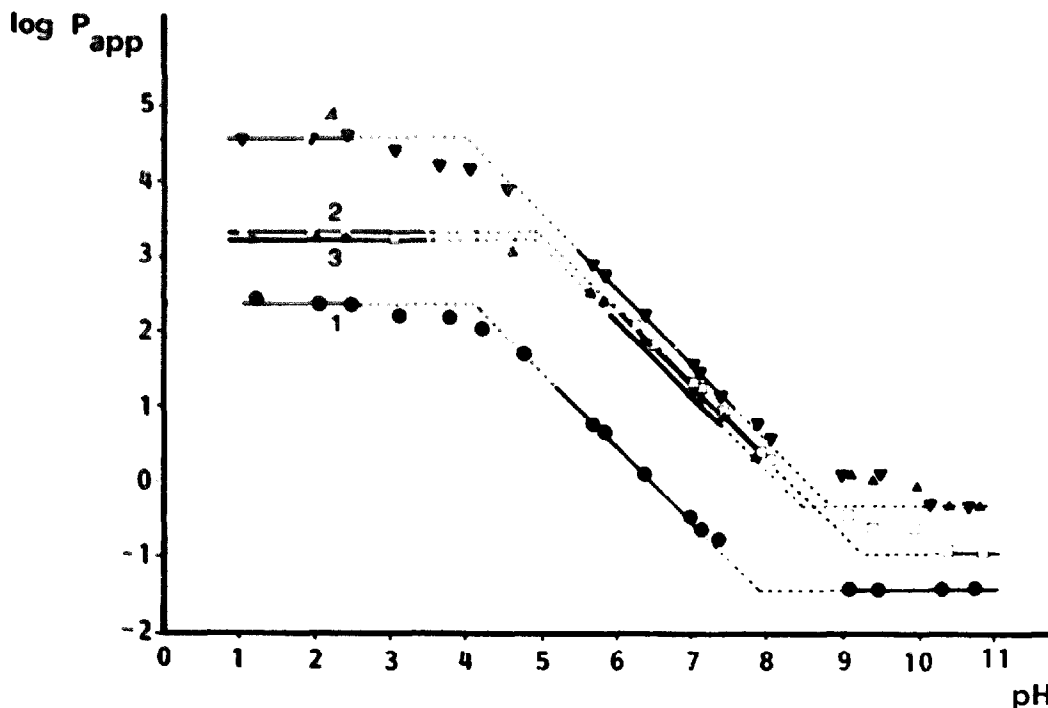[h] Number of points from pH profile; each point is the mean of 3 determinations.

Fig. 2. Log P_{app}–pH profiles of monobasic 4-hydroxycoumarin derivatives. 1: 4-hydroxycoumarin; 2: warfarin; 3: acenocoumarin; 4: phenprocoumon. The points are the mean of 3 determinations. The lines have been drawn with slopes of −1 and 0. The dashed lines are the extrapolated parts of the drawn lines.

The log P_{app} corresponding to the intersections at high pH is therefore a conditional constant.

At pHs around the pK_a Eqns. 7 and 8 are not valid; then Eqn. 5 must be used to describe the pH profile. To prove the validity of this equation we calculated the log P_{app} values of 4-hydroxycoumarin in the pH region 1.25–7.11 using Eqn. 5. A value of 2.37 was taken for log P and for the pK_a a value of 4.13. The standard error of estimate of log P_{app} turned out to be 0.044 (n = 12), which means that the log P_{app}–pH profile found experimentally is in good agreement with theory.

For dibasic acids the log P_{app}–pH profile is more complicated because multiple ionization steps might occur. Depending on pH, the species H_2A, HA^- and A^{2-} may be found in the aqueous phase. In the organic phase, H_2A, $NaHA$ and in principle Na_2A may occur. In Fig 3 the results are shown for the dibasic compounds 5 and 6 when log P_{app} is plotted against pH. The various characteristic parts have been denoted as I, II, III and IV to facilitate discussion. The pK_a values will be called $pK_{a,1}$ and $pK_{a,2}$.

