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Structure–gastrointestinal absorption relationship of penicillins *

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Summary

Factors influencing the gastrointestinal absorption of penicillins were characterized in order to rationally design orally active penicillins. Acid stability in the gastric juice was correlated with sum of the Taft's inductive substituent constants (σ_I values) of the side-chain at the 6-amino group, and a $\Sigma\sigma_I$ value of equal or greater than 0.34 was found necessary for orally active penicillins. A good relation between the lipophilicity, log P and the peak plasma level or the relative bioavailability after oral administration of penicillins was observed in mice and rats, and the penicillin having a log P value between 1.9 and 3.3 showed a relatively higher plasma level or relative bioavailability. From these results, it is suggested that a $\Sigma\sigma_I$ value of the side-chain of equal or greater than 0.34 and a log P value between 1.9 and 3.3 are necessary for penicillins to be absorbed well from the gastrointestinal tract after oral administration.

Introduction

A number of penicillins have been synthesized by chemical modification of 6-aminopenicillanic acid (6-APA) and some have been used perorally in clinical practice. These orally active penicillins (Bergan, 1978; Nayler, 1973), have two types

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of side-chains attached to the amino group of 6-APA: (a) a phenylglycine analog (e.g. ampicillin (D(−)-α-aminobenzylpenicillin, AB-PC, XIV) and amoxicillin (D(−)-α-amino-*p*-hydroxybenzylpenicillin, AM-PC)); and (b) acyl analog (e.g. propicillin, D,L-α-phenoxypropylpenicillin, PP-PC, V) and dicloxacillin (5-methyl-3-(2, 6-dichlorophenyl)-4-isoxazolyl-penicillin, MDI-PC)). Recently, the ester prodrugs, e.g. carfecillin (α-phenoxycarbonylbenzylpenicillin, CF-PC) (Clayton et al., 1975; Wilkinson et al., 1975) have been used clinically as orally active penicillins.

For the oral absorption of penicillins, except the phenylglycine analog, stability in the gastrointestinal tract (GI tract) and lipophilicity are well known important factors (Bergan, 1978; Hou and Poole, 1971; Bird and Bayler, 1971; Nayler, 1973). However, these physicochemical properties have not been characterized quantitatively for the purpose of rational design of orally active penicillins including a prodrug such as CF-PC.

In order to design orally active penicillins, characterization of physicochemical properties such as stability and lipophilicity was attempted by investigating the structure–gastrointestinal absorption relation of penicillins in mice and rats.

Materials and Methods

Materials

The following penicillins were used. Dihydropenicillin-F (dihydro PC-F; I), penicillin-K (PC-K; II), penicillin-G (PC-G; III), sulbenicillin (SB-PC; IV), propicillin (PP-PC; V), D,L-α-hydroxybenzylpenicillin (VII), N-acetylampicillin (VIII), penicillin-V (PC-V; IX), 2,4-dichlorobenzylpenicillin (X), N-methansulfonylampicillin (XI), cyclacillin (AC-PC; XII), D,L-α-chlorobenzylpenicillin (XIII) and ampicillin (AB-PC; XIV) were prepared at Takeda Chemical Industries and phenethicillin (PE-PC; VI) (Banyu Pharmaceuticals) was obtained commercially.

Methods

Acid stability

Each penicillin (50 mg) was added to a mixture of 0.2 N aqueous hydrochloric acid (25 ml) and ethanol (25 ml) maintained at 35°C; aliquot portions of each solution were withdrawn at appropriate intervals and the penicillin contents were determined by iodometry (Mundell et al., 1946).

Lipophilicity

Log P values between 1-octanol and water of II, III, V, VI, VII and X were used from literature values (Bird and Marshall, 1967). Log P of IV was calculated from the following equation.

$$\log P = 1.253R_M + 0.854 \quad (1)$$

$n = 7,\ r = 0.945$

In this equation, R_M value was measured according to the procedure by Biagi et al. (1969 and 1970) using silica impregnated with silicone oil and aqueous acetone as the mobile phase. This equation was obtained through the least-squares analysis from R_M values and log P values of III, IX, VI, methicillin (2,6-dimethoxyphenylpenicillin), cloxacillin (5-methyl-3-o-chlorophenyl-4-isoxazolylpenicillin), oxacillin (5-methyl-3-phenyl-4-isoxazolylpenicillin) and dicloxacillin. I, X and XIV were calculated from Hansch-Fujita method (Hansch, 1971; Tute, 1971).

