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Hydrophobicity of β-lactam antibiotics

Explanation and prediction of their behaviour in various partitioning solvent systems and reversed-phase chromatography

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ABSTRACT

β-Lactam antibiotics tend to undergo self-association in hydrophilic organic solvents, which leads to a strong dependence of their experimentally observable log *P* values on the partitioning conditions. As a result, most of the earlier obtained log *P* values for β-lactam antibiotics cannot be applied as a common hydrophobicity measure, but they proved to be linearly related to each other and to a large body of reversed-phase chromatographic data. The retention of cephalosporins on reversed-phase liquid chromatographic columns is complicated by silanophilic interactions. However, under elution conditions that eliminate these silanophilic interactions, good correlations with log *P* data are observed, and a unified hydrophobicity scale for 90 penicillin and cephalosporin compounds could be evaluated. The Hansch and Leo additive scheme was shown to be valid for the calculation of hydrophobicities for penicillin and cephalosporin C-6(7) substituents, but it failed when applied to the prediction of cephalosporin C-3-substituent hydrophobicities. The hydrophobic increments for the sixteen most common cephalosporin C-3-substituents were empirically evaluated from literature data, and a simple equation was derived for an overall β-lactam antibiotic hydrophobicity calculation. The proposed scale is valid for predicting the partitioning of most β-lactam antibiotics in both hydrophilic and lipophilic organic–water systems, although it should be used with caution when applied to antibiotics containing additionally charged side-chains.

INTRODUCTION

It is essential to identify relationships between the physico-chemical properties of β-lactam antibiotics and their biological activity when searching for new pharmaceuticals of this class. As far back as 1963 Hansch and Fujita [1,2] established the need to proceed from the hydrophobicities of investigated substances when tackling the problem of any structure–activity relationships. In fact, the biological activity of β-lactam antibiotics and their hydrophobicities have been shown to be interrelated in numerous studies [3–21]. Meanwhile, the problem of determining the hydrophobic properties of penicillins and cephalosporins is still far from being solved completely.

First, in spite of a number of studies devoted to this problem [5–8,17–29], many of them considered the partitioning of antibiotics under different conditions, which makes their joint analysis complicated. As a result, at present only limited and often contradictory data sets are available in the literature on the partitioning for a maximum of about twenty antibiotics in each set. However, many publications devoted to the chromatographic separation and determination of β-lactams have appeared but have not yet been analysed in order to characterize the hydrophobicity of antibiotics.

Second, so far the generally accepted rules of hydrophobicity calculations suitable for other classes of compounds have been thought to be inapplicable to penicillins and cephalosporins [23,30,31].

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A rough estimate of log P reported [8] for a series of cephalosporins is obviously unsatisfactory and, as we show in this paper, contradicts the experimental results obtained in other work. Therefore, the aim of this study was to survey the available data on the hydrophobic properties of β-lactam antibiotics, and to demonstrate the possibility of their theoretical estimation according to the conventional rules of Hansch and Leo [32].

THEORETICAL

To establish a common hydrophobicity scale characterizing the partitioning of solutes in various organic–water systems, the free energies of partition should be linearly related:

$$\Delta G_i = \alpha \Delta G_j + \beta \quad (1)$$

where $\Delta G_{i(j)}$ is the free energy of the solute transition from water to the $i(j)$ organic phase. Depending on the system studied, $\Delta G_{i(j)}$ is proportional to the logarithm of partition coefficient, log P, the capacity factor, log k, or the value of $R_M = \log (1/R_F - 1)$.

In 1951, Collander [33] found that the eqn. 1 was valid for substances of various classes partitioned between water and different alcohols. Later, Leo showed [34] that in a more general case the linear dependence in eqn. 1 is observed only separately for hydrophilic (*e.g.*, alcohols and ketones) and lipophilic (*e.g.*, alkanes, benzene and chloroform) organic solvents. Seiler [35], however, asserted that the partition coefficients in lipophilic and hydrophilic organic systems were likewise related:

$$\log P_{\text{octanol}} = \log P_{\text{alkane}} + \sum I_{\text{H}} + \beta \quad (2)$$

where I_{H} denotes the additive increment to hydrogen bonding by a solute molecular segment in hydrophilic organic solvent.

Eqn. 2 is obviously transformed into the single-parameter dependence in eqn. 1 only for certain classes of substances with $\sum I_{\text{H}} = \text{constant}$. Indeed, Leo [34] showed log P for octanol–water partition to be linearly dependent on log P for partition in lipophilic solvents only for proton-acceptor (H-acceptor) or proton-donor (H-donor) solutes separately.

It is convenient to consider the β-lactam antibiotics as properly substituted nuclei (Table I). It is reasonable to assume that for β-lactam antibiotics with substituents similar in their H-donor or -acceptor ability, the linear dependence in eqn. 1 between their partition in hydrophilic and lipophilic systems will be observed as the nucleus hydrogen-bonding increment is always the same or very similar. Indeed, our own experience confirms that this is so for the majority of solutes listed in Table I.

An analysis of literature data shows that in the absence of side-group ionization in β-lactam antibiotics, eqn. 1 is valid irrespective of the temperature change. Thus, the log k values for six penicillins (2, 4, 5, 13, 23 and 29 in Table I) at five different temperatures have been reported [36]. Using these data in the form of the Van 't Hoff equation, we were able to calculate values proportional to the enthalpy and entropy of sorption for each solute:

$$\ln k = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} + \phi \quad (3)$$

where ΔH, ΔS and T represent the enthalpy, entropy and temperature of sorption on the bonded reversed-phase, respectively, ϕ is the volume ratio of the stationary and mobile phases and R is the universal gas constant. The slope is equal to $\Delta H/R$ and the intercept to $\Delta S/R + \phi$. The latter effective values proved to be linearly interrelated:

slope $= -387 \times$ intercept $- 638$; $n^a = 5$, $r = 0.9776$, $s = 201$

where n, r and s represent the number of correlated points, correlation coefficient and standard deviation, respectively. Having analogously processed the data from ref. 37 where log k values are reported for four cephalosporins (51, 56, 64 and 76 in Table I), we obtained:

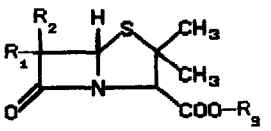
slope $= -248 \times$ intercept $+ 1283$; $n^a = 3$, $r = 0.9999$, $s = 12.2$

A theoretical analysis performed by Melander *et al.* [38] showed that the above-mentioned compensation relationships between ΔH and ΔS account for the linear dependence in eqn. 1 irrespective of temperature.

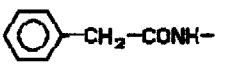
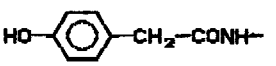
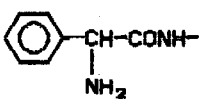
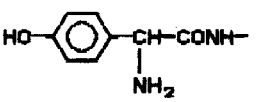
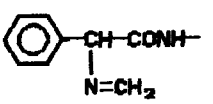
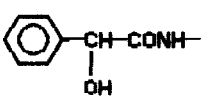
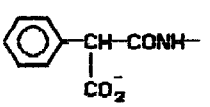
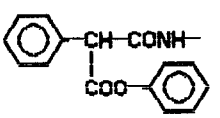
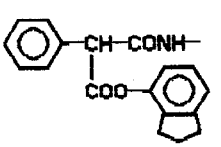
[a] Log k values of amoxicillin from ref. 36 and deacetylcephapirin from ref. 37 have been excluded from the common sets as they largely depended on determination of the column void volume.

TABLE I

PENICILLIN AND CEPHALOSPORIN STUCTURES



Penicillins

No.	Generic name or code	R_1	R_2	R_3^a
1	6-Aminopenicillanic acid (6-APA)	NH_2	H	H
2	Benzylpenicillin (penicillin G)		H	H
3	*p*-Oxybenzylpenicillin (penicillin X)		H	H
4	Ampicillin		H	H
5	Amoxicillin		H	H
6	Methylenampicillin (metampicillin)		H	H
7	α-Oxybenzylpenicillin		H	H
8	Carbenicillin (anion)		H	H
9	Carbenicillin phenyl (carfecillin)		H	H
10	Carbenicillin indanyl		H	H

(Continued on p. 6)

TABLE I *(continued)*

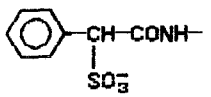
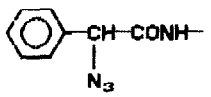
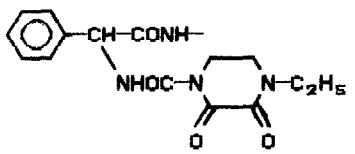
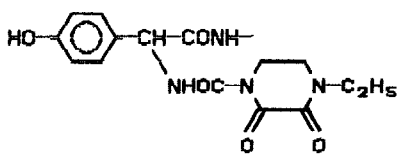
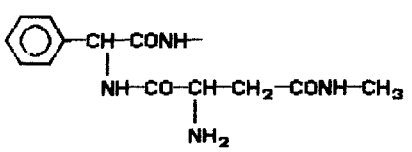
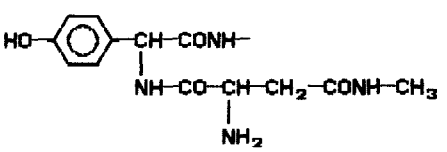
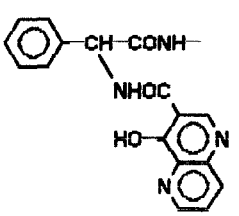
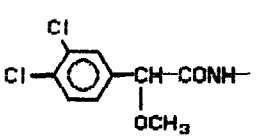
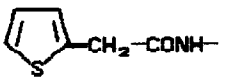
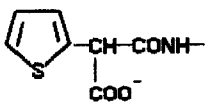
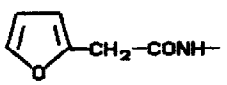
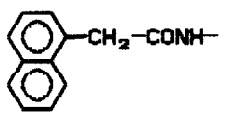
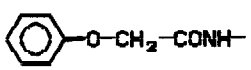
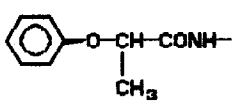
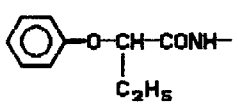
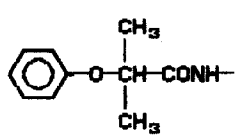
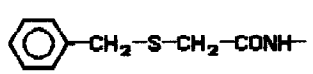
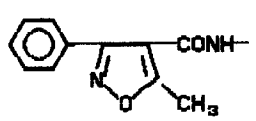
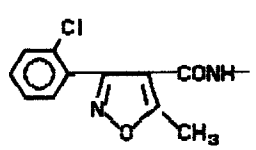
No.	Generic name or code	R_1	R_2	R_3^a
11	Sulbenicillin (anion)		H	H
12	Azidocillin		H	H
13	Piperacillin		H	H
14	*p*-Oxypiperacillin		H	H
15	Desoxyaspoxillin		H	H
16	Aspoxillin		H	H
17	Apalcillin		H	H
18	Clometocillin		H	H

TABLE I *(continued)*

No.	Generic name or code	R_1	R_2	R_3^a
19	2-Thienylmethylpenicillin		H	H
20	Ticarcillin (anion)		H	H
21	2-Furylmethylpenicillin		H	H
22	1-Naphthylmethylpenicillin		H	H
23	Phenoxymethylpenicillin (penicillin V)		H	H
24	Pheneticillin (phenoxyethyl-penicillin)		H	H
25	Propicillin (phenoxypropyl-penicillin)		H	H
26	Phenoxyisopropylpenicillin		H	H
27	Benzylthiomethylpenicillin		H	H
28	Oxacillin		H	H
29	Cloxacillin		H	H

(Continued on p. 8)

