Antiarrhythmic Activity of *p*-Hydroxy-*N*-(2-diethylaminoethyl)benzamide (the *p*-Hydroxy Isostere of Procainamide) in Dogs and Mice

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p-Hydroxy-*N*-(2-diethylaminoethyl)benzamide (**2**), the *p*-hydroxy isostere of procainamide (1), shows antiarrhythmic activity against acontine-induced atrial arrhythmia and lowers mean arterial blood pressure after iv infusion in dogs. In isolated canine Purkinje fibers, phenolic **2** in a bath concentration of 20 μg/ml significantly reduced the rate of phase 0 depolarization, prolonged the repolarization time, and reduced automaticity. These in vitro and the above in vivo activities of phenolic **2** were similar to those observed for procainamide (1). Bioisosteres, phenolic **2** and procainamide (1), have almost identical respective ^{13}C NMR chemical shifts indicating that electron densities on the respective carbons are very similar. This may explain their similar antiarrhythmic and hypotensive effects. Phenolic **2** and procainamide (1) therapeutic ratios in ICR male mice (acute LD_{50}/ED_{50} against chloroform hypoxia induced ventricular fibrillation) are 2.1 and 1.8, respectively. Procainamide analogues with electron-donating groups [OH, NH_2, $NHC(=O)CH_3$] on the aromatic ring possess more antiarrhythmic activity in mice than the analogue with an electron-withdrawing group (NO_2). This indicates that a shift in electron density toward the amide region in the former analogues, as determined by ^{13}C NMR spectroscopy, is one of the factors influencing antiarrhythmic potency in this series.

The concept of bioisosterism involves the modification of a prototype drug for the purpose of developing a new drug that shows a similar spectrum of desired activities as the prototype but hopefully less of the prototype's undesired effects. The modification involves replacing the group in question with an isostere.[1] Isosteres are groups that have identical peripheral layers of electrons and are similar sterically. Hydroxy (OH) and amino (NH_2) groups are isosteric (seven valence electrons) and both are strong electron donors when situated on an aromatic ring. Procainamide (1), used clinically as an effective agent in the treatment of cardiac arrhythmias,[2-4] contains an aromatic amino group that is suspected to be the cause of some of the toxic effects of procainamide therapy (the reasons to be given in a subsequent section). The present investigation, therefore, was undertaken to study the antiarrhythmic activity in dogs and mice of *p*-hydroxy-*N*-(2-diethylaminoethyl)benzamide (**2**), the isostere formed by replacing the procainamide aromatic amino group with a hydroxy group (see Table I for chemical structures), and to present a structure–antiarrhythmic activity relationship for analogues of procainamide in mice.

Results

Phenolic **2** effectively converted aconitine-induced atrial arrhythmias to sinus rhythms with slowing of the atrial rates and lowered mean arterial blood pressure after iv infusion in mongrel dogs (Table II, Figure 1). In five of seven of these dogs, a sinus tachycardia with 1:1 conduction or normal sinus rhythm was obtained. Similar effects were seen after infusion of procainamide (1) (Table II). The mean phenolic **2** and procainamide (1) plasma levels, present when this response was observed, were 137 and 118 μg/ml, respectively. These levels seem high, but they were determined when drug infusion was terminated which was when the arrhythmia responded. This was before drug distribution was complete. After the infusion of both drugs was completed, the atrial rate increased or atrial fibrillation returned. Infusion of *p*-acetoxy-*N*-(2-diethylaminoethyl)benzamide (**3**) caused conversion of the aconitine arrhythmia to a sinus rhythm with 1:1 conduction in each of four dogs (Table II). Analysis of a plasma sample withdrawn from each dog when the arrhythmia responded showed less than 0.5 μg/ml of ester **3** present after analysis as compared to a mean phenolic **2** level of 97 μg/ml. Ester **3** (20 μg/ml) was 60–70% hydrolyzed after standing in fresh human plasma 5 min at room temperature. Adding physostigmine (10^{-5} M) to the plasma or dissolving the ester **3** in saline significantly reduced the hydrolysis.

