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METAL COMPLEXES OF TETRACYCLINES

I. Complexes with alkaline -earth and transition metal ions

Macroscopic ionization constants for seven protonated tetracycline antibiotics and stability constants for their complexes with alkaline earth and transition metals are presented. For the alkaline earth metals variations in stability are irregular and are difficult to explain in terms of the rather small alterations in the structure of the antibiotics, in positions which are not close to the coordinating sites; for the transition metals variations are more regular and follow the Irving-Williams' order of stability.

1 — INTRODUCTION

Since 7-chlorotetracycline, the first member of the tetracycline antibiotics family, was isolated in 1947 (1), the importance of these compounds as profilatic and therapeutic agents for a wide range of diseases has stimulated the efforts of many investigators who have been trying to elucidate their inhibiting effect on the process of bacterial reproduction (2-5).

The mechanism of this effect has not been established so far; various authors express the opinion that the ability of these compounds to act as ligands by forming metal complexes with several ions may be involved in the process (6, 7, 8).

In 1950, LOOMIS (9) noticed that 7-chlorotetracycline uncoupled the oxidative phosphorylation in mammals, thus inhibiting the formation of ATP (adenosine triphosphate), the primary energy source for cellular functions.

Other authors (10) suggested, furthermore, that this effect could be due to the complexing by the tetracyclines of the magnesium ions of certain enzymes without removing them from their positions in these compounds.

This agrees with the observations of BRODY (11), who demonstrated that the uncoupling of oxidative phosphorylation was prevented if an excess of magnesium ions was added to the culture media.

Several investigators (11, 12) also found that divalent cations suppressed the inhibition caused by tetracycline antibiotics in mammalian mitochondria, and this led them to postulate that phosphorylation uncoupling by tetracyclines was due to the interaction of these compounds with heavy metals bonded to enzymes.

In 1952, MIURA (13) demonstrated that tetracyclines also inhibit the oxidative phosphorylation in intact bacteria cultures and this fact, coupled with the observation that metal ions reversed again the effect of the tetracyclines (14, 15), reinforced ALBERT's view (16) that complexation with metal ions does play an important role in the mechanism of action of these antibiotics.

In 1953, SAZ and MORMUR (17) found that the reduction of nitroaromatic groups by cell-free extracts of *E. coli* was inhibited by 7-chlorotetracycline and once again other investigators (18) demonstrated that inhibition was suppressed if

manganese ion was added to the culture medium; these findings lead these authors to admit that the activity of the antibiotic could be related to its capacity to alter metabolism by combining with cations existant in bacterial cells.

In the same line of reasoning, other authors (19) demonstrated that reduction of cytochrome-*c* in cultures of *E. coli*, catalised by a mamal metal-flavoenzyme, was inhibited by tetracycline antibiotics, and they proposed that this effect could be partly due to the chelation of enzyme bound iron by those compounds. Since the flavine prostetic group (isoaloxazine) in metaloenzymes of bacterian origin dissociates easily from the proteic part of the enzyme, the above mentioned authors postulated that, in such enzymes, the tetracycline antibiotics may remove the flavine group and compete with it for the protein bound iron; this leads to the dissociation of the enzyme which thereby loses its activity. Even so, one cannot exclude the hypothesis of direct bonding between the tetracyclines and cytochrome-*c*.

In agreement with these studies, COLAIZZI and *al.* (19) advanced the idea that tetracyclines may inhibit growth and reproduction of bacteria by blocking the action of metaloenzymes responsible for oxidative phosphorylation; according to the same investigators, the selective toxicity of tetracyclines towards microorganisms and host animals is due to the fact that in enzymes of bacterial origin the flavine prostetic group is far more easily dissociable than in mamal enzymes.

Several authors reported that besides the uncoupling of respiratory chain and oxidative phosphorylation, the tetracyclines may cause a wide variety of metabolic disturbances in bacteria, such as the inhibition of protein and nucleic acid synthesis, as well as interference with the electron transport system, vitamin K metabolism and synthesis of cell wall membranes. Nevertheless, the primary mode of action of these compounds in their inhibition of growth and reproduction of bacterial cells has not been precisely established so far. This is why several investigators are still interested in the subject and, recently, the idea of an inhibition of biosynthesis of proteins as primary mode of action has been gaining support.

The synthesis of proteins by living organisms is the result of a series of highly coordinated processes;

indeed, the proteines are synthesised in the ribosomes, which act as sites for the reactions involving the ribosomes themselves and several surrounding compounds; there are transitory associations between these compounds and the ribosomes in the various steps and it is obvious that any inhibitor either of the surrounding compounds or of the ribosomes should affect the reaction between them.

