Physicochemical and Pharmacological Studies of Some 8-Substituted Decahydroisoquinolines

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Several compounds of a diastereoisomeric series of 8-substituted 2-methyldecahydroisoquinolines have been found to be equipotent or more potent than quinidine in prolonging the refractory period of isolated guinea pig atria. The effects of selected compounds on the intracellularly recorded transmembrane action potential of isolated canine Purkinje fibers indicated a mechanism of action similar to quinidine in causing a large decrease in the rising velocity of rapid depolarization. Acid dissociation constants and percentage buccal membrane absorption have been determined, in an attempt to correlate these physicochemical properties and the stereochemistry of the derivatives with the observed antiarrhythmic potency. Compounds with high lipophilicity and the trans ring-juncture stereochemistry generally appear to possess superior potency.

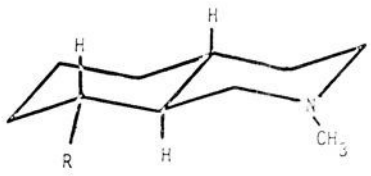
During the course of investigations directed to the development of new and safer antiarrhythmic drugs, Mathison and co-workers[2] prepared some derivatives of 5-amino- and 5-hydroxy-2-methyldecahydroisoquinolines which possessed significant antiarrhythmic potency and therapeutic indexes superior to quinidine, a standard antiarrhythmic drug.[3] As an extension of that work, a diastereoisomeric series of derivatives of 8-amino- and 8-hydroxy-2-methyldecahydroisoquinolines (see Figure 1) has been prepared. The synthesis and stereochemistry of the intermediate 8-amino- and 8-hydroxy-2-methyldecahydroisoquinolines and the synthesis and pharmacological screening of some 22 target compounds have been reported.[4] All the compounds in the study (see compounds **1–21** in Table I) exhibited significant antiarrhythmic potency in the screening test, and several possessed therapeutic indexes two to four times greater than that observed with quinidine. In the current report, the results of some physical measurements (pK_a and buccal absorption) and further pharmacological tests are reported, aimed at detecting any obvious relationships between physicochemical properties and antiarrhythmic potency in addition to ascertaining the cardiopharmacological effects of the more potent compounds in anticipation of providing insight into the mechanism of action of these novel antiarrhythmic compounds at a molecular level.

Experimental Section

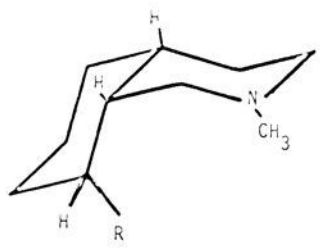
Determination of Acid Dissociation Constants. A 1×10^{-3} M solution of the respective analytically pure compound was prepared in twice-distilled (from potassium permanganate solution) CO_2-free water. In cases of difficultly soluble organic bases, a minimal amount (5%) of methanol was used to promote dissolution. Each sample (15 ml) was titrated with a 0.01 M solution of potassium hydrogen phthalate in a jacketed thermostatically controlled (Heto Ultrathermostat) vessel. The reaction media were stirred with a stirring motor (Radiometer) and nitrogen was slowly bubbled into the media throughout the titration. The titrations were carried out on Radiometer equipment consisting of a pH meter (PHM 28), an automatic titrator (TTT 11), a titrigraph recorder (SBR 2c), an autoburet (ABU 1c), a saturated calomel electrode (Radiometer type K401), and a glass electrode (Radiometer type G202C). Four titrations were conducted on each compound and the pK_a values were determined from the average of the pH values at one-half neutralization. The neutralization points were obtained from the titration curve (pH vs. volume of titrant added) by drawing parallel tangents to the curve on each side of the inflection point. The midpoint of a line connecting the two points of the tangent was taken as the neutralization point. The point at which a line drawn from the point on the curve representing the volume at one-half neutralization crossed the pH coordinate was taken as the pK_a.

Initial titrations were performed using standardized 0.01 N hydrochloric acid as titrant. This procedure was subsequently amended by using 0.01 M potassium hydrogen phthalate. No precedent could be found in the literature for using potassium hydrogen phthalate as a titrant against amines, so the accuracy of the method was checked in the following ways: (a) the pK_a for morpholine was determined to be 8.34 ± 0.01, agreeing closely with the recently reported values of 8.33 at 25°[5] and 8.36 at 25°;[6] and (b) pK_a values for compounds **9** and **10** were found to be 9.02 ± 0.03 and 8.77 ± 0.04, respectively, with 0.01 M potassium hydrogen phthalate and 9.04 ± 0.06 and 8.77 ± 0.04, respectively, with 0.01 N HCl. These results appeared to justify the use of potassium hydrogen phthalate as titrant and eliminated the necessity of preparing a standard solution of NaOH necessary to standardize the hydrochloric acid titrant.