Phenolic **2** in bath concentrations of 5 and 20 μg/ml produced significant alterations in the single cell action potentials of isolated His Purkinje fibers (Table III). These effects consisted of reductions in the rate of phase 0 depolarization (dV/dt) and a prolongation of the repolarization time. A significant reduction in spontaneous rate was also observed following exposure to 20 μg/ml (Figure 2) but not 5 μg/ml. In some experiments rate actually increased following exposure to the lower concentration. Neither concentration significantly altered the maximum diastolic potential or the total height of the action potential (Table III). However, action potential height was reduced in several experiments in association with the reduction in dV/dt.

The dose–response study results for 28–35 day-old ICR male mice (av wt 25 g) protected by phenolic **2** against chloroform-induced ventricular fibrillation can be seen in Figure 3. The ED_{50} is 157 mg/kg with 95% confidence limits of 122–200 mg/kg as determined by the method of Litchfield and Wilcoxon.[5] At a dose of 240 mg/kg of compound **5**, all mice had seizures, but none died. However, only 38% of the mice were protected at this dose. At a dose of 233 mg/kg of compound **6**, all mice had seizures and died. A dose of 100 mg/kg of compound **6** offered no protection and caused seizures in one out of eight mice. The LD_{50} for phenolic **2** and procainamide (**1**) in mice is 335 and 255 mg/kg, respectively (Figure 3).

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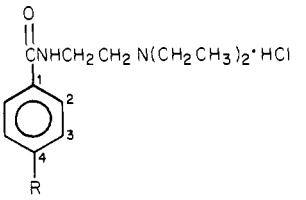
Table I. Carbon-13 NMR Chemical Shift Values and Assignments for Para-Substituted Analogues of Procainamide[a]



Compd	R	Chemical shifts, ppm								
		C-1	C-2	C-3	C-4	C=O	$NHCH_2$	CH_2N	CH_2CH_3	CH_3
2	OH	125.1	130.4	116.3	160.6	171.1	35.9	52.1	49.1	9.2
1	NH_2	122.2	130.1	115.6	152.3	171.3	35.9	52.2	49.1	9.2
4	$NHC(=O)CH_3$[b]	128.8	129.1	120.7	142.1	170.4	35.8	51.8	48.9	9.1
6	H	133.3	129.6	127.9	133.3	171.5	35.7	51.5	48.8	9.0
5	NO_2	139.5	129.3	124.5	150.4	169.1	36.0	51.7	49.2	9.1

[a] Spectra were determined as 0.9 M solutions of the hydrochloride salt in D_2O containing atrace of dioxane as an internal reference. Values are reported in parts per million downfield from Me_4Si, using the dioxane-Me_4Si conversion of 67.4 ppm as per the convention of Johnson and Jankowski.[27] [b] Additional shift values occur at 173.0 (acetyl carbonyl) and 24.1 ppm (acetyl methyl).

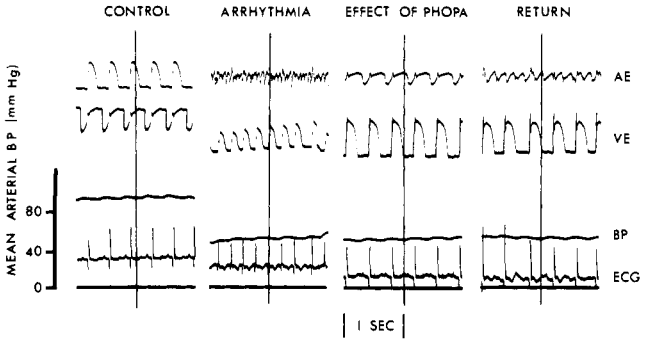


Figure 1. Bipolar electrogram changes produced by PHOPA [p-hydroxy-N-(2-diethylaminoethyl)benzamide (2)] on aconitine-induced arrhythmia in dog 14. The control reading was taken just prior to introduction of aconitine, the effect was determined 7 min after the end of the infusion, and the return was determined 20 min after the end of the infusion. Abbreviations used are AE, atrial electrogram; VE, ventricular electrogram; BP, blood pressure; ECG, electrocardiogram.

Confidence limits (95%) were 309–374 and 220–307 mg/kg, respectively.

The ^{13}C NMR chemical shifts and assignments for para-substituted analogues of procainamide can be seen in Table I.

