# Conformation of antibiotic X-537A (lasalocid-A) and its calcium complex in acetonitrile solvent using circular dichroism spectroscopy

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The calcium binding characteristics of antibiotic X-537A (lasalocid-A) in a lipophilic solvent, acetonitrile ($CH_3CN$), have been studied using circular dichroism (CD) spectroscopy. The analysis of the data indicated that in this medium polar solvent, X-537A forms predominantly the charged complexes of stoichiometries 2:1 and 1:1, the relative amounts of the two being dependent on $[Ca^{2+}]$. The conformations of the complexes, arrived at on the basis of the data, seem to indicate a rigid part encompassing $Ca^{2+}$, liganded to 3 oxygens of the molecule, viz., the carbonyl, the substituted tetrahydrofuran ring and the substituted pyran ring oxygens (apart from, possibly, the liganding provided by nitrogen atoms of the solvent molecules), and a flexible part consisting of the salicylic acid group of the molecule.

*Circular dichroism spectroscopy* *X-537A (lasalocid A)* *Conformation* *Carrier ionophore* *Sandwich complex* *Transmembrane ion-transport*

## 1. INTRODUCTION

Among the ion-carrier antibiotics, X-537A (lasalocid-A; fig.1) [1] a carboxylic ionophore, has been the subject of extensive studies because of its diverse biological applications, in particular, in the understanding of the role of biologically important divalent cations (viz., $Ca^{2+}$) in various physiological systems [2–4]. The ionophore has been shown to bind to both biological and artificial membranes, to form lipophilic complexes with both monovalent and divalent metal ions, rare earth and transition metal ions, the $H^+$ and biogenic amines and to facilitate the membrane transportation of cations by increasing the permeability for these ions [5–9]. Circular dichroism (CD) [5,6] and nuclear magnetic resonance (NMR) [9–15] studies have shown that the ionophore exists as a monomer in polar solvents [11] and aggregates into a dimer in non-polar solvents [12]. It forms neutral as well as charged complexes [2,5] both in monomeric and dimeric forms with different cations depending on the solvent, the bound cation and the deprotonation at the carboxylate group of the ionophore. Monomeric and dimeric forms of the ionophore and its complexes exist in the solid state [16–18]. While complexes of X-537A with a variety of monovalent and divalent metal ions have been reported, no detailed study has been done on the calcium complex of the ionophore. We now report on the CD spectroscopic studies on the calcium complex of X-537A in acetonitrile, and suggest a role of non-stoichiometric charged complexes in its calcium transporting behaviour.

## 2. MATERIALS AND METHODS

Sodium salt of X-537A and calcium perchlorate ($Ca(ClO_4)_2 \cdot 6\,H_2O$) were obtained from Sigma Chemical Co. Salt-free X-537A was obtained by extracting it from a benzene solution of the sodium

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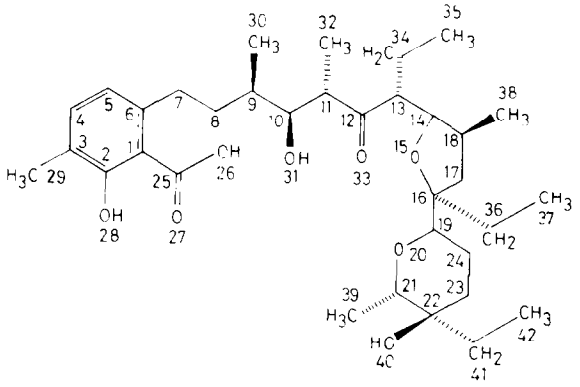

Fig.1. Structure and numbering scheme of X-537A.

salt, through repeated treatment with 0.1 N sulfuric acid [6]. (The extracted X-537A gave identical proton NMR spectra in deuterated chloroform as reported for free X-537A in the same solvent [13].) Freshly prepared solutions of the recrystallised and vacuum dried X-537A in Analar grade dry acetonitrile were used for the CD studies. Perfectly dry calcium perchlorate was added to the acetonitrile solution containing known amounts of free X-537A so that a stock solution containing the ionophore and $Ca^{2+}$ at a stoichiometry of 1:15 was obtained. Solutions of intermediate stoichiometries were obtained by mixing this in appropriate quantities with free X-537A solution of exactly equal ionophore concentration. The CD spectra were recorded on Jasco J-20A spectropolarimeter using optical pathlengths of 0.1–1 mm. All the CD spectral data reported are for neutral pH solution, unless otherwise stated, and are the average of 2–3 obs. UV spectra were recorded on Shimadzu-UV-210A spectrometer using curvettes of 1 cm pathlength and solutions of 0.15 mM ionophore.

