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COMMUNICATIONS

Stoichiometric ratio and stability constants of complexes of some aromatic amino acids with caffeine and procaine hydrochloride

G. M. ELMAHROUK, P. ROHDEWALD, *Institut für Pharmazeutische Chemie, Hittorfstr. 58–62, 4400 Münster, Germany*

Vapour pressure osmometry was used in our studies of complex formation between caffeine and the hydrochlorides of aromatic amines (Rohdewald et al 1981) and of the sodium salts of aromatic carboxylic acids (Rohdewald & Baumeister 1969). The continuous variation method of Job (1928) and the 'straight line' method of Asmus (1960) were adapted for the determination of the stoichiometry of these complexes. In the present study both the stoichiometric ratio and the stability constants of complexes between the hydrochlorides and sodium salts of three aromatic amino acids and caffeine or procaine hydrochloride were determined by the method of Asmus (1960).

Data for the binding strength between aromatic amino acids and drugs may show preferential binding sites of proteins and may help to explain food-drug interactions after the simultaneous intake of protein and drugs.

Materials and Methods

Materials. Caffeine, procaine hydrochloride and the amino acids were obtained from Merck, Darmstadt and were used without further purification. All solutions were made just before the measurements.

Instrumentation. The vapour pressure osmometer was that described by Wesselmann & Rohdewald (1978). It consists mainly of parts of the vapour pressure osmometer of Knauer, Oberursel, the thermostatic bath 7603 A of LKB with proportional controller 7602 A, a Hewlett-Packard 419 A galvanometer and a Phillips digital-multimeter.

Methods. Series of mixtures of 10 ml (v_a) of a 0·1 M solution of substance A (a_o), caffeine or procaine HCl, and increasing volumes (v_b) of a 0·5 M solution of substance B (b_o), salts of the amino acids, were prepared and diluted to a final volume of 100 ml (V) with water.

A second series of solutions was prepared with the same concentrations and volumes of B, but without the addition of substance A. In the vapour pressure osmometer, the difference in the resistance between the thermistor holding the solution of 0·01 M substance A and the thermistor holding water, was measured (ΔR_a). The differences in the resistance between the solution of B containing A and equal concentrated solutions of B only were also registered (ΔR_m).

The change in resistance, $\Delta\Delta R$, as the consequence of the formation of a complex, and the corresponding reduction in the number of individual molecules or ions, is obtained by subtracting ΔR_m from ΔR_a.

$\Delta\Delta R$ is used as the physical property for the indication of complex formation instead of the absorbance of the complex used by Asmus (1960). Following his method for the simultaneous determination of the stoichiometry as well as for the stability constant of the complexes, the absorbance in the original equation of Asmus (1960) is replaced by $\Delta\Delta R$:

$$\frac{1}{v_b^n} = \frac{a_o \cdot v_o \cdot b_o^n}{V^{n+1} \cdot K_c} \cdot \frac{1}{\Delta\Delta R} - \left(\frac{b_o}{V}\right)^n \cdot \frac{1}{K} \qquad (1)$$

The plot of the reciprocal of $\Delta\Delta R$ as function of the reciprocal potency of the added volume v_b gives a straight line under the condition that n in v_b^n is identical with the stoichiometric factor of B in the complex. So plots with different exponents for v_b are constructed until a straight line relationship is obtained.

From equation 1 it is obvious that from the intercept $-(b_o/V)^n \cdot 1/K_c$ the stability constant can be calculated, because b_o, V and n are given.

Results and discussion

The linearity of the plot of $1/(\Delta\Delta R)$ against $1/(v_b^n)$ demonstrates that the ratio drug to amino acid is 1:1 in all cases (Fig. 1a–d). The sodium and the hydrochloride salts of tryptophan show a much stronger binding to caffeine than the corresponding salts of phenylalanine and histidine (Table 1).

The differences in respect to the stability constants between sodium- and hydrochloride salts of the amino acids are very small, so that the interaction between the cation or anion and caffeine is of equal strength. The formation of complexes of caffeine with the hydrochlorides of aromatic amines (Rohdewald et al 1981) as well as with sodium salts of aromatic carboxylic acids (Rohdewald & Baumeister

* Correspondence.

Table 1. The stoichiometry and the stability constants of the complexes of aromatic amino acids and caffeine or procaine hydrochloride in water at 25°C.

Substance A	Substance B	Stoichiometric ratio	K_c litre mol^{-1}
Caffeine	Tryptophan hydrochloride	1:1	16·6
	Phenylalanine hydrochloride	1:1	3·2
	Histidine hydrochloride	1:1	3·0
	Tryptophan sodium	1:1	15·0
	Phenylalanine sodium	1:1	3·4
	Histidine sodium	1:1	2·4
Procaine hydrochloride	Tryptophan hydrochloride	1:1	20·0
	Phenylalanine hydrochloride	1:1	29·0

1969), has demonstrated the possibility of caffeine interacting both with negatively or positively charged aromatics.

