Synthesis and Study of Conformationally Defined Enantiomers of Local Anesthetics and Conformationally Defined Enantiomers of Fluorescent Dyes Designed to Label Electrically Excitable Membranes

Henry G. Mautner,* Carol Lorenc, Paul Quain, Judith K. Marquis,

Department of Biochemistry and Pharmacology, Tufts University School of Medicine, Boston, Massachusetts 02111

and Ichiji Tasaki

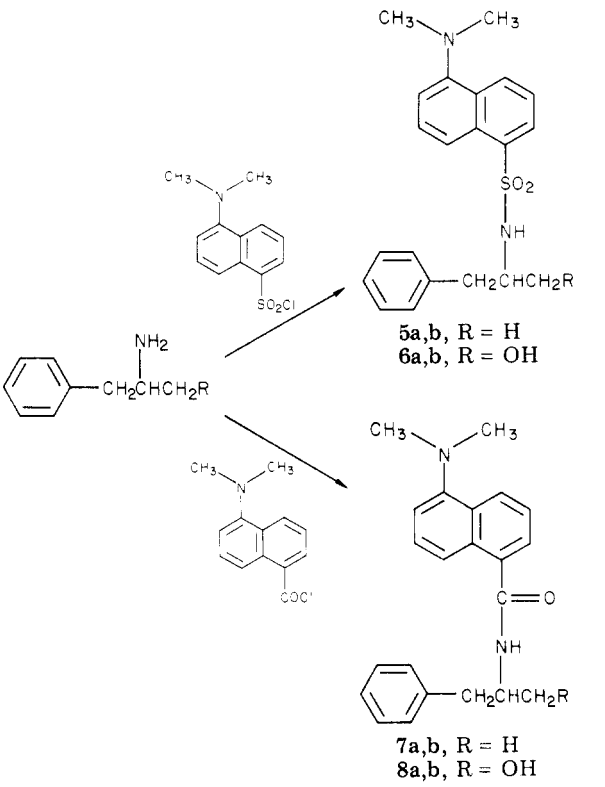
Laboratory of Neurobiology, National Institute of Mental Health, Bethesda, Maryland 20014. *Received August 20, 1979*

Conformationally defined enantiomeric local anesthetics and fluorescent dyes were synthesized. Neither the local anesthetics nor the fluorescent probes showed stereospecificity in interacting with nerve membranes. The fluorescence signals generated by the dyes showed excellent correlation with the time course and shape of the nerve action potential.

While the literature dealing with the mechanisms of action of local anesthetics is enormous, the details of the mechanism of action of this class of compounds remain controversial. It is generally accepted that local anesthetics block axonal conduction by interfering with the influx of sodium ions during the early part of the action potential without interfering with the resting potential.[1-5] However, it is not clear whether local anesthetics exert their effects by relatively nonspecific interactions with axonal membranes causing membrane expansion,[6] changes in surface charge,[7] by displacement of calcium ions,[7,8] or by interaction with specific receptors.[9] While it is generally accepted that local anesthetics in the cationic form interact with negatively charged binding sites, it is not known whether the latter are carried by proteins or by lipids.

It is not clear whether local anesthetics are stereospecific in their action, although attachment with a specific receptor would, presumably, involve considerable structural specificity and stereospecificity in the ligands interacting with it. Several attempts have been made to detect stereospecificity in local anesthetics. As early as 1923, Gottlieb[10] reported that the enantiomers of cocaine were equipotent local anesthetics. Similarly, several other reports failed to detect stereospecificity in the action of local anesthetics.[11-13] However, it was reported by other workers that certain local anesthetics can exhibit stereospecificity[14-16] and that the differences in the biological activities of the enantiomers are not due to differences in their abilities to reach their site of action.[17]

Scheme I



5a,b, R = H
6a,b, R = OH

7a,b, R = H
8a,b, R = OH

In view of these discrepancies, it was of interest to prepare enantiomeric, conformationally defined local anesthetics, as well as a series of enantiomeric, conformationally defined fluorescent molecules designed to probe axonal membranes.

