J. Enzyme Inhibition, 1993, Vol. 6, pp. 283–291
Reprints available directly from the publisher
Photocopying permitted by license only

CHOLINESTERASE STUDIES WITH (*R*) (+)- AND (*S*)(−)-5-(1,3,3-TRIMETHYLINDOLINYL)-*N*-(1-PHENYLETHYL)CARBAMATE*

C.N. LIESKE†, R.T. GEPP, D.M. MAXWELL, J.H. CLARK, C.A. BROOMFIELD, P. BLUMBERGS** and C.C. TSENG**

*US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, Maryland 21010, USA, and **Ash-Stevens Inc., Detroit, Michigan 48202, USA*

(*Received 5 March, 1992*)

A limited number of carbamates have been found useful for treatment of cholinergic symptoms with pyridostigmine and physostigmine being the main focus. In recent years 5-(1,3,3-trimethylindolinyl)*N*,*N*-dimethylcarbamate (**I**) has received considerable attention in the Chinese literature for a similar role. We report on the first synthesis of stereoisomers of an analog of (**I**). The isomers prepared were (*R*)(+)-5-(1,3,3-trimethylindolinyl)-*N*-(1-phenylethyl)carbamate (**II**) and (*S*)(−)-5-(1,3,3-trimethylindolinyl)-*N*-(1-phenylethyl)carbamate (**III**). The pK_a value for each isomer was 6.8. Eel acetylcholinesterase inhibition studies were carried out at 25.0°C over the pH range of 6.0 to 9.0. They reflect the first pH profiles using enantiomorphs of a cholinesterase inhibitor. The inhibition potencies for (**II**) and (**III**) over the range examined were similar. At pH 7.60 the k_i for **II** = 7.38×10^3 M^{-1} min^{-1} (SD = 398) and for (**III**) the $k_i = 6.67 \times 10^3$ M^{-1} min^{-1} (SD = 355). In accord with the findings of Wilson and Bergmann[20] on physostigmine our results indicate that the protonated form of (**II**) and (**III**) is the more potent inhibitor.

KEY WORDS: Carbamates, stereoisomers, acetylcholinesterase, inhibition.

INTRODUCTION

Regulation of acetylcholine turnover and concentration in synaptic junctions and neurons may perform an important role in a number of neurologic disorders including myasthenia gravis, Alzheimer's disease, Huntington's disease, tardive dyskinesia and organophosphorus poisoning from pesticides and nerve agents. Two of the carbamates most frequently used to treat these disorders are pyridostigmine and physostigmine.[1] In recent years 5-(1,3,3-trimethylindolinyl)*N*,*N*-dimethylcarbamate (**I**) has received considerable attention in the Chinese literature for an analogous role.[2–6] Each of these compounds is an inhibitor of acetylcholinesterase through carbamylation of the esteratic site of the enzyme.[7] This inhibition is subsequently reversed upon decarbamylation.[8] We recently reported on the eel acetylcholinesterase inhibition constants of five analogs of 5-(1,3,3-trimethylindolinyl)*N*,*N*-dimethylcarbamate (**I**).[9] This parent carbamate, which has structural similarities to physostigmine, was first prepared by Ahmed and Robinson in 1965.[10] Since that time the only references to it, with the exception of reference 9, are found in the Chinese literature where it is frequently referred to as Cui Xing Ning. In this paper we report on the

*The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of Defense.

†Correspondence.