Log P value of other penicillin was measured by the following method. The solution of 0.2 and 1 mmol of a penicillin was prepared using an isotonic buffer of pH 3.7, 5.0 and 7.4 (0.1 M) and 15 ml of the solution was put into a test tube with glass stopper containing an equal volume of 1-octanol. The test tubes were shaken vigorously at 25°C. When an equilibrium was achieved, the aqueous layer was separated by centrifugation and the concentration of penicillin in it was measured by cylinder-plate method using *B. subtilis* PCI 219P as the test organism. Log P value was calculated from the observed partition coefficient P′ at various pHs following Eqn. 2 and averaged.

$$\log P = \log P' + \log(10^{pH-pK_a} + 1) \quad (2)$$

pK_a value of VIII, IX and XIII measured by the titration method was 2.71, 2.73 and 2.70, respectively.

Absorption studies

A penicillin, at a dose of 100 mg/kg was administered orally or subcutaneously to a group of 3 male SLC-ICR mice (4 weeks old), starved overnight. Blood was taken from the inferior vena cava at 0.25, 0.5, 1 and 2 h after dosing. The relative bioavailability was calculated from the area under the plasma level–time curve (AUC_{oral}) after oral administration and that (AUC_{sc}) after subcutaneous administration. AUC value was calculated by trapezoidal method.

A group of 3 male Sprague–Dawley rats (5 weeks old), starved overnight, were also given the same dose orally. Blood was taken from the inferior vena cava at 0.5, 1, and 2 h after dosing.

Assay

The blood specimens were centrifuged at 3000 rpm for 10 min. The plasma penicillin levels were assayed by the cylinder-plate method using *B. subtilis* PCI 219P as the test organism.

Results and Discussion

Acid stability

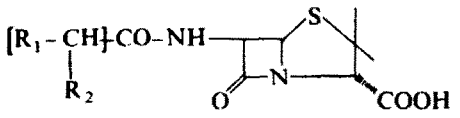
The β-lactam ring of a penicillin is known to be highly reactive and very sensitive to electrophiles or nucleophiles. Thus, stability of a penicillin in the gastric juice is a prerequisite for its GI absorption. The acid stability, the degradation rate constants

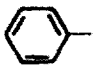
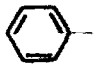
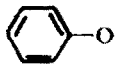
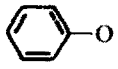
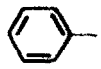
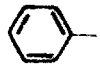
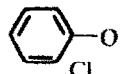
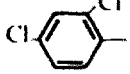
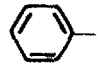
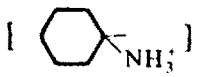
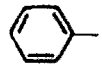
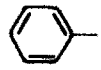
constants (k), and half-lives ($t_{1/2}$) of 14 penicillins determined in 50% aqueous ethanol at pH 1.3 and 35°C are shown in Table 1.

The reaction took place following pseudo-first-order kinetics. The half-lives of

TABLE 1

$\Sigma\sigma_I$-VALUES AND ACID STABILITY OF PENICILLIN



No.	R_1	R_2	$\sigma_I(R_1)$ [a]	$\sigma_I(R_2)$	$\Sigma\sigma_I$	k (min^{-1}) [c]	$t_{1/2}$ (min) [d]
I	n-C_4H_9	H	−0.04	0.0	−0.04	0.462	1.5
II	n-C_6H_{13}	H	−0.04	0.0	−0.04	0.578	1.2
III		H	0.10	0.0	0.10	0.138	5.0
IV		SO_3^-	0.10	0.13	0.23	0.0224	31
V		C_2H_5	0.39	−0.05	0.34	0.00385	180
VI		CH_3	0.39	−0.05	0.34	0.0033	210
VII		OH	0.10	0.25	0.35	0.0082	84
VIII		$NHCOCH_3$	0.10	0.28	0.38	0.00495	140
IX		H	0.39	0.0	0.39	0.00552	126
X		H				0.00155	446
XI		$NHSO_2CH_3$	0.10	0.32	0.42	0.00212	326
XII			−0.07 [b]	0.60	0.53	0.00108	638
XIII		Cl	0.10	0.47	0.57	0.00212	326
XIV		NH_3^+	0.10	0.60	0.70	0.00059	1160

