6,7-dibenzyloxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (12) dissolved in 100 mL of anhydrous ethanol and 25 mg of 10% palladium-on-carbon catalyst was hydrogenated at 40 psi for 8 h. The solution was gravity filtered, concentrated in vacuo, and allowed to crystallize in a saturated Et_2O chamber. After recrystallization from methanol–Et_2O 53 mg (76.6%) of an off-white crystalline 2, mp 264–265 °C, was obtained. Anal. ($C_{19}H_{24}O$-NCl·CH_3OH) C, 58.04; H, 6.82; N, 3.38. Found: C, 58.00; H, 6.46; N, 3.49. Mass spectrum (EI) m/e 345; mass spectrum (CI) m/e 346.

Method B. A solution containing 200 mg (0.36 mmol) of 4-(3,4,5-trimethoxybenzyl)-6,7-dibenzyloxy-3,4-dihydroisoquinoline hydrochloride (11) dissolved in 250 mL of anhydrous ethanol and 100 mg of 10% palladium-on-carbon catalyst was hydrogenated on a Parr apparatus at 25 °C for 16 h at an initial H_2 pressure of 48 psi. The solution was gravity filtered, concentrated in vacuo and filtered through a cotton plug. The solution was further concentrated in vacuo and filtered through a cotton plug. The solution was further concentrated to ca. 3 mL under a stream of argon and allowed to crystallize in a saturated Et_2O chamber. After recrystallization from methanol–Et_2O, 136 mg (83.6%) of off-white 2, mp 264–265 °C, was obtained.

Biological Testing. Guinea pigs of either sex weighing 300–500 g were used in these experiments. The procedures for the pharmacological testing of each compound in isolated tracheal strip and right atrial preparations were identical with those previously described.[2a] In all biological experiments the ED_{50} values represent the concentration of each agonist required to produce a response equal to one-half of the maximal response in the appropriate system.

Drug solutions were prepared in normal saline containing 0.05% sodium metabisulfite.

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Isoquinolines. 5.[1] Synthesis and Antiarrhythmic Activity of Benzylisoquinoline Derivatives[2]

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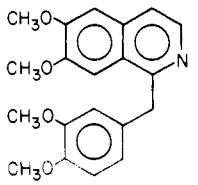
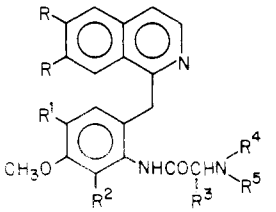
The synthesis of a series of benzylisoquinolines **2–9** containing aminoacetamide side chains is described. The method involved reduction of the appropriately substituted nitrobenzylisoquinolines followed by acylation to the chloroacylamide derivatives. Amination with the appropriate amine yielded the desired secondary and tertiary amines. The primary amines were prepared via the phthalimides. Two acetanilides **14** and **15** are also described and compared with the benzylisoquinoline derivatives. All compounds were evaluated for their ability to protect against chloroform-induced ventricular fibrillation in mice. The active compounds **6** and **7** were tested for their effect against ventricular arrhythmias in dogs with myocardial infarction. All compounds with the exception of **5** and **12** exhibited some antiarrhythmic effect. The most potent compound, 1-[2-(2-ethylaminoacetyl)amino-3,4-dimethoxybenzyl]isoquinoline (**7**), showed greater antiarrhythmic potency, was considerably less toxic than lidocaine, and is a candidate for further evaluation.

Presently, the most widely used antiarrhythmic drugs are quinidine, procainamide, diphenylhydantoin, lidocaine, and propranolol.[3] In order to improve the therapeutic ratio of such amide-type antiarrhythmic agents as procainamide and lidocaine, we wished to examine certain derivatives of papaverine (**1**), a drug which has been well recognized for its spasmolytic and coronary vasodilator activity and, to a lesser extent, for its antiarrhythmic effects.[4] The synthesis of a series of benzylisoquinolines **2–9**, structurally related to lidocaine, containing an amino group partially ionized at physiological pH, and an amide function, was thus undertaken. The tetrahydroisoquinoline **13** was also prepared and compared with (±)-laudanosine (**12**), a related isoquinoline lacking the amine and amide functional groups. Two additional compounds **14** and **15**, which can be considered as dissected analogues of **2** and **5**, lacking

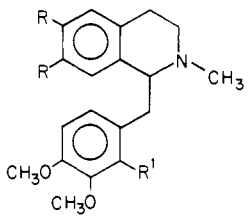
the isoquinoline moiety, were also prepared. All of these (2–15) were evaluated for antiarrhythmic activity and compared with papaverine (1) and lidocaine. Mathison and Morgan[5] recently reported that benzoyloxy and benzamido derivatives of 8-substituted 2-methyldecahydroisoquinolines were active antiarrhythmic agents with therapeutic indices considerably higher than that of quinidine.