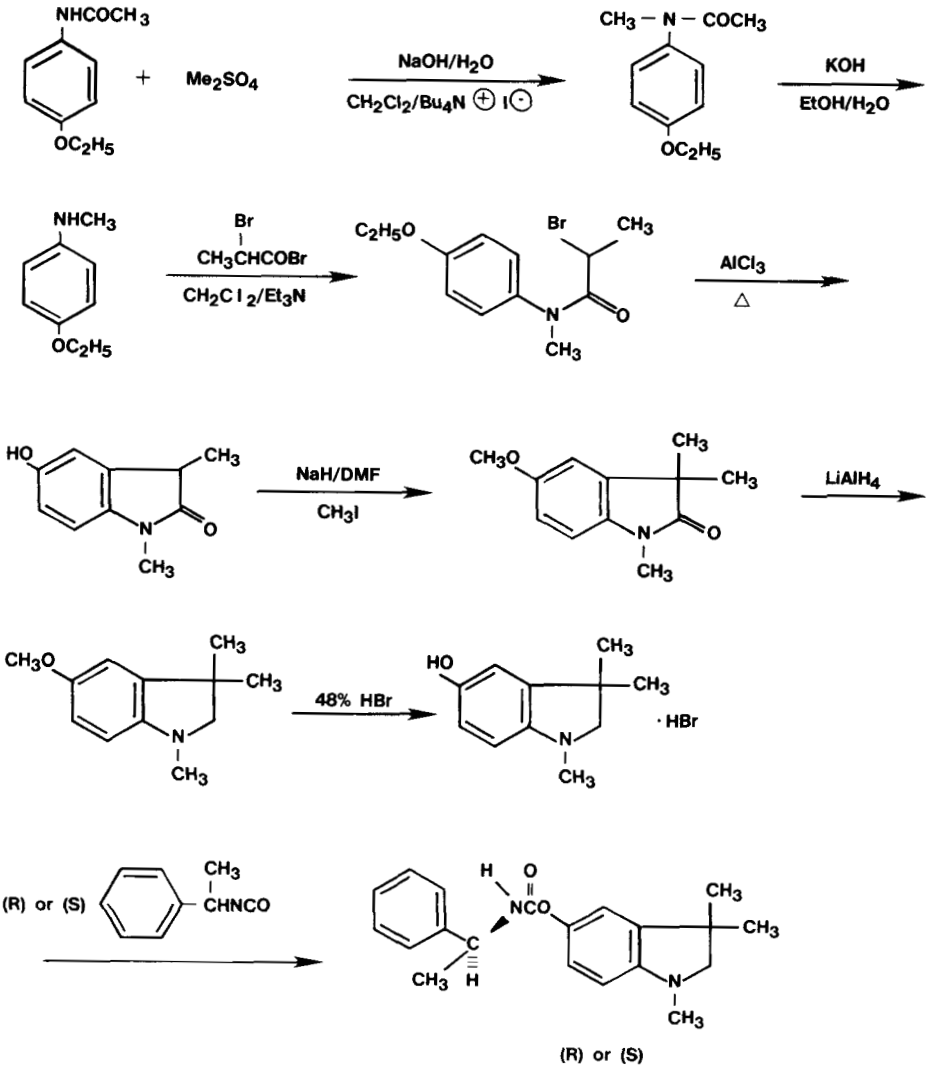

FIGURE 1 Flowsheet for the preparation of (*R*)(+)-5-(1,3,3-trimethylindolinyl)-*N*-(1-phenylethyl)-carbamate (**II**) and (*S*)(−)-5-(1,3,3-trimethylindolinyl)-*N*-(1-phenylethyl)carbamate (**III**).

first synthesis of stereoisomers of an analog of (**I**). These are (*R*)(+)-5-(1,3,3-trimethylindolinyl)-*N*-(1-phenylethyl)carbamate (**II**) and (*S*)(−)-5-(1,3,3-trimethylindolinyl)-*N*-(1-phenylethyl)carbamate (**III**). Figure 1 shows the synthetic steps. In addition to physio-chemical characterization of (**II**) and (**III**), we determined their eel acetylcholinesterase (AChE) inhibition constants over the pH range of 6.0 to pH 9.0. Inhibition constants are among the initial parameters frequently evaluated when searching for therapeutically functional cholinesterase antagonists.