Determination of Buccal Membrane Absorption. The general method for conducting the buccal absorption test followed closely that reported by Beckett and co-workers[7] and the analytical technique employed was modified from Bickel and Weder.[8] Stock solutions of the less soluble organic bases were prepared by dissolving 36 mg in 0.2 ml of 5% HCl and 5.8 ml of distilled water. A 0.5-ml sample, containing 3 mg, was transferred to a beaker containing 24.5 ml of the appropriate buffer. Phosphate buffers (0.1 M) of pH 5.50 and 7.50 and a sodium tetraborate buffer of pH 9.30 were used in the experiment. After swishing 25 ml of the buffered solution of the test compound in his mouth for 5 min, the subject expelled the solution into a beaker and immediately rinsed his mouth with 10 ml of distilled water and emptied it into the same beaker. The pH of the solution was recorded before and after the test. The expelled solution was made up to 50 ml with distilled water and mixed well, and a 10-ml aliquot was adjusted to pH 10 with 10% Na_2CO_3 solution. This was extracted with 3 × 10 ml portions of peroxide-free ether. A 10-ml aliquot of the combined ether extracts was extracted with 10 ml of 0.1 N HCl solution and the uv absorbance determined at the λ_{max} of the respective compound. The concentration was obtained from previously constructed Beer's law plots, and the percentage of the compound not recovered and assumed to have been absorbed through the buccal membrane was calculated. Identical experiments were conducted at the three buffer pH values and plots were constructed of the average pH of the buffer solutions before and after the test vs. the percentage buccal absorption (see Figure 2). Three compounds were tested in three subjects, seven in two subjects, and the remaining compounds in one subject. It was established that quantitative recovery of the test compound could be obtained using the cited procedure. A sample of buffer was swirled in the mouth using the technique



trans-8,9,10-*H*-8-substituted 2-methyldecahydroisoquinolines



cis-8,9,10-*H*-8-substituted 2-methyldecahydroisoquinolines

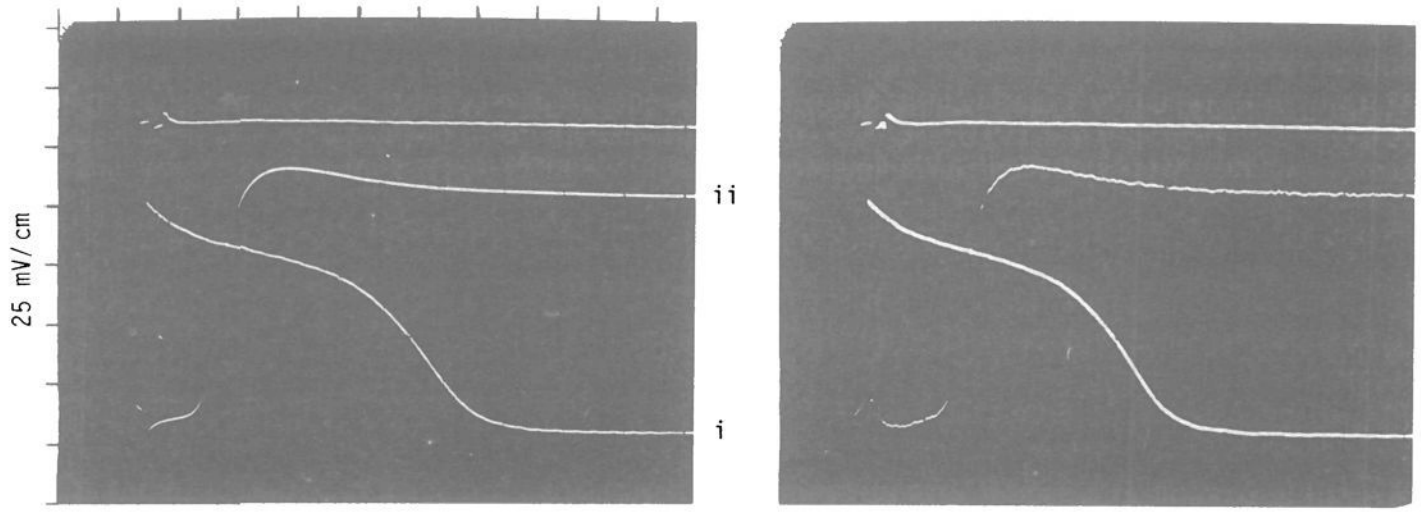
R = -NHX or -OX; X = substituted benzoyl grouping

Figure 1. Structure and stereochemistry of the diastereoisomeric 8-substituted 2-methyldecahydroisoquinolines.

described above. The test compound was then added to the expelled solution and subjected to extraction as noted. Quantitative recovery of the compound was obtained.

