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Contribution of carboxylic acids to the permeability of imipramine

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Summary

The influence of 3 series of acids (monocarboxylic and bicarboxylic aliphatic, and monosubstituted aromatic) on the permeability of imipramine through a dimethylpolysiloxane membrane was determined. All the series of acids give an increase of the permeability as a consequence of an ion-pair formation. Hansch and Free-Wilson statistical approaches were applied to correlate permeability constants with physicochemical and structural parameter, respectively, of the acids. Hansch analysis shows the importance of hydrophobic factors. Free-Wilson analysis allows to calculate the contributions of structural features to the ion-pair permeability.

Introduction

The effect of carboxylic acids on the permeation of chlorpromazine through a dimethylpolysiloxane non-polar membrane was previously investigated (Gasco et al., 1982). The permeability of the diffusate increased considerably in the presence of carboxylic acids or of phosphate, probably due to the formation of an ion-pair between the relative anions and chlorpromazine; the selected carboxylic acids were dietary or physiological and therefore had different structures. The effect of the structure of the pairing ion on the formation of ion-pair has received considerable attention (Davis et al., 1974). Partitioning of ion-pair complexes was reported to be influenced by the molecular weight of the organic ions, by the branching effects of

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the amine cations (Divata and Biles, 1961) and the organic solvents (Harris et al., 1973). The use, with some limitations, of ion-pair data to obtain group contribution values was demonstrated (Fung and Ow, 1972). The functional group contribution of bile salt molecules to ion-pair partitioning of a quaternary ammonium derivative of propranolol was shown (Lee et al., 1978). Correlation of permeation data with structural features of ion-pairs do not appear to have been performed.

The present investigation studies the interaction between imipramine and 3 series of carboxylic acids (monocarboxylic aliphatic, bicarboxylic aliphatic and monosubstituted aromatic acids); imipramine is a tricyclic amine having antidepressant activity. The aim of the study was to verify the eventual increase of the permeability of imipramine as a consequence of an ion-pair formation and to investigate possible correlations between the increase of permeability and the physicochemical and structural properties of the acids.

The group contribution data relative to the enhanced permeability of imipramine as ion-pair might be used to design a system to increase the bioavailability of antidepressants.

There are two main kinds of approaches developed in the study of quantitative structure relationships. In the Hansch (1978) approach the biological activity of a set of congeners is correlated with some physicochemical properties. The Free-Wilson (1964) method of analysis is a statistical approach independent of physicochemical properties based on the assumption that each substituent makes an additive, constant contribution to the activity of the drug independently of other substituents in the molecule. Thus for a congeneric series of N compounds, the activity (A) for each member n of the set under equivalent conditions of assay can be expressed as

$$A_n = \sum_p \sum_s a_{n,ps} + \mu$$

$$n = (1, 2, \ldots, N)$$

where μ represents the activity of the constant portion (parent structure) of the series, a the activity contribution of the substituent s, dependent on the position p. Hansch's linear regression model and the modified form of the Free-Wilson additive model have shown to be theoretically interrelated and numerically equivalent, due to the fact that the individual group contributions can be interpreted as a weighted sum of several physicochemical constants of the substituents.

Materials and Methods

Materials

Imipramine hydrochloride (Geigy); aliphatic monocarboxylic acids C_1- C_8 (Fluka), aliphatic bicarboxylic acids C_2–C_{11} (Fluka), aromatic monosubstituted acids Cl, CH_3, NO_2, OH (*o*, *m*, *p*) (Merck). Non-reinforced dimethylpolysiloxane (Silastic) sheeting in a labeled thickness of 5 mil ($12{,}5 \times 10^{-3}$ cm), thoroughly rinsed and

treated as previously described (Gasco et al. 1982) was used. 1-Octanol grade 1 was the organic phase in the partition studies.

Apparatus

A pH-meter (Orion mod. 701/A), a spectrophotometer (Perkin-Elmer EPS-3T), and a Dognon-Abribat plate tensiometer were used.