Discussion

Phenolic **2**, the p-hydroxy isostere of procainamide (1), and procainamide (**1**) show comparable antiarrhythmic activity in the following models.

(1) Aconitine-induced atrial arrhythmias in dogs. There is no statistical difference in dose or plasma levels of phenolic **2** and procainamide (1) needed to produce a similar atrial fibrillation reduction (Table II).

(2) Canine His Purkinje fibers. Phenolic **2** in a bath concentration of 20 μg/ml significantly reduced automaticity, prolonged the duration of the action potential, and reduced the rate of phase 0 depolarization. These changes are equivalent to those observed in this laboratory with similar concentrations of procainamide (**1**) (Table III). The relationship between these drug-induced electrophysiological alterations and the antiarrhythmic actions of procainamide has been reviewed recently.[6]

(3) Chloroform hypoxia induced ventricular fibrillation in mice. Phenolic **2** and procainamide (1) therapeutic ratios (acute LD_{50}/ED_{50}) are 2.1 and 1.8, respectively (Figure 3). The latter value is in agreement with the earlier observation of Lawson[7] who found a procainamide therapeutic ratio of 1.5.

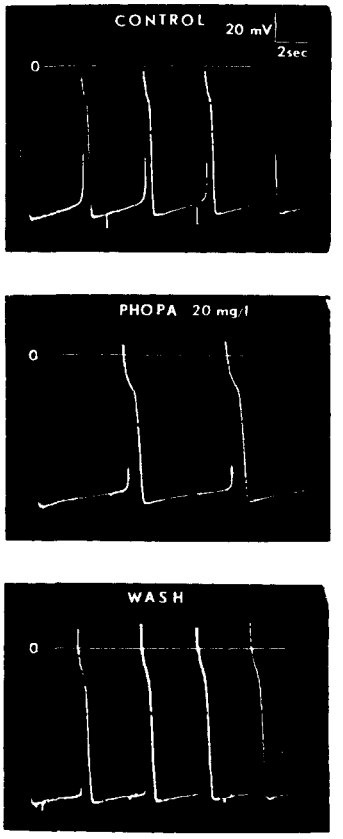


Figure 2. Effects of PHOPA [p-hydroxy-N-(2-diethylaminoethyl)benzamide (2)] on phase 4 depolarization of spontaneously depolarizing Purkinje cells.

(4) Isolated, ligated guinea pig hearts.[8] The phenolic **2** and procainamide (**1**) therapeutic ratios (decrease in force of ventricular contraction/decrease in ventricular rate) are analogous.

Bioisosteres phenolic **2** (Table II) and procainamide (1)[9,10] (Table II) also cause a fall in mean arterial blood pressure after iv infusion in dogs. One probable explanation for the corresponding antiarrhythmic and hypotensive effects of procainamide (**1**) and its p-hydroxy isostere is that the bioisosteres have very similar electron densities on respective carbons as indicated by nearly

Table II. Effects of *p*-Hydroxy-*N*-(2-diethylaminoethyl)benzamide (2), Procainamide (1), and *p*-Acetoxy-*N*-(2-diethylaminoethyl)benzamide (3) on Aconitine-Induced Atrial Fibrillation and on Mean Arterial Blood Pressure in Dogs[a]

	Control		Aconitine arrhythmia			Drug administration[b]		Effect at end of infusion[b]			Postinfusion		
Compd (n)	NSR, beats/min	MABP, mmHg	A rate, beats/min	V rate, beats/min	MABP, mmHg	Iv dose,[c] mg/kg	Plasma [drug], μg/ml	A rate, beats/min	V rate, beats/min	MABP, mmHg	Time,[d] min	A rate, beats/min	V rate, beats/min
2 (7)	143 ± 29	103 ± 33	Fibrillation	237 ± 42	85 ± 33	59 ± 25	137 ± 34	210 ± 36[e]	197 ± 42	64 ± 16	16 ± 10	278 ± 57[f]	160 ± 26
1 (4)	170 ± 19	103 ± 39	Fibrillation	255 ± 25	72 ± 25	48 ± 38	118 ± 17[g]	225 ± 71[h]	203 ± 48	68 ± 33	17 ± 16	283 ± 95[f]	215 ± 46
3 (4)	163 ± 33	101 ± 28	Fibrillation	159 ± 32	82 ± 6	50 ± 24	<0.5[j]	173 ± 37[i]	173 ± 37	70 ± 7	20 ± 3	373 ± 126[f]	183 ± 46
Saline (2)	140 ± 30	85 ± 23	Fibrillation	200 ± 60	77 ± 7	28 ml infused at rate of 3.82 ml/min		Fibrillation	205 ± 65	70 ± 8	58 ± 6	Fibrillation	205 ± 65