Fluorescent dyes have proved useful for obtaining information about biopolymers, such as the dielectric constant of dye-binding sites, distances between such sites, and conformational changes brought about by ligand attachment. Labeling nerve membranes with fluorescent dyes provides a method for studying the changes that occur during electrical excitation[18-22] of nerves, since the process

(1) R. E. Taylor, *Am. J. Physiol.*, **196**, 1071 (1959).
(2) B. Hille, *Nature (London)*, **210**, 1220 (1966).
(3) J. M. Ritchie, *Br. J. Anaesth.*, **47**, 191 (1975).
(4) B. Hille, *J. Gen. Physiol.*, **69**, 497 (1977).
(5) T. Narahashi, D. Frazier, and M. Yamada, *J. Pharmacol. Exp. Ther.*, **171**, 32 (1977).
(6) P. Seeman in "Permeability and Function of Biological Membranes", J. Bolis, A. Katchalsky, and R. Keynes, Eds., North Holland, Amsterdam, 1970.
(7) M. B. Feinstein, *J. Gen. Physiol.*, **48**, 357 (1964).
(8) M. P. Blaustein and D. E. Goldman, *J. Gen Physiol.*, **49**, 1043 (1966).
(9) G. Strichartz, *Anesthesiology*, **45**, 421 (1976).
(10) R. Gottlieb, *Arch. Exp. Pathol. Pharmakol.*, **97**, 113 (1923).
(11) H. King, *J. Chem. Soc.*, **125**, 41 (1924).
(12) F. H. Schultz and P. H. Barbour, *J. Pharmacol. Exp. Ther.*, **76**, 295 (1942).
(13) M. Lokhandwala, D. B. Patel, H. Patel, P. C. Merker, A. Shafi'ee, and G. Hite, *J. Pharm. Sci.*, **60**, 685 (1971).
(14) B. Åkerman, G. Comougis, and R. Sandberg, *Acta Pharmacol. Toxicol.*, **25**, 52 (1967).
(15) F. P. Luduena, *Annu. Rev. Pharmacol.*, **9**, 503 (1969).
(16) B. Åkerman, *Acta Pharmacol. Toxicol.*, **32**, 97 (1973).
(17) B. Åkerman, *Acta Pharmacol. Toxicol.*, **32**, 225 (1973).
(18) I. Tasaki, A. Watanabe, R. Sandlin, and L. Carnay, *Proc. Natl. Acad. Sci. U.S.A.*, **61**, 883 (1968).
(19) L. B. Cohen, R. D. Keynes, and B. Hille, *Nature (London)*, **218**, 438 (1968).
(20) I. Tasaki, A. Watanabe, and M. Hallett, *J. Membr. Biol.*, **8**, 109 (1972).
(21) A. Waggoner, *J. Membr. Biol.*, **27**, 317 (1976).
(22) W. N. Ross, B. M. Salzberg, L. B. Cohen, A. Grinvald, H. V. Davila, A. S. Waggoner, and C. H. Wang, *J. Membr. Biol.*, **33**, 141 (1977).

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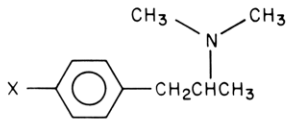
Table I. Effects of (*R*)-*p*-Nitro- (*S*)-*p*-Nitro-, (*R*)-*p*-Amino- and (*S*)-*p*-Amino[2-(dimethylamino)propyl]benzene on the Excitability of Spider Crab Walking Leg Nerve Bundles[a]



X	config	% B[b]	% R[c]
$-NO_2$	*R* (**2b**)	93 ± 6	46 ± 11
	S (**2a**)	84 ± 4	35 ± 8
$-NH_2$	*R* (**3b**)	28 ± 2	100
	S (**3a**)	33 ± 8	100

[a] The data represent the mean plus or minus the standard error of between four and six experiments in which the drug was applied in the external bath at a concentration of 1 mM for 20 min. [b] % B is the percentage decrease in peak action potential amplitude, recorded by a pair of external Ag–AgCl electrodes. [c] % R is the percentage recovery to control peak action potential amplitude upon washing in physiological saline for 20 min.

of depolarization is associated with transient changes in light absorption, fluorescence emission, and the polarization of emitted light.