For various years it has been currently accepted that the synthesis of proteins in microorganisms is effectively inhibited by tetracyclines (20) but it wasn't known if this effect corresponded or not to a primary mode of action.

In 1964, a research team conducted several studies on the effect of antivirotics of the tetracycline type on 'stafilococcus aureus' strains (21) and concluded that for these family of compounds the reactions inhibited were specifically those involved in protein synthesis; these authors were unable to elucidate which were those particular reactions, but they suggested a mechanism of interference with the metabolism of glutamic acid as a possibility.

In 1965, other authors (22, 23) proposed that the effect of tetracyclines in protein synthesis was the result of the inhibition of the fixation of aminoacyl-t-RNA to the ribosome-m-RNA complex. Indeed, these authors (23) have found that the synthesis of polyphenylalanine (a synthetic protein) ruled by polyuracil (a synthetic m-RNA) was completely inhibited by tetracyclines, but this effect could be reversed when the concentration of ribosomes and polyuracil in the reaction medium was increased and not when the aminouracil-t-RNA concentration was increased, showing that the antibiotic may bond itself to one or more active sites of the ribosomes involved in the fixation of aminoacyl-t-RNA.

Recent work shows that in ribosomes 70S there exists two sites for fixation of aminoacyl-t-RNA, and since it has been found that the tetracyclines inhibit the fixation reaction to the extent of about 50 %, it is reasonable to assume that the antibiotics bond to ribosomes and block one of the above mentioned two sites. These results have since then been confirmed by various other authors (24-27).

CONNAMACHER and MANDEL (24) and DAY (28-30) have also studied the bonding of tetracyclines to

ribosomes and concluded that this bond blocked the fixation of aminoacyl-t-RNA to the ribosome-m-RNA complex.

A result of considerable interest was reported by BODLEY and ZIEVE (31), who have shown that the fixation of phenylalanine-t-RNA to the ribosome-m-RNA complex in the presence of tetracycline depends on the concentration of Mg^{2+} ion in the reaction media; hence, for low Mg^{2+} concentrations (< 0.01 M), tetracycline does not inhibit the fixation, whereas for higher concentrations, inhibition is very marked. These findings are consistent with the results of other authors (32), according to which for low Mg^{2+} concentration (< 0.005 M) phenylalanine-t-RNA bonds only to one of the ribosomal sites whereas for higher Mg^{2+} concentration (> 0.013 M) both ribosomal sites are occupied.

The results obtained so far stimulate the interest on a deeper study of the interaction of tetracycline compounds with different types of metal ions and of the study of mixed complexes involving tetracyclines, metal ions and model compounds for ribosomes. The study of flavines or cytochromes, as the second ligand, is also of interest to test the hypothesis of the uncoupling of oxidative phosphorylation.

In the present series of papers we will present the results of studies of the simple complexes formed by several tetracyclines with different metal ions and attempt to relate the activity of the antibiotics with the formation constants of those complexes. In a subsequent series we will report work on mixed complexes and further studies on the interaction between tetracyclines and metal ions.

2 — RESULTS AND DISCUSSION

In the present paper we report results obtained with a series of six active and one inactive tetracyclines namely: tetracycline (TC), chlorotetracycline (CTC), oxytetracycline (OTC), demethylchlorotetracycline (DCTC), metacycline (MC), doxycycline (DXC) and anhydrotetracycline (ATC).

The structural formulae of these antibiotics are represented below without taking into account the possible existence of different isomers. When

this was the case (e. g. in the case of doxycycline) the most active isomer was used in the present study (α -isomer for doxycycline)