Diffusion studies

The apparent constant D of imipramine in the absence and in the presence of carboxylic acid was determined at pH 6.0. The diffusion cell was constructed according to the one used previously (Gasco et al., 1982). The glass cell consisted of donor and receptor compartments and of a membrane (available area 3.14 cm^2) placed between them. The concentration of diffusing solutions were always corrected with sodium hydroxide, since previous work (Gasco et al., 1982) showed that phosphate anions of the buffer can change drug permeation considerably. Twenty-two ml of the test solution was placed in one arm and an equal volume of 0.01 N HCl was added to the other arm to maintain diffused imipramine in a dissociated form.

All solutions were warmed in a jacketed container maintained at 37 ± 0.1°C. The contents of each compartment was rotated by a magnet attached to an electric motor, the rotating speed was ≃ 300 rpm.

Analytical methods

At scheduled times an aliquot (0.5 ml) of the receptor solution was pipetted out for UV determination and the same volume of 0.01 N HCl was added to the receptor compartment to replace the reduced volume. The concentration of imipramine was determined spectrophotometrically at 250 nm ($\log \epsilon = 3.92$).

Determination of the apparent diffusion constants

Eqn. 1, derived by Garret and Chemburkar (1968), for a steady-state diffusion was used to obtained the apparent diffusion constant D

$$D = \frac{C_1 X V}{t C_2 s} \tag{1}$$

where C_1 is the concentration of diffusate in the desorbing solution, X is the thickness of the membrane, s is the available area of membrane, V is the volume of the desorbing solution and C_2 is the concentration of the diffusate.

Diffusion studies of imipramine in the presence of carboxylic acids

The diffusing solutions contained $0.5–3.0 \times 10^{-3}$ M of imipramine and a carboxylate concentration 50 times the molarity of the drug. This molar ratio was selected because, in these conditions, the diffusion of imipramine is independent from the concentration of the different acids.

Determination of log P

The partition studies relative to some acids (azelaic and sebacic acids) not

reported in literature were performed according to the method indicated by Leo et al. (1971).

Solid complexes of imipramine with bicarboxylic acids

25 ml of imipramine 10^{-2} M were added to 25 ml of bicarboxylic acids 50×10^{-2} M; a precipitate was formed, washed with some drops of water and successively dried. The analysis on weighted aliquots of the solid complexes was performed by spectrophotometric determination of imipramine. The ratio was 1:2 for all the examined acids.

Determination of critical micelle concentration (CMC) in the absence and in the presence of bicarboxylic acids

The CMC of imipramine in the presence of bicarboxylic acids was determined by measurement of surface tension with a plate tensiometer at 37 ± 0.5°C in a 50×10^{-3} M solution of acid at pH 6.0. The ionic strength was maintained at 0.148 M by adding sodium chloride.

Calculations

For each series of acids correlations were sought between log D_{IP} (permeability constant of imipramine in the presence of the acid) and some parameters of the acids: log P (Leo et al., 1971), log pK_a (Kortum et al., 1961), log MW, log α and for aromatic acids also with some properties of the substituents σ, π, RM, E_s.

The Free-Wilson statistical approach, modified by Fujita and Ban (1971) was also applied and the substituents and parent structure contributions were calculated.

Technique

The stepwise procedure of the multiple regression program of the SPSS library was used. Calculations were performed on a OH-5560 Olivetti-Hitachi computer.

Results of diffusion studies

The apparent permeability constant D of imipramine at pH 6.0 at 37°C, in the absence of acids, is 0.6×10^{-8} l/cm · min. In the presence of acids the apparent permeability constant D_{IP} greatly increases from a minimum of 1.5 times to a maximum of nearly 10 times. A typical set of data for permeation of imipramine in the presence and in the absence of carboxylic acids is plotted in Fig. 1 according to Eqn. 1. The apparent permeability constants D_{IP} were computed from the slopes of the lines and they are summarized in Tables 1, 2 and 3. If one compares the effect of the acids on the permeability of imipramine, it can be observed that the effect on the permeation varies among the 3 considered series of acids. For the aromatic acids the increase is lower for the meta-substituted and higher for the para-substituted. The chlor- and the methyl-substituted aromatic acids give the highest rise of permeability of imipramine (Table 1).

A regular increase of D_{IP} with the increase of the CH_2 number can be noted for monocarboxylic acids; this behaviour can be related to the log P of the acids as it can be noted from Fig. 2.

