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CORRELATIONS BETWEEN THE DISTRIBUTION COEFFICIENTS AND THE CAPACITY FACTORS IN REVERSE HPLC OF N-PYRROLYL DERIVATIVE ACIDS

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ABSTRACT

The distribution coefficients in octanol-water of the dissociated and non-dissociated forms for eleven N-pyrrolyl derivative acids which possess analgesic properties and plaquetary antiaggregating agents are determined. Likewise the capacity factors have been determined by reverse HPLC, and a study has been made of relationship between the two types of coefficients.

INTRODUCTION

The N-pyrrolyl derivative acids here studied are characterized by their analgesic properties and plaquetary antiaggregating agents (1) (2).

In biopharmaceutical studies, the determination of the distribution coefficients between two non-miscible phases (3) (4) is of interest because it is very often an important physico-chemical property related to adsorption, tissular distribution, renal reabsorption and other phenomena in which a through-membrane transport exists.

The purpose of this paper is to determine the apparent distribution coefficients in two non-miscible phases (octanol-water), as a pH function, in the following acids :

- (I).- 3-(phenyl)-2-(1-[1H]pyrrolyl) propanoic
- (II).- 3-(4-hydroxyphenyl)-2-(1-[1H]pyrrolyl) propanoic
- (III).- 3-(4-fluoro phenyl)-2-(1-[1H]pyrrolyl) propanoic
- (IV).- 3-(4-chloro phenyl)-2-(1-[1H]pyrrolyl) propanoic
- (V).- 2-(1-[1H]pyrrolyl) ethanoic
- (VI).- 2-phenyl-2-(1-[1H]pyrrolyl) ethanoic
- (VII).- 2-[4-(1-[1H]pyrrolyl) phenyl] ethanoic
- (VIII).- 2-(2-fluoro phenyl)-2-(1-[1H]pyrrolyl) ethanoic
- (IX).- 2-(4-chloro phenyl)-2-(1-[1H]pyrrolyl) ethanoic
- (X).- [2-(1-[1H]pyrrolyl) phenyl] metanoic
- (XI).- [4-(1-[1H]pyrrolyl) phenyl] methanoic

and also determine the apparent distribution coefficients in two non-miscible phases (octanol-water) as a function of pH with a discussion of the correlations between the said coefficients and the capacity factors determined by HPLC in reverse phase, previously determined by us (1).

MATERIAL AND METHODS

Reagents

The acids studied by us were synthesized and characterized using IR and ^1H -RMN spectrophotometry and elementary composition, the values obtained (table I) being identical with those mentioned in the literature (2). The purity of each acid studied was determined by alkalimetry. The values obtained are shown in Table I.

All the other reagents used were commercial ones of analytical grade and were not purified before use.

Determination of apparent pK_a .

The potentiometric method (5) was used, determinations being made at 25°C, in a nitrogen atmosphere, with aqueous solutions of each acid, 2×10^{-3} M, at constant ionic strength, 0.1 M (NaCl).

The values obtained are shown in Table II.

Determination of apparent distribution coefficients.

Solutions (0.125 mg/ml) of each acid in a 0.04 M phosphate buffer, ionic strength 0.1 M (NaCl). and pH : 7.0 , 6.5 , 6.0 , 5.5 , 5.0 were prepared; 10 ml of each of these solutions with 10 ml of

TABLE I
Analytical Data Concerning the Identification and Characterization of the Acids Studied.