1

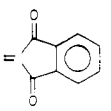


2, R = R^1 = OCH_3; R^2 = R^3 = H; R^4, R^5 = Et
3, R = R^1 = OCH_3; R^2 = R^3 = R^4 = H; R^5 = Et
4, R = R^2 = R^3 = H; R^1 = OCH_3; R^4, R^5 = Et
5, R = R^1 = R^2 = R^3 = H; R^4, R^5 = Et
6, R = R^1 = R^3 = H; R^2 = OCH_3; R^4, R^5 = Et
7, R = R^1 = R^3 = R^5 = H; R^2 = OCH_3; R^4 = Et
8, R = R^1 = R^3 = R^4 = R^5 = H; R^2 = OCH_3
9, R = R^1 = R^4 = R^5 = H; R^2 = OCH_3; R^3 = CH_3

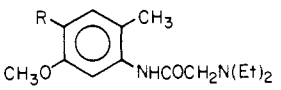


12, R = OCH_3; R^1 = H
13, R = H; R^1 = $NHCOCH_2N(Et)_2$

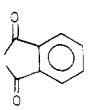
10, R = R^1 = R^3 = H; R^2 = OCH_3; R^4, R^5 =



11, R = R^1 = H; R^2 = OCH_3; R^3 =



CH_3; R^4, R^5 =

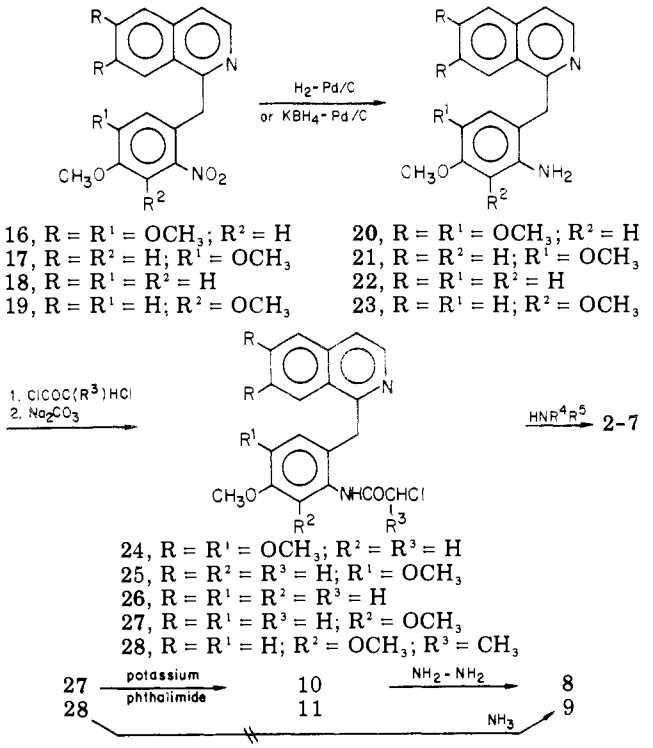


14, R = OCH_3
15, R = H

Chemistry. The synthesis of 2–11 involved the reduction of the appropriately substituted nitrobenzylisoquinolines 16–19 (Scheme I) either with potassium borohydride in ethanol using palladium on charcoal as a catalyst or with hydrogen over palladium on charcoal. The resulting amines 20–23 were acylated in ethyl acetate solution with either chloroacetyl chloride or 2-chloropropionyl chloride to give the corresponding chloroacylamido hydrochlorides, which were converted to the free bases 24–28 with aqueous sodium carbonate. Amination of 24–27 with the appropriate amine yielded compounds 2–7. 27 and 28 were allowed to react with potassium phthalimide to give 10 and 11, from which the primary amines 8 and 9 were obtained by treatment with hydrazine. The direct conversion of 27 and 28 with ammonia-saturated ethanol failed to yield the desired amines and resulted in the isolation of starting material. The nitrobenzylisoquinolines 16–19 were prepared either by nitration of the appropriate 1-benzylisoquinolines, i.e., 16 and 17,[6] or by the alkylation of 2-benzoyl-1,2-dihydroisoquinaldonitrile with a nitrobenzyl halide by recently published procedures[7,8] to yield 18 and 19.