In part II around pH 2.5, a straight line with a slope value of −1 has been drawn for compound 5. The exact slope value is given in Table 2. It will be clear that the distribution process is described here as predicted by Eqn. 8. In contrast to the situation described for compounds 1–4, the constant level at low pH, describing the distribution of H_2A, could not be reached here, due to the relative low $pK_{a,1}$ value (see Table 2). For compound 5 the dotted line which describes part I in Fig. 3 is a
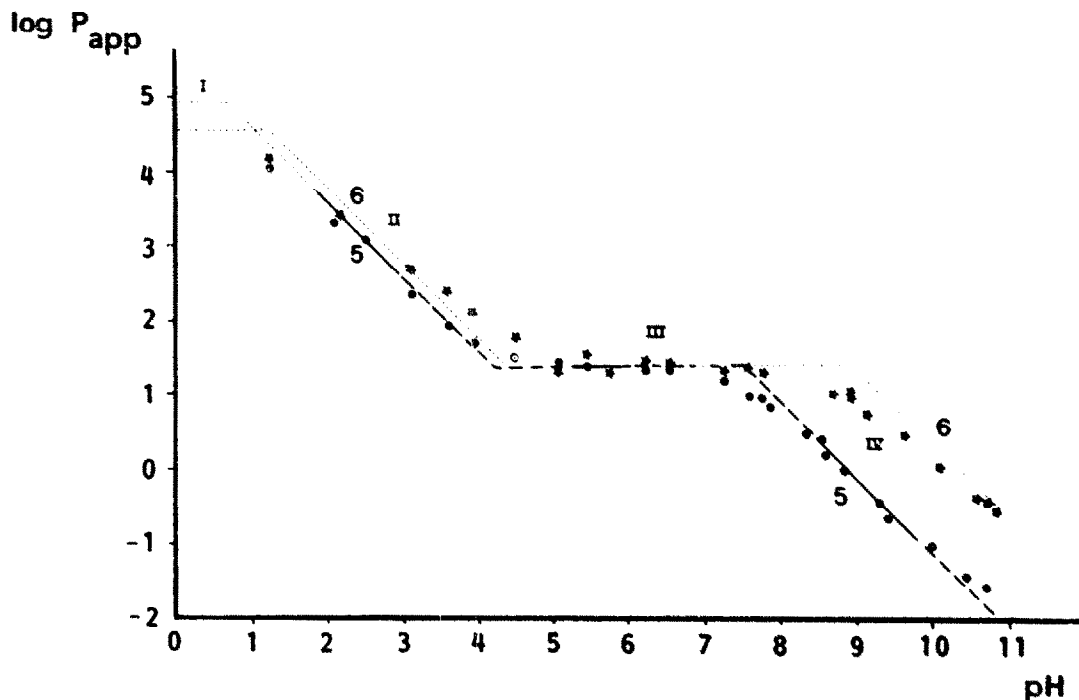
Fig. 3. Log P_{app}–pH profiles of dibasic 4-hydroxycoumarin derivatives. 5: ethylbiscoumacetate; 6: coumetarol. The points are the mean of 3 determinations. The lines have been drawn with slopes of −1 and 0. The dashed lines are the extrapolated parts of the drawn lines. The dotted lines are drawn as expected theoretically, taking the known pK_a values into account.

theoretical one, i.e. the straight line with slope −1 was extrapolated to the point $pH = pK_{a,1}$ and from this point a zero slope line was drawn.

In a small pH region at intermediate pH, indicated by III in Fig. 3, log P_{app} is independent of pH. In this region only partioning of HA^- occurs and the log P_{app} value depends on the kind of counterion, on the counterion concentration and on the lipophilicity of the ion-pair formed; a process occurs here analogous to that described for compounds 1–4 at high pH. As long as only HA^- is present in the aqueous phase, log P_{app} can be described in the same way as Eqn. 12, viz.

$$\log P_{app} = \log P_{NaHA} - pNa + pK_{NaHA} \tag{13}$$

When pNa is constant, log P_{app} must be constant too. Part IV describes the pH region where HA^- dissociates into A^{2-}, whereas in the organic phase NaHA will still be found. This part of the pH profile is given by

$$\log P_{app} = \log P_{NaHA} - pNa + pK_{NaHA} - pK_{a,2} - pH \tag{14}$$

So, as long as pNa is constant, log P_{app} against pH will result in a straight line with slope −1. It should be noted that in the pH profile the point of intersection of the straight lines extrapolated according to Eqns. 13 and 14 corresponds to $pH = pK_{a,2}$. Table 2 shows that these predictions have been confirmed experimentally especially

for compound 5. The results for compound 6 are not in full agreement with theory, probably because some decomposition has occurred. The theoretical graph for compound 6 is given by the dotted line and is based on the known pK_a values.