Unfortunately, most of the dyes used in such work show little specificity and are bound largely to electrophysiologically unresponsive sites.[23] In recent years, several attempts have been made to prepare active-site-directed fluorescent ligands for electrically excitable membranes.[24-27]

Fluorescent molecules with local anesthetic activity, such as quinacrine,[28] have also been used to study conformational transitions in synaptic membranes. However, neither with synaptic membranes nor with axonal membranes does there appear to be any information regarding the stereospecificity of binding of fluorescent ligands. The present article describes the synthesis of a series of enantiomeric conformationally defined fluorescent molecules and their study in isolated nerve bundles. The same nerve preparation was used to study the enantiomeric local anesthetics discussed previously.

The synthetic scheme used for the preparation of the enantiomeric fluorescent molecules is outlined in Scheme I. The enantiomers of amphetamine were used as the synthetic starting material since their absolute configuration is known, with (+)-amphetamine being in the *S* configuration.[29,30] The enantiomers of phenylalaninol (**8a,b**), prepared by the reduction of (*R*)- and (*S*)-phenylalanine,[31] were converted to the dansyl (**6a,b**) and danthoyl derivatives (**8a,b**) by reaction with 5-(dimethylamino)naphthalenesulfonyl chloride (dansyl chloride) and with its acyl chloride analogue (danthoyl chloride).[32] Similarly, (*R*)- and (*S*)-amphetamine were used for the synthesis of **5a,b** and **7a,b**.

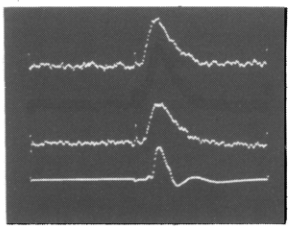


Figure 1. Representative experiments of the computer oscillograph tracings recorded from a crab leg nerve bundle stained with (*S*)-(−)-danthoylamphetamine (**7a**). Averaged fluorescence signals are shown in the top two traces, and the third trace is the extracellularly recorded compound action potential.

Table II. Optical Responses Recorded from Spider Crab Walking Leg Nerve Bundles Stained with the Enantiomeric Dansyl and Danthoyl Derivatives of Amphetamine and Phenylalaninol[a]

		response amplitude $\Delta I/I$,[b] mV × 10^{-4}	
no.	*P*, *A*[c]	*R*	*S*
5a,b	⊥,0	0.85 ± 0.08	0.7 ± 0.07
7a,b	⊥,0	3.5 ± 0.4	3.8 ± 0.5
6a,b	⊥,0	1.5 ± 0.2	1.8 ± 0.09
8a,b	∥,0	2.5 ± 0.3	2.4 ± 0.3

[a] The data are presented as the mean plus or minus the standard error of between two and eight experiments at dye concentrations of 25–50 μM. [b] $\Delta I/I$ is the relative fluorescence intensity, where ΔI is the change in the intensity of the fluorescent light associated with nerve excitation and I is the intensity of the fluorescent light emitted from the stained nerve at rest. [c] *P* and *A* indicate the axes of the polarizer and analyzer filters relative to the longitudinal axis of the nerve bundle.

The local anesthetics (*R*)- and (*S*)-1-(4-nitrophenyl)-2-(dimethylamino)propane (**2a,b**) were prepared by the nitration of (*R*)- and (*S*)-amphetamine,[33] followed by methylation of the amino group by the procedure of Gruber and Gunsalus.[34] The *p*-amino derivatives (**3a,b**) were prepared by the reduction of the *p*-nitro compounds.

