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CORRELATION OF SERUM BINDING OF PENICILLINS WITH PARTITION COEFFICIENTS

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Abstract—The extent of binding of penicillins to human serum is shown to be dependent on the hydrophobic character of their side-chains, as expressed by a partition coefficient function $\Sigma\pi$, calculated using the substituent constant π developed by Hansch *et al.* Regression analysis gives a good correlation with $\Sigma\pi$ for the binding of seventy nine penicillins. The binding of seven of these penicillins is also correlated with their measured partition coefficients and this correlation indicates some anomalies in the $\Sigma\pi$ values. These anomalies are discussed in terms of interaction between functional groups in the side-chains and either the amide group or the β-lactam ring. The relevance of the correlations to the mechanism of binding of penicillins to serum albumin is discussed.

HANSCH *et al.*[1–3] have developed a substituent constant, π, defined as log (K_X/K_H) where K_H is the partition coefficient between *n*-octanol and water for the parent molecule of a series and K_X is that of a derivative. The π values have been used successfully by their originators[4–6] in numerous correlations of structure with biological properties. One of the less successful correlations was with the minimum inhibitory concentrations (MIC) of two series of penicillins.[7–8] The correlations with MIC values determined in the absence of serum were poor while those for MIC in the presence of serum were reasonably good. The difference between MIC values in the presence and absence of serum is related to the reversible binding of the antibiotic to the albumin fraction of serum.[9] Hansch *et al.*[7] analysed their results in a rather complicated way to indicate a correlation between π and the extent of serum binding of the penicillins. In this paper we present a correlation of serum binding with calculated π values for seventy nine penicillins and with measured partition coefficients for seven of these penicillins.

METHODS

Serum binding measurements

Serum binding results were obtained by an ultrafiltration method[9] using pooled human serum adjusted to pH 7·4. They are expressed as per cent penicillin bound to serum, defined as:

$$B = \frac{\text{(Initial penicillin concentration} - \text{penicillin concentration in ultrafiltrate) } 100}{\text{Initial penicillin concentration}}$$

Measurements were made at initial penicillin concentrations in serum up to 200 μg/ml. The concentration chosen was dependent on the activity of the particular penicillin

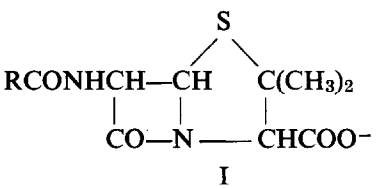
against the test organism used for assay. For our purpose the binding measurements should ideally be made at the same molar concentration for each penicillin, because the total concentration of any bound substance can affect the per cent bound.[10] However, it has been shown[9] for some penicillins in serum that this effect is small at concentrations below about 200 μg/ml. This concentration corresponds to only about one mole of penicillin per mole of albumin in serum, so that saturation of binding sites seems unlikely. Consequently we believe our use of the per cent bound figures is justified. The binding results for twenty nine penicillins are averages of two or more determinations and those for the rest are from one determination.

Partition coefficient measurements

Partition coefficients of penicillins between *n*-octanol and water were measured by a modification[11] of Brändström's method.[12]

Partition coefficient functions

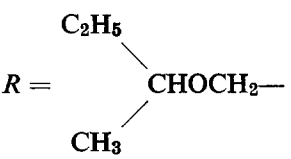
$\Sigma\pi$ values for the penicillin side-chains (*R* in I) were calculated from published partition coefficients of the parent compounds and group π values.[2, 3, 6] Phenoxyacetic acid group π values were used for substituents in quinoline and thiophen rings



I

and for most of those in benzene rings. Where phenoxyacetic acid π values are not available (NH_2, $CONH_2$, and SO_2NH_2 groups) the values for the substituent in benzene were used. The following examples illustrate the calculation of side-chain $\Sigma\pi$ values.