MATERIALS AND METHODS

Prepation of (R)(+)-*5*-(*1,3,3-Trimethylindolinyl*)-N-(*1-phenylethyl*)*carbamate* (**II**)

1,3,3-Trimethyl-5-hydroxyindole hydrobromide[3,11,12] (23 g, 89.1 mmol) was dissolved in water (200 ml) and covered with ether (300 ml). With ice-cooling, sodium carbonate (4.72 g, 44.55 mmol) was added to the solution. After shaking, the aqueous layer was separated, and additional sodium carbonate (4.72 g, 44.55 mmol) was added. The resultant aqueous solution was extracted with ether (2 × 300 ml). The combined ether layer was washed with water (2 × 300 ml) and dried ($MgSO_4$). The solvent was removed at reduced pressure (aspirator) and the residue, 1,3,3-trimethyl-5-hydroxyindole free base, was dissolved in anhydrous benzene (200 ml) containing (*R*)(+)-1-phenylethyl isocyanate (15.7 g, 106.9 mmol) and a small piece of sodium metal (ca. 160 mg). The mixture was stirred at room temperature for 64 h, cooled in an ice bath and diluted with ether (100 ml) previously saturated with water. The solution was filtered and the filtrate was evaporated under reduced pressure (aspirator) to give a light pink solid (30.5 g). The solid was chromatographed over silica gel (300 g) with chloroform/ethyl acetate (9:1) as eluent. The product-containing fractions were concentrated and the white residual solid (24.4 g) was recrystallized three times from a mixture of ether/petroleum ether (100 ml:300 ml) to yield 15.7 g (54%) of pure title compound as white crystals, mp 84–86°C. $[\alpha]_D$ + 89.1° (c = 1.01, $CHCl_3$). *Anal.* Calc. for $C_{20}H_{24}N_2O_2$ (324.42): C, 74.05; H, 7.46: N, 8.63%. Found: C, 74.03; H, 7.54; N, 8.64%. The NMR spectrum was in agreement with the assigned structure.

Preparation of (S)(−)-*5*-(*1,3,3-trimethylindolinyl*)-N-(*1-phenylethyl*)*carbamate* (**III**)

To an ice-cold, stirred mixture of 1,3,3-trimethyl-5-hydroxyindole hydrobromide (16 g, 61.97 mmol), water (140 ml), and ether (200 ml) was added sodium carbonate (3.28 g, 31 mmol) and the layers were separated. Additional sodium carbonate (3.28 g) was added to the aqueous layer which was extracted again with ether (2 × 200 ml). The combined ether layer was washed with water (2 × 200 ml) and dried ($MgSO_4$). After filtration the solvent was removed under reduced pressure (aspirator). The residue was dissolved in anhydrous benzene (250 ml) containing (*S*)(−)-1-phenylethyl isocyanate (9.58 g, 65.07 mmol) and sodium metal (about 50 mg). The mixture was stirred at room temperature under nitrogen for 14 days, filtered, and the filtrate was poured into ice water (150 ml). The layers were separated and the aqueous layer was extracted with ether (1 × 150 ml). The ether solution was washed with water (3 × 150 ml) and dried ($MgSO_4$). It was concentrated under reduced pressure (aspirator) to near dryness and the residue was chromatographed over silica gel (1 kg) using chloroform/ethyl acetate (9:1) as eluent. Fractions containing product were combined and evaporated under reduced pressure to give a light yellow solid. One recrystallization from a mixture of ether (50 ml) and petroleum ether (200 ml) gave 13.2 g of product as white crystals. Thin layer chromatography of this material showed a minor impurity. Accordingly, the solid was dissolved in ether (400 ml) and the solution was extracted with 1 N HCL (2 × 40 ml) and 3 N HCl (1 × 40 ml). The combined hydrochloric acid extract was washed with ether (2 × 250 ml) and made alkaline with 50% sodium hydroxide solution (16 g). The precipitated product was extracted with ether (2 × 250 ml). The combined ether extract was washed with water (3 × 250 ml) and dried ($MgSO_4$).

The solvent was removed under reduced pressure (aspirator) to give a white solid. One recrystallization from a mixture of ether (60 ml) and petroleum ether (240 ml) gave 9.0 g (45%) of pure product as white crystals, mp 85–86°C. $[\alpha]_D - 88.4°$ (c = 1.01, $CHC_{.3}$). *Anal.* Calc. for $C_{20}H_{24}N_2O_2$ (324.42): C, 74.05; H, 7.46: N, 8.63%. Found: C, 73.98; H, 7.37; N, 8.80%. The NMR spectrum was in agreement with the assigned structure.