Results

The blocking activities of the *R* and *S* enantiomers of *p*-amino- (**3a,b**) and *p*-nitro[2-(dimethylamino)propyl]-benzene (**2a,b**) on the excitability of spider crab walking leg nerves are summarized in Table I. In the control experiments, incubation in 1 mM procaine hydrochloride for 30 min decreases the compound action-potential amplitude by 30 ± 4% (n = 4), and incubation of 5 mM procainamide hydrochloride for 30 min induces a 46 ± 5% (n = 3) block. The effect of both control compounds is fully reversed on washing with physiological saline. It can be seen that, when the nerves are exposed to 1 mM solutions of the *p*-nitro and *p*-amino derivatives, the *p*-nitro derivative is the more potent nerve-blocking agent. Its effects are only partly reversible on washing, while the *p*-amino compounds, including procaine and procainamide,

(23) I. Tasaki, M. Hallett, and E. Carbone, *J. Membr. Biol.*, **11**, 353 (1973).
(24) G. Weber, D. Borris, E. de Robertis, F. J. Barrantes, F. J. La Torre, and M. de Carlin, *Mol. Pharmacol.*, **7**, 530 (1971).
(25) M. Martinez-Carrion and M. A. Raftery, *Biochem. Biophys. Res. Commun.*, **55**, 1156 (1973).
(26) F. J. Barrantes, B. Sakmann, R. Bonner, H. Eibl, and T. M. Jovin, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3097 (1975).
(27) G. Waksman, M. C. Fournié-Zaluski, B. Roques, T. Heidmann, H. H. Grünhagen, and J.-P. Changeux, *FEBS Lett.*, **67**, 335 (1976).
(28) H. H. Grünhagen and J.-P. Changeux, *J. Mol. Biol.*, **106**, 497 (1976).
(29) W. Leithe, *Ber. Dtsch. Chem. Ges.*, **65**, 660 (1932).
(30) P. Karrer and K. Ehrhardt, *Helv. Chim. Acta*, **34**, 2202 (1951).
(31) V. S. Venkateswaran and T. J. Bardos, *J. Org. Chem.*, **32**, 1256 (1967).
(32) P. Quain and H. G. Mautner, "Abstracts of Papers", 172nd National Meeting of the American Chemical Society, San Francisco, Calif., Aug. 1976, American Chemical Society, Washington, D.C., 1976, Abstr. BIOL 199.
(33) T. M. Patrick, E. T. McBee, and H. B. Hass, *J. Am. Chem. Soc.*, **68**, 1153 (1946).
(34) W. Gruber and I. C. Gunsalus, *J. Org. Chem.*, **21**, 1024 (1956).
(35) I. Tasaki and K. Sisco, *Methods Membr. Biol.*, **5** 163 (1975).

induce effects which are fully reversible. Neither the *p*-amino- nor the *p*-nitro-substituted enantiomers showed stereospecificity at any dose investigated.

Figure 1 shows time-averaged fluorescence signals from a nerve bundle stained with danthoylamphetamine (**7a,b**). The top two traces show the fluorescence signals, while the bottom trace shows the extracellularly recorded compound action potential. It can be seen that the time course of the optical signals parallels that of the electrical signal. Here, the peak value of the ratio $\Delta I/I$ is 2.3×10^{-4}. ΔI represents the change in light intensity associated with nerve excitation, while I represents the intensity of the fluorescence emission from the stained nerve at rest.

As summarized in Table II, all of the dyes used in this investigation showed an increase in fluorescence emission of about 10^{-4} times the background intensity when the bundle was stimulated electrically, with the signals associated with staining with the carboxamide derivatives exceeding those induced by the sulfonamides by a factor of 2–3. At dye concentrations that induce no detectable effect on the excitability of the nerve bundles (25–50 μM), the fluorescent molecules used to stain nerve membranes exhibited no stereospecificity.