From the results described so far it will be clear that log P values of ionizable compounds can only be calculated from pK_a and log P_{app} values if the log P_{app} values have been obtained at pHs where ion-pair extraction did not occur. Unger (1980) also made a remark to this effect. The method proposed by Ezumi and Kubota (1980) for determining simultaneously P and K_a values from P_{app} values at pHs around the pK_a using Eqn. 9 does not make it possible to check whether ion-pair extraction occurs; this method therefore can yield erroneous values for P and K_a. Possible ion-pair extraction can be examined by establishing whether Eqn. 8 is valid for the compound in the solvent system under study. The pK_a values can be obtained from pH profiles, as shown in Figs. 2 and 3. This can be interesting when it is not possible to determine pK_a values by spectrophotometry or by potentiometric titration.

Terada et al. (1981) did not consider the problem of electroneutrality in a two-phase system and they calculated a value for the partition coefficient of the anion and of two different ion-pairs of 2,4-dinitrophenol. Unfortunately, these authors did not give a statistical analysis of their data, but we assume that if the given value of the partition coefficient of the anion (3.5×10^{-3}) is indeed significantly larger than 0, small amounts of cationic impurities might be involved.

Comparison of calculated and experimental values of log P_{HA}

Table 3 gives the log P_{HA} values found experimentally. It is of interest to investigate if these values can be calculated using the system of the hydrophobic fragmental constant of Rekker (Rekker, 1977; Rekker and De Kort, 1979). The results are presented in Table 3. The calculation of log P_{HA} values according to the rules given by Rekker gives far too low values except in the case of coumarin, the

TABLE 3

EXPERIMENTAL AND CALCULATED log P_{HA} VALUES

Compound	log P_{exp}	log P_{calc} [a]	log P_{calc} [b]	log P_{calc} [c]
coumarin	1.43	1.37		
4-hydroxycoumarin	2.37	2.37		
warfarin	3.28	2.45	3.94	3.49
acenocoumarin	3.24	2.21	3.71	3.25
phenprocoumon	4.36	4.09	5.59	
ethylbiscoumacetate	4.93	1.69	4.68	
coumetarol	4.55	1.35	4.34	

[a] Calculated by means of the hydrophobic fragmental constant system using f values from column IV of Table 2 of Rekker and De Kort (1979).
[b] Calculated with f value of 4-hydroxycoumarin skeleton of $2.37 - f(H) = 2.188$.
[c] As b, but now hemiketal structure is assumed (Valente and Trager, 1978).

calculated value of which is in reasonable agreement with the value found experimentally. Re-calculating the log P_{HA} values for 5 compounds, starting with the experimentally found value for 4-hydroxycoumarin, gives considerably higher values, but these are not yet in accordance with the experimentally found values. The correct value of log P for acenocoumarol is found only if a hemiketal structure is assumed for this compound (Valente and Trager, 1978). If, however, warfarin is assumed to have a hemiketal structure, the calculated value of log P still deviates from the experimental value.

Many examples are known for which the f-system yields good agreement between calculated and experimentally obtained log P values. It should be pointed out that the experimentally found log P_{HA} values for compounds 1–4 and log P_{HA} values obtained by extrapolating for compounds 5 and 6 correlate very well with the R_{M_w} values in a reversed-phase thin-layer chromatographic system in which oleyl alcohol is used as lipid phase (van der Giesen and Janssen, 1982), as shown in Eqn. 15 [1]