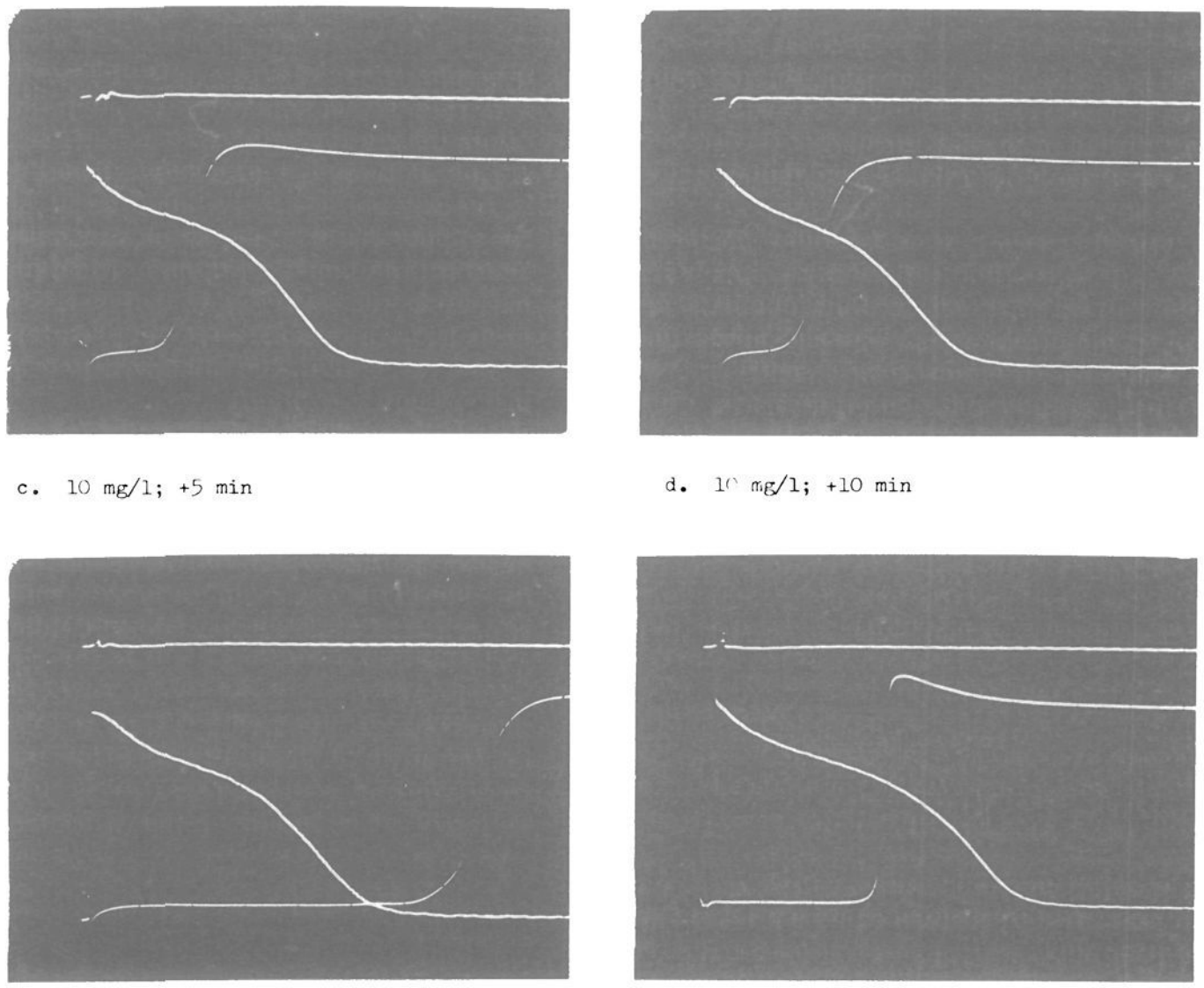
In conducting the buccal absorption tests, it was of interest to find that compounds **15–19** exhibited topical local anesthesic properties, all three subjects experiencing considerable numbness in the areas of the mouth on which the test solution (about 5×10^{-5} M) had contact. The anesthesia persisted for 15–20 min.

Determination of Chronotropic Effect and Effects on Maximum Driving Rate (MDR) in Isolated Guinea Pig Atria. Some years ago Dawes[9] published a method which attempted to estimate the effect of compounds on the refractory period in atrial tissue. Although the test is carried out in an indirect manner by measuring the shift in the maximum rate of stimulation that isolated atria will follow, the maximum follow



a. control

b. 10 mg/l; +2 min post-drug exposure



c. 10 mg/l; +5 min

d. 10 mg/l; +10 min

e. 10 mg/l; +20 min; flow rate increased and washout started

f. recovery at + 50 min; fast sweep 2 ms/cm

Figure 2. The effects on transmembrane action potentials obtained from the experiment ($K^+ = 5.4$ mM) conducted on *trans*-8,9,10-*H*-8-(3,4,5-trimethoxybenzoyloxy)-2-methyldecahydroisoquinoline (**16**) at a concentration of 10 mg/l. The slow sweep (i) was recorded at a sweep speed of 50 ms/cm whereas the fast sweep (ii) was recorded at a sweep speed of 1 ms/cm, except for the recovery (f), which was 2 ms/cm. The upper tracing is an electrogram (see text).

frequency or maximum driving rate is considered to be the reciprocal of the refractory period (refractory period, ms, = 1/MDR, in cycles per second × 1000). The present study utilized the modification proposed by Wojciechowski and Lawson[10] and was undertaken to investigate the effects of the 8-substituted decahydroisoquinolines on the frequency of spontaneously beating isolated atria and on the refractory period by measuring changes in the maximum driving rate.

Young adult guinea pigs, derived from the Hartley strain, weighing between 400 and 700 g were sacrificed by a sharp blow on the head and exsanguinated by cutting the throat. The chest was opened by diagonal cuts, lateral to either side of the sternum; the heart was quickly excised and transferred to a beaker containing oxygenated Krebs–Hensleit solution. Atria were freed of ventricular muscle, fat, and connective tissue and mounted in a 15-ml working volume overflow isolated organ bath (Metro Scientific, Inc.) containing Krebs–Hensleit bicarbonate solution maintained at 30° and aerated with a gas mixture of 95% O_2–5% CO_2. The bathing medium had the following composition (mM/l.): NaCl, 118.4; KCl, 4.7; $CaCl_2 \cdot 2H_2O$, 2.5; KH_2PO_4, 1.2; $MgSO_4 \cdot 7H_2O$, 1.2; $NaHCO_3$, 26.0; dextrose anhydrous, 11.5. The pH of the solution was 7.4. Fine suture threads were tied to the tip of each auricle. The right atrium was tied onto the glass aerating support tube and platinum-tipped stimulating electrodes were just inserted into the tissue. The whole assembly was transferred to the overflow isolated organ bath. The end of the thread from the left atrium was tied to a Grass FT 0.03 force displacement transducer mounted in an adjustable isometric tension clamp (Harvard Apparatus Co., Inc.) and a resting tension of 1g was placed on the preparation. The amplitude of isometric contractions and the rate of spontaneous beating were recorded on one channel of a Grass Model 7 polygraph. Changes in atrial rate were simultaneously recorded on a second channel of the polygraph by means of a tachograph preamplifier (Grass Model 7P4A). This arrangement allowed continuous monitoring of individual contractions as well as rate. Before any compounds were tested, the preparation was allowed to stabilize until the spontaneous rate did not change by more than five to six beats per minute during a 10-min observation period; this usually required about 1 h. During this equilibration phase, the fluid bathing the atria was changed every 10 min and the initial tension on the atria was maintained by vernier adjustment when necessary.