TABLE I *(continued)*

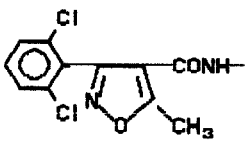
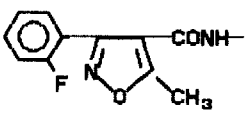
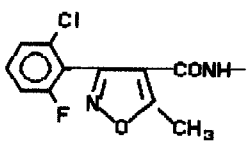
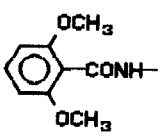
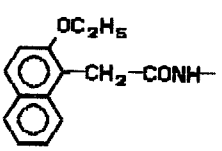
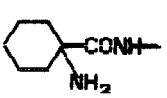
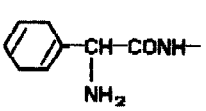
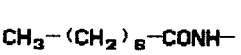
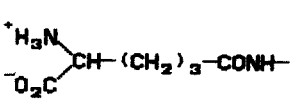
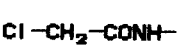
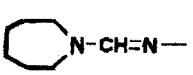
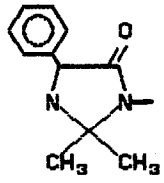
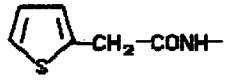
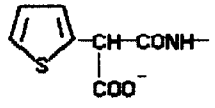
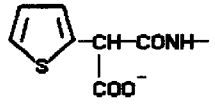
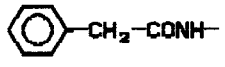
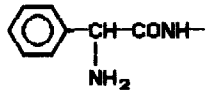
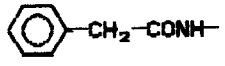
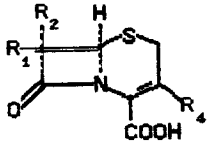
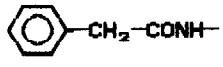
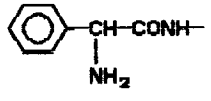
No.	Generic name or code	R_1	R_2	R_3^a
30	Dicloxacillin		H	H
31	Fluoxacillin (floxacillin)		H	H
32	Flucloxacillin		H	H
33	Methicillin		H	H
34	Nafcillin		H	H
35	Cyclacillin		H	H
36	Epicillin		H	H
37	*n*-Heptylpenicillin (penicillin K)		H	H
38	D-α-(δ-Aminoadipyl)penicillin (penicillin N)		H	H
39	Chlormethylpenicillin		H	H
40	Mecillinam (amdinocillin)		H	H

TABLE I *(continued)*

No.	Generic name or code	R_1	R_2	R_3^a
41	Hetacillin		H	H
42	BRL 20 153		OCH_3	H
43	Temocillin (anion)		OCH_3	H
44	BRL 26 277 (anion)		SCH_3	H
45	Penamecillin		H	CH_2OAc
46	Pivampicillin		H	$CH_2OOCC(CH_3)_3$
47	Penethacillin		H	$C_2H_4NEt_2$



Cephalosporins

No.	Generic name or code	R_1	R_2	R_4
48	7-Aminocephalosporanic acid (7-ACA)	NH_2	H	CH_2OAc
49	Cephaloram		H	CH_2OAc
50	Cephaloglycin		H	CH_2OAc

(Continued on p. 10)

TABLE I *(continued)*

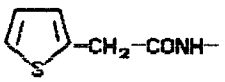
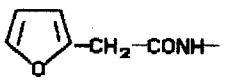
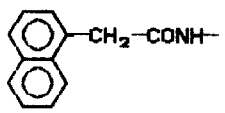
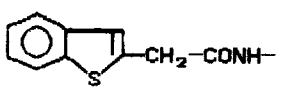
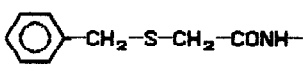
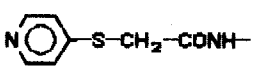
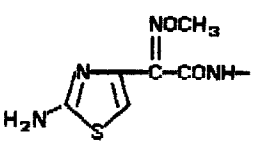
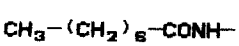
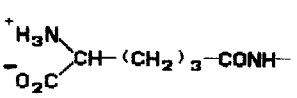
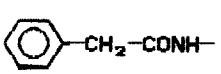
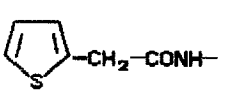
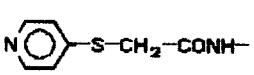
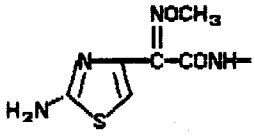
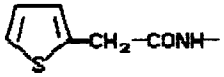
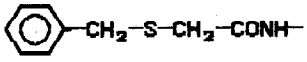
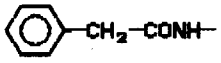
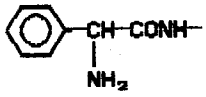
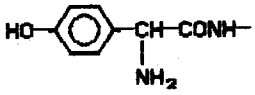
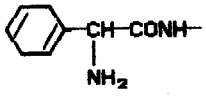
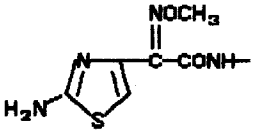
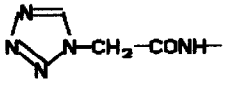
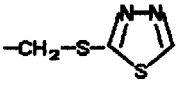
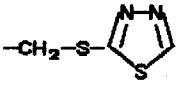
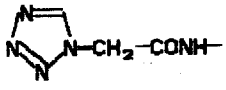
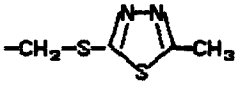
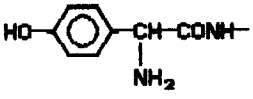
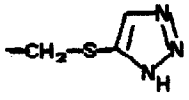
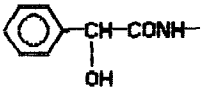
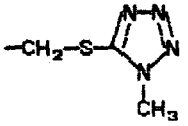
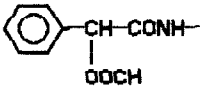
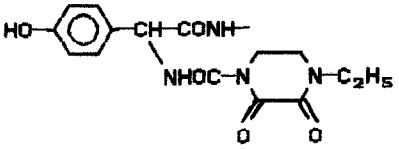
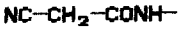
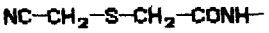
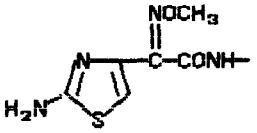
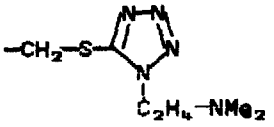
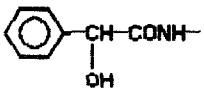
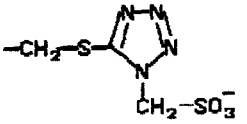
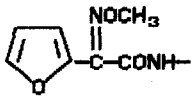
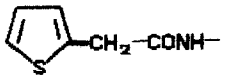
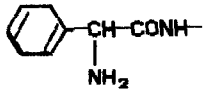
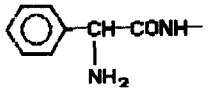
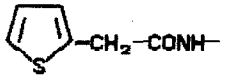
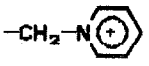
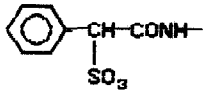
No.	Generic name or code	R_1	R_2	R_4
51	Cephalothin		H	CH_2OAc
52	2-Furylacetamidocephalosporin		H	CH_2OAc
53	1-Naphthylacetamidocephalosporin		H	CH_2OAc
54	Benzothienylacetamidocephalosporin		H	CH_2OAc
55	Benzylthioacetamidocephalosporin		H	CH_2OAc
56	Cephapirin		H	CH_2OAc
57	Cefotaxime		H	CH_2OAc
58	Caprylacetamidocephalosporin		H	CH_2OAc
59	Cephalosporin C		H	CH_2OAc
60	Cephacetrile	NC–CH_2–CONH–	H	CH_2OAc
61	Chloracetamidocephalosporin	Cl–CH_2–CONH–	H	CH_2OAc
62	Deacetylcephaloram		H	CH_2OH
63	Deacetylcephalothin		H	CH_2OH
64	Deacetylcephapirin		H	CH_2OH

TABLE I *(continued)*

No.	Generic name or code	R_1	R_2	R_4
65	Deacetylcefotaxime		H	CH_2OH
66	Deacetoxy-3′-azidocephalothin		H	CH_2N_3
67	7-(Benzylthioacetyl)aminodeacetoxy-3′-azidocephalosporanic acid		H	CH_2N_3
68	7-Aminodeacetoxycephalosporanic acid (7-ADCA)	NH_2	H	CH_3
69	Deacetoxycephaloram		H	CH_3
70	Cephalexin		H	CH_3
71	Cefadroxil		H	CH_3
72	Cefradine		H	CH_3
73	Ceftizoxime		H	H
74	Ceftezole		H	
75	C 49 753 (Ciba Geigy)	$NC-CH_2-CONH-$	H	
76	Cefazolin		H	
77	Cefatrizine		H	

(Continued on p. 12)

TABLE I *(continued)*

No.	Generic name or code	R_1	R_2	R_4
78	Cefamandole		H	
79	Cefamandole nafate		H	As No. 78
80	Cefoperazone		H	As No. 78
81	C 49 288 (Ciba Geigy)		H	As No. 78
82	7-H-Cefmetazole		H	As No. 78
83	Cefotiam		H	
84	Cefonicid		H	
85	Cefuroxime		H	CH_2OCONH_2
86	Cefoxitin		OCH_3	CH_2OCONH_2
87	Cefrodaxin		H	OCH_3
88	Cefaclor		H	Cl
89	Cephaloridine (cation)		H	
90	Cefsulodin (zwitterion)		H	

[a] The relative hydrophobicity values for penicillins do not depend on the cation near the C-3 carboxyl anion (except in the case of protonation), therefore the R_3 radical conventionally is denoted as H. Ac = Acetyl; Me = methyl; Et = ethyl.

As far as the aqueous phase properties are concerned, the situation seems to be much more complicated. It follows from the results of many studies that varying the mobile phase composition in reversed-phase liquid chromatography (RP-LC) does not necessarily lead to a linear change in log k. It may be assumed, however, that such unpredictable behaviour during chromatography is due to eluate interactions with free silanol groups on the column [39]. Indeed, the data from ref. 40 indicate that under conditions eliminating silanophilic interactions, the variation of the organic solvent content or pH of the eluent leads to linear log k changes (eqn. 1) for cephalosporins without C-7 and C-3 ionogenic groups. Analogously, the mobile phase variation in refs. 20–22 led merely to linear changes in R_M for cephalosporins and penicillins in RP thin-layer chromatography (TLC).

The evidence cited above testifies that by the linear transformations in eqn. 1 the literature partition data of at least the majority of β-lactam antibiotics can be brought into a single hydrophobicity scale.

RESULTS AND DISCUSSION

A unified penicillin hydrophobicity scale

It is most convenient to choose partitioning between water and octanol as standard conditions when reducing a large body of literatue data to a common scale, as in this instance the hydrophobicity of individual antibiotics might be calculated according to well known rules [32,41]. However, the log P values of β-lactam antibiotics for octanol–water partitioning are not convenient owing to their self-association in hydrophilic media (see the last section). We believe that ignoring this fact was the main reason why earlier attempts to predict the partitioning of penicillins [30,89] were unsuccessful, and only poor correlations between calculated and experimental log P values were achieved ($r < 0.94$). In fact, at present no octanol–water partition data on β-lactam antibiotics free from the self-association contribution are available. Therefore, we tried to find an "ideal" hydrophobicity scale that would satisfy the following requirements: it should describe the partitioning of β-lactam antibiotics adequately, and it should be free from contributions of various secondary equilibrium processes, so that the hydrophobicity for the simplest structures could be easily calculated.

Log P values for octanol–water partitioning of penicillin R_1-substituent amides proved to meet these requirements. The calculated log P values for single amides correlated well with experimental data for the proper penicillins from refs. 19–22, with a correlation coefficient always exceeding 0.99. A detailed calculation of these log P values is given in the Appendix. Tables II and III list the standardized penicillin hydrophobicity values, f_{Common}, obtained by linear transformations of the literature data to the log P scale of the R_1-substituent amides. The correlation parameters of these transformations are listed in Table IV for each case.

Methicillin appeared to contain an R_1 substituent with a large H-acceptor ability in comparison with other penicillins. Therefore, the two individual f_{C6} values were necessary for it in hydrophilic and lipophilic organic phases.

As the antibiotics 43–47 in Table I contain modified penicillin nuclei, their f_{Common} values should be regarded as a sum of hydrophobicity increments, f_{C6} for the C-6 substituent (or f_{C7} in the case of cephalosporins) and f_{Nuclei} for the modified nucleus:

$$f_{\text{Common}} = f_{C6(C7)} + f_{\text{Nuclei}} \quad (4)$$

Such an additive hydrophobicity representation is permissible, as the distance between the various substituents in the solute molecules is large enough, and their intramolecular interactions, according to ref. 32, can be neglected. The corresponding values of f_{Common} and f_{Nuclei} are given in Table III.

