washed with water. Recrystallization of the residue from dilute ethanol gave 1.3 g. (94%) of IX, m.p. 114–115°. IR: 1620 cm.$^{-1}$ (C=O), 3340 cm.$^{-1}$ (—NH—), 700 cm.$^{-1}$ (—S—), and 3017 cm.$^{-1}$ (cyclohexyl).

Anal.—Calc. for $C_{23}H_{28}N_2OS$: C, 72.59; H, 7.41; N, 7.36; S, 8.43. Found: C, 72.53; H, 7.25; N, 7.28; S, 8.55.

The hydrochloride was similarly prepared as described for IV, m.p. 169–170°.

2-Chloro-3,4-cyclohexenothiaxanthone (X)—Diazotization of VIII and treatment of the diazonium solution with cuprous chloride, as described for III, gave X. After recrystallization from ethanol, it melted at 186–187°.

Anal.—Calc. for $C_{17}H_{13}ClOS$: C, 67.88; H, 4.36; S, 10.66. Found: C, 68.41; H, 4.67; S, 10.73.

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Preparation and Activity of β-Substituted Acetylcholine Iodides

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Abstract □ The enantiometers of (−)- and (+)-acetyl β-ethylcholine iodide were prepared following the resolution of 1-dimethylamino-2-butanol from (+)-tartaric acid and (+)-bromocamphorsulfonic acid for the (−)- and (+)-isomers, respectively. The absolute configuration of the (−)-enantiomer was determined by the synthesis of (−)-acetyl β-ethylcholine iodide from R(−)-2-hydroxybutyric acid. The optically active acetyl β-phenylcholine iodides were prepared from the optically active mandelic acids. These enantiomers show that the R(−)-isomers have greater affinity for the acetylcholinesterase receptor and that the S(+)-isomers are more potent agonists on guinea pig ileum.

Keyphrases □ Acetylcholine iodides, β-substituted—preparation, activity □ Acetyl β-ethylcholine iodide, enantiomers—preparation, absolute configuration, activity

It was shown previously (1, 2) that acetylcholinesterase and the smooth muscle of guinea pig ileum each reacts best with only one of the enantiomers of acetyl β-methylcholine. Cocolas *et al.* (3) also pointed out that the enantiomers of acetyl β-methylcholine likely assume a conformation in which the interface between them and the corresponding susceptible receptor area, during interaction, is the same as that for acetylcholine; that is, the facet of the choline fragment facing the receptor is the side that resembles acetylcholine.

We sought to extend the study of stereochemical requirements at the acetylcholinesterase and the smooth muscle receptor in guinea pig ileum by examining the activity of β-ethyl and β-phenyl acetylcholines. Some investigators (1, 4, 5) assumed that S(+)-acetyl β-methylcholine has better affinity for acetylcholinesterase than the R(−)-enantiomer. It might be expected that acetylcholinesterase would produce a greater disparity between the action of the enantiomers of β-ethyl- and β-phenyl-substituted acetylcholines, since the increase in bulk would produce a more remote possibility of similar accommodation of the enantiomeric pairs. An examination of the enzymatic activity of each optical isomer of β-ethyl and β-phenyl acetylcholine iodide was undertaken to test the hypothesis of enantiomeric stereoselectivity of the acetylcholinesterase receptor area. The potency of these enantiomeric pairs on guinea pig ileum was also investigated as a measure of their biologic activity on the muscarinic receptor site.