After 1 h, the stimulation threshold was determined by delivering square wave pulses of 1-ms duration for 10 sec at increasing voltage intensity at a frequency 50% above the spontaneous rate. Threshold intensity (volts) was reached and noted when the atria followed the electrical stimulus. Approximately 5 min later, the threshold was redetermined. In anticipation of any elevation of this threshold value that might occur as a result of the experimental procedure or intervention, the stimulus voltage was increased 50% above threshold to ensure that each time the stimulator was turned on, the atria would be electrically driven. With the stimulus intensity set at the determined suprathreshold voltage level (i.e., 50% above threshold), control maximum driving rates (MDR's) were obtained by electrically driving the atria with square wave pulses of 1-ms duration for periods of 10 s every 30 s. The pulses were delivered through the platinum electrodes to the atria by a Grass Model S8 stimulator through a stimulus isolation unit (Model S1V4678). Stimulation frequencies were increased in a stepwise fashion until the atria failed to follow the stimulus. The inability of the atria to follow the electrical stimulus was manifested by "escape" beats of greater amplitude following the dropped beat in the tracing recorded on the first channel of the polygraph, while the same end point in the tachograph recording channel was observed as an abrupt change in rate. The stimulation frequency just preceding the failure point was taken as the maximum driving rate. A second pair of determinations was made 15 min later and the control MDR was taken as the mean value of the last two determinations. In this way, a minimum of two stable control values was obtained before any compounds were added to the isolated tissue chamber.

Solutions of the compounds under investigation were prepared just prior to use and added to the bath usually in volumes of 0.2 ml to give the desired final bath concentration. At the end of a 5-min period the MDR was determined in the presence of the drug and once the measurements were made, the compound was washed from the bath. After washout, two MDR's were determined each 15 min for up to 2 h in order to follow the reversibility of the response and the recovery time of the tissue toward control levels. During this phase also, the fluid bathing the atria was changed every 10 min. Each compound was tested in at least two atrial preparations, generally at a single screening dosage level. Quinidine sulfate (calculated as the base) was used as the standard of comparison. Response curves for quinidine were determined in three different bath concentrations using six to seven atrial preparations at each bath concentration.

Mean responses of both chronotropic effects and shifts in MDR were expressed as percentage changes from their respective control values. The data tabulated in Table I reflect the values calculated at the peak response time, generally 5–30 min after the compounds had been introduced into the bath.

Determination of the Effect of Selected Compounds on the Transmembrane Action Potential (TMAP) of Isolated Canine Purkinje Fibers. Adult dogs weighing from 10 to 15 kg were anesthesized with 30 mg/kg of sodium pentobarbital (iv). A lateral incision was made in the fourth intercostal space and the heart was rapidly excised. Purkinje tissue (false tendons) was carefully dissected along with adjacent connected myocardium. The configuration of the preparation anatomically resembled a barbell. The preparations were placed in a lucite chamber filled with oxygenated (95% O_2–5% CO_2) Tyrode solution maintained at 37 °C. Compositions of the Tyrode solution in millimoles per liter were NaCl, 137.0; $NaHCO_3$, 12.0; NaH_2PO_4, 0.18; $MgCl_2$, 0.5; KCl, 2.7; $CaCl_2$, 2.7; and glucose, 5.5. Some experiments were performed with the potassium level at 5.4 mM/l. (see later). The chamber was rectangular and contained 20 ml of bathing solution. The flow rate of perfusate through the chamber was adjusted to 1 ml/min.

The Purkinje fibers were stimulated at a rate of 1.2 cps with a small bipolar electrode. The stimulus consisted of 4–10-V square wave pulses of 1-ms duration delivered by a Grass stimulator and isolation unit. An electrogram was obtained with a small bipolar electrode from the functional distal muscle mass of the preparation with respect to the proximal stimulating electrode. TMAP's were obtained with glass capillary microelectrodes (resistance, 5–10 MΩ) filled with 3 M KCl by boiling under vacuum. The first-stage input consisted of high impedance, capacitance neutralized amplifiers (NF-1, Bioelectric Instruments). The indifferent electrode was placed in the recording chamber near the preparation. Liquid–metal junctions were made with Ag–AgCl electrodes. A square wave voltage calibrator was placed between solution and ground. Time marks were supplied by a Tetronix time-mark generator. The experiments were taped on a four-channel analog tape recorder (HP no. 3960) and records were filmed with a Polaroid oscilloscope camera from a conventional oscilloscope (Tetronix 65) both directly and from the tape at various voltage gains and sweep speeds for more defined measurements. The experiments were performed under a dissecting microscope (12.5× magnification) for definition of the fiber groups which were impaled by micromanipulation of the microelectrodes.

Solutions of the test compounds **10**, **14**, and **16** were administered to the bath by a bolus injection of appropriate volume to give the desired final bath concentration. Each compound was tested in two tissue preparations at two or three concentrations using normal Tyrode solution (KCl, 2.7 mM/l.) and a modified Tyrode solution (KCl, 5.4 mM/l.). The higher potassium concentration is more equivalent to normal physiological levels and recently Singh[11] and Vaughn Williams[12] have suggested that more accurate interpretation of the effects of antiarrhythmic agents on the TMAP may be obtained at the higher potassium level. In each experiment a filmed record of the control TMAP was obtained and similar records were made at 2-, 5-, 10-, and 20-min intervals after administration of the compound (see Figure 2). After 20 min, the flow rate was increased in order to wash out the compound, and in all cases the tissue was recovered from the effects of the compound from 30 min to 1 h after the wash-out was begun.