The problem of the correct determination of the column void volume often arises when reversed-phase high-performance liquid chromatographic (RP-HPLC) data have to be correlated. For instance, the eluent retention time obtained in refs. 54 and 66 exceeds that of the first antibiotics. As the accuracy of t_0 determination greatly affects the log k value for the most hydrophilic antibiotics, in some instances it has been estimated by us proceeding from the correlation standard error minimization. The chromatographic data in refs. 43 and 47 cannot be described by the f_{Common} scale, indicating large non-hydrophobic interactions between the eluted penicillins and the stationary phases. Amino-containing compounds are known to be especially disposed to enhanced silanophilic interactions [39].

TABLE II

STANDARD PENICILLIN HYDROPHOBICITY VALUES NORMALIZED TO COMMON OCTANOL–WATER PARTITION LOG *P* SCALE OF THEIR 6-SUBSTITUENT AMIDES

No.[a]	Standard hydrophobicity value, f_{C6}[b]	References which confirm the f_{C6} value[c]
1	−0.800[d] (H, L)[e]	21, 26, 45, 56
2	0.451 (H, L)	9, 17–19, 21–25, 28, 36, 43–45, 48, 51–57, 59, 60, 63, 64, calc.[f]
3	−0.220 (H, L)	48, 53, calc.
4	−0.098 (H, L)	9, 17, 20–22, 25, 26, 36, 42–45, 47, 49, 52, 55–57, 59, 60, 64, calc.
5	−0.743 (H, L)	9, 17, 25, 26, 36, 42, 44–46, 49, 57, 59, 60, calc.
6	−0.573 (L)	20, 21, 64
7	−0.060 (H)	19, calc.
8	−0.820 (L)	9, 20–22, 44, 45, 47, 54, 57, 58
9	2.250 (H, L)	24, 25, 44, 63
10	3.230 (H)	24
11	−1.125 (L)	9, 25
12	1.230 (H, L)	18, 44, 45, 57
13	0.450 (H, L)	36, 42, 46, 47, 54, calc.
14	−0.220 (L)	42
15	0.070[g] (L)	42
16	−0.510[g] (L)	42
17	−0.110[g] (L)	46
18	2.142 (L)	44
19	0.289 (H, L)	21, calc.
20	−1.013 (L)	9, 44, 46, 47, 54, 58, 61
21	−0.042 (H, L)	21, calc.
22	1.557 (H, L)	21, calc.
23	0.890 (H, L)	17–28, 36, 43–45, 48, 52, 53, 55–57, 59, 60, 63, calc.
24	1.101 (H, L)	17–25, 28, 44, 45, 55, 56, 63, calc.
25	1.683 (H, L)	17, 19, 23–25, 28, 43–45, calc.
26	1.580 (H)	19, calc.
27	1.226 (H, L)	21, calc.
28	1.189 (H, L)	9, 17, 18, 20–28, 43–45, 49–51, 59, 60, 62, 64
29	1.467 (H, L)	9, 17, 18, 20–27, 36, 44, 45, 49–51, 60, 62–65
30	1.950 (H, L)	9, 17, 21–25, 27, 44, 45, 49–51, 60, 62, 65
31	1.588 (H, L)	17, 24, 25, 43
32	1.640 (L)	20, 27, 44, 45, 49, 63–65
33	0.385 (L)	9, 21, 22, 27, 44, 45, 53, 60, 62
	−0.250 (H)	18, 19, 23, 24
34	1.545 (L)	21, 22, 45, 46, 58–60
35	0.613 (H, L)	17, 25, 45, 57
36	−0.010 (H, L)	17, 44, 57
37	1.943 (H, L)	21, calc.
38	−1.242 (L)	21
39	−0.690 (H, L)	21, calc.
40	0.552[d,g] (L)	57
41	0.060[d] (L)	45, 49

[a] The numbers correspond to those in Table I.

[b] For penicillins $f_{C6} = f_{Common}$ (see text).

[c] References in which predicted hydrophobicity differed from the standard f_{Common} value by less than 0.1 are included here.

[d] These antibiotics possess a non-traditional penicillin structure, therefore the hydrophobic values cannot be experimentally measured as log *P* for the proper amides.

[e] The letters in parentheses indicate the type of organic–water system for which the given value was shown to be valid: H = hydrophilic; L = lipophilic.

[f] Calculated in Appendix.

[g] These are uncertain values.

TABLE III

STANDARD HYDROPHOBICITY VALUES FOR THE PENICILLINS WITH MODIFIED NUCLEI (f_{Common})

No.[a]	f_{Common}	f_{C6}[b]	f_{Nuclei}	References which confirm the f_{Common} value[c]
42	0.176 (L)[d]	0.289	−0.113	61
43	−1.126 (L)	−1.013	−0.113	44, 61
44	−1.040 (L)	−1.013	−0.027	61
45	2.300 (L)	0.450	1.850	45
46	2.370 (L)	−0.098	2.468	45
47	1.820 (L)	0.450	1.370	45

[a,c,d] See footnotes *a*, *c* and *e*, respectively, in Table II.
[b] The f_{C6} values have been taken from Table II.

This appears to be the reason why the f_{C6} values of penicillins 4, 5, 6, 35 and 36 most often deviate from the relationships established in Table IV.

In refs. 38–40, the conditions for virtually complete elimination of silanophilic interactions between the eluate and RP stationary phases were enumerated: an enhanced ionic strength and pH, a moderate concentration of organic cosolvent and the presence of a hydrophobic alkylamine in the eluent. In fact, the chromatographic parameters from refs. 20–22, 25 and 49, where these requirements for elution were satisfied, correlate perfectly with the hydrophobic parameters in Table II.

Retention of cephalosporins in reversed-phase chromatography

In contrast to penicillins, no data are available on relations between the chromatographic behaviour of cephalosporins and their partition in water–organic solvent systems. Moreover, studying retention of fifteen cephalosporins on different RP-HPLC columns, Wouters and co-workers [66,67] came to the conclusion that no relationships among the structure of antibiotics, their elution time and different properties of RP-HPLC columns could be established. However, if reassessed, these data indicate that such relationships do in fact exist.

Table V presents a matrix of correlation coefficients between log k of all the cephalosporins reported in refs. 66 and 67 (top right part of the table) and between log k of selected cephalosporins with the same C-3 substituent (bottom left part of the table) on different RP-HPLC columns. Considering the relationships between log k values of the cephalosporins with different C-3 radicals, it will be easy to see that all the columns listed in this table can be divided into two groups: columns 1–8 and columns 10–13; column 9 occupies an intermediate position. The retention of cephalosporins on the columns inside each group can be interrelated by the eqn. 1, with the slope being close to 1. Log k values for cephalosporins on columns from different groups are poorly correlated. Of all the columns, Zorbax C_8 is distinguished as the log k values in this instance cannot be linearly related to those on other columns.

According to the theory of Horváth and co-workers [38,39,68], a least two types of forces contribute to solute–stationary phase interactions, hydrophobic and silanophilic. Therefore, in order to describe the interrelation of the log k values of cephalosporins on different columns, eqn. 1 should be supplemented by taking account of the additional type of interaction:

$$\begin{aligned} \Delta G_i &= a_i \Delta G_{\text{Hydroph}} + b_i \Delta G_{\text{Non-hydroph}} + c_i \\ \Delta G_j &= a_j \Delta G_{\text{Hydroph}} + b_j \Delta G_{\text{Non-hydroph}} + c_j \end{aligned} \quad (5)$$

where $\Delta G_{\text{Hydroph}}$ and $\Delta G_{\text{Non-hydroph}}$ are, respectively, the free energies of hydrophobic and silanophilic interactions between the eluate and the stationary phase. $\Delta G_{i(j)}$ is the total free energy of eluate sorption on the column, so it is directly proportional to log k; $b_{i(j)}$ is a certain characteristic of the column which depends on the number of free silanol groups. $\Delta G_{\text{Hydroph}}$ and $\Delta G_{\text{Non-hydroph}}$ values depend only on the eluate properties, and $a_{i(j)}$, $b_{i(j)}$ characterize only the stationary and mobile phases. The equation system 5 is reduced to the single-parameter eqn. 1 in the following cases:

(a) For all the eluates either $\Delta G_{\text{Hydroph}}$ = constant or $\Delta G_{\text{Non-hydroph}}$ = constant. Since this would mean the existence of the linear relationships in eqn. 1 between the log k values of cephalosporins on any of the two columns, irrespective of their division into separate groups, this case is not observed here.

(b) For columns from the same group the following condition is satisfied:

$$\frac{a_i}{a_j} = \frac{b_i}{b_j} = \alpha \quad (6)$$

TABLE IV

CORRELATION PARAMETERS OF THE LINEAR RELATIONSHIPS IN EQN. 1 BETWEEN THE LITERATURE DATA (ΔG_i) AND THE COMMON HYDROPHOBICITY SCALE OF PENICILLINS, f_{C6} OR f_{Common}, FROM TABLES II AND III (ΔG_j)

Ref.	Organic phase (stationary phase)	Aqueous phase (mobile phase)	Ionic form of antibiotic[a]	Correlated value[b] (ΔG_i)	Slope	Intercept	n	r	s	Included points[c]	Excluded points[c]
	n-Octanol	Water[d]	–	Log P	0.993	0.017	17	0.9976	0.061	2–5, 7 14, 19, 21–27, 35, 37, 39	–
18	*n*-Octanol	Acetate buffer (pH 4.0)		Log P^e	0.838	1.297	7	0.9953	0.049	2, 12, 23, 24, 28, 29, 33	8, 30
19	*n*-Octanol	pH 3–4	Un-ionized form	Log P^e	0.772	1.438	7	0.9947	0.066	2, 7, 23–26, 33	–
23	*n*-Octanol	pH 3–4	Un-ionized form	Log P^e	0.751	1.364	8	0.9960	0.051	2, 23–25, 28–30, 33	–
23	*n*-Octanol-impregnated TLC plate	pH 3.0		R_M[d,f]	0.779	−0.356	8	0.9958	0.055	2, 23–25, 28–30, 33	–
24	*n*-Octanol	pH 3–6, 0.15 *M* KCl	Un-ionized form	Log P^e	0.725	1.415	11	0.9953	0.069	2, 9, 10, 23–25, 28–31, 33	–
24	*n*-Octanol	pH 7.4, 0.15 *M* KCl	Anion	Log P	0.731	−2.098	6	0.9708	0.158	9, 10, 23, 25, 30, 31	–
25	*n*-Octanol		Un-ionized form; estimated	Log P	0.735	1.413	12	0.9986	0.043	2, 4, 5, 9, 11, 23–25, 28–31	35
17	Isobutanol	pH 7.4, 0.15 *M* KCl	Anion	Log P	0.535	−0.411	11	0.9967	0.039	4, 5, 23–25, 28–31, 35, 36	–
24	Isobutanol	pH 7.4, 0.15 *M* KCl	Anion	Log P^e	0.434	−0.323	11	0.9791	0.088	2, 9, 10, 23–25, 28–31, 33	–
24	Isobutanol	pH 7.4, 0.15 *M* KCl	Anion	Log P^e	0.512	−0.393	9	0.9858	0.064	2, 23–25, 28–31, 33	9, 10
26	*n*-Butanol	0.5 *M* KCl, 37°C	Anion	Log P	0.352	−0.296	5	0.9701	0.102	1, 4, 5, 23, 29	–
27	Chloroform	Teorell buffer	Anion	Log P	1.306	0.506	6	0.9972	0.061	23, 28–30, 32, 33	–
28	Chloroform	0.1 *M*, 25°C	Un-ionized form	(Log P + pK_a)	1.208	3.318	5	0.9753	0.142	2, 23–25, 28	–
28	Chloroform	0.1 *M*, 25°C	Un-ionized form	(Log P + pK_a)	1.522	3.067	4	0.9955	0.059	2, 23, 24, 28	25
28	Chloroform	0.1 *M*, 25°C	Anion	Log K_{QX}[g]	1.266	1.609	5	0.9713	0.161	2, 23–25, 28	–
28	Chloroform	0.1 *M*, 25°C	Anion	Log K_{HAX}[h]	1.228	3.195	5	0.9529	0.203	2, 23–25, 28	–
28	Chloroform	0.1 *M*, 25°C	Un-ionized form	Log K_{Ass}[i]	−0.144	2.054	3	−0.9455	0.028	23, 25, 28	24
9	Silica gel 60 F_{254}[m] (Merck)	Acetate–veronal buffer + 20% CH_3OH, pH 7.0	Anion	R_M	0.614	−0.118	10	0.9844	0.129	2, 4, 5, 8, 11, 20, 28–30, 33	–
20	Silica gel G[m]	Extrapolated to 0% acetone, pH 7.4	Anion	R_M	0.776	0.166	8	0.9993	0.029	4, 6, 8, 23, 24, 28, 29, 32	5
21	Silica gel G[m]	Extrapolated to 0% acetone, pH 7.4	Anion	R_M	0.773	0.172	11	0.9991	0.030	2, 4, 6, 8, 23, 24, 28–30, 33, 34	–
21	Silica gel G[m]	Extrapolated to 0% acetone, pH 7.4	Anion	R_M[j]	0.720	0.211	10	0.9975	0.057	1, 2, 4, 19, 21, 22, 27, 37–39	–
22	Silica gel G[m]	Extrapolated to 0% acetone, pH 9.4	Anion	R_M	0.710	0.129	10	0.9956	0.060	2, 4, 8, 23, 24, 28–30, 33, 34	–
22	Silica gel G[m]	Extrapolated to 0% acetone, pH 2.6	Un-ionized form	R_M	0.807	0.363	10	0.9810	0.142	2, 4, 8, 23, 24, 28–30, 33, 34	–

