4'-HYDROXYPHENYLCARBAMATES OF (3a*S*)-ESEROLINE AND (3a*S*)-*N*(1)-NORESEROLINE: POTENTIAL METABOLITES OF THE ALZHEIMER'S ANTICHOLINESTERASE DRUG PHENSERINE

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Abstract- 4'-Hydroxyphenylcarbamates of (3a*S*)-eseroline (**3**) and (3a*S*)-*N*(1)-noreseroline (**4**), as predicted metabolites of phenserine (**1**), were synthesized. Biological evaluation showed that **3** and **4** possessed potent activities for inhibition of acetylcholinesterase and butyrylcholinesterase *in vitro*. In contrast the intermediates, 4'-benzyloxyphenserine (**8**) and 4'-benzyloxy *N*(1)-benzylphenserine (**12**), demonstrated unusually potent and selective activities against butyrylcholinesterase.

Phenserine (**1**), the phenylcarbamate of (3a*S*)-eseroline, is a superior selective and long-acting inhibitor of acetylcholinesterase, AChE, with little action on butyrylcholinesterase, BChE, and details of its synthesis and biological properties have been reported.[1,2] In brief, it is a physostigmine (**2**) analogue that dramatically improves memory in animals and alters the processing of β-amyloid precursor protein away from the production of the toxic Alzheimer peptide, β-amyloid.[3,4] In light of forthcoming clinical trials of phenserine in patients with Alzheimer's disease it seemed appropriate to prepare and characterize the potential metabolites (**3**) and (**4**). The former results from aromatic hydroxylation of **1** and the latter by aromatic hydroxylation and oxidative *N*-demethylation (**Figure 1**), two reactions commonly encountered as routes of drug metabolism.[5,6]

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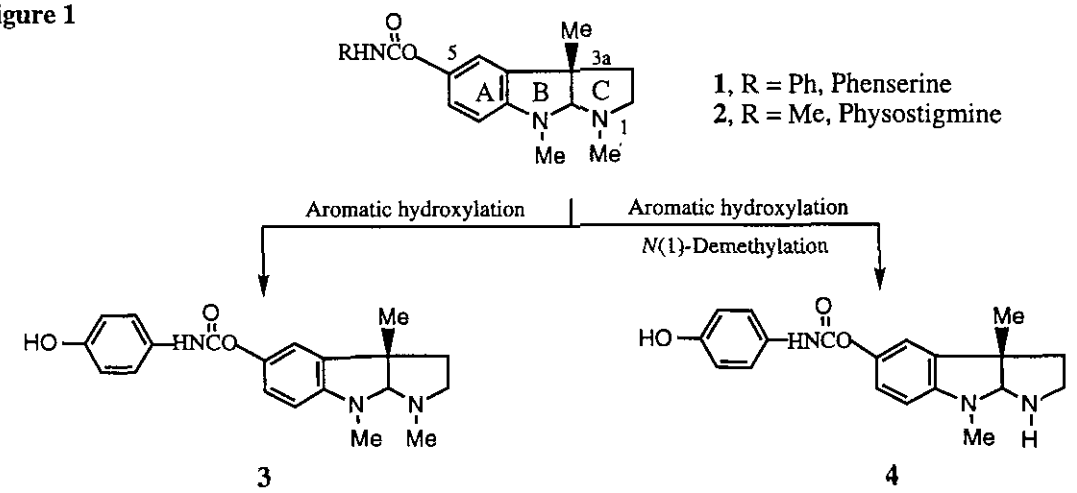
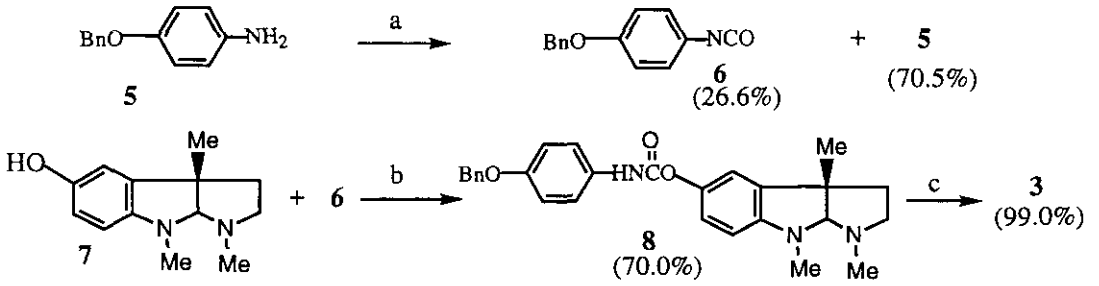
Chemistry. 4-Benzyloxyphenyl isocyanate (**6**), made by reaction of 4-benzyloxyphenylaniline hydrochloride (**5**) with phosgene in toluene,[7] was reacted with eseroline (**7**),[8] following a known procedure,[9] to form 4'-benzyloxyphenserine (**8**). This latter compound (**8**) then underwent debenzylation over the catalyst palladium hydroxide on carbon to give 4'-hydroxyphenserine (**3**) in high yield (**Scheme 1**). Additionally, the known *N*(1)-benzylesermethole (**10**) [10] was synthesized directly from eseroline (**7**) following a procedure of introducing hetero-atoms into its ring C position[1,2,11,12] in 51.2% yield. Demethylation of esermethole (**10**) gave the phenol (**11**) which, when reacted with isocyanate (**6**), afforded 4'-benzyloxy-*N*(1)-benzylphenserine (**12**). Then *O*-debenzylation together with *N*-debenzylation of compound (**12**) gave 4'-hydroxy-*N*(1)-norphenserine (**4**) in excellent yield (**Scheme 2**).

