125. Total Synthesis of Racemic and Optically Active Compounds Related to Physostigmine and Ring-C Heteroanalogues from 3-[2′-(Dimethylamino)ethyl]-2,3-dihydro-5-methoxy-1,3-dimethyl-1*H*-indol-2-ol

by **Xue-Feng Pei**[1])* and **Nigel H. Greig**

Laboratory of Neuroscience, National Institute on Aging, NIH, Bethesda, MD 20892, USA

and **Judith L. Flippen-Anderson**

Laboratory of the Structure of Matter, Naval Research Laboratory, Washington, DC 20375, USA

and **Sheng Bi** and **Arnold Brossi**[a])

Department of Chemistry, Georgetown University, Washington, DC 20057, USA

[a]) Scientist Emeritus, National Institutes of Health, Bethesda, MD 20892, USA

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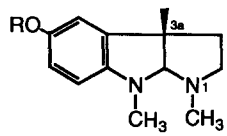
Oxindole **11**, obtained on 3-[2′-(dimethylamino)ethyl]alkylation of oxindole **12**, yielded, on stereoselective reduction with sodium dihydridobis(2-methoxyethoxy)aluminate, aminoalcohol **8** (*Scheme 2*). The quaternary methiodide **10**, obtained from **8** with MeI, gave, in nucleophilic displacements concurring with a *Hofmann* elimination, (±)-esermethole **6**, (±)-5-*O*-methylphysovenol (**14**), (±)-5-*O*-methyl-1-thiaphysovenol (**15**), and (±)-1-benzyl-1-demethylesermethole (**16**). Syntheses of (±)-1-benzyl-1-demethylphenserine (**18**), (±)-1-demethylphenserine (**19**), and (±)-phenserine (**4**) from **6** and **16** are described. Optically active **8a** and **8b**, obtained by chemical resolution, similarly gave the enantiomers **6a** and **14a**–**16a** of the (3a*S*)-series (prepared earlier from physostigmine (**1a**)) and their (3*R*)-enantiomers. The anticholinesterase activity of (±)-**4**, (±)-**18**, and (±)-**19** was compared with that of their optically active enantiomers.

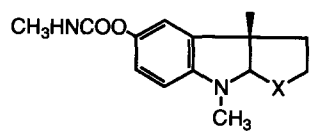
Introduction. – The alkaloid physostigmine (**1a**)[2]), isolated from the seeds of *Physostigma venenosum* named *Calabar* beans [1], is used in medicine to treat glaucoma, and it seems beneficial in the treatment of other disorders which affect the bioconversion of acetylcholine into choline [2]. In our search to find longer-acting and less toxic compounds related to **1a**, we recently reported on several carbamate analogs of natural physovenine (**2a**) [4] and its thiacongener **3a** [4] which significantly inhibited cholinesterase *in vitro*. Another interesting compound related to **1a** is the known phenserine (**4a**), the phenylcarbamate of eseroline (**5a**) [5]. Phenserine (**4a**) shows a rather specific inhibition of acetylcholinesterase (AChE) and has a wide therapeutic window, suggesting that it might be useful in *Alzheimer*'s disease [6]. The (3a*S*)-configuration present in these compounds is a critical point in studying structure-activity relationships in this series since the unnatural (3a*R*)-enantiomers of *Calabar* alkaloids were found to be largely inactive in assays measuring the inhibition of cholinesterases [7].

[1]) *X.-F. P.* is also a postodoctoral fellow at the Department of Chemistry, Georgetown University.

[2]) The lowercase letters **a** and **b** used in the key numbers of compounds refer to optically pure compounds having (3a*S*)- and (3a*R*)-configuration , respectively. Compounds lacking these letters are racemic mixtures.



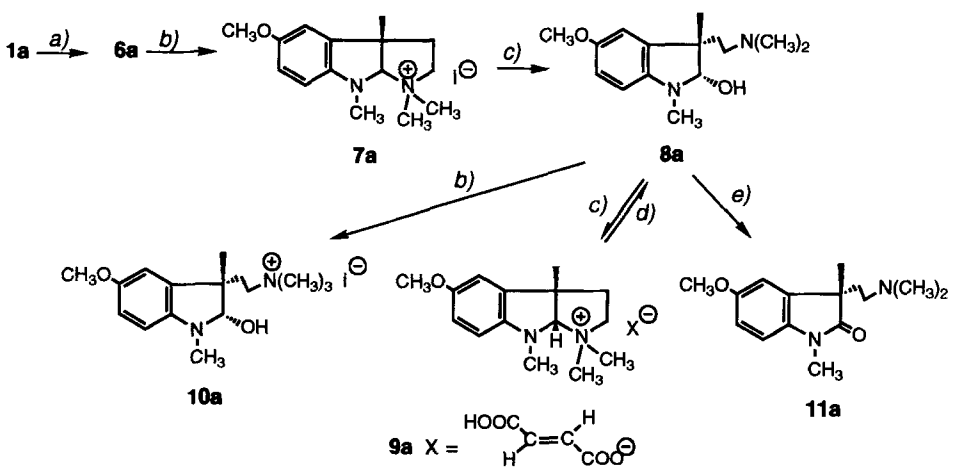
1a R = $CONHCH_3$
4a R = CONHPh
5a R = H
6a R = CH_3



2a X = O
3a X = S

The biologically interesting carbamates **2a** and **3a** were first prepared from physostigmine (**1a**) without isolation of the intermediates [3] [4]. Based on experiments perfected earlier in the ethyl-ether series [8], it is believed that the chemical steps in accomplishing these transformations proceed, as shown in *Scheme 1* for the methyl-ether analogs, from **1a**→**5a**→**6a**→**7a**→**8a**→**10a**. The methiodide **10a** of aminoalcohol **8a** is believed to be the ultimate precursor of the tricyclic ethers **6a**, **14a**, **15a**, and **16a** used to prepare *Calabar*-alkaloid congeners and ring-C heteroanalogs [1] [3] [4]. Aminoalcohol **8a**, therefore, plays a pivotal role in correlating oxindoles (= 1,3-dihydro-1*H*-indol-2-ones) with physostigmine and its congeners, and a simple synthesis of **8**, and perfected with its resolution to **8a**, could offer an attractive alternative route to racemic and optically *Calabar* alkaloids and ring-C heterocongeners. We report here a successful completion of this task.