EXPERIMENTAL[1]

1-Dimethylamino-2-butanol—To a solution of 50 g. 1-nitro-2-butanol (6) in 50 ml. 90% formic acid and 125 ml. formalin (40%)

[1] Melting points were taken on a Mel-Temp apparatus and are uncorrected. Specific rotations were taken on a Cary 60 spectropolarimeter. The IR spectra were run on a Perkin-Elmer model 257 spectrophotometer. Microanalyses were carried out by M-H-W Laboratories, Garden City, Mich.

was added 2.0 g. 10% palladium–charcoal catalyst. The mixture was shaken in an atmosphere of hydrogen at an initial pressure of 75 p.s.i.g. on a Parr apparatus until the theoretical amount of hydrogen was taken up (24–36 hr.). The catalyst was removed, 75 ml. concentrated HCl solution was added, and the solvent was removed on a rotary evaporator with the aid of a hot water bath. The residue was dissolved in 100 ml. water and made alkaline with NaOH solution, and the product was extracted with ether. The ethereal solution was dried over anhydrous Na_2SO_4 and distilled, yielding 47.0 g. of a fraction, b.p. 138° [lit. (7) b.p. 142–143°]; n_D^{25} 1.4125; $\nu_{max.}^{CHCl_3}$ 3465 cm.$^{-1}$, broad (OH).

Anal.—Calc. for $C_6H_{15}NO$: C, 61.50; H, 12.90. Found: C, 61.28; H, 13.19.

Resolution of (+)- and (−)-1-Dimethylamino-2-butanol—The methods of Major and Bonnett (8) were used to separate the enantiomers.

(−)-1-Dimethylamino-2-butanol—The salt prepared from 40 g. racemic 1-dimethylamino-2-butanol and 52.5 g. (+)-tartaric acid was recrystallized 16 times from 96% ethanol, using 6 ml. solvent for each gram of salt and finally collecting 14.0 g. of a diastereoisomeric salt which showed no change in rotation from the previous recrystallization; $[\alpha]_{589.3\ nm.}^{25°}$ +0.5° (c 10.0, H_2O). The free base was obtained by dissolving the salt in a minimum amount of water, making the solution alkaline with 20% NaOH, and separating the optically active 1-dimethylamino-2-butanol. The product was dried over anhydrous Na_2SO_4 and distilled, b.p. 143–144°; n_D^{25} 1.4245; $[\alpha]_{589.3\ nm.}^{25°}$ −16.8° (c 1.0, 1 *N* HCl).

Anal.—Calc. for C, 61.50; H, 12.90. Found: C, 61.38; H, 12.89.

(+)-1-Dimethylamino-2-butanol—The ammonium salt (161 g.) of (+)-bromocamphorsulfonic acid (9) in 1 l. water was passed through a Dowex-50W resin (H^+ form), and the eluate was concentrated to a faint-yellow viscous liquid of (+)-bromocamphorsulfonic acid. The acid was mixed with 300 ml. ethanol and 61.0 g. racemic 1-dimethylamino-2-butanol, and the solution was stirred until homogeneous. The solution was neutral. The ethanol was removed on a rotary evaporator under reduced pressure, and the residue was treated with 500 ml. ethyl acetate. Then 204 g. of crystals was collected; $[\alpha]_{589.3\ nm.}^{25°}$ +69.0° (c 1.0, H_2O). The solid was recrystallized three times using 6 ml. of a 10:1 mixture of EtOAc–EtOH to each gram of solid, at which time a constant rotation for the two recrystallizations was observed; $[\alpha]_{589.3\ nm.}^{25°}$ +74.0° (c 1.0, H_2O). The free base was obtained as described for the (−)-isomer. (+)-1-Dimethylamino-2-butanol was distilled, b.p. 141–142°; n_D^{25} 1.4241; $[\alpha]_{589.3\ nm.}^{25°}$ +17.0° (c 1.0, 1 *N* HCl).

Anal.—Calc. for C, 61.50; H, 12.90. Found: C, 61.33; H, 13.12.

(−)-Acetyl β-Ethylcholine Iodide—(−)-1-Dimethylamino-2-butanol (200 mg.) was mixed with 2 ml. 2-propanol and 1.0 g. acetic anhydride. After 25 min., 1.0 g. methyl iodide was added, and the solution was allowed to remain 5 min. more at room temperature. Anhydrous ether was slowly added to initiate precipitation. The collected product (235 mg.) was recrystallized from 2-propanol, m.p. 181–182°; $[\alpha]_{589.3\ nm.}^{25°}$ −19.6° (c 1.0, H_2O); $\nu_{max.}^{KBr}$ 1740 cm.$^{-1}$ (C=O).