Figure 1

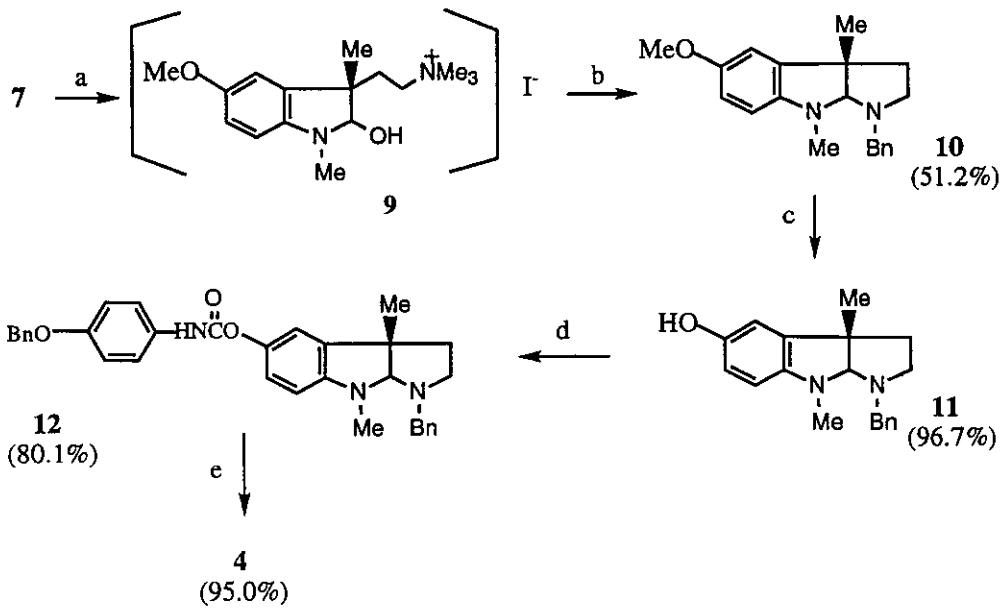


Scheme 1



Reagents: (a) $COCl_2$ / toluene (20%); (b) ether, Na; (c) $Pd(OH)_2$ / C, MeOH.

Scheme 2



Reagents: (a) DMSO, KOH, MeI; (b) $BnNH_2$; (c) BBr_3, CH_2Cl_2; (d) Compound (**6**), ether, Na; (e) $Pd(OH)_2$ / C, MeOH.

