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# BINDING OF ORGANIC ANIONS TO A MACROCYCLIC ALKALOID *d*-TUBOCURARINE

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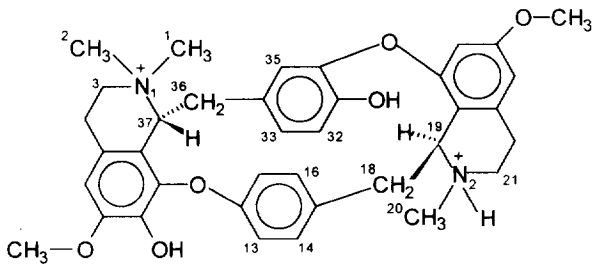
**Abstract:** An alkaloid *d*-tubocurarine in aqueous solution binds 8-anilino-1-naphthalenesulfonate and anions of substituted benzoic, aliphatic dicarboxylic, and N-acetyl-α-amino acids. The binding constants vary from ca. 50 to 3300 $M^{-1}$ depending on the anion structure, charge and hydrophobicity. The binding of N-acetyl-α-amino acids is enantiospecific.

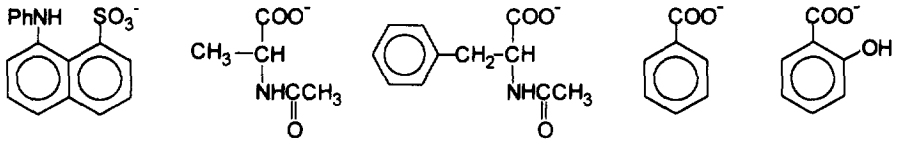
*d*-Tubocurarine, a member of the bisbenzylisoquinoline family of alkaloids,[1] finds an application in medicine as a muscle relaxant due to its ability to block the acetylcholine receptor.[2] An interesting feature of the structure of this alkaloid is its macrocyclic nature, which has no apparent relation to its biological function. The structure of *d*-tubocurarine is remarkably similar to that of cyclophanes, a group of synthetic macrocycles which attract at present much attention as host molecules.[3,4] In view of this structural similarity one can expect *d*-tubocurarine, and other alkaloids of this family, to be capable of the non-covalent binding of various guest molecules, including drugs and important metabolites.




**The chemical structure of dication of *d*-tubocurarine**

Such a property of *d*-tubocurarine can be of interest for biomedical studies with this alkaloid. It is of interest also in the study of molecular recognition by cyclophane-type hosts because *d*-tubocurarine possesses several structural characteristics, such as chiral centers and unsimmetrical distribution of substituents, hardly accessible by synthetic routes. The conformation of *d*-tubocurarine macrocycle is

fairly flexible. It becomes more open on passing from dichloride[5a] to dibromide[5b] salts in the solid state and changes from an elongated shape to a "cup"-shape when the alkaloid binds itself to the acetylcholine receptor in an aqueous solution.[6] The structural study of the complexation of various guests with *d*-tubocurarine will be the matter of further research. It is the purpose of this communication to test the ability of *d*-tubocurarine to bind some organic anions specifically. The structures of anions chosen for this study are shown in Chart 1.



**1** 3300±250 $M^{-1}$



**2** D-**2**: 80±20 $M^{-1}$ L-**2**: <20 $M^{-1}$



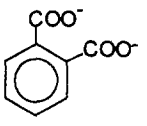
**3** D-**3**: 320±70 $M^{-1}$ L-**3**: <20$M^{-1}$



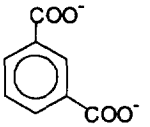
**4** 100±20 $M^{-1}$



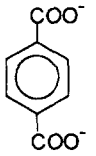
**5** 90±2 $M^{-1}$



**6** 59±6 $M^{-1}$

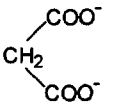


**7** 200±30 $M^{-1}$

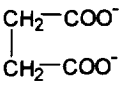


**8** 780±80 $M^{-1}$

**9** 88±2 $M^{-1}$



**10** 256±2 $M^{-1}$



**11** 180±3 $M^{-1}$

**Chart 1**. Chemical structures and binding constants of anions tested as the guests for *d*-tubocurarine.

The binding of anions **1**-**11** to *d*-tubocucarine ((+)-tubocurarine chloride pentahydrate, Aldrich) was followed by fluorometry (a FluoroMax SPEX spectrofluorometer), conductometry (an YSI-31 conductance meter) or $^1$H NMR titration (a GN-500 NMR spectrometer). All solutions were prepared in purified (Milli-Q Reagent Water System) water or in $D_2O$ for NMR studies. Conductance was measured in pure water and fluorescence in 0.03 M phosphate buffer solution pH 6.0. Under these conditions *d*-tubocurarine exists in the dicationic protonated form because its first $pK_a$ equals[7] 7.6.