## 3. RESULTS

CD spectra of free X-537A and of solutions containing ionophore and calcium in different molar concentration ratios are shown in fig.2. As seen in the figure, the CD curve for free X-537A has an intense negative band around 295 nm ($[\theta]_{295} \simeq -9300$ deg.cm$^2$.dmol$^{-1}$) and a weaker, also negative, band around 245 nm ($[\theta]_{245} \simeq -3500$ deg.dmol$^{-1}$). On gradual addition of the

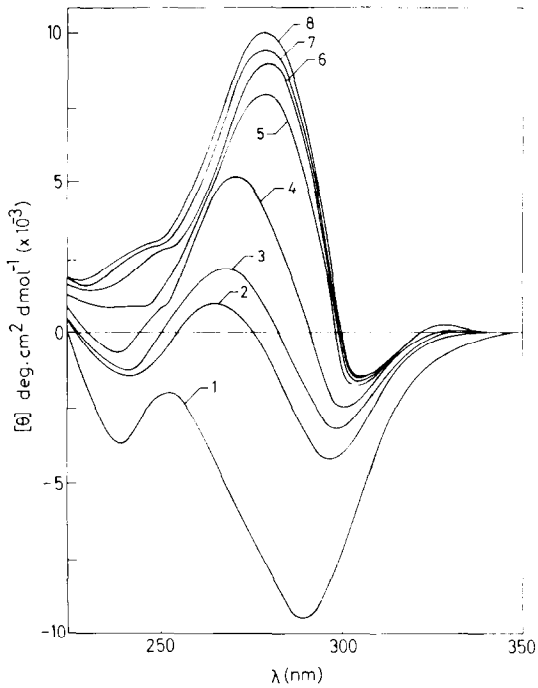


Fig.2. CD spectra of X-537A–$Ca^{2+}$ in acetonitrile at various molar concentration ratios of ionophore to salt: [X-537A] = 17.59 mM; [$Ca^{2+}$]/[X-537A] = (1) 0; (2) 0.1; (3) 0.2; (4) 0.3; (5) 0.4; (6) 0.63; (7) 1.3; (8) 2.8.

stock solution to the free X-537A solution, the CD band centered around 295 nm at first diminishes in intensity but soon separates into two bands, one of a small negative molar ellipticity around 304 nm and the other around 284 nm, the intensity of which continuously changes, becomes zero and then gradually becomes positive. The $[\theta]_{max} \simeq$ 10100 deg.cm$^2$.dmol$^{-1}$ is reached at $\lambda$ = 284 nm for a ratio 1:1.5 (X-537A:$Ca^{2+}$) beyond which no CD spectral changes are observed. It is clear that addition of $Ca^{2+}$ drastically changes the 284 nm band, while the changes in 304 nm and 245 nm bands are rather gradual. In fact, the weak 245 nm band for free also changes in sign and becomes a weak positive CD band appearing as a broad shoulder on the positive CD band around 284 nm. The CD titration graph showing the variation in molar ellipticity at 284 nm with respect to that of free at the same wavelength, $\Delta[\theta]_{284}$ *vs* [$Ca^{2+}$]/[X-537A] is shown in fig.3. Also shown are the variations of the resolved components (section 4) of total apparent $\Delta[\theta]_{284}$, pertaining to the contributions from the component species of com-