[a] Values = mean ± SD. Abbreviations used are as follows: A, atrial; V, ventricular; NSR, normal sinus rhythm; MABP, mean arterial blood pressure. [b] There is no statistical difference when comparing dose or plasma levels of 1, 2, and 3 needed to produce a similar atrial fibrillation reduction or blood pressure lowering. The data were analyzed using the Mann–Whitney U test. [c] Infusion rate, 11.5 ± 0.5 (S.D.) mg/kg/min. [d] After end of infusion. [e] In five of these dogs a sinus tachycardia with 1:1 conduction or normal sinus rhythm was obtained. [f] Fibrillation recurred in one dog. Therefore, the atrial rate could not be determined. [g] Excluding a high plasma level (585 μg/ml) for one of the dogs. [h] In three of these dogs a sinus rhythm with 1:1 conduction was obtained. [i] In each dog a sinus rhythm with 1:1 conduction was obtained. [j] In addition, a mean phenolic 2 level of 97 ± 45 μg/ml was observed.

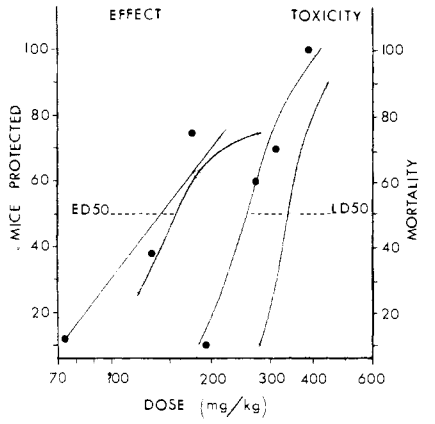


Figure 3. Dose–response curves for *p*-hydroxy-*N*-(2-diethylaminoethyl)benzamide (2) (open circles) and for procainamide (1) (close circles) injected ip in mice. Effect is protection against ventricular arrhythmia produced by chloroform and hypoxia. Toxicity is the observed acute mortality. The procainamide (1) antiarrhythmic activity data were taken from Drayer et al.[23] Eight mice were used at each dosage interval in the antiarrhythmic study and ten mice were used at each dosage interval in the toxicity study.

identical respective ^{13}C NMR chemical shifts (Table I). Other examples of bioisosteres formed by replacing an NH_2 with an OH group are the following: the *p*-hydroxy isostere of procaine has 0.6 the local anaesthetic potency of procaine;[11] *p*-hydroxyephedrine and *p*-aminoephedrine have similar blood pressure action;[12] the *p*-hydroxy isostere of *p*-aminobenzamide has 0.1–0.3 the bacteriostatic activity of *p*-aminobenzamide.[13] However, the *p*-hydroxy isostere of sulfanilamide has very little bacteriostatic activity.[14] The full scope of the bioisosterism concept has been reviewed by Korolkovas,[1] Burger,[15] and Ariens.[16]

Phenolic **2** is potentially a more desirable therapeutic agent than procainamide (**1**) because it has similar antiarrhythmic activity but would be expected to be less toxic. The decrease in toxicity would be for those toxic effects caused by aromatic primary amino groups. Methemoglobinemia and hemolytic anemia in people with congenital erythrocyte hemoglobin reductase deficiencies such as glucose-6-phosphate dehydrogenase deficiency are examples of this type of toxicity. We think that the development of antinuclear antibody and systemic lupus erythematosus-like illness in procainamide-treated patients is another example of primary aromatic amine toxicity. Our reason is that slow acetylators of procainamide (**1**) are more likely to develop the lupus-like illness than fast acetylators.[17–19] Slow acetylators have higher plasma levels of procainamide to *N*-acetylprocainamide than rapid acetylators,[20] suggesting that the primary amine is etiologically related to the lupus. Since phenolic **2** has no primary aromatic amine, we think that it will not cause the hematologic toxicity or systemic lupus erythematosus with the same frequency that these are caused by procainamide (**1**). Comparative trials will have to be carried out to test this hypothesis.