Table 1. 50% Inhibitory Concentration (IC_{50}, nM) ± SEM* of Compounds versus Human Erythrocyte AChE and Plasma BChE

	Compound	IC_{50} AChE (nM)	IC_{50} BChE (nM)
1	(-)-Phenserine	24.0 ± *6.0	1300.0 ± *85.0
2	(-)-Physostigmine	27.9 ± 2.4	16.0 ± 2.9
3	(-)-4'-Hydroxyphenserine	77.9 ± 8.4	66.1 ± 29.1
4	(-)-4'-Hydroxy-*N*(1)-norphenserine	59.2 ± 3.2	28.2 ± 6.3
8	(-)-4'-Benzyloxyphenserine	58.5 ± 4.0	7.1 ± 0.9
12	(-)-4'-Benzyloxy-*N*(1)-benzylphenserine	NDA#	24.3 ± 5.8

*Standard error of mean of at least 4 assays

NDA#: No detectable activity at a concentration of $3x10^{-5}$ M

Biological evaluation and discussion: Compounds (**3**, **4**, **8** and **12**) were tested for *in vitro* inhibitory activity of human erythrocyte AChE and plasma BChE. The results, shown in Table 1, are compared with

(-)-phenserine (**1**) and (-)-physostigmine (**2**), whose activities we have previously characterized.[1,2,13] (-)-Phenserine (**1**), as a consequence of its unsubstituted phenylcarbamate, is highly selective for AChE versus BChE inhibition by some 55-fold. Potential aromatic hydroxylation together with oxidative *N*-demethylation, two common hepatic phase I metabolic processes,[5,6] would result in (-)-4'-hydroxyphenserine (**3**) and (-)-4'-hydroxy-*N*(1)-norphenserine (**4**). These possessed potent anticholinesterase inhibitory action that lacked selectivity between AChE and BChE; closely related enzymes with temporal, spacial and biological divergence. The loss of selectivity of **3** and **4** is in accord with our previous studies which have demonstrated that substitutions in the 4'-position of phenserine can alter the selectivity of resulting compounds away from AChE.[1,2,13] For example, 4'-methyl substitution results in a loss of (-)-phenserine's (**1**) AChE selectivity from 55-fold to 2-fold,[13] whereas 4'-isopropyl or 4'-methoxy substitutions result in potent compounds with a 15- and 12-fold selectivity for BChE, respectively.[13,14] In comparison, 4'-hydroxy substitution (**3**) resulted in a complete lack of selectivity for either AChE or BChE, whereas 4'-benzyloxy substitution (**8**) favored an 8-fold selectivity for BChE.

We have also previously reported that specific modifications in the *N*(1) position of both phenserine (**1**) and physostigmine (**2**) provide compounds with potent anticholinesterase action and a modified selectivity to favor either AChE or BChE.[1,2,11-14] In this regard, (-)-*N*(1)-norphenserine (IC_{50}: AChE 13.8 $\pm$ 0.7, BChE 612.0 $\pm$ 0.4; 44-fold AChE selectivity) and (-)-*N*(1)-norphysostigmine (IC_{50}: AChE 21.0 $\pm$ 1.0, BChE 2.0; 10-fold BChE selectivity) possess a modest altered selectivity away from AChE and toward BChE inhibitory action, compared to (-)-phenserine (**1**) (55-fold AChE selectivity) and (-)-physostigmine (**2**) (2-fold BChE selectivity).[10,15] In accord with this, (-)-4'-hydroxy-*N*(1)-norphenserine (**4**) demonstrated an equal potency, compared to **3**, for AChE and a greater activity, by 2-fold, for BChE inhibition. Large lipophilic *N*(1) position substitutions, such as with a phenethyl or benzyl moiety, provide a high degree of selectivity for BChE inhibition.[14,16] For example, (-)-*N*(1)-benzylnorphysostigmine (IC_{50}: AChE 330.0 $\pm$ 100, BChE 10.4 $\pm$ 4.0) has a 32-fold selectivity for BChE inhibitory action. The combination of this BChE-favoring *N*(1) position modification with a BChE-favoring 4'-benzyloxy substitution (**8**), resulted in (-)-4'-benzyloxy-*N*(1)-benzylnorphenserine (**12**) which possessed a high potency and selectivity (>1200-fold) for BChE inhibitory action.