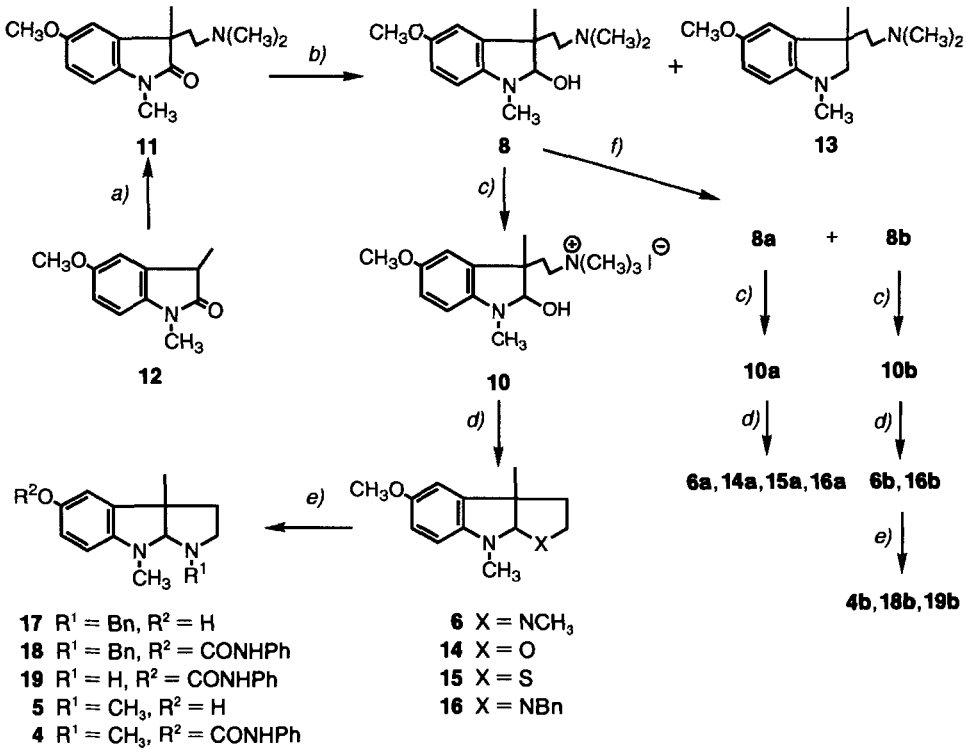
Scheme 1



a) Na, EtOH, TsOMe. *b*) MeI, Et_2O. *c*) Fumaric acid, $EtOH/Et_2O$. *d*) 5% NaOH soln. *e*) $K_3[Fe(CN)_3]$, KOH.

Chemistry. – Aminoalcohol **8a**, prepared from physostigmine (**1a**) as shown in *Scheme 1*, on oxidation with $K_3[Fe(CN)_6]$, was reported to give oxindole **11a** [9], and it cyclized spontaneously, as reported here, on treatment with fumaric acid to the quaternary fumarate **9a**. Aminoalcohol **8a** and its methiodide **10a**, like its ethyl-ether analogs [8], are dextrorotatory (see *Exper. Part*) while the fumarate **9a** is levorotatory also

Scheme 2



a) $ClCH_2CH_2NMe_2$, $NaNH_2$, toluene. *b*) *Vitride*, toluene. *c*) MeI/Et_2O. *d*) **6**: $MeNH_2$, MeCN; **14**: 7N NaOH; **15**: 7N NaSH; **16**: $BnNH_2$, MeCN. *e*) **17** and **5**; BBr_3, CH_2Cl_2; **18** and **4**: PhNCO, Na, Et_2O; **19**: H_2, $Pd(OH)_2$, MeOH. *f*) 2,3-Di-*O*-(*p*-toluoyl)-D-tartaric acid, EtOH.

suggesting that it has a tricyclic structure. The structure of **9a**, which reconverted on addition of NaOH to **8a**, is secured by an X-ray analysis (see below). The *cis*-arrangement of the 2-OH group and the 3-(2′-dimethylamino)ethyl group in **8a**, is also confirmed by an X-ray analysis (see below).

Compounds **8a**–**11a** are crucial intermediates in our total synthesis. With their structural information on hand, we turned our work to the total synthesis. The oxindole **11** was obtained from the known oxindole **12** [10] [11] on alkylation with 2-chloro-*N*,*N*-dimethylethanamine hydrochloride [12] (*Scheme 2*). Stereoselective reduction of **11**, accomplished with sodium dihydridobis(2-methoxyethoxy)aluminate in toluene (*Vitride*), readily afforded aminoalcohol **8** whose spectral data were identical with those of **8a**. Purification of **8** which was contaminated with small amounts of the 2,3-dihydro-1*H*-indole **13** was effected with fumaric acid yielding the quaternary fumarate **9**. Optical resolution of oxindole **11** proved very difficult with many resolving agents, and only an imperfect resolution was achieved with 2,3-di-*O*-(*p*-toluoyl)tartaric acid. But the resolution of aminoalcohol **8** was easily perfected with (+)-2,3-di-*O*-(*p*-toluoyl)-D-tartaric acid. Aminoalcohol **8a**, obtained from the lesser soluble salt, was identical with the material

prepared from **1a** and, therefore, had the (3a*S*)-configuration. The (3a*R*)-enantiomer **8b** was isolated from the mother liquor of the chemical resolution and can be used to prepare compounds of the unnatural series [3] [13]. Methiodide **10**, obtained from **8** with MeI, afforded **14** in the presence of NaOH, **15** in the presence of NaSH, and **16** when reacted with benzylamine ($BnNH_2$). The (±)-esermethole (**6**) was also obtained from **10** when heated with $MeNH_2$ in a sealed tube. The optically active series was similarly developed from aminoalcohol **8a** and yielded ethers **6a** [9], **14a** [3], **15a** [4], and **16a** [14–16], prepared earlier from natural physostigmine. Their (3a*R*)-antipodes were also prepared from **8b**.

The use of **6** and **16** for making carbamates of the racemic physostigmine series is shown in *Scheme 2* and follows a protocol elaborated earlier with the (3a*S*)-enantiomers prepared by different routes [15] [17]. The chemical reactions included their *O*-demethylation to phenol **5** and **17**, formation of *N*-phenylcarbamates **4** and **18** with phenyl isocyanate, and reductive debenzylation of **18** to (±)-1-demethylphenserine (**19**). The (3a*R*)-enantiomers were prepared in the same way.