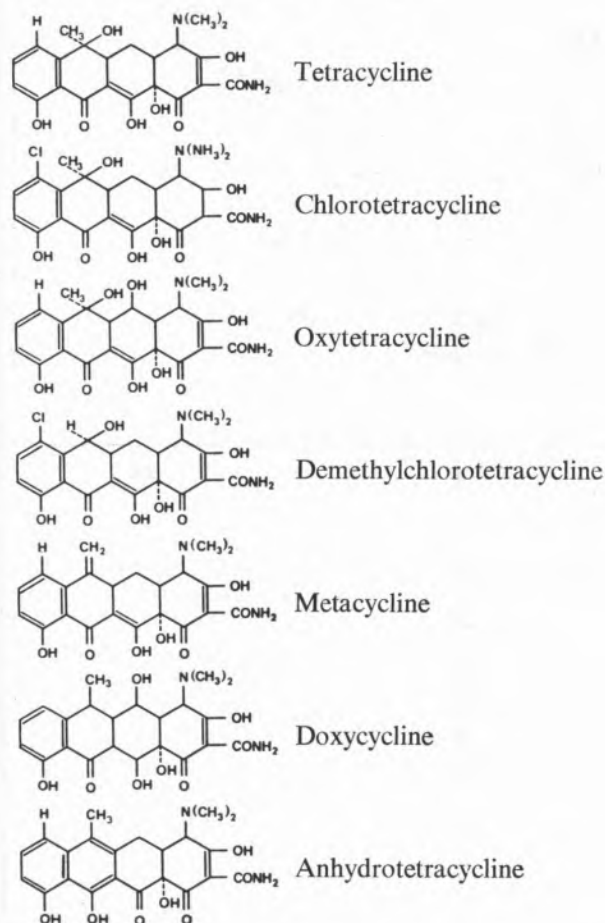


Fig. 1
Structural formulae of the various tetracyclines studied

All the antibiotics were used in the form of their respective hydrochlorides or in the form of free bases to which the equivalent amount of hydrochloric acid was added.

The macroscopic ionization constants⁽¹⁾ of the various species were determined by potentiometric titrations with a standard alkali solution, as described under the experimental section.

Some examples of titration curves of the protonated bases are presented in Figs. 1 and 2, as well as those obtained in the presence of several metal ions.

All the fully protonated antibiotics behave as tribasic acids and the values obtained by standard

(1) See Part II of the present series of papers.

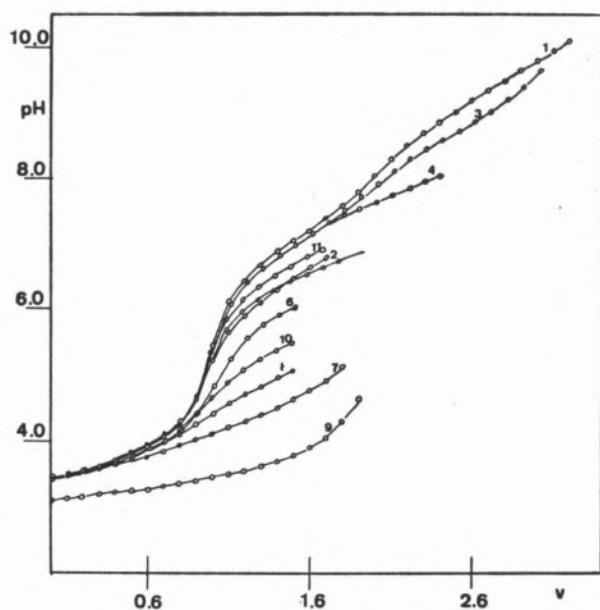


Fig. 2

Titration curves of demethylchlorotetracycline (HCl) in the absence and presence of metal ions.

1 — DCTC. (HCl); 2 — DCTC. (HCl) — Mg^{2+} ;
3 — DCTC. (HCl) — Ba^{2+} ; 4 — DCTC. (HCl) — Sr^{2+} ;
5 — DCTC. (HCl) — Ca^{2+} ; 6 — DCTC. (HCl) — Mn^{2+} ;
7 — DCTC. (HCl) — Ni^{2+} ; 8 — DCTC. (HCl) — Co^{2+} ;
9 — DCTC. (HCl) — Cu^{2+} ; 10 — DCTC. (HCl) — Zn^{2+} ;
11 — DCTC. (HCl) — Cd^{2+} .

calculation procedures for the ionization constants are presented in Table I.

As it was mentioned the values reported are the so called «macroscopic» ionization constants, relative to overall ionization processes; it can be shown that, for our purpose, macroscopic constants may be used instead of «microscopic» ionization constants (33). Discussion on the physical meaning of the two types of constants and on the mechanism of the ionization can be found in the work of REILLEY *et al.* (34).