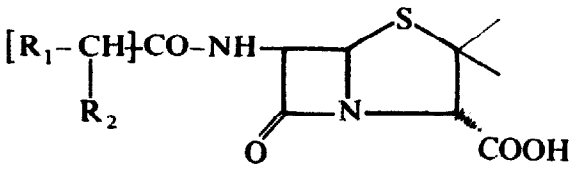
[a] See Charton (1964).

[b] Estimated from $\sigma(C_2H_5) = -0.05$ and $\sigma(C_3H_7\text{-}n) = -0.02$

[c] Pseudo-first-order rate constant for the acid hydrolysis of penicillin in 50% aqueous ethanol at pH 1.3 and 35°C.

[d] Half-life

dihydropenicillin-F (pentylpenicillin, dihydro PC-F; I), penicillin-K (heptylpenicillin, PC-K; II) and that of parenteral penicillins, such as penicillin-G (benzylpenicillin, PC-G; III) and IV were 1.5, 1.2, 5 and 31 min, respectively, whereas those of orally active penicillins such as V and XIV was 180 and 1160 min, respectively.



Scheme 1

As the structural differences among these penicillins are only the substituents (R_1 and R_2) in the side-chain, the decomposition rate constants, k, must depend on the physicochemical properties of this substituents. Doyle et al. (1961, 1963) pointed out that substitution with a strong electron-withdrawing group in the side-chain, particularly in the α-position of III, could stabilize the penicillin markedly in an acid solution. Bird and Nayler (1971) found a linear relation between pK_a value of a carboxylic acid moiety of the side-chain measured in 50% aqueous ethanol at pH 1.3 and 35°C and the half-life observed under the same conditions.

In Fig. 1, the sum of the Taft's inductive substituent constant (σ_I) (Charton, 1964) of R_1 and R_2 in the side-chain, $\Sigma\sigma_I$ values, are plotted against half-life; a good

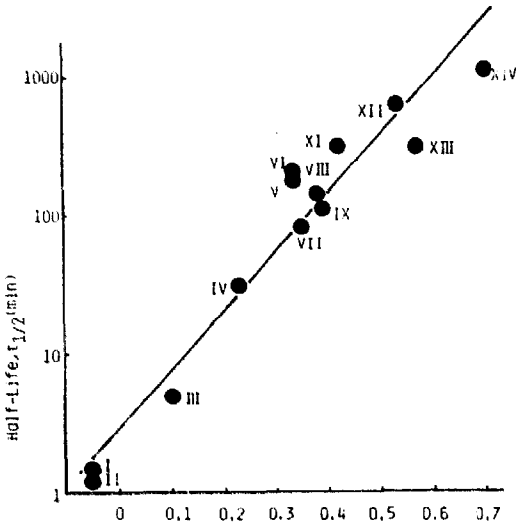


Fig. 1.

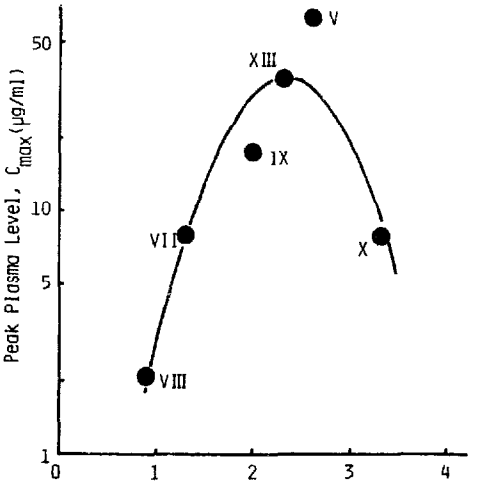


Fig. 2.