Pharmacological Evaluation. Protection against Chloroform-Induced Ventricular Fibrillation in Mice. Ventricular fibrillation was produced in female Swiss albino mice essentially according to the method

Scheme I



16, R = R^1 = OCH_3; R^2 = H
17, R = R^2 = H; R^1 = OCH_3
18, R = R^1 = R^2 = H
19, R = R^1 = H; R^2 = OCH_3

20, R = R^1 = OCH_3; R^2 = H
21, R = R^2 = H; R^1 = OCH_3
22, R = R^1 = R^2 = H
23, R = R^1 = H; R^2 = OCH_3

24, R = R^1 = OCH_3; R^2 = R^3 = H
25, R = R^2 = R^3 = H; R^1 = OCH_3
26, R = R^1 = R^2 = R^3 = H
27, R = R^1 = R^3 = H; R^2 = OCH_3
28, R = R^1 = H; R^2 = OCH_3; R^3 = CH_3

described by Lawson.[9] For preliminary studies of antiarrhythmic effect, a dose of 100 mg/kg of drug in 0.9% saline was administered subcutaneously to a group of ten randomly selected mice. During 20 min the animals were observed for signs of toxicity. After 20 min they were exposed to chloroform vapors and investigated for the presence or absence of ventricular fibrillation. When fibrillation was not observed, the mice were considered protected. When neither protection nor toxicity was observed, the dose was raised to 200 mg/kg.

For full tests, three log-spaced doses of drug were chosen to give a low, intermediate, and high degree of protection against fibrillation. Each dose was administered subcutaneously to ten mice. The data obtained from the three-point assay were computer-analyzed using Berkson's minimum logit χ^2 analysis.[10] The program was designed to give ED_{50}'s with 95% Fieller limits. Potency figures were calculated as the reciprocal of the ED_{50} in millimoles per kilogram and standardized to give a potency of 1 for compound 2.

Effect against Ventricular Arrhythmias in Dogs with Myocardial Infarction. Myocardial infarction was produced in female beagle dogs by ligation of the anterior descending coronary artery in two steps according to the method of Harris.[11] On the first day after surgery, animals demonstrating ventricular arrhythmias with 85–100% beats of abnormal origin were used for testing.

The test solution was infused intravenously via a fore limb vein at a rate of 0.5 mg/kg/min (calculated as base, in 0.9% saline, adjusted to pH 6–7) until clearing occurred. Clearing was defined as reduction of ventricular ectopic activity to 5% or less for a consecutive 5 min. During the infusion of test drug, and for a reasonable period after clearing, EKG and other vital physiological signs were observed.

Results and Discussion

The physical properties of the target compounds as well as their antiarrhythmic and toxic effects in mice are summarized in Table I. Preliminary information on

Table I. Physical Properties of Benzylisoquinoline Derivatives and Antiarrhythmic and Toxic Effects in Mice

Compd	Mp, °C	Formula[a]	Dose,[b] mg/kg	Protection[c]	Toxicity at.[d]	Toxicity c.[e]	Toxicity l.r.r.[f]	Toxicity d.[g]	ED_{50} for protection in mg/kg[l]	Potency[h]
Lidocaine			100	10	10	10	10	0	62[i] ± 21	2
1			100	2	2	0	0	0		
2	179-180	$C_{26}H_{33}N_3O_5$	100	6	0	0	0	0	211 (124-360)	1
3	168-169	$C_{24}H_{29}N_3O_5$	100	5	9	0	0	0	142 (61-330)	1
4	147-148	$C_{24}H_{29}N_3O_3$	100	2	7	0	0	0		
5	106-107	$C_{23}H_{27}N_3O_2$	100	0	0	0	0	0		
			200[j]	0	0	0	0	0		
6	103-104	$C_{24}H_{29}N_3O_3$	100	6	0	0	0	0	146 (103-436)	1
7	101-102	$C_{22}H_{25}N_3O_3$	100	7	0	0	0	0	54 (38-86)	3
8	141.5-142	$C_{20}H_{21}N_3O_3$	100	1	0	0	0	0		
9	152-153	$C_{21}H_{23}N_3O_3$	100	1	0	0	0	0	257 (153-634)	1
10	242-243	$C_{26}H_{23}N_3O_5$	k							
11	206-207	$C_{29}H_{25}N_3O_5$	k							
12	113-113.5	$C_{21}H_{27}NO_4$	100	0	10	10	0	10		
			47	0	0	6	0	5		
13·2HCl	203-204	$C_{25}H_{37}Cl_2N_3O_3$	138	5	0	0	0	0	147 (100-204)	1
14·HCl	147-148	$C_{15}H_{25}ClN_2O_3$	100	0	0	0	0	0		
			200[j]	4	0	0	0	0		
15·HCl	162-162.5	$C_{14}H_{23}ClN_2O_3$	200	3	0	0	0	0		

[a] Analyses for C, H, and N where formula is given. [b] Subcutaneous administration. [c] Number of animals protected against fibrillation induced by chloroform. Ten animals tested in all cases. [d-g] Numbers of animals with ataxia, convulsions, loss of righting reflex, and death of ten tested. [h] Calculated on a molar basis. [i] Mean and standard deviation of 72 ED_{50} values determined according to the method described. [j] Highest dose tested. [k] Insoluble. [l] 95% Fieller limits in parentheses.

