of which bear two different N-substituents. From Table V, it is clear that log DEA is markedly dependent on lipophilicity, with log P_H and $f_{RR'}$ each accounting for ~50–60% of the variance (~30% for Subject 3). Equations based on log P_H or $f_{RR'}$ alone thus are below acceptable levels of significance and are not reported. The inclusion of quadratic terms or of NH did not improve the correlation.

Good levels of significance were obtained by including a second parameter that served as a descriptor of the N-substituent removed by dealkylation. This approach results in Eqs. 20–28 (Table VIII). Equations 20, 21, 23, 24, 26, and 27 indicate that the rate of the first dealkylation increases with increasing lipophilicity of the molecules (as assessed by log P_H or $f_{RR'}$) and with decreasing volume of the substituent that is split off. There is an apparent contradiction between these two trends, allowing an interesting insight into the molecular factors controlling N-dealkylation. The equations may mean that a high lipophilicity favors affinity to cytochrome P-450 and, hence, a fast reaction (2). On the other hand, the bulkier the substituent to be split off, the less favorable appears the initial C_α-hydroxylation ultimately leading to N-dealkylation.

Such an interpretation is strengthened by Eqs. 22, 25, and 28, which show that the rate of dealkylation increases with the number of hydrogen atoms on the α-carbon. In other words, increasing the steric bulk on the α-carbon being hydroxylated decreases the rate of N-dealkylation, suggesting that, in terms of the reaction mechanism, the C_α-hydroxylation by oxene transfer is the rate-limiting step in the overall N-dealkylation reaction.

Table VIII shows that the utility of the three sets of independent variables (log P_H and V_R, $f_{RR'}$ and V_R, and $f_{RR'}$ and $C_\alpha H$) varies from one subject to another. The set of log P_H and V_R yields the best correlations for Subjects 1 and 3 but not for Subject 2. This situation outlines the interest in quantitative structure–activity relationship studies of having a variety of parameters and descriptors available. The fourth possible set of variables, log P_H and $C_\alpha H$, does not yield significant equations, despite the orthogonality of the two variables (Table V).

Experimental log DEA_i values are compared in Table IV with the values calculated from the best equations in Table VIII.

CONCLUSION

The present study indicates that careful pharmacokinetic studies can yield biological data suitable for quantitative structure–activity relationship treatment and that following pharmacokinetic calculations with quantitative structure–activity relationship calculations can lead to new insights into, and a better understanding of, drug metabolism and disposition. This study also shows that the excretion of amphetamines decreases with increasing lipophilicity of the molecules and that, given the same lipophilicity, tertiary amines are excreted faster than secondary amines, which are excreted faster than primary amines. Also, the correlation equations indicate that the rate of N-dealkylation increases with increasing lipophilicity of the substrates (enhanced enzyme affinity) but decreases with increasing bulk of the N-substituent that is split off (steric hindrance of C_α-hydroxylation).

The quantitative nature of the correlations obtained will allow interesting comparisons with other series of drugs when data become available.

REFERENCES

(1) Y. C. Martin, "Quantitative Drug Design," Dekker, New York, N.Y., 1978.

(2) C. Hansch, *Drug. Metab. Rev.*, **1**, 1 (1972).

(3) G. L. Tong and E. J. Lien, *J. Pharm. Sci.*, **65**, 1651 (1976).

(4) B. Testa and P. Jenner, in "Concepts in Drug Metabolism," vol. 1, P. Jenner and B. Testa, Eds., Dekker, New York, N.Y., 1980, pp. 143–168.

(5) B. Testa, *Pharm. Acta Helv.*, **53**, 143 (1978).

(6) M. Donike, R. Iffland, and L. Jaenicke, *Arzneim.-Forsch.*, **24**, 556 (1974).

(7) R. F. Rekker and H. M. De Kort, *Eur. J. Med. Chem.*, in press.

(8) R. F. Rekker, "The Hydrophobic Fragmental Constant," Elsevier, Amsterdam, The Netherlands, 1977.

(9) A. Bondi, *J. Phys. Chem.*, **68**, 441 (1964).

(10) N. H. Nie, C. H. Hull, J. G. Jenkins, K. Steinbrenner, and D. H. Bent, "SPSS, Statistical Package for the Social Sciences," 2nd ed., McGraw-Hill, New York, N.Y., 1975.

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Interactions of Cephalosporins and Penicillins with Nonpolar Octadecylsilyl Stationary Phase

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Received June 18, 1979, from the *Departamento Interfacultativo de Bioquimica, Cátedra de Química, Facultad de Veterinaria, Leon, Spain.* Accepted for publication November 26, 1979.

Abstract □ The capacity factors of several penicillins and cephalosporins, as well as those of 7-aminocephalosporanic acid, 6-aminopenicillanic acid, and 7-aminodeacetoxycephalosporanic acid, were determined at pH 2.5–7.5 with different methanol concentrations in the mobile phase. The influence of ionic strength on activity factors also was studied. Some theoretical equations providing a quantitative description of the influence of the mobile phase pH on the retention of penicillins and cephalosporins by an octadecylsilyl stationary phase were established. The analysis of experimental data by a nonlinear least-squares fit to theoretically deduced equations permitted determination of the capacity factors of anionic, cationic, zwitterion, and undissociated forms of the substances studied.

