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Stereochemical Studies on Medicinal Agents. 10.[1,2] The Role of Chirality in α-Adrenergic Receptor Blockage by (+)- and (−)-Phenoxybenzamine Hydrochloride[3]

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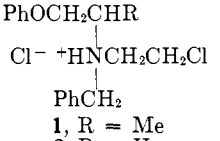
The synthesis and absolute configurational assignment of (+)- and (−)-phenoxybenzamine·HCl (**1**) were accomplished by utilization of (*R*)- and (*S*)-alanine as starting materials. (*R*)-(+)-**1** was 14.5 times more potent than (*S*)-(−)-**1** while the desmethyl analog **2** possessed intermediate α-adrenergic blocking activity. Evidence is presented which suggests that the potency difference between enantiomers is due to an affinity difference for the receptor rather than to a difference in intrinsic alkylating capacity. The differences in affinity between (*R*)-(+)-**1**, (*S*)-(−)-**1**, and **2** are postulated to be related to the abilities of the aziridinium species derived from these compounds to achieve a negative synclinal conformation and to the binding energy afforded by a properly oriented methyl group.

Although stereostructure–activity relationships of ligands acting at α-adrenergic receptors have been investigated extensively,[5] there is relatively little information[6-8] available concerning the effects of stereoisomerism in nonequilibrium[9] adrenergic blockade by β-haloethylamines (β-HEAs).

Since it is known that many β-HEAs which are capable of forming aziridinium ions are ineffective as alkylators of adrenergic receptors,[8,10] it is apparent that both chemical reactivity and complementarity between antagonist and receptor are of major importance. This was elaborated upon by Belleau[11] who proposed that the phenyl ring and alkylating carbon of the aziridinium species derived from β-HEAs are required to fit a phenethylamine pattern conducive to alkylation of an anionic site on the receptor. If this is the case, it would be expected that β-HEAs which possess a *critically positioned* chiral center should show a high degree of stereoselectivity.

We report herein the synthesis and biological evaluation of phenoxybenzamine·HCl (**1**)[12-14] enantiomers in an effort to shed light on this aspect of β-HEAs.



1, R = Me
2, R = H

Chemistry.—Enantiomers of **3** were prepared from (*R*)- and (*S*)-alanine by modification of a known[15] procedure. Treatment of **3** with $SOCl_2$ afforded **4** which was converted *in situ* into aziridine **5** by the action of NaOPh. The aziridine could be isolated in pure form by treatment with base followed by distillation. It is noteworthy that reaction of **5** with HCl readily gave **4** rather than **5**·HCl. Other aziridines also have been reported[16] to exhibit this property. Intermediate **6** was obtained by prolonged refluxing of an ethanolic solution of **5** and NaOPh. Under these conditions a substantial amount of unreacted **5** was

(1) We gratefully acknowledge support of this work by Public Health Service Grant GM09402.

(2) Part 9 of this series: P. S. Portoghese and J. G. Turcotte, *J. Med. Chem.*, **14**, 288 (1971).

(3) Presented in part at the 160th National Meeting of the American Chemical Society, Chicago, Ill., Sept 1970, MEDI-39.

(4) Predoctoral trainee (1967–1969), National Institute of Health Training Grant GM11769.

(5) P. N. Patil, J. B. LaPidus, and A. Tye, *J. Pharm. Sci.*, **59**, 1205 (1970); P. S. Portoghese, *Annu. Rev. Pharmacol.*, **10**, 51 (1970); D. J. Triggle, "Chemical Aspects of the Autonomic Nervous System," Academic Press, London, 1965.

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(7) B. Belleau and D. J. Triggle, *J. Med. Pharm. Chem.*, **5**, 636 (1962).

(8) G. E. Ullyot and J. F. Kerwin, *Med. Chem.*, **2**, 234 (1956).

(9) M. Nickerson, *Pharmacol. Rev.*, **1**, 27 (1949).

(10) J. D. P. Graham, *Progr. Med. Chem.*, **2**, 132 (1962).

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(12) J. F. Kerwin, G. C. Hall, F. J. Milnes, I. H. Witt, R. A. McLean, E. Macko, E. J. Fellows, and G. Ullyot, *J. Amer. Chem. Soc.*, **73**, 4162 (1951).

(13) N. Nikerson and G. M. Nomaguchi, *J. Pharmacol. Exp. Ther.*, **101**, 379 (1951).