Table II. Antiarrhythmic Effect and Toxicity in Dogs

Compd	Control values[a] % vent. ect	Control values[a] Vent. rate	Control values[a] MABP	Clearing values % vent. ect	Clearing values Vent. rate	Clearing values MABP	Dose (mg/kg) to cause Clearing	Dose (mg/kg) to cause Toxicity
6	95	189	80	9	153	100	46.5	12.5[b]
	93	221	92	0	135	90	52	6.25[b] 15.75[c]
7	84	126	115	1	147	125	10	
	98	186	100	0	123	85	45	

[a] Values, before start of drug infusion, of percent ventricular ectopic beats; ventricular rate (beats per minute); and mean arterial blood pressure. [b] Profound head tremors. [c] Convulsions.

protection against fibrillation and CNS toxicity was obtained for all compounds except the insoluble phthalimides **10** and **11**. All compounds exhibited some antiarrhythmic effect except **5** and **12**. CNS toxicity was not evident or restricted to ataxia except with (±)-laudanosine (**12**), which lacks the aminoacetamide function characteristic of this series. Lidocaine, in contrast, caused severe CNS toxicity. In six cases, the ED_{50} for protection against chloroform-induced fibrillation was determined and relative potencies were calculated on a molar basis.

The most potent compound **7** and the corresponding tertiary amine **6** were tested in coronary-ligated dogs (Table II). Both abolished severe ventricular arrhythmias (85–100% ectopic beats). CNS toxicity was observed with **6** at doses well below the ones required for clearing. The secondary amine **7**, however, did not cause CNS toxicity at effective doses. Like lidocaine,[12] the papaverine derivatives appear to be free of cardiovascular toxicity. The duration of **7**, defined as the interval between the end of the infusion at clearing and return of 50% of ectopic beats, was 30 min or less, similar to results with lidocaine.

Based on the limited biological information, only tentative conclusions regarding structure–activity relationships can be drawn. Papaverine (**1**), the starting point for this investigation, showed only little antiarrhythmic activity in the chloroform mouse test. Introduction of the aminoacetamide side chain, characteristic of lidocaine, increased the antiarrhythmic effect in the case of the papaverine series (**2**) and markedly decreased the central nervous system toxicity in the *N*-methyltetrahydroisoquinoline series (**13**). Alteration of substitution of the benzyl ring from 4′,5′-dimethoxy (**4**) to 3′,4′-dimethoxy substituents (**6**) yielded a drug of superior properties. Modification of the tertiary to a secondary amine, often of little interest (compare **2** with **3**), resulted in the 3′,4′-dimethoxybenzylisoquinoline series in a promising compound (**7**). It showed greater antiarrhythmic potency than both its congener **6** as well as lidocaine and was considerably less toxic than lidocaine.

Further studies are underway to investigate the potential of compound **7** as an antiarrhythmic drug.

Experimental Section

Melting points were determined with a Thomas-Hoover apparatus and are uncorrected. Elemental analyses (indicated by C, H, and N when within ±0.4% of theoretical values) were performed by Midwest Microlab, Ltd., Indianapolis, Ind. Infrared spectra were recorded on a Perkin-Elmer 700 spectrophotometer. The NMR spectra were determined on a Varian T-60 spectrophotometer with Me_4Si as the internal standard.

6′-Nitropapaverine (16). Papaverine (**1**) was liberated as the free base from 37.6 g (0.1 mol) of **1**·HCl by stirring with aqueous sodium carbonate solution and then nitrated by a known procedure[13] with nitric acid in acetic acid to afford 35.2 g (0.091 mol, 91%) of pure 6′-nitropapaverine (**16**), mp 178–179 °C (lit.[13] mp 186–187 °C).