In view of Pfeiffers's rule[36] that higher affinity for receptors is required for stereospecificity of action, it is possible that the low stereospecificity of local anesthetics may be related to the relatively high concentrations required for nerve blockade.

Our findings, coupled with the relatively low stereospecificity seen even in cases where the local anesthetic potency of enantiomers was claimed to differ,[14-17] do not provide support for the existence of specific receptors for local anesthetics.

The lack of stereospecificity of the fluorescent dyes also provides no support for their interaction with well-defined binding sites. However, the very good correlation of the fluorescence signals generated by the amphetamine analogues with the time course and shape of the nerve action potential suggests that these dyes may be useful probes of the changes undergone by excitable membranes during electrical excitation.

Experimental Section

The melting points were determined with a Gallenkamp (capillary) apparatus and are uncorrected. All compounds gave IR and NMR spectra consistent with the assigned structures. Optical rotations were obtained with a Zeiss 0.01° polarimeter. UV spectra were recorded with a Cary 15 recording spectrophotometer. No effort was made to optimize yields. Analyses were performed by the Baron Consulting Co., Orange, Conn., and are within 0.4% of the theoretical values.

(*S*)-(+)- (1a) and (*R*)-(−)-1-(4-Nitrophenyl)-2-aminopropane Hydrochloride (1b). The general procedure of Patrick et al.[33] was followed, except that during the addition of (*S*)-(+)-amphetamine (30.0 g) to 150 mL of fuming (*d* 1.50) HNO_3 over a period of 2.5 h the temperature of the reaction mixture was not permitted to rise above −30 °C. The crude product base was dissolved in 30 mL of Et_2O–EtOH (2:1) and saturated with dry HCl to yield 24.1 g (52%) of product. A sample recrystallized from EtOH had mp 197–201 °C, lit.[34] 197–199 °C; UV (95% EtOH) λ_{max} 273 nm (ϵ 10 900); $[\alpha]^{23}_D$ +19.3° (*c* 2.54, H_2O), lit.[34] $[\alpha]^{20}_D$ +22.0°. **1b** was obtained in analogous fashion in 54% yield, mp 197–199 °C; $[\alpha]^{23}_D$ −18.8° (*c* 2.50, H_2O).

(*S*)-(+)- (2a) and (*R*)-(−)-1-(4-Nitrophenyl)-2-(dimethylamino)propane Hydrochloride (2b). **2a** was prepared by the procedure of Gruber and Gunsalus:[14] mp 214–216 °C, lit.[14] 224–226 °C; $[\alpha]^{23}_D$ +9.0° (*c* 2.53, H_2O), lit.[34] $[\alpha]^{20}_D$ +78°. **2b** was prepared by the analogous procedure: mp 212–213 °C; $[\alpha]^{23}_D$ −8.4° (*c* 2.50, H_2O).

(*S*)-(+)- (3a) and (*R*)-(−)-1-(4-Aminophenyl)-2-(dimethylamino)propane Hydrochloride (3b). The (*S*)-(−)-amine was prepared by catalytic reduction of the (*S*)-(+)-nitro compound, following the procedure of Gruber and Gunsalus,[34] and converted to the (*S*)-(+)-hydrochloride: mp 198–202 °C; $[\alpha]^{23}_D$ +11.1° (*c* 1.21, H_2O). The (*R*)-(−) enantiomer was prepared by the analogous procedure: mp 198–201 °C; $[\alpha]^{23}_D$ −12.0° (*c* 1.20, H_2O).

(*R*)-(+)- (4a) and (*S*)-(−)-Phenylalaninol (4b). These were prepared by the procedure of Venkateswaran and Bardos[31] from the corresponding phenylalanine enantiomers. Melting points and rotations agreed with literature values.