The order of basicity of the several antibiotics studied in the present work, on the basis of the value of pk_3 is:

tetracycline > demethylchlorotetracycline >
> doxycycline > metacycline >
chlorotetracycline, oxytetracycline >
> anhydrotetracycline

but if one takes $pk_2 + pk_3$ to represent the basicity, the order will be

tetracycline > doxycycline > metacycline >
> oxytetracycline,
chlorotetracycline > demethylchlorotetracycline >
> anhydrotetracycline

One would expect that for each metal ion a correspondent order of stabilities of the respective complexes would be obtained, but this expectation

Table I

Macroscopic ionization constants (pk) of protonated forms of tetracyclines ($T = 25.0^\circ C$, $\mu = 0.1 M KNO_3$)

Antibiotics		pk_1	pk_2	pk_3
Tetracycline	1	3.42	7.52	9.07
	2	3.69 3.30	7.63 7.68	9.24 9.69
Chlorotetracycline	1	3.26	7.20	8.77
	2	3.66 3.30	7.40 7.44	9.06 9.27
Oxytetracycline	1	3.26	7.25	8.73
	2	3.60 3.27	7.42 7.32	9.05 9.11
Demethylchloro- tetracycline	1	3.46	6.96	8.96
	2	3.85	7.31	9.23
Metacycline	1	3.17	7.29	8.83
Doxycycline	1	3.11	7.62	8.94
Anhydrotetra- cycline	1	4.08	6.21	8.37

(1) Present work.

(2) Values reported in the literature.

Table II

Stability constants of the complexes of the several antibiotics studied with alkaline-earth and transition metals
($T = 25.0^\circ\text{C}$, $\mu = 0.1 \text{ M KNO}_3$)

Metal ion		TC. (HCl)		CTC. (HCl)		OTC (HCl)		DCTC. (HCl)		MC. (HCl)		DXC. (HCl)		ATC. (HCl)	
		log KML	log KMHL	log KML	log KMHL	log KML	log KMHL	log KML	log KMHL	log KML	log KMHL	log KML	log KMHL	log KML	log KMHL
Mg^{2+}	1	5.42	3.82	4.68	3.34	5.19 ± 0.04		5.15	3.47	6.00	3.93	6.08	4.30	N. F.	
	2*			4.41				4.17		4.19					
Ca^{2+}	1	5.63 ± 0.09		5.94	3.82	4.92 ± 0.09		5.52	3.29	p. p.	p. p.	p. p.	p. p.	N. F.	
Sr^{2+}	1	4.08 ± 0.04		3.81	2.38	3.99 ± 0.09		4.06 ± 0.15		4.08 ± 0.05		4.07 ± 0.07		N. F.	
Ba^{2+}	1	3.12 ± 0.05		3.07	2.68	N. F.		3.11 ± 0.06		2.86 ± 0.03		2.89 ± 0.05		N. F.	
Mn^{2+}	1	6.07	4.05	5.78	3.84	5.76	4.16	6.33	3.88	6.52	3.81	6.46	4.23	4.94	3.79
Co^{2+}	1	8.04	5.54	8.24	4.62	8.02	5.01	8.49	5.02	9.01	5.88	9.24	6.08		4.98 ± 0.06
Ni^{2+}	1	9.00	5.81	8.87	5.36	8.50	5.69	9.26	5.76	10.27	6.62	10.76	6.87		5.9 ± 0.1
	2		6.1 6.0		5.7 5.8		5.9 5.8		6.4						
Cu^{2+}	1	12.23	7.58 ± 0.008	10.71	7.46 ± 0.02	11.85	7.83 ± 0.05	12.14	7.66 ± 0.04	p. p.	8.08 ± 0.02	14.10	8.93 ± 0.04		6.8 ± 0.1
	2		7.5 7.8		7.5 7.6		7.6 7.2		6.9						
Zn^{2+}	1	7.15	4.55	7.09	4.33	6.69	4.73	7.56	4.48	7.77	5.31	7.81	5.43	5.72	4.26
	2		5.1 4.9		4.8 4.5		4.7 4.6		5.3						
Cd^{2+}	1	5.18 ± 0.04	3.32	5.12 ± 0.04		4.81	3.30	4.84	3.07	5.19 ± 0.03		5.25 ± 0.02		N. F.	N. F.

(¹) Present work; when no standard deviations are mentioned, the constants were obtained by an iterative method.—(²) J. T. Doluisio and A. N. Martin, *J. Med. Chem.*, **6**, 16 (1963).

(^{2*}) J. Benbough, G. A. Morrison, *J. Pharm.*, **17**, 409 (1965).—N. F.—No complexes are formed.—p. p.—The complexes precipitate at relatively low pH.

was not fulfilled in the majority of the cases, showing that it is not the basicity alone which controls the order of stabilities.