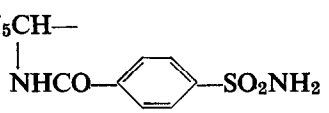
Penicillin 8



$$\Sigma\pi = \pi_{iso\text{-}Pr.} + \pi_{Me} + \pi_{al.\text{-}OMe}$$
$$= 1{\cdot}32 + 0{\cdot}5 - 0{\cdot}98$$
$$= 0{\cdot}84$$

The three terms are π values for the isopropyl group, methyl group and methoxy group attached to aliphatic carbon.

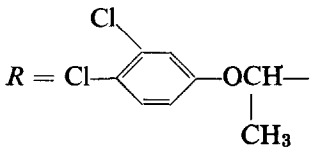
Penicillin 35



$$\Sigma\pi = \log P_{tol.} + \pi_{CONH_2} + \log P_{bz.} + \pi_{SO_2NH_2}$$
$$= 2{\cdot}69 - 1{\cdot}49 + 2{\cdot}13 - 1{\cdot}82$$
$$= 1{\cdot}51$$

$P_{tol.}$ and $P_{bz.}$ are the partition coefficients for toluene and benzene and the two π terms are from the monosubstituted benzene series of group π values.

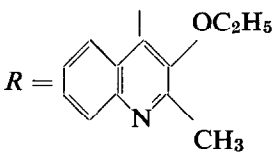
Penicillin 58



$$\Sigma\pi = \log P_{an.} + \pi_{Me} + \pi_{p-Cl} + \pi_{m-Cl}$$
$$= 2{\cdot}11 + 0{\cdot}5 + 0{\cdot}70 + 0{\cdot}76$$
$$= 4{\cdot}07$$

$P_{an.}$ is the partition coefficient for anisole, π_{p-Cl} and π_{m-Cl} are from the phenoxyacetic acid series of group π values.

Penicillin 79



$$\Sigma\pi = \log P_{quin.} + \pi_{o-OMe} + 2\pi_{Me}$$
$$= 2{\cdot}03 - 0{\cdot}33 + 1{\cdot}0$$
$$= 2{\cdot}70$$

$P_{quin.}$ is the partition coefficient for quinoline and the methoxy group π is from the phenoxyacetic acid series.

Use of side-chain $\Sigma\pi$ values for correlation of penicillin properties involves the assumption that they are directly proportional to the penicillin partition coefficients. This assumption has two parts; (a) that $\Sigma\pi$ values accurately represent the partition coefficients of the side-chains, and (b) that the nucleus and amide bond contribute a constant amount to the partition coefficient of all penicillins. Both parts are justifiable by the principle of additivity by group π values, which has been established by Hansch *et al.*[2, 3] for many compounds where strong group interactions are absent. The structures of the side-chains are such that strong interactions between their groups are unlikely. However, a definite possibility exists of interaction between the amide bond and the side-chain in many of the penicillins. This is discussed below.

RESULTS AND DISCUSSION

The function log (B/F), where B is per cent penicillin bound and F is per cent free, is used for correlation of the serum binding results with $\Sigma\pi$. Hansch *et al.*[13] used log B for correlation of the data of Weinbach and Garbus[14] on binding of phenols to mitochondrial protein. (B/F) is clearly a more rational choice than B for this type of correlation because it is directly analogous to an organic solvent/water partition

coefficient. The superiority of the (B/F) form of correlation is shown by comparison of equations (1) and (2), which were calculated from the data in Table 3.

	n	r	s^2	
$\log B = 0{\cdot}169\ \Sigma\pi + 1{\cdot}399$	79	0·850	0·018	(1)
$\log (B/F) = 0{\cdot}488\ \Sigma\pi - 0{\cdot}628$	79	0·924	0·065	(2)

where n is the number of points, r is the correlation coefficient and s^2 is the residual variance. The z-transformation of the correlation coefficients[15] shows that the coefficient for (2) is significantly greater than that for (1) (P = 0·025).

The method of least squares used to obtain (2) assumes that the variance in log (B/F) is homogeneous. The variances from multiple determinations of B on some penicillins are given in Table 1. A test of these values by Bartlett's method[16a] shows that this set of variances is homogeneous.