Ultraviolet Spectra of (**II**) *and* (**III**)

Stock solutions of (**II**) and (**III**) (1×10^{-3} M) in acetonitrile were prepared. Following addition of 10.0 μl of stock solution to 0.990 ml of acetonitrile in a UV cell (25.0°C) the spectrum was recorded from 400 nm to 200 nm using a Beckman DU-70 spectrophotometer. Three independent scans were made for each compound. Both (**II**) and (**III**) exhibited peaks at 256 and 308 nm. For (**II**), $\varepsilon_{256} = 1.40 \times 10^4$ and $\varepsilon_{308} = 3.63 \times 10^3$. For (**III**), $\varepsilon_{256} = 1.37 \times 10^4$ and $\varepsilon_{308} = 3.50 \times 10^3$.

Alkaline Hydrolysis of (**II**) and (**III**)

The alkaline hydrolysis of (**II**) and (**III**) was monitored spectrophotometrically at 290 nm. Reaction was initiated by introducing, with rapid mixing, 10.0 μl of a 1×10^{-2} M solution of (**II**) or (**III**) in acetonitrile into a UV cell containing 1.00 ml of 0.20 M phosphate buffer of pH 11.0 at 25.0°C. The A_∞ value was taken at 7.0 h. Three replicates were completed with each compound. Linear first-order kinetics were observed for >2.5 half-lives (correlation coefficient = 0.99). For (**II**), $k_{obsd} = 0.0148\ min^{-1}$ (SD = 0.00023); for (**III**), $k_{obsd} = 0.0145\ min^{-1}$ (SD = 0.00023).

Dissociation Constants (pK_a) *of* (**II**) *and* (**III**)

The dissociation constants were determined by direct titration (in triplicate) in a Radiometer automatic titrimeter. A 250 μl aliquot of a 1×10^{-2} M solution of the carbamate in acetonitrile was added to 3.0 ml of 0.15 M NaCl in the titration chamber. The solution was titrated from pH 3.85 to pH 10.0 under a nitrogen atmosphere using carbonate-free NaOH (1×10^{-2} M). A baseline curve was obtained by titrating 250 μl of acetonitrile in the same manner. The titration curve of the carbamate was obtained by subtracting the baseline curve from the raw titration data at increments of 0.1 pH unit. The inflection point of the resulting curve was taken as the pK_a for the compound. The pK_a for (**II**) and (**III**) was 6.8 (± 0.2).

Preparation of Buffer Solutions

All buffers for the enzyme studies were 0.10 M in the buffering component and contained 0.01 M Mg^{++} ($MgCl_2$), 0.002% sodium azide, and 0.01% bovine serum albumin. MES [2-(*N*-morpholino)ethanesulfonic acid] buffer was used in the pH 6.00 and pH 6.50 studies. MOPS [3-(*N*-morpholino)propanesulfonic acid] buffers were used in the pH 7.00 and pH 7.60 studies, and Bicine [*N*,*N*′-bis(2-hydroxyethyl)glycine] buffers were used in the pH 8.00, pH 8.50, and pH 9.00 studies. All pH values were determined at 25.0°C using a Beckman Model 70 digital pH meter.

TABLE I
Kinetic constants for the inhibition of eel acetylcholinesterase by (**II**) and (**III**), pH 6.00 to 9.00, 25.0°C