Results and Discussion

Acid Dissociation Constants (pK_a). The pK_a's for 17 compounds were determined (see Table I), and in all cases where comparisons could be made between the two iso-

Table I. Physical and Pharmacological Data on the 8-Substituted Decahydroisoquinolines

R =	Isomer	No.	pK_a	% buccal absorption at pH 9.3	Isolated atria test: Bath concn, μM/l.	Isolated atria test: % change in MDR	Isolated atria test: % change in spont rate	Mouse tests[a]: ED_{50}, μM/l.	Mouse tests[a]: LD_{50}, μM/l.	LD_{50}/ED_{50}
Quinidine					61.6	−36.3	−36.1			
					30.8	−22.8	−28.6	160	533	3.3
					15.4	−13.3	−18.8			
C_6H_5CNH-	Cis	1	9.18 ± 0.04	40.6	73.4	−15.1	−7.2	242	1430	5.9
	Trans	2	8.99 ± 0.04	42.9	73.4	−17.6	−19.3	246	1450	5.9
$2\text{-}OCH_3C_6H_4CNH-$	Cis	3	9.11 ± 0.06	35.1	66.1	−23.2	−25.5	116	847	7.3
	Trans	4	8.81 ± 0.05	41.2	66.1	−19.8	−25.0	175	761	4.3
$4\text{-}OCH_3C_6H_4CNH-$	Cis	5	9.19 ± 0.05	30.6	66.1	−15.1	−44.2	268	1560	5.8
	Trans	6		34.3	66.1	−19.8	−21.5	195	1490	7.6
$3,4\text{-}(OCH_3)_2C_6H_2CNH-$	Cis	7		17.6	60.2	−9.71	−16.9	199	1770	8.9
	Trans	8	8.89 ± 0.04	27.1	60.2	−13.8	−20.2	164	1600	9.8
$3,4,5\text{-}(OCH_3)_3C_6H_2CNH-$	Cis	9	9.02 ± 0.03	23.8	55.2	−7.00	−9.8	160	1170	7.3
	Trans	10	8.75 ± 0.05	31.1	55.2	−13.3	−14.4	124	835	6.7
$4\text{-}ClC_6H_4CNH-$	Cis	11		43.2	65.2	−28.5	−13.1	209	909	4.4
	Trans	12	8.83 ± 0.03	54.4	65.2	−20.0	−57.4	200	952	4.8
$3,4\text{-}(Cl)_2C_6H_3CNH-$	Cis	13	9.05 ± 0.01	60.6	58.6	−34.1	−21.7	73	712	9.7
	Trans	14		59.3	58.6	−45.0	−41.2	104	1304	12.7
$3,4,5\text{-}(OCH_3)_3C_6H_2CO-$	Cis	15	8.91 ± 0.01	70.7	55.0	−32.3	−53.2	162	421	2.6
	Trans	16	8.82 ± 0.05	69.5	55.0	−54.5	−42.4	74	338	4.5
$4\text{-}ClC_6H_4CO-$	Cis	17	8.90 ± 0.03	86.5	65.0	−53.3	−52.3	257	1200	4.7
					32.5	−32.2	−48.6			
	Trans	18	8.75 ± 0.03	84.7	65.0	−55.8	−60.5	146	757	5.2
C_6H_5CO-	Cis	19	9.05 ± 0.02	81.8	73.2	−55.5	−57.8	245	900	3.7
					36.6	−24.5	−23.0			
$C_6H_5SO_2NH-$	Cis	20	9.17 ± 0.03	30.4				282	1460	5.2
	Trans	21	8.87 ± 0.02	37.1				347	1113	3.3

[a] See ref 4b for method.

mers, the cis-8,9,10-*H*-8-substituted isomers possessed higher pK_a values than the corresponding trans-8,9,10-*H*-8-substituted isomers by 0.1–0.3 pK units (see Figure 1 for a depiction of the stereoisomers included in the discussion). The protonated cis-8,9,10-*H* isomer forms a strong intramolecular H bond between the acidic proton on the ring nitrogen and the amido nitrogen (stronger than the intramolecular H bond between the amido proton and the ring nitrogen in the free base), thus causing the cis isomers to be of higher basicity than the trans isomers, in which no intramolecular H bonding is possible. As a result of the small differences in pK_a (0.3 pK) for the entire series of compounds, this physicochemical property has been ignored in assessing structure–activity relationships, since the percentages of the compounds un-ionized at physiological pH would differ only by a small amount.

Buccal Absorption Test. Buccal absorption is a measure of the ability of the drug to pass through these oral membranes in an in vivo situation and is considered to be representative of the lipid solubility of the drug. In fact, several workers have shown that the ranking of the degree of lipid solubility rarely changes when results of the buccal absorption test and standard partition coefficients are compared.[7b,8,13]

The degree of absorption is usually reported as the percentage of the drug absorbed by the membrane from a buffered solution over a 5-min period. Since all the test compounds possessed pK_a values between 8.7 and 9.2, the percentage ionized at the different buffer pH values (5.5, 7.5, and 9.3) used in the experiment varies greatly. This is reflected by a sharp increase in the percentage buccal absorption as pH increases. The complete data obtained in the experiment are presented in Table II. Since the percentage absorption was highest in the buffer of pH 9.3, and the difference in the compounds was accentuated at this pH, the buccal absorption data obtained at this pH were used in attempts to correlate lipophilicity, stereochemistry, and antiarrhythmic activity.