TABLE 1.

s^2 is the variance of log (B/F) about its mean value, which is calculated from n determinations of serum binding, where B is per cent bound and F is per cent free.

Penicillin*	Log (B/F)	n	$s^2 \times 10^3$
17	0·188	14	17
26	−0·646	5	60
42	1·060	13	17
47	0·589	9	27
48	0·645	14	19
50	0·792	9	16
68	0·920	6	21

* See Table 3 for structures.

A further assumption involved in the normal least squares method used to obtain (2) is that the variance in $\Sigma\pi$ is negligible with respect to that in log (B/F). This is unlikely to be correct because of the assumption involved in calculation and use of $\Sigma\pi$. If this latter assumption is accurately applicable, there should be an accurate linear relation between $\Sigma\pi$ and the logarithm of the penicillin partition coefficients. Equation (3) was calculated from the data for seven penicillins in Table 2.

	n	r	s^2	
$\Sigma\pi = 1{\cdot}272 \log K - 0{\cdot}302$	7	0·888	0·180	(3)

where K is the n-octanol–water partition coefficient of the penicillin free acid.

If we assume that the variance in log K is negligible, then s^2 from (3) is an estimate of the variance in $\Sigma\pi$. This is of limited value because it is obtained from a very small sample of penicillins, but it is greater than the variance in log (B/F) (*ca.* 0·03, from Table 1). Thus the assumption of negligible variance in $\Sigma\pi$ with respect to that in log (B/F) is almost certainly not valid.

In view of the above discussion the following equations have been derived assuming equal variance in log (B/F) and $\Sigma\pi$. The least squares method of calculation in this

case[17] involves minimizing the sums of the squares of the deviations of both variables, so that estimated values of both log (B/F) and $\Sigma\pi$ are obtained. The results for the 79 penicillins obtained with equation (4) are given in Table 3 (the estimated log (B/F) values have been transformed into B values). The derivation of (4), giving equal weight

	n	r	s^2	
$\log (B/F) = 0{\cdot}504\,\Sigma\pi - 0{\cdot}665$	79	0·924	0·066	(4)

to $\Sigma\pi$ and log (B/F), is more rigorous than that of (2) where infinite weight is given to $\Sigma\pi$, so that (4) is probably the better estimator of log (B/F).

The correlation coefficient is satisfactorily high in view of the wide range of structures covered and the assumption involved in use of $\Sigma\pi$. Nevertheless there are several penicillins where a large discrepancy is found between observed and estimated B values. The cause of these discrepancies is unlikely to be inaccurate experimental values of B, because duplicate measurements were made on those penicillins where a large discrepancy occurs.

Discrepancies would occur if the binding is governed by a factor (or factors) in addition to partition coefficient. The good correlation with $\Sigma\pi$ for most of the penicillins makes requirement of an additional factor in the correlation unlikely. However,

TABLE 2.

Comparison of log K with $\Sigma\pi$ where K is the n-octanol–water partition coefficient of the penicillin free acid and $\Sigma\pi$ is the partition coefficient function of the penicillin side-chain. Estimated $\Sigma\pi$ values were calculated from equation (4).

Penicillin*	log K	Calculated $\Sigma\pi$	Estimated $\Sigma\pi$
15	1·22	1·47	1·25
17	1·83	2·69	2·03
37	1·40	0·89	1·48
47	2·09	2·11	2·36
48	2·28	2·61	2·60
49	2·76	3·11	3·21
50	2·65	3·11	3·07

* See Table 3 for structures.

the assumption involved with $\Sigma\pi$ values makes it desirable to test this postulate by reference to a correlation with measured partition coefficients. Equation (5) was calculated for the 7 penicillins in Table 2 from the log K values in Table 2 and the relevant B values in Table 3.

	n	r	s^2	
$\log (B/F) = 0{\cdot}680 \log K - 0{\cdot}905$	7	0·971	0·012	(5)

The residual variance of (5) is lower than the variance of measurement of log (B/F), given in Table 1. Consequently addition of another factor to the log K correlation would have no practical significance with log (B/F) data of this precision.