(*S*)-(+)- (5a) and (*R*)-(−)-*N*-Dansylamphetamine (5b). (*S*)-(+)-Amphetamine (0.34 g, 2.5 mmol) was substituted for **4a** in the following procedure, and the reaction mixture was stirred for 2.5 h and filtered. CH_3CN was removed from the filtrate in vacuo and the remaining aqueous slurry was extracted with Et_2O. Evaporation of the dried ($MgSO_4$) extract and recrystallization from C_6H_{12} yielded **5a**: yield 0.41 g (46%); mp 93–94 °C; $[\alpha]^{29}_D$ +51.7° (*c* 4.80, CH_2Cl_2); UV (0.1 N HCl in 95% EtOH) λ_{max} 278 nm (ϵ 5900), 288 (6950), 298 (5300), 320 (1800). Anal. ($C_{21}H_{24}N_2O_2S$) C, H, N. The (*R*)-(−) enantiomer was prepared in 71% yield by the analogous procedure: mp 94–95 °C; $[\alpha]^{28}_D$ −50.1° (*c* 30.0, CH_2Cl_2). Anal. ($C_{21}H_{24}N_2O_2S$) C, H, N.

(*R*)-(+)- (6a) and (*S*)-(−)-*N*-Dansylphenylalaninol (6b). Dansyl chloride (0.68 g, 2.5 mmol) was added to a mixture containing 50 mL of CH_3CN, 10 mL of H_2O, 10 mL of saturated aqueous $NaHCO_3$, and 0.38 g (2.5 mmol) of **4a**. The orange solution was stirred for 1 h, concentrated in vacuo to a slurry, and refrigerated overnight. Filtration yielded 0.56 g (60%) of yellow crystals: mp 136–137 °C; $[\alpha]^{28}_D$ +83.7° (*c* 3.8, CH_2Cl_2); UV (0.1 N HCl in 95% EtOH) λ_{max} 278 nm (ϵ 5100), 290 (6600), 302 (5100), 322 (1660). Anal. ($C_{21}H_{24}N_2O_3S$) C, H, N. **6b** was prepared analogously from **4b**: mp 136–137 °C; $[\alpha]^{27}_D$ −78.0° (*c* 4.0, CH_2Cl_2). Anal. ($C_{21}H_{24}N_2O_3S$) C, H, N.

(*S*)-(−)- (7a) and (*R*)-(+)-*N*-Danthoylamphetamine (7b). To 0.70 g (3.0 mmol) of danthoyl chloride in 40 mL of dry CH_2Cl_2 was added 0.41 g (3.0 mmol) of (*S*)-(+)-amphetamine, followed by 0.61 g (6.0 mmol) of Et_3N and overnight stirring at room temperature. After solvent and excess Et_3N were removed in vacuo, the residue was suspended in H_2O and extracted with EtOAc. Evaporation of the dried ($MgSO_4$) extract yielded 0.95 g (85%) of crude **7a**, mp 129–132 °C, which was recrystallized from Et_2O–EtOH (9:1): mp 133–134 °C; $[\alpha]^{28}_D$ −9.9° (*c* 7.1, CH_2Cl_2); UV (0.1 N HCl in 95% EtOH) λ_{max} 275 nm (ϵ 7700), 283 (8600), 298 (6800), 317 (1100). Anal. ($C_{22}H_{24}N_2O$) C, H, N. The (*R*)-(+) enantiomer was prepared in an analogous fashion in 79% yield: mp 131–132 °C; $[\alpha]^{28}_D$ +8.3° (*c* 1.94, CH_2Cl_2). Anal. ($C_{22}H_{24}N_2O$) C, H, N.