X-Ray Analysis. – The results of the X-ray studies on **8a** and **9a** are illustrated in *Figs. 1* and *2*. Both structures were solved by direct methods with the aid of program

Table 1. *Crystal and Refinement Data*

	Molecule **8a**	Molecule **9a**
Formula	$C_{15}H_{24}N_2O_2$	$C_{15}H_{23}N_2O^+ \cdot C_4H_3O_4^- \cdot H_2O$
Formula weight	264.4	380.4
Crystal color, habit	colorless, irregular	colorless, prism
Crystal dim. [mm]	0.58 × 0.21 × 0.34	0.48 × 0.46 × 0.40
Crystal system	orthorhombic	orthorhombic
Space group	$P2_12_12_1$	$P2_12_12_1$
a [Å]	8.572 (3)	7.910 (2)
b [Å]	11.290 (3)	11.191 (2)
c [Å]	16.108 (5)	23.072 (5)
V [Å^3]	1558.9 (8)	2042.4 (8)
Z	4	4
ρ (calc.) [g cm^{-3}]	1.126	1.237
μ, absorption coeff. [mm^{-1}]	0.60	0.09
Temp. [°C]	22	22
Diffractometer	*Siemens R3m/V*	*Siemens R3m/V*
Cell determination reflections, 2θ range	25, 56–66	25, 19–40
λ, wavelength [Å]	CuK_α, 1.54184	*Mo*K_α, 0.71073
2θmax., scan mode	115, $\theta/2\theta$	*50, Wykoff*
Total reflections measured	1318	2155
Unique data	1253	2086
	refinement on F^2 using all data	
Observed data ($I > 2\sigma I$)	1152	1548
R_{int}	0.017	0.009
Parameters refined	176	254
R^a), wR^b), S^c) (for obs. data)	0.040, 0.128, 1.04	0.044, 0.112, 1.04
R^a),wR^b), S^c) (for all data)	0.044, 0.133, 1.04	0.063, 0.129, 1.04
Extinction parameter (x)^d)	0.0072 (11)	0.014 (2)
Data/parameter ratio	7:1	8:1
Final Δ_{max}/σ	0.11	0.01
Fourier excursions [e Å^{-3}]	0.15, −0.16	0.13, −0.13

a) $\Sigma|\Delta|/\Sigma|F_o|$. b) $[\Sigma(w\Delta^2)^2/\Sigma(wF_o^2)^2]^{1/2}$. c) $[\Sigma w(\Delta^2)^2/(N_o - N_p)]^{1/2}$. d) $F_c = F_c\{k[1 + 0.001(x)F_c^2\lambda^3/\sin(2\theta)]^{-1/4}\}$.

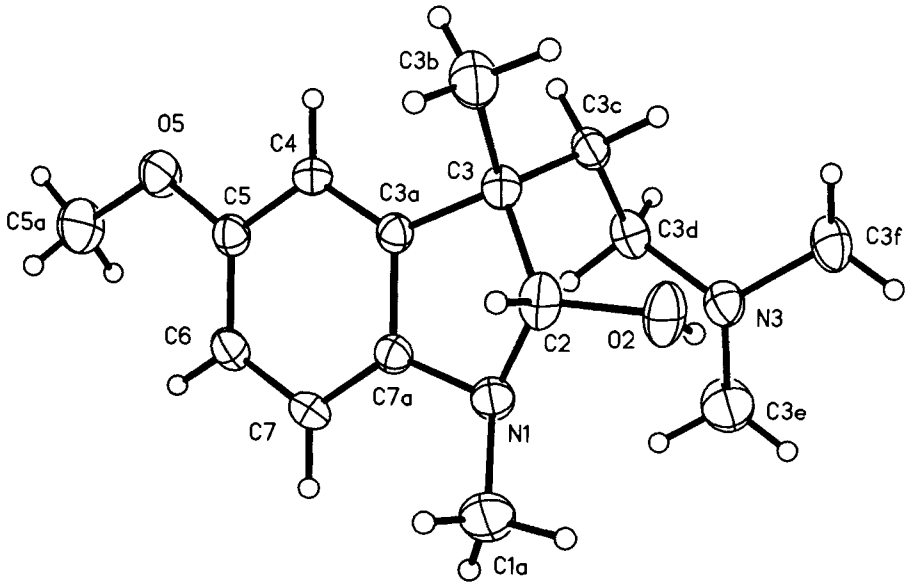


Fig. 1. *X-Ray structure of* **8a**. The figure is drawn from the final refined coordinates with the thermal ellipsoids displayed at the 20% probability level.

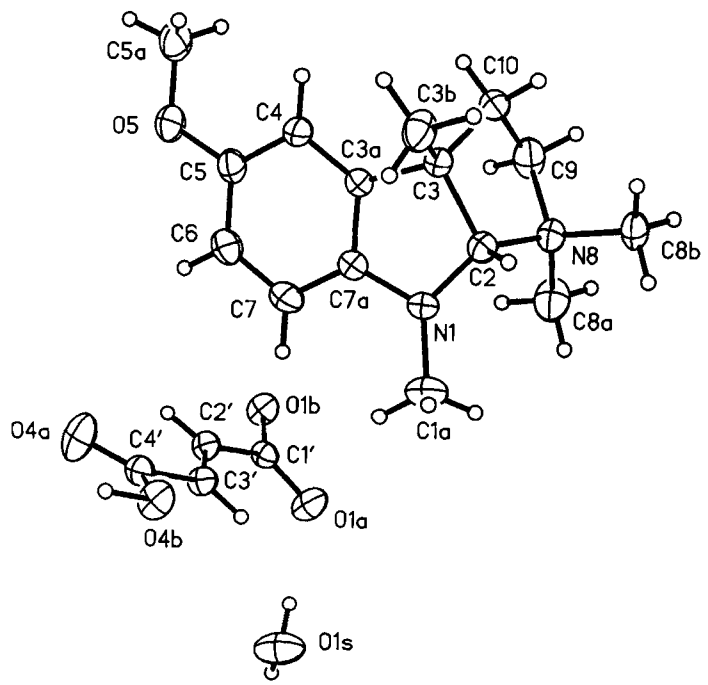


Fig. 2. *X-Ray structure of* **9a** *showing the full contents of the asymmetric unit*. The figure is drawn from the final refined coordinates with the thermal ellipsoids displayed at the 20% probability level. Arbitrary numbering.