Anal.—Calc. for $C_9H_{20}INO_2$: C, 35.77; H, 6.99. Found: C, 35.69; H, 7.08.

(+)-Acetyl β-Ethylcholine Iodide—This compound was prepared according to the method already described. The product melted at 182–183°; $[\alpha]_{589.3\ nm.}^{25°}$ +19.8° (c 1.0, H_2O); $\nu_{max.}^{KBr}$ 1740 cm.$^{-1}$ (C=O).

Anal.—Calc. for $C_9H_{20}INO_2$: C, 35.77; H, 6.99. Found: C, 35.67; H, 6.78.

Resolution of R(−)-2-Hydroxybutyric Acid—2-Hydroxybutyric acid (1) was resolved with (−)-morphine, according to the method of Levene and Haller (10). The barium salt had a specific rotation of +17.5 at 589.3 nm. The free acid was a very hygroscopic, low melting solid and was purified by distillation, b.p. 85°/0.4 mm. Hg; $\nu_{max.}^{mull}$ 1730 cm.$^{-1}$ (C=O).

Anal.—Calc. for $C_4H_8O_3$: C, 46.60; H, 7.74. Found: C, 45.85; H, 8.20.

Determination of Absolute Configuration of (−)-Acetyl β-Ethylcholine Iodide R(+)-2-Acetoxybutyric Acid—R(−)-2-Hydroxybutyric acid (9.0 g.) was mixed with 20 g. of acetyl chloride at room temperature. When evolution of hydrochloric acid subsided, the excess acetyl chloride was removed by distillation and the product was distilled, b.p. 86°/0.2 mm. Hg; wt. 10.8 g.; $[\alpha]_{589.3\ nm.}^{25°}$ +41.0° (c 1.0, CH_3OH); $\nu_{max.}^{mull}$ 1725 cm.$^{-1}$ (ester C=O).

Anal.—Calc. for $C_6H_{10}O_4$: C, 49.33; H, 6.90. Found: C, 49.21, H, 7.07.

R(+)-*N,N*-Dimethyl-2-acetoxybutyramide—R(+)-2-Acetoxybutyric acid (10.8 g.) was mixed with 50 g. of thionyl chloride, and the mixture was refluxed for 30 min. The excess thionyl chloride was removed in a rotary evaporator, and the residue was dissolved in 200 ml. anhydrous Et_2O. This solution was added dropwise to an anhydrous ethereal solution containing 10 g. of dimethylamine. The precipitate was removed, and the filtrate was distilled to give 11.0 g. of product, b.p. 81°/0.4 mm. Hg; n_D^{25} 1.4490; $[\alpha]_{589.3\ nm.}^{25°}$ +4.87° (c 3.2, CH_3OH); $\nu_{max.}^{KBr}$ 1735 cm.$^{-1}$ (ester C=O), 1650 cm.$^{-1}$ (amide C=O).

Anal.—Calc. for $C_8H_{15}NO_3$: C, 55.43; H, 8.66. Found: C, 55.32; H, 8.90.

R(−)-1-Dimethylamino-2-butanol—To a suspension of 7.5 g. $LiAlH_4$ in 200 ml. anhydrous ether was added 11.0 g. of R(+)-*N,N*-dimethyl-2-acetoxybutyramide in 100 ml. ether. The reaction was refluxed for 3 hr., cooled, and treated with 50 ml. of a 40% potassium and sodium tartrate solution. The ether was filtered from the precipitate, dried over anhydrous Na_2SO_4, and distilled to yield 4.0 g. of the optically active aminoalcohol, b.p. 145°; n_D^{25} 1.4242; $[\alpha]_{589.3\ nm.}^{25°}$ −17.0° (c 2.5, 1 *N* HCl).