TABLE 3.

Data used for regressions of log (B/F) on $\Sigma\pi$, where B is per cent penicillin bound to serum, F is per cent penicillin free and $\Sigma\pi$ is the partition coefficient function of the penicillin side-chain. Estimated B and $\Sigma\pi$ values were obtained by the least squares regression procedure which gave equation (4). Calculated $\Sigma\pi$ values were obtained by summation of group π values, as explained in the text.

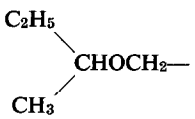
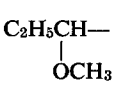
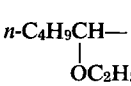
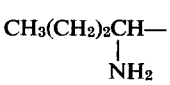
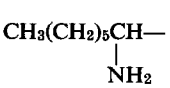
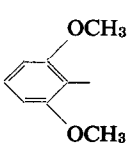
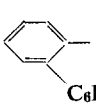
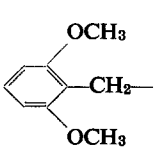
Penicillin	R*	Observed B(%)	Estimated B(%)	Calculated Σπ	Estimated Σπ
1	H—	18·0	17·8	0	0
2	CH_3—	15·0	24·8	0·50	0·36
3	$CH_3(CH_2)_6$—	92·4	92·6	3·50	3·49
4	$(n\text{-}C_3H_7)_3C$—	93·3	97·5	4·68	4·46
5	CH_3OCH_2—	7·2	10·1	−0·48	−0·56
6	$C_2H_5OCH_2$—	28·0	19·9	0·02	0·12
7	$n\text{-}C_4H_9OCH_2$—	58·8	44·9	1·02	1·14
8		47·0	38·5	0·84	0·92
9		20·0	26·5	0·52	0·44
10		74·0	70·3	2·02	2·06
11	$C_2H_5O(CH_2)_2$—	25·0	27·6	0·52	0·49
12		33·0	22·7	0·15	0·26
13		66·2	63·1	1·75	1·78
14	C_6H_5—	87·0	75·7	2·13	2·30
15		49·0	53·3	1·47	1·43
16		91·0	95·1	4·02	3·88
17	$C_6H_5CH_2$—	60·7	79·5	2·69	2·49
18		77·0	71·2	2·03	2·10

TABLE 3.—*continued*

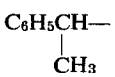
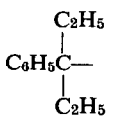
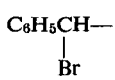
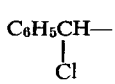
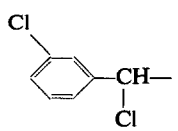
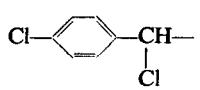
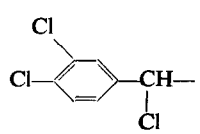
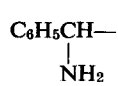
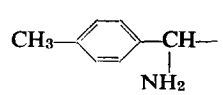
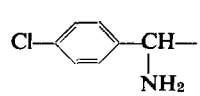
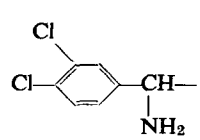
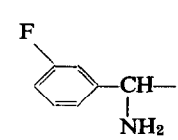
Penicillin	R*	Observed B(%)	Estimated B(%)	Calculated Σπ	Estimated Σπ
19		68·0	86·8	3·19	2·94
20		93·6	97·5	4·69	4·48
21		82·5	83·5	2·73	2·71
22		78·0	80·3	2·56	2·53
23		94·0	91·7	3·31	3·39
24		94·0	91·3	3·26	3·35
25		97·0	96·1	4·02	4·08
26		18·0	32·1	0·84	0·62
27		60·0	52·5	1·34	1·41
28		55·0	56·1	1·54	1·53
29		82·2	77·2	2·30	2·37
30		26·0	36·9	0·97	0·86