42	Silica gel 60 F_{254}[m] (Merck)	0.05 *M* phosphate buffer, pH 7.0	Anion	R_M	1.492	0.556	6	0.9814	0.138	4, 5, 13–16	–
43	HPTLC plates F_{254}[m] (Merck)	0.01 *M* KH_2PO_4 + 61.5% CH_3OH + 7.7% CH_3CN, pH 4.1		R_M	0.258	−0.247	6	0.8325	0.132	2, 4, 23, 25, 28, 31	–
44	Silica gel layer[m]	Average of 22 mobile phases, pH ≈ 5.0		R_M[f]	0.445	−0.149	18	0.9855	0.088	2, 4, 5, 8, 9, 12, 18, 20, 23–25, 28–30, 32, 33, 36, 43	–
44	Silica gel layer[m]	Average of 22 mobile phases, pH ≈ 5.0		R_M[f]	0.412	−0.087	15	0.9981	0.029	2, 8, 9, 12, 18, 20, 23–25, 28–30, 32, 33, 43	4, 5, 36
45	Silica gel HF_{254}[m]	Phthalate buffer + 20% acetone, pH 6.0; first elution		R_M	0.759	−0.468	18	0.9872	0.119	1, 2, 4, 5, 8, 12, 23–25, 28–30, 32–34, 36, 41, 47	6, 35
45	Silica gel HF_{254}[m]	As above, second elution		R_M	0.839	−0.762	18	0.9901	0.114	2, 4, 5, 8, 12, 23–25, 28–30, 32–34, 36, 41, 45, 47	6, 35
45	Silica gel HF_{254}[m]	As above, third elution		R_M	0.851	−0.925	19	0.9893	0.124	2, 4, 5, 8, 12, 23–25, 28–30, 32–34, 36, 41, 45–47	6, 35
25	Zorbax-ODS	0.035 *M* NH_4Cl + 30% CH_3OH	Anion	Log *k*	0.755	−0.380	13	0.9710	0.198	2, 4, 5, 9, 11, 23–25, 28–31, 35	–
25	Zorbax-ODS	0.035 *M* NH_4Cl; extrapolated to 0% CH_3OH, pH 7.4	Anion	Log *k*	0.712	1.000	10	0.9987	0.043	2, 4, 5, 9, 11, 23, 24, 29–31	–
36	Spherisorb-ODS	0.05 *M* phosphate buffer + 20% isopropanol, pH 7.25, 15°C	Anion	Log *k*, t_0 = 1.8	0.607	0.073	6	0.9939	0.058	2, 4, 5, 13, 23, 29	–
46	LiChrosorb RP-18	0.01 *M* sodium acetate + 20% CH_3CN, pH 3.15		Log *t*, t_0 = 0	0.492	0.638	5	0.9857	0.099	5, 13, 17, 20, 34	–
47	Ultrasphere-ODS	6 m*M* MOPS–TEA buffer + 40% CH_3OH, pH 6.7	Anion	Log *t*, t_0 = 0	−0.106	0.795	4	−0.2896	0.289	4, 8, 13, 20	–
48	RP-8, Brownlee Labs.	0.05 *M* phosphate buffer + 53% CH_3OH, pH 3.5		Log *t*, t_0 = 0	0.283	0.766	3	0.9938	0.032	2, 3, 23	–
	μBondapak C_{18}	0.1 *M* borate buffer + 0.6% cetyltrimethylammonium bromide + 23% *n*-propanol, pH 8.5	Anion	Log *k*, t_0 = 2.5	0.332	0.427	7	0.9995	0.001	4, 5, 28–30, 32, 41	–
50	RP-2, Brownlee Labs.	0.05 *M* phosphate buffer + 20% CH_3CN, pH 4.5		Log *t*, t_0 = 0	0.559	−0.827	3	0.9996	0.009	28–30	–

(Continued on p. 18)

TABLE IV *(continued)*

Ref.	Organic phase (stationary phase)	Aqueous phase (mobile phase)	Ionic form of antibiotic[a]	Correlated value[b] (ΔG_i)	Slope	Inter-cept	n	r	s	Included points[c]	Excluded points[c]
51	Develosil-ODS-10	50% CH_3OH, pH 2.5		Log k	0.357	0.523	4	0.9997	0.006	2, 28–30	4, 5, 8, 35
52	Anion-exchange resin	0.02 *M* $NaNO_3$ + 0.01 *M* borate buffer, pH 9.15	Anion	Log k, t_0 = 4.7	0.741	−0.328	3	0.9993	0.019	2, 4, 23	–
53	RP-HPLC column	Linear gradient: 0.01 *M* NaH_2PO_4 + 0.01 *M* EDTA + 16.5–31.5% CH_3OH		Log t, t_0 = 0	0.427	0.837	4	0.9469	0.081	2, 3, 23, 33	–
54	Ultrasphere-ODS	0.01 *M* NaH_2PO_4 + 15% CH_3CN, pH 4.7, 45°C		Log k	1.231	0.364	4	0.9862	0.201	2, 8, 13, 20	–
55	Bondapak C_{18}	0.15 *M* buffer + 30% CH_3OH	Anion	Log k	0.784	0.470	4	0.9969	0.040	2, 4, 23, 24	–
56	Styrene–divinyl-benzene copolymer	0.15 *M* buffer + 50% CH_3OH	Anion	Log k	0.996	0.660	5	0.9930	0.106	1, 2, 4, 23, 24	–
57	LiChrosorb RP	0.1 *M* phosphate buffer + 30% CH_3OH, pH 8.0	Anion	Log k	0.824	0.542	9	0.9598	0.181	2, 4, 5, 8, 12, 23, 35, 36, 40	–
58	Bondapak phenyl (Waters Assoc.)	0.01 *M* ammonium acetate, pH 7.0	Anion	Log t, t_0 = 0	0.328	0.561	3	0.9836	0.121	8, 20, 34	–
59	Spherisorb S5-ODS	1 m*M* acetate buffer + 10% CH_3CN, pH ≈ 4.0		Log k	1.095	0.208	5	0.9921	0.124	2, 4, 5, 23, 28	–
59	Spherisorb S5-ODS	1 m*M* acetate buffer + 20% CH_3CN, pH ≈ 4.0		Log k	0.427	−0.330	4	0.9923	0.030	2, 23, 28, 34	–
60	Chromegabond C_{18}	0.01 *M* KH_2PO_4 + CH_3OH + CH_3CN		Log k, t_0 = 3.2	0.689	−0.432	9	0.9926	0.078	2, 4, 5, 23, 28–30, 33, 34	–

61	μBondapak C_{18}	0.1 *M* phosphate buffer + 10% CH_3OH, pH 7.0	Anion	Log t, $t_0 = 0$	0.654	1.669	4	0.9912	0.066	20, 42–44	–
62	C_8	Extrapolated to 0% CH_3CN		Log k	0.740	0.998	3	0.9991	0.018	28–30	33
62	C_8	Extrapolated to 0% CH_3CN		Log k^k	0.728	1.990	4	0.9991	0.024	28–30, 33	–
63	–	Water, 30°C		Log CMC[l]	−0.473	−0.189	6	−0.9644	0.091	2, 9, 23, 24, 29, 32	–
63	–	0.15 *M* KCl, 30°C		Log CMC[l]	−0.655	−0.166	6	−0.9895	0.067	2, 9, 23, 24, 29, 32	–
64	μBondapak C_{18}	0.015 *M* phosphate buffer + 30% CH_3OH, pH 7.0	Anion	Log t, $t_0 = 0$	0.491	0.826	6	0.9946	0.039	2, 4, 6, 28, 29, 32	5
65	Hypersil-ODS	0.01*M* Na_2HPO_4, extrapolated to 0% CH_3CN and pH 0, 28°C	Un-ionized form	Log k^f	0.122	0.850	3	0.9983	0.002	29, 30, 32	–

[a] The ionic form of antibiotic is given here when it was especially indicated in the original references or in cases when it was possible to define the ionic state from the partitioning conditions (pH of aqueous phase).

[b] P, k and t are the partition coefficient, capacity factor and retention time of antibiotics, respectively; t_0 is retention time of the eluent (min).

[c] The numbers correspond to those in Table I. Excluded points refer to antibiotics with additionally ionized side-chains under partition conditions or those which diverge sharply from established relationships.

[d] Calculated in Appendix for the proper R_{C6} amides.

[e] In these instances the hydrophilic partition value f_{C6} of −0.250 for methicillin was used. In all the other instances the lipophilic value of 0.385 was accepted (see Table II).

[f] Data for the other mobile and stationary phases given in these papers correlated analogously.

[g] K_{QX} is the coefficient of extraction by the tetrabutylammonium cation: $K_{QX} = [Q^+X^-]/([Q^+][X^-])$, where Q^+ is the tetrabutylammonium cation and X^- is the penicillin anion.

[h] K_{HAX} is the coefficient of extraction by dodecylammonium cation: $K_{HAX} = [HA^+X^-]/([HA^+][X^-])$, where HA^+ is the dodecylammonium cation and X^- is the penicillin anion.

[i] K_{Ass} is the dimerization constant of protonated penicillin (see Scheme 1).

[j] In this instance the data for C-3′-acetylcephalosporins were correlated.

[k] In this instance the data for the {[(1,4-dihydro-1-methyl-3-pyridinyl)carbonyl]oxy}methyl esters of the proper penicillins were correlated (see Table VIII).

[l] CMC is critical micelle concentration (mol kg^{-1}) of the penicillins in aqueous solutions.

[m] Impregnated with silicone oil or other reversed-phases.