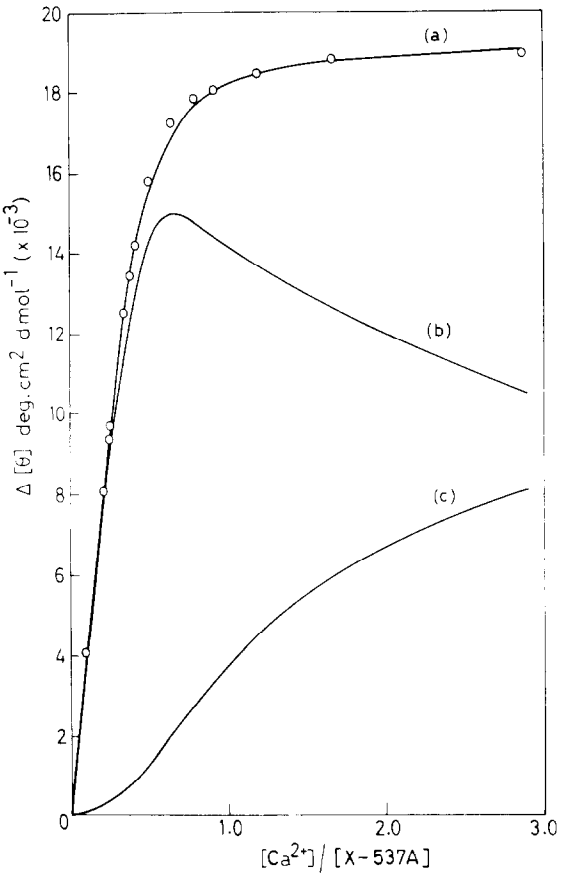


Fig.3. CD titration graph of X-537A–$Ca^{2+}$ in acetonitrile; (a) 4 parameter fit curve (——); experimental points (○—○). Computed contributions from the individual species: 2:1 complex (b) and 1:1 complex (c).

plexes in co-existence. The UV absorption spectra of X-537A at pH 7 and pH 4 (controlled by 1 mM $HClO_4$) as well as those of complex with calcium (1:15) all are near identical and show two absorption peaks: 313 nm ($\epsilon$ = 8200) and 247 nm ($\epsilon$ = 5000).

## 4. DISCUSSION

The UV absorption spectrum for X-537A in acetonitrile resembles that for a species of the type XH (undissociated X-537A) in methanol [5,6]. Observed CD bands for free or the complexed X-537A are accountable in terms of asymmetry in the molecular environment of the two chromophores, viz., the ketonic ($^{12}C=^{33}O$) group and salicylic acid carboxyl group. The CD band at 295 nm has got contribution for molar ellipticity from both the chromophoric groups while that at 245 nm has from only the salicylic acid group [5,6]. The larger negative band at 295 nm and a smaller negative band at 245 nm for free X-537A, indicate the lesser involvement of the salicylic group, in this solvent, in defining the secondary structure of the molecule. Thus, apart from the stronger ($^{27}O\ldots H^{28}O$) hydrogen bond common in salicylic acid moieties, we can expect only a weaker ($H^{26}O\ldots H^{31}O$) hydrogen bond. The other possible hydrogen bond, viz., ($^{27}O\ldots H^{40}O$), which puts a heavier constraint on the salicylic acid group leading to a large ellipticity for 245 nm band also, possibly does not exist as indicated by a very small ellipticity value for this band. Hence, in the notation of [5], conformation II (> I in propensity) is the most preferred one for X-537A in the medium polar, less basic acetonitrile solvent. Also, the CD spectral nature is in agreement with XH form X-537A consistent with UV spectral observation (see above). The reversal of the sign and large changes in the ellipticities of 284 nm band assignable to the ketonic group indicate that ion interacts rather strongly with the ligand $^{12}C=^{33}O$, possibly changing the orientation of it to a large extent to bind to the cation, thus affecting the ketonic optical activity considerably. With gradual salt addition, the changes observed in the CD band (which finally stabilized in complex at 304 nm) are only apparent as the overlapping CD band of the ketone moiety (stabilized in complex at 284 nm) gets only gradually separated leaving behind the CD band of the salicylic acid moiety. Another component band of the salicylic acid group with a small positive ellipticity, centered at 245 nm in the complex, also gives an indication of the possible undissociated XH form for the complex also, so far as the ionophore molecule is concerned (the small positive molar ellipticity values around 245 nm are generally associated with XH form [5]). Also, it is interesting to note that, from conductivity measurements across phospholipid membranes, it has been observed that X-537A transports $Ca^{2+}$ as a charged complex [19]. The weak band at 304 nm, practically unaffected during the salt addition, is indicative that complexation with $Ca^{2+}$ does not affect the salicylic acid

ring. However, the change of 245 nm band to small positive molar ellipticity gives an indication that the conformation of the ionophore in the complex could possibly change such that the salicylic acid ring is more flexible; i.e., it goes from conformation II to I [5].