Keyphrases □ Cephalosporins—interactions with a nonpolar octadecylsilyl stationary phase □ Penicillins—interactions with a nonpolar octadecylsilyl stationary phase □ Capacity factors—cephalosporins and penicillins, interactions with a nonpolar octadecylsilyl stationary phase □ Antibiotics—cephalosporins and penicillins, interactions with a nonpolar octadecylsilyl stationary phase, capacity factors

Penicillins and cephalosporins constitute a large family of antibiotics of generalized use and similar structure. All of these compounds have at least one carboxylic group, and some possess one or more amino groups. Therefore, depending on the medium, they can be in undissociated, anionic, cationic, or zwitterion form.

There have been several studies of nonpolar stationary phases for the separation of certain cephalosporins and penicillins (1–4). However, a more systematic study of the influence of the ionization of penicillins and cephalosporins on interactions with nonpolar octadecylsilyl stationary phases was desired to elucidate the factors in the chro-

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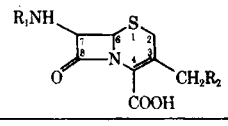
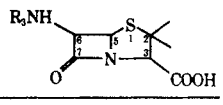
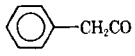
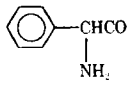
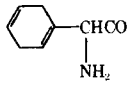
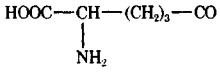
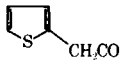
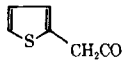
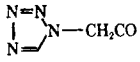
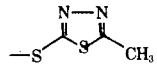
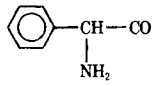
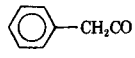
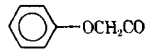
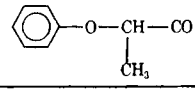
Table I—Structure of Investigated Substances





Compound	Substituents R_1	R_2	R_3
I 7-Aminodeacetoxycephalosporanic acid	H	H	—
II 7-Phenylacetamidodeacetoxycephalosporin		H	—
III Cephalexin		H	—
IV Cephradine		H	—
V 7-Aminocephalosporanic acid	H	$OCOCH_3$	—
VI Cephalosporin C		$OCOCH_3$	—
VII Cephalothin		$OCOCH_3$	—
VIII Cephaloridine			—
IX Cefazolin			—
X 6-Aminopenicillanic acid	—	—	H
XI Ampicillin	—	—	
XII Penicillin G	—	—	
XIII Penicillin V	—	—	
XIV Phenoxypropylpenicillin	—	—	

matographic process and to determine the conditions necessary for optimum separation. It also may help to define the hydrophobic properties of the various penicillins and cephalosporins and their possible relationships to pharmacological characteristics.

EXPERIMENTAL

Materials—The penicillins, cephalosporins, 7-aminocephalosporanic acid, 6-aminopenicillanic acid, and 7-aminodeacetoxycephalosporanic acid were used as supplied[1]. The chemicals were of the highest commercial grade available and were used without further purification.

Phosphate buffer solutions were used at a concentration of 0.05 *M*; sodium sulfate was used to obtain the desired ionic strength in each case. The mixtures of methanol and the buffer solution are expressed in volume percent.

Chromatographic Conditions—A 2.5 × 600-mm steel column, fitted with suitable adaptors and a 10-μm filter, was packed using a dry-packing technique with 30–70-μm silica with bonded octadecylsilane[2]. After packing, the column was conditioned with 200 ml of dimethylformamide at 40° followed immediately by 300 ml of methanol. The liquid chromatograph was fitted with a pump[3], a 20-μl sample valve injector[4], a 250-nm UV detector[5], and a strip-chart recorder[6].

[1] Courtsey of Antibióticos, S.A., Madrid, Spain.
[2] Bondapak C_{18}, Waters Associates, Milford, Mass.
[3] Constametrick II, Laboratory Data Control.
[4] Model 7120, Rheodyne.
[5] UV III monitor, Laboratory Data Control.
[6] Model XER, Sargent-Welch Scientific Co., Skokie, Ill.

The solutions of the chromatographed substances were prepared in the mobile phase at a concentration of 0.5 mg/ml, except for the penicillins, which were dissolved at a concentration of 3 mg/ml. In each case, 20 μl of a sample solution was injected. The flow rate was 1 ml/min, and the sensitivity of the UV detector was set between 0.004 and 0.128 aufs according to the coefficient of extinction of the analyzed product.

The capacity factors, k', were calculated using $k' = (V_R - V_0)/V_0$, where V_R is the elution volume of the chromatographic peak and V_0 is the column void volume, determined as the elution volume of a nonretained peak.

Determination of pKa—The literature does not contain pKa values of the drugs determined under appropriate conditions of the mobile phase. Therefore, the apparent pKa values were determined by a potentiometric method (5) for the ionic strength and methanol content of each mobile phase at room temperature.