TABLE 3.—*continued*

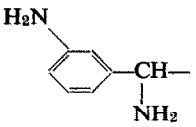
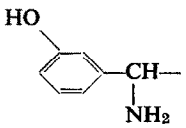
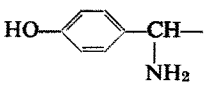
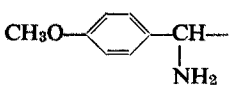
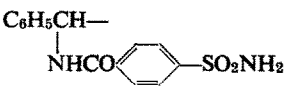
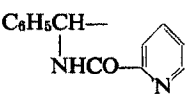
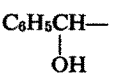
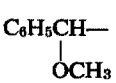
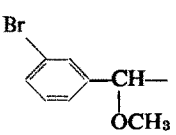
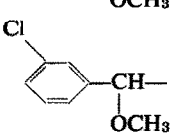
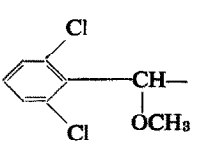
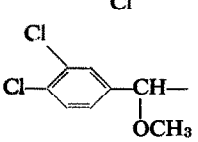
Penicillin	*R**	Observed *B*(%)	Estimated *B*(%)	Calculated Σπ	Estimated Σπ
31		12·0	12·1	−0·39	−0·39
32		16·8	22·8	0·35	0·27
33		21·0	23·8	0·35	0·32
34		38·0	35·9	0·80	0·82
35		42·0	52·5	1·50	1·41
36		63·0	64·5	1·85	1·84
37		53·2	40·8	0·89	1·00
38		62·0	61·3	1·71	1·72
39		88·0	83·7	2·65	2·73
40		83·0	80·0	2·47	2·51
41		84·0	85·7	2·89	2·86
42		92·0	90·1	3·17	3·22

TABLE 3.—*continued*

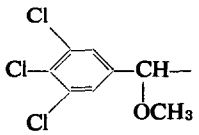
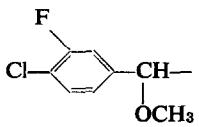
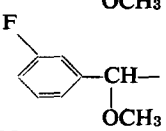
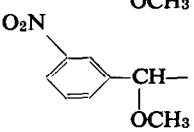
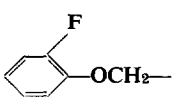
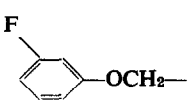
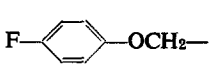
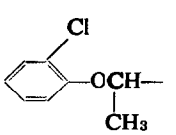
Penicillin	*R**	Observed *B*(%)	Estimated *B*(%)	Calculated Σπ	Estimated Σπ
43		96·5	95·6	3·93	3·98
44		83·0	81·0	2·54	2·57
45		65·0	64·7	1·84	1·84
46		60·0	63·3	1·82	1·79
47	$C_6H_5OCH_2$—	79·5	73·2	2·11	2·19
48	$C_6H_5OCH(CH_3)$—	81·5	81·7	2·61	2·61
49	$C_6H_5OC(CH_3)_2$—	92·5	89·7	3·11	3·19
50	$C_6H_5OCH(C_2H_5)$—	86·1	88·4	3·11	3·07
51	$C_6H_5OCH(C_6H_5)$—	97·2	96·8	4·24	4·27
52		91·5	77·3	2·12	2·37
53		83·5	76·5	2·24	2·34
54		81·5	76·3	2·26	2·33
55		96·0	91·6	3·20	3·37