Compound	pH	K_d (M)	k_i (M^{-1} min^{-1})	k_2 (min^{-1})
II	6.00	8.81×10^{-6}	1.93×10^4	0.17
		(2.37)[a]	(0.11)	(0.05)
III	6.00	1.53×10^{-5}	1.83×10^4	0.28
		(1.38)	(0.25)	(0.19)
II	6.50	8.73×10^{-6}	1.26×10^4	0.11
		(1.91)	(0.20)	(0.01)
III	6.50	1.50×10^{-5}	1.13×10^4	0.17
		(0.90)	(0.25)	(0.06)
II	7.00	1.17×10^{-5}	9.39×10^3	0.11
		(0.12)	(0.86)	(0.01)
III	7.00	6.30×10^{-6}	1.27×10^4	0.08
		(1.83)	(0.15)	(0.02)
I[b]	7.60	3.47×10^{-7}	2.88×10^5	0.10
		(0.52)	(0.23)	(0.01)
I	7.60	1.63×10^{-5}	7.38×10^3	0.12
		(0.44)	(0.40)	(0.03)
III	7.60	3.75×10^{-5}	6.67×10^3	0.25
		(0.33)	(0.36)	(0.01)
II	8.00	2.28×10^{-5}	5.27×10^3	0.12
		(0.61)	(0.92)	(0.01)
III	8.00	3.91×10^{-5}	5.12×10^3	0.20
		(1.77)	(0.99)	(0.05)
II	8.50	1.81×10^{-5}	6.08×10^3	0.11
		(0.45)	(0.49)	(0.02)
III	8.50	5.13×10^{-5}	5.07×10^3	0.26
		(2.83)	(0.66)	(0.09)
II	9.00	3.99×10^{-5}	4.76×10^3	0.19
		(0.73)	(0.34)	(0.03)
III	9.00	1.59×10^{-4}	2.96×10^3	0.47
		(1.21)	(0.26)	(0.28)

[a]Standard deviation of n = 4.
[b]Cui Xing Ning; included for comparison purposes.

Inhibition of Eel Acetylcholinesterase (Eel AChE) by (**II**) *and* (**III**), *and Evaluation of Inhibition Constants* (k_i, k_2, *and* K_d)

See Lieske *et al.*, 1991.[9] The results for the inhibition constants and the corresponding standard deviations for (**II**) and (**III**) at the selected pHs are shown in Table I.

RESULTS AND DISCUSSION

Pyridostigmine and physostigmine have a long history as the most prominent carbamates for counteracting a variety of cholinergic problems.[1] However, during the past decade at least two new potential contenders have emerged in this carbamate arena. They are (**I**) (Cui Xing Ning) and [5*R*-(5a,9b,11E)]-5-amino-11-ethylidene-5,6,9,10-tetrahydro-7-methyl-5,9-methanocycloocta[b]pyridin-2(1H)-one(**IV**, huperzine A). Publications on the former are found largely in the Chinese literature[2–6,9] while those on the latter are more diversified in origin.[13–18]

We have synthesized both stereoisomers of an analog of (**I**) and evaluated their anticholinesterase activity over the pH range 6.0 to 9.0. Inhibition constants are among the initial parameters sought in the identification of therapeutically functional cholinesterase antagonists. The isomers prepared were (*R*)(+)-5-(1,3,3-trimethylindolinyl)-*N*-(1-phenylethyl)carbamate (**II**) and (*S*)(−)-5-(1,3,3-trimethylindolinyl)-*N*-(1-phenylethyl)carbamate (**III**). They were characterized by elemental analysis, NMR, optical rotation, hydrolytic stability at pH 11.0, and dissociation constant. The latter values, which correspond to a $pK_a = 6.8 \pm 0.2$ for (**II**) and (**III**), are important in interpreting the pH profiles for AChE inhibition exhibited by the isomers.

The inhibition of AChE by carbamates involves carbamylation of the active site serine and proceeds by the mechanism shown in Eq. (1).[19]

$$\text{AChE} + \text{C—X} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{AChE}\cdot\text{C—X} \xrightarrow{k_2} \text{AChE—C} + \text{X} \qquad (1)$$

The characteristics of such an inhibitor are expressed in terms of its dissociation constant, K_d (i.e., k_{-1}/k_1), and the subsequent unimolecular bonding constant, k_2, to form the inhibited enzyme, AChE—C. An overall rate of inhibition is commonly stated in terms of a bimolecular reaction constant, k_i (i.e., k_2/K_d). The results from our inhibition experiments at selected pHs with carbamates (**II**) and (**III**) are shown in Table I. For comparison purposes we have included in the Table our results with (**I**) at pH 7.60.