Ester **3** when infused in dogs acts as a "prodrug" by being almost completely hydrolyzed to phenolic **2** (Table II). This hydrolysis also takes place in vitro in plasma and is significantly reduced by the addition of 10^{-5} M physostigmine. Therefore, it appears that serum pseudocholinesterase is the enzyme catalyzing the hydrolysis.

The structure–antiarrhythmic activity relationship for para-substituted analogues of procainamide (**1**) in mice (Table IV) indicates that compounds containing electron-donating groups [OH, NH_2, or $NC(=O)CH_3$] on the

Table III. Effects of p-Hydroxy-N-(2-diethylaminoethyl)benzamide (2) and Procainamide (1)[a] on Canine Purkinje Fiber Single Cell Potentials[b]

Compd	Max diastolic potential, −mV (n)	Action potential, mV (n)	dV/dt, V/s (n)	Repolarization time, ms (n) 50%	Repolarization time, ms (n) 90%	Intrinsic rate, beats/min (n)
Control	93 ± 2	127 ± 2	439 ± 47	200 ± 6	282 ± 8	19 ± 4
Phenolic 2, 5 μg/ml	95 ± 2 (10)	127 ± 2 (7)	396 ± 41[c] (7)	250 ± 18[c] (5)	344 ± 19[c] (5)	20 ± 5 (10)
Control	93 ± 2	128 ± 3	478 ± 67	236 ± 9	302 ± 12	22 ± 5
Phenolic 2,[d] 20 μg/ml	94 ± 2 (9)	126 ± 3 (9)	423 ± 54[c] (6)	280 ± 10[c] (9)	381 ± 12[c] (9)	16 ± 4[c] (15)
Control			491 ± 40	244 ± 15	274 ± 30	33 ± 5
Procainamide (1), 10 μg/ml			421 ± 42[c] (11)	265 ± 16[c] (10)	334 ± 18[c] (10)	24 ± 6[c] (5)
Control			474 ± 57	223 ± 11	284 ± 12	35 ± 7
Procainamide (1), 20 μg/ml			411 ± 53 (8)	239 ± 11 (7)	316 ± 17 (7)	23 ± 5[c] (9)

[a] Procainamide values were taken from Bagwell et al.[10] [b] Values = mean ± SE. [c] $p < 0.05$ compared to control. [d] The changes in intrinsic rate, repolarization time, and rate of phase 0 depolarization (dV/dt) are equivalent to those produced by similar procainamide concentrations.

Table IV. Structure–Antiarrhythmic Activity Relationship for Analogues of Procainamide in Mice[a]

Drug	Para substituent	ED_{50}, mg/kg
4[d]	$NHC(=O)CH_3$	90 } 110[b]
4[e]	$NHC(=O)CH_3$	130 }
1[d]	NH_2	140 }
1[e]	NH_2	120 } 137[b]
1[f]	NH_2	150 }
Phenolic 2	OH	157
5	NO_2	38% of mice protected at dose of 240 mg/kg[c]

[a] See text for experimental details. [b] Average. [c] All eight mice had seizures at this dose; therefore, higher doses could not be used. [d] See ref 23. [e] See ref 29. [f] See ref 28.

aromatic ring possess strong antiarrhythmic activity, whereas the analogue containing the electron-withdrawing group (NO_2) seemed to have less activity. The central nervous system toxicity of compound 6 precluded a determination of the antiarrhythmic activity of this unsubstituted analogue in the chloroform mouse arrhythmia model. A similar structure–local anesthetic activity relationship exists for analogues of procaine.[11,21,22] The ^{13}C NMR chemical shift for the aromatic carbon bearing the amide group (C-1 in Table I) was shifted upfield in the procainamide analogues with electron-donating substituents relative to that in the H and NO_2 analogues. This increase in electron density in the amide region of the former analogues probably through resonance interactions is, therefore, one of the factors influencing antiarrhythmic potency in this series.

