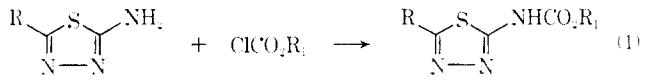

Antimicrobial Activity.—The compounds listed in Table I were tested *in vitro* against *Escherichia coli*, *Bacillus anthracis*, and *Staphylococcus aureus*, using a tube dilution method and the disk diffusion method.[3] Of all the compounds tested, *N*-[5-trifluoromethyl-1,3,4-thiadiazol-2-yl]carbamic acid *n*-butyl ester (**22**) at 200 μg/ml inhibited the growth of *B. anthracis*, after 24 hr incubation at 37°. All other compounds showed only slight antimicrobial activity probably due to low solubility and/or poor diffusion.

Experimental Section[4]

***N*-[5-Trifluoromethyl-1,3,4-thiadiazol-2-yl]carbamic Acid *n*-Butyl Ester (22).**—2-Amino-5-trifluoromethyl-1,3,4-thiadiazole[2] (1.67 g, 0.01 mole) in 15 ml of dry $CHCl_3$, was refluxed for 5 hr with 1.64 g (0.012 mole) of *n*-butyl chloroformate. Removing the solvent followed by crystn of the residue from 50% aq EtOH, gave 2.42 g (90%) of **22**, white crystals, mp 158°. The other compds listed in Table I were prepared similarly.

Acknowledgments—The authors gratefully acknowledge the constant encouragement of Dr. A. Alikhani of Tehran University.

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(4) Melting points were taken on a Kofler hot stage microscope and were uncorrected. The ir spectra were determined with a Leitz Model III spectrograph. Nmr spectra were obtained on a Varian A60A instrument.

A New Series of Antiarrhythmic Agents. The 2-Aminotetralins[1a]

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The synthesis and analgetic properties of some substituted 2-aminotetralins were previously reported by Martin, *et al.*[2] Subsequent pharmacological investigations have shown these compounds to possess marked negative chronotropic and mild negative inotropic activities in spontaneously beating guinea pig atria.[3] In order to determine the relationship between the toxicity and the antifibrillatory activity of the series of 2-aminotetralins *in vivo* a screening method developed by Lawson[4] was used. This procedure enables rapid screening of the compounds with a large number of mice and a relatively small quantity of drug. The mice are pretreated with the test compounds at a set period before induction of arrhythmias with $CHCl_3$. During this period symptoms of acute toxicity (ataxia, convulsions, etc.) can be noted. The mouse is then subjected to a $CHCl_3$ atmosphere until respiration ceases. This procedure induces a high incidence of ventricular fibrillation in the animals. In the exposed hearts the fibrillatory movements can be observed and the cardiac rate counted. The protection afforded by pretreatment with an antiarrhythmic compound can then be determined.

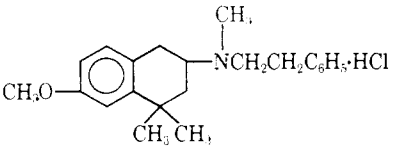
Results and Discussion

N-Methyl-*N*-phenylethyl-1,2,3,4-tetrahydro-6-methoxy-4,4-dimethyl-2-naphthylamine hydrochloride (**1**) had previously been shown to possess potent myocardial depressant activity.[2] Because of its past indications of antiarrhythmic activity and because of its availability **1** was investigated in detail. The results are shown in Table I. The procedure used to induce

TABLE I
CHLOROFORM-INDUCED CARDIAC ARRHYTHMIAS BEFORE AND AFTER TREATMENT WITH **1**

Dose (mg/kg)	Number of mice	% protected	Average rate (beats/min ± S.E.)	% toxic	% fatal
Controls	40	0.3	351.91 ± 16.61	0.0	0.0
50	40	5.0	289.62 ± 10.63	0.0	0.0
75	40	32.5	228.00 ± 14.83	0.0	0.0
100	40	55.0	166.08 ± 13.19	0.0	0.0
125	40	72.5	146.88 ± 13.04	15.0	0.0
150	40	92.5	136.09 ± 7.74	47.5	0.0
175	40	100.0	124.24 ± 6.75	69.4	7.5

arrhythmias *in vivo* resulted in the production of arrhythmia and fibrillation in 100% of the nontreated control animals. The ED_{50} of **1** was 94.41 mg/kg