The effect of pH on the second-order inhibition constants (k_i) can be more readily appreciated from a plot of k_i *vs* pH. As shown in Figure 2, which is a composite of the k_i values for (**II**) and (**III**) *vs* pH, the inhibition rate increases with decreasing pH. Since the pK_a for both (**II**) and (**III**) is 6.8, the cationic (protonated) form is clearly a more potent inhibitor than the free base. However, at pH 9.0 essentially the

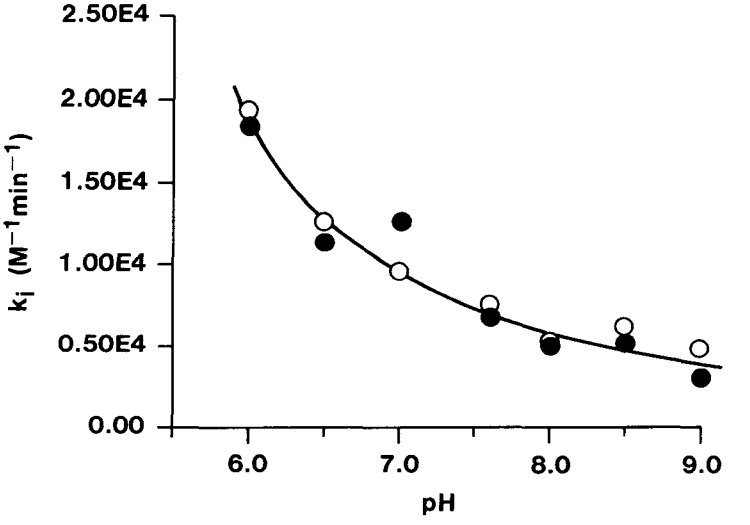

FIGURE 2 Composite of k_i *vs* pH for (*R*)(+)-5-(1,3,3-trimethylindolinyl)-*N*-(1-penylethyl)carbamate (**II**) (○) and (*S*)(−)-5-(1,3,3-trimethylindolinyl)-*N*-(1-phenylethyl)carbamate (**III**) (●).

entire inhibition rate (99.4%) must be ascribed to the free base. Our observed changes in k_i values with pH are in concert with those obtained by Wilson over 40 years ago.[20] Using eel AChE he found over the pH range of 6.0 to 10.0 that the physostigmine cation is a stronger inhibitor than the free base (pK_a of physostigmine = 7.9).

In 1967 somewhat contradictory results were published by Reiner and Aldridge.[21] For the positively charged carbamates neostigmine, monomethylneostigmine, and phosphostigmine, the k_i–pH plots were bell-shaped with a pH optimum between 7.5 and 9.0. These results with charged compounds are inconsistent with Wilson's earlier findings. Before assigning the discrepancy to the difference in enzyme sources it should be noted that both eel AChE and bovine erythrocyte AChE show approximately the same pH optimum of 8.0 for the hydrolysis of the charged substrate acetylcholine.[22,23]. Using yet another AChE, Chattopadhyay *et al.*[24] reported on the inhibition of chicken brain AChE by phosphamidon (a neutral phosphate ester) and physostigmine. Over the pH range of 5.4 to 8.0 both compounds showed an inhibition maximum at pH 7.6. These results are inconsistent with Wilson's results on physostigmine and our results with (**II**) and (**III**).

For the benefit of future investigators, we elected to present k_i *vs* pH (Figure 2) rather than k_2 or K_d. Several authors[25,26] have pointed out that while k_i values are quite reproducible from different laboratories, widely diverse k_2 and K_d values are not uncommon. However, from the results obtained under our experimental conditions it is quite clear that the effects of pH on the k_i values for (**II**) and (**III**) are primarily the result of changes in K_d. The K_d values progress over about a four-fold range for (**II**) and a ten-fold range for (**III**) whereas for each the k_2 values vary no more than two-fold. Though not of the same order of magnitude, our observations on the effect of pH on K_d are not unlike the effect of structural changes on K_d as observed by Nishioka *et al.*[26] and O'Brien *et al.*[27] Nishioka observed that changes in substituents in 53 phenyl *N*-methyl carbamates produced 10^3 to 10^4 changes in K_d which were accompanied by a less than ten-fold change in k_2. One explanation is that changes in the protonation of carbamates (**II**) and (**III**) produce effects on AChE inhibition by a mechanism(s) similar to the mechanism(s) for changes in AChE inhibition resulting from alterations in the electronic, steric, and hydrophobic properties of substituents.