From other investigations using data from ORD and partition studies (Nakano & Higuchi 1968), the stability constants for tryptophan - caffeine are reported to be 30·0 and 26·0 litre mol^{-1} and for phenylalanine-caffeine 6·3 litre-mol^{-1}. These values indicate a stonger complex formation for the free amino acid by a factor of two compared with the salts. However, the ratio of the stability constants for the caffeine complexes of tryptophan and phenylalanine is in both cases 5:1, so that the same difference in the binding

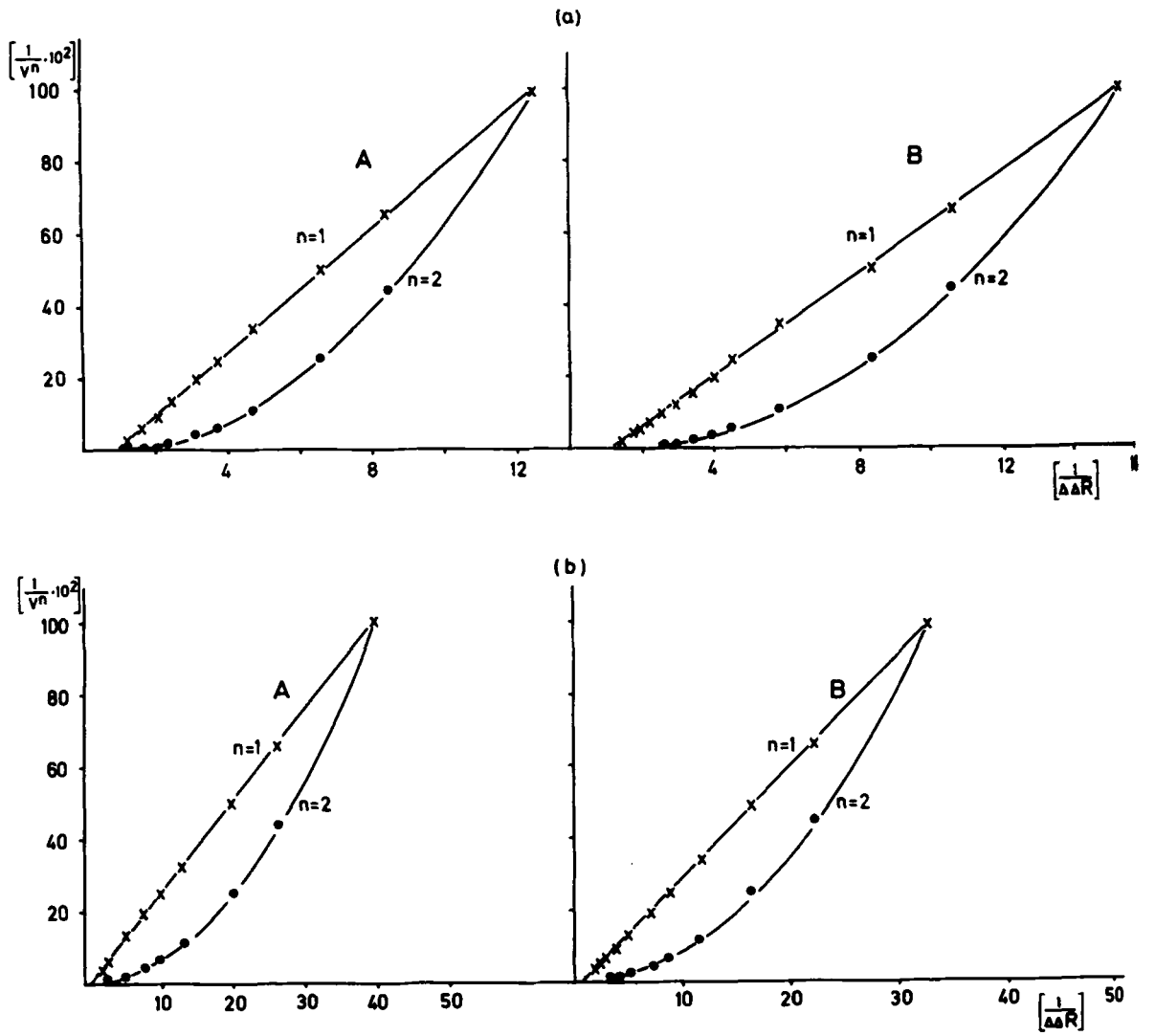
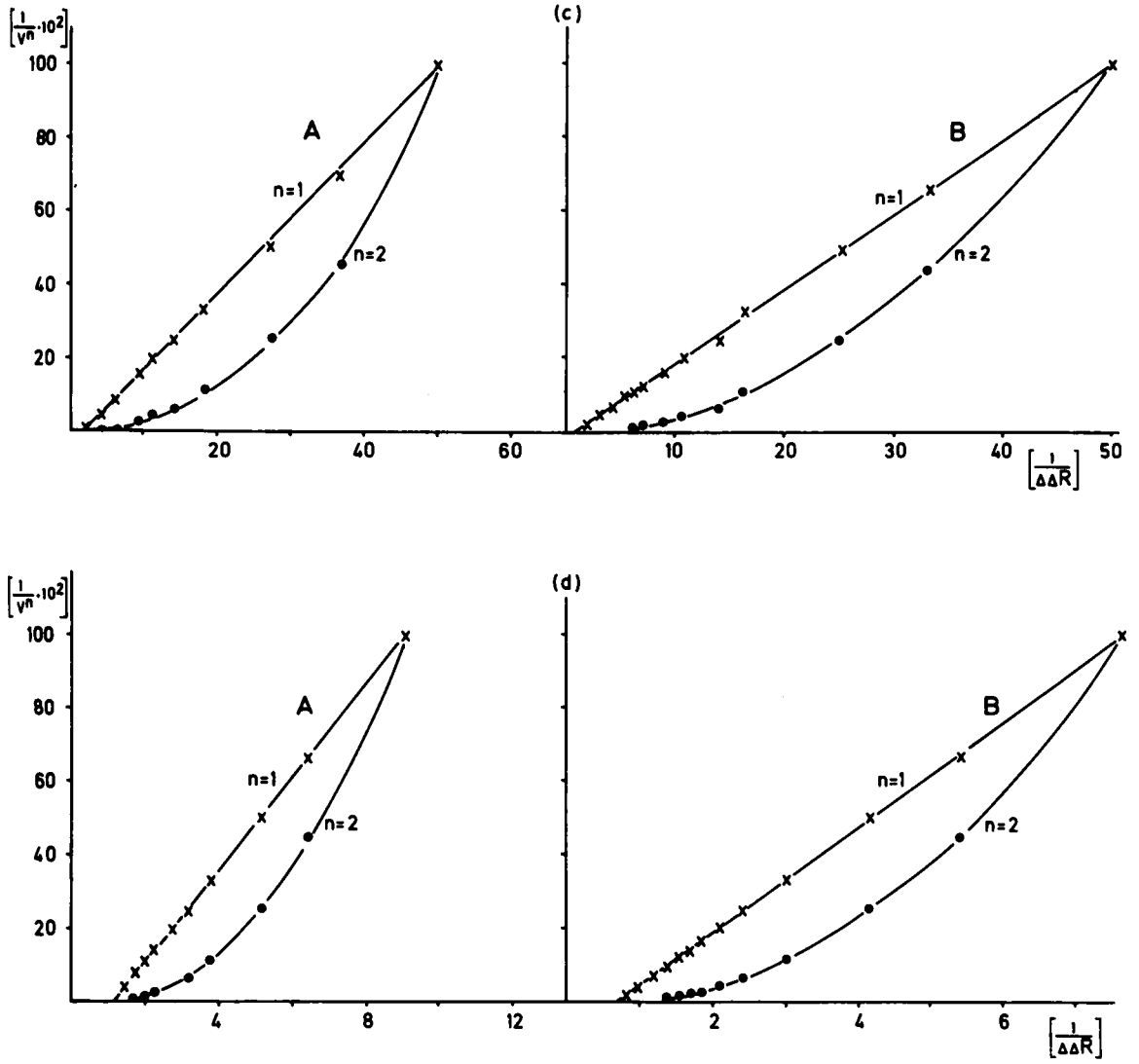