1

(0.262 mmole/kg). The average heart rate corresponding to this dose was graphically estimated to be 160 beats/min. No toxicity was noted at this ED_{50} dose. Compound **1** showed a good degree of antiarrhythmic activity in relation to its toxicity. Only at the higher doses, approaching 100% protection, does significant toxicity result, generally manifested as mild ataxia. Convulsions and death occurred only at the highest dose studied. This compound was used as a standard for comparison of all the other analogs tested. The doses used for the other compounds were the equimolar equivalents of the ED_{50} dose for **1** (0.262 mmole/kg). The results are shown in Table II. The analogs **2–5** have chemical structures most closely resembling **1**. These compounds involve only substitutional changes on N atom. They varied greatly in activity ranging from 0 to 100% protection from fibrillation. The

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(1) (a) This research was supported in part by a Washington State Heart Association Grant No. 6970-6. (b) National Science Foundation Fellow, 1969–1970.

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TABLE II: ANTIARRHYTHMIC 2-AMINOTETRALINS[a]

No.	X	Y	R_1	R_2	R_3	R_4	Number of mice	Equimolar dose, (mg/kg)	Average rate (beats/min ± S.E.)	% protected	% toxic	% fatal
1	H	OCH_3	CH_3	$(CH_2)_2C_6H_5$	H	CH_3	240	94.41	160.00	50.0	0.0	0.0
2	H	OCH_3	H	CH_3	H	CH_3	40	67.24	325.13 ± 10.58	0.0	0.0	0.0
3	H	OCH_3	H	$(CH_2)_3CH_3$	H	CH_3	40	78.35	125.74 ± 25.40	100.0	48.0	32.5
4	H	OCH_3	H	$(CH_2)_2C_6H_5$	H	CH_3	10	91.25	97.30 ± 12.04	100.0	100.0	70.0
5	H	OCH_3	H	H	H	CH_3	30	63.56	271.19 ± 19.24	20.0	0.0	0.0
6	H	OCH_3	H	H	H	C_6H_5	30	79.90	196.88 ± 15.81	46.7	0.0	0.0
7	H	OCH_3	CH_3	CH_3	H	C_6H_5	30	87.31	184.02 ± 10.91	66.7	83.3	0.0
8	H	H	CH_3	CH_3	H	CH_3	10	63.08				100.0
9	OCH_3	H	H	H	H	CH_3	20	63.56	286.54 ± 23.54	0.0	0.0	0.0
10[b]	H	OH	H	CH_3	H	CH_3	30	75.34	301.01 ± 11.22	6.7	0.0	0.0
11	H	H	H	H	CH_3	CH_3	30	59.38	147.52 ± 13.42	73.3	0.0	0.0
12	H	H	H	CH_3	CH_3	CH_3	30	63.08	184.03 ± 7.02	56.7	80.0	0.0
13	H	H	CH_3	CH_3	CH_3	CH_3	30	66.78	139.88 ± 17.66	76.9	100.0	13.3
14	H	OCH_3	H	H	CH_3	CH_3	15	67.26	222.68 ± 24.64	26.6	0.0	0.0
15	H	H	H	H	$CH_2C_6H_5$	CH_3	30	79.42	164.44 ± 13.82	69.0	93.1	3.3
16	H	H	H	CH_3	$CH_2C_6H_5$	CH_3	30	83.13	142.96 ± 19.49	84.2	84.2	36.7
17	H	H	CH_3	CH_3	$CH_2C_6H_5$	CH_3	30	86.83	275.92 ± 11.36	10.0	73.3	0.0

[a] Employed as HCl salts with the exception of **10**. [b] HBr salt.

