treated glass fiber filter strips, followed by rapid washing with ice cold saline. The filters are counted for radioactivity. All data points represent duplicate determinations. Binding curves are fit to the data points using the SCAFIT routine and a two-site model. Reported binding constants are for the lower affinity site and are generally presumed to represent A-receptor binding.

Cyclic GMP Assays. Confluent monolayers of BTAEC or rVSMC are incubated in assay medium with a test compound at 37 °C for 2 h. After termination of the incubation by acidification with $HClO_4$ to 1.6%, the supernatant is collected by centrifugation. Cyclic GMP was determined after acetylation by radioimmunoassay. Data are expressed relative to the maximum response (=100%) observed for ANP[5-28]. All data points represent duplicate determinations.

In Vivo Studies. All rat experiments are performed using male Sprague-Dawley rats. The animals are anesthetized with Inactin (100 mg/kg, ip) and catheters placed in the femoral artery and vein for measurement of arterial blood pressure and infusion of drugs, respectively. A bladder catheter is inserted for timed collections of urine. After completion of surgical procedures the animals are allowed to equilibrate for 90 min. Test agonists are studied via a stepped-dose protocol, in which eight half-log incremental doses of peptide are infused sequentially for 15-min intervals. Mean arterial pressure, urine volume, and urinary sodium and cGMP are measured over each infusion period. Alternatively, renal and hemodynamic parameters are recorded following bolus injection of a single dose of peptide.

Male purpose-bred beagle dogs are anesthetized with pentobarbital sodium (30 mg kg^{-1} min^{-1}, iv, followed by 5 mg kg^{-1} h^{-1}), thermostatically regulated, and surgically prepared with femoral artery and vein catheters for blood pressure measurement and drug infusion respectively, ureteral cannulae for bilateral urine collection, and electromagnetic blood flow probes on both renal arteries. Inulin was continuously infused for measurement of glomerular filtration rate. Following equilibration for 1 h, two control clearance periods (30 min each) are followed by three drug infusion periods (0.03, 0.3, and 3.0 μg kg^{-1} min^{-1}, iv) and two recovery periods, in sequence. An arterial blood sample is taken at the midpoint of each clearance period for determination of plasma inulin, renin activity, and aldosterone levels. Urine samples are collected over each 30-min clearance period and are assayed for inulin, sodium, potassium, and cGMP.

Use of animals was in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health and was approved by Abbott's Institutional Animal Care and Use Committee.

Acknowledgment. The authors particularly thank Diane M. Beno, Terry P. Dillon, Mark A. Holst, Carol A. Marselle, Eugene I. Novosad, and Siobhan Yeh for valuable discussions and invaluable assistance.

Supplementary Material Available: Chemistry experimentals and analytical data for the compounds investigated in this article (13 pages). Ordering information is given on any current masthead page.

Synthesis and Biological Action of the Aminotetrahydroisoquinocarbazoles and Related Compounds: A New Class of Compounds with Antiarrhythmic Activity

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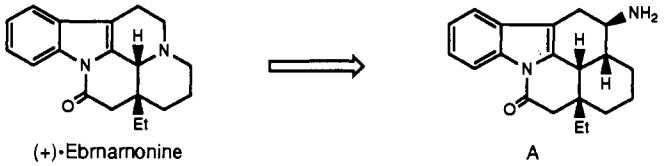
A series of 12-aminotetrahydroisoquinocarbazoles and related compounds were synthesized using an intramolecular Diels-Alder reaction and screened for antiarrhythmic activity in chloroform-induced ventricular arrhythmias in mice. Several compounds showed more potent activity than disopyramide. There was some correlation between substituents on aromatic ring and angular position, and antiarrhythmic activity. An amino group or some functional groups containing an amino group on C-12 seemed to be essential to exhibit the activity. Ring size also influenced the activity. The compound (+)-10 (RS-2135) had the most favorable combination of antiarrhythmic activity and toxicity and was selected for further evaluation.

The problem of the risk of sudden death following myocardial infarction has not been resolved though many antiarrhythmic agents have been developed for the treatment of arrhythmia. Current antiarrhythmic therapy is far from satisfactory, because it is often ineffective and accompanied by serious adverse effects. The development of new antiarrhythmic agents that are effective and safe in the treatment of ventricular arrhythmia has been a major goal of cardiovascular research and development of pharmaceutical firms.[1] In the course of synthetic studies on carbazole derivatives, we found that some pentacyclic fused carbazole derivatives (for example A) which were prepared[2] as eburnamonine analogues[3,4] by an intramolecular Diels-Alder reaction[5] had potent antiarrhythmic activity. Afterward we synthesized a series of related fused carbazole derivatives and tested their antiarrhythmic activities to clarify the structure-activity relationships. In this paper, we wish to report our results on the chemistry and pharmacology of a new class of antiarrhythmic agents, aminotetrahydrocarbazoles, and related compounds.



(+)-Eburnamonine A

Chemistry

A series of aminoisoquinocarbazole compounds was

(1) Steinberg, M. I.; Lacefield, W. B.; Robertson, D. W. Class I and III Antiarrhythmic Drugs. *Annu. Rep. Med. Chem.* 1986, *21*, 95.

(2) Shimoji, Y.; Saito, F.; Sato, S.; Tomita, K.; Morisawa, Y. Synthesis of Fused Aminotetrahydrocarbazole Compounds. *Heterocycles*. Submitted.