m. p., °C	Purity	IR (cm ⁻¹)	¹ H-NMR (δ)
I..... 95-97	97.3	3000, 1710, 1490, 1280, 1090, 1070, 930.	3.3(m,2H); 4.8(m,1H); 6.2(t,2H) 6.7(t,2H); 7.2(m,5H); 9.7(s,1H)
II..... 150-152	97.0	3440, 3240, 1710, 1500, 1260, 1200, 1050, 1070, 820, 720.	3.3(m,2H); 4.7(s,1H); 6.0(t,2H) 6.6(t,2H); 6.8(m,2H).
III... 115-117	89.0	2860, 1720, 1690, 1500, 1430, 1280, 1210, 1090, 920, 720.	3.3(m,2H); 4.7(m,1H); 6.1(t,2H) 6.6(t,2H); 6.9(d,2H); 8.6(s,1H)
IV.... 128-129	94.5	2940, 1690, 1270, 1220, 1100, 1090, 1020, 930, 820, 760.	3.3(m,1H); 4.7(m,1H); 6.1(t,2H) 6.3(t,2H); 6.9(d,2H); 10.0(s,1H)
V..... 87-89	95.5	3050, 1710, 1280, 1090.	4.5(s,2H); 6.1(t,2H); 6.7(t,2H) 10.0(s,1H).
VI.... 124-126	95.5	3000, 1710, 1230, 1280, 1170, 1090, 1070, 1030, 790, 690.	5.8(s,1H); 6.1(t,2H); 6.7(t,2H) 7.3(s,5H); 8.6(s,1H).
VII... 176-177	89.9	3000, 1710, 1690, 1520, 1280, 1250, 1120, 1070, 920, 810.	3.6(m,2H); 6.4(t,2H); 7.1(t,2H) 7.3(s,4H).
VIII.. 143-144	85.9	2900, 1710, 1480, 1440, 1280, 1240, 1220, 1160, 1090, 730.	4.3(s,1H); 6.0(t,2H); 6.5(t,2H) 7.3(m,5H).
IX.... 129-129	89.8	3000, 2500, 1700, 1430, 1290, 1110, 1030, 740.	5.9(s,1H); 6.2(t,2H); 6.5(t,2H) 7.2(m,2H).
X..... 105-107	90.5	3000, 1680, 1500, 1280, 1090, 1060, 1010, 820, 760, 730.	6.2(t,2H); 6.9(t,2H); 7.2(s,2H)
XI.... 202-204	99.5	3000, 1680, 1520, 1290, 1200, 1070, 920, 850, 730.	6.3(t,2H); 7.4(t,3H); 7.7(d,2H) 8.0(d,2H).

TABLE II

Values of pKa, Capacity Factors (k') determined by HPLC.

	pKa	k'				
		pH				
		7.0	6.0	5.0	3.5	2.5
I.....	3.3	10.7	11.0	13.0	44.3	---
II.....	3.8	---	1.7	1.7	11.3	13.0
III.....	3.8	---	10.3	13.0	---	---
IV.....	4.3	19.3	19.0	21.3	62.3	---
V.....	3.9	---	0.0	0.0	1.0	1.0
VI.....	3.5	4.7	5.0	5.6	16.3	26.3
VII.....	5.9	9.3	10.7	19.0	60.6	---
VIII.....	3.2	5.3	5.0	6.0	17.3	25.7
IX.....	3.1	19.3	19.0	21.3	62.3	---
X.....	3.5	---	1.3	1.7	10.0	15.3
XI.....	5.5	---	4.7	13.0	---	---

octanol, previously saturated in water (3) were mixed. The mixture was stirred for two hours at 25 ± 0.1 °C; after which the concentrations of acids : I, III, IV, V, VI and IX were determined by UV spectrophotometry, at 250 nm, and the remainder at 300 nm.

The P_{ap} apparent distribution coefficients are determined by the equation :

$$P_{ap} = \frac{C_o \times V_w}{C_w \times V_o} \quad (\text{Equation I})$$

where C_o and C_w are the acid concentrations in the organic (octanol) and aqueous phases respectively, and V_o and V_w are the volumes of each phase used in the test. The values obtained are shown in Table III.

Determination of capacity factors.

This parameter has been determined by reverse phase HPLC, at room temperature. Conventional chromatographic equipment was used, its important components being a μ -Bondapack C-18 column, irregular

10 μ particles packed in a Waters Associates Inc. "Z-Module" radial compression module, and a Waters 441 semivariable wave length detector (254, 229 nm).

The values of k' capacity factors were determined at the following pH : 2.5, 3.5, 5.0, 6.0, 7.0, and are shown in Table II. To this end, mobile phases composed of 0.05 M phosphate buffer and methanol (70/30 v/v) were used.

The equation :

$$k' = \frac{k'_o + k'_{-1} \frac{K_a}{|H_3O^+|}}{1 + \frac{K_a}{|H_3O^+|}} \quad (\text{Equation II})$$

was used to determine the capacity factors of the k'_{-1} ionized form and k'_o non-ionized forms of the acids studied (1) (6) (7).

RESULTS AND DISCUSSION.

Since the acids studied can be found dissociated or non-dissociated in aqueous solution, it would be of interest to discover the distribution of each dissociated or non-dissociated form between the two non-miscible phases (octanol-water) (3) (4).

P_i is the distribution coefficient for the ionized form, defined by this expression :

$$P_i = \frac{(A^-)_o}{(A^-)_w} \quad (\text{Equation III})$$

P_u is the apparent distribution coefficient of the non-ionized form :

$$P_u = \frac{(HA)_o}{(HA)_w} \quad (\text{Equation IV})$$

P_i and P_u are calculated from the basis of P_{ap} values experimentally determined at several pH according to this equation :

$$P_{ap} \frac{(H_3O^+)}{K_a} + 1 = P_u \frac{(H_3O^+)}{K_a} + P_i \quad (\text{Equation V})$$

Table III shows the values obtained for P_u and P_i , as well as those for P_{ap} at several pH, for all compounds studied.