(*R*)-(+)- (8a) and (*S*)-(−)-*N*-Danthoylphenylalaninol (8b). **8a** was prepared from (*R*)-(+)-phenylalaninol by the method given for the amphetamine analogue. The crude product (oil) was purified by column chromatography on Florisil, eluted with $CHCl_3$ and $CHCl_3$–EtOAc (1:1), followed by recrystallization from C_6H_{12}–EtOAc (5:1). The product was obtained in 27% yield: mp 130 °C; $[\alpha]^{28}_D$ +29.1° (*c* 6.8, CH_2Cl_2); UV (0.1 N HCl in 95% EtOH) λ_{max} 275 nm (ϵ 7700), 283 (8600), 293 (6800), 317 (1100). Anal. ($C_{22}H_{24}N_2O_2$) C, H, N. The enantiomer **8b** was prepared similarly in 70% yield: mp 130–131 °C; $[\alpha]^{28}_D$ −26.1° (*c* 3.0, CH_2Cl_2). Anal. ($C_{22}H_{24}N_2O_2$) C, H, N.

Testing of Local Anesthetic Activity. Spider crabs (*Libinia emarginata*) were purchased from the Marine Biological Laboratory in Woods Hole, Mass. The claw nerves were dissected as described previously.[35]

Solutions of the compounds tested for nerve-blocking activity were prepared in physiological saline (416 mM NaCl, 10 mM KCl, 11 mM $CaCl_2$, 55 mM $MgCl_2$, 2.5 mM $NaHCO_3$, pH 8.0). For external electrophysiological recordings, the nerve was placed on five Ag–AgCl electrodes (two recording, two stimulating, one ground) in a 5-mL Lucite chamber containing the test solution. The external bathing medium was removed at measured intervals for recording of the compound action potential. The nerve was stimulated briefly with pulses of 0.4-ms duration and threshold intensity using a Grass S4 stimulator with an SIU 4678 stimulus isolation unit. Responses were recorded on a Tektronix 564 storage oscilloscope via a 3A3 dual-trace differential amplifier. Experiments were carried out at 18–20 °C. Control experiments were

(36) C. C. Pfeiffer, *Science*, **124**, 29 (1956).

run with 1 mM procaine hydrochloride and 5 mM procainamide hydrochloride.

Testing of Fluorescent Probes. The nerve bundles were immersed in artificial sea water (423 mM NaCl, 9 mM KCl, 10 mM $CaCl_2$, 23 mM $MgCl_2$, 25.5 mM $MgSO_4$ buffered to pH 8 with Tris) containing the fluorescent probe in concentrations ranging from 25 to 50 μM for a period of 30 min. The poor solubility of some of the dyes in water could be overcome by dissolving the compounds in 0.1 mL of 95% EtOH and then diluting to 100 mL with artificial sea water. This concentration of EtOH had no measurable effect on conduction. Fluorescence emission was studied using the apparatus described by Tasaki et al.[20] The stained nerves were exposed to quasimonochromatic light (365 ± 10 nm) from a 200-W xenon–mercury lamp through a Kodak interference filter. The fluorescence emission from the nerve was detected at right angles to the incident light with a photomultipilier tube (RCA C70109E) through an absorption filter (Wratten 2A). A polarizer (Polaroid MBP'B) could be inserted between the interference filter and the nerve and an analyzer (HN38 or KN36) between the nerve and the absorption filter. Averaging (64 to 256 scans) was carried out with a Nicolet 1070 signal averaging computer to visualize fluorescence signals. All experiments were carried out at 8 °C.

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Antihypertensive Indole Derivatives of Phenoxypropanolamines with β-Adrenergic Receptor Antagonist and Vasodilating Activity

William E. Kreighbaum,* W. Lesley Matier, Ronald D. Dennis, Joseph L. Minielli, David Deitchman, James L. Perhach, Jr., and William T. Comer

Pharmaceutical Research, Mead Johnson Pharmaceutical Division, Mead Johnson & Company, Evansville, Indiana 47721.
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A series of 25 aryloxypropanolamines containing the 3-indolyl-*tert*-butyl [i.e., 1,1-dimethyl-2-(1*H*-indol-3-yl)ethyl] or substituted 3-indolyl-*tert*-butyl moiety as the N substituent is reported. These compounds have been tested for antihypertensive activity in spontaneously hypertensive rats (SHR), β-adrenergic receptor antagonist action in conscious normotensive rats, vasodilating activity in ganglion-blocked rats with blood pressure maintained by angiotensin II infusion, and for intrinsic sympathomimetic action (ISA) in reserpinized rats. Some of the compounds exhibit antihypertensive activity in combination with β-adrenergic receptor antagonist and vasodilating action. The structure–activity relationships in these tests are discussed.

