PRACTICAL SYNTHESIS OF UNNATURAL (+)-PHYSOSTIGMINE AND CARBAMATE ANALOGUES

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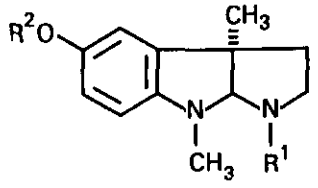
Abstract - Details of a synthesis of unnatural (+)-physostigmine (5) prepared from urea 1 via (+)- eseroline (4) are given. Preparation of (+)-octylcarbamate 6, (+)-benzylcarbamate 7, (+)-phenylcarbamate 8 and (+)-N-methylphysostigmine (9) from (+)-eseroline (4) is also described.

(+)-Physostigmine (5), the unnatural antipode of the alkaloid (-)-physostigmine from Calabar beans[1] inhibits acetylcholinesterase in vitro more than 100 times less than the natural alkaloid[2], but blocks the open channel of the nicotinic acetylcholine receptor similarly.[3] Several analogs of (-)-physostigmine with different carbamate side chains showed even higher inhibitory potency of cholinesterases and are presently being further evaluated[4]. These findings mark Calabar alkaloids as very interesting biochemical tools and an efficient chemical synthesis of compounds of the unnatural (+)-series was therefore, highly desirable. This seemed particularly warranted since the original synthesis of 5 was only accomplished by a tedious separation of optical isomers at an intermediate stage, affording optically pure 5 in low yield only.[5] Urea 1, obtained as the faster moving diastereomer after reaction of (±)-N1-O-methylnoreseroline with S-(-)-1-phenylethyl-isocyanate (silica gel, CH_2Cl_2/MeOH)[6], and converted into 2 in refluxing butanol in the presence of sodium butoxide, represents a key intermediate in our synthesis of (+)-physostigmine (5) and carbamate analogues. Although preparation of (+)-eseroline (4) from 1 was reported[7], the present procedure using pentanol instead of butanol and isolating intermediates as fumarate salts, constitutes a much superior method to prepare these compounds and is, therefore, presented in detail. Decomposition of urea 1 in refluxing pentanol in the presence of sodium pentoxide prepared in situ, afforded the fumarate salt of 2 in high yield. Reductive N-methylation of the fumarate salt of 2 with

formaldehyde and sodium borohydride afforded the fumarate of 3 in 62% yield. O-Demethylation of 3 with boron tribromide afforded 4 (85%). (+)-Eseroline (4) prepared from its fumarate salt in the usual way ($NaHCO_3/Et_2O$) afforded by reaction with methylisocyanate 5 isolated as the salicylate (74%). The free base of 5 prepared from its salicylate was in every respect identical to natural (-)-physostigmine except for its opposite optical behavior.[8] Reaction of 4 with octylisocyanate, benzylisocyanate and phenylisocyanate afforded carbamates 6, 7 and 8 respectively and (+)-N-methylphysostigmine (9) was obtained from 4 by reaction with dimethylcarbamoyl chloride by the procedure given for preparing its (-)-enantiomer.[9]



	R^1	R^2
1,	CH3 ▼ CONHCHPh	CH_3
2,	H	CH_3
3,	CH_3	CH_3
4,	CH_3	H
5,	CH_3	$CONHCH_3$
6,	CH_3	$CONH(CH_2)_7CH_3$
7,	CH_3	$CONHCH_2C_6H_5$
8,	CH_3	CONHPh
9,	CH_3	$CON(CH_3)_2$

EXPERIMENTAL

Melting points were determined on a Fisher-Johns melting point apparatus, and optical rotations were measured on a Perkin-Elmer 241 MC polarimeter. ^{1}H-nmr spectra were measured on a Varian XL-300 (300 MHz) spectrometer, and chemical shifts are reported in δ with tetramethylsilane as the internal reference. Mass spectra were taken on a Finnigan 1015 D instrument (C). Flash column chromatography was done with grade 60, 60 Å silica gel (Merck) (from Aldrich Chemical Company, Inc.)