The role of chiral centers in organophosphorus inhibitors in irreversible cholinesterase inhibition was first suggested in 1955 by Michel.[28] The multiplicity of studies in this area in subsequent years have been nicely chronicled by de Jong and Benschop in a 1988 review article.[29] Curiously, not even this rather extensive literature documents a pH profile for an organophosphorus inhibitor with a chiral center.

The literature on cholinesterase inhibition studies using carbamates containing a chiral center is much more limited. In 1970 Dale and Robinson reported that both (+)-physostigmine and (+)-physovenine had lower erythrocyte AChE inhibiting activities than the corresponding (−)-isomers.[30] Sixteen years later Brossi *et al.* showed that (+)-physostigmine is a poorer inhibitor of eel AChE than natural (−)-physostigmine.[31] Atack[32] expanded these studies in 1989 and found the same to be true for human cortex, caudate, and erythrocyte AChE, as well as human cortex and plasma BuChE. Bores *et al.*[33] prepared the (*R*)- and (*S*)-1-methylbenzyl derivatives of (−)-physostigmine and found that at pH 7.2 and 37°C the (*R*)-isomer was a 5 times better inhibitor of rat striata AChE and a 28 times better inhibitor of human serum BuChE. As the resulting (−)-physostigmine derivative each have two

optical centers, the compounds they studied were the (+)(−)- and (−)(−)-isomers. Their results contrast with our studies on the derivatives of 5-(1,3,3-trimethylindolinyl)*N*,*N*-dimethylcarbamate prepared from the same optically active isocyanates. We found that with eel AChE the inhibition potencies for (**II**) and (**III**) were very similar over the pH-range of 6.0 to 9.0 (Figure 2).

Our results reflect the *first* pH profiles for enantiomorphs of an AChE inhibitor. They are quite different from the results obtained by Bores at pH 7.2. Unlike Bores' carbamates, which contain an optical active center in both the carbamylating moiety and the leaving group, with (**II**) and (**III**) we are dealing with only a single optical center in each that is located in the carbamylating moiety. A recent excellent publication by Sussman *et al.*[34] identifies the active site of an AChE as lying near the bottom of a deep and narrow gorge. The conventional simplistic picture[19,23] of a negatively charged 'anionic site' is replaced by 14 aromatic residues that line the gorge. Possible participation by this many residues in the binding of inhibitors with multiple stereoisomers and/or conformers precludes a simple model of enzyme reactivity.