Analysis of Experimental Data—The numerical analysis of the experimental data was carried out on a calculator[7] specially programmed for a nonlinear least-squares fit.

RESULTS AND DISCUSSION

The structures of the studied substances are given in Table I. The apparent pKa values, determined at room temperature at an ionic strength of 0.15 and at different methanol concentrations, are given in Table II.

Influence of pH—Due to the low stability of the penicillins, cephalosporins, and the stationary phase in strongly acidic or alkaline media, the studies were conducted within the pH 2.5–7.7 range.

[7] Texas Instruments TI-59.

Table II—Apparent pKa Values of Investigated Substances, Determined Potentiometrically at 20°, Ionic Strength of 0.15, and Various Methanol Concentrations

Compound	pKa_1 0% (v/v) Methanol	pKa_1 10% (v/v) Methanol	pKa_1 20% (v/v) Methanol	pKa_1 30% (v/v) Methanol	pKa_2 0% (v/v) Methanol	pKa_2 10% (v/v) Methanol	pKa_2 20% (v/v) Methanol	pKa_2 30% (v/v) Methanol
I	3.63	—	—	—	5.16	—	—	—
II	—	3.67	3.85	4.08	—	—	—	—
III	—	3.52	3.93	4.02	—	7.29	7.23	7.16
IV	—	4.00	4.17	4.37	—	7.56	7.56	7.54
V	3.49	—	—	—	4.71	—	—	—
VII	—	3.59	3.76	3.94	—	—	—	—
VIII	—	3.53	3.70	3.88	—	—	—	—
IX	—	3.03	3.18	3.38	—	—	—	—
X	3.04	—	—	—	4.90	—	—	—
XI	—	3.60	3.74	3.93	—	7.31	7.21	7.16
XII	—	—	3.82	4.10	—	—	—	—
XIII	—	—	—	4.00	—	—	—	—
XIV	—	—	—	4.21	—	—	—	—

Figure 1 shows the capacity coefficients for I, V, VI, and X at room temperature at different pH values and with a mobile phase of 0.05 *M* phosphate buffer at an ionic strength of 0.15. An octadecylsilyl stationary phase was used.

The capacity factors for II–IV, VI–IX, and XI at the various pH values are shown in Fig. 2. The mobile phase was a 10:90 mixture of methanol and the corresponding 0.05 *M* phosphate buffer at an ionic strength of 0.05. Figure 3 shows the capacity factors of the same substances as well as XII at different pH values when a mobile phase of 20 volumes of methanol and 80 volumes of 0.05 *M* phosphate buffer and an ionic strength of 0.15 was used. Figure 4 shows the capacity factors of the described penicillins and cephalosporins along with those of XII and XIV for different pH values with a mobile phase that was a 30:70 mixture of methanol and 0.05 *M* phosphate buffer at an ionic strength of 0.15. All tests were carried out at room temperature.

The physicochemical interactions between the solutes and the nonpolar stationary phase may be interpreted in accordance with the solvophobic theory (6); therefore, the influence of the solute ionization on chromatographic retention may be justified.

Depending on the pH of the medium, I, V, X, and penicillins and cephalosporins with amino and carboxyl groups may be found in solution in cationic ($^+H_3NPCOOH$), anionic (H_2NPCOO^-), or dipolar ($^+H_3NPCOO^-$) form. The dissociation equilibria are:

$$^+H_3NPCOOH \rightleftharpoons {}^+H_3NPCOO^- + H^+$$

Scheme I

$$K_{a_1} = \frac{[^+H_3NPCOO^-][H^+]}{[^+H_3NPCOOH]} \qquad \text{(Eq. 1)}$$

$$^+H_3NPCOO^- \rightleftharpoons H_2NPCOO^- + H^+$$

Scheme II

$$K_{a_2} = \frac{[H_2NPCOO^-][H^+]}{[^+H_3NPCOO^-]} \qquad \text{(Eq. 2)}$$

The chromatographic process may be considered as a reversible association of the solute, in any of its forms, with the ligand, L, formed by the octadecyl radical joined covalently to the surface of the silica. The process essentially is governed by solvophobic interactions, and no ionic or hydrogen bond is formed between the solute and the stationary phase. The equilibria governing these processes and their respective constants are:

$$^+H_3NPCOO^- + L \rightleftharpoons L^+H_3NPCOO^-$$

Scheme III

$$K_{L^+HP} = \frac{[L^+H_3NPCOO^-]}{[^+H_3NPCOO^-][L]} \qquad \text{(Eq. 3)}$$

$$H_2NPCOO^- + L \rightleftharpoons LH_2NPCOO^-$$

Scheme IV

$$K_{LP^-} = \frac{[LH_2NPCOO^-]}{[H_2NPCOO^-][L]} \qquad \text{(Eq. 4)}$$

$$^+H_3NPCOOH + L \rightleftharpoons L^+H_3NPCOOH$$

Scheme V

$$K_{L^+HP} = \frac{[L^+H_3NPCOOH]}{[^+H_3NPCOOH][L]} \qquad \text{(Eq. 5)}$$

The retention of a solute is expressed by the capacity factor, k', defined as the coefficient of distribution of the solute between the mobile and stationary phases. In a column where the relationship between the volume of the stationary phase and that of the mobile phase is constant and equal to ϕ, the distribution ratio is given by:

$$k' = \phi \frac{[L^+H_3NPCOOH] + [LH_2NPCOO^-] + [L^+H_3NPCOO^-]}{[^+H_3NPCOOH] + [H_2NPCOO^-] + [^+H_3NPCOO^-]} \qquad \text{(Eq. 6)}$$