$$\log P_{HA} = 0.859(\pm 0.034)R_{M_w} + 2.187(\pm 0.074) \tag{15}$$

$$n = 6 \qquad r^2 = 0.9938 \qquad s = 0.088 \qquad F = 664$$

$$0.787 < \text{slope}^b < 0.931 \qquad 2.030 < \text{intercept}^2 < 2.344$$

When we restrict our discussion to the experimentally found log P values, it becomes clear that the lipophilic behaviour of the 4-hydroxycoumarins is quite unusual. Introducing a hydroxyl group in position 4 of the coumarin skeleton increases the lipophilicity enormously, whereas a considerable decrease was expected regardless of whether the hydroxyl group should be considered as being aromatic or aliphatic. Introducing a p-nitro group into warfarin, which results in acenocoumarol, does not give the expected clear decrease in the lipophilicity. Also the difference between the log P values of warfarin and phenprocoumon (one carbonyl group) is smaller than expected. Finally the differences between, for instance, the log P values of warfarin and ethylbiscoumacetate and between warfarin and coumetarol are much larger than expected. The large discrepancy between calculated and experimentally obtained log P values suggests that the structures of the coumarins in the aqueous phase or in the organic phase or in both are significantly different from the structures that coumarins are generally assumed to have. It might be that dimerization takes place in the organic phase via hydrogen bond formation. However, this possibility seems to be ruled out because we did not observe any concentration dependence of the P values. Complications may also be caused by the tautomeric behaviour of the 4-hydroxycoumarins. This type of compound may occur in a keto and in an enol form. The exact concentration of a given tautomeric form will be governed by the tautomeric equilibrium constant defined here as $K_T = [\text{keto}]/[\text{enol}]$.

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[1] The standard deviation is given in parentheses; n = number of compounds; r = correlation coefficient; s = standard error of estimate or $s_{y,x}$; F = value of the F-test of significance.

[2] 90% confidence interval.

It is well known that such a K_T depends strongly on the medium (Albert, 1968). This means that K_T is different for the aqueous and for the organic phase ($K_{T,w}$ and $K_{T,o}$, respectively). It can easily be demonstrated that $K_{T,o}$ and $K_{T,w}$ are linked: $K_{T,o} = (P_{keto}/P_{enol}) \cdot K_{T,w}$. In this expression P_{keto} and P_{enol} represent the partition coefficient of the keto and enol form, respectively. This means that the keto and enol form must have a different lipophilicity. This has direct consequences for the measured partition coefficient, which is now given by $P = (P_{keto} \cdot K_{T,w} + P_{enol})/(K_{T,w} + 1)$. The value of P lies somewhere between the extreme values P_{keto} and P_{enol}, and will depend on K_T. It is reasonable to assume that the changes in the P values of both the keto and enol form, due to chemical modification, will follow the usual pattern. However, chemical modification will also cause a change in K_T, which gives complications. We suggest that such phenomena may explain the unusual lipophilic behaviour of 4-hydroxycoumarins. Unfortunately it is very difficult to study the tautomerism of these compounds (Elguero et al., 1976) and no data for tautomeric constants are available. In addition one should realize that for the series of compounds in this study a third tautomeric form having an acid proton on position 3 is possible as well (O'Neal et al., 1982). Another possible explanation for the very high log P values of the dicoumol compounds 5 and 6 is that intramolecular hydrogen bond formation occurs between the carbonyl and the enolic hydroxyl groups of the adjacent ring systems (Hutchinson and Tomlinson, 1969); if this occurs, then these polar groups will be less available for interaction with the water molecules, and the lipophilicity will increase. The role of intramolecular hydrogen bond formation has been mentioned already in literature (Hansch and Leo, 1979a).

pNa profiles

The pNa-dependent partitioning of compounds 1–4 has been studied at pH = 10.0 and of compounds 5 and 6 at pH = 6.0. In Fig. 4 the results are given when log P_{app} is plotted against pNa. For the compounds 2–4 straight lines with slope values very close to −1 are found; the exact slope values are given in Table 4. The validity of Eqn. 12 is evident, and provides proof for the ion-pair formation in the organic phase. For compounds 5 and 6 straight lines were obtained too. The slope values, however, deviate from −1 (see also Table 4). This is surprising especially since the log P_{app}–pH profile of compound 5 is in full agreement with the theory. For the moment we do not have a sound explanation for this anomalous behaviour. In any case, these results suggest that ion-pair formation occurs in the organic phase for the dicoumol compounds too.

The slope value −0.507 found for compound 1 is in contradiction to Eqn. 12. This value of −0.5 suggests a transfer of a 1:2 complex from the water phase to the organic phase. Since only a neutral complex can pass to the other phase, this would suggest the involvement of a complex with bruto formula $NaHA_2$. However, the partitioning of such a complex should be pH-dependent at high pH as well, but this in fact is not the case, see Fig. 2.