The stability constants of the complexes of the several antibiotics with Mg^{2+} , Ca^{2+} , Sr^{2+} , Ba^{2+} , Mn^{2+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} and Cd^{2+} , obtained from potentiometric titrations are presented in Table 2 and it is apparent that pronounced discrepancies were found in some cases relatively to values previously reported by other investigators. It must be pointed that our results are the average of several titrations and are reproducible to ± 0.05 log units; besides, many of the values previously reported were obtained by less accurate methods or calculated using wrong assumptions about the nature and number of the several species which may coexist in solution. On the other hand some authors quote K_{ML} as K_{MHL} values and this casts some serious doubts on the conclusions derived from them.

It should be noticed that we have used in our work a fixed ionic strength ($\mu = 0.1\text{M KNO}_3$) whereas in most of the previous studies the authors tried to keep the medium similar to that found in biological fluids ($\mu \simeq 0.01\text{M}$). Since we were more interested in relative differences of behaviour it was thought preferable to work under conditions insuring better precision in the measurements since the concentration of the free ligands and metal ions was always less than 10^{-3}M and their variations were then completely swamped by the ionic background.

The results obtained in this work present some interesting peculiarities; for the alkaline-earth metals, for instance, the order of stabilities of the complexes formed with the several antibiotics is quite irregular:

Mg^{2+} : DXC, MC > TC > OTC, DCTC > CTC

Ca^{2+} : CTC > TC > DCTC > OTC

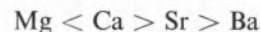
Sr^{2+} : TC, MC > DXC > DCTC > OTC > CTC

Ba^{2+} : TC, DCTC > CTC > DXC, MC > OTC

This means that the order of stabilities is strongly conditioned by the size of the ions, although it is difficult to see how much this may be the result of differences in conformation of the ligands,

arising from relatively small structural alterations in the molecules.

Apparently the ionic radius of Ca^{2+} is the most favourable since that in the majority of the cases one obtains



and it is interesting to see that with metacycline and doxycycline precipitates of the calcium complexes are obtained at low pH values.

As to the transition metal ions the irregularities are not so pronounced and the order of stabilities seems to be the IRVING-WILLIAM's natural order, *e.g.*



It should be stressed that in the case of copper-metacycline and copper-anhydrotetracycline only K_{MHL} values could be measured because the complexes precipitate at relatively low pH; K_{ML} values would be, in both cases, much higher.

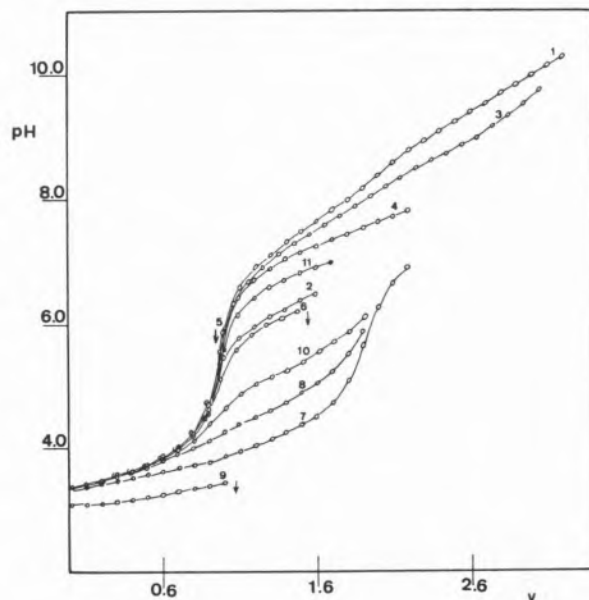


Fig. 3
Titration curves of methacycline (HCl) in the absence and presence of metal ions.

- 1 — MC. (HCl); 2 — MC. (HCl) — Mg^{2+} ;
3 — MC. (HCl) — Ba^{2+} ; 4 — MC. (HCl) — Sr^{2+} ;
5 — MC. (HCl) — Ca^{2+} ; 6 — MC. (HCl) — Mn^{2+} ;
7 — MC. (HCl) — Ni^{2+} ; 8 — MC. (HCl) — Co^{2+} ;
9 — MC. (HCl) — Cu^{2+} ; 10 — MC. (HCl) — Zn^{2+} ;
11 — MC. (HCl) — Cd^{2+} .