(+)-N(1)-O-Methylnoreseroline (2): Sodium (8 g) was dissolved in pentanol (350 ml), and after its disappearance urea 1 (7.86 g, 21.5 mmol) added, and the reaction mixture refluxed for 2 h in nitrogen atomosphere. After evaporation of the solvent in vacuo the residue was dissolved in H_2O (150 ml) and extracted with Et_2O (2 x 200 and 2 x 100 ml). The ether extracts were combined, washed with brine (50 ml), dried (Na_2SO_4) and concentrated. A saturated ethanolic solution of fumaric acid (3.0 g) was added to give the fumarate salt of 2 (6.78 g, 93.4%): mp 199-200°C; $[\alpha]_D$+73.0°(c=0.5, MeOH); ^{1}H-nmr(D_2O), 1.47(s, 3H, C1O-CH_3), 2.18-2.47(m, 2H, C3-H_2), 2.99(s, 3H, N8-CH_3), 2.92-3.48(m, 2H, C2-H_2), 3.79(s, 3H, O-CH_3), 5.11(s, 1H, C9-H), 6.60-6.64(m, 1H, C7-H), 6.87-6.97(m, 2H, C4-H and C6-H); ms(CI), m/z 219 (M^++1). Anal. Calc. for $C_{13}H_{18}N_2O.C_4H_4O_4$: C,61.06; H,6.63; N,8.38. Found: C,61.01; H,6.64; N,8.32.

(+)-O-Methyleseroline (3): The fumarate salt of 2 (6.48 g, 19.38 mmol) was dissolved in MeOH (100 ml) and Et_3N (6.48 ml) and CH_2O (9.72 ml) added. The reaction mixture was stirred for 3h at room temperature in nitrogen atmosphere, then cooled to 0°C and $NaBH_4$ (2.92 g) slowly added and the mixture stirred for 0.5h at room temperature. After evaporation of the solvent, HCl solution (2 M) was added to dissolve solid borane complexes. The acidic aqueous solution was washed once with Et_2O (30 ml), made basic with saturated Na_2CO_3 solution, then extracted with ether (2 x 100, 2 x 50). The ether solutions were combined, washed with brine (50 ml) and dried over Na_2SO_4. After evaporation of the solvent, the concentrated residue was added to a saturated alcoholic solution of fumaric acid (2.70 g), and left overnight in the refrigerator to give the fumarate salt of 3 (5.13 g, 62.10%): mp 137-138°C; $[\alpha]_D$+98.7°(c=0.8, MeOH); ^{1}H-nmr(D_2O), 1.49(s, 3H, C1O-CH_3), 2.39 (m, 2H, C3-H_2), 2.85(s, 3H, N1-CH_3), 3.09(s, 3H, N8-CH_3), 3.20-3.60(m, 2H, C2-CH_2). 3.79(s, 3H, O-CH_3), 5.09(s. 1H, C9-H), 6.67(m, 1H, C7-H), 6.87-6.95(m, 2H, C4-H and C6-H); ms(CI), m/z 232(M^++1). Anal. Calc. for $C_{14}H_{20}N_2O.\ 1\frac{1}{2}C_4H_4O_4$: C,59.11; H,6.45; N,6.89. Found: C,59.40; H,6.62; N,6.89.