TABLE V

CORRELATION MATRIX BETWEEN LOG k OF FOURTEEN CEPHALOSPORINS[a] WITH DIFFERENT C-3 SUBSTITUENTS, NOS. 50, 51, 56, 57, 59, 70, 71, 72, 76, 77, 78, 85, 86 AND 88 ACCORDING TO TABLE I (TOP RIGHT PART OF THE TABLE), AND BETWEEN LOG k OF FIVE CEPHALOSPORINS WITH THE SAME C-3 SUBSTITUENT NOS. 50, 51, 56, 57 AND 59 (BOTTOM LEFT PART OF THE TABLE) ON DIFFERENT RP-HPLC COLUMNS

No.[b]	Column[c]	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Methyl red adsorption value (mg/g)[d]
1	LiChrosorb RP-8, Hibar, 10 μm	1	0.990	0.974	0.983	0.990	0.996	0.983	0.991	0.985	0.962	0.934	0.954	0.888	0.968	ND
2	LiChrosorb RP-8, 10 μm	0.998	1	0.986	0.976	1.000	0.997	0.988	0.997	0.990	0.981	0.959	0.976	0.921	0.958	2
3	LiChrosorb RP-8, 5 μm	0.994	0.998	1	0.984	0.986	0.987	0.998	0.989	0.960	0.951	0.913	0.937	0.866	0.916	2
4	LiChrosorb RP-8[e,f], 10 μm	0.992	0.990	0.993	1	0.975	0.985	0.991	0.981	0.956	0.921	0.881	0.919	0.831	0.910	2
5	LiChrosorb RP-8[e,f], 10 μm, 2 years later	0.998	1.000	0.998	0.989	1	0.997	0.987	0.997	0.990	0.979	0.956	0.974	0.915	0.955	2
6	LiChrosorb, RP-8[e,f], Hibar, 10 μm	0.998	1.000	0.998	0.989	1.000	1	0.991	0.999	0.988	0.974	0.948	0.965	0.907	0.973	ND
7	LiChrosorb RP-8[e,f], 5 μm	0.996	0.998	1.000	0.996	0.998	0.998	1	0.992	0.964	0.948	0.910	0.936	0.860	0.924	2
8	μBondapak C_{18}, 10 μm	0.998	1.000	0.999	0.994	0.999	0.999	1.000	1	0.988	0.970	0.944	0.966	0.906	0.955	1
9	Nucleosil C_{18}, 10 μm	0.999	0.998	0.993	0.989	0.998	0.998	0.995	0.997	1	0.981	0.973	0.989	0.951	0.973	ND
10	Nucleosil C_8, 10 μm	0.985	0.987	0.977	0.957	0.986	0.988	0.976	0.982	0.988	1	0.991	0.984	0.961	0.967	13
11	Polygosil C_8, 10 μm	0.978	0.981	0.970	0.948	0.980	0.981	0.968	0.975	0.984	0.998	1	0.991	0.987	0.956	15
12	R-Sil C_{18} LL, 10 μm	0.992	0.993	0.986	0.976	0.995	0.993	0.986	0.990	0.997	0.992	0.993	1	0.982	0.952	92
13	Partisil ODS, 10 μm	0.977	0.978	0.965	0.948	0.979	0.978	0.965	0.972	0.984	0.993	0.998	0.995	1	0.918	ND
14	Zorbax C_8[f] 7 μm	0.986	0.989	0.981	0.960	0.989	0.990	0.979	0.984	0.988	1.000	0.996	0.991	0.990	1	0.5

[a] In all instances cephaloridine was excluded from the general cephalosporin set, its log k values being appearently outside the general relationships.

[b] The numbers in the row correspond to those in the column. Columns with the same $b_{i(j)}$ value are separated by continuous lines.

[c] If not stated otherwise, the data were taken from ref. 66. The mobile phase in all instances was 5% phosphate buffer containing 1.0–11.5% CH_3CN.

[d] Data from ref. 67. This value characterizes the overall number of residual silanol groups in the columns.

[e] Data from ref. 67. The mobile phase in all instances was 0.01 M phosphate buffer (pH 7.0) containing 8.5–12.0% CH_3CN.

[f] In these instances the values of eluent retention times (t_0) reported in the original papers exceeded those for the most hydrophilic eluates or they were not reported at all, therefore the following values of t_0 were accepted: No. 4, 2.80 min; No. 5, 2.50 min; No. 6, 2.30 min; No. 7, 2.65 min; No. 14, 1.80 min. In all other instances t_0 values reported in ref. 66 were used.

where $a_{i(j)}$, $b_{i(j)}$ are related to eqns. 5 and α to eqn. 1. Indeed, as shown in Table V, columns inside one group have roughly the same number of non-end-capped silanol groups, and therefore $b_i/b_j \approx 1$. The ratio a_i/a_j cannot noticeably differ from 1, as the reversed-phase was the same (bonded n-alkyl) and the eluent composition differed insignificantly in all instances. For columns from different groups, char-

acterized by large differences in the density of free silanol groups, $b_i/b_j \ll 1$ ($\gg 1$), and therefore the linear relationships in eqn. 1 are not observed in this instance. A small change in the composition of the eluent causes only a minor deviation of the ratios a_i/a_j and b_i/b_j from 1, and therefore, irrespective of the RP column properties, good correlations between $\log k_{\text{Eluent}_i}$ and $\log k_{\text{Eluent}_j}$ ($r > 0.99$) are obtained.

It is noteworthy that the amounts of free silanol groups listed for each column in ref. 67 (Table V) should be regarded only as an approximate characteristic of the silanophilic interaction ability of the stationary phase. Actually, the parameter $b_{i(j)}$ should be compared not with the total number of free silanol groups but rather with the proportion that readily interacts with analytes [69]. This is the probable reason why the poor correlation between $\log k$ on the Zorbax C_8 column and the columns inside the first group are observed.

Considering the cephalosporins with the same C-3 substituent, linear relationships (eqn. 1) between $\log k$ are observed for all the columns, independently of their division into separate groups (see the bottom left part of Table V). This demonstrates that if the C-3 radical is constant, the silanophilic interaction increment for different cephalosporins also appears to be constant. In accordance with the case (a) this leads to the linear relationships in eqn. 1 for any mobile and stationary phases, irrespective of their $a_{i(j)}$ and $b_{i(j)}$ characteristics. However, this does not mean that C-3 substituents themselves interact with the free silanol groups. Most likely, the C-4 carboxyl anion, common to all the cephalosporins, is involved in such an interaction, and different C-3 substituents appear to create a corresponding microenvironment for this group. Charged C-3 substituents exert an especially strong effect. Cephaloridine was reported [67] to be the most sensitive to silanophilic interactions among the studied cephalosporins. Indeed, $\log k$ of cephaloridine sharply deviated from the linear dependence in almost all the eluate sets examined by us (see below). Large positive changes were observed even with a minor change in the mobile phase composition with a constant stationary phase. Ion–ion interactions, extremely sensitive to changes in the bulk medium properties (*i.e.*, in the organic co-solvent concentration in the eluent) appear to take place between the positively charged C-3′ pyridinium substituent and the deprotonated C-4 carboxyl group.

The proposed interactions between the C-3 substituent and the C-4 carboxyl anion apparently make the chromatographic behaviour of cephalosporins

TABLE VI

STANDARD CEPHALOSPORIN HYDROPHOBICITY VALUES

No.[a,b]	f_{Common}	f_{C7}[c]	f_{Nuclei}[d]	References which confirm f_{Common} value[e]
48	−0.801 (L)[f]	−0.800	−0.001	21, 22, 56, 72, 81
49	0.424 (L)	0.451	−0.027	21, 22
50	−0.101 (H, L)	−0.098	−0.003	21, 25, 45, 66, 71, 72, 78, 84
51	0.315 (H, L)	0.289	0.026	6, 9[g], 21, 22, 25, 37, 40, 45, 49, 54–56, 66, 71, 73–76, 79, 88
52	−0.050 (L)	−0.042	−0.008	21, 22
53	1.550 (L)	1.557	−0.007	21, 22
54	1.626 (H)	1.600[h]	0.026	8
55	1.219 (L)	1.226	−0.007	21, 22
56	−0.420 (L)	−0.450[h]	(0.030)	37, 49, 81
		−0.420[i]		
57	−0.407 (L)	−0.407[i]		40, 54, 66, 71, 74–77, 79, 80, 82, 86–88
58	1.936 (L)	1.943	−0.007	21, 22
59	−1.241 (L)	−1.242	0.001	9[g], 21, 22, 66, 71, 81, 84
60	−0.837 (H, L)	−1.460[h]	(0.624)	6, 8, 9[g], 49, 81
		−0.837[i]		
61	−0.698 (L)	−0.690	−0.008	21

(Continued on p. 22)

TABLE VI *(continued)*

No.[a,b]	f_{Common}	f_{C7}[c]	f_{Nuclei}[d]	References which confirm f_{Common} value[e]
62	−0.367 (L)	0.451	−0.818	21
63	−0.508 (L)	0.289	−0.797	72, 79
64	−1.230 (L)	−0.42[i]	−0.810	37
65	−1.210 (L)	−0.407[i]	−0.803	77, 86, 88
66	0.240 (L)	0.289	−0.049	21, 22
67	1.219 (L)	1.226	−0.007	21, 22
68	−1.110 (L)	−0.800	−0.310	56, 72, 81
69	0.107 (L)	0.451	−0.344	55, 56
70	−0.414 (H, L)	−0.098	−0.316	17, 25, 26, 45, 66, 71, 72, 74, 76, 77, 80, 83–85, 87, 88
71	−1.040 (H, L)	−0.743	−0.297	9[g], 17, 66, 74, 80
72	−0.288 (H, L)	−0.010	−0.278	17, 25, 45, 49, 71, 74, 83, 84, 86
73	−0.630 (L)	−0.407[i]	−0.223	74, 82
74	−0.747 (L)	−0.959[i]		9[g]
75	−0.625 (H)	−0.837[i]	0.212	6
76	−0.370 (H, L)	−0.959[i]	0.589	6, 8, 9[g], 25, 37, 40, 49, 54–56, 66, 71, 73, 74, 76, 77, 80, 81, 84, 88
77	−0.546 (L)	−0.743	0.197	66, 71, 83
78	0.111 (H, L)	−0.060	0.171	8, 9[g], 40, 54, 66, 71, 73–76, 78, 79, 82
79	0.504 (L)	0.252[h]	0.252	71, 74
80	0.060 (L)	−0.220	0.280	54, 73, 74, 80
81	−0.620 (H)	−0.837[i]	0.217	6
82	−0.906 (H)	−0.822[h] −1.136[i]	(−0.084)	8
83	0.238 (L)			40, 85
84	−0.630 (L)	−0.060	−0.570	54
85	−0.404 (H, L)	−0.014[i]	−0.390[k]	8, 40, 66, 71, 75, 78, 79
86	−0.215 (H, L)	0.289	−0.504	8, 9[g], 54, 66, 71, 74–80, 82
87	−0.153 (L)	−0.010	−0.143	40, 85
88	−0.451 (L)	−0.098	−0.353	66, 71, 74, 76, 80, 87
89	−2.051 (L)	0.289	−2.340	26, 49, 81, 88
90	−1.279[j] (L)	−1.125	−0.154[j]	9[g], 40, 85

[a,e,f,h,j] See footnotes *a*, *c*, *e*, *f* and *g*, respectively, in Table II.

[b] The antibiotics with the same nuclei (*i.e.*, the same C-3 substituent except in the case of cefoxitin) are separated by horizontal lines.

[c] If not stated otherwise, the f_{C7} values are taken from Table II.

[d] For cephalosporins (except cefoxitin) f_{Nuclei} values represent relative hydrophobicities of the C-3 substituents. The mean f_{Nuclei} values for all the nuclei are given in Table VIII. The f_{Nuclei} values in parentheses were not taken into account when calculating the mean values.

[g] In this instance the mean values of the data for 22 antibiotics from refs. 9–16 were correlated. The main part of these data (for sixteen solutes) was given in ref. 9.

[i] These f_{C7} values were calculated from reliable f_{Nuclei} values as follows: $f_{C7} = f_{Common} - f_{Nuclei}$.

[k] Calculated from the f_{Nuclei} value of cefoxitin by postulating that the hydrophobic increment of 7-OCH_3 was the same as for penicillins (−0.113).

distinctly different from that of penicillins. For the latter silanophilic interactions may be expected always to be identical, as the surrounding of the C-3 carboxyl in their nuclei is always the same. Therefore, in this instance condition (a) for reducing the equation system 5 to eqn. 1 always applies irrespective of the $a_{i(j)}$ and $b_{i(j)}$ values. Hence any set of log k or R_M values will always correlate with the unified hydrophobicity scale. This is true, of course, only if no specific interactions between the stationary phase and the R_1 substituent are present and in the absence of an R_4 substituent. For cephalosporins the linear relationships in eqn. 1 between log k or R_M will be observed only on stationary phases with roughly the same number of free silanol groups. However, a unified set of log k or R_M values obtained by those linear transformations will not necessarily correlate with the common hydrophobicity scale. Probably this is the reason why up to now there has been no published evidence for the correlation between the chromatographic behaviour of cephalosporins and their water–organic solvent partition, whereas for penicillins such relationships have been reported on many occasions.