1-(4,5-Dimethoxy-2-nitrobenzyl)isoquinoline (17) was prepared by a modified literature procedure.[6] Thus concentrated nitric acid (35 mL) was cooled in an ice–ethanol bath and 5 g of 1-(3,4-dimethoxybenzyl)isoquinoline[6] (0.018 mol) was added portionwise. After the addition was complete the mixture was stirred in an ice bath for 1 h. Ammonium hydroxide was cau-

tiously added to the clear brown solution until the solution became basic. The resulting precipitate was stirred for 30 min and then filtered and air-dried to give crude **17** (5.7 g). The compound was further recrystallized from methanol to give the product **17**: 5.3 g (91%); mp 155–156 °C (lit.[6] mp 157–158.5 °C).

1-(4-Methoxy-2-nitrobenzyl)isoquinoline (18) was prepared by known procedures[7] from 2-benzoyl-1,2-dihydroisoquinaldonitrile and α-bromo-4-methoxy-2-nitrotoluene, mp 161–162 °C (lit.[7] mp 164–165 °C).

1-(3,4-Dimethoxy-2-nitrobenzyl)isoquinoline (19) was prepared by the published procedure[8] from 2-benzoyl-1,2-dihydroisoquinaldonitrile and α-chloro-3,4-dimethoxy-2-nitrotoluene, mp 127–128 °C (lit.[8] mp 129–130 °C).

General Procedures for the Reduction of 1-Nitrobenzylisoquinoline Derivatives. Method A. 1-(2-Amino-3,4-dimethoxybenzyl)isoquinoline (23). To the nitro compound **19** (13.6 g, 0.042 mol) dissolved in 400 mL of hot ethanol and 80 mL of water was added 300 mg of 10% palladium on carbon. Then KBH_4 was added in four 1.5-g batches in 10-min intervals. The mixture was stirred at room temperature for 2 h and then filtered through a Celite pad. The Pd/C was washed thoroughly with methylene chloride. The organic layer was separated and concentrated to dryness. The residue was recrystallized from ethanol to afford 10.4 g (83%) of **23**: mp 90–91 °C; λ_{max}^{KBr} 3380 cm^{-1} (–NH); $\delta_{Me_4Si}^{CDCl_3}$ 1.10 (br s, 2, NH_2), 3.53 (s, 3, OCH_3), 3.60 (s, 3, OCH_3), 4.27 (s, 2, CH_2), 6.00 (d, J = 8.0 Hz, 1, aromatic), 6.80 (d, J = 8.0 Hz, 1, aromatic), 7.00–7.50 (m, 4, aromatic), and 8.00–8.25 (m, 2, aromatic). Anal. ($C_{18}H_{18}N_2O_2$) C, H, N.

Method B. 1-(2-Amino-4-methoxybenzyl)isoquinoline (22). Catalytic hydrogenation of the nitro compound **18** (2.2 g, 7.5 mmol) over Pd/C afforded 1.65 g (84%) of the amine **22**, mp 102–103 °C. Anal. ($C_{17}H_{16}N_2O_2$) C, H, N.

1-[2-(2-Chloroacetyl)amino-3,4-dimethoxybenzyl]isoquinoline (27). The amine **23** (5.9 g, 0.02 mol) was dissolved in 400 mL of ethyl acetate and was stirred for 2 h with 15 mL of chloroacetyl chloride. The precipitate which formed was filtered and dried to yield 7.1 g (87%) of the hydrochloride salt, mp 193–194 °C, which was suspended in 200 mL of water and stirred for 10 min with 10% Na_2CO_3 solution. The free base was extracted with chloroform, and the organic layer was washed with water, dried (sodium sulfate), and concentrated to yield a residue which was crystallized from methanol to give pure **27**: mp 166–167 °C; λ_{max}^{KBr} 1650 cm^{-1} (amide C=O); $\delta_{Me_4Si}^{CDCl_3}$ 3.75 (s, 3, OCH_3), 3.90 (s, 3, OCH_3), 4.20 (s, 2, CH_2Cl), 4.55 (s, 2, CH_2), 6.63–7.00 (m, 2, aromatic), 7.23–7.85 (m, 4, aromatic), 8.20–8.45 (m, 2, aromatic), and 9.80 (br s, 1, NH). Anal. ($C_{20}H_{19}ClN_2O_3$) C, H, N. Similarly prepared were **1-[2-(2-chloroacetyl)amino-4,5-dimethoxybenzyl]-6,7-dimethoxyisoquinoline (24)**, mp 168–169 °C, from **20** and **1-[2-(2-chloroacetyl)amino-4,5-dimethoxybenzyl]isoquinoline hydrochloride (25)**, mp 214 °C dec, from **21**. Anal. ($C_{20}H_{20}Cl_2N_2O_3$) C, H, N.