(+)-Eseroline (4): The fumarate salt of 3 (4.58 g, 11.29 mmol) was dissolved in H_2O (20 ml), basified by $NaHCO_3$ solution, extracted with Et_2O(2x100/and 2x50 ml). After washing with brine (20 ml), the extract was dried by Na_2SO_4 to give, after evaporation of the ether 3. Base 3 was dissolved in CH_2Cl_2 (50 ml) and BBr_3 (99.99%, 5ml) dissolved in CH_2Cl_2(50 ml) was added dropwise to the above solution with stirring. Stirring was continued for 2 h at room temperature in nitrogen atmosphere. After evaporation of solvent, the residue was dissolved in MeOH (20 ml) and stirred for 10 min. Evaporation of the MeOH gave a residue which was dissolved in H_2O (20 ml), basified with a saturated aqueous solution of $NaHCO_3$, extracted by

Et_2O (2x200+50 ml), washed by brine (50 ml) and dried over Na_2SO_4. Evaporation of Et_2O gave a brown oily base 2 which crystallized after staying overnight in the refrigerator. Washing the crystals with cold Et_2O gave crystalline 4 (2.23 g, 85.02%): mp 125-126°C; $[\alpha]_D$ + 112°(c=0.45, MeOH). Anal. Calc. for $C_{13}H_{18}N_2O$: C,71.52; H,8.31; N,12.84. Found: C,71.44; H,8.36; N,12.70. Fumarate Salt: Crystalline base 4 (100 mg) was dissolved in Et_2O(5 ml), then added to a saturated alcohol solution of fumaric acid (63.8 mg) to afford the fumarate of 4 (150 mg, 98.0%): mp 200-202°C; $[\alpha]_D$ +103.0° (c=0.3, MeOH); ^{1}H-nmr(D_2O),1.48(s, 3H, C10-CH_3), 2.17-2.47(m, 2H, C3-H_2), 2.80(s, 3H, N1-CH_3), 3.07(s, 3H, N8-CH_3), 3.24-3.52(m, 2H, C2-H_2), 5.05(s, 1H, C9-H), 6.63(m, 1H, C7-H), 6.77-6.80(m, 2H, C4-H and C6-H); ms(CI), m/z 219(M^++1). Anal. Calc. for $C_{13}H_{18}N_2O \cdot C_4H_4O_4$: C,61.06; H, 6.63; N,8.38. Found: C,60.98; H,6.67; N,8.37.

(+)-Physostigmine (5): (+)-Eseroline (4) (500 mg, 2.30 mmol) was dissolved in anhydrous Et_2O (50 ml) and a small piece of sodium (about 5 mg) added. The resulting solution was stirred for 1.5 min. at room temperature in nitrogen atmosphere. Methylisocyanate (156 mg, 2.74 mmol) was added dropwise and stirring continued for 5 min. The solvent was moved in vacuo and the residue dissolved in Et_2O (50 ml), washed by brine (20 ml) and dried over Na_2SO_4. The residue obtained after evaporation of ether was added to an ether solution (10 ml) of salicylic acid (378 mg) to give a white precipitate which was crystallized from EtOH to give crystalline salicylate of 5 (720 mg, 74.35%): mp 183-184°C; $[\alpha]_D$ +75.0°(c=0.5, EtOH)).(+) Physostigmine (5) prepared from the salicylate salt in the usual way and crystallized from ether: mp 84-85°C; $[\alpha]_D$ + 75.0° (c=0.5,$CHCl_3$); ir, ^{1}H-nmr, ms: identical with those of (-) physostigmine.

(+)-Octylcarbamoyleseroline (6): (+)-Eseroline (4) (150 mg, 0.687 mmol) was dissolved in anhydrous Et_2O(15 ml) and a small piece of sodium (4) (about 5 mg) added. After stirring for 1.5 min. at room temperature in nitrogen atmosphere, octylisocyanate (128 mg, 0.826 mmol) was added dropwise. After the addition was complete, the solvent was evaporated immediately. The residue was flash chromatographed on a silica gel column (CH_2Cl_2/MeOH, 100:1-100:2) to give 6 as an oil (160 mg, 62.0%): $[\alpha]_D$+53.2°(c=1.2, $CHCl_3$); ^{1}H-nmr($CDCl_3$), 0.88(t, 3H, J=7, terminal CH_3), 1.28-1.60(m, 12H, -(CH_2)-), 1.42(s, 3H, C10-CH_3), 1.94(m, 2H, C3-H_2), 2.53(s, 3H, N1-CH_3), 2.60-2.73(m, 2H, C2-H_2), 2.91(s, 3H, N8-CH_3), 3.25(m, 2H, N-CH_2-), 4.11(s, 1H, C9-H), 6.32(d, 1H, J=8, C7-H), 6.75-6.81(m, 2H, C6-H and C4-H); ms(CI), m/z 374 (M^++1). Anal. Calc. for $C_{22}H_{35}N_3O_2$: C.70.78;H.9.45;N,11.26. Found:C,70.74;H,9.50;N,11.21.