Another possible explanation for the slope value −0.507 might be a dimerization in the organic phase. However, dimerization was already excluded to occur, see
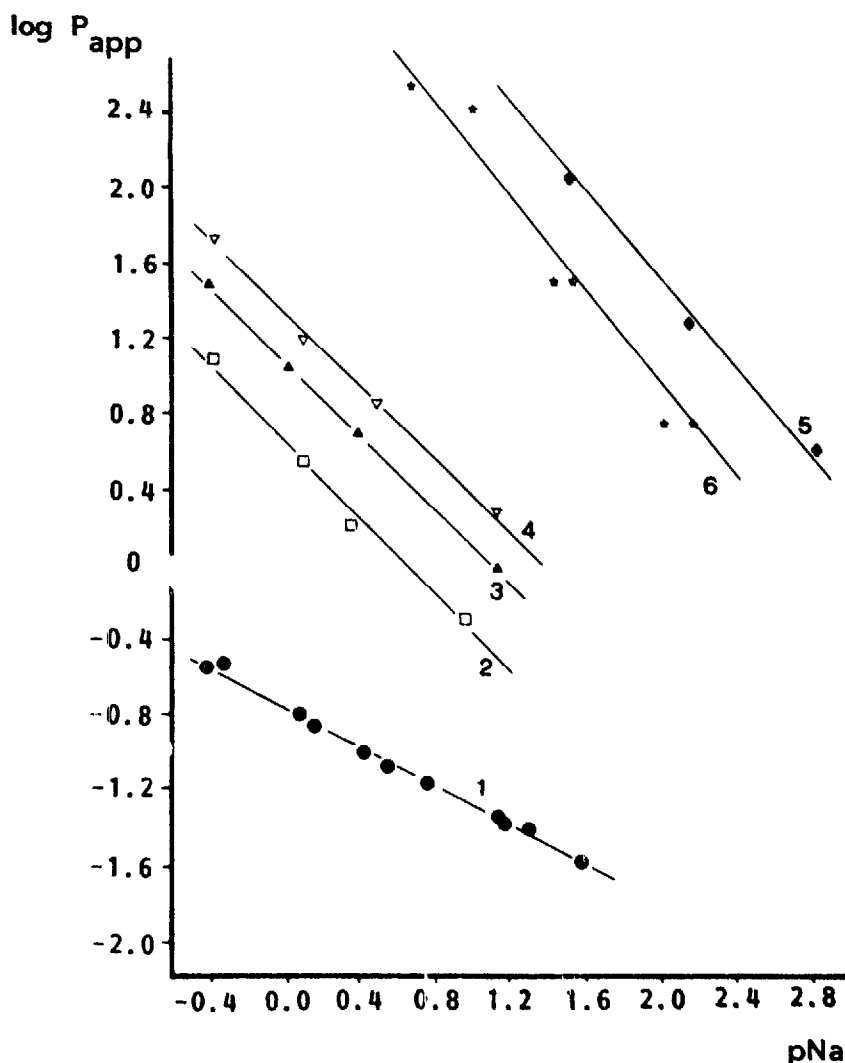
Fig. 4. Log P_{app}-pNa profiles of 4-hydroxycoumarin derivatives. 1: 4-hydroxycoumarin; 2: warfarin; 3: acenocoumarin; 4: phenprocoumon; 5: ethylbiscoumacetate; 6: coumetarol. The points are the mean of 3 determinations. The drawn lines are calculated from experimental points using the least-squares method for linear regression, see also Table 4. The values for compounds 1-4 were obtained at pH = 10.0, for compounds 5 and 6 at pH = 6.0.

above. We think that dissociation of the ion-pair in the organic phase as described by Johansson and Schill (1980) for the tetrabutylammonium ion-pair of octyl sulfate in 1-pentanol might offer an explanation for this effect. Partitioning of a weak acid at high pH including dissociation of the sodium ion-pair in the organic phase can be represented by Scheme c in Fig. 1. The various equilibria in this scheme can be defined as follows. The dissociation constant of the ion-pair in the water phase K_{NaA}

$$K_{NaA} = \frac{[A^-]_w [Na^+]_w}{[NaA]_w} \qquad (16)$$

TABLE 4

CHARACTERISTICS OF THE pNa PROFILES OF THE PARTITIONING OF 4-HYDROXY-COUMARIN DERIVATIVES

No.	Compound	Slope [a]	n [b]	$\log P_{NaA}$ [c]	$\log P_{HA}$ $-\log P_{NaA}$	$\log P_{app}$ [d]	$\log P_{HA}$ $-\log P_{app}$
1	4-hydroxycoumarin	−0.507 (±0.011)	12			−1.28	3.65
2	warfarin	−1.002 (±0.072)	4	⩾2.55	<0.73	−0.38	3.66
3	acenocoumarin	−0.970 (±0.023)	4	⩾2.98	<0.26	0.08	3.16
4	phenprocoumon	−0.966 (±0.041)	4	⩾3.25	<1.31	0.38	4.22
5	ethylbiscoumacetate	−1.17	3				
6	coumetarol	−1.22	6				