Fig. 1. Relation between inductive effects, $\Sigma\sigma_I$ of acyl moiety, R of penicillin and half-life ($t_{1/2}$) in 50% aqueous ethanol at 35°C and pH 1.3.

Fig. 2. Relation between log P and peak plasma level after oral administration of penicillins to mice at a dose of 100 mg/kg.

linear relation following Eqn. 3 derived by least-squares analysis was observed.

$$\log t_{1/2} = 4.223\Sigma\sigma_I + 0.465 \quad (3)$$

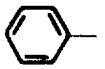
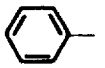
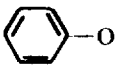
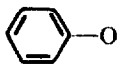
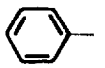
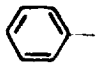
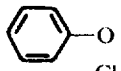
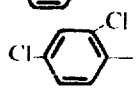
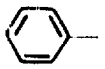
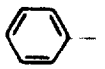
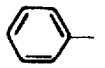
$n = 13$, $r = 0.966$, $s = 0.249$,

$F_{1,11} = 153.56$ ($F_{1,11;\alpha=0.005} = 12.2$)

where n = number of studies; r = correlation coefficient; s = standard deviation; F = F-statistic

TABLE 2

PEAK PLASMA LEVEL AND RELATIVE BIOAVAILABILITY AFTER ORAL ADMINISTRATION OF PENICILLINS TO MICE AND RATS

No.	R_1	R_2	log P [a]	Mice		Rats
				C_{max} [b] (μg/ml)	BA [c] (%)	C_{max} [b] (μg/ml)
I	n-C_4H_9	H	2.37			5.0 (0.4) [d]
II	n-C_6H_{13}	H	3.37			3.2 (0.3)
III		H	1.76			5.0 (1.0)
IV		SO_3^-	−0.22	2.3	1.9	0.2 (0.0)
V		C_2H_5	2.58	62.4	62.6	16.1 (1.3)
VI		CH_3	2.20			15.0 (1.2)
VII		OH	1.31	8.0	8.4	3.7 (0.4)
VIII		$NHCOCH_3$	0.90	2.1	6.1	0.7 (0.0)
IX		H	2.01	17.6	60.0	13.8 (1.0)
X		H	3.30	7.9	21.5	10.9 (0.9)
XI		$NHSO_2CH_3$	0.95			1.2 (0.2)
XIII		Cl	2.30	35.2	56.7	15.3 (0.8)
XIV		NH_3^+	0.57			4.6 (0.3)

[a] P = partition coefficient of penicillin between 1-octanol and water.
[b] C_{max} = peak plasma level after oral administration of various penicillins at a dose of 100 mg/kg.
[c] See Table 3.
[d] S.E. in parentheses.

TABLE 3

PLASMA LEVEL AFTER ORAL (p.o.) OR SUBCUTANEOUS (s.c.) ADMINISTRATION OF PENICILLIN TO MICE AT A DOSE OF 100 mg/kg

No.	Dosing route	Plasma level (μg/ml) (mean ± S.E.)				AUC_0^{2h} (μg·h/ml)	Bioavailability (%)
		1/4 h	1/2 h	1 h	2 h		
IV	s.c.	129.6 ± 10.2	57.0 ± 4.5	8.6 ± 2.6	4.1 ± 0.0	62.4	100.0
	p.o.	2.3 ± 0.3	1.6 ± 0.0	-	-	1.2	1.9
V	s.c.	86.6 ± 4.8	90.3 ± 10.3	58.4 ± 14.5	9.2 ± 2.8	103.9	100.0
	p.o.	51.6 ± 8.4	62.4 ± 2.9	33.2 ± 8.9	7.6 ± 1.4	65.0	62.6
VII	s.c	129.5 ± 13.3	56.4 ± 8.9	6.3 ± 0.7	1.1 ± 0.5	58.8	100.0
	p.o.	8.0 ± 2.0	4.0 ± 1.1	1.9 ± 0.1	-	4.9	8.4
VIII	s.c.	76.2 ± 3.1	55.7 ± 7.9	8.9 ± 4.4	1.2 ± 0.0	47.2	100.0
	p.o.	2.0 ± 0.4	2.1 ± 0.4	1.3 ± 0.1	1.2 ± 0.0	2.9	6.1
IX	s.c.	43.9 ± 1.5	41.1 ± 4.5	3.4 ± 0.9	0.3 ± 0.1	29.1	100.0
	p.o.	17.6 ± 0.8	15.3 ± 5.6	8.7 ± 1.9	1.6 ± 0.2	17.5	60.0
X	s.c.	30.6 ± 6.0	17.3 ± 3.1	3.4 ± 0.8	0.1 ± 0.0	16.7	100.0
	p.o.	7.9 ± 2.6	3.4 ± 1.1	0.4 ± 0.1	0.1 ± 0.0	3.6	21.5
XIII	s.c.	62.0 ± 11.7	36.7 ± 3.2	8.1 ± 1.5	0.8 ± 0.4	36.0	100.0
	p.o.	35.2 ± 3.0	22.7 ± 3.2	3.8 ± 0.9	0.5 ± 0.1	20.4	56.7