SHELXS-86 [18] and refined by full-matrix least squares on F^2 values using program SHELXL-93 [19]. *Table 1* provides a listing of the experimental data collection and refinement parameters (see also *Exper. Part*).

In both molecules, the MeO moiety is nearly coplanar with the aromatic ring; however, in **8a** it is extended away from the aromatic ring (C(4)–C(5)–O(5)–C(5a) = –177.4 (4)°) while in **9a** it is folded back towards the fused ring system (C(4)–C(5)–O(5)–C(5a) = 5.5 (8)°). In **8a**, the 5-membered heterocyclic ring has an envelope conformation with C(2) being 0.45 Å out of the plane formed by the other four ring atoms. There is an intramolecular H-bond between the H-atom on O(2) and N(3) (O(2)–H = 0.95 (5) Å, O(2)···N(3) = 2.70 (1) Å, and angle O(2)–H···N(3) = 154.2 (15)°). Both the OH group and the 3-[2′-(dimethylamino)ethyl] group are on the same side of the fused ring system (O(2)–C(2)–C(3)–C(3c) = 33.7 (4)°) such that they are properly positioned to form the third ring during the production of **9a**. In **9a**, the central five-membered ring is now planar (average absolute value for the ring torsion angles = 9.3 (4)°) as opposed to being in the envelope conformation it displayed in **8a**. The terminal five-membered ring in **9a** is in an envelope conformation with C(10) being 0.55 (2) Å out of the plane of the other four ring atoms. The thermal parameters for the MeO group and C(1a) are larger than for the other atoms in both molecules. To a certain extent this is to be expected due to normal thermal motion of these groups. When the thermal motion is largest in one direction, it is normal to check for possible disorder in the crystal. This was done for both molecules **8a** and **9a**, and in neither case did the experimental data support refinement of either the MeO group or N(1)–C(1a) as disordered entities. Even if there is a small amount of unresolvable disorder in the 5-membered heterocyclic ring, it would not change the overall conformation of the ring. Compound **9a** crystallized as a fumarate salt with one molecule of H_2O in the asymmetric unit. The fumarate ions are linked into infinite chains by a strong O(4b)···O(1b) intermolecular H-bond (O(4b)–H = 1.14 (5) Å, O(4b)···O(1b) = 2.44 (1) Å, and angle O(4b)–H···O(1b) = 174.1 (15)°). The chains of fumarate ions are linked by H-bonds to the H_2O molecule (O(1s)–H distances were fixed at 0.85 Å, O(1s)···O(1a) = 2.83 (1) Å, O(1s)···O(4a) = 2.92 (1) Å, angle O(1s)–H···O(1a) = 176 (2)° and angle O(1s)–H···O(4a) = 179 (2)°) to form folded sheets which flow between stacks of **8a** cations. Except for the H-bonded contacts between the fumarate ions and the H_2O molecules in **9a**, there are no intermolecular approaches less than *van der Waals* distances.

Biological Assay. – The availability of compounds **4**, **18**, and **19** of the racemic series and their (3a*R*)-enantiomers suggested a comparison with their (3a*S*)-enantiomers [17] and with physostigmine (**1a**) in assays measuring inhibition of cholinesterases. A classical enzyme-inhibition assay was undertaken to quantitate the anticholinesterase activity of these compounds against acetyl- and butyrylcholinesterase, AChE and BChE, respectively. Activity was determined against human erythrocyte AChE and plasma BChE in 0.1M Na_3PO_4 buffer (pH 8.0), using the spectrophotometric method of *Ellman et al.* [20], as modified by *Atack et al.* [7]. The pharmacological activity of each compound was expressed as an IC_{50}, which is defined as the concentration, in nmol, required to inhibit 50% of the enzyme activity of AChE and BChE, separately. A comparison of the IC_{50} values of **4**, **18**, and **19** to those of their (3a*S*)- and (3a*R*)-enantiomers and of **1a** is shown in *Table 2*.

Table 2. *50% Inhibitory Concentration (IC_{50}) of Phenserine, 1-Benzyl-1-demethylphenserine, and 1-Demethylphenserine* vs. *Human Erythrocyte AChE and Human Plasma BChE*[a])

		IC_{50} [nM]	
		AChE	BChE
1a	(−)-physostigmine	28	16
4a	(−)-phenserine	22	1552
4	(±)-phenserine	75	5610
4b	(+)-phenserine	3500	> 10,000
18a	(−)-1-benzyl-1-demethylphenserine	466	4809
18	(±)-1-benzyl-1-demethylphenserine	969	> 10,000
18b[b])	(+)-1-benzyl-1-demethylphenserine	3538	> 10,000
19a	(−)-1-demethylphenserine	25	623
19	(±)-1-demethylphenserine	47	1659
19b[b])	(+)-1-demethylphenserine	93	4136

[a]) Minimum of 4 measurements per compound; s.e. *ca.* 10%.
[b]) (3a*R*)-isomer e.e. 90%.

The (−)-phenserine (**4a**) and (−)-1-demethylphenserine (**19a**), as we reported before [17], proved equipotent to (−)-physostigmine (**1a**) in inhibiting AChE but were dramatically less effective against BChE. Substitution on N^1 with benzyl (**18a**, **18**, **18b**) resulted in reduced activities for both AChE and BChE inhibition. As we expected, the (±)-phenserine (**4**), (±)-1-benzyl-1-demethylphenserine (**18**), and (±)-1-demethylphenserine (**19**) showed 1- to 4-fold reduced activity compared to their (−)-enantiomers. The (+)-phenserine (**4b**) and (+)-1-benzyl-1-demethylphenserine (**18b**) showed insignificant activity for AChE and were inactive to BChE. Interestingly, the (+)-1-demethylphenserine (**19b**) still demonstrated moderate potency against AChE. Considering that (+)-physovenine was also active in inhibiting AChE and BChE [3], the activity of (+)-1-demethylphenserine is not surprising.