In summary, synthesis of the novel compounds (-)-4'-hydroxyphenserine (**3**) and (-)-4'-hydroxy-*N*(1)-norphenserine (**4**), two likely metabolites of hepatic phase I aromatic hydroxylation and oxidative *N*-demethylation of the Alzheimer drug phenserine (**1**), allowed characterization of their *in vitro* anticholinesterase action. Both **3** and **4** demonstrated potency for cholinesterase inhibitory action, but with a loss of the AChE selectivity afforded phenserine (**1**). Additionally, synthesis and biological characterization of the intermediate, (-)-4'-benzyloxy-*N*(1)-benzyl- norphenserine (**12**), resulted in a highly potent and selective inhibitor of BChE as a valuable tool to explore the role of this specific enzyme in health, aging and disease.

EXPERIMENTAL SECTION

Chemistry. Melting points (uncorrected) were measured with a Fisher-Johns apparatus; ^{1}H NMR were recorded on a Bruker (Billerika, MA) AC-300 spectrometer; MS (m/z) were recorded on a Hewlett-Packard 5890 6-c-ms GCMS (EI) and on a Finnigan-1015D mass spectrometer. Optical rotations were measured on a JASCO, Model DIP-370 (Japan, Spectroscopic Co., LTD.); elemental analyses were performed by Atlantic Microlab, Inc. Unless otherwise indicated, all separations were carried out using flash column chromatography (silica gel 60, 230-400 mesh) with the described solvents. All reactions involving nonaqueous solutions were performed under an inert atmosphere.

4-Benzyloxyphenyl isocyanate (6). 4-Benzyloxyaniline hydrochloride (**5**.HCl) (2.35 g, 10 mmol) and phosgene in toluene (20%) (11.07 mL, 21.5 mmol) were heated and refluxed for 2 h. After cooling, filtration gave recovered starting material (**5**) (1.66 g, 70.5%) as crystals and the filtrate was evaporated to give product (**6**) (0.6 g, 26.6%) directly as crystals: mp 118-132 °C (with the appearance of liquor crystals); ^{1}H NMR ($CDCl_3$) δ7.50 - 6.70 (m, 9H, Ar-H), 4.95 (s, 2H, Ar-CH_2-); EI-MS, m/z (relative intensity): 225 (M^+, 10), 91 (100).

(-)-(3aS)-Eseroline-4'-benzyloxyphenylcarbamate (8). Eseroline (**7**) (218 mg, 1.0 mmol) was dissolved in anhydrous ether (20 mL), and a piece of Na metal (approx. 1 mg) was added. The mixture was stirred at room temperature for 1 min, then isocyanate (**6**) (225 mg, 1.0 mmol) was added and stirred for 5 min. The reaction mixture was washed with dilute NaOH (2%) (2 x 20 mL), then brine, and then dried over Na_2SO_4. Evaporation of solvent provided a crude product which crystallized from ether to give **8** (310 mg, 70.0%) as crystals: mp 160 °C; $[\alpha]_D^{20}$ - 61.7° (c = 0.3, EtOH); ^{1}H NMR ($CDCl_3$): δ7.50-6.30 (m, 12H, Ar-H), 5.03 (s, 2H, Ph-CH_2-), 4.22 (s, 1H, C8a-H), 2.92 (s, 3H, N8-CH_3), 2.80-2.60 (m, 2H, C2-H_2), 2.52 (s, 3H, N1-CH_3), 1.95 (m, 2H, C3-H_2), 1.40 (s, 3H, C3a-CH_3); EI-MS, m/z (relative intensity): 225 (M^+ - eseroline, 6.4), 218 (3.0), 108 (31.4), 91 (100). Anal. Calcd for $C_{27}H_{29}N_3O_3$: C, 73.21; H, 6.59; N, 9.47. Found: C, 72.88; H, 6.57; N, 9.32.