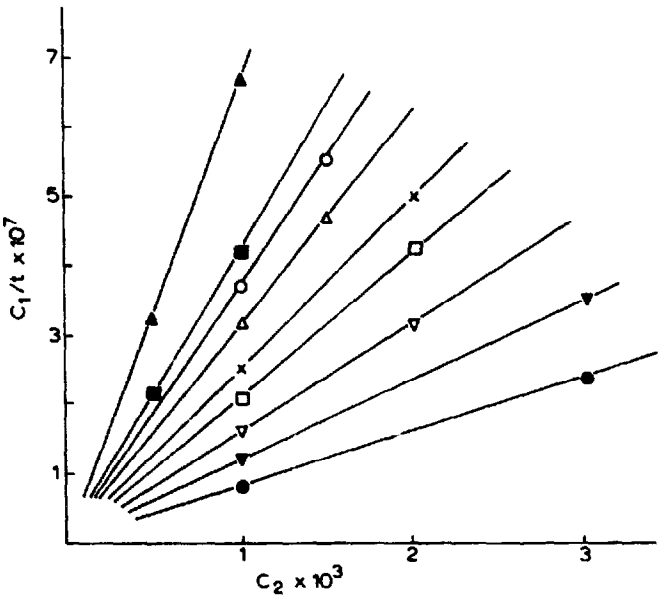

Fig. 1. Rates, C_1/t (moles·liter^{-1}·min^{-1}), of concentration increase in 22 ml of 0.01 N HCl desorbing solution against concentrations, C_2, (moles·liter^{-1}) of imipramine solution in the presence of carboxylic acids at a concentration 50 times the molarity of the drug. ●, none; ▲, formic; △, acetic; □, propionic; ◆, butyric; ▵, pentanoic; ○, esanoic; ■, heptanoic; ◢, octanoic.

Contrarily, in the series of bicarboxylic acids, no influence on D_{IP} values can be ascribed, for the first 10 acids, to the presence of CH_2 groups and to the log P of the acids (Fig. 2). The CMC of imipramine alone, at pH 6.0, is 16.4×10^{-3} M; the CMC of imipramine in the presence of bicarboxylic acids is practically the same for the

TABLE 1

MONOSUBSTITUTED AROMATIC ACIDS

Acid	log P [a]	pK_a [b]	$D_{IP} \times 10^8$ (l/cm·min)
o-methylbenzoic	2.11	3.91	4.5
m-methylbenzoic	2.37	4.27	4.5
p-methylbenzoic	2.27	4.37	6.3
o-hydroxybenzoic	2.23	pK_1 2.99, pK_2 13.09	3.0
m-hydroxybenzoic	1.50	pK_1 4.08, pK_2 9.28	2.6
p-hydroxybenzoic	1.58	pK_1 4.58, pK_2 9.38	5.5
o-chlorobenzoic	1.98	2.92	5.7
m-chlorbenzoic	2.68	3.82	2.9
p-chlorbenzoic	2.65	3.97	4.6
o-nitrobenzoic	0.26	2.2	3.8
m-nitrobenzoic	1.83	3.47	1.7
p-nitrobenzoic	1.89	3.44	3.5
benzoic	1.87	4.20	2.4

[a] Leo et al., 1971.

[b] Kortum et al., 1961.

TABLE 2

MONOCARBOXYLIC ALIPHATIC ACIDS

Acid	log P [a]	pK_a [b]	$D_{IP} \times 10^8$ (l/cm·min)
formic	−0.70	3.75	0.9
acetic	−0.31	4.75	1.2
propionic	0.32	4.87	1.6
butyric	0.79	4.82	1.9
pentanoic	1.46	4.84	2.4
esanoic	1.9	4.86	2.8
heptanoic	2.5	4.88	3.2
octanoic	3.3	4.89	5.2

[a] Leo et al., 1971.
[b] Kortum et al., 1961.