Conclusion. – Aminoalcohol **8** and its enantiomers now give easy access to racemic and optically active *Calabar* alkaloids and ring-C heterocongeners. The successful nucleophilic displacement of **10** and its enantiomers with benzylamine and $MeNH_2$ with concomitant cyclization to **6**, **16**, and their enantiomers of the physostigmine series of compounds is noteworthy. The use of *Vitride* instead of the hazardous $LiAlH_4$ used earlier for the reduction of oxindole [1] [9] offers another advantage and makes the aminoalcohol route attractive for a large-scale production of these compounds.

We would like to thank Dr. *Qian-sheng Yu*, Shanghai Institute of Organic Chemistry, Academia Sinica, China, who kindly provided us with the oxindole **12**, and Dr. *Yu-Long Pu*, Analytical Laboratory, NIDDK, NIH, for the measurements of the opical rotations.

Experimental Part

General. Vitride (= sodium dihydridobis(2-methoxyethoxy)aluminate = sodium bis(2-methoxyethoxy)aluminium dihydride) in toluene (70%) is commercially available from *Fluka Chemical Co.*, Ronkonkoma, N. Y., and from *Hexel Co.*, Zeeland, Mi., USA. TLC: silica gel *GHLF*, 250 μm, *Analtech Inc.* Column chromatography: silica gel *GHLF*, 250 μm, *Merck 60* (230–400 mesh). HPLC: *Rainin-81-2XM-Macintosh*-controlled HPLC system, *Chiralcel OD* column (*Daicel Chemical Industries, Ltd.*); eluent: i-PrOH/hexane 1:99 with 0.1% Me_2NH flow rate 1.0 ml/min; detector wavelength 254 nm. M.p. (uncorrected): *Fisher-Johns* apparatus. Optical rotations ($[\alpha]_D$): *Perkin-Elmer-241-MC* automatic polarimeter. IR Spectra (cm^{-1}): *MIDIC* FTIR instrument. ^{1}H-NMR (in $CDCl_3$ with Me_4Si as internal reference, δ in ppm, J in Hz): *Varian-XL-300-MHz* spectrometer. MS (*m/z*): for chemical ionization (CI), *Finnigan-1015D* mass spectrometer; for electron impact (EI), *V.G.-Micromass-7070* mass spectrometer.

(−)-(3aS)-1,2,3,3a,8,8a-Hexahydro-5-methoxy-1,3a,8-trimethylpyrrolo[2,3-b]indole (= *Esermethole;* **6a**) *and Its Methiodide* **7a**. Prepared as described in [9].

6a: Oil. $[\alpha]_D = -128.3$ ($c = 0.9$, EtOH). IR: 2957, 1497, 1277, 1032. ^{1}H-NMR: 6.59–6.62 (*m*, H–C(4), H–C(6); 6.28 (*d*, $J = 8.1$, H–C(7)); 3.99 (*s*, H–C(8a)); 3.67 (*s*, MeO); 2.81 (*s*, Me–N(8)); 2.49–2.69 (*m*, 2 H–C(2)); 2.46 (*s*, Me–N(1)); 1.85–1.90 (*m*, 2 H–C(3)); 1.36 (*s*, Me–C(3a)). EI-MS: 232 (M^+).

Fumarate: M.p. 141–142°. $[\alpha]_D = -99.1$ ($c = 0.63$, EtOH; [15]: M.p. 137–138°; $[\alpha]_D = -98.7$ ($c = 0.8$, MeOH)). Anal. calc. for $C_{14}H_{20}N_2O \cdot C_4H_4O_4$ (348.40): C 62.05, H 6.94, N 8.04; found: C 61.68, H 7.18, N 7.97.

7a: M.p. 165–169° ([9]: M.p. 169–170°). $[\alpha]_D = -140.9$ ($c = 1.14$, EtOH). Anal. calc. for $C_{15}H_{23}IN_2O$ (347.27): C 48.13, H 6.19, N 7.49, I 33.90; found: C 48.13, H 6.24, N 7.45, I 33.82.

(+)-(3S)-3-[2'-(Dimethylamino)ethyl]-2,3-dihydro-5-methoxy-1,3-dimethyl-1H-indol-2-ol (**8a**), *Its Methiodide* **10a**, *and (−)-(3aS)-1,2,3,3a,8,8a-Hexahydro-5-methoxy-1,1,3a,8-tetramethylpyrrolo[2,3-b]indol-1-ium Hydrogen (E)-Butanedioate* (**9a**). To a soln. of **7a** (320 mg, 0.85 mmol) in H_2O (5 ml) and EtOH (1 ml), 50% NaOH soln. (0.5 ml) was added. The mixture was extracted with Et_2O (3 × 10 ml) and the combined Et_2O layer washed with brine (20 ml), dried (Na_2SO_4), and evaporated: 200 mg of light yellow oil. The oil was dissolved in Et_2O (10 ml) and a sat. soln. of fumaric acid (93 mg, 0.8 mmol) in EtOH was added to give 262 mg (84%) of **9a**, White crystals. A sample for X-ray analysis was obtained by recrystallization from i-PrOH.

Fumarate **9a** was dissolved in H_2O and made basic by 50% NaOH soln., then extracted with Et_2O. The Et_2O layer was dried (Na_2SO_4) and evaporated: 190 mg of **8a**. Colorless crystals. A sample used for the X-ray analysis was obtained by recrystallization from $(i\text{-Pr})_2O$.

Methiodide **10a**, a white hygroscopic powder, was obtained quantitatively on addition of MeI to an Et_2O soln. of **8a**.

8a: M.p. 76–78°. $[\alpha]_D = +11.6$ ($c = 0.65$, EtOH). IR ($CHCl_3$): 2953, 1494. ^{1}H-NMR: 8.90 (br. *s*, OH); 6.58 (*dd*, $J = 2.5, 8.2$, H–C(6)); 6.48 (*d*, $J = 2.5$, H–C(4)); 6.26 (*d*, $J = 8.2$, H–C(7)); 4.33 (*s*, H–C(2)); 3.66 (*s*, MeO); 2.62 (*s*, Me–N(1)); 2.10 (*s*, Me_2N); 2.08–2.10 (*m*, CH_2CH_2); 1.20 (*s*, Me–C(3)). CI-MS: 265 ($[M + 1]^+$). Anal. calc. for $C_{15}H_{24}N_2O_2$ (264.37): C 68.14, H 9.15, N 10.60; found: C 68.22, H 9.15, N 10.59.