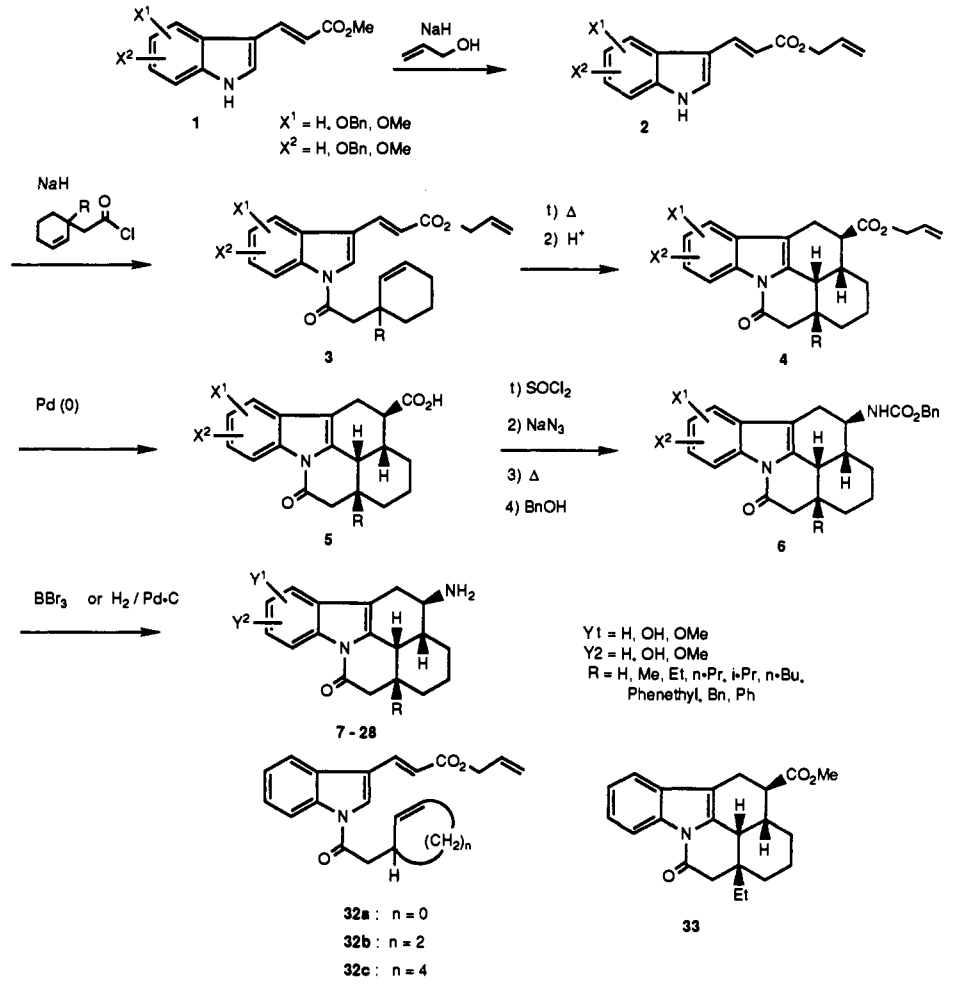
(3) Imanishi, T.; Miyashita, K.; Nakai, A.; Inoue, M.; Hanaoka, M. 1,6-Dihydro-3(2H)-Pyridinones as synthetic intermediates. Total synthesis of (±)-Eburnamonine. *Chem. Pharm. Bull.* 1982, *30*, 1521.

(4) Friedman, Y.; Meller, E.; Hallock, M. Effects of Conformationally Restrained Analogues of Serotonin on Its Uptake and Binding in Rat Brain. *J. Neurochem.* 1981, *36* (3), 931.

(5) Shimoji, Y.; Saito, F.; Sato, S.; Tomita, K.; Morisawa, Y. Intramolecular Diels-Alder Reaction of 3-(1H-indol-3yl)-2-Propenoate. Synthesis of Fused Indole Compounds. *Heterocycles* 1989, *29*, 1871.

0022-2623/92/1835-0816$03.00/0

Scheme I



prepared from indolylpropenoate via six steps using an intramolecular Diels–Alder[5] reaction and Curtius rearrangement, as shown in Scheme I.

Ester-exchange reaction of indolylpropenoate **1** with allyl alcohol in the presence of sodium hydride gave allyl ester **2**. Acylation of **2** by cyclohexenylacetyl chloride led to amide **3** in good yield. Intramolecular Diels–Alder reaction of **3** was carried out under reflux in mesitylene, followed by double bond migration in the presence of proton acid to give pentacyclic fused carbazole derivative **4** in good yield. Deprotection of ester function in **4** with Pd-$(PPh_3)_4$–PPh_3 led to carboxylic acid **5**. The stereochemistry of **5** was confirmed by comparison with compound **33**, of which stereochemistry had been determined by X-ray analysis.[5] Curtius rearrangement of the carboxylic azide which was prepared from the carboxylic acid by chlorination with $SOCl_2$ and azidation with NaN_3, followed by alcoholysis by benzyl alcohol, gave carbamate **6** in good yield. Deprotection of the hydroxy and amino groups was carried out by the two different methods. For the carbamates that had a methoxy group on the aromatic ring, the reaction of **6** with BBr_3 in CH_2Cl_2, followed by alkali and acid treatment, afforded the amine as a salt. On the other hand, the carbamates that did not have a methoxy group were hydrogenated in the presence of 10% Pd–C in EtOH containing a small amount of hydrochloric acid to give the amine as a hydrochloride salt, in quantitative yield.

Pyridocarbazole derivative **29**, cyclopentindroquinoline derivative **30**, and cycloheptindroquinoline derivative **31** were also synthesized from the amides (**32a–c**) according to the same procedure as the aminoisoquinocarbazole compound **7**.

The hydrazide, ester, and amide compounds were prepared as shown in Scheme II. Treatment of the mixed anhydride of **5a** with hydrazine, followed by hydrogenolysis in the presence of 10% palladium–charcoal gave 12-hydrazide **35**. *tert*-Aminoalkyl hydrazide or amide was obtained by the condensation of carboxylic acid **5b** with the corresponding hydrazine or amine using diethyl cyanophosphonate, followed by hydrogenolysis deprotection. Carboxylic acid **5b** was esterified by bromoethyl alcohol using dicyclohexylcarbodiimide and 4-(dimethylamino)-pyridine, and treated with *N*-methylpiperazine, followed by hydrogenolysis to afford aminoalkyl ester **39**. Indole compound **42**, which was not fused with another ring but had the same number of carbon atoms as **7**, was prepared as shown in Scheme III. Aldehyde **40** was treated with nitromethane and ammonium acetate, followed by hydrogenation to afford amine **42** as a hydrochloride salt.

Since compound **10** had the most favorable combination of antiarrhythmic activity and toxicity, optically active **10** was prepared by optical resolution via diastereomer separation to examine the activities of the resolved enatiomers. Racemic **10** was condensed with L-Boc-proline by the DEPC method, and diastereomers **43** and **44** were separated by silica gel column chromatography (Scheme IV). Each diastereomer was treated with 6 N hydrochloric acid to afford optically active (+)-**10** and (−)-**10**, respectively. The absolute configuration of (+)-**10** and (−)-**10** was determined by the synthesis of (+)-**10** using optically active (*R*)-2-(2-cyclohexenyl)acetyl chloride.[6]

(6) Fukazawa, T.; Hashimoto, T. Unpublished result.