A unified cephalosporin hydrophobicity scale

As has been stated above, it is most convenient to choose octanol–water partition log P for the R_1-substituent amides as standard conditions when reducing the literature data to a single hydrophobicity scale (Table VI). Table VII gives the correlation parameters of these transformations for each case. The zero value for a standard f_{Common} scale was chosen in order to correlate jointly data for penicillins and cephalosporins. In the previous section we have demonstrated that the linear relationships in eqn. 1 may be fulfilled even when the retention of cephalosporins is noticeably affected by silanophilic interactions. Therefore, reducing all RP chromatographic data to a common scale, we paid most attention to their ability to correlate well with the literature data, where silanophilic interactions were minimal. Such data include, especially, log k and R_M from refs. 21, 22, 40, 49, 75 and 84, where the corresponding conditions of the eluent composition were generally fulfilled (for the enumeration of the indications of an "ideal" eluent, see above), and also log k on the stationary phases from the first group in Table V.

Table VII shows that the corresponding values for cephapirin (56) and cephaloridine (89) nearly always diverged from the established relationships (eqn. 1). This is probably due to their extraordinary ability for silanophilic interactions, and so f_{Common} values for these antibiotics were calculated proceeding from the most reliable data from ref. 49. Deviation of the retentions of other antibiotics from the established relationships in Table VII is probably due to the protonation–deprotonation ability of their ionogenic R_1 substituents or to the inaccurate column void volumes determined in the original work; this, in turn, badly distorts the log k values for the most hydrophilic eluates. Generally, the results summarized in Table VII indicate that the published data on the partitioning of β-lactam antibiotics are well described by the common hydrophobicity scale. Therefore, the absence of linear relationships between f_{Common} and the calculated values of log P from ref. 8 or experimental values from ref. 5 is probably indicative of incorrect results in those studies.

As the total hydrophobicity of cephalosporins, like that of penicillins, is a sum of hydrophobic increments of the C-7 radical and nucleus, Table VI adduces the corresponding values of f_{C7} (taken generally from Table II) and f_{Nuclei} (calculated according to eqn. 4). To make it more convenient for use in calculations, the mean hydrophobicity values of f_{Nuclei} are summarized in Table VIII.

Obviously, using eqn. 4, one can calculate the value of f_{Common} for all β-lactam antibiotics that have the $f_{C6(C7)}$ value for the corresponding R_1 radical in Tables II and VI, and the f_{Nuclei} value for the corresponding nucleus in Table VIII. Compounds containing both a nucleus with the functional R_2 substituent and a non-typical R_1 radical, *e.g.*, 7-amino-7-methoxycephalosporanic acid, form an exception, since in this instance the mutual intramolecular interactions of the variable fragments ought to be taken into account, and eqn. 4 cannot be applied. In all the other instances the variable molecular fragments are sufficiently distant from each other, and thus their intramolecular interactions may be neglected. Much care is needed in estimating hydrophobicity values of antibiotics with charged C-6(C-7) or C-3 radicals because of possible implications in their self-association (see the last section).

It is noteworthy that the $f_{C6(C7)}$ value may be

TABLE VII

CORRELATION PARAMETERS OF THE LINEAR RELATIONSHIPS IN EQN. 1 BETWEEN THE LITERATURE DATA (ΔG_i) AND COMMON HYDROPHOBICITY SCALE OF CEPHALOSPORINS AND PENICILLINS, f_{Common}, FROM TABLES II, III AND VI (ΔG_j)

Ref.	Organic phase (stationary phase)	Aqueous phase (mobile phase)	Ionic form of antibiotic[a]	Correlated value[b] (ΔG_i)	Slope	Inter-cept	n	r	s	Included points[c]	Excluded points[c]
5	*n*-Octanol	0.2 *M*, pH 7.4	Anion[d]	Log *P*	−0.707	−2.683	6	−0.3232	1.880	50, 51, 70, 72, 76, 89	–
7, 8	*n*-Octanol	0.1 *M* glycine–HCl buffer, pH 2–3, or 0.1 *M* citrate buffer, pH 4.0	Un-ionized form	Log *P*	0.909[h]	0.228	7	0.9921	0.108	54, 60, 76, 78, 82, 85, 86	49, 51, 80
8	*n*-Octanol	0.1 *M* glycine or citrate buffer, calculated	Un-ionized form	Log *P*	0.310	0.366	18	0.2529	0.964	49–51, 54, 57, 59, 60, 70, 73, 76, 78, 80, 82, 85, 86, 88–90	–
29	*n*-Octanol	0.2 *M*, pH 8.0	Anion[d]	Log *P*	0.414	−0.852	4	0.9991	0.019	50, 70, 72, 89	–
6	Isobutanol	0.02 *M* phosphate buffer + 0.9% NaCl, pH 7.4	Anion[d]	Log *P*	1.263	−0.869	7	0.9596	0.201	2, 4, 51, 60, 75, 76, 81	89
17	Isobutanol	pH 7.4, 0.15 *M* KCl	Anion	Log *P*	0.557	−0.439	14	0.9967	0.047	4, 5, 23–25, 28–31, 35, 36, 70–72	–
26	*n*-Butanol	0.5 *M* KCl	Anion[d]	Log *P*	0.465	−0.342	7	0.9608	0.171	1, 4, 5, 23, 29, 70, 89	–
9[e]	Silica gel 60 F_{254} (Merck)	Acetate–veronal buffer + 20% CH_3OH, pH 7.0	Anion[d]	R_M	0.636	−0.123	19	0.9863	0.105	2, 4, 5, 8, 11, 20, 28–30, 33, 51, 59, 60, 71, 74, 76, 78, 86, 90	70, 80, 89
21	Silica gel G[i]	Extrapolated to 0% acetone, pH 7.4	Anion[d]	R_M	0.741	0.200	24	0.9978	0.047	2, 4, 6, 8, 23, 24, 28, 29, 30, 33, 34, 48–53, 55, 58, 59, 61, 62, 66, 67	89
22	Silica gel G[i]	Extrapolated to 0% acetone, pH 9.4	Anion[d]	R_M[f]	0.700	0.218	20	0.9887	0.100	2, 4, 8, 23, 24, 28, 29, 30, 33, 34, 48–53, 55, 58, 59, 66, 67	89
45	Silica gel HF_{254}[i]	Phthalate buffer + 5% acetone, pH 6.0		R_M	1.104	−0.005	4	0.9973	0.032	50, 51, 70, 72	56, 89
45	Silica gel HF_{254}[i]	Phthalate buffer + 20% acetone, pH 6.0		R_M	0.744	−0.438	22	0.9859	0.119	1, 2, 4, 5, 8, 12, 23–25, 28–30, 32–34, 36, 41, 47, 50, 51, 70, 72	6, 35, 56, 89
71	Silica gel[i]	15% ammonium acetate + 15% CH_3OH, pH 6.2		R_M[f]	0.943	0.593	14	0.9893	0.067	50, 51, 57, 59, 70–72, 76–79, 85, 86, 88	56, 89
72	Silica gel 60 F_{254}[i] (Merck)	25% CH_3OH		R_M	0.937	0.090	4	0.9830	0.090	50, 63, 68, 70	51, 48, 89
25	Zorbax-ODS	0.035 *M* NH_4Cl + 30% CH_3OH, pH 7.4	Anion[d]	Log *k*	0.551	−0.101	16	0.9753	0.113	2, 4, 9, 23–25, 28–31, 35, 50, 51, 70, 72, 76	5, 11, 89
37	Octadecylsilane	0.01 *M* NaH_2PO_4 + 1 m*M* $[N(C_4H_9)_4]HSO_4$ + 1 m*M* $[N(C_2H_5)_4]OH$ + 5% CH_3CN		Log *k*	0.656	0.907	4	0.9695	0.128	51, 56, 64, 76	–

40	LiChrosorb RP-18	0.3 *M* Na_2HPO_4 + 15% CH_3OH, pH 8.0	Anion	Log *k*	1.132	1.485	8	0.9977	0.042	51, 57, 76, 78, 83, 85, 87, 90	–
49	μBondapak C_{18}	0.1 *M* borate buffer + 0.6% cetyltrimethylammonium bromide + 23% *n*-propanol, pH 8.5	Anion[d]	Log *k*, t_0 = 2.5	0.333	0.437	13	0.9950	0.040	4, 5, 28–30, 32, 41, 51, 56, 60, 72, 76, 89	–
54	Ultrasphere-ODS	0.01 *M* NaH_2PO_4 + 15% CH_3CN, pH 4.7		Log *k*	1.171	0.394	11	0.9826	0.118	2, 8, 13, 20, 51, 57, 76, 78, 80, 84, 86	56
54	Ultrasphere-ODS	0.01 *M* NaH_2PO_4 + 15% CH_3CN, pH 4.0		Log k^f	0.697	0.425	7	0.9968	0.021	51, 57, 76, 78, 80, 84, 86	56
55	Bondapak C_{18}	0.15 *M* buffer + 30% CH_3OH	Anion[d]	Log *k*	0.872	0.391	7	0.9924	0.062	2, 4, 23, 24, 51, 69, 76	70, 72, 89
55	Bondapak C_{18}	0.15 *M* buffer + 20% CH_3OH	Anion[d]	Log *k*	0.827	1.071	5	0.9825	0.059	2, 4, 51, 69, 76	70, 72, 89
56	Styrene–divinylbenzene copolymer	0.15 *M* buffer + 50% CH_3OH	Anion[d]	Log *k*	0.954	0.744	10	0.9868	0.123	1, 2, 4, 23, 24, 48, 51, 68, 69, 76	70, 72
66	μBondapak C_{18}	5% phosphate buffer + 10% CH_3CN		Log k^f, t_0 = 1.50	0.869	1.153	13	0.9852	0.065	50, 51, 57, 59, 70–72, 76–78, 85, 86, 88	56, 89
66	R-Sil C_{18} LL	5% phosphate buffer + 8% CH_3CN		Log k^f, t_0 = 2.20	0.874	0.931	13	0.9004	0.182	50, 51, 57, 59, 70–72, 76–78, 85, 86, 88	56, 89
66	Zorbax C_8	5% phosphate buffer + 1% CH_3CN		Log t^f, t_0 = 0	0.765	1.160	13	0.9080	0.152	50, 51, 57, 59, 70–72, 76–78, 85, 86, 88	56, 89
72	RP-2 (Merck)	25% CH_3OH		Log *k*	1.151	0.068	4	0.9691	0.158	48, 50, 68, 70	51, 63, 89
73	μBondapak C_{18}	5 m*M* acetate buffer + 25% CH_3OH, pH 4.5		Log *t*, t_0 = 0	0.597	1.016	4	0.9869	0.034	51, 76, 78, 80	56, 89
74	μBondapak C_{18}	0.02 *M* acetic acid + 30% CH_3OH, pH 3.2		Log *k*, t_0 = 3.1	0.898	0.652	12	0.9941	0.044	51, 57, 70–73, 76, 78–80, 86, 88	56
75	RP C_{18}	0.01 *M* Titrisol–phosphate buffer + 0.4% cetyltrimethylammonium bromide + 35% CH_3CN, pH ≈ 6.5	Anion	Log *t*, t_0 = 0	0.547	1.134	5	0.9890	0.030	51, 57, 78, 85, 86	80
76	C_{18}	Acetate buffer + 30% CH_3OH, pH 5.5		Log *t*, t_0 = 0	0.860	0.829	7	0.9908	0.039	51, 57, 70, 76, 78, 86, 88	56
77	μBondapak C_{18}	0.007 *M* H_3PO_4 + 15% CH_3CN		Log *k*, t_0 = 3.4	2.151	1.072	5	0.9956	0.092	57, 65, 70, 76, 86	–
78	Stearic acid bonded to polysilicate	0.2% $(NH_4)_2CO_3$ + 9% C_2H_5OH		Log *k*, t_0 = 2.0	1.239	0.054	4	0.9849	0.086	51, 78, 85, 86	76, 80
79	μBondapak C_{18}	0.01 *M* acetate buffer + 15% CH_3OH, pH 4.8		Log *t*, t_0 = 0	1.287	1.646	6	0.9753	0.107	51, 57, 63, 78, 85, 86	76, 80

(Continued on p. 26)