TABLE 3.—*continued*

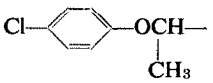
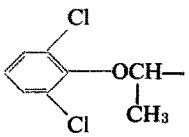
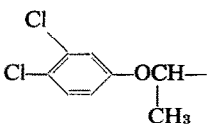
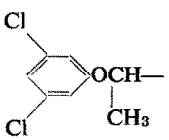
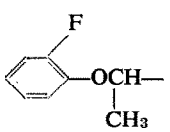
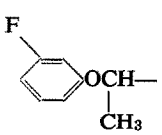
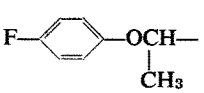
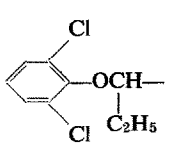
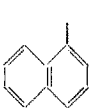
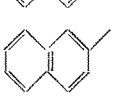
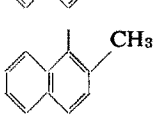
Penicillin	R*	Observed B(%)	Estimated B(%)	Calculated Σπ	Estimated Σπ
56		94·8	91·9	3·31	3·41
57		96·0	94·9	3·79	3·84
58		97·4	96·4	4·07	4·15
59		97·0	96·1	4·03	4·09
60		86·0	82·8	2·62	2·67
61		84·0	83·9	2·74	2·74
62		80·0	83·4	2·76	2·71
63		95·2	96·6	4·29	4·21
64		86·0	90·6	3·37	3·27
65		95·6	92·6	3·37	3·49
66		82·1	93·5	3·87	3·62

TABLE 3.—*continued*

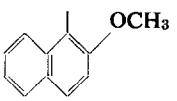
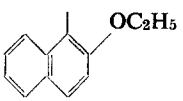
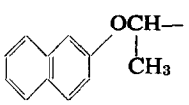
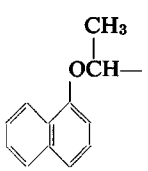
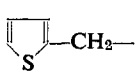
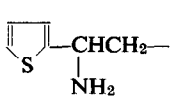
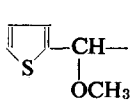
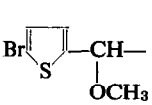
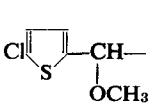
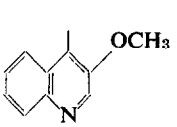
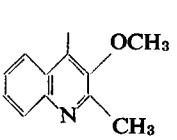
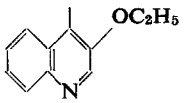
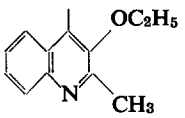
Penicillin	R*	Observed B(%)	Estimated B(%)	Calcalated Σπ	Estimated Σπ
67		80·0	86·7	3·04	2·93
68		89·3	92·3	3·54	3·46
69		94·7	94·9	3·85	3·84
70		97·4	95·6	3·85	3·97
71		58·0	72·8	2·31	2·17
72		32·0	38·1	0·96	0·90
73		59·0	52·1	1·33	1·39
74		89·7	78·9	2·27	2·46
75		83·6	74·0	2·09	2·22
76		57·0	60·1	1·70	1·67
77		61·7	71·3	2·20	2·10

TABLE 3.—*continued*

Penicillin	R*	Observed B(%)	Estimated B(%)	Calculated Σπ	Estimated Σπ
78		69·7	72·8	2·20	2·17
79		74·5	81·7	2·70	2·61

* See structure I.

The *B* values estimated from (5) are compared in Table 4 with those from equations (4) and (6). Equation (6) is the correlation with Σπ for the seven penicillins in Table 2, and was calculated from the relevant *B* and Σπ values in Table 3. It is included as a direct comparison with (5). The results in Table 4 show that (5) gives the best estimate

$$\log (B/F) = 0{\cdot}392\,\Sigma\pi - 0{\cdot}418 \qquad n = 7 \quad r = 0{\cdot}802 \quad s^2 = 0{\cdot}073 \qquad (6)$$

of binding for most of the seven penicillins, and in particular it gives by far the best estimate for benzylpenicillin (*17*) and its α-hydroxy derivative (*37*).

Reference to the data in Table 2 shows that benzylpenicillin (*17*) is apparently less hydrophobic and its α-hydroxy derivative (*37*) is more hydrophobic than the calculated Σπ values allow. These results may be explained by consideration of interaction between the side-chain and the amide group or the penicillin nucleus. Interaction between groups, either by inductive electronic effects or by hydrogen bonding, is known[2, 3] to cause non-additivity of group π values. The side-chain structures of six

TABLE 4

Serum binding of seven penicillins estimated from regressions of log (*B*/*F*) on log *K* (equation 5) and on Σπ (equations 4 and 6) where *B* is per cent penicillin bound, *F* is per cent free, *K* is the *n*-octanol–water partition coefficient of the penicillin free acid and Σπ is the partition coefficient function of the penicillin side chain. See Table 2 for log *K* and Σπ values.