Scheme II

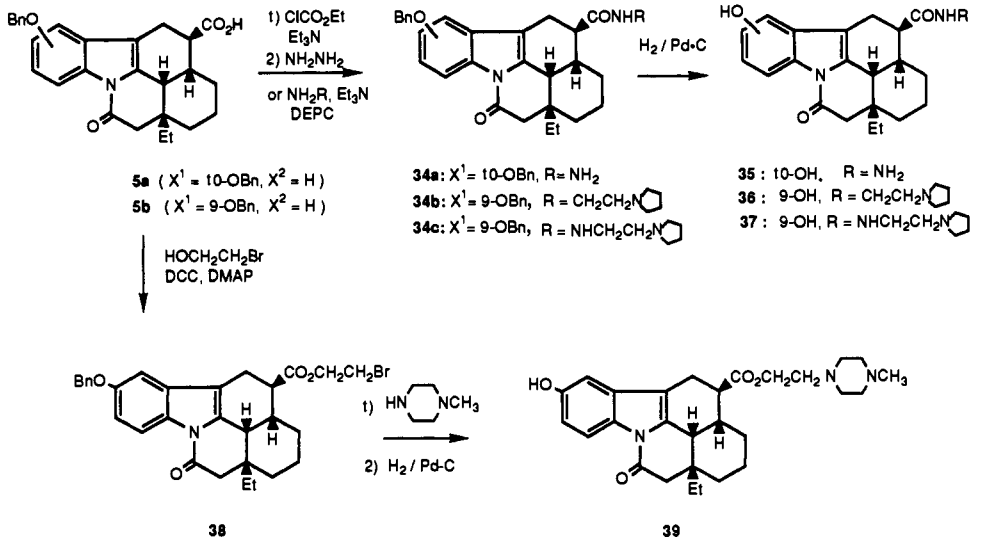
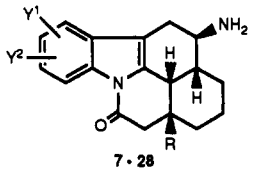


Table I. Physical and Biological Properties of 12-Aminoisoquinocarbazole



no.	Y^1	Y^2	R	salt	mp, °C	recrystn solvent	formula[a]	ED_{50},[b] mg/kg
7	H	H	H	maleate	221–222 dec	EtOH–AcOEt	$C_{18}H_{20}N_2O \cdot C_4H_4O_4$	32.0
8	H	H	Et	maleate	216–216.5 dec	EtOH–AcOEt	$C_{20}H_{24}N_2O \cdot C_4H_4O_4$	14.0
9	H	H	Pr	maleate	224–225 dec	EtOH	$C_{21}H_{26}N_2O \cdot C_4H_4O_4$	3.6
10	10-OH	H	H	HCl	260–262 dec	EtOH–AcOEt	$C_{18}H_{20}N_2O_2 \cdot HCl \cdot 1.5H_2O$	10.0
(+)-10	10-OH	H	H	HCl	260–265 dec	MeOH-*i*-Pr_2O	$C_{18}H_{20}N_2O_2 \cdot HCl \cdot 1.5H_2O$[c]	5.0
11	10-OH	H	Me	HCl	>300	EtOH–AcOEt	$C_{19}H_{22}N_2O_2 \cdot HCl \cdot 1.5H_2O$	8.0
12	10-OH	H	Et	HCl	>300	EtOH–MeOH	$C_{20}H_{24}N_2O_2 \cdot HCl \cdot 0.5H_2O$	6.0
13	10-OH	H	*n*-Pr	HCl	>300	EtOH	$C_{21}H_{26}N_2O_2 \cdot HCl \cdot 0.5H_2O$	20.0
14	10-OH	H	$PhCH_2CH_2$		250–252	EtOH	$C_{26}H_{28}N_2O_2$	>40
15	9-OH	H	H		235–236	EtOH	$C_{33}H_{32}N_2O_2$	>40
16	9-OH	H	Me		292–294	EtOH	$C_{19}H_{22}N_2O_2$	>40
17	9-OH	H	Et		270–273	dioxane	$C_{20}H_{24}N_2O_2 \cdot 0.5H_2O$	22.5
18	9-OH	H	*n*-Pr		>300	DMF–EtOH	$C_{21}H_{26}N_2O_2$	4.2
19	9-OH	H	*i*-Pr		306–308	EtOH	$C_{21}H_{26}N_2O_2 \cdot 0.5H_2O$	8.4
20	9-OH	H	*n*-Bu		287–290	dioxane	$C_{22}H_{28}N_2O_2$	12.0
21	9-OH	H	Bn		282–284	dioxane	$C_{25}H_{26}N_2O_2 \cdot 0.5H_2O$	>40
22	9-OH	H	$PhCH_2CH_2$		274–275	dioxane	$C_{26}H_{28}N_2O_2 \cdot H_2O$	>40
23	9-OH	H	Ph		288–290	dioxane	$C_{24}H_{24}N_2O_2$	12.0
24	9-OH	8-OH	H	HCl	>300	EtOH	$C_{18}H_{20}N_2O_3 \cdot HCl \cdot 0.5H_2O$	>40
25	9-OH	8-OH	Me	HCl	>300	MeOH	$C_{19}H_{22}N_2O_3 \cdot HCl \cdot H_2O$	34.0
26	9-OH	8-OH	Et	HCl	>300	MeOH	$C_{20}H_{24}N_2O_3 \cdot HCl$	7.0
27	9-OH	8-OH	*n*-Pr	HCl	>300	MeOH	$C_{21}H_{26}N_2O_3 \cdot HCl \cdot H_2O$	20.0
28	9-MeO	8-MeO	Et	HCl	297–298 dec	EtOH	$C_{22}H_{28}N_2O_3 \cdot HCl \cdot 0.5H_2O$	34.0
							disopyramide	11.8

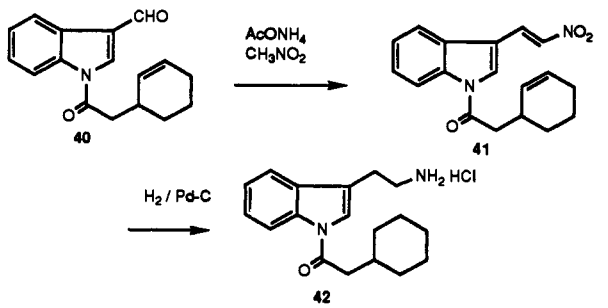
[a] Compounds were analyzed for C, H, and N, and results agreed to ±0.4% of the theoretical values. [b] Antiarrhythmic activity in chloroform-induced arrhythmias in mice. [c] $[\alpha]^{23}_D$ +66.44 (c = 0.45, MeOH).

Biological Tests

The synthesized compounds were screened for antiarrhythmic activity in chloroform-induced ventricular arrhythmia in mice. The tested compounds were intraperitoneally administered 15 min before mice were exposed to chloroform vapor. Antiarrhythmic activity of the tested compounds was evaluated according to Block's methods[7] and ED_{50} was calculated (Tables I–III).