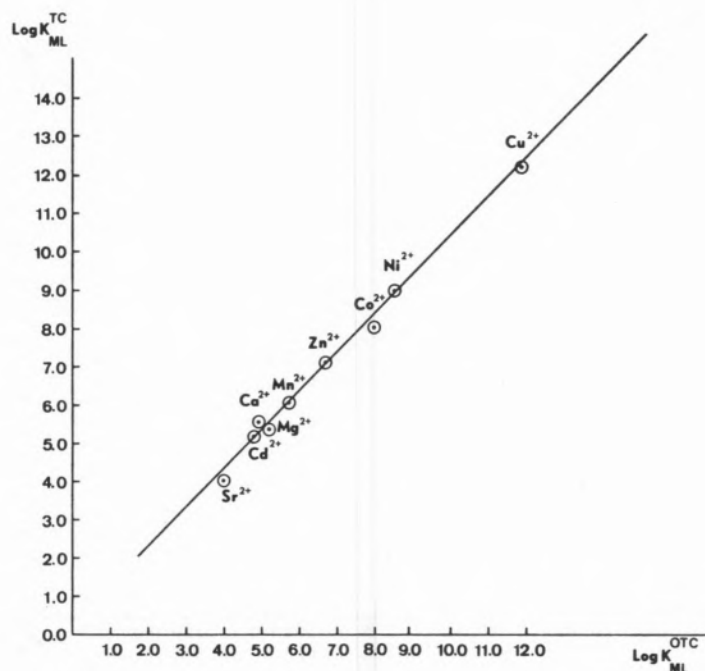


Fig. 4

Correlation between the stability constants ($\log K_{ML}$) of the metal-complexes of TC and OTC.

Relatively to the ligands the following orders of stability are obtained:

Mn^{2+} :

MC > DXC > DCTC > TC > CTC, OTC > ATC

Co^{2+} :

DXC > MC > DCTC > CTC > TC, OTC > ATC

Ni^{2+} :

DXC > MC > DCTC > TC > CTC > OTC > ATC

Cu^{2+} :

DXC > TC > DCTC > OTC > CTC > ATC

Zn^{2+} :

DXC > MC > DCTC > TC > CTC > OTC > ATC

Cd^{2+} :

DXC > MC, TC > CTC > DCTC, OTC

The reason for these orders of stabilities is not apparent but may be related to the stereochemical requirements of each one of the metal ions as it happened with the alkaline-earth metals since it is unlikely that they are bonded to the various antibiotics in different coordinating sites. Indeed

it seems that the substitutions in the skeleton of molecules of the ligands causes slight changes in conformation which result in the modification of their coordinating capabilities or different packing effects; illustrative examples are those of the stability or of the solubility of the calcium complexes on passing from tetracycline to metacycline and doxycycline and of the barium complexes on passing from tetracycline to oxytetracycline, although the substituents are all far apart from the most likely coordinating sites.

In several cases, however, good correlations may be established between the stability constants ($\log K_{ML}$ or $\log K_{MHL}$) of the complexes of any two of the antibiotics — figs. 4, 5 and 6.

From these figures an approximate order of complexing power of the several antibiotics can be established, which is quite general for $\log K$ values above 5. That order is the following:

Metacycline > Doxycycline > Demethylchlorotetracycline > Tetracycline > Chlorotetracycline > Oxytetracycline > Anhydrotetracycline

It is interesting to notice that the order of complexing power parallels that of the biological

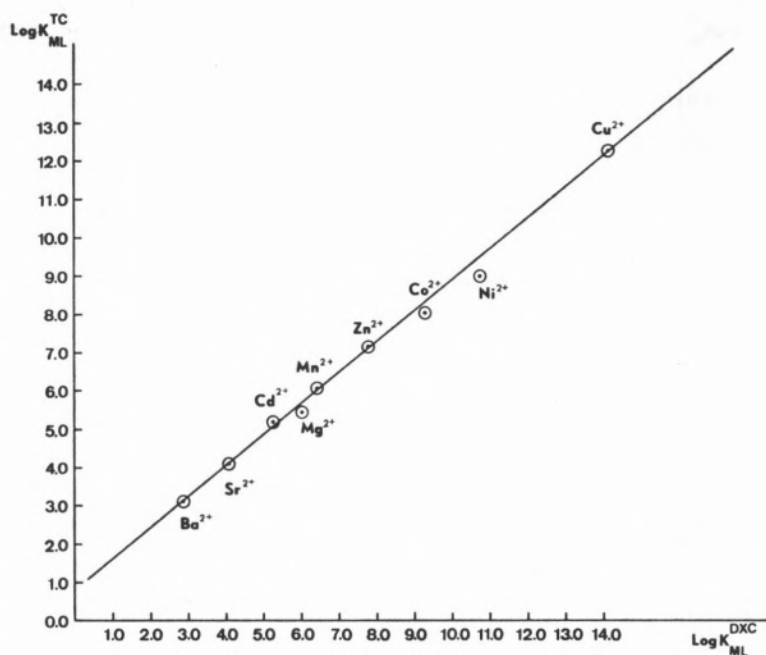


Fig. 5

Correlation between the stability constants ($\log K_{ML}$) of the metal-complexes of TC and DXC.