TABLE 3

BICARBOXYLIC ALIPHATIC ACIDS

Acid	log P [a]	pK_1 [b]	pK_2 [b]	$D_{IP} \times 10^8$ (l/cm·min)
oxalic	−1.70	1.32	4.28	2.1
malonic	−0.90	2.83	5.09	2.1
succinic	−0.59	4.19	5.62	2.2
pentandioic	−0.40	4.34	5.42	1.6
esandioic	0.08	4.42	5.41	2.4
eptandioic	0.56	4.49	5.36	2.2
octandioic	1.01	4.52	5.36	2.2
nonandioic	1.57	4.54	5.37	2.3
decandioic	1.84	4.57	5.45	2.2
undecandioic	2.35	4.59	5.49	4.5

[a] Leo et al., 1971.
[b] Kortum et al., 1961.

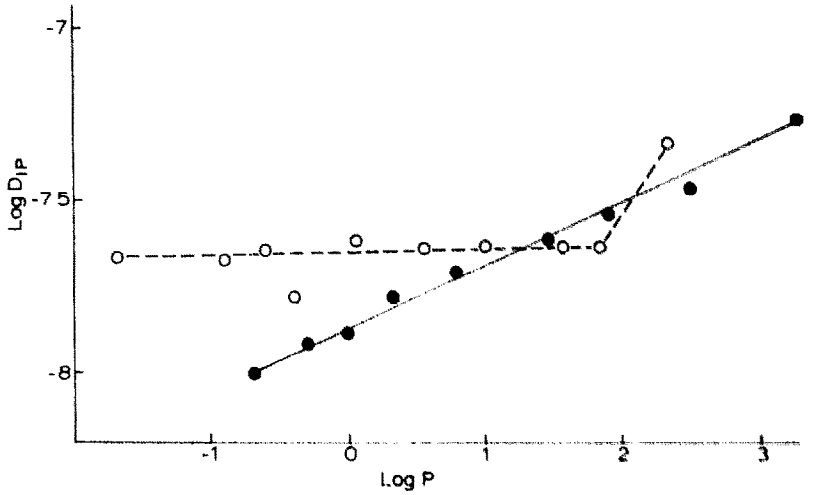

Fig. 2. Log apparent diffusion constant D_{IP} (liter·cm^{-1}·min^{-1}) of imipramine in the presence of monocarboxylic aliphatic acids (●———●) and bicarboxylic aliphatic acids (○· · · · · ○) into 0.01 N HCl desorbing solution against log P_a of the corresponding acids.

TABLE 4

FREE-WILSON MATRIX AND CALCULATED VALUES OF log D_{1P} AND RESIDUALS

Compound no.	Ph	CH_3	OH	Cl	NO_2	I-1 [a]	I-2 [b]	n-CH_2	COO	2nd-COOH	log D_{1P} calc.	Δ log D_{1P} [c]
1	1	1							1		−7.373	0.023
2	1	1							1		−7.373	0.023
3	1	1				1			1		−7.153	−0.046
4	1		1						1		−7.527	0.017
5	1		1						1		−7.526	−0.063
6	1		1			1			1		−7.307	0.047
7	1			1					1		−7.277	0.037
8	1			1			1		1		−7.567	0.027
9	1			1					1		−7.277	0.027
10	1				1				1		−7.453	0.033
11	1				1		1		1		−7.743	−0.027
12	1				1				1		−7.453	−0.007
13	1								1		−7.620	0.000
14									1		−8.026	−0.024
15								1	1		−7.926	0.006
16								2	1		−7.827	0.036
17								3	1		−7.727	0.007
18								4	1		−7.628	0.008
19								5	1		−7.528	−0.022
20								6	1		−7.429	−0.061
21								7	1		−7.321	0.049
22									1	1	−7.641	−0.039
23									1	1	−7.641	−0.039
24									1	1	−7.641	−0.019
25									1	1	−7.641	−0.159
26									1	1	−7.641	0.021
27									1	1	−7.641	−0.019
28									1	1	−7.641	−0.019
29									1	1	−7.641	0.001
30									1	1	−7.641	−0.019
31									1	1	−7.641	0.291
32											−8.220	0.000

[a] I-1 = *p*-Me and *p*-OH.

[b] I-2 = *m*-NO_2 and *m*-Cl.

