Fluorimetric Complexing Constants and Circular Dichroism Measurements for Antibiotic X-537A with Univalent and Bivalent Cations

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Summary Complexing constants for Li^+, Na^+, K^+, Rb^+, Cs^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+} with antibiotic X-537A in methanol have been determined fluorimetrically; fluorescence enhancements and most of the large augmentations of circular dichroic absorption due to the cations are correlated with the complexing constants.

THE growing use of macrocyclic complexing agents (macrolide antibiotics, polycyclic ethers[1]) in biological studies requires the most sensitive possible means for the detection of the transformations and transfers involved. This led us to measure the fluorescence of various mixtures of X-537A (I) with metal ions in methanol to establish the compositions

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and formation constants of the dominant complexes. The circular dichroism of the same systems was also measured. Caswell and Pressman[2] recently reported complexing constants for X-537A in 80% ethanol for Mg^{2+} and Ca^{2+} only and found no change in fluorescence with Sr^{2+} in their studies of partition and transport.

TABLE

Complexing constants, relative fluorescence yields, and c.d. absorption coefficients for combinations of univalent and bivalent metal ions with antibiotic X-537A *at* 23 °C *in methanol containing* Bu^n_3N

Metal ion	Binding constant M^{-1}	Relative fluorescence yield	C.d. absorption coefficient[c,d] $-\Delta\epsilon$ 240 nm	290 nm
—[a]	—	(1·0)	0·7	1·1
Li^+	48	1·15	b	b
Na^+	$3·7 \times 10^2$	1·9	3·2	4·2
K^+	$3·8 \times 10^3$	2·3	4·9	5·4
Rb^+	$3·7 \times 10^3$	2·2	5·5	5·7
Cs^+	$2·7 \times 10^3$	2·1	5·3	5·0
Mg^{2+}	$6·7 \times 10^3$	0·64	0·7	2·3
Ca^{2+}	$3·7 \times 10^4$	1·3	1·2	2·5
Sr^{2+}	$3·0 \times 10^5$	2·8	1·7	4·6
Ba^{2+}	$2·9 \times 10^6$	2·9	4·5	7·8

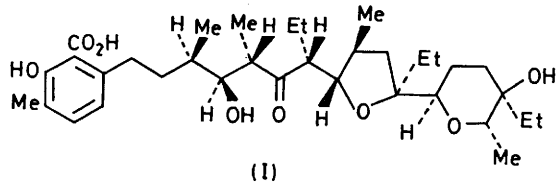
[a] Bu^n_3N salt. [b] In this case the spectrum is somewhat different and thus, these two coefficients are not directly comparable with the others. [c] The definition of this coefficient is given, for example, by L. Velluz, M. Legrand, and M. Grosjean, *Compt. rend.*, 1963, **256**, 1878. [d] Estimated from data for mixtures of $2—5 \times 10^{-4}$M X-537A with 0·01—0·05M metal ion. In the case of Na^+ the metal ion concentration was varied over a wide range; these coefficients are due to a 1:1 complex. In the case of Ba^{2+}, coefficients are unchanged if chloride is replaced by acetate ion and if up to 10 mol. % H_2O is added.

Water has an effect on the fluorescence yield of X-537A as well as on the complexing constants, fluorescence level decreasing smoothly by a factor of more than ten in changing from methanol to water. Complexing constants in methanol were determined by fluorimetric titrations of X-537A with metal ion salts (excitation, 310 nm; emission, 420 nm; anions: Cl^-, Br^-, or ClO_4^-). Redistilled Bu^n_3N (2×10^{-4}M) was present in all solutions. The concentration of antibiotic was in the range $1—5 \times 10^{-6}$M. Constants for Ca^{2+} and Li^+ (fluorescence yield not very different from uncomplexed X-537A) were evaluated using competition with other ions: a plot of $F_0/F - F_0$ *vs.* $[M^2]^{-1}$ yields a straight line [equation (1)]. F = fluorescence intensity; $F_0 = F$ in absence of M^2; M^1, M^2 ions, one maintained at constant concentration; K_1, K_2 complexing constants, one known; ϕ_1, ϕ_2, relative quantum yields, one known.

$$F_0/(F - F_0) = \frac{(1 + K_1[M^1])/(K_2[M^2]) + 1}{\phi_2(1+K_1[M^1])/(1+\phi_1 K_1[M^1]) - 1} \quad (1)$$

The unknown binding constants and relative fluorescent yields are evaluated from the x and y intercepts, respectively. The concentration dependence of fluorescence indicated a 1:1 stoicheiometry and monomeric character for these complexes. The greater fluorescence intensity for these complexes compared to X-537A or its anion may be attributed to cation co-ordination by the carboxy-group of the salicylate unit.

Maxima appear in the c.d. spectra at 290 and 240 nm. The c.d. band at 290 nm may be attributed to the $n \rightarrow \pi^*$ transition of the aliphatic carbonyl. The variations in c.d. may be explained by differences in the asymmetry of the environment of the carbonyl and salicylate groups in the



(I)

various forms of free and complexed X-537A. Some possible arrangements are suggested by the crystal structures reported for the complexes with Ba^{2+} and Ag^{2+}.[3]

The c.d. bands (Table) show striking changes in the intensity ratio as the ion is varied. The ratio is higher for Group II ions. The composition dependences of the c.d. spectra have not yet been investigated over a wide enough range (except in the case of Na^+) to rule out the possibility that there are significant contributions from complexes other than 1:1.

These observations show that complexes of X-537A and various ions can be studied in detail in homogeneous media by fluorimetry and c.d. Other antibiotics could be studied by competition with X-537A. Finally, it is likely that the fluorescence method can be applied directly to corresponding studies for vesicles and in synthetic and natural membranes.

We thank Mrs. Ruth Panigel for technical assistance, Dr. J. Berger, Hoffman LaRoche, for supplying antibiotic X-537A, Professor S. Simon, Biology Department, S.U.N.Y, for aid with the c.d. spectra, and Professor N. Hirota, Chemistry Department, S.U.N.Y., for advice.

(*Received, 12th December* 1972; *Com.* 2056.)

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