9a: M.p. 150–151°. $[\alpha]_D = -154.1$ ($c = 0.55$, EtOH). Anal. calc. for $C_{19}H_{28}N_2O_5$ (364.44): C 62.61, H 7.74, N 7.69; found: C 62.53, H 7.89, N 7.64.

10a: M.p. 110–115°. $[\alpha]_D = +8.0$ ($c = 0.57$, EtOH). Anal. calc. for $C_{15}H_{24}N_2O_2 \cdot 1.2$ MeI (434.70): C 44.76, H 6.40, I 35.03, N 6.45; found: C 44.56, H 6.48, I 35.28, N 6.51.

(−)-(3S)-3-[2'-(Dimethylamino)ethyl]-1,3-dihydro-5-methoxy-1,3-dimethyl-2H-indol-2-one (**11a**). Prepared as described in [9]. Its fumarate was prepared on addition of a saturated EtOH soln. of fumaric acid to an Et_2O soln. of **11a**.

11a: Oil. $[\alpha]_D = -40.8$ ($c = 0.95$, EtOH). IR (film): 2946, 1713. CI-MS (NH_3): 263 ($[M + 1]^+$). ^{1}H-NMR ($CDCl_3$): 6.57–6.75 (*m*, H–C(4), H–C(6), H–C(7)); 3.65 (*s*, MeO); 3.05 (*s*, Me–N(1)); 1.95 (*s*, Me_2N); 1.65–2.10 (*m*, CH_2CH_2); 1.320 (*s*, Me–C(3)).

11a·Fumarate: M.p. 169–172°. $[\alpha]_D = -30.8$ ($c = 0.68$, EtOH). Anal. calc. for $C_{19}H_{26}N_2O_6$ (378.43): C 60.30, H 6.93, N 7.40; found: C 60.46, H 7.02, N 7.34.

(±)-3-[2'-(Dimethylamino)ethyl]-1,3-dihydro-5-methoxy-1,3-dimethyl-2H-indol-2-one (**11**). Oxindole **12** (2.637 g, 13.8 mmol) was dissolved in dry toluene (140 ml), and $NaNH_2$ (1.615 g, 41.4 mmol) was added. The mixture was stirred at r.t. under N_2 for 1 min, then 2-chloro-*N*,*N*-dimethylethanamine hydrochloride (3.972 g, 27.6 mmol) was added. The mixture was refluxed with stirring under N_2 for 1 h, cooled to r.t., and extracted with 1N HCl (2 × 60 ml) and the aq. soln. washed with Et_2O (2 × 50 ml), basified with 50% NaOH soln., and extracted with Et_2O (3 × 60 ml). The combined Et_2O layers were washed with brine (80 ml), dried (Na_2SO_4), and evaporated:

2.943 g of brown oil. Chromatography (CH_2Cl_2/MeOH 20:1) gave 2.60 g (73%) of light yellow oil. IR, MS, ^{1}H-NMR: identical with those of **11a** as described above.

*(±)-3-[2'-(Dimethylamino)ethyl]-2,3-dihydro-5-methoxy-1,3-dimethyl-1*H*-indol-2-ol* (**8**) *and Its Fumarate* **9** *and Methiodide* (**10**). To a soln. of **11** (859 mg, 3.27 mmol) in dry toluene (20 ml), *Vitride* (0.92 ml, 3.27 mmol) was added. The mixture was stirred at r.t. under N_2 for 1 h and then extracted with 2N HCl (2 × 20 ml). The aq. soln. was washed with Et_2O (2 × 30 ml), basified with 50% NaOH soln., and extracted with Et_2O (3 × 30 ml). The combined Et_2O layers were washed with brine (50 ml), dried (Na_2SO_4), and evaporated: 889 mg of crude **8** as a brown oil. The oil was dissolved in Et_2O (20 ml) and a sat. EtOH soln. of fumaric acid (390 mg, 3.36 mmol) added to give 1.055 g (89%) of **9** as light yellow crystals. A small amount of 2,3-dihydro-1*H*-indole **13** (compared with a standard sample on TLC) remained in the mother liquor. Pure **8**, a colorless oil, was obtained from **9** and converted to **10** quantitatively as hygroscopic powder. Spectra of **8**: identical with those of **8a** (see above).

Chemical Resolution of **8**. Crude **8** (311 mg, 1.18 mmol) was dissolved in Et_2O (50 ml) and (+)-2,3-di-*O*-(*p*-toluoyl)-D-tartaric acid (455 mg, 1.18 mmol) in Et_2O (10 ml) added dropwise. The salt was collected by filtration and recrystallized from EtOH (7 ml) to give white powder (306 mg) as the 1st crop. The mother liquor was concentrated to 3 ml to give colorless prisms (265 mg) as the 2nd crop. The 1st crop was the (3a*S*)-**8**·di-*O*-(*p*-toluoyl)-D-tartrate. After 1 recrystallization from EtOH, it gave the pure salt. Total yield 32%. M.p. 135–137°. $[\alpha]_D = +9.7$ ($c = 0.42$ MeOH). Anal. calc for $C_{35}H_{40}N_2O_9 \cdot H_2O$ (650.73): C 64.60, H 6.51, N 4.31; found: C 64.45, H 6.47, N 4.33.

The 2nd crop was impure (3a*R*)-**8**·di-*O*-(*p*-toluoyl)-D-tartrate. After 2 recrystallization; from EtOH, it gave a salt containing 5% of (3a*S*)-**8** and 95% of (3a*R*)-**8** (e.e. 90%). Total yield 33%. M.p. 131–133°. $[\alpha]_D = +154$ ($c = 0.40$, MeOH). Anal. calc. for $C_{35}H_{40}N_2O_9 \cdot 0.5\,H_2O$ (641.72): C 65.50, H 6.44, N 4.37; found: C 65.54, H 6.66, N 4.26.

Pure (3a*R*)-**8** (= **8b**) was obtained by converting the salt to free base on addition of 5% NaOH and extraction with Et_2O and then converting the free base to its 2,3-di-*O*-(*p*-toluoyl)-L-tartrate and recrystallizing the salt from EtOH. M.p. 135–137°. $[\alpha]_D = -10.6$ ($c = 0.39$, MeOH).