toxicity also varied greatly. In general, the most active analogs were the most toxic. The externally observable toxic responses in mice to the 2-aminotetralins appeared similar to amphetamine toxicity. The animals first exhibited nervousness that was followed by skeletal muscle tremors. In the cases of more acute toxic responses, the tremors progressed to frank convulsions with loss of coordinated respiration and often death. Loss of equilibrium or ataxia typically preceded the convulsive state. Compound **2** showed no activity or toxicity, whereas **3** and **4** resulted in significant toxicity in the doses used. Compound **5** showed 20% protection with the lack of any observed toxicity. Compounds **6** and **7** possess substitutions on N and the substitution of Ph for a Me at the 4 position on the saturated ring. Both of these compounds exhibited favorable protection. Compound **6** appeared somewhat less effective than **1** in producing antifibrillatory protection, 46.7% compared with 50% for **1**, and lacks toxicity at this dose. Compound **7** gives better antifibrillatory protection, 66.7%, but ataxia and mild convulsions resulted in 83.3% of the animals.

Compounds **8** and **9** have substitutions on both the N and the aromatic portion of the ring system. These compounds exhibited very little antiarrhythmic efficacy. Compound **8** exhibited very high toxicity resulting in death in 100% of the animals. Compounds **9** and **10** both showed very low antifibrillatory effect. Compounds **11–14** differ from **8** and **9** by the introduction of Me at C-2. All of these compounds show antiarrhythmic activity. In fact, **11**, **12**, and **13** exhibited considerable toxicity. Compound **11** showed good protection but no toxicity. Compounds **15–17** lack substitution on the aromatic ring but possess a benzyl group at C-2. The protection provided by these agents ranged from 10 to 84.21%. All of these compounds possessed a high degree of toxicity. Compounds **15** and **16** may show some antiarrhythmic effectiveness at lower doses, however the unfavorable toxicity:protection ratio for **17** and **18** would appear to eliminate them as possible antiarrhythmic agents.

Four standard antiarrhythmic agents (quinidine, *dl*-propranolol, procainamide, and lidocaine) were included in the study for comparative purposes. The results are shown in Table III.

The results obtained for the standard antiarrhythmic agents compare favorably with those reported by Lawson.[4] The ED_{50} dose reported for quinidine·SO_4 was 72 mg/kg, *dl*-propranolol·HCl, 35 mg/kg, and procainamide·HCl, 102 mg/kg. The dose which was used was equimolar to the ED_{50} dose for **1**. This dose gave the following results: quinidine·SO_4, 118 mg/kg, gave 83.33% protection, *dl*-propranolol·HCl, 77 mg/kg, gave 60.00%, and procainamide·HCl, 59 mg/kg, gave 13.33% protection. The toxicity varied greatly with quinidine and procainamide being nontoxic in the dose used but lidocaine showed toxicity in 86.7% of the animals and *dl*-propranolol in 46.7%. None of the 4 compounds caused fatalities to occur.

Although the limited number of 2-aminotetralins available restricts the development of any thorough structure–activity theory for antifibrillatory activity at this time, some important observations may be made which should aid in the development of additional analogs with antiarrhythmic activity. Consideration of

TABLE III
CHLOROFORM-INDUCED CARDIAC ARRHYTHMIAS AFTER TREATMENT WITH 4 COMMON, CLINICALLY USED ANTIARRHYTHMIC AGENTS

Drug	Number of mice	Equimolar dose (mg/kg)	Average rate (beats/min ± S.E.)	% protected	% toxic	% fatal
Quinidine·SO_4	40	118.47	129.05 ± 13.42	83.3	0.0	0.0
dl-Propranolol·HCl	40	77.47	185.61 ± 12.45	60.0	46.0	0.0
Procainamide·HCl	40	58.92	291.57 ± 17.11	13.3	0.0	0.0
Lidocaine·HCl	40	70.58	182.77 ± 14.78	56.7	86.7	0.0

