Steric and Physicochemical Studies of Decahydroisoquinolines Possessing Antiarrhythmic Activity

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Abstract □ Distribution coefficients and acid dissociation constants were determined for some diastereoisomers of 5-substituted decahydroisoquinolines possessing significant antiarrhythmic activity. It was found that the distribution coefficients of the compounds may be correlated with antiarrhythmic potency. The correlations of steric structure, lipid–water distribution, and antiarrhythmic potency are discussed in an attempt to advance the knowledge concerning the molecular events and requirements in the action of antiarrhythmic drugs.

Keyphrases □ Decahydroisoquinolines, 5-substituted, diastereoisomers—distribution coefficients calculated, correlated with antiarrhythmic activity, acid dissociation constants determined □ Antiarrhythmic agents, decahydroisoquinolines—steric and physicochemical studies, structure–activity relationships □ Structure–activity relationships—diastereoisomers of 5-substituted decahydroisoquinolines □ Distribution coefficients—5-substituted decahydroisoquinolines, correlated with antiarrhythmic activity

The established interest of these laboratories in structure–activity relationships of antiarrhythmic drugs has more recently developed into physicochemical studies of active compounds in an attempt to elucidate molecular parameters in the action of antiarrhythmic agents. In earlier publications (1–3), it was reported that both stereoisomeric amido and ester derivatives of decahydroisoquinolines possess significant antiarrhythmic properties and the presence of a stereospecific receptor was suggested as a result of the superior activity of the *trans*-ring junction compounds as opposed to the *cis*-conformational isomers. Antiarrhythmic studies were carried out in intact animals (1, 2) and in isolated atrial preparations (3); in both instances, significant antiarrhythmic activity was noted as well as the implication of a stereospecific receptor.

It was, therefore, of great interest from a molecular viewpoint to investigate whether the differences observed between the stereoisomers were indeed a result of the presence of a stereoselective receptor or due to some physicochemical property of the individual isomers. Both the distribution (partition) coefficients and the dissociation constants of the stereoisomeric pairs of compounds reported by Mathison *et al.* (1) were determined; the results are discussed with the view to shedding some light on the molecular requirements and/or molecular events during the action of this class of antiarrhythmic agents.

EXPERIMENTAL

The following analytically pure compounds were prepared according to procedures outlined by Mathison *et al.* (1, 4): *cis*-5,9,10-*H*,5-(3,4,5-trimethoxybenzoyloxy)-2-methyldecahydroisoquinoline (M-29), *trans*-9,10-*trans*-5*H*,5-(3,4,5-trimethoxybenzoyloxy)-2-methyldecahydroisoquinoline (M-31), *cis*-5,9,10*H*,5-(3,4,5-trimethoxybenzamido)-2-methyldecahydroisoquinoline (M-30), and *trans*-9,10-*trans*-5*H*,5-(3,4,5-trimethoxybenzamido)-2-methyldecahydroisoquinoline (M-32).

Table I—Physical Properties of New Antiarrhythmic Drugs

Compound Number	Pc	pKa
M-29	2.98 ± 0.17	9.03 ± 0.04[a]
M-31	3.83 ± 0.03	8.98 ± 0.03[b]
M-30	0.23 ± 0.04	9.19 ± 0.04[a]
M-32	0.50 ± 0.01	9.19 ± 0.04[a]

[a] Ethanol added to aid dissolution. [b] Methanol added to aid dissolution.

Determination of Partition Coefficient (Pc)—*Preparation of System*—In all cases, partitioning was carried out in an octanol–water system at 26.2 ± 0.3°. It was necessary, therefore, to prepare water saturated with octanol and octanol saturated with water for the system.

The octanol was successively washed with equal volumes of 0.1 *N* sulfuric acid, 0.1 *N* sodium hydroxide, and distilled water. The washed octanol was distilled and then allowed to equilibrate with an equal volume of distilled water.