(14) E. H. Fellows, R. A. McLean, E. Macko, J. F. Kerwin, G. C. Hall, F. J. Milnes, I. H. Witt, and G. E. Ullyott, *ibid.*, **110**, 462 (1954).

(15) J. H. Hunt and D. McHale, *J. Chem. Soc.*, 2073 (1957).

(16) P. E. Fanta, in "Heterocyclic Compounds with Three- and Four-Membered Rings," Part I, Chapter II, A. Weissberger, Ed., Interscience, New York, N. Y., 1964, pp 524–564.

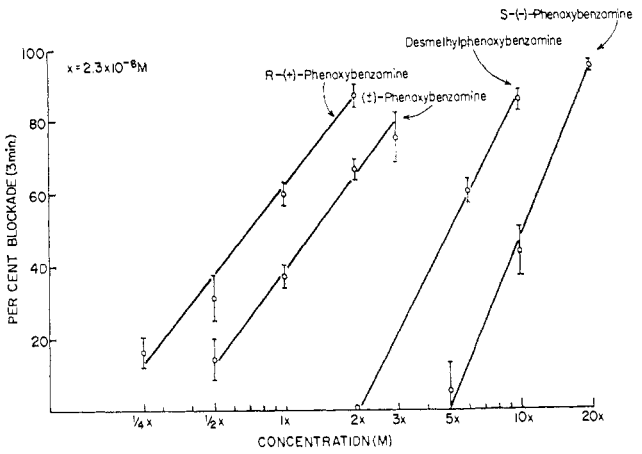
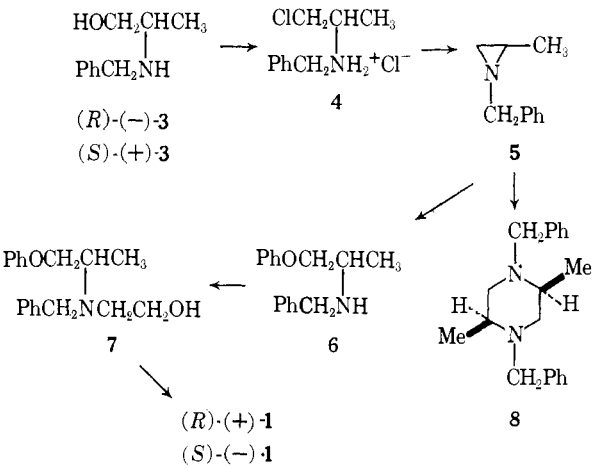


Figure 1.—The per cent blockade of the response to norepinephrine (5×10^{-5} M) on the vas deferens of the rat after a 3-min exposure to several different concentrations of various β-HEAs.

recovered. When DMF, PhOH, and (*S*)-**5** were employed, the piperazine **8**, $[\alpha]D$ +125°, was obtained in addition to a poor yield of **6**. The cis stereochemistry is assigned to **8**, as the trans isomer should be optically inactive by virtue of its center of symmetry. Reaction of **6** with ethylene oxide yielded **7** which then was converted into optically active **1** with $SOCl_2$.



Since the stereochemical integrity of the chiral center was maintained during the reaction sequence, (+)- and (−)-**1** possess the same chiralities as (*R*)- and (*S*)-alanine, respectively, from which they were derived.

Pharmacology.—Figure 1 shows the per cent blockade produced by various concentrations of the compounds after a 3-min period of exposure. The dose–response curves were parallel and the relative potencies for a 50% blockade were: (*R*)-(+)-phenoxybenzamine (1.0), (±)-phenoxybenzamine (1.9), desmethylphenoxybenzamine (7.2), and (*S*)-(−)-phenoxybenzamine (14.5). In this ranking the most potent compound has a value of 1.0. The blocking activity of the racemate can be accounted for by the amount of (*R*)-(+) isomer present. As expected the racemic mixture has about one-half the activity of the (*R*)-(+) form. The quantity of (*S*)-(−) isomer present in concentrations of the racemate needed for complete blockade would be expected to produce essentially no activity. The activity of desmethylphenoxybenzamine (**2**) was about twice that of (*S*)-(−)-phenoxybenzamine but considerably less potent than the (*R*)-(+) isomer.

The time required to produce a 50% blockade in the presence of various concentrations of the blocking agents is shown in Figure 2. The curves are parallel and the relative potencies after various exposure times are essentially the same as those previously given for 3 min.