R_2) in the side-chain is more stable under acidic conditions. Orally active penicillins, e.g. V, VI and XIV, have $\Sigma\sigma_I$ values of equal or greater than 0.34 and this seems to be prerequisite for penicillin stability in the GI tract.

Oral absorption

Peak plasma level (C_{max}) after oral administration of various penicillins at a dose of 100 mg/kg to mice and rats are shown in Table 2. The area under the plasma level–time curve for 0–2 h (AUC oral) after oral administration and that (AUC s.c.) value after subcutaneous administration at the same dose to mice are also shown in Table 3. The ratio (AUC oral/AUC s.c.) is used as relative bioavailability (BA).

Peak plasma levels in the range of 2–60 μg/ml were observed in mice. A typically orally active penicillin, V, gave a higher peak plasma level, 60 μg/ml, whereas a parenteral penicillin such as IV had levels of only 2.3 μg/ml. In addition, V and penicillin V (6-phenoxyacetamidopenicillanic acid, PC-V; X) showed higher bioavailability than IV after oral administration.

In rats, peak plasma levels were found in the range of 0.2–16.1 μg/ml. Here again, the orally active penicillins, such as IX, phenethicillin (DL-α-phenoxyethylpenicillin, PE-PC; VI) and V, had higher levels, whereas parenteral penicillins, such as III and IV, had lower values.

It has been well established that absorption of a drug through the GI tract is correlated to the partition coefficient P of the drug molecule (Lien, 1975). Log P values of penicillins are plotted against peak plasma level or relative bioavailability after oral administration to mice and rats as shown in Fig. 3, 4 and 5.

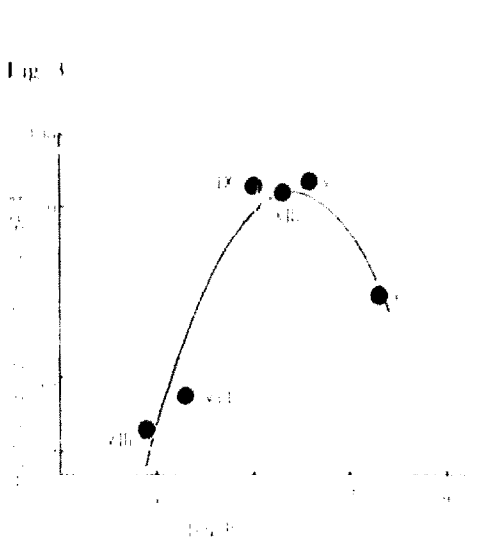


Fig. 3

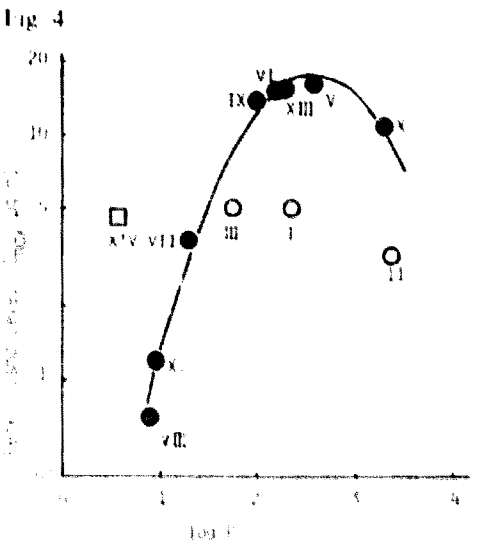


Fig. 4

Fig. 3. Relation between log P and bioavailability after oral administration of penicillins to mice at a dose of 100 mg/kg.