Experimental Section

Procainamide was obtained from K and K Laboratories, Inc. Melting points were obtained on a Mel-Temp capillary apparatus and are uncorrected. Satisfactory ^1H NMR spectra (60 MHz) were obtained for all compounds in D_2O using $(CH_3)_3SiCH_2CH_2CO_2Na$ as the internal standard. ^{13}C NMR spectra were determined on a Bruker HFX 90 spectrometer at an operating frequency of 22.623 MHz, with proton decoupling, and using the deuterium resonance of D_2O for an internal lock. Spectra were taken in the Fourier transform mode with the aid of a Nicolet 1085 data system. Generally, from 1K to 4K FID's with an acquisition time of 0.4096 s were collected in 4K of core. This gives a maximum error of ±0.11 ppm.

p-Hydroxy-N-(2-diethylaminoethyl)benzamide Hydrochloride (2). Phenolic 2 was supplied by Aldrich Chemical Co., Inc., as a custom synthesis. This compound, made by reacting N,N-diethylethylenediamine with excess methyl p-hydroxybenzoate, is a white crystalline solid and has mp 128.5–130 °C. Anal. ($C_{13}H_{21}N_2O_2Cl$) C, H, N, Cl.

p-Acetoxy-N-(2-diethylaminoethyl)benzamide Hydrochloride (3). Ester 3 was supplied by Aldrich Chemical Co., Inc., as a custom synthesis and is obtained as follows. p-Hydroxybenzoic acid was allowed to react with acetic anhydride to yield p-acetoxybenzoic acid (mp 182–184 °C). This acid was converted into an acid chloride by reaction with PCl_5 in diethyl ether. The ether and $POCl_3$ were removed on a rotoevaporator and the acid chloride was distilled on a Kügelrohr apparatus at 12 mm, 140–150 °C (air bath). The acid chloride was allowed to react with N,N-diethylethylenediamine in ether at room temperature. Ester 3 precipitated out of solution and is a white crystalline solid (mp 133 °C). Anal. ($C_{15}H_{23}N_2O_3Cl$) C, H, N.

p-Acetamido-N-(2-diethylaminoethyl)benzamide Hydrochloride (4). Compound 4, the internal standard for the quantitation of phenolic 2 by TLC densitometry, was prepared as previously described.[23]

p-Nitro-N-(2-diethylaminoethyl)benzamide Hydrochloride (5). A solution of N,N-diethylethylenediamine (0.0368 mol) and p-nitrobenzoyl chloride (0.0370 mol) in chloroform was stirred for 3.5 h at room temperature to yield compound 5 (80%). The melting point of this white crystalline solid (recrystallized from 2-propanol) is 163–165 °C (lit.[24] 164–165 °C). N-(2-Diethylaminoethyl)benzamide hydrochloride (6) was synthesized in a similar manner.

Determination of in Vivo Pharmacologic Activity in the Dog. The antiarrhythmic activity of phenolic 2, ester 3, and procainamide (1) and their effect on mean arterial blood pressure were determined in anesthetized open-chest mongrel dogs weighing between 11 and 13.7 kg as previously described.[23] Stated briefly, aconitine was injected into the right atrial wall to produce the arrhythmia.[25] In this study no attempts were made to produce circus movement flutter first. The animals then received infusions of drug in saline iv at a rate shown in Table II. When the atrial rate slowed, the infusion was discontinued and plasma was withdrawn for analysis. In addition, two dogs injected with aconitine were infused with only saline. These animals were followed for 1 h and served as controls against spontaneous conversion to a normal rhythm (Table II).

Effects of Phenolic 2 on Canine Purkinje Fiber Single Cell Potentials. Twenty-four mongrel dogs were used for this study. Sodium pentobarbital (30 mg/kg iv) was injected and the hearts were exposed through a right lateral thoractomy. After heparin treatment, the hearts were rapidly removed and the Purkinje fibers (false tendons) excised from both ventricles. The fibers were placed in a 3-ml Lucite chamber and perfused with an oxygenated (95% O_2–5% CO_2) Tyrode's solution (NaCl 137 mM, KCl 4mM, NaH_2PO_4 1.8 mM, $CaCl_2$ 2.7 mM, $MgCl_2$ 0.5 mM, dextrose 5.5 mM, $NaHCO_3$ 12 mM) at a rate of 10–15 ml/min. The temperature was maintained at 37 °C. The preparations were stimulated at 90 beats per minute with a voltage twice threshold and a duration of 1 ms. The stimuli were delivered through polyethylene-coated silver bipolar electrodes from a Model S88 Grass stimulator and isolated from ground with a stimulus isolation unit (Grass Model SLU5).