It is of interest to note that both of the isomers of phenoxybenzamine inhibitied the uptake of norepinephrine by the isolated auricles of the rabbit at concentrations of 7×10^{-6} M for 20 min. The inhibition of transport was about the same for the two isomers. The concentration required to inhibit transport was much greater than that for adrenergic blockade inasmuch as exposure of the vas deferens to a concentration of 2.3×10^{-8} M (*R*)-(+)-phenoxybenzamine for 10 min produced almost complete adrenergic blockade, but did not alter the uptake of norepinephrine by this tissue. Thus, the receptor site for adrenergic blockade and for catecholamine transport differ with respect to the specificity and affinity of the optical isomers.

Discussion

The lack of stereoselectivity for (*R*)-(+)- and (*S*)-(−)-**1** at the norepinephrine uptake sites rules out the remote possibility that differential inhibition of catecholamine transport is responsible for the large potency difference between enantiomers. Moreover, the difference in rate of receptor blockade appears to be independent of the rate of cyclization to an aziridinium species, since the rates of aziridinium ion formation for (*R*)-(+)- and (*S*)-(−)-**1** are identical by virtue of their enantiomeric relationship. As it is expected that enantiomers should have very similar, if not identical, access to the biophase, it is therefore very probable that the observed potency difference represents events at the receptor level.

The high stereoselectivity suggests that multiple-point interaction rather than 2-point contact[17] of the aziridinium species with the receptor is required for complex formation. The reported[7] lack of optical stereoselectivity in the case of *N,N*-dimethyl-2-bromo-2-phenethylamine may be due to the possibility that the chiral center is not located in a part of the molecule which is sensitive to steric effects in the receptor interaction. Lower stereoselectivity also may have been due to the specific testing procedure which was employed.[18]

Recent studies[19] have provided evidence that the action of β-HEAs is biphasic in nature. The initial phase of blockade, being competitive and reversible, is presumed to arise from formation of a reversible complex between the aziridinium species and receptor. The second phase is characterized by irreversible blockade and is a consequence of receptor alkylation. Thus it appears reasonable that the rate of irreversible blockade may be dependent on receptor affinity and on proper juxtaposition of the aziridinium ring with a nucleo-

(17) B. Belleau, *Ann. N. Y. Acad. Sci.*, **139**, 580 (1967).

(18) The *in vivo* test methods[8] usually involve the measurement of the blood pressure response to epinephrine after administration of the β-HEA. If the interval between administration of the β-HEA and measurement of epinephrine-induced response is long (0.5–1 hr) it is possible that the test procedure may not detect a moderate difference in potency between enantiomers, particularly when the onset of adrenergic blockade is rapid.

(19) J. F. Moran, C. R. Triggle, and D. J. Triggle, *J. Pharm. Pharmacol.*, **21**, 38 (1969).

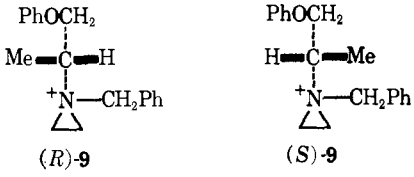
philic group on the receptor so that alkylation can take place.

The biphasic feature suggests two interesting alternative possibilities concerning the stereoselectivity of α-adrenergic receptor alkylation by the reactive ions, (*R*)-**9** and (*S*)-**9**, derived from (*R*)-(+)-**1** and (*S*)-(−)-**1**, respectively.

(1) The enantiomeric alkylating agents possess similar affinities but have greatly different abilities to alkylate the receptor after reversible complex formation. This implies that the enantiomers have different modes of receptor interaction which result in the placement of the alkylating center at different distances from the nucleophilic group on the receptor. The model predicts that the less active isomer should offer some protection against alkylation by the more potent isomer.

(2) The enantiomeric alkylating agents differ substantially in receptor affinity and alkylate the receptor at similar rates after reversible complex formation. This model implies that (*R*)-**9** and (*S*)-**9** both form reversible complexes which result in near-equal accessibility of the alkylating carbon to the nucleophilic component on the receptor. Accordingly, the rates of receptor alkylation would be dependent on the magnitude of the ligand–receptor dissociation constant and no receptor protection should be afforded by the less potent isomer.

A combination of cases 1 and 2 presents a third possibility, but for the sake of brevity we shall dwell on the simpler models as a first approximation.