The free bases **8a** and **8b** were obtained on addition of 10% NaOH to the salts and extraction with Et_2O. The optical purity was analyzed directly by HPLC on a *Chiralcel OD* column[3]).

*(±)-3,3a,8,8a-Tetrahydro-5-methoxy-3a,8-dimethyl-2*H*-furo[2,3-b]indole* (= *5-O-Methylphysovenol;* **14**). To a soln. of **10** (341 mg, 0.84 mmol) in H_2O (10 ml), 7N NaOH (1 ml) was added. The mixture was refluxed under N_2 for 4 h. After cooling to r.t., the aq. mixture and the condensor washing (Et_2O) were extracted with Et_2O (2 × 10 ml). The combined Et_2O layers were dried (Na_2SO_4) and evaporated: 108 mg (58.6%) of **14**. Colorless syrup. Spectra: identical with those of **14a** [3].

Optically active **10a**, on similar treatment, afforded **14a**.

*(±)-3,3a,8,8a-Tetrahydro-5-methoxy-3a,8-dimethyl-2*H*-thieno[2,3-b]indole* (= *5-O-Methyl-1-thiaphysovenol;* **15**). To a soln. of **10** (406 mg, 1.0 mmol) in H_2O (8 ml), 7N NaSH (3 ml) was added. The mixture was refluxed under N_2 for 24 h. After cooling to r.t., the mixture was extracted with Et_2O (3 × 10 ml). The combined Et_2O layers were washed with 10% citric acid (3 × 8 ml) and brine (10 ml), dried (Na_2SO_4), and evaporated: 180 mg of light yellow syrup. Chromatography on a short column (silica gel, hexane/Et_2O 3:1) gave 154 mg (65%) of **15**. Light yellow syrup. Spectra: identical with those of **15a** [4].

Optically active **10a**, on similar treatment, afforded **15a**.

(±)-1-Benzyl-1,2,3,3a,8,8a-hexahydro-5-methoxy-3a,8-dimethylpyrrolo[2,3-b]indole (= *1-Benzyl-1-demethylesermethole;* **16**). To a soln. of **10** (1.77 g, 4.35 mmol) in MeCN (12 ml), benzylamine (932 mg, 0.95 ml, 8.7 mmol) was added. The mixture was refluxed under N_2 for 5 h. After cooling to r.t., the mixture was poured to H_2O (25 ml) and extracted with Et_2O (2 × 40 ml), the combined Et_2O layer washed with brine (50 ml), dried (Na_2SO_4), and evaporated, and the residue chromatographed on a short column (silica gel, hexane/Et_2O 6:1): 733 mg (55%) of **16**. Colorless syrup. Spectra: identical with those of **16a** [15].

Optically active **10a** or **10b**, on similar treatment, afforded **16a** or **16b**, resp.

(±)-1,2,3,3a,8,8a-Hexahydro-5-methoxy-1,3a,8-trimethylpyrrolo[2,3-b]indole (= *Esermethole;* **6**). A soln. of **10** (131 mg, 0.32 mmol) in MeCN (2 ml) and 40% $MeNH_2/H_2O$ (2 ml) was heated (oil-bath, 100°) under Ar in a sealed tube for 24 h. After cooling to r.t., H_2O (5 ml) was added, the mixture extracted with Et_2O (3 × 5 ml), and the combined Et_2O layer washed with brine (10 ml), dried (Na_2SO_4), and evaporated: 60 mg (81%) of **6**. Light yellow syrup. Spectra: identical with those of **6a**.

Optically active **10a** or **10b**, on similar treatment, afforded **6a** or **6b**, resp.

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[3]) Details on the conditions for the HPLC analysis will be published elsewhere.

(±)-1,2,3,3a,8,8a-Hexahydro-1,3a,8-trimethylpyrrolo[2,3-b]indol-5-ol (= *Eseroline*; **5**). Prepared from **6** as described in [18]. Spectra: identical with those of **5b** [13].

Optically active **6b**, on similar treatment, gave **5b**.

(±)-1,2,3,3a,8,8a-Hexahydro-1,3a,8-trimethylpyrrolo[2,3-b]indol-5-yl N-*Phenylcarbamate* (= *(±)-(Phenylcarbamoyl)eseroline* = *Phenserine*; **4**). Prepared from **5** as described for the preparation of **4a** from **5a** [17]. Spectra: identical with those of **4a** [17].

Optically active **5b**, on similar treatment, gave **4b**.

(±)-1-Benzyl-1,2,3,3a,8,8a-hexahydro-3a,8-dimethylpyrrolo[2,3-b]indol-5-ol (= *1-Benzyl-1-demethyleseroline*; **17**). Prepared from **16** as its (–)-enantiomer described in [15]. Spectra: identical with those of **17a** [17].

Optically active **16b**, on similar treatment, gave **17b**.

(±)-1-Benzyl-1,2,3,3a,8,8a-hexahydro-3a,8-dimethylpyrrolo[2,3-b]indol-5-yl N-*Phenylcarbamate* (= *(±)-1-Benzyl-5-O-(phenylcarbamoyl)-1-demethyleseroline* = *1-Benzyl-1-demethylphenserine*; **18**). Prepared from **17** as described for the preparation of **18a** [17]. Spectra: identical with those of **18a** [17].

Optically active **17b**, on similar treatment, gave **18b**.

(±)-1,2,3,3a,8,8a-Hexahydro-3a,8-dimethylpyrrolo[2,3-b]indol-5-yl N-*Phenylcarbamate* (= *(±)-5-O-(Phenylcarbamoyl)-1-demethyleseroline* = *1-Demethylphenserine*, **19**). Prepared from **18** as described for the preparation of **19a** [17]. Spectra: identical with those of **19a** [17].

Optically active **18b**, on the same treatment, afforded **19b**.