[a] Slope of the straight lines obtained when $\log P_{app}$ is plotted against pNa, the standard deviation is given in parentheses.
[b] Number of points from pNa profile, each point is the mean of 3 determinations.
[c] Obtained from extrapolating to Eqn. 12.
[d] Experimental value under the conditions pNa = 1 and pH = 10.

The dissociation constant of the ion-pair in the octanol phase K_{NaA_0}

$$K_{NaA_0} = \frac{[A^-]_0[Na^+]_0}{[NaA]_0} \tag{17}$$

The partition coefficient of the undissociated ion-pair P_{NaA}

$$P_{NaA} = \frac{[NaA]_0}{[NaA]_w} \tag{18}$$

Since sodium salts are normally completely dissociated in aqueous solutions, the distribution coefficient for this situation can be represented by:

$$P_{app} = \frac{[NaA]_0 + [A^-]_0}{[A^-]_w} \tag{19}$$

To express P_{app} in the measurable quantities $[Na^+]_w$ and $[A^-]_w$, it is convenient to use an extraction constant (Schill, 1978) K_{ex}

$$K_{ex} = \frac{[NaA]_0}{[Na^+]_w[A^-]_w} = \frac{P_{NaA}}{K_{NaA}} \tag{20}$$

Combining Eqns. 17 and 20 results in:

$$[A^-]_0[Na^+]_0 = K_{ex} \cdot K_{NaA_0}[Na^+]_w[A^-]_w \tag{21}$$

If NaA is the only ion-pair that passes to the organic phase, then $[Na^+]_0 = [A^-]_0$ and hence Eqn. 22 is valid

$$[A^-]_0 = \left(K_{ex} \cdot K_{NaA_0}[Na^+]_w[A^-]_w\right)^{1/2} \tag{22}$$

Combining of Eqns. 19, 20 and 22 results in:

$$P_{app} = [Na^+]_w\left(K_{ex} + \left(K_{ex} \cdot K_{NaA_0} \cdot [Na^+]_w^{-1}[A^-]_w^{-1}\right)^{1/2}\right) \tag{23}$$

If K_{NaA_0} is zero, then $P_{app} = [Na^+]_w \cdot K_{ex}$, which is equivalent with Eqn. 12. Now P_{app} has been expressed in terms of $[Na^+]_w$ and $[A^-]_w$. Eqn. 23 can be written as

$$P_{app}[Na^+]_w^{-1} = K_{ex} + \left(K_{ex} \cdot K_{NaA_0} \cdot [Na^+]_w^{-1} \cdot [A^-]_w^{-1}\right)^{1/2} \tag{24}$$

If $P_{app} \cdot [Na^+]_w^{-1}$ is plotted against $([Na^+]_w[A^-]_w)^{-1/2}$, then a straight line is obtained with an intercept K_{ex} and slope value of $(K_{ex} \cdot K_{NaA_0})^{1/2}$. Doing this for 4-hydroxycoumarin does indeed result in a straight line with a slope value of 3.698 (± 0.118) $\cdot 10^{-3}$ ($n = 8$; $F = 976$) and since this value is significantly larger than zero, this proves the validity of the model presented here. This method does not give reliable values for K_{ex} of 4-hydroxycoumarin due to the wide-ranging extrapolation that is needed ($K_{ex} = 3.16(\pm 11.1) \cdot 10^{-3}$ was found). The slope of -0.5 in the log P_{app}–pNa profile can now be explained by assuming that K_{ex} in Eqn. 23 or 24 is much smaller than $K_{ex} \cdot K_{NaA_0} \cdot [Na^+]_w^{-1}[A^-]_w^{-1}$. The logarithmic form of Eqn. 23 then becomes:

$$P_{app} = 0.5\left(\log K_{ex} - pK_{NaA_0}\right) - 0.5(pNa - \log[A^-]_w) \tag{25}$$

Plotting log P_{app} against pNa $-$ log $[A^-]_w$ will result in a straight line with a slope value of -0.5. Applying this to 4-hydroxycoumarin gives a straight line with a slope of 0.504 (± 0.016). The conclusion is that Eqn. 25 is valid owing to the considerable dissociation of the sodium ion-pair of 4-hydroxycoumarin in the octanol phase.