structural requirements for substitution on N is unclear since good protection resulted from compounds having primary, secondary, and tertiary amine groups. In almost all cases, the primary amine derivatives caused greatly reduced toxicity when compared with the analogous substituted compounds (see **5**, **6**, **11**, **14**). In the comparison of the secondary amine analogs, an increase in the lipid solubility or hydrophobic character of the substituent increased both the antifibrillatory protection and the toxicity (see **3**, **4**). The substitution of more hydrophobic groups at any position on the saturated ring increased the antifibrillatory activity and also the toxicity (see **6**, **7**, and **9**–**17**). This may be due to an enhanced transport capability related to the relative partition characteristics of the molecule, as described by Levy,[5] rather than any specific receptor requirement.

From the results it is apparent that some of the 2-aminotetralins tested possess very good antifibrillatory protection properties compared with the commonly used antiarrhythmic agents and show little or no acute toxicity in effective doses. The relationship between toxicity and antiarrhythmic efficacy is very important. Although many agents possessing relatively potent antiarrhythmic activity have been developed, a great number of them have exhibited untoward toxic properties. Thus the absence of noticeable toxicity in dosage levels resulting in good antiarrhythmic activity in the screening experiments in highly desirable.[6]

Experimental Section

Adult male mice (Carworth strain CF 1) (22–28 g were weighed and injected ip with the test compound and placed in separate glass containers. The injections were given to groups of animals with 2-min intervals between individual animal treatments. After injection each animal was observed for toxic symptoms. Exactly 10 min after treatment each animal was transferred to a 250-ml beaker that held a cotton pad saturated with 20 ml of $CHCl_3$. The animal was removed immediately after respiratory arrest and the heart was quickly exposed by removing the anterior thoracic wall without touching the heart with the surgical instruments. The heart rate and fibrillatory movements were recorded with the aid of a stop watch and a binocular dissecting microscope. Any animal showing fibrillation or ventricular rates in excess of 200 beats/min was defined as unprotected. All animals exhibiting rates below 200 beats/min were reported as protected from fibrillation. In all cases where sufficient drug was available groups of 40 mice were used. Values for the percentage of animals protected were used to calculate an ED_{50} according to Litchfield and Wilcoxon.[7] All other statistical calculations were performed according to Steel and Torrie.[8]

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Antimalarial and Other Biological Activities of Some 2′-Alkyl and 2′-Aryl Derivatives of Cinchona Alkaloids[1]

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We report an investigation undertaken primarily to compare with activities of 2′-alkyl and 2′-aryl derivatives of quinine, quinidine, and their 10,11-dihydrides against experimental malarias caused by *Plasmodium berghei and P. gallinaceum* in mice and chicks, respectively, in the framework of studying the effect of blocking the 2 position of the quinoline nucleus.[2-4] The substances were also examined for cardiac antiarrythmic and antibacterial activity.

The 2′-alkyl and 2′-aryl derivatives were prepared from the alkaloid ar-*N*-oxides and appropriate Grignard or Li organometallic reagents (*cf.* ref 5). Satisfactory yields were obtained with simple primary alkyl Grignard reagents. *i*-BuMgBr and *sec*-RMgBr yielded predominantly the parent diamine along with a low yield of the corresponding 2′-alkyl derivative. The reaction failed with *t*-BuMgBr. However, 2′-*t*-Bu and 2′-cyclopropylquinidine were obtained from quinidine ar-*N*-oxide and the corresponding Li alkyl. The 10,11-dihydroquinidines and dihydroquinines were obtained by catalytic hydrogenation of the appropriate quinidine and quinine derivative I and II, respectively. Under the conditions used, no loss of halogen from the 2′-halophenyl derivatives was observed. Physical constants for the new compounds and other data are listed in Tables I–IV.

Antimalarial Activity.—Activity was measured against *P. berghei* in mice and *P. gallinaceum* in chicks by previously described methods.[6] The data in Table I show that 2′-alkyl substituents lower the maximum tolerated dose (MTD) thereby restricting testing to low dosage levels. Also, 2′-aryl substituents confer in-

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