(*R*)-**9** (*S*)-**9**

The finding that the racemic **1** is about half as potent as (*R*)-(+)-**1** is consistent with the second model, inasmuch as it is evident from the dose–response curves (Figure 1) that the less active isomer [(*S*)-(−)-**1**] shows no blocking action at a concentration equal to that found in the racemate at 50% blockade. Hence, *the potency difference between enantiomers is attributed to an affinity difference rather than to a difference in intrinsic alkylating capacity.* The fact that parallel slopes are obtained when concentrations of the β-HEAs are plotted against "minutes for 50% blockade" (Figure 2) also is consistent with this interpretation and suggests that, in the concentration ranges employed, the mechanism of receptor inactivation essentially is the same for the enantiomers and the desmethyl compound **2**.

According to the interpretation above, the order of blocking potencies [(*R*)-(+)-**1** > **2** > (*S*)-(−)-**1**] should parallel the order of affinities in reversible complex formation. Since **2** possess a potency twice that of (*S*)-(−)-**1** and 0.07 times that of (*R*)-(+)-**1**, it appears that the methyl group attached to the chiral center is enhancing complex formation in (*R*)-**9** and hindering this process in (*S*)-**9**.

It has been proposed[6,11] that aziridinium ions derived from phenoxyethyl-β-HEAs assume a gauche OCCN conformation which enables the ligand to fit the α-adrenergic receptor. Conformations of (*R*)-**9** and

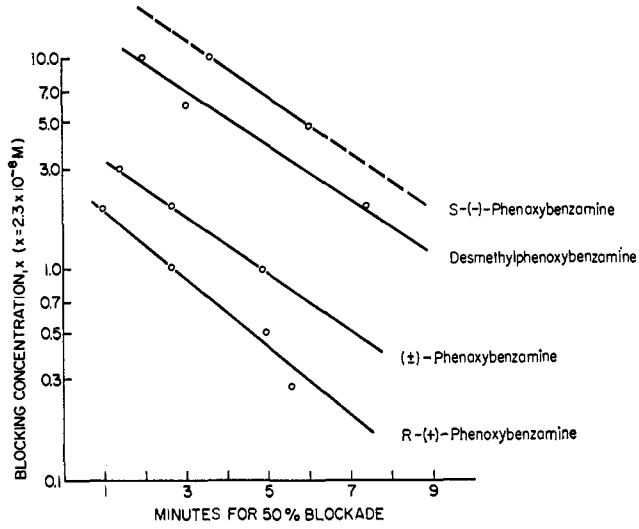
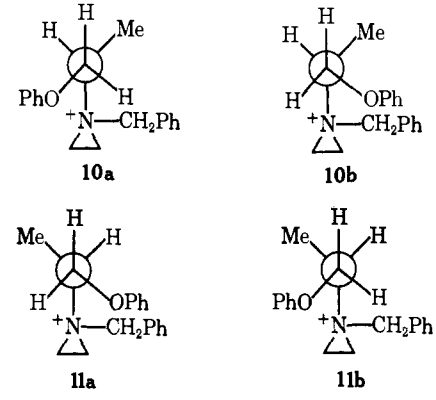


Figure 2.—Time required to produce 50% blockade of the response of the vas deferens to norepinephrine after exposure to various concentrations of β-HEAs.

(*S*)-**9** that fulfill this gauche relationship are depicted by projection formulas **10** and **11**, respectively. In the more active isomer ((*R*)-**9**), **10a** should be more stable than **10b** because of the gauche PhO–Me interaction in the latter. Similarly, **11a** should possess greater stability than **11b** in the less active isomer ((*S*)-**9**). It



can be noted that **10a** and **11b** both possess a negative synclinal (− sc)[20] torsion angle while **10b** and **11a** are positive synclinal (+ sc).

If it is assumed that the more active isomer (*R*)-**9** binds to the receptor in conformation **10a**, the difference in blocking potencies can be rationalized on the basis that the less active isomer (*S*)-**9** must complex as the less stable conformer **11b** in order to possess a torsion angle (− sc) identical with that of **10a**. As a result, **11b** should form a weaker reversible complex with the receptor and consequently (in accordance with case 2) alkylate the receptor at a slower rate.

In addition to the possibility that C-1 chirality influences activity by governing the equilibrium (−sc ⇄ +sc) among gauche conformers in (*R*)-**9** and (*S*)-**9**, it appears likely that the methyl group at this chiral center also influences reversible complex formation by *direct involvement* in the receptor interaction. Thus, the methyl group in the more active isomer (*R*)-(+)-**1** may contribute to receptor affinity through hydrophobic bonding and/or van der Waals attraction. This is

(20) W. Klyne and V. Prelog, *Experientia*, **16**, 521 (1960).

consistent with our observation that the desmethyl compound **2** is substantially less potent than (*R*)-(+)-**1** despite the fact that aziridinium species derived from **2** must possess equal amounts of −sc and −sc conformations due to its symmetry. The intermediate activity of **2** consequently may be the result of two factors.