The numerator of Eq. 6 is the sum of the concentrations of each solute form bound to the stationary phase; the denominator is the sum of the concentrations of each solute form in the mobile phase.

By substituting into Eq. 6 the values of the concentrations of each form found in Eqs. 1–4 and defining the capacity factor of the dipolar form, k'_0, as $k'_0 = \phi[L]K_{L^+HP^-}$, the capacity factor of the anionic form, k'_{-1}, as $k'_{-1} = \phi[L]K_{LP^-}$, and the capacity factor of the cationic form, k'_{+1}, as $k'_{+1} = \phi[L]K_{L^+HP}$, Eq. 6 finally may be transformed into:

$$k' = \frac{k'_0 + k'_{-1}\frac{K_{a_2}}{[H^+]} + k'_{+1}\frac{[H^+]}{K_{a_1}}}{1 + \frac{K_{a_2}}{[H^+]} + \frac{[H^+]}{K_{a_1}}} \qquad \text{(Eq. 7)}$$

Therefore, Eq. 7 should explain the influence of pH on the capacity factors of the amphoteric penicillins and cephalosporins as well as those of I, V, and X.

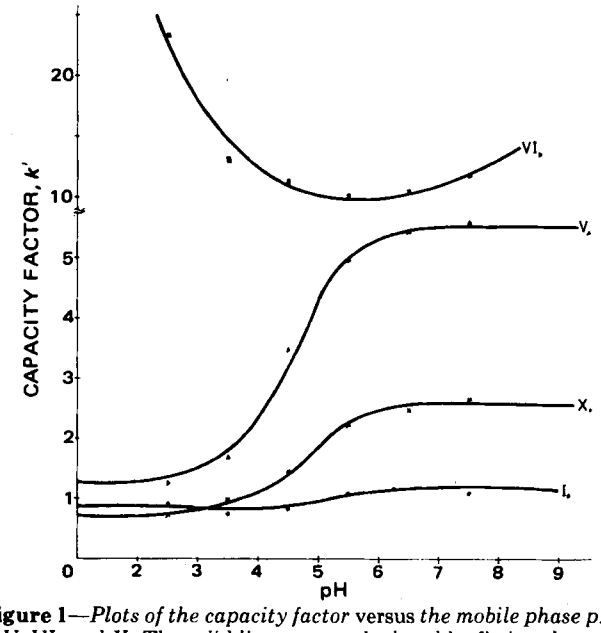


Figure 1—*Plots of the capacity factor* versus *the mobile phase pH for I, V, VI, and X. The solid lines were calculated by fitting the experimental data to Eq. 7.*

Table III—Capacity Factors of Molecular Forms

Compound	0% (v/v) Methanol			10% (v/v) Methanol			20% (v/v) Methanol			30% (v/v) Methanol		
	k'_0	k'_{-1}	k'_{+1}	k'_0	k'_{-1}	k'_{+1}	k'_0	k'_{-1}	k'_{+1}	k'_0	k'_{-1}	k'_{+1}
I	0.76	1.14	0.84									
X	0.95	2.58	0.69									
V	2.10	5.52	1.25									
III				6.40	20.3	18.6	2.10	7.70	6.10	0.70	1.90	2.00
XI				7.50	30.9	25.6	3.20	11.8	9.30	0.87	2.58	3.23
IV				9.40	35.3	27.3	3.70	10.9	9.20	1.00	3.40	3.00
VIII				11.4	—	10.3	4.10	—	3.80	1.30	—	1.40
IX				40.7	21.3	—	8.50	5.40	—	2.10	1.00	—
VII				83.7	63.7	—	27.2	20.3	—	7.60	4.20	—
II				111	31.1	—	38.6	13.1	—	9.90	3.10	—
XII							65.8	29.0	—	22.3	6.00	—
XIII										35.5	15.7	—
XIV										47.0	21.3	—

Each dissociated and undissociated form of penicillins and cephalosporins with only one ionizable group can interact with the stationary phase, giving rise to equilibria similar to those discussed previously.

By proceeding in the same way as for amphoteric penicillins and cephalosporins, the following is obtained:

$$k' = \frac{k'_0 + k'_{-1}\dfrac{K_a}{[H^+]}}{1 + \dfrac{K_a}{[H^+]}} \qquad \text{(Eq. 8)}$$

where k'_0 and k'_{-1} represent the capacity factors of the undissociated and anionic forms of the penicillins and cephalosporins, respectively.