«activity» of the several compounds, taking as a measure of this activity the inhibitory rate constant of the reactions of cell division and of protein and nucleic acid synthesis, studied with strains of «*Escherichia coli* W.» by various authors (35-37), except for the inversion of the values relative to tetracycline and chlorotetracycline.

Unfortunately no correspondent values of «activities» have been reported for metacycline and doxycycline to allow a more extensive comparison but it is apparent that the range of stability constants considered ($\log K > 5$) corresponds to the complexes of transition metals and Mg^{2+} .

We will deal with this problem as well as with that of the conformation of the antibiotics in subsequent papers.

3 — EXPERIMENTAL

3.1 — TETRACYCLINES

The samples of the antibiotics used in the present work were kindly provided by ATRAL-CIPAN, SARL, Lisbon.

Fresh solutions were prepared every day since they decompose quite rapidly at room temperature.

3.2 — POTASSIUM HYDROXIDE

Carbonate free solutions of potassium hidroxide were prepared from commercial standards diluted with previously boiled deionised water and checked by titration against standard perchloric acid.

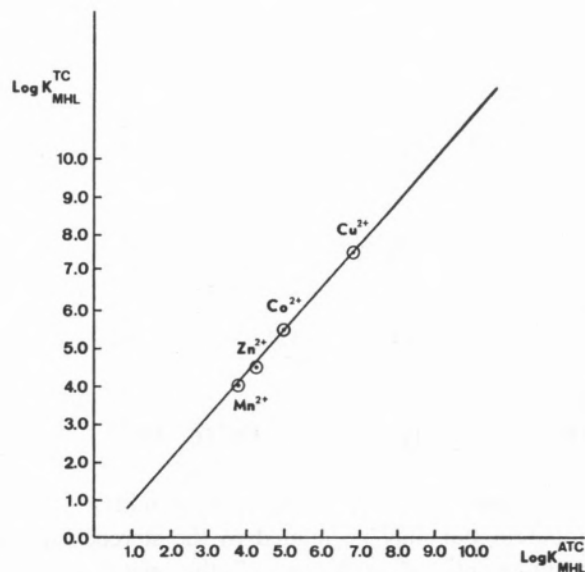


Fig. 6

Correlation between the stability constants ($\log K_{MHL}$) of the metal-complexes of TC and ATC

3.3 — METAL SOLUTIONS

Metal nitrates of «Analar» grade were used and solutions were standardised by complexometric titrations or electrolytic deposition (copper).

3.4 — IONIC BACKGROUND

Potassium nitrate of «Analar» grade was used as background salt.

3.5 — DE-IONISED WATER

Laboratory distilled water was passed through a mixed-bed ion exchange resin.

3.6 — INSTRUMENTS

pH measurements and titrations were made using a Radiometer pHM4, fitted with a Radiometer glass electrode type G2025 B and a Radiometer saturated calomel reference.

All the measurements were carried out in double-walled cells thermostatically controlled at $25.0 \pm 0.1^\circ\text{C}$ by circulating water through the jacket.

The glass electrode was calibrated in $-\log[\text{H}^+]$ values by titrating a sample of perchloric acid in 0.1M KNO_3 at 25.0°C temperature.

3.7 — TECHNIQUE

The determination of ionization constants and stability constants of the metal complexes by pH titrations has been described in previous papers (38).

4 — CALCULATIONS

4.1 — IONIZATION CONSTANTS OF PROTONATED TETRACYCLINES

In the case of tetracyclines hydrochlorides $\text{LH}_3^+ \cdot \text{Cl}^-$, the species LH_3^+ behave as a weak tribasic acid and their successive macroscopic ionization constants are of the order of 3, 7 and 9 (pk values).

The values of the constants for each antibiotic considered can be calculated from the results of the potentiometric titrations using expressions derived from mass and charge balances as described in previous papers (38):

$$k_1 = \frac{R[\text{H}^+]}{(1-R)} \quad (1)$$

and

$$-\frac{(R-2)}{(R-3)}[\text{H}^+] = \frac{(R-1)[\text{H}^+]^2}{(R-3)} \cdot \frac{1}{k_2} + k_3 \quad (2)$$

where

$$R = \frac{a C_a + [\text{H}^+] - [\text{OH}^-]}{C_a} \quad (3)$$

and a is the number of equivalents of base added per mole of the ligand (concentration C_a). The second and third constants are obtained by graphic resolution of equation 2.