The electrophysiological effects of compound (+)-10 were evaluated in isolated canine Purkinje fibers. The transmembrane potentials were recorded by using the standard microelectrode technique.

Scheme III



(7) Block, A. J. Prevention of Chloroform-induced Ventricular Tachycardia in Mice as a Index of Antiarrhythmic Activity. *Life Sci.* 1981, *28*, 2623.

Scheme IV

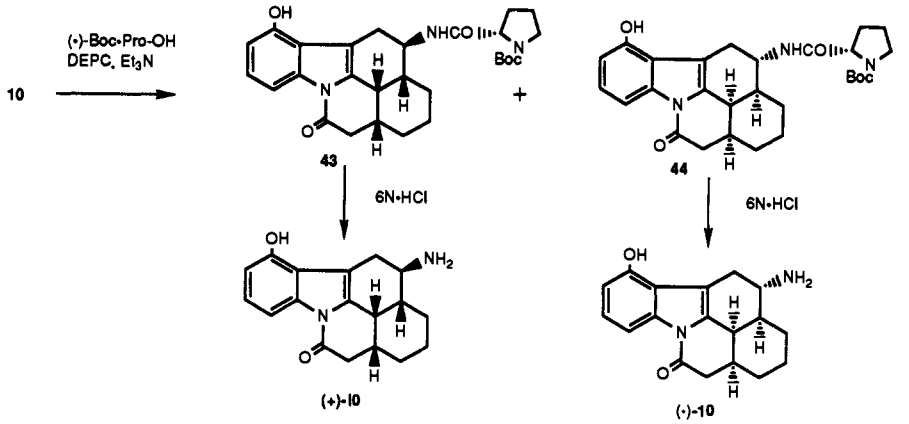
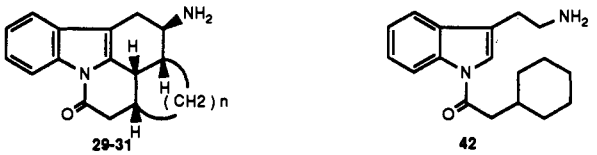


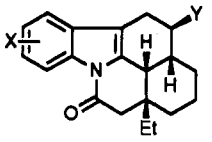
Table II. Physical and Biological Properties of the Compounds Which Have Skeleton Other Than Isoquinocarbazole



no.	*n*	salt	mp, °C	recrystn solvent	formula[a]	ED_{50},[b] mg/kg
29	0	maleate	221–222 dec	EtOH	$C_{15}H_{16}N_2O \cdot C_4H_4O_4$	>40
30	2	maleate	197–198 dec	EtOH–AcOEt	$C_{17}H_{18}N_2O \cdot C_4H_4O_4$	40
31	4	maleate	213 dec	EtOH–iPr_2O	$C_{19}H_{22}N_2O \cdot C_4H_4O_4$	80
42		HCl	>250	*i*-PrOH	$C_{18}H_{24}N_2O \cdot HCl$	48

[a] Compounds were analyzed for C, H, and N, and results agreed to ±0.4% of the theoretical values. [b] Antiarrhythmic activity in chloroform-induced arrhythmias in mice.

Table III. Physical and Biological Properties of the Compounds Which Have Modified Function at C-12 of Isoquinocarbazole



no.	X	Y	mp, °C	recrystn solvent	formula[a]	ED_{50},[b] mg/kg
35	10-OH	$CONHNH_2$	280–281 dec	80% EtOH	$C_{21}H_{25}N_3O_3$	>40
36	9-OH	$CONHCH_2CH_2N$⟨⟩	amorphous		$C_{27}H_{35}N_3O_3 \cdot 4H_2O$[c]	>40
37	9-OH	$CONHNHCH_2CH_2N$⟨⟩	amorphous		$C_{27}H_{36}N_4O_3 \cdot 0.5H_2O$	22.5
39	9-OH	$COOCH_2CH_2N$⟨⟩NCH_3	amorphous		$C_{28}H_{37}N_3O_4 \cdot 2H_2O$[c]	34.0
45[d]	H	H	187–189	AcOEt	$C_{20}H_{23}NO$	>40

[a] Compounds were analyzed for C, H, and N, and results agreed to ±0.4% of the theoretical values. [b] Antiarrhythmic activity in chloroform-induced arrhythmias in mice. [c] Hygroscopic, M^+ peak was detected in mass spectrum. [d] Compound **45** was prepared by Barton's method.[2]

Results and Discussion

The results of antiarrhythmic test using chloroform-induced arrhythmias in mice are summarized in the tables. Several compounds were more potent than disopyramide. Substituents on the aromatic ring and angular position greatly influenced activity. However, the relationships between the substituents and the potency of antiarrhythmic activity were complicated. When the compound had no substituent on the aromatic ring (A type), the order of potency in antiarrhythmic activity was as follows: R = *n*-Pr > *n*-Bu > Et > H. But the compounds with substituents on the aromatic ring showed a different tendency: (10-hydroxy compounds, B type) R = Et > Me > H > *n*-Pr > phenethyl; (9-hydroxy compounds, C type) *R* = *n*-Pr > *i*-Pr > *n*-Bu, Ph > Et > H, Me, phenethyl, Bn; (8,9-dihydroxy compounds, D type) R = Et > *n*-Pr > Me > H, phenethyl. As shown in Figure 1, there is some correlation between the length of the carbon chain of substituent R and the antiarrhythmic activity: all A, B, C, and D type compounds showed a letter V like pattern. In both B and D groups, the activity became maximum when R was ethyl and declined as R became larger. On the other hand, in both A and C groups the activity became maximum when R was propyl and was reduced a little but still remained at high levels when R was butyl. In the B group the antiarrhythmic activity was very high when R was smaller than propyl. However, in both C and D groups the activity was reduced greatly when R was smaller than an ethyl group.