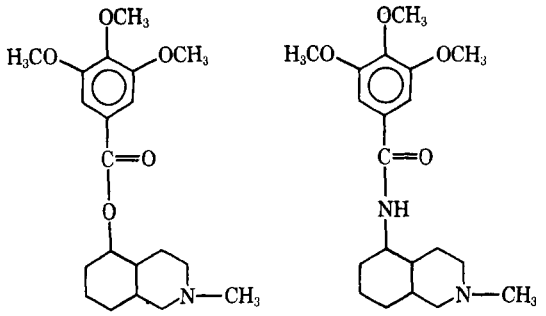
Dissolution, Preparation of Beer's Law Plot, and Partitioning—Approximately 10–15 mg. of compound was dissolved in 250 ml. of water-saturated octanol. The more difficultly soluble compounds were aided by long periods of vigorous shaking. From this stock solution, a sample was taken and the UV spectrum was determined using a UV visible spectrophotometer[1]. Various dilutions of the stock solution were made, and their absorbances were measured on a spectrophotometer[2] at a wavelength of maximum absorption ascertained previously[3] (214 nm. for Compounds M-30 and M-32; 215 nm. for Compounds M-29 and M-31); the absorbance values were used to establish a Beer's law plot for each compound.

Partitioning was then accomplished by shaking 10 ml. of stock solution with 50 ml. of octanol-saturated water for 2 hr. in a constant-temperature bath. The partitioning flasks were then allowed to stand for 1 hr., after which the octanol layer was drawn off and centrifuged to remove excess water. Each partitioning experiment consisted of five flasks containing compound and one blank flask (without compound). Duplicate runs were made for each compound. A second determination of the partition coefficient was made at a different octanol–water ratio, using 5 ml. of stock solution and 50 ml. of octanol-saturated water.

The absorbance of the octanol layer, after centrifuging, was redetermined on the spectrophotometer[2]. The concentration (in moles per liter) of compound remaining in the octanol layer following partition could thus be calculated from the Beer's law plot for the compound. The amount of compound partitioned into the water was determined (by subtraction) and, taking into account the difference between the partitioning volumes of water and octanol used, the partition coefficient was calculated as the ratio of the molarity of compound remaining in the octanol to that of the molarity of compound partitioned into the water layer. The values shown in Table I are the averages of the determinations made at both solvent volume ratios.

Determination of Acid Dissociation Constant (pKa)—A 1×10^{-3} *M* aqueous solution of the respective compound was pre-

[1] Perkin-Elmer 202.
[2] Gilford 240.
[3] On the Perkin-Elmer spectrophotometer.



M-29 M-30
M-31 M-32

pared. In the cases where the compounds were difficultly soluble, minimal quantities (4%) of methanol or ethanol were added to promote dissolution. These solutions of the organic bases were titrated with 1×10^{-2} *M* HCl using an automatic titrigraph[4]. Six titrations were carried out for each compound. The acid dissociation (pKa) constant was calculated from the expression:

$$\text{pKa} = \text{pH at } \tfrac{1}{2} \text{ neutralization point} \qquad \text{(Eq. 1)}$$

The neutralization point was determined from the titration curve by the standard graphical method.

RESULTS

The data obtained from the experimental procedures are summarized in Table I. In all cases, a maximum of 4% solvent was used to solubilize the compound in water prior to titration with the acid.

DISCUSSION

As already indicated, 5-substituted decahydroisoquinolines (possessing the structural formulas shown here) were previously demonstrated to possess significant antiarrhythmic properties (1). In both the amides and esters, it was shown that the *trans*-ring junction compounds possessed superior activity to the *cis*-analogs, and it was apparent that the amido derivatives were less toxic and less potent and possessed higher therapeutic indexes compared to the esters. Since the functional groupings at position 5 of the ring system were in the equatorial conformation in all cases, structure–activity correlations with regard to steric features may be related to the differences in stereochemistry about the ring junction (*i.e.*, *cis* or *trans*).