Similarly prepared was **1-[2-(2-chloroacetyl)amino-4-methoxybenzyl]isoquinoline hydrochloride (26·HCl)** from **22** and chloroacetyl chloride to give 82% of product [mp 193–194 °C (EtOH–Et_2O). Anal. ($C_{19}H_{18}Cl_2N_2O_2$) C, H, N] and **1-[2-(2-chloropropionyl)amino-3,4-dimethoxybenzyl]isoquinoline (28)** from **23** and 2-chloropropionyl chloride to give 6.2 g (92%) of **28·HCl**, mp 199–200 °C, from which was liberated 4.5 g (73%) of **28** [mp 162–163 °C. Anal. ($C_{21}H_{21}ClN_2O_3$) C, H, N].

1-[2-(2-Ethylaminoacetyl)amino-3,4-dimethoxybenzyl]isoquinoline (7). A mixture of 1.85 g (0.005 mol) of **27**, ethylamine (20 mL), and benzene (150 mL) was allowed to reflux for 3 h, the solvent was removed, and the pale yellow residue which remained was triturated with hexane and filtered to yield 1.4 g of a colorless powder. The crude product was then triturated with water, filtered, washed with water, and dried. Recrystallization from 2-propanol gave 1.3 g (68%) of **7**: mp 107–108 °C; λ_{max}^{KBr} 3280 (NH) and 1670 cm^{-1} (amide C=O); $\delta_{Me_4Si}^{CDCl_3}$ 1.00 (t, 3, CH_2CH_3), 1.75 (br s, 1, NH), 2.60 (q, 2, CH_2CH_3), 4.60 (s, 2, CH_2), 6.70 (s, 2, aromatic), 7.37–7.80 (m, 4, aromatic), 8.10–8.45 (m, 2, aromatic), and 8.70 (br s, 1, CONH) (Table I). Similarly prepared from **24–27** and the appropriate amines were **2–6** (Table I).

1-[2-(2-Phthalimidoacetyl)amino-3,4-dimethoxybenzyl]isoquinoline (10). To a solution of 6.6 g (0.018 mol) of the chloride **27** in 700 mL of dimethylformamide at 90 °C was slowly added 4.8 g (0.026 mol) of potassium phthalimide. Stirring with heating (90 °C) was continued for 4 h. The suspension was poured into ice–water, and the precipitate was filtered off, washed with 5% sodium hydroxide solution and with water, and finally dried to yield 7.0 g (81%) of **10**: mp 242–243 °C (Table I) (EtOH); λ_{max}^{KBr} 1710 and 1680 cm^{-1}; $\delta_{Me_4Si}^{CDCl_3}$ 3.73 (s, 3, OCH_3), 3.93 (s, 3, OCH_3), 4.53 (s, 2, CH_2), 4.67 (s, 2, $COCH_2$), 6.50–6.97 (m, 2, aromatic), 7.70 (s, 4, aromatic), and 9.60 (br s, 1, NH). Similarly prepared from **28** was **11** (Table I).

1-[2-(2-Aminoacetyl)amino-3,4-dimethoxybenzyl]isoquinoline (8). A mixture of 2.0 g (4.1 mmol) of **10**, aqueous hydrazine, and 100 mL of ethanol was allowed to reflux for 1 h, cooled to room temperature, and concentrated to dryness. The residue was extracted with chloroform and was washed with water. The chloroform layer was concentrated to yield fine crystals. Recrystallization from benzene–ether afforded 0.6 g (42%) of **8**: mp 141.5–142 °C; λ_{max}^{KBr} 3250 (NH) and 1660 cm^{-1} (amide C=O); $\delta_{Me_4Si}^{CDCl_3}$ 1.63 (br s, 2, NH_2), 3.46 (s, 2, $COCH_2$), 3.78 (s, 3, OCH_3), 3.87 (s, 3, OCH_3), 4.53 (s, 2, CH_2), 6.70 (m, 2, aromatic), 7.35–7.80 (m, 4, aromatic), 8.13–8.43 (m, 2, aromatic), and 8.95 (br s, 1, CONH) (Table I).

Similarly prepared was **1-[2-(2-aminopropionyl)amino-3,4-dimethoxybenzyl]isoquinoline (9)** from **28** via **11**.