[c] Δ = observed − calculated value. Observed values are reported in Tables 1, 2 and 3.

different acids (14×10^{-3} M). The CMC of oxalic, decandioic and undecandioic acids could not be determined, because the ion-pairs precipitated.

Results of the correlation analysis

(A) Hansch linear multiple regression model

Monosubstituted aromatic acids. The analysis was effected on 13 acids with CH_3, OH, Cl, NO_2 substituents in *o*, *m*, *p* positions. Log P values of benzoic acids show an anomalous behaviour for *o*-substituted acids, due to mutual interactions between nearly substituents which generally lower the log P, except for the *o*-OH derivative where the hydrogen bonding between OH and COOH groups increases log P (Hansch and Leo, 1979). Excluding *o*-substituted acids, log P is well correlated with π.

$$\log P = 0.738\pi + 2.019 \quad (2)$$

($r^c = 0.96932$, $s^d = 0.115$, $P^f < 0.001$)

Correlations were sought between log D_{IP} and the parameters pK_a, log α, σ, π, MR, E_s. Omitting *m*-nitro, *m*-chlor, *p*-hydroxy and *p*-methyl derivatives, the best equation obtained was:

$$\log D_{IP} = 0.131\pi^* + 0.029MR^* - 7.583 \quad (3)$$

n [a]	K [b]	r [c]	s [d]	r^2 [e]	F [f]
9	2	0.9176	0.058	84.2	15.9 ($P < 0.005$)

* Values of π and MR for aromatic substituents are from Hansch and Leo (1979).
[a] Number of compounds used for regression analysis.
[b] Number of explanatory variables entered in the regression equation. The explanatory variables were allowed to enter the regression if they exceeded an *F*-values of 0.1.
[c] Simple or multiple correlation coefficient.
[d] Standard deviation of the estimate.
[e] Percentage of the variance of the dependent variable explained by the regression equation.
[f] *F*-ratio and significance level.

From Eqn. 3 the hydrophobic and steric parameters appear to be the most significant.

Monocarboxylic aliphatic acids. The analysis was performed on 8 acids. The number was limited due to the low solubility of the superior homologues of octanoic acid. Correlations were tried between log D_{IP} values and log P, log MW, log α, pK_a. The best equation obtained was:

TABLE 5

CALCULATED GROUP CONTRIBUTIONS OF MONOCARBOXYLIC AND BICARBOXYLIC ALIPHATIC, MONOSUBSTITUTED AROMATIC ACIDS

Substituent	C	s	F	r^2 change
ϕ	0.405	0.098	17.28 $P < 0.001$	29.37
COOH (2^{nd})	0.385	0.059	42.60 $P < 0.01$	17.14
Cl	0.343	0.098	12.37 $P < 0.01$	4.59
Me	0.247	0.098	6.38 $P < 0.025$	2.36
I_1	0.220	0.071	9.59 $P < 0.01$	3.96
COO^-	0.194	0.098	3.96 $P < 0.1$	17.95
NO_2	0.167	0.098	2.91 $P < 0.25$	0.85
CH_2	0.100	0.013	61.83 $P < 0.001$	8.09
OH	0.093	0.037	0.91 $P < 0.5$	0.37
I_2	−0.290	0.071	16.67 $P < 0.001$	6.87
μ (parent structure)	−8.220			

n = 32; K = 10; $r = 0.95681$; $r^2 = 91.6$; SEE = 0.082; $F = 22.7$, ($P < 0.001$).

$$\log D_{IP} = 0.179 \log P - 7.884 \quad (4)$$

n	K	r	s	r^2	F
8	1	0.9919	0.033	98.4	368 ($P < 0.001$)

From Eqn. 4 the fundamental role of the hydrophobic factor is noted, the other parameters are not statistically significant.

Bicarboxylic aliphatic acids. The almost constant values of log D_{IP} do not allow any equation to be formulated.

(B) Free-Wilson statistical analysis

The 3 series of acids were analyzed together introducing two indicator variables, I_1 and I_2, for aromatic acids with *p*-Me, *p*-OH and *m*-Cl, *m*-NO_2 substituents, respectively. For bicarboxylic acids the contribution of CH_2 groups was assumed to be zero, because of the independence of the permeability data from the length of the aliphatic chain. The 32 structures were encoded in the matrix of Table 4 where the calculated values of permeability of each ion-pair and the residuals are reported. In Table 5 the contributions of the structural parameters are listed together with the statistical tests.