The addition of *d*-tubocurarine enhances and shifts to shorter wavelengths the fluorescence maximum of **1** which is indicative of the binding of **1** to a hydrophobic region of the macrocycle.[8] Fig. 1 shows the change in the fluorescence intensity ($\Delta I$) at the fixed emission wavelength as a function of *d*-tubocurarine concentration. The fitting[9] of this and other similar plots obtained in the wavelength range

500-530 nm to equation (1)

$$\Delta I = \Delta I_{\infty} K [d\text{-tubocurarine}] / (1 + K [d\text{-tubocurarine}]) \quad (1)$$

where $\Delta I_{\infty}$ is the difference in the fluorescence intensity of free and bound anion **1** and K is the binding constant, allowed us to calculate the value of K given in Chart 1. Equation (1) is correct under the conditions [*d*-tubocurarine] >> [**1**] used in this experiment.

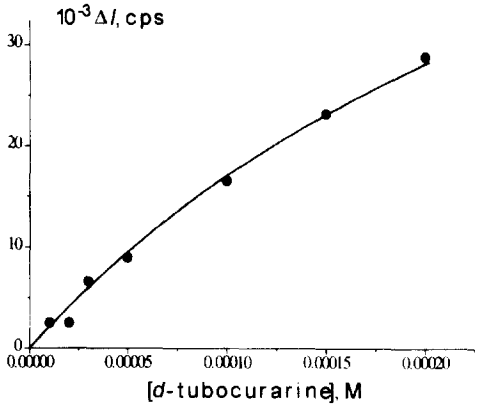



**Figure 1**. The change of fluorescence intensity of 2 μM **1** at 515 nm vs. *d*-tubocurarine concentration. The curve is the theoretical profile calculated from eq. (1) with K=3300 $M^{-1}$ and $\Delta I_{\infty}$=70500 cps

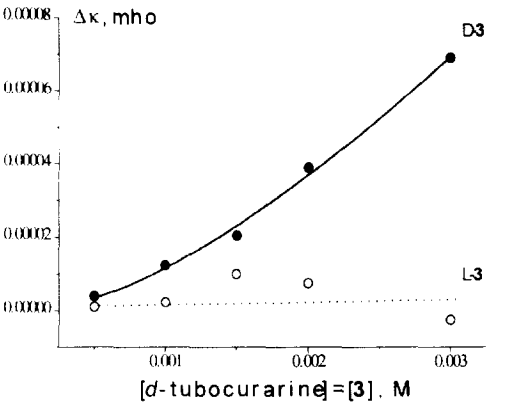



**Figure 2**. Conductances of the mixtures of *d*-tubocurarine with enantiomers of **3** in coordinates of eq. (2). The solid curve is the theoretical profile calculated from eq. (2) with K=320 $M^{-1}$.

The binding of anions **2-11** was studied by conductometry by using a modified procedure of Ref. 10. Conductances (κ) of sodium salts of carboxylate anions **2-11** and *d*-tubocurarine chloride were measured in interval of their concentrations 0-0.003 M in water. In all cases the molar conductivity (Λ) was a linear function of the square root of the ionic strength (μ) in accordance with the limited form of the Onsager equation.[11] The molar conductivity of *d*-tubocurarine chloride at the infinite dilution ($\Lambda_O$) equaled 140 $\Omega^{-1}cm^{-1}$. Values of $\Lambda_O$ for monocarboxylates were in the range 65 - 80 $\Omega^{-1}cm^{-1}$ and those for dicarboxylates, in the range 140-170 $\Omega^{-1}cm^{-1}$. These values of $\Lambda_O$ are very close to those for chemically related ammonium and carboxylic acid salts.[10,11] Then the conductances of 1:1 mixtures of *d*-tubocurarine chloride and sodium salts of the anions were measured in the same concentration interval, and the differences between calculated and observed conductances of these mixtures (Δκ) were plotted vs. *d*-tubocurarine total concentration and fitted to the theoretical equation (2)

$$\Delta\kappa = \Delta\Lambda_O([d\text{-tubocurarine}]_t + 0.5/K - ([d\text{-tubocurarine}]_t/K + 0.25/K^2)^{1/2}) + f(\mu) \quad (2)$$

where K is the binding constant, $f(\mu)$ is a correction factor for the ionic strength effect, and $\Delta\Lambda_O$ is the

sum of molar conductivities of *d*-tubocurarine cation and a carboxylate anion at the infinite dilution from which the molar conductivity of the complex at the infinite dilution is subtracted. Fig. 2 shows typical $\Delta\kappa$ vs. *d*-tubocurarine concentration profiles which demonstrate a relatively tight binding of D-**3** and the absence of any binding of L-**3** in the given concentration interval. The fitting[9] of these and other similar results to eq. (2) allowed us to calculate the binding constants of guests **2, 3,** and **6-11**, Chart 1. The results with anions **4** and **5** were poorly reproducible. The binding constants of these guests were calculated from NMR titration data, see below.

All binding constants found in this study are presented in Chart 1. In general, binding constants of **1-11** to *d*-tubocurarine lie within the limits of published values of binding constants of these guests to synthetic cyclophanes under similar conditions.[12] The binding of N-acetyl-α-amino acids is enantioselective with the D/L discrimination factor of ca. 10, as is seen in Chart 1. It is worth noting that the development of enantioselective receptors for amino acids and their derivatives attracts much attention at present.[13]

A comparison of the binding constants for D-**3** and D-**2** shows that the phenyl group of D-**3** contributes to the binding probably by means of hydrophobic interactions with aromatic groups of *d*-tubocurarine macrocycle. These interactions should be responsible also for the tight binding of anion **1**.