The size of the southeast ring in the pentacyclic compounds also influenced the activity. The potency of activity decreased in the following order: 6-membered ring > 5-membered ring > 7-membered ring > no ring (Table III). Interestingly, indole compound **42**, which was not

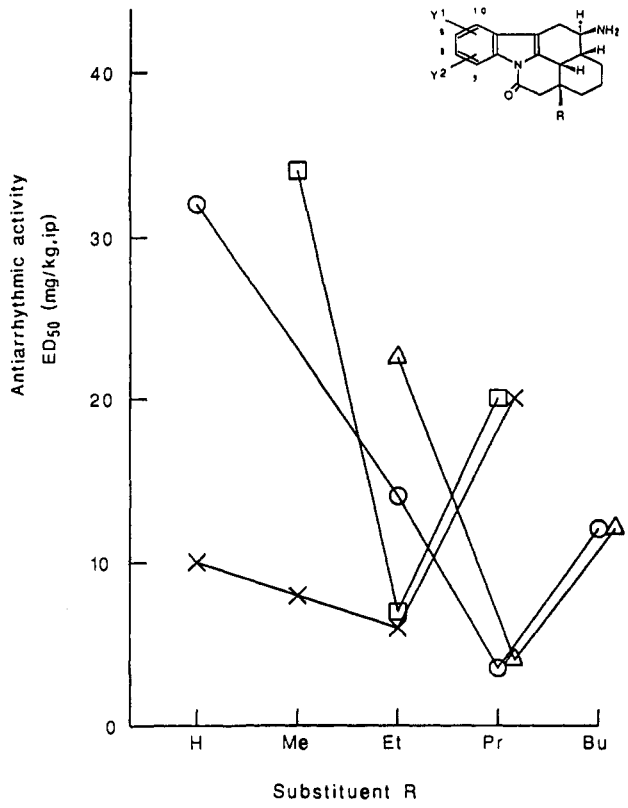


Figure 1. Relationship between substituents R and antiarrhythmic activity: (A) Y^1 = H, Y^2 = H, ○; (B) Y^1 = 10-OH, Y^2 = H, ×; (C) Y^1 = H, Y^2 = 9-OH, △; (D) Y^1 = 9-OH, Y^2 = 8-OH, □.

fused with another ring, also showed moderate activity. Dimethoxy compound **28** showed relatively lower activity than the corresponding diol compound. Compound **45** which did not have any function at C-12 showed no activity. However, a *tert*-aminoalkyl ester and *tert*-aminoalkyl hydrazide at C-12 gave relatively high activity (Table III).

The (+)-optical isomer of **10** was twice as potent as racemic **10**, and the (−)-optical isomer did not show the activity.

In canine Purkinje fibers, compound (+)-**10** (RS-2135) reduced the maximum upstroke velosity ($\dot{V}_{max}$) and shortened action potential duration (APD_{50} and APD_{90}) in a concentration-related manner (0.3–3 μM). These effects occurred without affecting resting membrane potentials. Flecainide, a class Ic antiarrhythmic agent, also decreased $\dot{V}_{max}$ and APD at concentrations of 3 and 10 μM. These findings suggest that RS-2135 can be classified as a class Ic antiarrhythmic drug and that the depressant action of $\dot{V}_{max}$ may be involved in antiarrhythmic activity of RS-2135, in a way similar to other "local anesthetic"-type antiarrhythmic agents.

In conclusion, we found antiarrhythmic agents in newly synthesized aminotetrahydroisoquinocarbazoles and described structure–activity relationships. Among the compounds tested, compound (+)-**10** (RS-2135) had the most potent antiarrhythmic activity with relatively low toxicity and was selected for further evaluation. Electrophysiological studies on canine Purkinje fibers revealed that the agent had a class Ic profile. The details of the electrophysiology and the pharmacology of RS-2135 will be published soon elsewhere.

Experimental Section

All melting points are uncorrected. IR spectra were measured on a JASCO A-102 spectrometer. ^{1}H NMR spectra were recorded with a Varian T-60A (60 MHz) and EM-390 (90 MHz) spectrometers, and the chemical shifts are expressed in ppm from tetramethylsilane as an internal standard: s, singlet; d, doublet; t, triplet; q, quartet; b, broad. Mass spectra were obtained with a JEOL JMS-01SG or JMS-G300 mass spectrometer. Merck silica gel (Kieselgel 60 Art. 7734) was employed for column chromatography.

Chemistry. Allyl (*E*)-3-[5-(Benzyloxy)-1*H*-indol-3-yl]-2-propenoate (2, X^1 = 9-OBn, X^2 = H, R = Et). To a solution of methyl (*E*)-3-[5-(benzyloxy)-1*H*-indol-3-yl]-2-propenoate (1) (122.93 g, 0.4 mol) in allyl alcohol (400 mL) and toluene (400 mL) was added 55% NaH (17.45 g, 0.4 mol) with ice cooling. The mixture was refluxed with stirring for 20 h and concentrated in vacuo. The residue was purified by silica gel column chromatography with AcOEt–hexane (1:4) to give **2** (100.12 g, 75.1%) as colorless crystals. Mp: 73–77 °C.

Allyl (*E*)-3-[5-(Benzyloxy)-1-[2-(1-ethyl-2-cyclohexen-1-yl)acetyl]-1*H*-indol-3-yl]-2-propenoate (3, X^1 = 9-OH, X^2 = H, R = Et). To a solution of **2** (23.23 g, 70 mmol) in DMF (100 mL) was added 55% NaH (3.36 g, 77 mmol) and the mixture was stirred at room temperature for 30 min. After cooling the mixture with ice, 2-(1-ethyl-2-cyclohexen-1-yl)acetyl chloride (14.00 g, 75 mmol) was added and the mixture was stirred for 0.5 h. The reaction mixture was the poured into ice–water and extracted with CH_2Cl_2. The extract was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by silica gel column chromatography with AcOEt–hexane (1:9) to give **3** (24.48 g, 72.3%) as colorless crystals. Recrystallization from isopropyl ether gave colorless needles. Mp: 68–72 °C. IR (KBr) cm^{-1}: 1690. ^{1}H NMR ($CDCl_3$) δ: 0.84 (3 H, t, J = 7 Hz), 1.25–2.20 (8 H, m), 2.85 (2 H, dd, J = 18, 14 Hz), 4.74 (2 H, dd, J = 5, 1 Hz), 5.12 (2 H, s), 5.20–6.30 (5 H, m), 6.50 (1 H, d, J = 16 Hz), 7.08 (1 H, dd, J = 9, 2 Hz), 7.20–7.60 (6 H, m), 7.70 (1 H, s), 7.80 (1 H, d, J = 16 Hz), 8.47 (1 H, d, J = 9 Hz). MS m/z: 483 (M^+). Anal. ($C_{31}H_{33}NO_4$): C, H, N.