Discussion

The linear dependence of the diffusion rates for the same membrane area, thickness and volume of the diffusion solution shows that rate of diffusion through

the membrane is directly proportional to the concentration of the diffusing drug. The lag times are negligible. The rates of imipramine increase (C_1/t) are shown in Fig. 1 to be a function of the respective concentration C_2 of the drug in the diffusing solution. The formation of an ion-pair between imipramine and carboxylic acids, more lipophilic than imipramine hydrochloride, causes the increase of the diffusion of the drug. The comparison among the 3 series of acids shows that the highest effect on permeability of imipramine is produced by aromatic acids. Free-Wilson analysis indicates that the sequence of the contribution of the substituents is the same as that of their hydrophobic parameters. The Hansch analysis shows that also steric and polarizability parameters have some importance on the stability of the ion-pair. One might deduce that stereochemical structure of the counter-ion can be important for the formation of an ion-pair between imipramine and aromatic acids. From the Hansch analysis of aromatic acids, pK_a parameters do not seem relevant for the formation of the ion-pair. For monocarboxylic aliphatic acids the contribution of the chain length is important for increasing the permeability of imipramine. From the Hansch analysis the most relevant effect seems to come essentially from the hydrophobicity of the acid, due to the presence of CH_2 groups. For bicarboxylic aliphatic acids, the importance of the second carboxylic group is to be noted. These results are different from those previously obtained for another tricyclic amine, chlorpromazine (Gasco et al., 1982), which gives, with the studied bicarboxylic acids, different D_{IP} values. On the contrary the D_{IP} values of imipramine in the presence of bicarboxylic acids are quite constant, having only a little deflection for glutaric acid and a net rise for undecandioic acid (Table 3). This behaviour could show that D_{IP} is not dependent on the methylenic chain, until it becomes a sufficient length. On the contrary, log P of bicarboxylic aliphatic acids rise with the increase of CH_2 group number and in consequence they cannot be related to log D_{IP} of the relative ion-pair, as in the case of monocarboxylic aliphatic acids. The Free-Wilson analysis (Table 5) shows that the second carboxylic group gives a positive contribution; this fact seems to indicate that the second acid function is not free, otherwise its contribution should be negative for its hydrophilicity. The relatively high permeability of the ion-pair of oxalic acid, having a negative log P, could be due to the presence of a lipophilic ion-pair, where the higher lipophilicity is probably connected with the presence of two molecules of the tricyclic amine (log P imipramine = 4.62) (Leo et al., 1971). To understand this behaviour, the apparent CMC of imipramine, in the presence of bicarboxylic aliphatic acids, was determined, as previously related for chlorpromazine (Gasco et al., 1982). The CMC describes the lipophilicity of the amphiphilic substance; the CMC values of imipramine in the presence of acids could be an indirect measure of the lipophilicity of the ion-pair. For chlorpromazine an almost linear correlation was noted between apparent diffusion constant D_{IP} and log CMC. For imipramine, on the contrary, almost constant values of CMC in the presence of bicarboxylic aliphatic acids were obtained, as in the case of the diffusion constants D_{IP}. In the case of bicarboxylic acids, the structure of tricyclic amine seems to play a fundamental role on the stability of the ion-pair.

One of the goals of the present research was to study the possibility of a method for the prediction a priori of the structural and chemicophysical parameters,

determining the formation and the stability of the ion-pair with imipramine.

The results of the statistical analysis of the substituent effect on the increase of permeability of imipramine in the presence of carboxylic acids demonstrate the validity of the Free-Wilson additive model for the set of acids considered. The quantification of subtistuent effects makes it possible to suggest the most active substituents in terms of producing a sufficiently stable ion-pair. The use of permeability data of ion-pair to obtain information about the effect of the structural features is adequately shown. These data might be useful for the development of dosage forms. Furthermore, a more favorable absorption in GI lumen of imipramine could be due to the presence of dietary acids.

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