Table II. Buccal Absorption of the 8-Substituted Decahydroisoquinolines at Buffer pH 5.50, 7.50, and 9.30 Expressed as Percent Buccal Absorption[a]

No.	Buffer pH 5.50	Buffer pH 7.50	Buffer pH 9.30	Uv λ_{max}, nm
1	9.30	16.0	40.6	227
2	11.1	15.9	42.9	228
3	1.20	5.30	35.1	205
4	5.20	12.8	41.2	206.5
5	4.70	7.00	30.6	252
6	7.40	9.00	34.3	252
7	7.00	10.4	17.6	256
8	16.8	18.9	27.1	257
9	8.10	14.2	23.8	256
10	5.50	18.1	31.1	256
11	6.70	9.3	43.2	238
12	8.20	12.4	54.4	239
13	20.1	25.1	60.6	240
14	20.5	25.7	59.3	240
15	21.2	38.2	70.7	264
16	17.1	19.7	69.5	265
17	13.7	43.7	86.5	242.5
18	17.4	42.7	84.7	243
19	29.7	46.0	81.8	232
20	14.9	17.7	30.4	222
21	11.9	20.4	37.1	221

[a] Tests on five compounds were repeated and the average intrasubject variation was 2.5% while the average intersubject (three subjects) was 6.8%.

As can be seen from the data in Table I, the ester derivatives **15–19** were considerably more lipid soluble than

the amide derivatives. In both series, as expected, the chloro compounds were more lipid soluble than the unsubstituted derivatives whereas the methoxy compounds exhibited lower lipid solubility. It was noted that in the pairs of isomeric amides, excepting the 3,4-dichloro pair **13** and **14**, the trans isomer tended to be more lipophilic than the corresponding cis isomer.

Isolated Guinea Pig Atria Test. The results obtained in this test are recorded in Table I. Quinidine was chosen as the standard of comparison since it remains one of the most widely used antiarrhythmic drugs.[3] Additionally, it has been reported by many investigators that quinidine lengthens or prolongs the refractory period which is manifested in this test by a reduction in the maximum driving rate.[9,10,17] In the present study, quinidine produced a dose-dependent reversible decrease in MDR which suggests prolongation of the refractory period since MDR measures this indirectly. Depending on the bath concentration of quinidine, control values were reattained after some 75–120 min. When the mean percent change in MDR from control is calculated at the peak response times it can be seen (Table I) that bath concentrations of 5, 10, and 20 mg/l. (15.4, 30.8, and 61.6 μM/l.) produced decreases in MDR of 13.3, 22.8, and 36.3%, respectively, changes which were significantly different from mean control values ($p < 0.001$). Administration of quinidine also decreased the frequency of spontaneously beating atria. It will be noted that the depression, a negative chronotropic effect, was also dose dependent, ranging from −18.8% at the lowest bath concentration to −36.1% at the highest bath concentration utilized. All of the 8-substituted decahydroisoquinolines tested produced decreases in MDR and in the frequency of spontaneously beating atria. Following washout, the effects on MDR and spontaneous rate were reversible during the experimental period. Where varying bath concentrations of the decahydroisoquinolines were studied (compounds **17** and **19**, Table I) responses were dose dependent. At the highest bath concentrations, all of the ester compounds except compound **15** produced changes in MDR which were about 1.5 times greater than that produced by an equivalent bath concentration of quinidine (61.6 μM/l.). The effect of compound **15** on MDR was essentially equivalent to that noted for quinidine.

In attempting to draw correlations between physicochemical properties and antiarrhythmic activity, there appears to be a distinct relationship between the degree of lipophilicity and the magnitude of the decreases in both MDR and spontaneous rate. The most active compounds in the series were the highly lipophilic esters **15–19** and the chloro-substituted benzamides **11–14**. With the exception of the 2-methoxybenzamides (**3** and **4**) and the 4-chlorobenzamides **11** and **12**, the trans isomers generally produced a greater decrease in MDR than the corresponding cis isomers, even in the isomeric pairs in which no significant difference in lipid solubility existed. This suggests stereoselectivity at the site of action favoring the rigid trans ring-juncture stereochemistry.

Effects on the Transmembrane Action Potential (TMAP) of Isolated Canine Purkinje Fibers. The primary objective of this aspect of the study was to determine the effect that three of the more active compounds in the other antiarrhythmic tests had on the various constituents of the TMAP. Experiments were designed to record the TMAP of a single cardiac cell by measuring the changes in potential across the cell membrane by means of a glass capillary microelectrode embedded in the cell. Photographs taken during the experiments were analyzed for changes in the amplitude of the action potential (millivolts), duration of the action potential at 100, 90, and 50% repolarization (in milliseconds), the average rising velocity (ARV) of phase 0 (in volts per second), and activation time (in milliseconds).

Activation time represents the time required for an applied impulse to travel through the tissue from the point of contact of the stimulating electrode to the cell in which the recording microelectrode is embedded. This parameter is dependent on the distance between the two electrodes, the strength of the impulse, and the conduction properties of the tissue pathway through which the impulse travels. Therefore, if the applied impulse voltage and the distance between electrodes is maintained constant, changes in activation time upon exposure of the tissue to the test compound reflect the effect the compound has on conduction velocity. In general, an increase in activation time is indicative of increased conduction delay which is one postulated mechanism considered a desirable effect of antiarrhythmic action.[18]

Perhaps the most important effect of antiarrhythmic drugs, as proposed recently by Vaughn Williams,[12] is a reduction in the rate of rise (ARV) of phase 0 of the TMAP, caused by a decreased membrane permeability of the cardiac cell to sodium influx during the phase of rapid depolarization (phase 0). This appears to be the most basic effect of "quinidine-like", membrane-depressant antiarrhythmic drugs and is the most probable cause for the other changes seen in the TMAP.