(+)-Benzylcarbamoyleseroline (7): Similarly prepared from (+)-eseroline (4) with benzylisocyanate (EtOAc,41.4%): mp 84-85°C; $[\alpha]_D$ +56.9°(c=0.5, $CHCl_3$); ^{1}H-nmr ($CDCl_3$), 1.42(s, 3H, C10-CH_3), 1.91-1.96(m, 2H, C3-H_2), 2.54(s, 3H, N1-CH_3), 2.60-2.75(m, 2H,C2-H_2), 2.91(s, 3H, N8-CH_3), 4.10(s, 1H, C9-H), 4.40(d, 2H, J=4, Ph-CH_2-), 6.34(d,1H, J=8, C7-H), 6.78-6.84(m, 2H, C4-H and C5-H), 7.30-7.35(m, 5H, Aromatic H); ms(CI), m/z 352 (M^++1). Anal. Calc. for $C_{21}H_{25}N_3O_2$: C,71.76; H,7.17; N,11.96: Found. C,71.89; H,7.20; N,11.91.

(+)-Phenylcarbamoyleseroline (8): Similarly prepared from (+)-eseroline (4) with phenylisocyanate (EtOAc, 64.7%): mp 140-142°C; $[\alpha]_D$ + 69.8° (c=0.5, $CHCl_3$), ^{1}H-nmr ($CDCl_3$), 1.44(s, 3H, C10-CH_3), 1.95 (m, 2H, N8-CH_3), 2.55 (s, 3H, N1-CH_3, 2.70 (m, 2H, C2-H_2), 2.93(s, 3H, N8-CH_3), 4.13 (s, 1H, 69-H), 6.36 (d, 1H, J=7, (7-H), 6.85 (m, 2H, C4-H and C6-H), 7.06-7.45 (m, 5H, Aromatic H); ms (CI), m/z 338 (M^++1). Anal. Calc. for $C_{20}H_{23}N_3O_2$: C,71,19; H, 6.87; N, 12.46; Found: C, 71.12; H, 6.92; N, 12.43.

(+)-N-Methylphysostigmine (9): Prepared from (+)-eseroline by reaction with dimethylcarbamoyl chloride in the presence of pyridine as described for the (-)-enantiomer[9] (62.9%): The gum-like material was crystallized from ether-hexane (62.9%: mp 73-75°C; $[\alpha]_D$+76.1° (c=0.5, $CHCl_3$); ^{1}H-nmr ($CDCl_3$), 1.43(s, 3H, C10-H), 1.92-1.97(m, 2H, C3-H_2), 2.54(s, 3H, N1-CH_3), 2.59-2.76(m, 2H, C2-H), 2.91(s, 3H, N8-CH_3), 2.99(s, 3H, N-CH_3), 3.07(s, 3H, N-CH_3'), 4.12(s, 1H, C9-H), 6.34(d, 1H, J=8, C7-H), 6.75-6.80(m, 2H, C4-H and C6-H); ms(CI), m/z 290 (M^++1). Anal. Calc. for $C_{16}H_{23}N_3O_2$: C,66.41; H,8.01; N,14.51. Found: C,66.51; H,8.05; N,14.51.

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