α-Phenyl-β-(3,4-dimethoxy)phenethylamines: Novel Inhibitors of Choline Acetyltransferase from *Torpedo* Electric Organ

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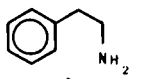
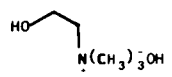
Abstract □ Some derivatives of α-phenyl-β-(3,4-dimethoxy)phenethylamine that might bear a certain conformational resemblance to choline were prepared. The *in vitro* inhibition of choline acetyltransferase from *Torpedo* electric organ was investigated. These compounds gave variable degrees of inhibition; the most potent inhibitor was, *N,N,N*-trimethyl-α-phenyl-β-(3,4-dimethoxy)phenethylammonium iodide, with an I_{50} of 1.3×10^{-5} M. The inhibition of choline acetyltransferase from *Spodoptera littoralis* larval brains was also determined for comparative study. The aforementioned compound has an I_{50} of 9×10^{-6} M on choline acetyltransferase from this source.

Keyphrases □ α - Phenyl -β- (3,4 - dimethoxy)phenethylamines—synthesis, inhibition of choline acetyltransferase □ Choline acetyltransferase—inhibition, α-phenyl-β-(3,4-dimethoxy)phenethylamines

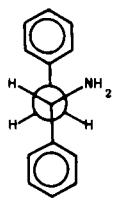
The phenethylamine (I) skeleton is an integral moiety for many naturally occuring compounds which possess remarkable biological properties, *e.g.*, morphine (1), ephedrine (2), epinephrine (3), norepinephrine (3), and papaverine (4, 5). The structural feature of choline base (II), the β-hydroxy ethylamine derivative, bears some conformational resemblance to I. It has been shown (6) that the most favorable conformation of II, at physiological pH, is the *gauche* disposition (IIa). The latter might be the most suitable conformer for interaction with the choline acetyltransferase (E.C. 2.3.1.6) surface prior to acetylation (7, 8). A bioisosteric *gauche* arrangement (Ia) would be assured by introducing a phenyl group in the α-position. This would also increase lipophilicity. Therefore, we prepared some derivatives (III) of α-phenyl-β-(3,4-dimethoxy)phenethylamine and evaluated them as inhibitors of choline acetyltransferase.



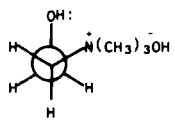
I



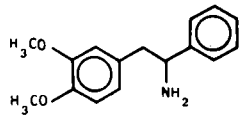
II



Ia



IIa



III

Choline acetyltransferase catalyzes the synthesis of acetylcholine, and both O'Brien (9) and Yu and Booth (10) recognized the potential of this enzyme as a target for new drugs and insecticides. For biochemical studies, choline acetyltransferase from *Torpedo* electric organ was used, and the results were compared with those recorded for choline acetyltransferase from *Spodoptera littoralis* larval brains.

Few potent inhibitors of choline acetyltransferase are known, and those that have been tested have not proved useful as insecticides (10). Other reports dealing with mammalian inhibitors of this enzyme have been published, *e.g.*, 5-hydroxy-1,4-naphthalenedione and 4-(1-naphthylvinyl)pyridine (11), halogenoacetylcholines (12), and (2-benzoylethyl)trimethylammonium chloride (13, 14). Most of these compounds are either esters or aromatic ketones which are relatively unstable (12, 14). On the other hand the compounds described here are neither esters nor ketones. Some clear differences existed between the choline acetyltransferases of mites, houseflies, and rats toward their substrate specificity (9). It was also reported that quite marked variation in sensitivity between insect and mouse choline acetyltransferases were displayed when both enzymes were tested against known inhibitors, such as the styrylpyridines (10).

The synthetic approach followed is outlined in Scheme I. 3,4-Dimethoxy-α-nitrostilbene (VI) was prepared using a modification of the method described by Owari (15). α-Phenyl-β-(3,4-dimethoxy)phenethylamine (III) was obtained by reduction of VI with lithium aluminum hydride in ether. The *N*-formyl derivative (VIIa) was obtained from III using the method of Sam *et al.* (16). Treatment of III with ethyl chloroformate in the presence of pyridine afforded the *N*-ethoxycarbonyl derivative (VIIb). The *N*-methyl derivative (VIII) was prepared either from VIIa or VIIb by reduction with lithium aluminum hydride in tetrahydrofuran or ether, respectively. The *N,N*-dimethyl derivative (IX) was obtained by applying an analogous procedure reported by Shafik *et al.* (5) for the preparation of similar compounds. The quaternarization of IX to give the trimethylammonium derivative (X) was carried out by using an excess of methyl iodide in ether.