The continuous lines in Figs. 1–4 were obtained by a nonlinear least-squares fit of the experimental data to Eq. 7 for I, V, and X as well as for the amphoteric penicillins and cephalosporins (III, IV, VIII, and XI). For penicillins and cephalosporins having only one carboxyl group (II, VII, IX, and XII–XIV), the lines were obtained with Eq. 8. The analysis of the experimental data by a nonlinear least-squares fit to Eq. 7 permitted calculation of the capacity factors for the zwitterion (k'_0), anionic (k'_{-1}), and cationic (k'_{+1}) forms of I, V, X, and amphoteric penicillins and cephalosporins (Table III). Similarly, a nonlinear least-squares fit to Eq. 8 was used to calculate the capacity factors of the undissociated (k'_0) and dissociated (k'_{-1}) forms of penicillins and cephalosporins with only one carboxyl group (Table III).

The acylation of the amino group of I, V, and X gave cephalosporins and penicillins that were retained more by the octadecylsilyl column. In general, cephalosporins were retained less than were penicillins.

For the nucleus of penicillins and cephalosporins, the hightest k' values were for V and the lowest ones were for I; X had intermediate values (Fig. 1). The greater retention of V may be attributed to its greater hydrophobicity due to the introduction of the acetoxy group in position 3.

For I, V, X, and the amphoteric penicillins and cephalosporins, the capacity factors of the anionic forms (k'_{-1}) (Table III) had the highest values, possibly due to the protective effect of the water molecules (7), which form hydrogen bonds with the protonated amino groups; this protection is smaller in the anionic forms, thus favoring hydrophobic interactions with the stationary phase. For I, V, and X, the capacity factors of the zwitterion form (k'_0) were similar to, but somewhat higher than, those of the cationic form (k'_{+1}). On the other hand, for amphoteric penicillins and cephalosporins, the capacity factors of the zwitterion form

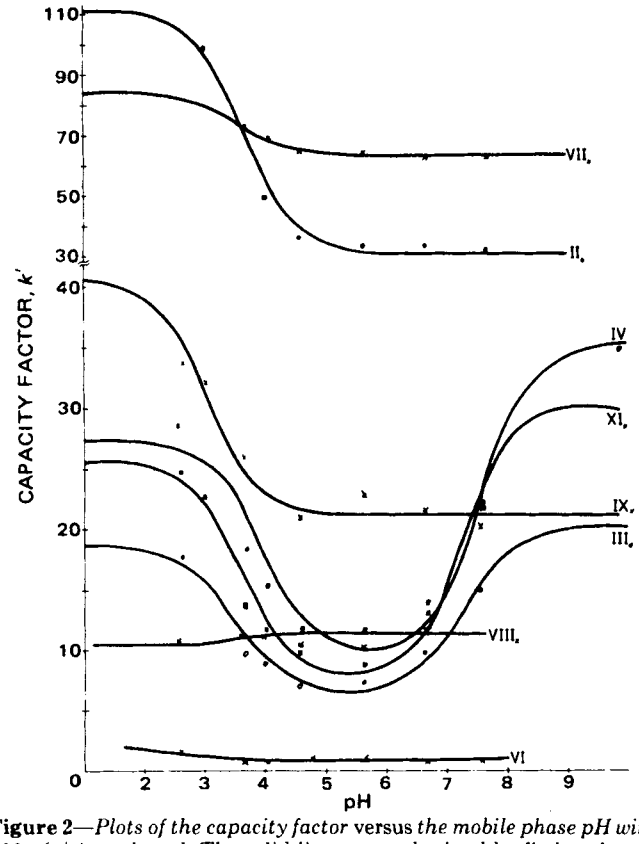


Figure 2—*Plots of the capacity factor* versus *the mobile phase pH with 10% (v/v) methanol. The solid lines were obtained by fitting the experimental data for III, IV, and XI to Eq. 7 and for II and VII–IX to Eq. 8.*