The past few years have seen greatly expanded use of β-adrenergic receptor antagonists (β blockers) for the treatment of essential hypertension. Limitations to this form of therapy, for patients in whom they are not contraindicated, lie mainly in the fact that (a) β blockers are effective in only about 50% of hypertensive patients, (b) onset of action is often slow, and (c) therapy may be, at least initially, accompanied by an increase in peripheral vascular resistance. Clinically it has been shown that addition of a vasodilator to β-blocker therapy tends to overcome these shortcomings of β-blocker therapy alone and increases the controlled population to about 70%.[1]

Several recent reports describe compounds that combine β-blocking and vasodilating activity in single molecules.[2-4] We now report the synthesis and biological properties of a new series of agents, VI, which exhibit this dual action. These compounds have a 1,1-dimethyl-2-(1*H*-indol-3-yl)-ethyl group, referred to as 3-indolyl-*tert*-butyl, for the nitrogen substituent.

Chemistry. The indole derivatives in Table I (compounds **1–25**) were prepared by the general reaction sequence shown in Scheme I. The aniline derivative **13** was obtained by catalytic reduction of the corresponding nitro compound **12**.

The phenolic precursors I were either available commercially or, in the case of 2-methyl-4-(methylsulfonyl)-phenol,[5] prepared by the literature procedure. Alkylation of the appropriate I with epichlorohydrin was followed by treatment with alkali to afford the epoxide II, which was then aminated with an indolyl-*tert*-butylamine, V, to produce the product VI.

Indolyl-*tert*-butylamines Va–d were obtained by conversion of the appropriate commercially available indole derivative to the corresponding gramine[6] IIIa–d which was, in turn, transformed by a two-step sequence[7] to the desired material. The N^1-methyl derivative Ve of indolyl-*tert*-butylamine was obtained from Va by a general procedure for the N-alkylation of indoles.[8]

Biology. The compounds in Table I were tested for β-blocking, intrinsic sympathomimetic (ISA), antihypertensive, and vasodilator activities as shown in Table II.

(1) Veterans Administration Cooperative Study Group on Antihypertensive Agents, J. R. Thomas (Chariman), *J. Am. Med. Assoc.*, **237**, 2303 (1977).
(2) J. J. Baldwin, R. Hirschmann, P. K. Lumma, W. C. Lumma, Jr., G. S. Ponticello, C. S. Sweet, and A. Scriabine, *J. Med. Chem.*, **20**, 1024 (1977).
(3) R. C. Dage, H. C. Cheng, C. P. Hsieh, and J. K. Woodward, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **37**, 633 (1978).
(4) R. E. Philion, D. K. Phillips, S. C. Laskowski, D. C. Schlegel, R. R. Lorenz, P. H. Hernandez, and H. E. Lape, "Abstracts of Papers", 176th National Meeting of the American Chemical Society, Miami Beach, FL, Sept 11–14, 1978, MEDI 24.
(5) J. Yates and E. Haddock, British Patent 1 128 217, Sept 1968, to Shell Internationale Research Maatschappii, NV; *Chem. Abstr.*, **70**, 19804*g* (1969).
(6) Arvid Ek and Bernhard Witkop, *J. Am. Chem. Soc.*, **76**, 5579 (1954).
(7) H. R. Snyder and L. Katz, *J. Am. Chem. Soc.*, **69**, 3140 (1947).
(8) H. Heaney and S. V. Ley, *J. Chem. Soc., Perkin Trans 1*, 499 (1973).

0022-2623/80/1823-0285$01.00/0