1-[2-(2-Diethylaminoacetyl)amino-3,4-dimethoxybenzyl]-2-methyl-1,2,3,4-tetrahydroisoquinoline Dihydrochloride (13·2HCl). 1-(2-Amino-3,4-dimethoxybenzyl)-2-methyl-1,2,3,4-tetrahydroisoquinoline[8] was converted to **1-[2-(2-chloroacetyl)amino-3,4-dimethoxybenzyl]-1,2,3,4-tetrahydroisoquinoline** in ethyl acetate and isolated as the hydrochloride, mp 229–230 °C (EtOH). Anal. ($C_{21}H_{26}Cl_2N_2O_3$) C, H, N. The hydrochloride was converted to the free base which was converted to **13** with diethylamine in benzene. The oily product **13** formed a hygroscopic dihydrochloride salt which was recrystallized from EtOH–Et_2O to give pure **13·2HCl** (Table I).

2-Diethylamino-4′,5′-dimethoxy-2′-methylacetanilide (14). Hydrogenation (Parr) of 3.0 g (15 mmol) of 4,5-dimethoxy-2-nitrotoluene[14] over Pd/C yielded 1.67 g (10 mmol, 67%) of 4,5-dimethoxy-2-methylaniline, mp 108–108.5 °C. Acetylation of the aniline with chloroacetyl chloride in ethyl acetate afforded 1.8 g (7.4 mmol, 74%) of 2-chloro-4′,5′-dimethoxy-2′-methylacetanilide, mp 125–125.5 °C, which on further treatment with 10 mL of diethylamine in 100 mL of refluxing benzene yielded **14** as an oil. The oily **14** was dissolved in anhydrous ether and HCl gas was passed through the solution. The precipitate was filtered and recrystallized from ethanol to yield 1.3 g (4.1 mmol, 56%) of **14·HCl**, mp 147–148 °C. Anal. ($C_{15}H_{25}ClN_2O_3$) C, H, N.

2-Diethylamino-5′-methoxy-2′-methylacetanilide (15). Hydrogenation of 5.01 g (0.03 mol) of 4-methoxy-2-nitrotoluene[7] over Pd/C afforded 2.2 g (0.016 mol, 54%) of 5-methoxy-2-methylaniline, mp 43–44 °C. Acetylation of 2.05 g (0.015 mol) of the aniline with chloroacetyl chloride gave 2.7 g (12.6 mol, 84%) of 2-chloro-5′-methoxy-2′-methylacetanilide, mp 89–89.5 °C. Further treatment of 2.13 g (0.01 mol) of the chloride with diethylamine yielded an oily product which was converted to 2.09 g (7 mmol, 70%) of **15·HCl**, mp 162–162.5 °C. Anal. ($C_{14}H_{23}ClN_2O_2$) C, H, N.

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Synthesis of Octanoyl[8-leucyl]angiotensin II, a Lipophilic Angiotensin Antagonist

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Octanoyl[8-leucyl]angiotensin II (oct-LAT) was synthesized with the aim of obtaining a longer acting angiotensin (AT) inhibitor. The new compound, with a partition coefficient $K = 7.00$ in *n*-BuOH–HOAc–H_2O, was compared with [Leu^8]angiotensin II (LAT, $K = 0.18$) as an AT antagonist in two isolated smooth muscle preparations and in the rat blood pressure assay. The two compounds were equally potent in the rat uterus, but LAT was more effective in the guinea pig ileum and the in vivo assay. LAT's effect was longer lasting in the smooth muscles, but the duration of in vivo inhibition was the same for the two compounds. It is concluded that partitioning between external medium and biophase is not a limiting factor for antagonistic potency and permanence of effects of 8-substituted AT derivatives.

The important finding that [Ala^8]angiotensin II is a competitive inhibitor of angiotensin II[1] (AT) has led to the description of numerous antagonistic AT analogues with aliphatic side chains in position 8.[2] These antagonists have proved to be useful tools in the study of the role of the renin–angiotensin system in human hypertension[3] and have potential uses as diagnostic and therapeutic agents. However, the compounds hitherto described have the disadvantage of a short in vivo half-life, and attempts to prolong their action by the introduction of enzyme-resistant peptide linkages have not been successful.[4]

An alternative way for obtaining longer acting angiotensin antagonists would be to increase the permanence of these compounds in the biophase[5] by increasing their lipophilicity. Lipophilic derivatives would also be of use for depot administration. In order to test these possibilities we have synthesized a derivative of [Leu^8]-AT[6] (LAT) in which the N-terminal amino group was acylated by *n*-octanoic acid (oct-LAT). The amino group was chosen for anchoring the lipophilic moiety because its acylation does not affect the pressor action of AT[7] and protects the peptide against attack by aminopeptidases. The results of the study of oct-LAT as an antagonist of the myotropic and pressor activities of AT are presented in this paper.