The filmed record of the changes in the TMAP caused by *trans*-8,9,10-*H*-8-(3,4,5-trimethoxybenzoyloxy)-2-methyldecahydroisoquinoline (**16**) at the 5.4 mM/l. potassium concentration is shown in Figure 2. As can be seen from the series of photographs and Table III, compound **16** produced marked decreases in ARV and marked increases in activation time which are indicative of an increased delay of conduction. These and the photographs for compounds **10** and **14** were analyzed for percentage changes from control and the data obtained are included in Table III.

In comparing results obtained at the high and low potassium levels, the repolarization phase was affected to a greater degree at the low (2.7 mM/l.) concentration, manifested by a shortening of the duration of the TMAP at 100, 90, and 50% repolarization. The duration was affected very little by the compounds at the 5.4 mM/l. potassium level. All three compounds (**10**, **14**, and **16**) produced marked depression of ARV, -24, -30, and -50%, respectively, at 10 mg/l. concentration in the 5.4 mM/l. potassium level experiment. Consistent with Singh's[11] and Vaughn Williams'[12] findings, the ARV was depressed significantly more in the experiment using the higher potassium level. Compounds **14** and **16** (10 mg/l.) produced a dramatic increase in conduction delay as reflected by the increase in activation times of 18 and 530%, respectively. The same two compounds also reduced the amplitude approximately 10%. Compound **10**, on the other hand, at the same concentration and potassium level (5.4 mM/l.) caused negligible change in amplitude and activation time, although as noted above, it caused a 24% decrease in ARV.

The results of these experiments indicate that the series of 8-substituted 2-methyldecahydroisoquinolines constitutes a novel class of antiarrhythmic agents possessing quinidine-like membrane-depressant properties by virtue of the fact that the three representative compounds significantly reduced the rate of rise of phase 0 of the TMAP. Agents possessing this type of activity are referred to as

Table III. Effects of Compounds 10, 14, and 16 on the Transmembrane Action Potential Parameters Expressed as Percentage Change from Control after 20 min of Exposure of the Tissue to the Test Compound[f]

No.	Bath concn K^+, mM/l.	Bath concn Compd, mg/l.	Amplitude	Activation time	ARV[a]	Duration at[b] 100%	Duration at[b] 90%	Duration at[b] 50%
16	2.7	10	−12 (119)[c]	+131 (12.4)[d]	−6 (175)[e]	−11 (366)[d]	−14 (304)[d]	−40 (205)[d]
	2.7	20	−15 (119)	+389 (12.4)	−58 (175)	+2 (366)	−14 (304)	−25 (205)
	5.4	10	−7 (107)	+530 (1.0)	−50 (216)	0 (322)	−6 (275)	−8 (200)
	5.4	20	−17 (111)	+300 (5.5)	−71 (296)	−5 (316)	−12 (281)	−11 (180)
14	2.7	30	−23 (116)	+81 (4.8)	−72 (543)	−14 (389)	−16 (292)	−35 (155)
	5.4	10	−11 (100)	+18 (6.2)	−30 (172)	+2 (370)	+6 (308)	+5 (204)
	5.4	20	−5 (92)	+42 (6.7)	−58 (170)	−6 (394)	−5 (344)	−10 (288)
10	2.7	10	0 (122)	+2 (4.1)	−9 (259)	+6 (377)	+2 (305)	−5 (189)
	2.7	25	−2 (122)	+23 (4.1)	−19 (259)	−6 (377)	−5 (305)	−22 (189)
	2.7	50	−8 (122)	+105 (4.1)	−44 (259)	+5 (377)	−1 (305)	−30 (189)
	5.4	10	0 (116)	−4 (7.2)	−24 (375)	0 (400)	+4 (337)	−7 (167)
	5.4	20	0 (116)	−1 (7.2)	−29 (375)	+1 (400)	0 (337)	−4 (167)
	5.4	40	−6 (114)	−1 (6.2)	−50 (233)	−3 (394)	0 (339)	+2 (150)

[a] Average rising velocity. [b] Duration of action potential at % repolarization indicated. [c] Control value in millivolts. [d] Control value in milliseconds. [e] Control value in volts per second. [f] Control values for each experiment are in parentheses.

"quinidine-like" since experiments have shown that this prototype of antiarrhythmic drugs expresses its primary effect by slowing ARV.[12] The possible mechanisms by which this effect prevents impending arrhythmias or converts existing arrhythmias to normal sinus rhythm have been discussed more fully in ref 14 and citations therein.

General Discussion of Structure–Activity Relationships. Antiarrhythmic data have been obtained on three levels: (1) the cellular level from the TMAP experiments, (2) the organ level from the atrial experiments, and (3) the previously reported[4b] whole animal studies involving the ability to prevent chloroform-induced fibrillation in mice and acute toxicity tests.[15] The ED_{50}, LD_{50}, and therapeutic indexes obtained in these latter experiments were reported earlier[4b] but for convenience in discussing SAR are included in Table I. It is interesting to note the similar ranking of the compounds in comparing the potencies of the mouse test and the MDR of the atrial test.

The most apparent correlation between physicochemical properties and activity in the mouse test and the isolated atria test was that as the lipophilicity increased in the series, the potency and toxicity generally increased. Also in the pairs of cis–trans isomers in which a significant difference in lipid solubility existed, the trans isomer was more lipophilic and more toxic. In the ester series, greater toxicity and potency appears to be related to the trans stereochemistry, since no differences were observed in their lipid solubility. In considering the isomeric pairs of the entire series, it is difficult to ascertain if enhanced potency and toxicity are a manifestation of the higher lipophilicity or stereoselectivity at the site of action of the trans isomers.