(-)-(3aS)-Eseroline-4'-hydroxyphenylcarbamate (3). Compound (**8**) (50 mg, 0.11 mmol) was dissolved in MeOH (1 mL) and $Pd(OH)_2$/C (Pd 20%) (5 mg) was added. The reaction mixture was stirred under hydrogen atatmospheric pressure and rt for 30 min, and then the catalyst was filtered through Celite. Evaporation of solvent gave compound **(3)** (39.5 mg, 99.0%) as crystals: mp 168-170 °C; $[\alpha]_D^{20}$ -73.9 °C (c= 0.2, EtOH); ^{1}H NMR ($CDCl_3$): δ7.25-6.30 (m, 7H, Ar-H), 4.05 (s, 1H, C8a-H), 2.85 (s, 3H, N8-CH_3), 2.75-2.55 (m, 2H, C2-H_2), 2.47 (s, 3H, N1-CH_3), 1.90 (m, 2H, C3-H_2), 1.35 (s, 3H, C3a-CH_3); EI-MS, m/z (relative intensity): 218 (MH^+-135, 98), 174 (100); CI-MS (NH_3) m/z: 354 (MH^+). Anal. Calcd for $C_{20}H_{23}N_3O_3$: C, 67.97; H, 6.56; N, 11.89. Found: C, 67.97; H, 6.64; N, 11.51.

(-)-(3a*S*)-1-Benzyl-3a,8-dimethyl-1,2,3,3a,8,8a-hexahydro-5-methoxypyrrolo[2,3-*b*]indole (10). Eseroline (**7**) (602 mg, 2.76 mmol) was dissolved in DMSO (5 mL) under atmospheric

nitrogen at rt, and powdered KOH (618 mg, 11.04 mmol) was added. After 5 min stirring, MeI (0.34 mL, 5.52 mmol) was added and stirring was continued for a further 1 h. An additional amount of MeI (0.68 mL, 11.04 mmol) then was added and the reaction mixture was again stirred for yet another 1 h. After removal of solid KOH and KI, the reaction mixture was added to benzylamine (5 mL), stirred and heated over an oil bath maintained at 100°C for 2 h. Evaporation of solvent by high vacuo gave a residue which was chromatographed to give **10** (435 mg 51.2%) as a colorless syrup: The optical rotation, ^{1}H NMR and MS were identical with those reported in the lit.[10]

(-)-(3a*S*)-1-Benzyl-3a,8-dimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-ol (11). Compound **(11)** was synthesized from **10** according to a procedure reported in the Lit.[9]

(-)-(3a*S*)-1-Benzyl-3a,8-dimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indol-5-yl-N-4'-benzyloxyphenylcarbamate (12). Compound (**11**) (186.6 mg, 0.64 mmol) was dissolved in ether (10 mL), and Na metal (approx. 1 mg) was added. The mixture was stirred at rt for 1 min, then isocyanate **(6)** (143 mg, 0.64 mmol) was added. After stirring for a further 5 min, evaporation of solvent gave a residue which was chromatographed (CH_2Cl_2 / MeOH = 20 / 1) and crystallized from ether to provide **12** (267 mg, 80.1%) as crystals: mp 158 °C; $[\alpha]_D^{20}$ -55.0° (c=0.3, EtOH); ^{1}H NMR ($CDCl_3$) δ 7.50-6.30 (m, 17 H, Ar-H), 5.03 (s, 2H, Ph-CH_2-O-), 4.37 (s, 1H, C8a-H), 4.00(m, 2H, Ph- CH_2-N), 2.80 (s, 3H, N8-CH_3), 2.80-2.60 (m, 2H, C2-H_2), 1.90 (m, 2H, C3-H_2), 1.46 (s, 3H, C3a-CH_3); EI-MS, m/z (relative intensity): 324 (MH^+ - 2 benzyl- CH_3, 31), 309 (100), 294 (36), 281 (31.4), 91 (90). Anal. Calcd for $C_{33}H_{33}N_3O_3$. 0.24 H_2O: C, 75.64; H, 6.44; N, 8.02. Found: C, 75.66; H, 6.48; N, 7.84.