As we can see, the distribution coefficients of the P_u non-dissociated form are much higher than those of the P_i dissociated form, as was to be expected. Most distribution coefficients of the non-dissociated form are over 100: acids I, III, IV, VIII, IX, X; acids II and VI have intermediate values and the only ones with a lower distribution coefficient for the non-dissociated form are V and VII. It should be noted that acid VIII has the highest distribution coefficient in its non-dissociated form together with the highest analgesic power of the whole series of acids studied (2).

Regarding the distribution coefficients of the P_i ionized form, only acids VII and VIII have values higher than unity, the others being lower, especially those of acids II and X.

A close relationship has been found to exist between the distribution coefficients of the non-ionized forms and those of the ionized forms for several of the acids studied, according to the equation :

$$\log P_u = 0.713 \log P_i + 2.304 \quad (A)$$

$$n = 3 ; r = 0.994 ; s = 0.072$$

where n is the number of data pairs used, r the correlation coefficient and s the estimated standard error.

The existence of this relationship can be explained by the lipophilous character of the molecules in their non-ionized form also being transmitted to their ionized form.

The values obtained for the P_{ap} apparent distribution coefficients, in octanol-water, have been linked with the capacity factors determined by HPLC (4) (8) with different mobile phases : k' (0%), that is without methanol in the mobile phase, k' (30%) with 30% methanol in the mobile phase. The ratios found by us are as follows :

$$\log P_{ap} = 1.735 \log k'(0\%) - 3.169 \quad (B)$$

$$n = 5 ; r = 0.945 ; s = 0.197$$

$$\log P_{ap} = 1.309 \log k'(30\%) - 1.684 \quad (C)$$

$$n = 5 ; r = 0.927 ; s = 0.292$$

$$\log P_{ap} = 1.018 \log k'(30\%) - 1.590 \quad (D)$$

$$n = 4 ; r = 0.988 ; s = 0.050$$

The data used to obtain these ratios are those obtained at pH = 7.0 for P_{ap} and k' , except those of equation (C) which correspond to determinations made at pH = 6.0.

The ratios between the capacity factors and the apparent distribution coefficients in octanol-water of the dissociated and non-dissociated forms of the N-pyrrolyl derivative acids studied were also determined, with these results :

$$\log k' = 1.587 \log P_i + 2.234 \quad (E)$$

$$n = 3 ; r = 0.995 ; s = 0.039$$

$$\log k' = 0.690 \log P_u - 0.966 \quad (F)$$

$$n = 3 ; r = 0.999 ; s = 0.010$$

$$\log k'_o = 0.806 \log P_u - 0.505 \quad (G)$$

$$n = 3 ; r = 0.994 ; s = 0.025$$

$$\log k'_{-1} = 0.684 \log P_i + 1.711 \quad (H)$$

$$n = 3 ; r = 0.999 ; s = 0.029$$

The above equations correspond to straight lines, which allows us to postulate that the distribution process in octanol-water is reasonably analogous to the one occurring in the chromatographic system constituted by a stationary phase of octadecilsilica and a mobile phase the polarity of which depends on cases and is constituted by a phosphate buffer and mixed with methanol.

Given the acceptable ratios found between P_{ap} and k' , we may deduce that the k' values may throw light on the lipophilous character of the N-pyrrolyl derivative acids here studied with the advantage that these parameters are easier to calculate than the corresponding values of the distribution coefficients in octanol-water.

REFERENCES

1. Salto, F., Negro, A., and Martín, J., J. Liq. Chromatogr.. 9, 2443, 1986.
2. Salto, F., Costa, L. and Fernández J., "A Process for Preparation of N-Pyrrolyl Acids Derivatives and Therapeutical Applications Thereof". Patent USA Nº 4563477.
3. Tsuji, A., Kubo, O., Miyamoto, E. and Yamana, T., J. Pharm. Sci., 66,1675, 1977.

4. Yamana, T., Tsuji, A., Miyamoto, E. and Kubo, O., J. Pharm. Sci., 66, 747, 1977.
5. Albert, A. and Sergeant, E., "The Determination of Ionization Constants". Chapman and Hall Eds. London. 1972.
6. Prieto, J., Barrio, J. and Zapico, J., J. Comput. Biol. Med., 14, 457, 1984.
7. Salto, F., Prieto, J. and Alemany, M. T., J. Pharm. Sci. 69, 501, 1980.
8. McCall, J., J. Chromatogr. Sci., 19, 549, 1975.