Fig. 4. Relation between log P and peak plasma level after oral administration of penicillins to rats at a dose of 100 mg/kg. ●, acid-stable penicillin; ○, acid-unstable penicillin; □, phenylglycine analog.

Through least-squares analysis good parabolic correlations following Eqns. 4, 5 and 6 were observed. [1]
In mice:

$$\log C_{max}(\mu g/ml) = -0.612(\log P)^2 + 2.865(\log P) - 1.802 \quad (4)$$

$n = 6$, $r = 0.946$, $s = 0.22$, $F_{2,3} = 12.77$ ($F_{2,3;\alpha=0.05} = 9.55$)

$$\log BA(\%) = -0.488(\log P)^2 + 2.350(\log P) - 1.072 \quad (5)$$

$n = 6$, $r = 0.954$, $s = 0.176$, $F_{2,3} = 15.2$

In rats:

$$\log C_{max}(\mu g/ml) = -0.464(\log P)^2 + 2.393(\log P) - 1.834 \quad (5)$$

$n = 8$, $r = 0.995$, $s = 0.061$, $F_{2,6} = 248.1$ ($F_{2,6,\alpha=0.005} = 14.5$)

The optimal log P values, $(\log P)_0$, setting $d(\log C_{max})/d(\log P) = 0$ and $d(\log BA)/d(\log P) = 0$, are 2.34 and 2.41 in mice; the value in rats is 2.58. Lien (1975) found that $(\log P)_0$ values giving the maximum absorption from the GI tract of rats were near 2 in several series of drugs. However, he did not find good correlations between log P and the intestinal absorption for acid drugs. Our results indicate a good relation between log P and the intestinal absorption of an acid drug, penicillins having approximately the same pK_a values at 2.7 (Bergan, 1978; Hou and Poole, 1971; Tsuji and Yamana, 1981). Log P values of orally active penicillins, e.g. IX and V are more than 2.0. Log P values of penicillins giving a relatively higher peak plasma level (more than 10 μg/ml) by oral administration are found between 1.40 and 3.29 in mice and 1.84 and 3.31 in rats. In addition, the penicillins having log P values between 1.65 and 3.17 show a relatively good bioavailability of more than 30% in mice.

As shown in Fig. 4, I, II and III, in spite of having sufficient log P values had lower peak plasma levels; this can be explained by the fact that these drugs are quite unstable in GI fluid. In addition, XIV with a low log P value, showed a higher peak plasma level in comparison with penicillins having the same log P value. Phenylglycine analogs, e.g. XIV, AM-PC and cyclacillin (α-amino cyclohexylpenicillin, AC-PC; XII) are known to be absorbed from the GI tract by a special mechanism

[1] If the plasma level is expressed as μmol/ml, the following equations were obtained.

$$\log C_{max}(\mu mol/ml) = -0.613(\log P)^2 + 2.871(\log P) - 4.388 \quad (4')$$

$n = 6$, $r = 0.947$, $s = 0.219$, $F_{2,3} = 12.96$

$$\log C_{max}(\mu mol/ml) = -0.464(\log P)^2 + 2.395(\log P) - 4.399 \quad (6')$$

$n = 8$, $r = 0.995$, $s = 0.086$, $F_{2,6} = 229.6$

Eqns. 4' and 6' are similar to Eqns. 4 and 6 except for the constant term.

(Bergan, 1978; Nayler, 1972; Tsuji and Yamana, 1981).

From these results, the optimal log P values producing a high plasma level or bioavailability by oral administration are in the range of 1.9–3.3; the phenylglycine analog and XII are exceptions to this rule.