(1) The energy barrier for attainment of the pharmacophoric −sc conformation of the aziridinium ion derived from **2** is greater than that for (*R*)-**9** and less than that for (*S*)-**9**.

(2) The binding energy afforded by a properly oriented methyl group is absent in **2**.

Lewis and Miller[21] have employed (±)-[^{3}H]phenoxybenzamine for investigation of α-adrenergic receptors. Since our studies indicate that (*R*)-(+)-**1** is considerably more potent that (*S*)-(−)-**1**, it is likely that (*R*)-(+)-**1** is more selective in its action and hence may be useful in receptor labeling experiments.

Experimental Section[22]

***N*-Benzyl-2-amino-1-propanol** (**3**).—Dried, recrystd samples of *N*-benzoylalanine[23] were reduced with LAH according to the procedure of Hunt and McHale[15] with the exception that THF was used as reaction solvent and higher yields (80–85%) of **3** were obtained.

***N*-Benzyl-1-chloroisopropylamine·HCl** (**4**).—A soln of 8.25 g (0.05 mole) of **3** in 75 ml of distd PhH (previously treated with HCl gas) was flushed with N_2 for 15 min followed by dropwise addn of 6.54 g (0.055 mole) of $SOCl_2$ in 25 ml of dry, distd PhH. The reaction mix was cooled in an ice bath during $SOCl_2$ addn. After addn was complete the mixt was allowed to warm to room temp and refluxed until a solid ppt formed. The ppt was filtered, washed with PhH, and crystd from Me_2CO–EtOH to yield a total of 8.85 g (80.5%) of (±)-**4**: mp 149–150°; (*R*)-**4**: mp 155–157°; $[\alpha]^{22}D$ +9.3° (*c* 4, EtOH); (*S*)-**4**: mp 158–159°; $[\alpha]^{22}D$ −9.8° (*c* 4, EtOH). *Anal.* [(±), (+), (−)-$C_{10}H_{14}NCl$·HCl] C, H, N.

***N*-Benzyl-1-phenoxyisopropylamine** (**6**).—An ethanolic soln (250 ml) contg 22.01 g (0.1 mole) of **4** and 23.2 g (0.2 mole) of NaOPh was refluxed for 120 hr. After cooling, the solvent was removed *in vacuo* and the oily residue was extd with Et_2O. The Et_2O was washed with equal vol of 2 *N* NaOH and satd NaCl soln. The Et_2O ext was dried (Na_2SO_4) and fractionally distd to yield 10.3 g (42.7%) of (±)-**6**: bp 155–159° (0.5 mm) [lit.[24] 150–152° (0.4 mm)]; (*R*)-**6**: bp 150–155° (0.2 mm); $[\alpha]^{22}D$ +10.2 (*c* 4, EtOH); (*S*)-**6**: bp 145–150° (0.15 mm); $[\alpha]^{22}D$ −10.6° (*c* 4, EtOH). *Anal.* [(+), (−)-$C_{16}H_{19}NO \cdot C_2H_2O_4$] C, H, N.†

***N*-Benzyl-2-methylaziridine** (**5**).—This intermediate was usually generated *in situ* by the action of NaOPh on **4** in the prepn of **6**. The aziridine could be isolated in quantitative yield by concn of the ethanolic reaction mix, extn with NaOH, and distn of the residue: (*R*)-**5**: bp 46–48° (0.15 mm); $[\alpha]^{22}D$ −10.9° (*c* 4, EtOH); (*S*)-**5**: bp 47–48° (0.15 mm); $[\alpha]^{22}D$ +11.5° (*c* 4, EtOH). *Anal.* [(±)-$C_{10}H_{13}N$] H, N, C.‡

***N*-Benzyl-*N*-(2-hydroxyethyl)-1-phenoxyisopropylamine** (**7**).—A mixt (0.021 mole) of **6**, 1.85 (0.042 mole) of ethylene oxide, and 0.4 g (0.0021 mole) of distd H_2O in a sealed tube was heated at 100° for 4 hr and at 120° for an addnl 6 hr. The cooled mixt was extd with Et_2O and the ext was dried (Na_2SO_4). Removal of Et_2O *in vacuo* yielded 5.1 g (84.5%) of (±)-**7**: bp 160–170° (0.3 mm) [lit.[12] 150–151° (0.25 mm)]. (*R*)-**7** was purified by column chromatog (silica gel, *n*-hexane–$CHCl_3$); $[\alpha]^{22}D$ +15.0 (*c* 4, EtOH); (*S*)-**7**: bp 155–165° (0.3 mm); $[\alpha]^{22}D$ −14.3° (*c* 4, EtOH). The ir and nmr spectra were identical with those obtained from authentic (±)-**7**.

