Fluorimetric Determination of Complexation Constants for the Ionophore Antibiotic X-537A with Biogenic Amines

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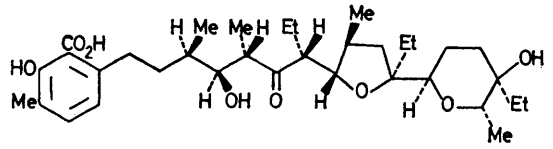
Summary The ionophore antibiotic X-537A (Lasalocid) is shown to bind strongly to biogenic amines in inert hydrocarbon solvents, but not in polar solvents such as methanol, in contrast to the complexation of metal ions which has been shown to occur in methanol and other polar solvent mixtures.

THE carboxylic acid ionophore antibiotic, X-537A, has been shown to transport metal ions and catecholamines directly across vesicle membranes.[1–3] Complexing constants for metal ions with X-537A in methanol and hexane have been measured.[4–6] Binding constants for norepinephrine and epinephrine with X-537A (in the solvent mixture 70% toluene–30% n-butanol) have been reported as 163 and 9·8,[1] respectively.

C.d. data have shown[5] that the linear open-chain conformation of X-537A predominates in polar solvents such as methanol. In nonpolar solvents, *e.g.* hexane, the



X-537A

cyclic conformation predominates in which the surface or exposed parts of the molecule are hydrophobic and all the hydrogen-bonding units are on the inside of the circular structure. This ring conformation is the structure assumed by the crystalline barium salt,[7] and is presumably also the structure of the ionophore–metal ion[8] and ionophore–catecholamine complex in solution. These results suggest that the cyclic conformation is stabilized either under the influence of a strongly complexing species or in a hydrophobic solvent environment. We have now determined the conditions required for the complexation of several biogenic amines, *i.e.* norepinephrine, epinephrine, dopamine, amphetamine, and phenylephrine, with the ionophore X-537A.

To this end, complexing constants were measured in pure methanol, octan-2-ol and iso-octane. Norepinephrine–ionophore complexation was also studied in octan-2-ol–iso-octane mixtures. Complexing constants were evaluated from measurements of the quenching of the native fluorescence of the sodium salt of X-537A at 37 °C on the addition of varying quantities of amine hydrochloride in methanol solution. Fluorescence intensity measurements were made at excitation and emission wavelengths of 310 and 420 nm, respectively.

There was no perceptable quenching of the fluorescence of the ionophore in methanol with any of the amines used in this study; the complexation of these amines by X-537A in methanol was, therefore, assumed to be negligible.