Anal.—Calc. for $C_6H_{15}NO$: C, 61.50; H, 6.99. Found: C, 61.42; H, 7.12.

R(−)-β-Ethylcholine Iodide—An ethereal solution of the optically active aminoalcohol was mixed with methyl iodide to precipitate the product. R(−)-β-Ethylcholine iodide was recrystallized from 2-propanol, m.p. 159–160°; $[\alpha]_{589.3\ nm.}^{25°}$ −17.5° (c 1.0, H_2O); $\nu_{max.}^{KBr}$ 3375 cm.$^{-1}$ (OH).

Anal.—Calc. for $C_7H_{18}INO$: C, 31.01; H, 6.69. Found: C, 31.28; H, 6.98.

R(−)-Acetyl β-Ethylcholine Iodide—This compound was prepared from R(−)-1-dimethylamino-2-butanol in the same manner as described for the resolved 1-dimethylamino-2-butanol, m.p. 182–183°; $[\alpha]_{589.3\ nm.}^{25°}$ −19.6° (c 1.0, H_2O). The IR spectrum was identical with that of the R(−)-isomer obtained from resolution with (+)-tartaric acid.

Anal.—Calc. for $C_9H_{20}INO_2$: C, 35.77; H, 6.99. Found: C, 35.99; H, 6.74.

R(−)-*N,N*-Dimethyl-*O*-acetylmandelamide—Ten grams of R(−)-mandelic acid[2] was treated with 30 g. of acetyl chloride for 30 min., at which time all the mandelic acid had dissolved and no more hydrochloric acid was being evolved. The excess acetyl chloride was removed under reduced pressure in a rotary evaporator, and the residue was mixed with 30 g. $SOCl_2$ and refluxed for 45 min. The excess $SOCl_2$ was removed by distillation, and the residue was dissolved in 100 ml. anhydrous ether. The ethereal solution was added dropwise to a cooled solution of 10 g. dimethylamine in dry ether. The dimethylamine hydrochloride was removed by filtration, and the filtrate was evaporated to give a solid residue of the crude product, 12.5 g., m.p. 90–92°. The crystals were purified by recrystallization from cyclohexane, m.p. 97–98°; $[\alpha]_{589.3\ nm.}^{25°}$ −189° (c 0.4, CH_3OH); $\nu_{max.}^{KBr}$ 1735 cm.$^{-1}$ (ester C=O), 1650 cm.$^{-1}$ (amide C=O).

Anal.—Calc. for $C_{13}H_{15}NO_3$: C, 65.14; H, 6.84. Found: C, 65.03; H, 6.99.

R(−)-*N,N*-Dimethyl-1-phenylethanolamine—A solution of 5.0 g. of R(−)-*N,N*-dimethyl-*O*-acetylmandelamide in 60 ml. tetrahydrofuran was added dropwise to a suspension of 5.9 g. $LiAlH_4$ in 100 ml. tetrahydrofuran, and the mixture was then refluxed for 4 hr. The excess $LiAlH_4$ was destroyed by ethyl acetate, and 50 ml. of 40% potassium and sodium tartrate solution was added. The ether solution was filtered from the precipitate, and the filtrate was dried over Na_2SO_4 (anhydrous) and distilled to give 3.0 g. of product, b.p. 58–60°/0.15 mm. Hg; n_D^{25} 1.5164; $[\alpha]_{589.3\ nm.}^{25°}$ −62.5° (c 1.0, CH_3OH); $\nu_{max.}^{mull}$ 3430 cm.$^{-1}$, broad (OH).

Anal.—Calc. for $C_{10}H_{15}NO$: C, 72.69; H, 9.15. Found: C, 72.48; H, 9.38.

R(−)-Acetyl β-Phenylcholine Iodide—This compound was prepared in a manner similar to that described for (−)-acetyl β-ethylcholine iodide. The product was recrystallized from a methanol–ether mixture, m.p. 195–196.5°; $[\alpha]_{589.3\ nm.}^{25°}$ −65.0° (c 1.0, H_2O).