Allyl (3a*β*,12*β*,12a*β*,12b*β*)-1,2,3,3a,4,5,11,12,12a,12b-Decahydro-9-(benzyloxy)-3a*β*-ethyl-5-oxoisoquino[2,1,8-*lma*]carbazole-12-carboxylate (4, X^1 = 9-OBn, X^2 = H, R = Et). A solution of **3** (24.00 g, 49.6 mmol) in mesitylene (200 mL) was refluxed with stirring for 12 h and then cooled. After adding 15% HCl/EtOH (20 mL) the reaction mixture was refluxed with stirring for 0.5 h and concentrated in vacuo. The residue was purified by silica gel column chromatography with AcOEt–hexane (1:9–1:4) to give **4** (21.00 g, 87.5%) as colorless crystals. Mp: 141–144 °C. Recrystallization from AcOEt–isopropyl ether gave colorless needles. Mp: 144–145 °C. IR (KBr) cm^{-1}: 1735, 1695. ^{1}H NMR ($CDCl_3$) δ: 0.73–2.00 (m), 2.34–2.36 (m), 4.60 (2 H, d, J = 5 Hz), 5.14 (2 H, s), 5.33 (2 H, dd, J = 11, 1 Hz), 5.70–6.20 (1 H, m), 6.87–7.62 (7 H, m), 8.30 (1 H, d, J = 10 Hz). MS m/z: 483 (M^+). Anal. ($C_{31}H_{33}NO_4$): C, H, N.

(3a*β*,12*β*,12a*β*,12b*β*)-1,2,3,3a,4,5,11,12,12a,12b-Decahydro-9-(benzyloxy)-3a*β*-ethyl-5-oxoisoquino[2,1,8-*lma*]carbazole-12-carboxylic Acid (5, X^1 = 9-OBn, X^2 = H, R = Et). A mixture of **4** (4.00 g, 8.27 mmol), tetrakis(triphenylphosphine)-palladium(0) (95 mg, 0.0822 mmol), triphenylphosphine (95 mg, 0.362 mmol), and 2-ethylhexanoic acid (3.02 g, 16.6 mmol) in AcOEt (70 mL) and $CHCl_3$ (30 mL) was stirred at room temperature for 8 h. Ether was added to the reaction mixture, and a crystalline solid was collected by filtration. The precipitate was dissolved in water. To this solution was added saturated citric acid and the resulting crystals were collected by filtration. The crystal was washed with water to give **5** (2.40 g, 65.4%) as colorless crystals. Mp: 237–239 °C. Recrystallization from dioxane–isopropyl ether gave colorless crystals. Mp: 240–242 °C. IR (KBr) cm^{-1}: 1728, 1698. ^{1}H NMR (DMSO-d_6) δ: 0.85 (3 H, t, J = 7 Hz), 1.12–1.85 (m), 2.18–3.25 (m), 5.18 (2 H, s), 6.97 (1 H, dd, J = 9, 2 Hz), 7.13 (1 H, d, J = 2 Hz), 7.30–7.60 (5 H, m), 8.17 (1 H, d, J = 9 Hz). MS m/z: 443 (M^+). Anal. ($C_{28}H_{29}NO_4$): C, H, N.

Benzyl *N*-[(3a*β*,12*β*,12a*β*,12b*β*)-9-(Benzyloxy)-1,2,3,3a,4,5,11,12,12a,12b-decahydro-3a*β*-ethyl-5-oxoisoquino[2,1,8-*lma*]carbazol-12-yl]carbamate (6, X^1 = 9-OBn, X^2 = H, R = Et). To a solution of **5b** (2.22 g, 5.01 mmol) and triethylamine (0.8 mL, 5.74 mmol) in acetone (50 mL) was added dropwise ethyl chloroformate (0.7 mL, 7.36 mmol) with ice cooling, and the mixture was stirred for 0.5 h. To the reaction mixture

was added a solution of sodium azide (0.5 g, 7.69 mmol) in water (5 mL) at 0 °C. After stirring for 30 min, the reaction mixture was poured into ice water and extracted with CH_2Cl_2. The extract was dried over $MgSO_4$ and concentrated in vacuo. Next, a solution of the residue in xylene (20 mL) was refluxed with stirring for 30 min. After benzyl alcohol (5 mL) was added, the reaction mixture was refluxed for 1 h and concentrated in vacuo. The residue was purified by silica gel column chromatography with AcOEt–hexane (1:4) to give **6** (1.78 g, 64.7%) as colorless crystals. Mp: 178–181 °C. Recrystallization from AcOEt–isopropyl ether gave colorless prisms. Mp: 181–183 °C. IR (KBr) cm^{-1}: 1713, 1687. ^1H NMR ($CDCl_3$) δ: 0.65–3.10 (m), 4.10 (1 H, m), 5.02 (2 H, s), 5.18 (2 H, dd, J = 15, 12), 5.62 (1 H, d, J = 11 Hz), 6.80–7.00 (2 H, m), 7.20–7.55 (10 H, m), 8.28 (1 H, d, J = 10 Hz). MS m/z: 548 (M^+). Anal. ($C_{35}H_{36}N_2O_4$): C, H, N.

(3aβ,12β,12aβ,12bβ)-12-Amino-3aβ-ethyl-2,3,3a,4,11,12,-12a,12b-octahydro-9-hydroxyisoquino[2,1,8-*lma*]carbazol-5(1*H*)-one (17). A mixture of **6** (5.05 g, 9.2 mmol) and 10% Pd–C (1.5 g) in DMF (50 mL) was stirred at room temperature under atmospheric pressure of hydrogen until the absorption of hydrogen ceased. The catalyst was filtered off and the filtrate was condensed under reduced pressure. The residue was washed with AcOEt to give **7** (2.68 g, 87.0%) as colorless crystals. Mp: 268–270 °C dec. Recrystallization from dioxane gave colorless crystals. Mp: 270–273 °C. Anal. ($C_{20}H_{24}N_2O_2 \cdot 0.5H_2O$) C, H, N.

(3aβ,12β,12aβ,12bβ)-10-(Benzyloxy)-1,2,3,3a,4,5,11,12,12a,-12b-decahydro-3aβ-ethyl-5-oxoisoquino[2,1,8-*lma*]carbazole-12-carboxylic Acid Hydrazide (34a). To a solution of **5a** (1.33 g, 3.00 mmol) and triethylamine (0.46 mL, 3.30 mmol) in CH_2Cl_2 (10 mL) was added dropwise ethyl chloroformate (0.34 mL, 3.57 mmol) with ice cooling, and the mixture was stirred for 0.5 h. To the reaction mixture was added a solution of 80% hydrazine hydrate at 0 °C. After stirring for 0.5 h, the reaction mixture was poured into ice–water and extracted with CH_2Cl_2. The extract was dried over $MgSO_4$ and concentrated in vacuo. The residue was crystallized from ether to give **34a** (0.95 g, 69.3%). Mp: 187–189 °C. ^1H NMR ($CDCl_3$) δ: 0.70–2.00 (11 H, m), 2.30–3.80 (8 H, m), 5.15 (2 H, s), 6.67–7.75 (7 H, m), 8.07 (1 H, d, J = 9 Hz).