Crystal-Structure Determination of **8a** *and* **9a**. Data collection and refinement parameters are given in *Table 1*. For both compounds, the intensities of three standard reflections, which were repeated after every 97 reflections, remained stable throughout the data collection. *Lorentz* and polarization, but not absorption, corrections were applied to the intensities. Both structures were refined using full-matrix least-squares on intensities using all the unique data. Coordinates and anisotropic thermal parameters were refined for all non-H-atoms. Coordinates for OH H-atoms in both molecules were refined. H-Atoms on the H_2O molecule in **9a** were fixed at an O–H distance of 0.85 Å. All other H-atoms were included using a riding model (coordinate shifts of C applied to attached H-atoms, C–H distance set to 0.96 Å, H angles idealized). Isotropic thermal parameters for all H-atoms were set to 1.1 U_{eq} (C) or, if Me, OH, or H_2O, to 1.2 U_{eq} (C or O). Corrections were applied for secondary extinction. There were no significant features in the final difference maps. It was not possible to determine the absolute configuration of **8a** or of **9a** from the X-ray intensities; therefore, in each case, the enantiomorph was chosen based on the known configuration of the reaction starting materials. Tables of coordinates and bond lengths and angles were deposited with the *Cambridge Crystallographic Data Centre*, 12 Union Road, Cambridge, CB2 1EZ, England.

Biological Assay. Freshly collected blood was centrifuged (6000*xg*, 10 min, at 4°), the plasma was separated and diluted 1:125 with 0.1M Na_3PO_4 (pH 7.4). Erythrocytes were washed 3 times in isotonic saline, lysed by the addition of 9 volumes of 0.1M Na_3PO_4, which contained 0.5% *Triton-X* (*Sigma Chemical Co.*, St Louis, MO) (pH 7.4 on ice for 30 min). This then was diluted with 19 volumes of 0.1M 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, resp. For each cholinesterase preparation, 25 µl of substrate and 25 µl of enzyme were added separately to a final incubation volume of 0.75 ml.

All compounds initially were dissolved in *Tween 80*/EtOH 3:1 (*v/v*, 75 µl total volume) and were then diluted with 0.1M Na_3PO_4, (pH 8.0) in half-log intervals to a final concentration range of between $1 \cdot 10^{-5}$ M and $0.3 \cdot 10^{-9}$ M, and were preincubated with enzyme (30 min at 21°) prior to addition of substrates. 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°, the absorbance of a yellow thionitrobenzoate anion product was measured with a spectrophotometer set to 412 nm wavelength. Nonspecific substrate hydrolysis was determined under condition of complete enzyme inhibition (by the addition of excess physostigmine, $1 \cdot 10^{-5}$ M), and the associated change in absorbance was subtracted from that observed with compounds measured. Furthermore, the activity of each compound was assessed alongside that of physostigmine, as an external standard, whose activity we reported previously [3] [7].

For determination of IC_{50} value of 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* values (*i.e.*, logit = ln(50/[100 – 50]) = 0) were determined only when correlation coefficients of less than –0.985 were achieved, and when more than 50% inhibition was achieved from duplicate samples. Each compound was analyzed on at least 4 occasions, in duplicate. A two-tailed *Student*'s *t*-test was performed to compare two

means [21]; when more than two means were compared, one-way analysis of variance and the *Bonferroni* multiple *t*-test were used [21]. Statistical significance was taken at the level of $P < 0.05$. The IC_{50} values are listed in *Table 2*.

REFERENCES

[1] S. Takano, K. Ogasawara, *The Alkaloids* **1989,** *36,* 225, and ref. cit. therein.
[2] J. A. Gearien, 'Medicinal Chemistry, Part II', 3rd edn., Ed. A. Burger, Wiley-Interscience, New York, 1970, p. 1296–1313.
[3] Q. S. Yu, C. Liu, M. Brzostowska, L. Chrisey, A. Brossi, N. H. Greig, J. R. Atack, T. S. Soncrant, S. I. Rapoport, H. E. Radunz, *Helv. Chim. Acta* **1991,** *74,* 761.
[4] X. S. He, N. H. Greig, S. I. Rapoport, A. Brossi, Y. Q. Li, Q. S. Yu, *Med. Chem. Res.* **1992,** *2,* 229.
[5] M. Polonovski, *Bull. Soc. Chim. Fr.* **1916,** *19,* 47, and summarized in 'Beilsteins Handbuch der Organischen Chemie', 2. Ergänzungswerk, Springer-Verlag, Berlin, 1954, Vol. 23, p. 333.
[6] S. Iijima, N. H. Greig, P. Garofalo, E. L. Spangler, B. Heller, A. Brossi, D. K. Ingram, *Psychopharmacology* **1993,** in press.
[7] J. R. Atack, Q. S. Yu, T. S. Soncrant, A. Brossi, and S. I. Rapoport, *J. Pharmacol. Exp. Ther.* **1989,** *249,* 194.
[8] F. J. Dale, B. Robinson, *J. Pharm. Pharmacol.* **1970,** *22,* 889, and ref. cit. therein.
[9] F. E. King, R. Robinson, *J. Chem. Soc.* **1932,** 326.
[10] P. L. Julian, J. J. Pikl, *J. Am. Chem. Soc.* **1935,** *57,* 755.
[11] B. Y. Lu, Q. S. Yu, X. F. Pei, *Heterocycles* **1994,** in press.
[12] E. C. Horning, M. W. Rutenberg, *J. Am. Chem. Soc.* **1950,** *72,* 3534.
[13] Q. S. Yu, A. Brossi, *Heterocycles* **1988,** *27,* 745.
[14] Y. Luo, Q. S. Yu, L. Chrisey, A. Brossi, *Heterocycles* **1990,** *31,* 283.
[15] Q. S. Yu, J. R. Atack, S. I. Rapoport, A. Brossi, *J. Med. Chem.* **1988,** *31,* 2297.
[16] Q. S. Yu, W. Luo, Y. Q. Li, A. Brossi, *Heterocycles* **1993,** *36,* 1279.
[17] M. Brzostowska, X. S. He, N. H. Greig, S. I. Rapoport, A. Brossi, *Med. Chem. Res.* **1992,** *2,* 238.
[18] G. M. Sheldrick, *Acta Crystallogr., Sect. A* **1990,** *46,* 467, 'SHELXS-86'.
[19] G. M. Sheldrick, submitted to *J. Appl. Crystallogr.*
[20] G. L. Ellman, K. D. Courtney, V. Jr. Andres, R. M. Featherstone, *Biochem. Pharmacol.* **1961,** *8,* 88.
[21] R. Miller, 'Simultaneous Statistical Inferences', McGraw-Hill, New York, 1966, p. 76.