Experimental Section

Materials. The chloromethylated copolymer of styrene and divinylbenzene (2% cross-linking, 0.9 mequiv/g) was from Bio-Rad (lot 6554) and the *tert*-butyloxycarbonyl (Boc) amino acid derivatives were from Bachem. Bradykinin, [Sar^1, Ala^8]-AT, and LAT[8] were synthetic products of this laboratory.

Peptide Synthesis. Oct-LAT was prepared by the solid-phase method.[9] Boc-Leu was attached to the polymer support,[10] and chain elongation was performed with the aid of an automatic peptide synthesizer.[11] CH_2Cl_2 was used as solvent for all reagents and *tert*-butyloxycarbonylamino acids with the exception of CF_3COOH, used as a 30% (v/v) solution in $CHCl_3$, and of Boc-Arg(Tos), which was dissolved in CH_2Cl_2–DMF (2:1). The amino acids with reactive side chains were used in the form of the following derivatives: Boc-His(Tos), Boc-Tyr(Bzl), Boc-Arg(Tos), and Boc-Asp(Bzl). All the coupling reactions, done with 2.5 equiv of *tert*-butyloxycarbonylamino acid and of DCI, were monitored with the ninhydrin reaction[12] and were completed in 6 h or less. The Boc groups were removed by treatment with 30% (v/v) CF_3COOH in $CHCl_3$ for 30 min. After the last cycle (coupling of *n*-octanoic acid), the peptide-resin was cleaved by treatment with anhydrous HF containing 5% (v/v) anisole for 45 min at 0 °C. After removal of HF and anisole, by vacuum distillation and washing with EtOAc, the peptide was extracted with glacial HOAc and lyophilized.

Table I. Comparison of Some Physical Properties of LAT and Oct-LAT

Compd	Partition coeff[a]	Electrophoretic migration[b] pH 2.8	pH 4.9	pH 9.9	R_f[c] A	B	C
LAT	0.18	0.75	0.44	0.43	0.24	0.57	0.44
Oct-LAT	7.00	0.48	0.21	0.18	0.55	0.70	0.71

[a] Obtained from countercurrent distribution in *n*-BuOH–HOAc–H_2O (4:1:5). [b] Relative to histidine (pH 2.8), arginine (pH 4.9), and picric acid (pH 9.9). [c] Solvent systems described in the Experimental Section.

The crude peptide was submitted to 200 transfers of countercurrent distribution in *n*-BuOH–HOAc–H_2O (4:1:5), followed by chromatography on a 10 × 1.8 cm carboxymethylcellulose column with a linear gradient between 0.01 M NH_4OAc (pH 5.0) and 0.5 M NH_4OAc (pH 8.6). The peptide-containing eluate was lyophilized until constant weight to remove NH_4OAc. Amino acid analyses were made on a Beckman 120C analyzer, after hydrolysis with 2 mL of 6 N HCl containing 0.1 mL of 10% (v/v) mercaptoethanol and 0.04 mL of 5% (v/v) phenol in nitrogen, for 72 h at 110 °C, yielding the following molar ratio: Asp, 1.01; Arg, 1.00; Val, 1.02; Tyr, 0.94; Ile, 0.98; His, 1.04; Pro, 1.01; Leu, 1.01. The peptide content was 88%, as expected from the diacetate form of the pure peptide. Only one spot was detected with Sakaguchi, Pauly, and hypochlorite reagents after TLC on silica gel (Eastman "Chromagram" plates, 0.1 mm) with the following solvent systems: (A) *n*-BuOH–HOAc–H_2O (5:1:1); (B) *n*-BuOH–EtOAc–HOAc–H_2O (1:1:1:1); (C) *n*-BuOH–pyridine–HOAc–H_2O (15:10:3:12). The R_f values are shown in Table I. Only one component, with the expected mobility, was detected after paper electrophoresis at 1000 V for 60 min in the following buffer systems: 1 M HOAc (pH 2.8), 0.1 M pyridine acetate (pH 4.9), and 0.2 M sodium carbonate–bicarbonate (pH 9.9). The relative mobilities, expressed as the ratio of the peptide's migration to that of a simultaneously run amino acid standard, are shown in Table I.

Bioassays. The preparations of the guinea pig isolated ileum,[13] rat isolated uterus,[14] and the rat blood pressure assay[15] were described in detail elsewhere. To avoid the interference of tachyphylaxis with our observations, in the case of the guinea pig ileum, the AT administrations were spaced at 15- to 20-min intervals to allow recovery from the tachyphylactic state.[16] In the rat uterus, the intervals between administrations could be