TABLE VII *(continued)*

Ref.	Organic phase (stationary phase)	Aqueous phase (mobile phase)	Ionic form of antibiotic[a]	Correlated value[b] (ΔG_i)	Slope	Inter-cept	n	r	s	Included points[c]	Excluded points[c]
80	Zorbax C_8	0.05 *M* lithium citrate buffer + 23% CH_3OH, pH 3.0		Log k, t_0 = 1.27	1.013	0.827	7	0.9676	0.096	57, 70, 71, 76, 80, 86, 88	–
81	DEAE-Sephadex A-25 (Cl^-)	0.2 *M* sodium acetate, pH 8.0	Anion[d]	Log k^g, V_0 = 9.0	0.329	1.175	7	0.9739	0.048	48, 56, 59, 60, 68, 76, 89	51, 70, 72
82	μBondapak C_{18}	0.5 *M* acetic acid + 13% CH_3CN		Log t, t_0 = 0	1.015	1.412	4	0.9906	0.055	57, 73, 78, 86	56, 70, 76, 89
83	R-Sil C_{18} LL	0.05 *M* NaH_2PO_4 + 20% CH_3OH, pH 7.5	Anion	Log k	1.815	1.076	3	0.9996	0.009	70, 72, 77	–
84	μBondapak C_{18}	0.03% $(NH_4)_2CO_3$ + 5% CH_3OH		Log k, t_0 = 2.67	1.427	0.007	5	0.9955	0.069	50, 59, 70, 72, 76	51
85	RP-8	0.09 *M* K_3PO_4 + 0.1 *M* $[N(C_4H_9)_4]HSO_4$ + 15% CH_3OH		Log t, t_0 = 0	0.310	1.292	4	0.9467	0.083	70, 83, 87, 90	–
86	RP-8	0.02 *M* NaH_2PO_4 + 23% CH_3OH, pH 4.5		Log t, t_0 = 0	0.425	1.257	3	0.9422	0.107	57, 65, 72	–
87	Bondapak C_{18}	0.15 *M* KH_2PO_4 + 18% CH_3OH, pH 6.5	Anion	Log t, t_0 = 0	1.416	1.697	4	0.9979	0.018	5, 57, 70, 88	4
88	Radial-Pak C_{18}	1 m*M* $[N(C_4H_9)_4]OH$ + 17% CH_3CN		Log k, t_0 = 1.80	0.947	0.810	6	0.9978	0.058	51, 57, 65, 70, 76, 89	–

[a,b,c,f,i] See footnotes *a*, *b*, *c*, *f* and *m*, respectively, in Table IV.

[d] Zwitterionic form for the cephaloridine and cefsulodine.

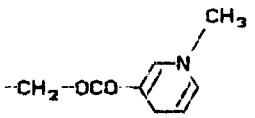
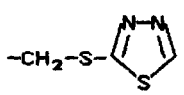
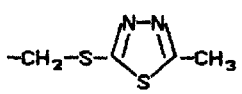
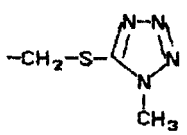
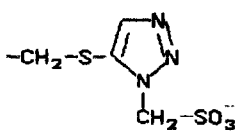
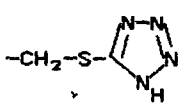
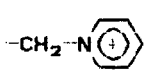
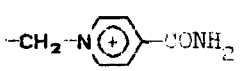
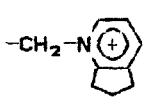
[e] In this instance the mean values of the data for 22 antibiotics given in refs. 9–16 under the same conditions were correlated. The main part of these data (for sixteen solutes) is given in ref. 9.

[g] In this instance the capacity factor k is expressed as follows: $k = (V - V_0)/V_0$, where V and V_0 are the elution volume of the antibiotic and the column void volume, respectively.

[h] In this instance the correlation parameters depended strongly on included or excluded points.

TABLE VIII

STANDARD HYDROPHOBICITY VALUES FOR PENICILLIN AND CEPHALOSPORIN NUCLEI

Nucleus[a]	R_2[b]	R_3	R_4	f_{Nuclei}[c]
I	H	–[d]	–	0.00 (H, L)
I	OCH_3	–[d]	–	−0.11 (L)
I	SCH_3	–[d]	–	−0.03 (L)
I	H	H	–	3.51[e] (H)
I	H	CH_2OAc	–	1.85 (L)
I	H	$CH_2OOCC(CH_3)_3$	–	2.47 (L)
I	H	$C_2H_4N(C_2H_5)_2$	–	1.37 (L)
I	H		–	0.88[f] (L)
II	H	–[d]	H	−0.22 (L)
II	H	–[d]	CH_3	−0.31 (H, L)
II	H	–[d]	CH_2OH	−0.81 (L)
II	H	–[d]	CH_2OAc	0.00 (H, L)
II	H	–[d]	CH_2OCONH_2	−0.39 (H, L)
II	H	–[d]	CH_2N_3	−0.03 (L)
II	H	–[d]		0.21 (H)
II	H	–[d]		0.59 (H, L)
II	H	–[d]		0.23 (H, L)
II	H	–[d]		−0.57[e] (L)
II	H	–[d]		0.20 (L)
II	H	–[d]		−2.34[e] (L)
II	H	–[d]		−0.15[e] (L)
II	H	–[d]		*ca.* 0[e,g] (H)

(Continued on p. 28)

TABLE VIII *(continued)*

Nucleus[a]	R_2[b]	R_3	R_4	f_{Nuclei}[c]
II	H	–[d]	OCH_3	−0.14 (L)
II	H	–[d]	Cl	−0.35 (L)
II	OCH_3	–[d]	CH_2OCONH_2	−0.50 (H, L)

[a] I denotes penicillin and II denotes cephalosporin nuclei according to Table I.
[b] The substituent designations correspond to those in Table I.
[c] Here the f_{Nuclei} values are mean values of those from Tables III and VI. Letters in parentheses have the same meaning as in Table II.
[d] C-3(4)-carboxyl anion.
[e] Uncertain value (see text).
[f] Calculated from ref. 62.
[g] From ref. 70, where log *P* of cefotaxime was stated to be almost the same as for cefpirome.

calculated using the generally accepted additive scheme of Hansch and Leo [32]. Hence theoretically f_{Common} values may be calculated for all β-lactam antibiotics which have one of the nuclei listed in Table VIII. At the same time the Hansch and Leo method does not allow one to estimate the influence of the C-3 radical on the value of f_{Nuclei}. This may be due both to the extremely complex intramolecular H-polar interaction between fragments in the cephalosporin nucleus and their C-3 substituents and to the spatial interaction of the latter with the C-4 carboxyl anion.

The hydrophobicity scale of f_{Common} can describe not only the retention of β-lactam antibiotics in RP chromatography but also their behaviour in ion-exchange and gel chromatography, and their aggregation [critical micelle concentration (CMC) or K_{Ass}] in different aqueous and organic media (Tables IV and VII). The existence of such correlations becomes clear if we assume that the total free energy of the investigated process, $\Delta G_{i(j)}$, can be described by the eqn. 5, where $\Delta G_{Non\text{-}hydroph}$ represents the ability antibiotics to undergo any non-hydrophobic interactions (*e.g.*, electrostatic), and $b_{i(j)}$ characterizes a corresponding property of the medium (*e.g.*, a dielectric constant). As the energy contribution of these non-hydrophobic interactions is constant for all the antibiotics studied, the system of eqns. 5 is simply reduced to linear the dependence in eqn. 1.

Characteristics of partitioning of β-lactam antibiotics in various organic–water systems

The f_{Common} scale shows good correlations with the partitioning of penicillins and cephalosporins in both hydrophilic and lipophilic systems. However, in a number of instances the correlation parameters obtained differ greatly from the expected values. Table IX compares the slopes of the correlations between different partitioning solvent systems for β-lactam antibiotics (α_1) and for various substances devoid of the β-lactam ring (α_2) from ref. 34. Such a discrepancy of these correlation slopes (especially for the octanol–water system) makes it impossible to apply the additivity schemes of Hansch and Leo [32] or Rekker [41] to calculate log *P* values of these antibiotics.

Earlier these methods were also considered to be inadequate for estimating log *P* values of penicillins. Attempts were made to explain this fact by various intramolecular interactions, *e.g.*, by shielding effects [23] or hydrogen bonding [31,89]. Meanwhile, the good correlations obtained in Tables IV and VII demonstrate that the observed effect is common for all β-lactams, and does not depend on particular intramolecular interactions in the antibiotic molecules. Moreover, the results in Table IX indicate that the ratio α_1/α_2 depends on the range of experimentally obtained log *P* values, which, in its turn, depend on the partitioning conditions, *e.g.*, the pH of the aqueous phase. All this is indicative of some secondary equilibrium processes taking place in organic–water systems in the course of partitioning of β-lactam compounds. Indeed, neutral penicillin molecules were shown to be capable of dimerization in chloroform [28] and butyl acetate [90]. The results in Table IX show that such dimerization is also typical of C-3(C-4) carboxyl anions. To describe the partitioning of β-lactam antibiotics, we adopted the following "minimal" scheme:

TABLE IX

DEPENDENCE OF SLOPE α IN EQN. 1 FROM TABLES IV AND VII ON THE PARTITION CONDITIONS

Ref.	Organic phase	Ionic form of antibiotic	Slope α_1 for β-lactam antibiotic[a]	Slope α_2 for non-β-lactam compounds[b]	Normalized slope, α_1/α_2	Range of log P_{obs}[c]	Predicted value of α_1/α_2[d]
7, 8	*n*-Octanol	Un-ionized form	(0.8–1.8)[e]	1.000	(0.8–1.8)[e]	−0.7[f] to +0.7	0.8–1.6
18	*n*-Octanol	Un-ionized form (pH 4.0)	0.838	1.000	0.838	1–3	0.8
19	*n*-Octanol	Un-ionized form	0.772	1.000	0.772	1–3	0.8
23	*n*-Octanol	Un-ionized form	0.751	1.000	0.751	1–3	0.8
24	*n*-Octanol	Un-ionized form	0.725	1.000	0.725	0–4	0.8
24	*n*-Octanol	Anion	0.731	1.000	0.731	0–4	0.8
6	Isobutanol	Anion	1.263	0.697	1.812	−2.0 to −0.2	1.6
17	Isobutanol	Anion	0.557	0.697	0.799	0–4	0.8
24	Isobutanol	Anion	0.512	0.697	0.735	0–1	0.8
26	*n*-Butanol	Anion	0.465	0.697	0.667	0–2	0.8
27	Chloroform	Anion	1.306	1.276	1.024	1–3	1.0
28	Chloroform	Un-ionized form	1.208	1.276	0.947	1–3	1.0
28	Chloroform	Anion + tetra-butylammonium	1.266	1.276	0.992	1–3	1.0

[a] From Tables IV and VII.
[b] Data taken from ref. 34.
[c] Ranges of experimentally obtained log P_{obs} values which were given in the original references.
[d] Calculated assuming that $K_{Ass} \approx P^{-0.4}$ in Scheme 1.
[e] In this instance the slope depended strongly on the points included or excluded and varied from 0.8 to 1.8.
[f] Estimated range; in the original references only the recalculated values of log P were given.

$$A_w \overset{P}{\rightleftarrows} A_{org} \overset{K_{Ass}}{\rightleftarrows} \frac{1}{2}(A_2)_{org}$$

Scheme 1.

where $P = [A_{org}]/[A_w]$ denotes the real partition coefficient, $K_{Ass} = [(A_2)_{org}]/[A_{org}]^2$ is the association constant, A_w and A_{org} are the neutral or the ionized antibiotic molecule, respectively, in aqueous or organic media, and $(A_2)_{org}$ designates the dimeric associates of antibiotics in an organic medium.

Apparently, the experimentally observed partition coefficient can be expressed as

$$P_{obs} = \frac{[A_{org}] + 2[(A_2)_{org}]}{[A_w]} \quad (7)$$

Generally, P_{obs} is not an independent thermodynamic value, as it depends on the solute concentration:

$$P_{obs} = P(1 + 2K_{Ass}[A_{org}]) \quad (8)$$

As the proposed dimerization should lead to energetically unfavourable partial solute desolvation, it would be reasonable to assume the value of K_{Ass} to be proportional to the value of P^x, where x varies from −1 to 0. In fact, the only available set of K_{Ass} values reported in the literature for penicillins in chloroform [28], as shown in Table IV, proved to be linearly related to $P^{-0.14}$. To interpret the results in Table IX, we assumed that in octanol and butanol K_{Ass} changes proportionally to $P^{-0.4}$. Accordingly, the following particular cases of a dependence between the real and observed partition coefficients can be distinguished:

(a) $[A_{org}] \gg [(A_2)_{org}]$ and $P_{obs} = P$;

(b) $[A_{org}] \ll [(A_2)_{org}]$ and $P_{obs} = 2\,P\,K_{Ass}[A_{org}]$.