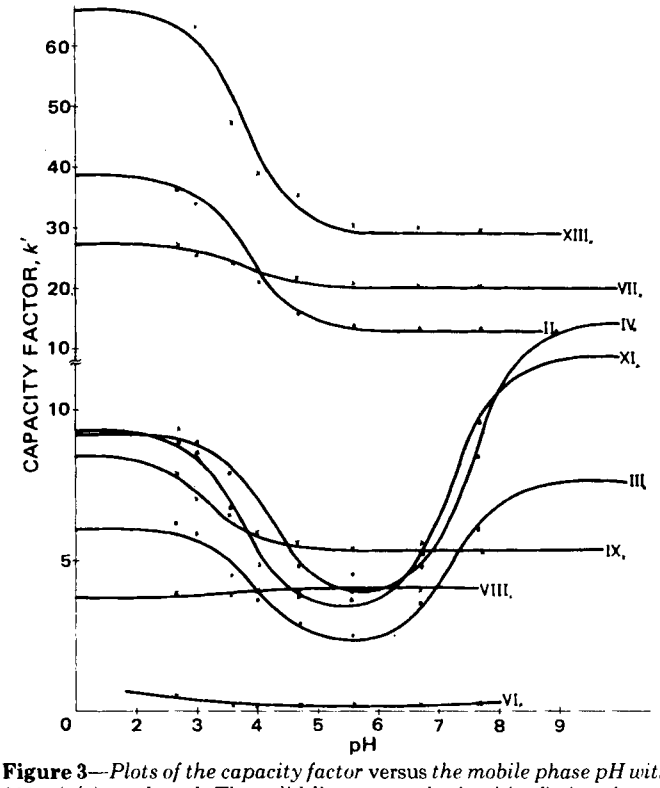


Figure 3—*Plots of the capacity factor* versus *the mobile phase pH with 20% (v/v) methanol. The solid lines were obtained by fitting the experimental data for II, IV, and XI to Eq. 7 and for II, VII–IX, and XII to Eq. 8.*

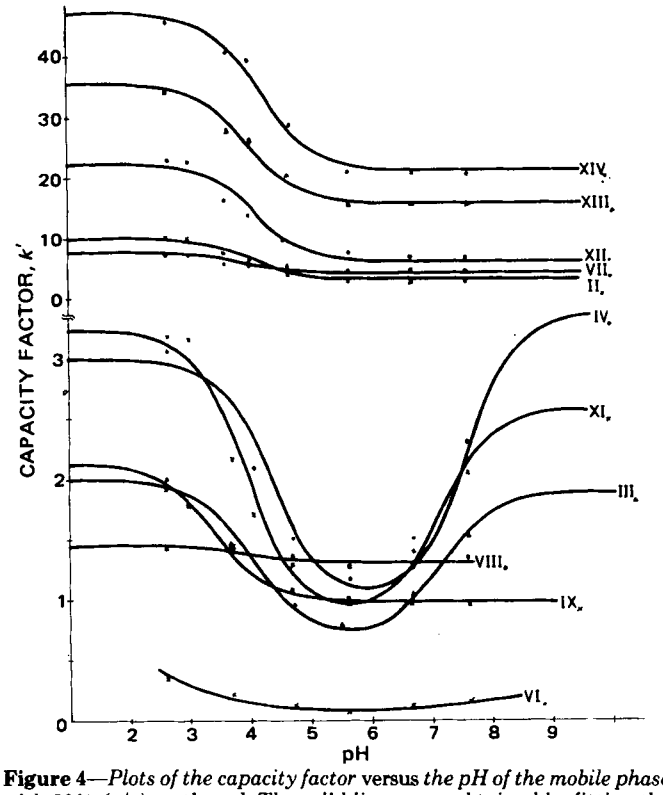


Figure 4—*Plots of the capacity factor* versus *the pH of the mobile phase with 30% (v/v) methanol. The solid lines were obtained by fitting the experimental data for III, IV, and XI to Eq. 7 and for II, VII–IX, and XII–XIV to Eq. 8.*

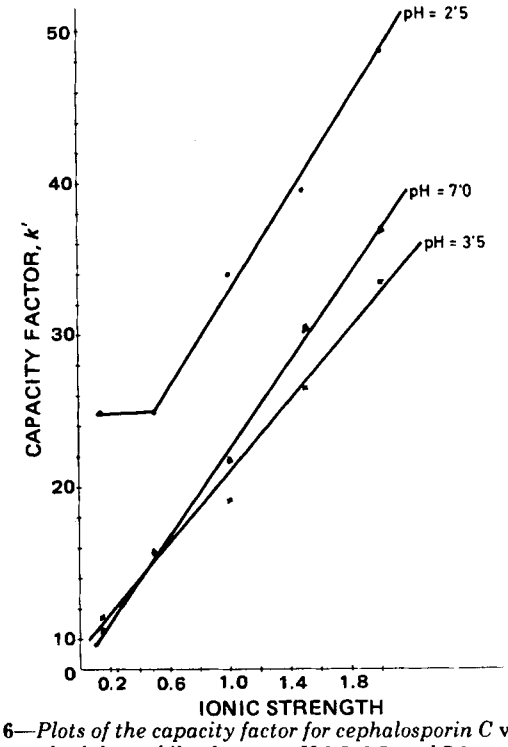


Figure 6—*Plots of the capacity factor for cephalosporin C* versus *the ionic strength of the mobile phase at pH 2.5, 3.5, and 7.0.*

(k'_0) were systematically lower than those of the other two (k'_{+1} and k'_{-1}). Therefore, in amphoteric cephalosporins and penicillins, the dipolar form interacts the least with the octadecylsilyl stationary phase, probably because although the form has no charge, there are a positive charge and a negative charge, which make the hydrophobic interaction with the stationary phase difficult.

In penicillins and cephalosporins with only one carboxyl group, as expected, the capacity factor of the undissociated acid form with no charge (k'_0) was greater than that of the negatively charged dissociated form (k'_{-1}).