Penicillin*	Observed *B* (%)	Estimated *B* equation (4) (%)	Estimated *B* equation (5) (%)	Estimated *B* equation (6) (%)
15	49·0	51·2	45·9	59·0
17	60·7	78·3	68·8	81·2
37	53·2	38·8	52·7	46·0
47	79·5	71·8	76·6	72·0
48	81·5	80·6	81·5	80·1
49	92·5	89·1	90·4	86·4
50	86·1	87·6	88·8	86·4

* See Table 3 for structures.

of the seven penicillins in Table 2 allow the possibility of hydrogen bonding. Five of the penicillins (*15*, *47–50*) have an ether link which could form a weak hydrogen bond to the amide NH, and the α-hydroxy group in *37* could bond to either the amide or the β-lactam carbonyl group. The effect of hydrogen bonding would be to make the compounds more hydrophobic than addition of group π values estimates. Thus relative to benzylpenicillin (*17*), where interaction with the amide group is unlikely, all six other penicillins would be more hydrophobic than is expected from their calculated $\Sigma\pi$ values. Correlation with log K (equation (3) and Table 2) would then show a spurious low hydrophobic character for benzylpenicillin. The results for the other penicillins then suggest that the effect of interaction is greatest with the α-hydroxy compound (*37*) and is smaller and roughly constant for the five ether penicillins.

This interpretation of the apparent low hydrophobic character of benzylpenicillin is supported by a correlation of the binding of seven penicillins (*1–4*, *17*, *19*, *20*) with hydrocarbon side-chains where the possibility of interaction with the amide group can be ignored. These seven penicillins give equation (7), calculated from the relevant B and $\Sigma\pi$ values in Table 3.

	n	r	s^2	
$\log(B/F) = 0{\cdot}432\,\Sigma\pi - 0{\cdot}832$	7	0·965	0·057	(7)

Equation (7) gives estimates of B for benzylpenicillin (*17*) and its α-methyl derivative (*19*) (67·1 and 76·5 per cent respectively), which are much closer to the observed values (60·7 and 68 per cent respectively) than those from the other $\Sigma\pi$ correlations.

Interaction between the amide group or the nucleus and a functional group in the side-chain might lead to different regression lines for penicillins containing different functional groups. Penicillins containing a primary amino group α- to the amide group (nos. *12*, *13*, *26–34*) give equation (8), those with an ether group α- to the amide (nos. *5–10*, *38–63*, *69*, *73–5*) give (9) and those with an ether group *ortho* to the bond between the amide and an aromatic ring (nos. *15*, *67*, *68*, *76–9*) give (10). Equations (8), (9) and (10) were calculated from the relevant B and $\Sigma\pi$ values in Table 3.

	n	r	s^2	
$\log(B/F) = 0{\cdot}586\,\Sigma\pi - 0{\cdot}765$	11	0·904	0·048	(8)
$\log(B/F) = 0{\cdot}522\,\Sigma\pi - 0{\cdot}588$	37	0·955	0·035	(9)
$\log(B/F) = 0{\cdot}427\,\Sigma\pi - 0{\cdot}647$	7	0·982	0·004	(10)

These equations have exceptionally low residual variance and the B values estimated from them (which are not reported) are closer to the observed values than those estimated from the general equation (4), for most of the penicillins in each group.

Statistical tests of (7)–(10) were made to determine if correlation of any of these groups separate from the total seventy nine penicillins is justified. This was done by a t-test comparison of the slope[18] and intercept[16b] of each equation with the slope and intercept of the equation calculated from those of the seventy nine penicillins which remain after removal of that particular group (subsequently referred to as the conjugate equation of a group). None of the differences were significant at the 95 per cent level. Thus although (7)–(10) are better estimators of B for their particular penicillins

than (4), there seems to be no statistical justification for separate correlation of these groups of penicillins.