FIG. 1. Asmus plot for the determination of the stoichiometric ratio of caffeine (a–c) or procaine hydrochloride (d) complexes.
(a)A Tryptophan hydrochloride. B Tryptophan sodium.
(b)A Phenylalanine hydrochloride. B Phenylalanine sodium.



(c) A Histidine hydrochloride. B Histidine sodium.
(d) A Phenylalanine hydrochloride. B Tryptophan hydrochloride.

strength exists between tryptophan and phenylalanine, regardless of the charge of the molecule.

The high value of K_c for the complex of the hydrochlorides of procaine and phenylalanine is unexpected in comparison with the interaction of caffeine with the amino acids. The system histidine hydrochloride-procaine hydrochloride was not investigated, because calorimetric and u.v.-spectrometric measurements gives no evidence for complex formation.

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REFERENCES

Asmus, E. (1960) Z. Anal. Chem. 178: 104–116

Job, P. (1928) Ann. Chim. (Paris) 9 (10): 113–203

Nakano, M., Higuchi, T. (1968) J. Pharm. Sci. 57: 1865–1868

Rohdewald, P., Wesselmann, G., Elmahrouk, G. M. (1981) Pharmazie in the press

Rohdewald, P., Baumeister, M. (1969) J. Pharm. Pharmacol. 21: 867–869

Wesselmann, G., Rohdewald, P. (1978) J. Chem. Res. (M). 2701–2719