[2] Aldrich Chemical Co., Inc.

Table I—Muscarinic and Enzymologic Activity of β-Substituted Acetylcholine Iodides

Iodide	Muscarinic Activity: Guinea Pig Ileum Potency of Acetylcholine	Muscarinic Activity: 95% Confidence Limits	Acetylcholinesterase Activity[a]: Hydrolysis, %	Acetylcholinesterase Activity[a]: K_m 10^{-4}	Acetylcholinesterase Activity[a]: K_i[b] 10^{-4}
Acetylcholine[c]	1.00	—	100	1.37	—
Acetyl β-methylcholine					
S(+)	0.802[d]	0.661–0.973[e]	19	—	No inhibition
RS	0.622	0.456–0.848[e]	11	—	—
R(−)	0.090	0.063–0.129[e]	0	—	5.5
Acetyl β-ethylcholine					
S(+)	0.0121	0.0081–0.018[f]	39.0	1.55	No inhibition
RS	0.0067	0.0051–0.0087[f]	28.0	—	—
R(−)	0.00028	0.00019–0.00041[f]	0	—	3.1
Acetyl β-phenylcholine					
S(+)	0.001	0.0007–0.0013[f]	11.0	—	No inhibition
RS	Inactive	—	8.0	—	—
R(−)	0.00001	0.000004–0.0000123[f]	0	—	6.3

[a] *Electrophorus electricus* acetylcholinesterase, Worthington Biochemical Corp., Freehold, N. J. [b] Physostigmine sulfate under similar conditions gave a K_i value of 7.74×10^{-8}. All inhibitors were competitive. [c] Used perchlorate salt for acetylcholinesterase study and bromide salt for guinea pig ileum study. [d] Reference 3. [e] Mean λ value for these assays is 0.123. [f] Mean λ value for these assays is 0.198.

Anal.—Calc. for $C_{13}H_{20}INO_2$: C, 44.71; H, 5.77. Found: C, 44.49; H, 5.72.

S(+)-*N*,*N*-Dimethyl-*O*-acetylmandelamide—This compound was prepared from S(+)-mandelic acid as described previously, m.p. 98–99°; $[\alpha]^{25^\circ}_{589.3\ nm.}$ +192° (c 0.4, CH_3OH).

Anal.—Calc. for $C_{13}H_{15}NO_3$: C, 65.14; H, 6.84. Found: C, 65.16; H, 6.85.

S(+)-*N*,*N*-Dimethyl-1-phenylethanolamine—This compound was prepared from the S(+)-mandelamide derivative as described previously, b.p. 58–60°/0.15 mm. Hg; $[\alpha]^{25^\circ}_{589.3\ nm.}$ +64.8° (c 2.2, CH_3OH); n^{25}_D 1.5171; $\nu^{mull}_{max.}$ 3430 cm.$^{-1}$, broad (OH).

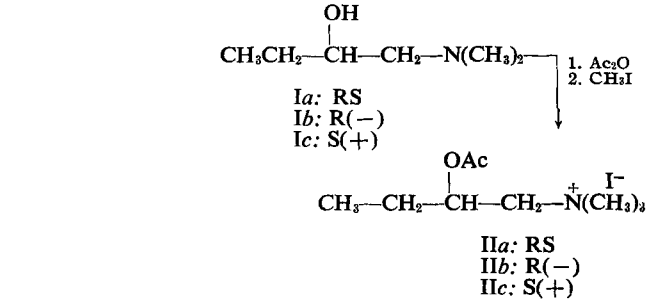
Anal.—Calc. for $C_{10}H_{15}NO_2$: C, 72.69; H, 9.15. Found: C, 72.89; H, 9.44.

S(+)-Acetyl β-Phenylcholine Iodide—This compound was prepared from S(+)-*N*,*N*-dimethyl-1-phenylethanolamine as described previously, m.p. 195–196.5°; $[\alpha]^{25^\circ}_{589.3\ nm.}$ +66.5° (c 1.0, H_2O).