A significant difference between equations in the above tests could indicate either the effect of interactions between the side-chain and the amide group, or a difference in the mechanism of binding. The results thus imply that all the seventy nine penicillins are bound by the same mechanism. As a further test of this, regression lines were calculated for penicillins which have the following features in their side-chain: no ring, one ring, two rings, heterocyclic ring and ArRCH— where *R* is non-aryl and Ar is phenyl or substituted phenyl. Comparison with the conjugate equations showed no statistically significant differences.

The correlations presented here show that the mechanism of binding of penicillins to serum involves the hydrophobic character of the penicillin as the most important determinant of the extent of binding. Several authors[19–22] have shown that penicillins are bound only to the albumin fraction of serum and recent work on the structure of serum albumin and its binding properties for anionic and non-ionic substances emphasizes the importance of hydrophobic bonding.[23] Foster[24] reviewed evidence for hydrophobic binding of some organic anions, particularly detergents, to albumin. Partly as a result of this he proposed a model of the albumin molecule consisting of four compact fragments with flexible links between them, held together in pairs by hydrophobic bonds. The two pairs so obtained are bonded electrostatically into a globular molecule which thus contains one hydrophilic and two hydrophobic interfaces. A model of this type has received general support from work on pepsin[25] and subtilisin[26] fragmentation, and acid expansion[27] of serum albumin. Fluorescence polarization measurements in Weber and Young's work[25] agree with other results[28–9] indicating that dye molecules adsorbed on serum albumin are removed from an aqueous phase to a medium of lower polarizability, and Weber and Young attributed their results to adsorption of the dye in a hydrophobic interface of the protein. Correlations between binding to serum albumin and partition coefficients and solubilities for phenols[13] and other non-ionic aromatic compounds[30] respectively, provide additional support for the occurrence of binding by hydrophobic interactions. Also this seems to be the only plausible mechanism for binding of hydrocarbons to proteins.[31–2]

In this context the correlation with partition coefficients suggests that penicillins are bound to serum albumin by interaction with a hydrophobic interface of the protein. The serum binding measurements were made at pH 7·4 where the penicillin carboxy group is almost completely ionized, so that this region of the molecule is hydrophilic and the side-chain is relatively much more hydrophobic. Consequently hydrophobic binding of penicillin to albumin must involve the side-chain as the main binding site on the penicillin molecule. The carboxy group may either, (a) project into the aqueous phase surrounding the protein molecule and not interact at all with the protein, or (b) bind to neighbouring groups on the protein surface, either by hydrogen bonds or ionically.

Kunin[33] and Fischer and Jardetsky[34] have previously suggested that penicillins are bound to albumin by their side-chain. However, Keen's[35] study of the variation of binding of benzyl and phenoxymethyl penicillins with pH led him to suggest that ionic binding through the penicillin carboxy group is probably necessary to enable Van der Waals binding of the side-chain to occur. The results in Table 5 for the

primary amides of three of the penicillins in our series show that it is possible for molecules very similar to penicillins to bind to serum when ionic binding is impossible. Also the very low levels of binding observed with some penicillins (e.g. *1*, *2*, *5*) show that the carboxy group alone can contribute very little to the extent of binding. Keen[35] found a sharp decrease in extent of binding of phenoxymethylpenicillin to

TABLE 5.

Serum binding of three penicillins and their primary amides.

Penicillin*	% bound	
	Penicillin	Primary amide
9	20·0	33·5
17	60·7	80·0
26	18·0	29·0

* See Table 3 for structures.

serum albumin below pH 4 and above pH 9. This is not inconsistent with a hydrophobic binding mechanism. The acid expansion of albumin which occurs at about pH 4 removes the hydrophobic interfaces in which it is postulated that binding occurs.[27] Structural changes of albumin at high pH have been studied less than those at low pH, but there is evidence, reviewed by Leonard *et al.*,[36] which suggests that a structural transformation in the pH 7–9 region may involve loss of the hydrophobic interfaces of the protein.

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