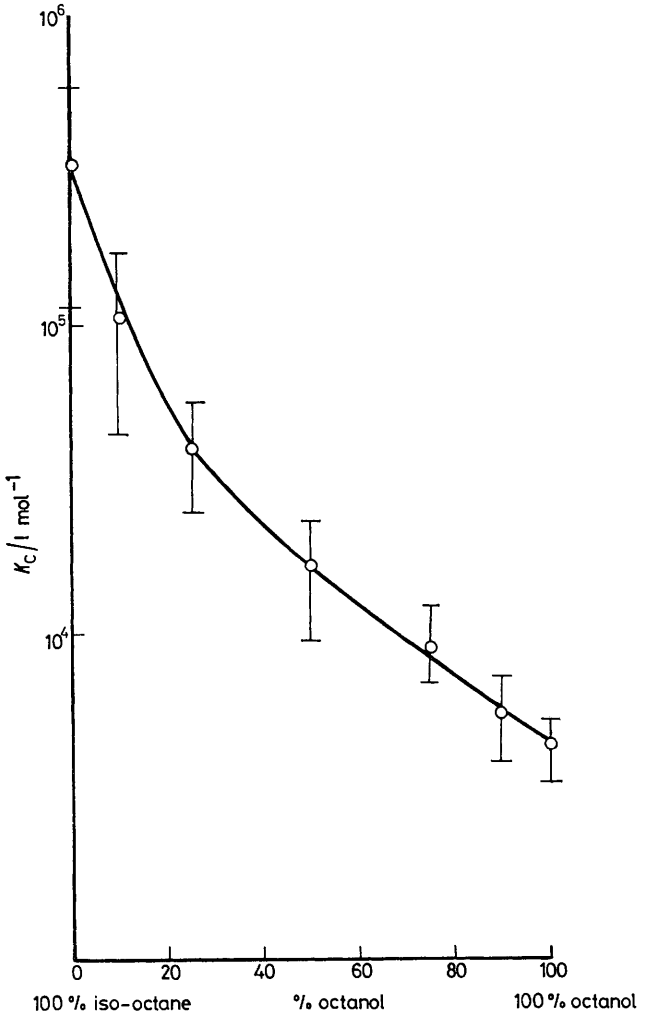


FIGURE. Complex formation constants, K_c, for norepinephrine–X-537A at 37 °C as a function of % octanol (v/v) in iso-octane solution. All solutions were adjusted to contain 1% methanol by volume.

For epinephrine and norepinephrine, binding is seen to be a strong function of solvent polarity (Table). Complexation constants of $1{\cdot}6 \times 10^5$ and $3{\cdot}4 \times 10^5$, respectively in iso-octane decrease by almost two orders of magnitude in octan-2-ol. The Figure shows that for norepinephrine the complexation constant decreases smoothly as the solvent polarity is increased by the addition of octan-2-ol to iso-octane. The decrease in complexation for amphetamine (Table) in octan-2-ol is less dramatic. If it is assumed that solvent hydrogen bonding to the biogenic amine reduces the extent of complexation, the decreased effect of the solvent polarity in the case of amphetamine is to be expected

TABLE. Complex formation constants (l mol^{-1}) for ionophore X-537A with biogenic amines.[a]

Amine	Iso-octane	Octan-2-ol
Epinephrine ..	$(1{\cdot}6 \pm 0{\cdot}8) \times 10^5$	$(6{\cdot}1 \pm 1{\cdot}3) \times 10^3$
Norepinephrine ..	$(3{\cdot}4 \pm 2{\cdot}2) \times 10^5$	$(4{\cdot}7 \pm 1{\cdot}0) \times 10^3$
Amphetamine ..	$(2{\cdot}1 \pm 1{\cdot}1) \times 10^5$	$(3{\cdot}6 \pm 1{\cdot}8) \times 10^4$
Phenylephrine ..	$(6{\cdot}2 \pm 1{\cdot}6) \times 10^3$	$(2{\cdot}7 \pm 1{\cdot}5) \times 10^3$
Dopamine	$(1{\cdot}1 \pm 0{\cdot}3) \times 10^4$	$(2{\cdot}2 \pm 1{\cdot}5) \times 10^3$

[a] Each measurement is the average of at least 10 determinations. The iso-octane and octan-2-ol solutions were adjusted to contain 1% methanol by volume. The ionophore and amine were added by micro syringe to the solvent in the fluorimeter cuvette as methanolic solutions. No perceptible complexation occurs in methanol.

since amphetamine has no hydroxy-groups to interact with the solvent. Dopamine is also less influenced by changes in solvent polarity than epinephrine and norepinephrine, since it, too, has less hydrogen bonding capabilities. No explanation is offered now for the considerably weaker complexation of phenylephrine as compared to epinephrine and norepinephrine. The complexation of phenylephrine is almost two orders of magnitude weaker in iso-octane and changes very little in octan-2-ol. A complete understanding of these phenomena must await further investigation. However, for each of the amines studied, strong complexation occurs in iso-octane and no perceptible complexation occurs in methanol.

These results support the proposed mechanism for the transfer of biogenic amines through lipid (nonpolar) membranes. The amine–ionophore complex is formed at the lipid membrane–aqueous solution interface; the complex remains stable within the nonpolar lipid phase allowing the amine to be transported through the membrane and dissociates on contact with a polar hydrogen bonding medium. This work thus provides *in vitro* support for the theoretical model for lipid membrane transport of biogenic amines.

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