EXPERIMENTAL SECTION[1]

3,4-Dimethoxy-α-nitrostilbene (VI)—To a solution of 16.6 g (0.1 mol) of veratraldehyde (IV) in 15 mL of methanol was added 13.7 g (0.1 mol) of phenylnitromethane (17) (V) and 0.5 mL of *n*-butylamine. The mixture was kept at room temperature for 72 h, and then the product was removed by filtration, dried, and recrystallized from ethanol to give 10.0 g (35% yield) of yellow needles, mp 105–107°C [lit (18) mp 109°C]; IR ν_{max}: 1365 and 1595 cm^{-1} (C—NO_2).

[1] Melting points were taken in open glass capillaries and are uncorrected. The IR spectra were scanned on a Beckman IR-4210 spectrophotometer with KBr pellets. The ^{1}H-NMR spectra were determined, for solutions in Me_2SO-d_6, on a Varian EM-390 NMR spectrometer, and are reported in δ values (ppm) relative to an internal standard of tetramethylsilane. Analyses were performed by members of the Microanalytical Unit, Faculty of Science, University of Cairo, Egypt.

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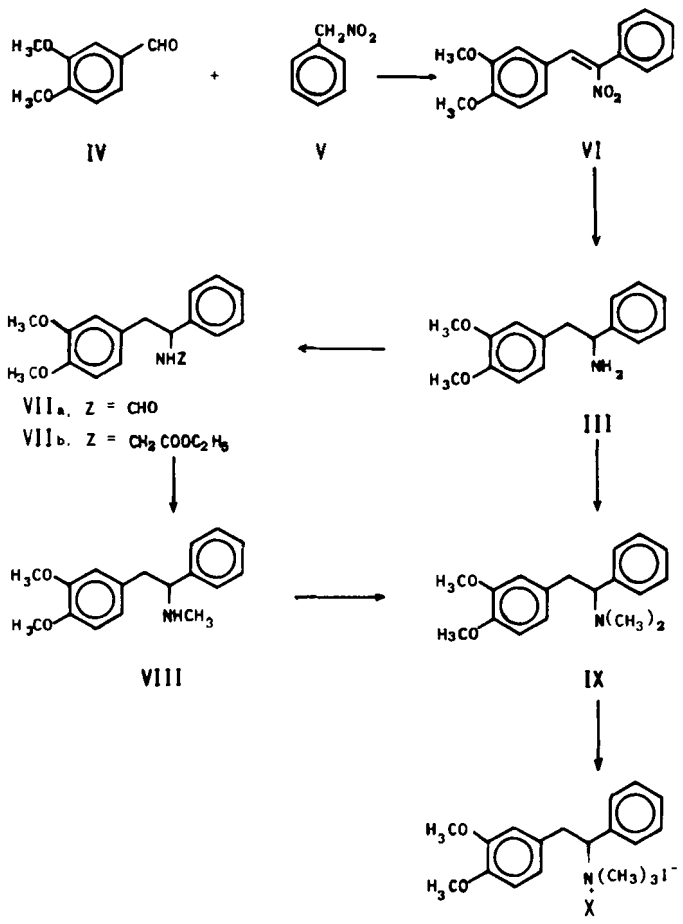

SCHEME I

α-Phenyl-β-(3,4-dimethoxy)phenethylamine (III)—To a stirred mixture of 1.9 g (0.05 mol) of lithium aluminum hydride and 300 mL of ether was added 2.8 g (0.01 mol) of VI. The mixture was refluxed for 72 h, and then chilled. The excess lithium aluminum hydride was destroyed with crushed ice and 20% aqueous NaOH, respectively. The product was isolated by ether extraction and was purified in the usual manner (5) by alternative pH adjustments to give 1.9 g (67% yield) of III as pale-yellow needles, mp 65°C [lit (15) mp 66–67°C]. The hydrochloride salt was obtained by passing dry hydrogen chloride into the ethereal solution of III to give white needles, which were recrystallized from methanol–ether to give 1.9 g (67% yield) of white crystals, mp 176–177°C [lit (19) mp 176–177°C]; IR ν_{max}: 2650 cm^{-1} (NH_3^+); ^{1}H-NMR (Me_2SO-d_6): δ 3.45 (s, 6), 3.55 (d, 2, J = 6 Hz), 4.4 (m, 1), 6.65 (m, 3, ArH), and 7.35 ppm (m, 5, ArH).