Conclusion

Acid stability in GI fluid and lipophilicity are important factors influencing the oral absorption of all penicillins except for the α-aminopenicillins, e.g. XIV and XII. The results of the present study indicate that an orally active penicillin should be designed to have a $\Sigma\sigma_I$-value of the side-chain equal to or greater than 0.34 for acid stability and a log P value between 1.9 and 3.3 for GI absorption. These values should be used as a guide to design the orally active penicillins.

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References

Bergan, T., Penicillins. Antibiot. Chemother., 25 (1978) 1–122 and refs. cited therein.

Biagi, G.L. Barbaro, A.M., Gamba, M.F. and Guerra, M.C., Partition data of penicillins determined by means of reversed-phase thin layer chromatography. J. Chromatogr., 41 (1969) 371–379.

Biagi, G.L., Guerra, M.C., Barbaro, A.M. and Gamba, M.F., Influence of lipophilic character on the antibacterial activity of cephalosporins and penicillins. J. Med. Chem., 13 (1970) 511–516.

Bird, A.E. and Marshall, A.C., Correlation of serum binding of penicillins with partition coefficients. Biochem. Pharmacol., 16 (1967) 2275–2290.

Bird, A.E. and Nayler, J.H.C., Design of penicillins. In Ariens, E.J. (Ed.), Drug Design, Vol. II, Academic Press, New York, 1971, pp. 277–318 and refs. cited therein.

Charton, M., Definition of 'Inductive' substituent-constants. J. Org. Chem., 29 (1964) 1222–1227.

Clayton, P.J., Cole, M., Elson, S.W. Hardy, K.D., Mizen, L.W. and Sutherland, R., Preparation, hydrolysis, and oral absorption of α-carboxy esters of carbenicillin. J. Med. Chem., 18 (1975) 172–177.

Doyle, F.P. Nayler, J.H.C., Smith, H. and Stove, E.R., Some novel acid-stable penicillins. Nature (London), 191 (1961) 1091–1092.

Doyle, F.P., Nayler, J.H.C., Waddington, H.R.J., Hanson, J.C. and Thomas, G.R., Derivatives of 6-aminopenicillanic acid. V. Analogues of 2,6-dimethoxy-phenylpenicillin with enhanced stability towards acids. J. Chem. Soc., (1963) 497–506.

Hansch, C., Quantitative structure–activity relationship in drug design. In Ariens, E.J. (Ed.), Drug Design, Vol. I, Academic Press, New York, 1971, pp. 271–342 and refs. cited therein.

Hou, J.P. and Poole, J.W., β-Lactam antibiotics: their physicochemical properties and biological activities in relation to structure. J. Pharm. Sci., 60 (1971) 503–532 and refs. cited therein.

Lien, E.J., Structure–absorption–distribution relationships: significance for drug design. In Ariens, E.J. (Ed.), Drug Design, Vol. V, Academic Press, New York, 1975, pp. 81–132.

Nayler, J.H.C., Advances in penicillin research. In Harper, N.J. and Simmonds, A.B. (Eds.), Adv. Drug Res., 7 (1973) 1–105.

Mundell, M., Fischbach, H. and Elbe, E.T., The chemical assay of penicillin. J. Am Med. Assoc. Sci. Edn., 35 (1946) 373–378.

Tsuji, A. and Yamana, T., Gastrointestinal absorption of β-lactam antibiotics. In Mitsuhashi, S. (Ed.), β-Lactam Antibiotics, Japan Sci. Soc. Press, Tokyo, 1981, pp. 235–258 and refs. cited therein.

Tute, M.S., Principles and practice of Hansch analysis: a guide to structure–activity correlation for the medicinal chemist. In Harper, N.J. and Simmonds, A.B. (Eds.), Adv. Drug Res., 6 (1971) 1–77 and refs. cited therein.

Wilkinson, P.J., Reeves, D.S., Wise, R. and Allen, J.T., Volunteer and clinical studies with carfecillin: a new orally administered esters of carbenicillin. Br. Med. J., ii (1975) 250–252.