***N*-Benzyl-*N*-(2-chloroethyl)-1-phenoxyisopropylamine·HCl** (**1**).—A soln of 4.5 g (0.016 mole) of **7** in 30 ml of $CHCl_3$ (satd with HCl gas) was treated with 2.38 g (0.002 mole) of $SOCl_2$ in 10 ml of $CHCl_3$ as previously described for the prepn of **4**. Crystn from hot EtOAc–EtOH followed by *in vacuo* drying (60°) provided 2.95 g (55%) of product. (*R*)-**1**: mp 123–124°; $[\alpha]^{22}D$ +18.01° (*c* 4, EtOH); (*S*)-**1**: mp 124–125°; $[\alpha]^{22}D$ −17.55° (*c* 4, EtOH). The ir and nmr spectra were identical with those obtained from authentic (±)-**1**.[12] *Anal.* [(+), (−)-$C_{18}H_{22}NCl$·HCl] C, H, N.

(2*S*,5*S*)-Dimethyl-1,4-dibenzylpiperazine (**8**).—A soln of 14.7 g (0.1 mole) of (*S*)-**5** and 37.6 g (0.4 mole) of PhOH in 100 ml of DMF was heated at 60° for 6 hr. The soln was concd *in vacuo* and the residue was extd with Et_2O. The ext was washed with 2 *N* NaOH and satd NaCl soln and dried (Na_2SO_4). Removal of Et_2O gave an oil which on fractional distn yielded 12.3 g of (*S*)-**5** and 4.3 g of crude **8**, bp 130–175° (0.15 mm). Chromatog of the latter oil (silica gel, PhH–Et_2O, 4:1) gave 1.3 g of (*S*)-**6** and 2.49 g of **8**: mp 64–66°; $[\alpha]D^{22}$ +125.35° (*c* 4, EtOH); nmr ($CDCl_3$) δ 1.05 (d, 6 H, CH_3), 2.45 (m, 6 H, piperazine ring CH), 3.55 (AB quartet, 4 H, benzylic CH_2), 7.45 (s, 10 H, aromatic CH). *Anal.* ($C_{20}H_{26}N_2$) C, H, N.

Pharmacological Testing.—The isolated vas deferens of the rat (~250 g) was suspended in modified Kreb's soln (37°) into which was bubbled O_2–CO_2 (95:5). Drug-induced contractions were recorded isometrically under 0.5-g tension on a Gilson polygraph. In the presence of norepinephrine (5×10^{-5} *M*) the vas deferens contracted to ~95% of maximum. At least 2 control responses were obtained with this standard dose. The tissue was washed until a stable base line was achieved, and a given dose of β-HEA was added to the bath fluid. After a specified interval the tissue was washed, norepinephrine was added, and the response was recorded. The per cent decrease in the contractile response was calculated and represents the degree of adrenergic blockade.

Acknowledgment.—The technical assistance of Mrs. Patricia Piheaja is gratefully acknowledged. We wish to express our appreciation to Dr. J. Stubbins and Dr. G. Ullyott, who provided us with specimens of racemic **1**, **2**, and **7**.

(21) J. E. Lewis and J. W. Miller, *J. Pharmacol. Exp. Ther.*, **154**, 46 (1966).

(22) All melting points (uncorrected) were determined with a Thomas-Hoover melting point apparatus. Microanalyses were performed by Schwarzkopf Microanalytical Laboratory, Woodside, N. Y. Ir spectra were obtained on a Perkin-Elmer 237B spectrophotometer. Optical rotations were obtained using a Perkin-Elmer 114 polarimeter and a 1-dm cell. Nmr spectra were obtained on a Varian A60D spectrometer.

(23) "Chemistry of the Amino Acids," Vol. 2, J. P. Greenstein and M. Winitz, Ed., Wiley, New York, N. Y., 1961, p 1267.

(24) J. van Dijk and H. D. Moed, *Recl. Trav. Chim. Pays-Bas*, **78**, 22 (1959).

† N for (*S*)-**6**: calcd, 65.24; found 65.03.

‡ C: 81.58; found 80.99.