It is remarkable that when log P_{app} of 4-hydroxycoumarin is plotted against pNa or against pNa $+$ log $[A^-]_w$ nearly the same slope value is found in both cases. The reason for this is that at relatively small values of P_{app} and/or at relatively small values of the volume ratio organic phase–aqueous phase, $[A^-]_w$ can be considered as a constant.

Estimation of log P_{NaA} from log P_{app}–pNa profiles

From Fig. 4 it can be seen that at least up to a pNa value of -0.4 the pNa profiles of compounds 2–4 are linear with a slope of -1. This means that the pK_{NaA} of these compounds is lower than -1.9, so the minimum value of log P_{NaA} can be estimated by extrapolating log P_{app} until pNa $= -1.9$ according to Eqn. 12. The obtained values are given in Table 4. This table also shows the differences between

log P_{HA} and log P_{NaA}. One comes to the remarkable conclusion that the lipophilicity of the undissociated ion-pair is not so very different from the lipophilicity of the undissociated acid HA. The results presented here, provided they have a general validity and also hold for biomembranes, suggest that the fact that ionized molecules are poorly adsorbed from the gastrointestinal tract is not due to the low lipophilicity of the ion-pair but to the low concentrations of (appropriate) counterions in the aqueous environment.

For compound 3 the smallest difference between log P_{HA} and log P_{NaA} has been found. If the hydrophobic fragmental constant of hydrogen f(H) is 0.18 (Rekker and De Kort, 1979), then f(Na) should be at least $-0.26 + 0.18 = -0.08$.

log P_{HA}, log P_{app} and log P_{NaA} in QSAR

In QSAR studies it would be interesting to use log P_{NaA} as hydrophobic parameter. However, it will generally be impossible to determine log P_{NaA}, because the counterion concentration cannot be chosen high enough to suppress the dissociation of the ion-pair in the aqueous phase. For applications in QSAR Hansch and Leo (1979b) have proposed the use of a hydrophobic parameter of ionized molecules, obtained at standardized conditions by measuring the distribution coefficients of the protolytes at a pH of the aqueous phase more than 4 pH units away from the pK_a (that is, above the pK_a of acids and below the pK_a of bases) and at a small inorganic counterion concentration of 0.1 M. This could be achieved by using 0.1 M NaOH or 0.1 M HCl as aqueous phase. For acids this means: P_{app} values obtained at very high pH at a pNa of 1. It is obvious from Fig. 2 and Eqn. 8 that log P_{app} at, for instance, pH = 6 depends on both log P_{HA} and pK_a. Analogous to this, log P_{app} depends on both log P_{NaA} and pK_{NaA} at high pH where P_{app} is independent of pH, see Fig. 4 and Eqn. 12. It therefore seems inadvisable to use such a log P_{app} value as a hydrophobic parameter, since its value is determined by constants of hydrophobic and electrostatic origin. To illustrate this point for the 4-hydroxycoumarins such standardized log P_{app} values are given in Table 4. In addition the differences are given between these log P_{app} values and the log P_{HA} values. It is clear from this table that complete dissociation of the 4-hydroxycoumarins does not lower the distribution coefficient to the same extent for the several compounds. Testa and Murset-Rossetti (1978) made similar observations for a series of antihistamines. A constant difference between the log P_{app} values under the standardized conditions mentioned above and the log P_{HA} values can only be expected if the dissociation constants of the various salts are the same.

It has become clear from this study that it is not possible to obtain an f-value (Rekker and De Kort, 1979) or a π-value (Hansch and Leo, 1979b) of carboxylate, protonated amine and quaternary ammonium using the standardized method proposed by Hansch and Leo, because such values are also dependent on the dissociation constants of the salts of which the ionized group is a fragment. It should be stressed that better insight into the lipophilic behaviour of protolytes can only be obtained from both log P_{app}-pNa profiles (or, in general, from log P_{app}-pX profiles, when X is the counterion) and log P_{app}-pH profiles, but not from log P_{app}-pH profiles only.

Acknowledgements

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