The value of $[A_{org}]$ for each separate antibiotic under the experimental conditions might be varied absolutely arbitrarily. Nevertheless, all the authors of the reports dealing with partitioning emphasized that the total concentration of partitioned penicillins and cephalosporins in the system was kept constant. Therefore, we might assume that the total antibiotic concentration $[A]_{Total}$ was constant. Then, when log $P_{obs} > 0$,

$$[A_{org}] \approx \left(\frac{[A]_{Total}}{2K_{Ass}}\right)^{0.5}$$

and hence

$$P_{obs} \approx P^{0.8} \sqrt{2[A]_{Total}}$$

and when $\log P_{obs} < 0$,

$$[A_{org}] \approx P\,[A]_{Total}$$

and hence

$$P_{obs} \approx P^{1.6} \cdot 2[A]_{Total}$$

Case (a) occurs in a chloroform medium, as here for different penicillins $\log K_{Ass} = 1.7$–2.0 [28]. This implies that in the most frequently used concentration range of $[A]_{Total}$ ($5 \cdot 10^{-5}$–$1 \cdot 10^{-3}$ M), in the organic phase the dominant form of antibiotics is A_{org}, and $\alpha_1/\alpha_2 = 1.0$ (see Table IX). However, in octanol and butanol media the associated form of $(A_2)_{org}$ seems to prevail, so the α_1/α_2 value changes depend on the partition conditions, being 0.8 at $\log P_{obs} > 0$ and 1.6 at $\log P_{obs} < 0$.

Owing to the proposed β-lactam antibiotics association, it should apparently be impossible to predict $\log P_{obs}$ values for the antibiotics with charged C-6(C-7) or C-3 substituents. The point is that the K_{Ass} value for additionally charged antibiotics, besides their total hydrophobicity, will depend on additional electrostatic repulsion. Indeed, the partition coefficients of cephaloridine and carbenicillin in hydrophilic systems given in refs. 6 and 18 showed large positive deviations from the established dependences in Tables IV and VII.

It should be stressed that Scheme 1 for equilibrium partitioning is only a "minimal" model, as it takes no account of the acid–base equilibrium of antibiotics and their association in the aqueous phase [63], or the possible interactions between neutral and ionized solutes in the organic phase. However, this is not of much significance, as any further elaboration of the partition model will certainly not interpret the results in Table IX any worse than Scheme 1 does. What is important here is the occurrence of secondary equilibrium processes demonstrating the non-stringency of the physical sense of $\log P_{obs}$ and, consequently, the conditionality of the most of the earlier investigations on β-lactam antibiotic partitioning. The partition data reported in refs. 5 or 7 and 8 can serve as an illustrative example thereof. The most probable reason for the disturbances of the linear dependence in these instances seems to result from the inconstancy of the experimental conditions, *e.g.*, from variations in $[A]_{Total}$ in the partitioning system.

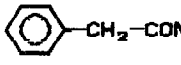
APPENDIX

Calculation of log P in octanol–water system by the fragment method of Hansch and Leo [32]

The method is based on the following equation:

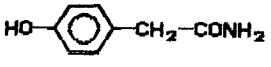
$$\log P = \sum_{1}^{n} a_n f_n + \sum_{1}^{m} b_m F_m \qquad (9)$$

Here $\log P$ is expressed as a sum of constant fragment values (f_n) and other factors (F_m) affecting the partition coefficients of complex substances. Below we give the calculation of $\log P$ only for amides with a "well characterizable" structure. This means that all functional fragments in the amide molecule are separated each from other by isolating carbon atoms (sp^3) and charged groups are absent. Quantitative values of $\log P$, f_n, F_m and their particular designations have been taken from ref. 32.

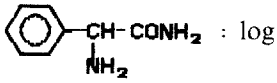


$$\log P = f_{C_6H_5} + f_{CH_2} + f^{1R}_{CONH_2} + F_b$$
$$1.90 + 0.66 - 1.99 - 0.12 = 0.45$$

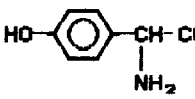
Experimentally observed $\log P = 0.45$



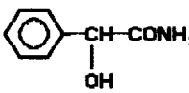
$$\log P = \log P_{Benzylamide} - f_H + f^{\phi}_{OH}$$
$$0.45 - 0.23 - 0.44 = -0.22$$



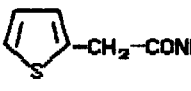
$$\log P = \log P_{Benzylamide} - f_H + f^{1R}_{NH_2} + F_b + F_{gBr} + F_{P1}$$
$$0.45 - 0.23 - 1.35 - 0.12 - 0.22 + 0.42\,(1.35 + 1.99) = -0.07$$



$\log P = \log P_{\alpha\text{-aminobenzylamide}} - f_H + f^{\phi}_{OH}$
$- 0.07 - 0.23 - 0.44 = -0.74$

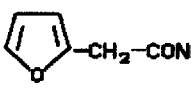


$\log P = \log P_{\text{Benzylamide}} - f_H + f^{1R}_{OH} + F_b + F_{gBr} + F_{P1}$
$0.45 - 0.23 - 1.34 - 0.12 - 0.22 + 0.42\,(1.34 + 1.99) = -0.06$



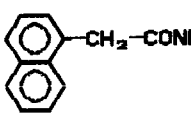
$\log P = \log P_{\text{Thiophene}} - f_H + f_{CH_2} + f^{1R}_{CONH_2} + F_b + F_{P2}$
$1.81 - 0.23 + 0.66 - 1.99 - 0.12 + 0.26\,(1.99 - 0.36) = 0.55;$

$\log P = \log P_{\text{Thiophene}} - f_H + f_{CH_2} + f^{1R}_{CONH_2} + F_b + F^{\phi}_{P2}$
$1.81 - 0.23 + 0.66 - 1.99 - 0.12 + \underline{0.08}\,(1.99 - 0.36) = 0.26$; accepted for correlations $\log P = 0.30$

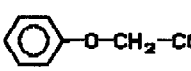


$\log P = \log P_{\text{Furan}} - f_H + f_{CH_2} + f^{1R}_{CONH_2} + F_b + F_{P2}$
$1.34 - 0.23 + 0.66 - 1.99 - 0.12 + 0.26\,(1.99 + 0.08) = 0.198;$

$\log P = \log P_{\text{Furan}} - f_H + f_{CH_2} + f^{1R}_{CONH_2} + F_b + F^{\phi}_{P2}$
$1.34 - 0.23 + 0.66 - 1.99 - 0.12 + \underline{0.08}\,(1.99 + 0.08) = -0.174$; accepted $\log P = -0.07$

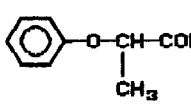


$\log P = \log P_{\text{Naphthalene}} - f_H + f_{CH_2} + f^{1R}_{CONH_2} + F_b$
$3.33 - 0.23 + 0.66 - 1.99 - 0.12 = 1.65$

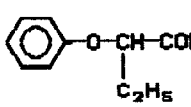


$\log P = f_{C_6H_5} + f^{\phi}_{O} + f_{CH_2} + f_{CONH_2} + 2F_b + F_{P1}$
$1.90 - 0.61 + 0.66 - 2.18 - 0.24 + 0.42\,(2.18 + 0.61) = 0.70;$

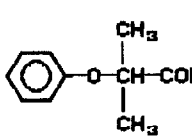
$\log P = f_{C_6H_5} + f^{\phi}_{O} + f_{CH_2} + f^{1R}_{CONH_2} + 2F_b + F_{P1}$
$1.90 - 0.61 + 0.66 - 1.99 - 0.24 + 0.42\,(1.99 + 0.61) = 0.81$; experimentally observed and accepted $\log P = 0.76$



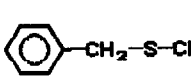
$\log P = \log P_{\text{Phenoxyacetamide}} + f_{CH_2} + F_b + F_{cBr}$
$0.76 + 0.66 - 0.12 - 0.13 = 1.17$



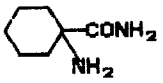
$\log P = \log P_{\text{Phenoxyacetamide}} + 2f_{CH_2} + 2F_b + F_{cBr}$
$0.76 + 1.32 - 0.24 - 0.13 = 1.71$



$\log P = \log P_{\text{Phenoxyacetamide}} + 2f_{CH_2} + 2F_b + 2F_{cBr}$
$0.76 + 1.32 - 0.24 - 0.26 = 1.58$



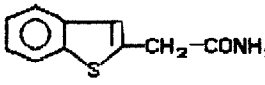
$\log P = f_{C_6H_5} + 2f_{CH_2} + f^{1R}_{S} + f_{CONH_2} + 3F_b + F_{P1}$
$1.90 + 1.32 - 0.64 - 2.18 - 0.36 + 0.42\,(0.64 + 2.18) = 1.22$



: $\log P = \log P_{\text{Cyclohexane}} - 2f_{H} + f_{NH_2} + f_{CONH_2} + F_{gBr} + F_{P1}$
$3.44 - 0.46 - 1.54 - 2.18 - 0.22 + 0.42\,(2.18 + 1.54) = 0.60$

$CH_3(CH_2)_6CONH_2$: $\log P = f_{H} + 7f_{CH_2} + f_{CONH_2} + 6F_b$
$0.23 + 4.62 - 2.18 - 0.72 = 1.95$

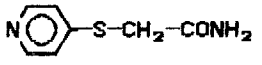
$ClCH_2CONH_2$: $\log P = f_{Cl} + f_{CH_2} + f_{CONH_2} + 2F_b + F_{H/SP}$
$0.66 - 2.18 - 0.24 + 1.05 = -0.65$; experimentally observed and accepted $\log P = -0.53$



: $\log P = \log P_{\text{Benzothiophene}} - f_{H} + f_{CH_2} + f^{1R}_{CONH_2} + F_b + F^{\phi}_{P2}$
$3.12 - 0.23 + 0.66 - 1.99 - 0.12 + 0.08$

$(1.99 - 0.36) = 1.57$;

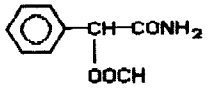
$\log P = \log P_{\text{Thionylacetamide}} - \log P_{\text{Thiophene}} + \log P_{\text{Benzothiophene}}$
$0.29 - 1.81 + 3.12 = 1.60$; accepted $\log P = 1.60$



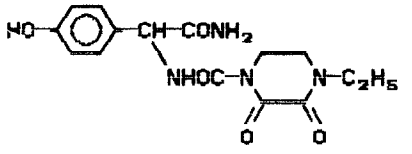
: $\log P = \log P_{\text{Pyridine}} - f_{H} + f^{\phi}_{S} + f_{CH_2} + f_{CONH_2} + 2F_b + F_{P1}$
$0.64 - 0.23 - 0.03 + 0.66 - 2.18 - 0.24 + 0.42$

$(2.18 + 0.03) = -0.450$

$NCCH_2CONH_2$: $\log P = f_{CN} + f_{CH_2} + f_{CONH_2} + F_b + F_{P1}$
$-1.27 + 0.66 - 2.18 - 0.12 + 0.42\,(2.18 + 1.27) = -1.46$



: $\log P = \log P_{\text{Benzylamide}} - f_{H} + f^{1R}_{OOCH} + F_b + F_{gBr} + F_{P1}$
$0.45 - 0.23 - 0.80 - 0.12 - 0.22 + 0.42\,(0.80 + 1.99) = 0.25$



: $\log P = \log P_{\text{Amide of piperacillin } C_6 \text{ radical}} - f_{H} + f^{\phi}_{OH}$
$0.45 - 0.23 - 0.44 = -0.22$

$NCCH_2SCH_2CONH_2$: $\log P = f_{CN} + 2f_{CH_2} + f_S + f_{CONH_2} + 3F_b + F_{P1} +$
$-1.27 + 1.32 - 0.79 - 2.18 - 0.36 + 0.42\,(1.27 + 0.79) +$
$+ F_{P1} + F_{P3}$
$+ 0.42\,(2.18 + 0.79) + 0.10\,(1.27 + 2.18) = -0.82$

As seen from Tables II, III and VI, inaccurately calculated log P values proved to be only those for cyanoacetamide and cyanomethylthioacetamide.

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