(3aβ,12β,12aβ,12bβ)-9-(Benzyloxy)-1,2,3,3a,4,5,11,12,12a,-12b-decahydro-3aβ-ethyl-5-oxo-*N*-[2-(1-pyrrolidinyl)-ethyl]isoquino[2,1,8-*lma*]carbazole-12-carboxamide (34b). To a solution of **5** (1.33 g, 3.00 mmol), 1-(2-aminoethyl)pyrrolidine (0.38 g, 3.33 mmol), and diethyl cyanophosphonate (0.54 g, 3.31 mmol) in DMF (10 mL) was added dropwise triethylamine (0.46 mL, 3.30 mmol) with ice cooling, and the mixture was stirred at room temperature for 5 h under nitrogen. The reaction mixture was poured into ice–water and extracted with CH_2Cl_2. The extracted was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by silica gel column chromatography with AcOEt to EtOH, to give **34b** (1.00 g, 61.7%). Mp: 140–170 °C. ^1H NMR ($CDCl_3$) δ: 0.70–3.80 (m), 5.03 (2 H, s), 6.70–7.50 (7 H, m), 8.18 (1 H, d, J = 9 Hz).

(3aβ,12β,12aβ,12bβ)-9-(Benzyloxy)-1,2,3,3a,4,5,11,12,12a,12b-decahydro-3aβ-ethyl-5-oxoisoquino[2,1,8-*lma*]carbazole-12-carboxylic Acid 2-[2-(1-Pyrrolidinyl)ethyl]hydrazide (34c). To a solution of **5** (1.33 g, 3.00 mmol), 1-(2-hydrazinoethyl)pyrrolidine (0.43 g, 3.33 mmol), and diethyl cyanophosphonate (0.54 g, 3.31 mmol) in DMF (10 mL) was added dropwise triethylamine (0.46 ml, 3.30 mmol) with ice cooling, and the mixture was stirred at room temperature for 0.5 h under nitrogen. The reaction mixture was poured into ice–water and extracted with CH_2Cl_2. The extract was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by silica gel column chromatography with AcOEt to EtOH, to give **34c** (1.10 g, 66.3%). Mp: 174–177 °C. ^1H NMR ($CDCl_3$) δ: 0.63–2.00 (m), 2.20–3.10 (m), 5.09 (2 H, s), 6.84–7.10 (2 H, m), 7.20–7.60 (5 H, m), 8.28 (1 H, d, J = 9 Hz).

(3aβ,12β,12aβ,12bβ)-1,2,3,3a,4,5,11,12,12a,12b-Decahydro-3aβ-ethyl-10-hydroxy-5-oxoisoquino[2,1,8-*lma*]carbazole-12-carboxylic Acid Hydrazide (35). A mixture of **34a** (0.95 g, 2.08 mmol) and 10% Pd–C (0.4 g) in DMF (10 mL) was stirred at room temperature under atmospheric pressure of hydrogen until the absorption of hydrogen ceased. The catalyst was filtered off and the filtrate was concentrated in vacuo. The residue was recrystallized from 80% EtOH to give **35** (385 mg, 50.4%) as colorless crystals. Mp: 280–281 °C. IR (KBr) cm^{-1}: 1710, 1670, 1625. ^1H NMR (DMSO-d_6) δ: 0.70–1.90 (11 H, m), 2.15–4.65 (m), 6.62 (1 H, d, J = 8 Hz), 7.01 (1 H, t, J = 8 Hz), 7.74 (1 H, d, J = 8 Hz), 9.03 (1 H, s), 9.54 (1 H, s). MS m/z: 367 (M^+). Anal. ($C_{21}H_{25}N_3O_3$): C, H, N.

2-Bromoethyl (3aβ,12β,12aβ,12bβ)-1,2,3,3a,4,5,11,12,12a,-12b-Decahydro-9-(benzyloxy)-3aβ-ethyl-5-oxoisoquino-[2,1,8-*lma*]carbazole-12-carboxylate (38). A mixture of **5a** (3.00 g, 6.76 mmol), 4-(dimethylamino)pyridine (83 mg, 0.679 mmol), 2-bromoethanol (1.01 g, 8.08 mmol), and 1,3-dicyclohexylcarboodimide (DCC) (1.67 g, 8.09 mmol) in dioxane (50 mL) was stirred at room temperature for 20 h. The precipitate was filtered off, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography with AcOEt–hexane (1:4) to give **38** (2.56 g, 68.8%) as colorless crystals. Mp: 161–163 °C.

2-(4-Methyl-1-piperazinyl)ethyl (3aβ,12β,12aβ,12bβ)-1,2,3,3a,4,5,11,12,12a,12b-Decahydro-9-hydroxy-3aβ-ethyl-5-oxoisoquino[2,1,8-*lma*]carbazole-12-carboxylate (39). A mixture of **38** (1.65 g, 3.00 mmol) and *N*-methylpiperazine (0.75 g, 7.49 mmol) in toluene (20 mL) was refluxed with stirring for 12 h. The reaction mixture was washed with water, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography with 5% triethylamine/EtOH to give 2-(4-methyl-1-piperazinyl)ethyl (3aβ,12β,12aβ,12bβ)-9-(benzyloxy)-1,2,3,3a,4,5,11,12,12a,12b-decahydro-3aβ-ethyl-5-oxoisoquino[2,1,8-*lma*]carbazole-2-carboxylate (1.54 g, 90.1%) as a pale red amorphous solid.

A mixture of the above benzyl ether (1.54 g, 2.70 mmol) and 10% Pd–C (0.8 g) in DMF (30 mL) was stirred at room temperature under atmospheric pressure of hydrogen until the absorption of hydrogen ceased. The catalyst was filtered off and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography with 5% triethylamine/EtOH to give **39** (1.16 g, 89.9%) as a pale brown amorphous solid. MS m/z: 479 (M^+). IR (KBr) cm^{-1}: 3380, 1725, 1690, 1630. ^1H NMR ($CDCl_3$) δ: 0.73–1.97 (11 H, m), 2.15–3.30 (17 H, m), 2.28 (3 H, m), 7.18 (1 H, bs), 8.18 (1 H, d, J = 8 Hz).

1-(2-Cyclohexen-1-ylacetyl)-1*H*-indole-3-carboxaldehyde (40). To a solution of indole-3-carboxaldehyde (5.81 g, 40 mmol) in DMF (50 mL) was added 55% NaH (1.92 g, 44 mmol) and the mixture was stirred at room temperature for 10 min. After cooling of the mixture with ice, 2-(2-cyclohexen-1-yl)acetyl chloride (6.98 g, 44 mmol) was added and the mixture was stirred for 10 min. The reaction mixture was poured into ice–water and extracted with AcOEt. The extract was washed with water, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography with AcOEt–hexane (3:7) to give **40** (5.77 g, 54.0%) as colorless crystals. Mp: 89–97 °C. Recrystallization from isopropyl ether gave colorless crystals. Mp: 99–101 °C. Anal. ($C_{17}H_{17}NO_2$): C, H, N.