As anticipated, the pKa values (Table I) for the pairs of isomers (*i.e.*, M-29 and M-31, and M-30 and M-32) do not differ significantly from each other. The possibility, therefore, of preferential ionization of one isomer at the site of antiarrhythmic action is not apparent since equal degrees of ionization would occur as a result of the similarity in pKa values. Thus, this factor is eliminated from consideration as a possible explanation for the differences in activity between isomers. Examination of the partition data, however, is more revealing (Table I). Of the ester compounds, M-29 and M-31, it is clear that the *trans*-isomer (M-31) possesses greater lipophilicity (*i.e.*, distribution favors the nonaqueous phase) than the *cis*-stereoisomer (M-29). By correlating this with biological (antiarrhythmic) activity, it will be recalled that M-31 was a significantly more potent compound and possessed a higher therapeutic index than the *cis*-isomer (1). Examination of the data for the amido derivatives, M-30 and M-32, reveals a similar trend in that the *trans*-isomer possesses greater lipophilic character and is also a more potent antiarrhythmic agent (1).

It must, therefore, be recognized that distribution phenomena may be a contributing factor in the mechanism of action of these agents in addition to steric considerations. The steric differences between the *cis*- and *trans*-isomers can be readily seen from the Dreiding molecular models shown in Fig. 1. It is well demonstrated in this figure that the rigid *trans*-compound possesses a more planar structure than the flexible *cis*-isomer and it is, therefore, difficult not to disregard this feature as opposed to a partition phenomenon alone accounting for the observed differences in antiarrhythmic activity. Precedents for steric phenomena are, of course, not unique to this series of compounds; quinine and quinidine and hydrocuprein and hydrocupreidine (5) demonstrate similar stereoselectivities in so far as antiarrhythmic activity is concerned.

Figure 1—*Dreiding molecular models of the* cis- *and* trans-*5-substituted decahydroisoquinolines. Left:* cis-*isomer. Right:* trans-*isomer. Sphere indicates the location of the 5-substituent.*

Luchi *et al.* (6) noted that the quinoline heteroatom in quinidine appears to play an important role in its antiarrhythmic action, possibly as a result of its ability to bind to the cardiac cell membrane, while the quinuclidine heteroatom is thought to hinder the transport of cations across the membrane as a result of its possible hydration (7) and/or repellency of cations. The present studies with substituted decahydroisoquinolines lend general support to this theory, since the decahydroisoquinoline molecule is capable of either: (*a*) binding at the cardiac cell membrane and thus altering permeability in some manner, or (*b*) preventing ionic transport as a result of the ionization of the tertiary amine *in vivo* (*i.e.*, at physiological pH) followed by hydration of the resulting cation. Since the difference in pKa values between the pairs of isomers is negligible, the possibility that the degree of ionization of the heteroatom is different between the isomers and thus is hindering cationic transport to a greater degree in one of the isomers is ruled out.

Examination of the partition data appears to lend support to the Luchi concept (6) of a binding phenomenon contributing to antiarrhythmic action. Lipophilicity would obviously aid binding of a compound to the cardiac cell membrane. It would, therefore, be anticipated that should the binding be a critical phenomenon in the mechanism of action of antiarrhythmics at a molecular level, then the more lipophilic a compound the better its antiarrhythmic activity. Since the more potent compounds, in both the amide and ester series studied, possessed a lipid-favoring distribution, support is given to the stated hypothesis. The possibility that the ionized heterocyclic nitrogen is involved in binding should not be disregarded. Anionic receptor sites are commonplace in theories of the action of medicinal agents, and the concept that the decahydroisoquinoline nitrogen binds to a protein receptor on the cardiac cell membrane and in some manner alters the permeability of the membrane to cations is a very realistic hypothesis for antiarrhythmic activity.

In addition, steric factors also must be considered. The planar structure of the *trans*-isomers would be anticipated to aid in this binding if more than one point of attachment is required for optimal antiarrhythmic activity. In the studies on these compounds, it has been shown that the benzoyl ester and benzoyl amide linkages were essential for activity in that the corresponding alcohols and acetamides possessed no noteworthy activities (1). It, therefore, appears at this time that in addition to the involvement of the heteroatom, either binding at the cell membrane or as a result of its hydration, a planar aromatic structure is also necessary for activity, a factor borne out by examination of the chemical structures of known antiarrhythmics such as procaine, procainamide, and quinidine. The importance of binding sites other than for the tertiary amine in quinidine was alluded to by Conn (7) and Conn and Luchi (8). Autoradiographic studies suggested that the binding sites appear to be located in the membranes and mitochondria (7) and are thought by some (9) to be involved in ion transfer. The present study seems to support the contention that more than one site is involved in antiarrhythmic action.