Another important type of intermolecular interactions between anions and *d*-tubocurarine is the electrostatic attraction. The monoanions are expected to form only one salt bridge with one of the ammonium centers of *d*-tubocurarine because the distance $N_1$-$N_2$ (9 Å)[6] is too large to allow the contact of $COO^-$ group of an anion with both $N^+$ groups of the host. Such interaction by itself should be very weak. Indeed, we did not detect any association of *d*-tubocurarine with simple monoanions like $Cl^-$ or $CH_3COO^-$ by any of the methods used in this study. Therefore the binding of aromatic monoanions **4** and **5** like that of **1** and **3** should involve a hydrophobic contribution from their phenyl rings. Anion **2**, which lacks the phenyl group, also forms a more stable complex than is expected for a simple monoanion. In this case an additional interaction can result from a hydrophobic binding of the methyl group.

The phthalate anions **6-8** are expected to form considerably more stable complexes than benzoate. This is the case, however, only for anion **8**, in which the carboxyl groups are separated by the distance $l$=5.8 Å. *Ortho*-phthalate ($l$=3.0 Å) forms an even less stable complex than benzoate and the *meta*- isomer ($l$=5.0 Å) possesses the binding constant only two times higher than that of benzoate (the Coulomb contribution of a single salt bridge to the binding free energy was evaluated as -5 kJ/mol, which corresponds to a factor of ca. 7 in the binding constant[14]). Clearly, the complementarity between positive

charges of *d*-tubocurarine and negative charges of the guest is important for the binding. Fraenkel, et al.[6] showed that the distance $N_1$-$N_2$ in *d*-tubocurarine decreases from 9 Å in the free molecule to 6 Å in its complex with a nicotinic acetylcholine receptor due to neutralization of the positive charges by the anionic binding site of the receptor. A similar shortening of the distance between the cationic centers of the host can be induced by anionic guests, and from this point of view the tight binding of anion **8** can result from an induced complementarity of the guest carboxyl groups to ammonium groups of the host.

The binding constants of anions **9-11** are typical of purely ionic association between a dianion and a dication. For example, the binding constants of anions **9** and **11** to $^{+}H_3N(CH_2)_3NH_3^{+}$ equal 151 and 130 $M^{-1}$ respectively.[10]

A detailed NMR study of the binding of anions **1-11** to *d*-tubocurarine in order to determine the geometry of the complexes in solution is in progress. Preliminary results show that bound anions are in contact with hydrogen atoms located in distant sites of *d*-tubocurarine molecule. Thus, the addition of **4** induced strong high-field shifts (0.5-0.6 ppm) of the signals, assigned as in ref. 6, of H37, H16 and H18a, and smaller shifts of H1-H3, H21, H33, H35 and H36. Similar effects were observed with **3** and **5**. In all cases the signals of all aromatic protons of the anions underwent high-field shifts.

In order to better envision possible complexation modes, we performed docking calculations[15] of the complex with benzoate anion **4**. Docking of the benzoate ring into the cavity lead to extremely high strain energy of the system (up to 100 kcal/mol) after initial energy minimization. Further minimization lead to the aromatic ring being extruded from the TB macrocycle. At the same time, the NMR results do indicate the interaction between hydrophobic parts of the host and the guest. Based on the complexation-induced shifts[16] of the signals in $^1$H NMR spectra of *d*-tubocurarine we have simulated a possible complex structure between **4** and *d*-tubocurarine. In this structure an electrostatic binding is provided by the formation of one salt bridge between the quaternary nitrogen of *d*-tubocurarine, which causes a shift of the neighboring H37 signal. The guest aromatic moiety is partially extracted from water by hydrophobic/ Van der Waals interactions with *d*-tubocurarine and probably becomes located near the H16 and H18a protons which experience the highest shielding effects.

The binding constants of **4** and **5** were calculated from the NMR data: spectra of $5\times10^{-4}$ M *d*-tubocurarine in $D_2O$ were recorded in the presence of increased concentrations (0-0.005 M) of **4** or **5** and the changes of chemical shifts of several protons were fitted to an equation similar to eq. (1), where [*d*-tubocurarine] was substituted for [anion] and $\Delta I_{\infty}$ for the difference of the chemical shifts ($\Delta\delta$) of the respective protons in free and complexed *d*-tubocurarine.

In conclusion we would like to point out that there are a variety of other macrocyclic natural products, others than widely used cyclodextrins and ionophoric antibiotics, such as macrocyclic alkaloids,[1] macrocyclic bis(bibenzyls)[17] and some antibiotics, e.g., rifamycins,[18] which possess suitable structural characteristics to function as host molecules. We hope that systematic testing of the binding ability of such compounds towards various guests can lead to a descovery of new highly specific recognition systems.

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