4.2 — STABILITY CONSTANTS OF THE COMPLEXES FORMED WITH METAL IONS

The necessary expressions can again be obtained from adequate mass and charge balances (38) for the several possible alternative cases:

4.2.1 — ONLY THE COMPLEX MHL IS FORMED

4.2.2 — ONLY THE COMPLEX ML IS FORMED

4.2.3 — MHL AND ML ARE FORMED SIMULTANEOUSLY

The following expressions were used:

4.2.1 —

$$K_{\text{MHL}} = \frac{[\text{MHL}^+]}{[\text{M}^{2+}][\text{LH}^-]} \quad (4)$$

where

$$[\text{MHL}^+] = C_a - [\text{LH}^-] \left(\alpha_H + \frac{k_3}{[\text{H}^+]} \right) \quad (5)$$

$$[\text{LH}^-] = \frac{(2 - a) C_a - [\text{H}^+] + [\text{OH}^-]}{\beta_H} \quad (6)$$

$$[\text{M}^{2+}] = C_m - C_a + [\text{LH}^-] \left(\alpha_H + \frac{k_3}{[\text{H}^+]} \right) \quad (7)$$

$$\alpha_H = 1 + \frac{1}{k_1 k_2} [\text{H}^+]^2 + \frac{1}{k_2} [\text{H}^+] \quad (8)$$

$$\beta_H = \frac{2}{k_1 \cdot k_2} [\text{H}^+]^2 + \frac{1}{k_2} [\text{H}^+] - \frac{k_3}{[\text{H}^+]} \quad (9)$$

C_m = total concentration of metal.

4.2.2 —

$$K_{\text{ML}} = \frac{[\text{ML}]}{[\text{M}^{2+}] [\text{L}^{2-}]} \quad (10)$$

where

$$[\text{ML}] = C_a - \alpha_H \cdot [\text{L}^{2-}] \quad (11)$$

$$[\text{L}^{2-}] = \frac{(3 - a) C_a - [\text{H}^+] + [\text{OH}^-]}{\beta_H} \quad (12)$$

$$[\text{M}^{2+}] = C_m - C_a + \alpha_H [\text{L}^{2-}] \quad (13)$$

$$\alpha_H = 1 + \frac{1}{k_1 \cdot k_2 \cdot k_3} [\text{H}^+]^3 + \frac{1}{k_2 k_3} [\text{H}^+]^2 + \frac{1}{k_3} [\text{H}^+] \quad (14)$$

$$\beta_H = \frac{3}{k_1 \cdot k_2 \cdot k_3} [\text{H}^+]^3 + \frac{2}{k_2 \cdot k_3} [\text{H}^+]^2 + \frac{1}{k_3} [\text{H}^+] \quad (15)$$

4.2.3 — The values of K_{MHL} and K_{ML} were obtained by an iterative resolution of the equation (16) using a program for the Hewlett-Pakard mod. 9820 A desk computer.

$$\frac{\bar{n}}{(1 - \bar{n}) [\text{L}^{2-}]} = K_{\text{ML}} + \frac{[\text{H}^+]}{k_3} \cdot K_{\text{MHL}} \quad (16)$$

where

$$\bar{n} = \frac{C_a - \alpha_H \cdot [\text{L}^{2-}]}{C_m} \quad (17)$$

and

$$[\text{L}^{2-}] = \frac{(3 - a) C_a - [\text{H}^+] + [\text{OH}^-]}{\beta_H + \frac{1}{k_3} \cdot [\text{H}^+] [\text{M}^{2+}] K_{\text{MHL}}} \quad (18)$$

α_H , β_H being given by expressions (14) and (15).

Iteration begins with $K_{\text{MHL}} = 0$; the value of K_{ML} obtained allows a first estimation of a better K_{MHL} and the process is repeated until successive values of $\log K_{\text{ML}}$ and $\log K_{\text{MHL}}$ do not differ by more than 0.05 units.

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RESUMO

Apresentam-se os valores das constantes de ionização macroscópicas das formas protonadas de 7 antibióticos da família da tetraciclina e as respectivas constantes de estabilidade dos complexos formados com um grupo de metais alcalino-terrosos e de transição. Para os metais alcalino-terrosos as ordens de estabilidade são irregulares e difíceis de explicar em termos de variações estruturais em posições não próximas dos centros de coordenação; para os metais de transição as variações são mais regulares e seguem a ordem natural de Irving-Williams.