N-Formyl-α-phenyl-β-(3,4-dimethoxy)phenethylamine (VIIa)—A solution of 2.6 g (0.01 mol) of III in a mixture of 15 mL of 98% formic acid and 18 mL of acetic anhydride was refluxed for 5 h and then diluted with 100 mL of water. The mixture was extracted with ether and washed with 10% NaOH, then 10% HCl, and water. The ethereal extract was dried (Na_2SO_4), and the solvent was removed under reduced pressure. The oily residue that solidified on standing was recrystallized from benzene–petroleum ether (bp 40–60°C) to give 1.2 g (43% yield) of white crystals, mp 120–121°C; IR ν_{max}: 3400 (NH), 1650, 1550, and 1250 cm^{-1} (amide bands).

Anal.—Calc. for $C_{17}H_{19}NO_3$: C, 71.6; H, 6.6; N, 4.9. Found: C, 71.5; H, 6.2; N, 5.3.

N-Ethoxycarbonyl-α-phenyl-β-(3,4-dimethoxy)phenethylamine (VIIb)—A solution of 2.7 g (0.025 mol) of ethyl chloroformate in 10 mL of benzene was added, dropwise, to a cold, stirred solution of 6.2 g (0.024 mol) of III in 30 mL of pyridine. The mixture was stirred for an additional 3 h and then was refluxed for 4 h. The mixture was poured onto 25 mL of 10% HCl and extracted with benzene. The organic phase was washed with 2 M NaOH and water, and dried (Na_2SO_4). The solvent was removed under reduced pressure to give an oily residue that solidified on standing at room temperature. Recrystallization from benzene–petroleum ether (bp 40–60°C) gave 1.6 g (47% yield) of pale-yellow crystals of VIIb, mp 107°C; IR ν_{max}: 3450 (NH), 1670, 1550, and 1265 cm^{-1} (amide bands).

Anal.—Calc. for $C_{19}H_{23}NO_4$: C, 69.3; H, 7.0; N, 4.3. Found: C, 69.1; H, 7.4; N, 4.3.

N-Methyl-α-phenyl-β-(3,4-dimethoxy)phenethylamine Hydrochloride (VIII)—*Method A*—To a stirred mixture of 1.9 g (0.05 mol) of lithium aluminum hydride and 200 mL of tetrahydrofuran was added 2.8 g (0.01 mol) of VIIa. The mixture was heated at reflux for 16 h, and the solvent was removed under reduced pressure. The residue was chilled to give (as in the preparation of III) the hydrochloride salt, 1.7 g (62% yield) as white crystals, mp 164–165°C.

Anal.—Calc. for $C_{17}H_{22}ClNO_2$: C, 66.3; H, 7.2; Cl, 11.5; N, 4.6. Found: C, 66.5; H, 7.2; Cl, 11.4; N, 4.2.

Method B—To a stirred mixture of 1.9 g (0.05 mol) of lithium aluminum hydride and 300 mL of ether was added 3.3 g (0.01 mol) of VIIb. The mixture was heated at reflux for 14 h. The hydrochloride salt was recrystallized from methanol–ether to give 2.4 g (72% yield) as white crystals, mp 164–165°C. Admixture of the products obtained by both methods (A and B) showed no melting point depression.

N,N-Dimethyl-α-phenyl-β-(3,4-dimethoxy)phenethylamine Hydrochloride (IX)—A mixture of 0.01 mol of the hydrochloride salt of III or VIII, 2.3 g (0.05 mol) of formic acid (98%), and 0.66 g (0.022 mol) of 38% formaldehyde was refluxed for 12 h. The mixture was transferred to an evaporating dish and heated on a water bath until most of the unreacted formic acid and formaldehyde were removed. The syrupy residue was treated with 0.6 mL of formic acid (98%) and 0.2 mL of 38% formaldehyde and allowed to evaporate on the water bath until dryness. The last traces of water were removed by azeotropic distillation with absolute ethanol and benzene. The residual hydrochloride salt was recrystallized from methanol–ether to give 2.0 g (78% yield) as white crystals, mp 218–220°C; IR ν_{max}: 2600 cm^{-1} (NH^+); ^{1}H-NMR (Me_2SO-d_6): δ 2.90 (s, 6), 3.30 (s, 6), 3.80 (d, 2, J = 5 Hz), 4.80 (m, 1), 6.90 (d, 3, J = 9 Hz, ArH), 7.60 ppm (m, 5, ArH).