Conclusions

From an overall analysis of the pharmacological data collected on the 8-substituted 2-methyldecahydroisoquinolines, we conclude that a novel series of experimentally effective antiarrhythmic agents has been synthesized and shown to possess greater potencies and superior margins of safety than that demonstrated by quinidine. The distribution of potency throughout the series of variously substituted benzamides, benzenesulfonamides, and benzoyloxy esters implies that the compounds probably do not interact with a highly stereoselective receptor site. This is substantiated further by earlier investigations of the 5-substituted[2b,c] 2-methyldecahydroisoquinolines and recently published work on the 6-substituted[16] 2-methyldecahydroisoquinolines, both of which have been shown to possess antiarrhythmic potency of a similar magnitude as the 8-substituted series. Indeed, all indications are that the compounds act similarly to nonspecific, quinidine-like drugs by diffusing into the cardiac cell membrane and suppressing sodium ion influx during depolarization.

The differences in potency observed in the series probably arise from differences in the ability of the compounds to (1) pass through membranes and be transported in the body fluids from the site of administration to the site of action, (2) be absorbed onto or into the cardiac cell membrane, and (3) cause disturbances in the conformational perturbations of macromolecular membrane constituents performing their function of varying the membrane permeability to ions, benefitting the heart as a whole by restoring or maintaining normal rhythm. Naturally, the optimum physicochemical properties and shape of the drug molecule determine its success in fulfilling these criteria, namely, high lipid solubility and, to some degree, trans ring-junction stereochemistry.

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References and Notes

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Etodolic Acid and Related Compounds. Chemistry and Antiinflammatory Actions of Some Potent Di- and Trisubstituted 1,3,4,9-Tetrahydropyrano[3,4-*b*]indole-1-acetic Acids

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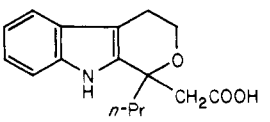
A series of 37 1-ethyl- and 1-*n*-propyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole-1-acetic acids bearing one, or two, substituents on the benzene ring has been synthesized via the acid-catalyzed condensation of a substituted tryptophol with ethyl propionylacetate or ethyl butyrylacetate. Antiinflammatory and ulcerogenic effects were examined and the results show that 1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole-1-acetic acid (etodolic acid, USAN) is a potent agent, particularly active against a chronic rat model of inflammation (ED_{50} 0.7 ± 0.1 mg/kg po in the adjuvant arthritis model) and which has a relatively low acute ulcerogenic potential in the same species.

In a previous publication[1] we have disclosed the antiinflammatory activities of a series of 1,3,4,9-tetrahydropyrano[3,4-*b*]indole-1-alkanoic acids bearing various hydrocarbon substituents on the pyrano ring and on the nitrogen atom. From that series, prodolic acid[2] was selected for further development, and detailed pharmacological studies have appeared.[3] The analog of prodolic acid which has an ethyl group[1] instead of an *n*-propyl group



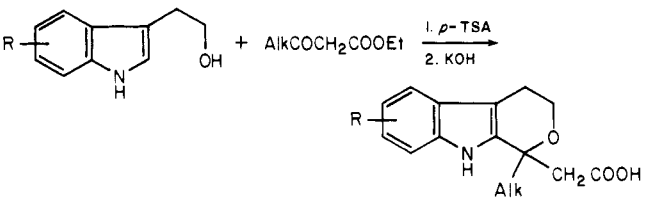
prodolic acid

at position 1 also possessed a high order of activity. In the present report we describe the syntheses and antiinflammatory activities of a series of 1-ethyl- and 1-*n*-propyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole-1-acetic acids which bear one or two substituents on the benzene ring.

Chemistry. The compounds prepared for antiinflammatory testing (Table I) were obtained by the previously described method for the synthesis of 1,3,4,9-tetrahydropyrano[3,4-*b*]indole-1-acetic acids.[1] Thus, mono- or disubstituted tryptophols were condensed, in the presence of an acid catalyst, with ethyl propionylacetate or with ethyl butyrylacetate, followed by alkaline hydrolysis, to afford 1-ethyl or 1-*n*-propyl derivatives (Scheme I).

Thirty-one substituted tryptophols were required in this study. Three of them have been previously reported, and 26 others were obtained by the appropriate segment of the pathway illustrated in Scheme II, starting with an aniline,

Scheme I



an isatin, or an indole. 7-*tert*-Butyltryptophol was synthesized from 7-*tert*-butylisatin, as shown in Scheme III, by the lithium aluminum hydride reduction of the 3-hydroxy-2-oxoindoline 3-acetate obtained from a Reformatsky reaction on the isatin with ethyl bromoacetate. 7-Cyclopropyltryptophol was prepared in one step from the reaction between 2-cyclopropylphenylhydrazine hydrochloride and 2,3-dihydrofuran using the method developed by Grandberg and Moskvina (Scheme IV).[4] The intermediates encountered in the course of the reactions shown in Schemes I–III were generally used without purification, but in a number of instances intermediates were characterized, principally by NMR spectroscopy (see Experimental Section).

The 37 mono- and disubstituted 1-ethyl- and 1-*n*-propyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole-1-acetic acids prepared are collected in Table I along with physical constants and analytical data. In addition, the source of the aniline, isatin, or indole starting material used for each of the compounds is documented in Table I.

Pharmacology. Methods. Compounds were tested orally for antiinflammatory activity in groups of six rats with established adjuvant arthritis ("therapeutic test") as described previously.[1,3,5] Treatment with compounds was