Anal.—Calc. for $C_{13}H_{20}INO_2$: C, 44.71; H, 5.77. Found: C, 44.47; H, 5.72.

Pharmacology—The muscarinic potency of each compound in relation to acetylcholine was determined by a bioassay on the isolated guinea pig ileum. The ileum was suspended in Krebs–Henseleit solution, aerated with 95% O_2–5% CO_2, and maintained at 37°. Acetylcholine and the unknown drug were tested at minimal, medium, and submaximal concentrations as determined prior to the actual assay. The compounds were run on separate days using different guinea pigs for each experiment. A 3 × 3 bioassay was used for the determinations.

Enzymology—Enzyme-catalyzed hydrolysis of the compounds and inhibition of acetylcholine perchlorate hydrolysis by eel acetylcholinesterase were determined at pH 7.5 by titration of the liberated acetic acid with NaOH solution (0.0005 *N*) using a Sargent pH-Stat. Concentrations of substrate varying from 1.0 to 5.0 × 10^{-4} *M* were used in a medium consisting of 0.02 *M* $MgCl_2$ and 0.1 *M* NaCl, and 0.25 unit of eel acetylcholinesterase for the kinetic determinations. The enzyme was diluted just prior to use by a solution mixture of 0.02 *M* $MgCl_2$, 0.1 *M* NaCl, and 0.005% bovine serum albumin. Inhibitor concentrations were 3.0 × 10^{-4} *M*. The reaction rates were measured at 25° for 5 min. The rate was linear. A graphic plot of *S*/*V* *versus* *S* provided K_m and K_i values. Comparisons of hydrolysis rates were carried out at a 5.0 × 10^{-4} *M* substrate concentration over the same reaction period as already described.

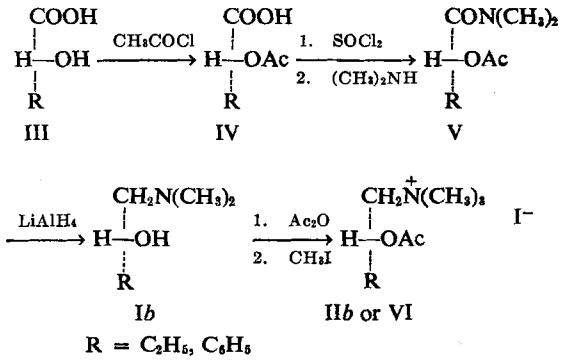


Scheme I

RESULTS AND DISCUSSION

Racemic 1-dimethylamino-2-butanol (I*a*) was prepared from 1-nitro-2-butanol. The (+)- and (−)-isomers of 1-dimethylamino-2-butanol (I*b* and I*c*, respectively) were resolved with (+)-tartaric acid and (+)-3-bromocamphorsulfonic acid, respectively, according to the methods of Major and Bonnett (8) for resolving 2-dimethylamino-1-propanol. The racemic and optically active aminoalcohols (I*a*, I*b*, and I*c*) were converted to the corresponding acetyl β-ethylcholine iodides (II*a*, II*b*, and II*c*) (Scheme I).

(−)-Acetyl β-ethylcholine (I*b*) was related to R(−)-2-hydroxybutyric acid (III: R = C_2H_5) by the stereospecific route shown in Scheme II. The assignment of the configuration of R(−)-2-hydroxybutyric acid was established by Levene and Haller (10) relating it to R(−)-lactic acid. Compound III was obtained by resolution of the racemic acid through its morphine salt. Compound III was acetylated to give R(+)-2-acetoxybutyric acid (IV: R = C_2H_5) which, when treated with thionyl chloride and then dimethylamine, gave R(+)-*N*,*N*-dimethyl-2-acetoxybutyramide (V: R = C_2H_5). Lithium aluminum hydride reduction of V gave R(−)-1-dimethylamino-2-butanol (I*b*), which was acetylated and treated with methyl iodide to give R(−)-acetyl β-ethylcholine iodide (II*b*); this compound was identical to the quaternary acetate obtained from the resolution of 1-dimethylamino-2-butanol with (+)-tartaric acid. (+)-1-Dimethylamino-2-butanol (I*c*), having the



Scheme II

same optical rotation but in the opposite direction, was assigned the S-configuration. It follows then that (+)-acetyl β-ethylcholine iodide (IIc) be assigned the S-configuration. The optically active R(−)-aminoalcohol was also used to prepare the quaternary alcohol derivative.