Among cephalosporins, the influence of the nature of the lateral chain is remarkable. Of the cephalosporins studied, cephalothin had the highest capacity factor and cephalosporin C had the lowest.

Cephalothin (VII) was more retained by the nonpolar stationary phase than was cephaloridine (VIII). This result probably was a consequence of the substitution in position 3 of the cephalothin thiazine ring of a charged pyridino group by the acetoxy group, thus increasing the polarity and consequently decreasing cephaloridine retention.

For deacetoxycephalosporins, the capacity factor values decreased in the order 7-phenylacetamidoacetoxycephalosporin > cephradine >

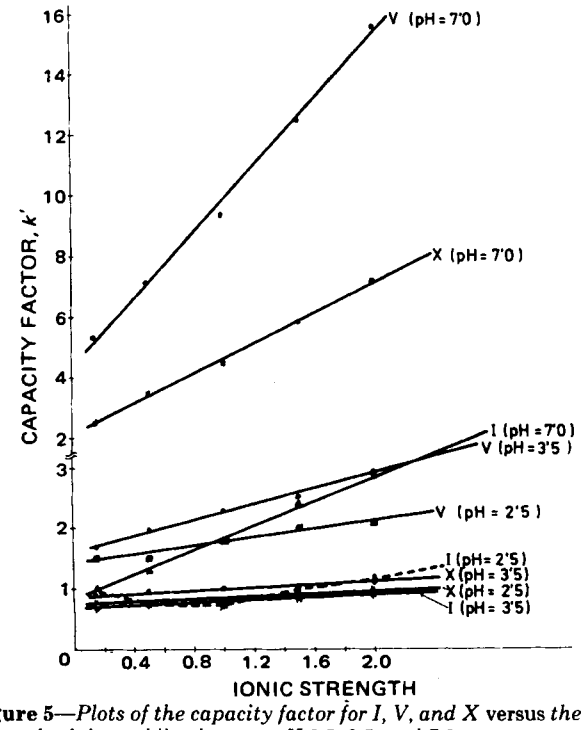


Figure 5—*Plots of the capacity factor for I, V, and X* versus *the ionic strength of the mobile phase at pH 2.5, 3.5, and 7.0.*

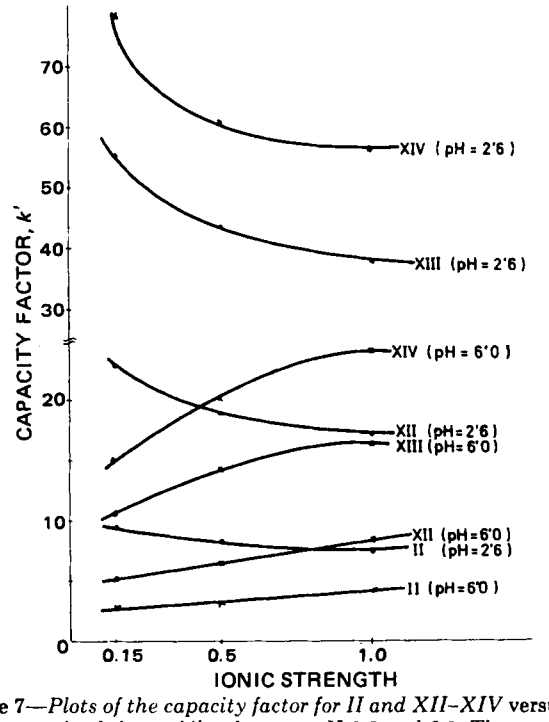


Figure 7—*Plots of the capacity factor for II and XII–XIV* versus *the ionic strength of the mobile phase at pH 2.6 and 6.0. The methanol content in the mobile phase was 20% (v/v).*

cephalexin. The introduction of an amino group in the lateral chain evidently reduced considerably the hydrophobicity of the molecule and, therefore, its retention by the octadecylsilyl column. In penicillins, capacity factors decreased in the order XIV > XIII > XII > XI.

Influence of Methanol Content in Mobile Phase—As expected, the retention of cephalosporins and penicillins by the nonpolar octadecylsilyl stationary phase decreased as the methanol concentration of the mobile phase increased. For the various penicillins and cephalosporins studied, the representation of $\log k'_0$, $\log k'_{-1}$, or $\log k'_{+1}$ *versus* the methanol concentration in the mobile phase, expressed in volume percent, shows a reasonable linear relationship with similar slopes.

Yamana *et al.* (8) used $\log k'$ as a lipophilic index for certain cephalosporins and penicillins, considering it to be analogous to the R_m index determined for cephalosporins and penicillins by means of reversed-phase TLC and comparable to $\log P$, the logarithm of the distribution ratio, determined in *n*-octanol–water systems, which is generally accepted as a lipophilic index. Similarly, $\log k'_0$, $\log k'_{-1}$, and $\log k'_{+1}$ could represent the lipophilic character of each form of penicillins and cephalosporins.