Single cell action potentials were obtained using standard electrophysiological techniques[26] and were displayed for analysis on a Model 1858 Honeywell visicorder. Control recordings were taken following a 30-min period of stimulation and measurements of total action potential voltage, maximum diastolic potential,

dV/dt, and time for repolarization were made. In experiments demonstrating spontaneous activity, the stimulator was cut off at the end of each test period and the fibers were allowed to beat spontaneously for 10 min at which time a regular rhythm was usually established. The spontaneous rates were used as a measure of automaticity. In addition, drug-induced changes in automaticity were studied in eight fibers which had not been stimulated. The results obtained from the two groups of fibers were not significantly different and the automaticity data were pooled.

After control observations, the fibers were perfused with Tyrode's solution containing phenolic **2** (5–20 μg/ml) for 30 min and the measurements repeated. The fibers were then washed for 30–60 min with plain Tyrode's and final measurements taken. The data were analyzed using a paired t test.

Determination of Antiarrhythmic Activity in Mice. The antiarrhythmic activity of phenolic **2** and compound **5** was determined in 28–35 day-old ICR male mice (av wt 25 g) as previously described.[23] Ten minutes after the drug was injected ip, the mice were put in a jar containing chloroform and anesthetized. Mice that did not show intermittent or continuous ventricular fibrillation following chloroform inhalation to the point of cessation of breathing were considered protected by the drug. Mice that showed definite ventricular fibrillation were considered unprotected even if the fibrillation subsequently changed to another rhythm. A dose–response relationship was ascertained using eight mice at each dosage interval.

The acute toxicity of phenolic **2** and procainamide (**1**) was determined in groups of ten ICR male mice (28–35 day old, av wt 26 g). After the drug was injected ip, the mice were observed at intervals of 10, 15, 30, 60, 120, and 210 min to determine the mortality caused by the drug.

Determination of Phenolic 2 in Plasma. To a 15-ml glass-stoppered centrifuge tube, 2 ml of plasma or diluted plasma, 8 μg of internal standard (compound **4**) and 500 mg of NaCl were added. After the pH was adjusted to 10 by the addition of 5% sodium carbonate solution, the aqueous phase was extracted with 10 ml of chloroform containing 5% isoamyl alcohol. The mixture was gently shaken until equilibrium was reached and then centrifuged. The organic phase was reduced to 20 μl on a Buchler rotary Evapo-Mix and 1.6 μl was spotted on a silica gel F_{254} TLC plate (Brinkmann). The R_f of phenolic **2** is 0.39 and that of internal standard is 0.52 when the plate is developed with a solution of benzene, 28% aqueous ammonia, acetone, and dioxane (5:8:80:5). After development, the plate was scanned with a Schoeffel Model SD 3000 spectrodensitometer in the reflectance mode illuminating with 260-nm light and measuring the total fluorescent emission to determine the amount of fluorescent quenching caused by these compounds. The area of peaks corresponding to phenolic **2** and internal standard were measured and plasma concentrations of phenolic **2** were determined from standard curves. The calibration curve for phenolic **2** was linear for the range of 0.1–0.8 μg of compound on the TLC plate with a standard deviation of the method of 8% for phenolic **2**.

Determination of Ester 3 in Plasma. This compound was measured in a manner similar to the determination of phenolic **2** in plasma except that no internal standard was used and the plasma extract containing ester **3** and phenolic **2** was spotted on a silica gel F_{254} TLC plate which was then developed with a solution of acetone and methanol (1:1). The R_f of phenolic **2** is 0.17 and that for ester **3** is 0.24. Extraction of ester **3** standards out of saline and plasma (with and without 10^{-5} M physostigmine) was performed.

Determination of Procainamide in Plasma. Procainamide (**1**) was quantitated by the TLC densitometric method previously described.[20]

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