A Scatchard plot [20] of the changes in apparent $\Delta[\theta]$ over the concentration range studied showed a non-linearity indicating that the complex is not of a simple 2:1 (X-537A:$Ca^{2+}$) stoichiometry. Analysis as in [21] also shows a non-simple binding. The observation that, although $\Delta[\theta]$ showed a sharp variation until around 1:0.5 (X-537A:$Ca^{2+}$) the stabilization occurred only at 1:1.5, also indicated the co-existence of other species. A four-parameter ($k_1$, $k_2$, $\Delta[\theta]_1$ and $\Delta[\theta]_2$)* computer fit [22] was tried which gave as indication of the co-existence of both 1:1 and 2:1 (X-537A:$Ca^{2+}$) complexes. From the best-fit of the curve, which was achieved for $k_1$ = 2.1 mM, $k_2$ = 0.045 mM, $\Delta[\theta]_1$ = 20000 deg.cm$^2$.dmol$^{-1}$ and $\Delta[\theta]_2$ = 19000 deg.cm$^2$.dmol$^{-1}$ (RMS error in $\Delta[\theta]$ is just 1800 deg.cm$^2$.dmol$^{-1}$), the apparent overall stability constants for the two species were obtained: $k_{1:1}$ = 4.8 × 10$^2$ mol$^{-1}$ and $k_{2:1}$ = 2.2 × 10$^4$ mol$^{-1}$. Fig.3 shows, as stated in results, the two component curves for the two species mentioned. The 2:1 charged complex is preferred at low [salt] when X-537A is comparatively in excess while for higher [salt], even 1:1 charged complex forms in comparable amounts (fig.3).

It is possible to propose models for the X-537A–$Ca^{2+}$ complexes based on the above observation and the conformational features observed in free and in various complexes of X-537A [11–18]. The model constructed, consistent with the above features and that carbonyl orientation changes by a very large amount (with its oxygen being drawn much closer to $Ca^{2+}$) in the formation of the complex (as against the smaller changes observed for other metal ion complexes), reveals that $H^{31}O$ is brought closer to $H^{40}O$ for a possible intramolecular hydrogen bond ($H^{31}O \ldots H^{40}O$), thus giving a pseudo-cyclic appearance to the molecule and a far rigid coordinating zone around $Ca^{2+}$. In 2:1 complex the overall conformations of the two individual ionophore molecules complexing with a single cation may be the same, the $Ca^{2+}$ having an identical coordination with $^{33}O$, $^{15}O$ and $^{20}O$ from each of the molecules. The complex as a whole may have a wide range of conformations varying from one in which the coordinating zones of the two individual ionophores overlap while their salicylic groups are placed farthest apart, in which the salicylic group of one molecule lies over that of the other. However, these conformations are indistinguishable from the CD data alone. In a 1:1 complex, we may expect very little change in the overall conformation as compared to that in 2:1 complex, as only small changes are observed in the concentration ranges where 1:1 also exists in substantial amounts. In this case, additional coordinations for the $Ca^{2+}$ may be provided by the solvent molecules and/or the anion ($ClO_4^-$). Fig.4 gives the schematic model depicting the conformation of 1:1 complex.

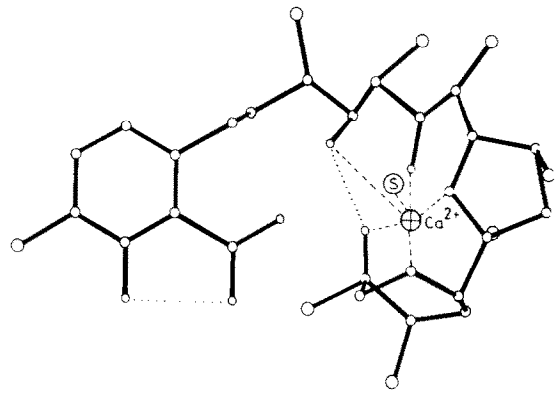


Fig.4. Schematic diagram showing the proposed model for 1:1 complex of X-537A with $Ca^{2+}$.

This work shows non-stoichiometric complexes, such as 2:1 (sandwich), for the ionophore X-537A in its calcium complex. These could be intermediates during ionophore-mediated transport across biological membranes.

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* $k_1$, $k_2$ are the apparent dissociation constants of reactions:

$$XH + Ca^{2+} \rightleftharpoons XH.Ca^{2+}$$

$$2XH + Ca^{2+} \rightleftharpoons (XH)_2 \cdot Ca^{2+}$$

and $\Delta[\theta]_1$, $\Delta[\theta]_2$ are the limiting molar ellipticity values of these two individual complex species, respectively

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