(-)-(3a*S*)-3a,8-Dimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-yl-N-4'-hydroxyphenyl carbamate (4). Compound (**12**) (45 mg, 0.087 mmol) was dissolved in MeOH (1 mL), and palladium hydroxide on carbon (Pd 20%) (4 mg) was added. The reaction mixture was stirred under hydrogen at atmospheric pressure and rt for 1 h, the palladium catalyst then was filtered through Celite and the solvent was evaporated by *vacuo*. The residue was chromatographed (CH_2Cl_2 / MeOH = 10 / 1) and crystallized from petroleum ether to give compound (**4**) (27.9 mg, 95.0%) as crystals: mp 132-135 °C; $[\alpha]_D^{20}$ -150.0° (c=0.2, EtOH); ^{1}H NMR ($CDCl_3$) δ 7.40-7.20 (m, 4 H, Ph-H), 6.85-6.70(m, 2H, C4-H, C6-H), 6.30 (d, J=10 Hz, 1H, C7-H), 4.55 (s, 1H, C8a-H), 2.85 (s, 3H, N8-CH_3), 2.70 (m, 2H, C2-H_2), 2.40 (m, 2H, C3-H_2), 1.45 (s, 3H, C3a-CH_3); EI-MS, m/z (relative intensity): 204 (MH^+ - HO-Phenyl-NHCO-, 34), 136 (50), 108 (36), 91 (100); CI-MS(NH_3) m/z: 340 (MH^+). Anal. Calcd for $C_{33}H_{33}N_3O_3$: C, 67.24; H, 6.24; N, 12.38. Found: C, 67.05; H, 6.42; N, 12.12.

Biological Assay. Freshly collected human blood was centrifuged (6000xg, 10 min, at 4°C) and the plasma then was separated and diluted 1/125 with 0.1 M Na_3PO_4 (pH 7.4). Erythrocytes were washed three times in isotonic saline, and lysed by the addition of 9 volumes of 0.1 M Na_3PO_4 containing 0.5 % Triton-X (Sigma Chemical Co., St. Louis, MO) (pH 7.4 on ice for 30 min). This then was diluted with 19 additional volumes of 0.1 M Na_3PO_4, (pH 7.4) to final dilution of 1/200. Acetyl-β-methylthiocholine (0.5 mM)

(Sigma) and *S*-butyrylthiocholine (0.5 mM) (Sigma) were used as specific substrates for the assay of AChE and BChE, respectively. For each cholinesterase preparation, 25 μLof substrate and 25 μL of enzyme were added separately to a final incubation volume of 0.75 mL.

For quantitative determination of their inhibitory action against AChE and BChE, compounds were initially dissolved in Tween 80/EtOH (3/1, v/v, 80 μL total volume) and then were diluted with 0.1 M Na_3PO_4, (pH 8.0) in half log-increments to a final concentration range that spanned $1x10^{-5}$ M to $0.3x10^{-9}$ M. Finally, they were preincubated with enzyme (30 min at rt) prior to addition of respective substrates for AChE and BChE. The Tween 80/EtOH was diluted to in excess of 1:1000 and did not affect either AChE or BChE activity, as determined in prior studies with physostigmine.

Following a 25 min incubation at 37°C with respective substrates for AChE and BChE and 5,5'-dithiobis-2-nitrobenzoic acid (0.5 mmol/L), the absorbency of a yellow thionitrobenzoate anion product was quantified by spectrophotometery set to 412 nm wavelength. Nonspecific substrate hydrolysis was determined under conditions of complete enzyme inhibition (following the addition of excess physostigmine, $1x10^{-5}$ M), and the associated change in absorbency was subtracted from that observed with the experimental compounds (**1**,**2**,**3**,**4**,**8**,**12**). Furthermore, the inhibitory activity of each compound was assessed alongside that of physostigmine, as an external standard, whose activity we have previously reported.[1,2,10-16]

For determination of an IC_{50} value for each compound, the enzyme activity at each concentration was expressed as a percent of that determined in the absence of compound. This then was transformed into a logit format, where *logit = ln(% activity / [100 - % activity])*, and was plotted as a function of the log concentration of the compound. IC_{50} values (i.e., logit = ln(50/[100-50]=0) were determined only when correlation coefficients of less than -0.99 were achieved, and when greater than 50 % inhibition was achieved from duplicate samples. Each compound was analyzed on at least 4 occasions, in duplicate. The IC_{50} values are listed in Table 1.

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