Influence of Salt Concentration—The effect of ionic strength on the capacity factor for I, V, and X was studied (Fig. 5). The mobile phases used were 0.05 *M* phosphate buffers at pH 2.5, 3.5, and 7.0; the ionic strength was varied from 0.51 to 2.0 for each pH value. The influence of the ionic strength on capacity factors at pH 2.5 and 3.5, where I, V, and X are in cationic or zwitterion forms, was very small. At pH 7.0, where these substances are in anionic form, this influence was important, especially for V; it decreased in the order V > X > I.

According to Horvath *et al.* (6), the increase in k' with ionic strength can be attributed to an increase in water surface tension when the salt concentration increases. On the contrary, the ionic form of cephalosporin C does not affect the influence of ionic strength on capacity factors. The k'/μ plots are straight lines of similar slope for each pH value studied, except at pH 2.5 and a very low ionic strength (Fig. 6).

The influence of ionic strength on the retention of II and XII–XIV also was studied at pH 2.6 and 6.0 (Fig. 7). The mobile phase was a mixture of 0.05 *M* phosphate buffer and methanol (2:1 v/v). Plotting k'/μ resulted in lines with negative slopes at the lowest acid pH, where the penicillins are in cationic or undissociated form. At pH 6, where the penicillins and the cephalosporins are in anionic form, the slopes of the k'/μ lines are positive.

It must be emphasized that the ionic strength of the mobile phase has a greater influence on the capacity factors of charged forms than on those of uncharged ones, and it is in the opposite direction. In fact, an increase of the ionic strength increases the retention of the charged forms while decreasing the retention of the uncharged acid forms.

CONCLUSION

The effect of cephalosporin and penicillin ionization on the chromatographic retention by nonpolar stationary phases can be interpreted in terms of hydrophobic interactions between the solute and the hydrocarbon function bound to the support surface. This quantitative study permitted determination of the capacity factors of each molecular form (k'_0, k'_{-1}, and k'_{+1}) of the investigated drugs.

The results given in this work will aid in the separation and analysis of some of the investigated substances.

The logarithms of the capacity factors of each molecular form, being independent of pH, probably will represent more adequately the lipophilic or hydrophobic characteristic of a compound having ionizable groups.

REFERENCES

(1) E. R. White, M. A. Carrol, J. E. Zarembo, and A. D. Bender, *J. Antibiot.*, **28**, 205 (1975).

(2) E. R. White, M. A. Carrol, and J. E. Zarembo, *ibid.*, **30**, 811 (1977).

(3) R. D. Miller and N. Neuss, *ibid.*, **29**, 902 (1975).

(4) F. Salto, *J. Chromatogr.*, **161**, 379 (1978).

(5) A. Albert and E. P. Serjeant, "The Determination of Ionization Constants," Chapman and Hall, London, England, 1971, chap. 2.

(6) C. Horvath, W. Melander, and I. Molnar, *Anal. Chem.*, **49**, 142 (1977).

(7) G. W.-K. Fong and E. Grushka, *ibid.*, **50**, 1154 (1978).

(8) T. Yamana, A. Tsusi, E. Miyamoto, and O. Kubo, *J. Pharm. Sci.*, **66**, 747 (1977).

Hypocholesterolemic Agents VII: Inhibition of β-Hydroxy-β-methylglutaryl-CoA Reductase by Monoesters of Substituted Glutaric Acids

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Abstract □ A series of 1-(4-biphenylyl)pentyl hydrogen 3-alkylglutarates and 3-hydroxy-3-alkylglutarates was synthesized and assayed for inhibition of rat liver β-hydroxy-β-methylglutaryl-CoA reductase. Limited solubility of the monoesters in the enzyme assay system prevented the determination of the I_{50} values. However, the limited data indicated no significant changes in the activity of the analogs when they were assayed at identical concentrations.

Keyphrases □ Hypocholesterolemic agents—inhibition of β-hydroxy-β-methylglutaryl-CoA reductase, monoesters of substituted glutaric acids, rat liver microsomes □ Microsomes, rat liver—inhibition of cholesterol biosynthesis, monoesters of substituted glutaric acids □ Enzyme inhibition—effect of monoesters of glutaric acids on cholesterol biosynthesis, rat liver microsomes

An approach to the design of cholesterol biosynthesis inhibitors as potential hypocholesterolemic agents was discussed previously (1, 2). The rationale for the inhibition of the enzyme β-hydroxy-β-methylglutaryl-CoA reductase was presented (1). Structure–activity relationships indicated that maximum activity was observed when R_1 = biphenylyl, R_2 = *n*-butyl, and n = 1–4 in a series of arylalkyl hydrogen alkanedioates (I). In addition, the incorporation of a β-hydroxy-β-methyl moiety into the acid portion of the glutarate analog provided II, which was seven times more active than the glutarate analog of I.

This paper describes the synthesis and assay of a series of 1-(4-biphenylyl)-*n*-pentyl hydrogen 3-alkylglutarates (III) and 3-hydroxy-3-alkylglutarates (IV). The 3-alkyl series (III) was designed specifically to determine if there was a hydrophobic binding site on the enzyme where the 3-alkyl group of the glutaric acid moiety could bind. The

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