References

1. Taylor, P. (1985) Anticholinesterase agents. In *The Pharmacological Basis of Therapeutics* (L.S. Goodman and A. Gilman, Eds), 7th edn, pp. 100–129. Macmillan Publishing Co., New York.
2. Sun, Q. (1981) *Beijing Yixue*, **3,** 170. [*CA*: **96,** 79719e (1981)].
3. Ji, Q. and Wei, Y. (1983) *Yiyao Gongye*, **8,** 7 [*CA*: **100,** 174587v (1984)].
4. Pang, Y. and Cui, Y. (1984) *Yaowu Fenxi Zazhi*, **4,** 25. [*CA*: **100,** 127004t (1984)].
5. Jin, J. and Huang, R. (1986) *Zhongguo Yaoli Xuebao*, **7,** 296. [*CA*: **105,** 108301z (1986)].
6. Shi, T., Xu, G., Qiao, Y. and Gao, Y. (1987) *Yaoxue Tongbao*, **22,** 279 (1987). [*CA*: **107,** 120966u (1987)].
7. Wilson, I.B., Hatch, M.A. and Ginsburg, S. (1960) *J. Biol. Chem.*, **235,** 2312.
8. Watts, P. and Wilkinson, R.G. (1977) *Biochem. Pharmacol.*, **26,** 757.
9. Lieske, C.N., Gepp, R.T., Clark, J.H., Meyer, H.G., Blumbergs, P. and Tseng, C.C. (1991) *J. Enz. Inhib.*, **5,** 215.
10. Ahmed, M. and Robinson, B. (1965) *J. Pharm. Pharmacol.*, **17,** 728.
11. Julian, P.L. and Pikl, J. (1935) *J. Am. Chem. Soc.*, **57,** 563.
12. Kolosov, M.N. and Preobrazhensky, N.N. (1953) *Zhur. Obshchei Khim.*, **23,** 1779. [*CA*: **49,** 295g (1955)].
13. Zhu, X.D. and Tang, X.C. (1988) *Acta Pharmacol. Sin.*, **9,** 492.
14. Guan, L.-C., Chen, S.-S. and Lu, W.-H. (1989) *Acta Pharmacol. Sin.*, **10,** 496.
15. Tand, X.-C., De Sarno, P., Sugaya, K. and Giacobini, E. (1989) *J. Neurosci. Res.*, **24,** 276.
16. Ayer, W.A., Browne, L.M., Orszanska, H., Valenta, Z. and Liu, J.-S. (1989) *Can. J. Chem.*, **67,** 1538.
17. Kozikowski, A.P. (1990) *J. Heterocyclic Chem.*, **27,** 97.
18. Kozikowski, A.P., Reddy, E.R. and Miller, C.P. (1990) *J. Chem. Soc. Perkin Trans.*, **1,** 195.
19. Main, A.R. (1979) *Pharmacol. Ther.*, **6,** 579.
20. Wilson, I.B. and Bergman, F. (1950) *J. Biol. Chem.*, **185,** 479.
21. Reiner, E. and Aldridge, W.N. (1967) *Biochem. J.*, **105,** 171.
22. Bergmann, F., Rimon, S. and Segal, R. (1958) *Biochem. J.*, **68,** 493.
23. Engelhard, N., Prchal, K. and Nenner, M. (1967) *Angew. Chem. Int. Ed.*, **6,** 615.
24. Chattopadhyay, R., Choudhuri, D.K. and Maity, C.R. (1986) *Indian. J. Med. Res.*, **83,** 435.
25. Aldrich, W.N. and Reiner, E. (1972) *Enzyme Inhibitors as Substrates.* American Elsevier Publishing Co., New York, New York.
26. Nishioka, T., Fujita, T., Kamoshita, K. and Nakajima, M. (1977) *Pestic. Biochem. Physiol.*, **7,** 107.
27. O'Brien, R.D., Hilton, B.D. and Gilmour, ●. (1966) *Mol. Pharmacol.*, **2,** 593.
28. Michel, H.O. (1955) *Fed. Proc. Fed. Am. Soc. Exp. Biol.*, **14,** 255.
29. de Jong, L.P.A. and Benschop, H.P. (1988). In *Stereoselectivity of Pesticides: Biological and Chemical Problems* (E.J. Ariens, J.J.S. van Rensen and W. Welling, Eds.), Chapter 4, p. 109. Elsevier Science Publishers B.V., Amsterdam, The Netherlands.

30. Dale, F.J. and Robinson, B. (1970). *J. Pharm. Pharmac.*, **22,** 889.
31. Brossi, A., Schonenberger, B., Clark, O.E. and Ray, R. (1986) *FEBS Lett.*, **201,** 190.
32. Atack, J.R., Yu, Q.-S., Soncrant, T.T., Brossie, A. and Rapoport, S.I. (1989). *J. Pharmacol. Exp. Ther.*, **249,** 194.
33. Bores, G.M., Huger, F.P., Hamer, R.R.L., Helsley, G.C. and Ellis, D.B. (1989) *FASEB J.*, **3,** A892.
34. Sussman, J.L., Harel, M., Frolow, F., Oefner, C., Goldman, A., Toker, L. and Silman, I. (1991) *Science*, **252,** 872.