In the light of these observations, studies are continuing, utilizing chemical and physicochemical tools, to elucidate some of the molecular requirements and events occurring in the action of antiarrhythmic agents.

[4] Radiometer model SBR2c/ABulc/TT11/PHM28.

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Determination of CMC from Liquid Junction Potential Measurements

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Abstract □ The CMC's of anionic and cationic surfactants were determined from diffusion potential measurements. The methodology used for the determinations is simple, capable of providing accurate results, and requires a minimum of very commonly available apparatus. The data also yield reliable transport number values for the surfactant ions.

Keyphrases □ CMC, surfactants—determined from diffusion potential measurements (liquid junction) □ Surfactants—determination of CMC using diffusion potential measurements (liquid junction) □ Transport numbers—determined using diffusion potential measurements (liquid junction), surfactants □ Diffusion potential measurements, liquid junction—determination of CMC surfactants, transport numbers

Surfactants are an important class of compounds with significant pharmaceutical applications as micellar solubilizing agents, stabilizers, antibacterials, *etc.* (1–6). One of the most important physical properties of a surfactant is its CMC. There are various methods of determining the CMC (7) which involve varying experimental difficulties and equipment requirements. The purpose of the present report is to describe a simple electrometric method for determining CMC values of ionic surfactants which requires a minimum of very commonly available apparatus. The method is demonstrated for two cationic detergents and an anionic detergent. In addition to CMC values, the transport number of the ions can be calculated from the data.

MATERIALS AND METHODS

Materials—Chromatographically pure dodecyltrimethylammonium bromide (I) was purchased[1]. Dodecylamine hydrochloride (II) was prepared from chromatographically pure dodecylamine in absolute ethanol and concentrated reagent grade hydrochloric acid using the procedure of Hutchinson and Winslow (8). Sodium lauryl sulfate (III) was also purchased[2]. Reagent grade potassium chloride was used to determine the conductivity apparatus cell constant. Double-distilled water was used to prepare all solutions.

Liquid Junction Potential Measurements—The liquid junction cell, together with the electrodes, syringe, and 50-ml. beakers for containing the reference and sample solutions, is shown in Fig. 1. The stem and arms of the Y-tube cell are each approximately 10 cm. in length with an inside diameter of 0.8 cm.; a 20-ml. syringe was employed. The electrodes are miniature saturated calomel electrodes (Corning) with fiber junctions. Potentials were recorded on a Sargent SR recorder. The different chart scales were utilized to achieve maximum precision for a given measurement on a given pair of solutions (reference and sample).

Fresh reference and sample solutions were prepared before each run. The surfactant concentration in the reference solutions for all of the surfactants was 0.0010 *M*. Surfactant concentrations in the sample solutions ranged from 0.0020 to 0.050 *M*. Approximately 50 ml. of a reference solution and 50 ml. of a sample solution were placed in the appropriate beakers (Fig. 1); the electrodes were placed into the solutions followed by the insertion of the arms of the Y-tube. The syringe was used to draw the two solutions into the arms of the Y-tube. When the two solutions came in contact to form a liquid junction at the Y-tube intersection, the ensuing liquid junction potential was recorded on the moving chart. Fresh reference solutions were used with each sample solution to minimize contamination effects due to cumulative leakage of potassium chloride through the reference electrode fiber junction. All potentials were corrected for small electrode asymmetry potentials, which were measured for each pair of solutions. The measurements were made at room temperature (25 ± 1°).

Conductometric Method—Conductivity measurements were made with a conductance bridge (Serfass), operating at a frequency of 1000 Hz., used in conjunction with a Washburn-type conductivity cell with platinized electrodes. A specific conductance value of 0.0129 ohm^{-1} $cm.^{-1}$ for 0.100 molal KCl at 25° (9) was used to calculate the cell constant (2.15×10^{-7} cm.). Solutions were pre-

[1] Lachat Chemical Co.

[2] Mann Chemical Co.