The synthesis of the enantiomers of acetyl β-phenylcholine (VI: R = C_6H_5) was carried out from the corresponding optically active mandelic acids, as described in Scheme II; *i.e.*, R = C_6H_5. S(+)-and R(−)-Mandelic acids gave S(+)-and R(−)-acetyl β-phenylcholine, respectively.

The results of the experiments in which the potency of the enantiomers of acetyl β-ethylcholine and acetyl β-phenylcholine iodide were determined in relation to acetylcholine bromide on guinea pig ileum are shown in Table I. The S(+)-acetyl β-ethylcholine and S(+)-acetyl β-phenylcholine enantiomers showed 44 and 100 times, respectively, greater muscarinic potency than their corresponding optical antipodes. This finding is consistent with the stereoselectivity by muscarinic receptors for S(+)-muscarine (11) and S(+)-acetyl β-methylcholine (1, 3) over their enantiomers. Although racemic acetyl β-phenylcholine did not give measurable results at the concentration levels used in the 3 × 3 assay, a very large concentration (1000 mcg./ml.) caused a 44-mm. contraction of guinea pig ileum. A 1000-mcg./ml. dose of R(−)-acetyl β-phenylcholine produced only a 27-mm. contraction on the same tissue. In all cases, the chemicals were found not to interfere with subsequent exposure of the tissue to acetylcholine.

Each enantiomer was examined as a substrate and inhibitor of acetylcholinesterase. The results of these studies are also presented in Table I. While none of the enantiomers showed any significant substrate activity during the 5-min. interval, both R(−)-acetyl β-ethylcholine and R(−)-acetyl β-phenylcholine were competitive inhibitors of acetylcholinesterase. The competitive inhibition can be interpreted as receptor affinity by these molecules (see Eqs. 1–5). The dissociation constant, K_s, for acetylcholine and eel acetylcholinesterase at pH 7.5 and 25° is 2.6×10^{-4} (12). The K_I values of 3.1×10^{-4} and 6.3×10^{-4} determined for the β-ethyl and β-phenyl compounds, respectively, indicate a favorable comparison of affinity for these inhibitors with acetylcholine for the active site on acetylcholinesterase. Both the S(+)-β-ethyl and S(+)-β-phenyl enantiomers showed some substrate activity, but no inhibition of acetylcholinesterase was observed. As expected, the racemic compounds had lower hydrolysis rates than the corresponding S(+)-isomers.

$$E + S \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} ES \qquad \text{(Eq. 1)}$$

$$K_s = \frac{k_{-1}}{k_{+1}} \qquad \text{(Eq. 2)}$$

$$E + I \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} EI \qquad \text{(Eq. 3)}$$

$$K_I = \frac{k_{-1}}{k_{+1}} \qquad \text{(Eq. 4)}$$

$$\text{affinity} = \frac{k_{+1}}{k_{-1}} \qquad \text{(Eq. 5)}$$

A comparison of the enzymatic and muscarinic activity of the enantiomers of acetyl β-ethylcholine and acetyl β-phenylcholine shows that the muscarinic receptor of guinea pig ileum and the receptor area of eel acetylcholinesterase each best accommodates one isomer of an enantiomeric pair. The area between the esteratic and anionic sites of the two receptor areas differs such that the muscarinic site accommodates the S-configuration and the enzyme receptor accommodates the R-configuration of β-substituted acetylcholines, as described previously by Cocolas *et al.* (3).

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