1-(2-Cyclohexen-1-ylacetyl)-3-(2-nitrovinylene)-1*H*-indole (41). A mixture of **40** (2.68 g, 10 mmol) and ammonium acetate (0.77 g, 9.99 mmol) in nitromethane (10 mL) was refluxed with stirring for 1 h. The reaction mixture was poured into ice–water. The resulting crystalline solid was collected by filtration and washed with isopropyl ether to give **41** (1.10 g, 35.5%) as yellow crystals. 152–154 °C. Recrystallization from benzene–MeOH gave yellow needles. Mp: 158–159 °C. IR (KBr) cm^{-1}: 1722, 1630. ^1H NMR ($CDCl_3$) δ: 1.10–2.25 (7 H, m), 2.92 (2 H, bs), 5.53–5.97 (2 H, m), 7.25–8.30 (5 H, m), 8.50–8.70 (1 H, m). MS m/z: 310 (M^+). Anal. ($C_{18}H_{18}N_2O_3$): C, H, N.

1-(2-Cyclohexylacetyl)-3-(2-aminoethyl)-1*H*-indole (42). A mixture of **41** (1.30 g, 4.19 mmol), PtO_2 (0.5 g), and concentrated HCl (1 mL) in EtOH (20 mL) and water (20 mL) was stirred at room temperature under atmospheric pressure of hydrogen until the absorption of hydrogen ceased. The catalyst was filtered off and the filtrate was concentrated in vacuo. The residue was recrystallized from isopropyl alcohol to give **42** (154 mg, 11.4%) as a colorless powder. Mp: >250 °C.

***tert*-Butyl (2*S*)-2-[[[(3a*R*,12*R*,12a*R*,12b*S*)-1,2,3,3a,4,5,11,12,12a,12b-Decahydro-10-hydroxy-5-oxoisoquino[2,1,8-*lma*]carbazol-12-yl]amino]carbonyl]-pyrrolidine-1-carboxylate (43) and *tert*-Butyl (2*S*)-2-[[[(3a*S*,12*S*,12a*S*,12b*R*)-1,2,3,3a,4,5,11,12,12a,12b-Decahydro-10-hydroxy-5-oxoisoquino[2,1,8-*lma*]carbazol-12-yl]-**

amino]carbonyl]pyrrolidine-1-carboxylate (44). To a solution of (±)-12-amino-2,3,3a,4,11,12,12a,12b-octahydroisoquino[2,1,8-*lma*]carbazol-5(1*H*)-one (**10**) (3.60 g, 10 mmol), L-Boc-proline (4.30 g, 20 mmol), and diethyl cyanophosphonate (5.15 g, 30 mmol) in DMF (50 mL) was added dropwise triethylamine (4.2 mL, 30.1 mmol) with ice cooling, and the mixture was stirred for 2 h under nitrogen. The reaction mixture was poured into ice–water. The resulting crystalline solid was collected by filtration and washed with water. The crystalline solid was fractionated by silica gel column chromatography with AcOEt–hexane (7:3) to give two isomers. The less polar isomer was recrystallized from EtOH–ether to give **43** (1.31 g, 25.6%) as colorless crystals. Mp: 178–180 °C. $[\alpha]_D$ +75.4° (c = 1.19, MeOH). Anal. ($C_{28}H_{35}N_3O_5 \cdot H_2O$): C, H, N.

The more polar isomer was also recrystallized from EtOH–ether to give **44** (1.30 g, 26.3%) as colorless crystals. Mp: 215 °C dec. $[\alpha]_D$ −84.4° (c = 1.21, MeOH). Anal. ($C_{28}H_{35}N_3O_5$): C, H, N.

(3a*R*,12*R*,12a*R*,12b*S*)-12-Amino-2,3,3a,4,11,12,12a,12b-octahydro-10-hydroxyisoquino[2,1,8-*lma*]carbazol-5(1*H*)-one Hydrochloride [(+)-10]. A mixture of **43** (1.20 g, 2.43 mmol) in concentrated HCl (20 mL) and dioxane (10 mL) was refluxed with stirring for 7 h. The reaction mixture was poured into ice–water and neutralized with $NaHCO_3$. The resulting crystalline solid was collected by filtration and washed with water. The crude solid was purified by silica gel column chromatography with 10% MeOH/CH_2Cl_2 and dissolved in EtOH. To this solution was added 4 N HCl–dioxane, and the resulting crystals were collected by filtration. The crystals was recrystallized from MeOH to give (+)-**10** (135 mg, 15.4%) as colorless crystals. Mp: 255–265 °C dec. $[\alpha]_D$ +67.6° (c = 0.74, H_2O).

Biological Activity: Antiarrhythmic Screening Using Chloroform-Induced Ventricular Arrhythmias in Mice.[7] Female mice (Charles River, ICR) weighing 20–25 g were used for the evaluation of antiarrhythmic activity. A glass chamber of 2-L volumes containing 150 mL of chloroform was warmed at 33 °C to vaporize chloroform. The mice were injected intraperitoneally with the tested compounds. After 15 min each mouse was placed into the chloroform chamber saturated with chloroform vapor. The mice were taken out 2 min later from the chamber and the chest was quickly opened. The electrocardiogram was recorded for 1 min via a pair of silver electrodes placed on the exposed surface of the ventricle.

The antiarrhythmic activity of the tested compounds was evaluated by ventricular rate measured just after the thoracotomy. If the ventricular rate was less than 400 bpm, a given dose of the tested compound was judged to be effective. For each dose level, 3–10 experiments were conducted and ED_{50} values for protection from ventricular tachycardia induced by chloroform were calculated. Mice that were not given drug showed a ventricular rate of 1000–1200 bpm.

Recording of Transmembrane Action Potential. Mongrel dogs were anesthetized with sodium pentobarbital 30 mg/kg iv and hearts were excised rapidly. The free-running false tendons were isolated from either ventricle. The tissues were mounted in an acrylic chamber filled with the oxygenated Krebs–Henseleit solution (37 °C). The myocardial preparations were stimulated using bipolar platinum electrodes at 1-ms duration and 3 times threshold voltage via an isolated stimulator at 1 Hz. The transmembrane action potential was recorded using the standard microelectrode technique. After the equilibration period of 1 h, RS-2135 (0.3–3 μM) or flecainide (3 and 10 μM) was applied and action potential was observed for 30 min.