Anal.—Calc. for $C_{18}H_{24}ClNO_2$: C, 67.2; H, 7.5; Cl, 11.0; N, 4.4. Found: C, 67.4; H, 7.1; Cl, 10.6; N, 4.0.

N,N,N-Trimethyl-α-phenyl-β-(3,4-dimethoxy)phenethylammonium Iodide (X)—To a solution of 2.9 g (0.01 mol) of IX in 100 mL of ether was added 1.7 g (0.012 mol) of methyl iodide. The mixture was allowed to stand at room temperature for 24 h. The crystalline material was removed by filtration and recrystallized from methanol–ether to give 1.8 g (63% yield) of yellow crystals, mp 228–230°C; ^{1}H-NMR (Me_2SO-d_6): δ 3.40 (s, 9), 3.75 (s, 6), 4.50 (m, 2), 6.90 (s, 1), 7.50 (m, 3, ArH), and 7.80 ppm (m, 5, ArH).

Anal.—Calc. for $C_{19}H_{26}INO_2$: C, 53.43; H, 6.1; N, 3.3. Found: C, 53.7; H, 5.9; N, 3.6.

Biochemical Assays—Membranes were prepared from the electric organ of *Torpedo ocellata* (collected near Alexandria, Egypt, and stored frozen at −90°C for up to 6 months) by homogenization (50%, w/v) in ice-cold 10 mM EDTA–20 mM Na_2HPO_4 buffer (pH 7.4). An emulsifier[2] (0.2%, v/v) was added to the homogenate, and the suspension was shaken lightly by hand for few seconds, filtered through cheesecloth, and centrifuged at 100,000×g for 30 min. The supernatant was used as the enzyme source. Head capsules of fourth instar *Spodoptera littoralis* larvae were homogenized in the aforementioned buffer (1 mL/10 head capsules) using a similar procedure. Protein was assayed by the method of Lowry *et al.* (20).

The radiochemical method was used for the determination of choline acetyltransferase activity (21). The incubation mixture contained 0.2 mM [1-^{14}C]acetyl CoA[3] (50 mCi/mmol), 300 mM NaCl, 50 mM Na_2HPO_4 buffer (pH 7.4), 8 mM choline bromide, 20 mM EDTA, and 0.1 mM physostigmine. The labeled acetyl CoA was diluted with the unlabeled compound[4]. In the inhibition studies of choline acetyltransferase activity, the enzyme preparation was incubated with 10 μL of the inhibitor solution for different intervals.

RESULTS AND DISCUSSION

The radioactivity assay was used to find the acetyl CoA concentration in the incubation medium and different protein concentrations for optimal *Torpedo* electric organ choline acetyltransferase activity. A range of 0.1–0.2 mM acetyl CoA was necessary to give near-optimal activities. Such high acetyl CoA concentrations gave linear activity curves over a greater range, maintaining linearity up to 120 μg of enzyme in the incubation mixture. Under these conditions the enzyme-specific activities of six different preparations were 14.60 ± 0.16 (mean ± SD) and 6.64 ± 0.12 λ_{max} 412·mg^{-1} of protein·h^{-1} for *Torpedo* electric organ and *Spodoptera* larval brain preparations, respectively. The differences in membrane binding might be due to differences among the surface charges of choline acetyltransferase from the two species.

As can be seen from the structures of I and II, the quaternary ammonium derivative (X) was the most potent inhibitor in a micromolar range for choline

[2] Triton X-100; Rohm and Haas.
[3] The Radiochemical Center, Amersham, England.
[4] Sigma Chemical Co., St. Louis, Mo.

Table I—*In Vitro* Inhibition of Choline Acetyltransferase Activity from Two Sources by α-Phenyl-β-(3,4-dimethoxy)phenethylamine Derivatives

Compound[a]	Inhibition Constants			
	Torpedo		*Spodoptera*	
	I_{50}, M	K_i, μM	I_{50}, M	K_i, μM
III	7.0×10^{-5}	60	2.7×10^{-5}	40
VIII	5.8×10^{-5}	45	2.5×10^{-5}	32
IX	4.0×10^{-5}	35	1.8×10^{-5}	25
X	1.3×10^{-5}	10	9.0×10^{-6}	6

[a] The I_{50} values were compared with those determined for diisopropylfluorophosphonate ($I_{50} = 5 \times 10^{-4}$ M from both sources). Lit. (10) data reported for diisopropylfluorophosphonate using choline acetyltransferase (house fly or mouse): $I_{50} = 1 \times 10^{-4}$ M.

acetyltransferase from both sources. From the I_{50} and K_i values obtained, the *Spodoptera* larval enzymes were shown to be more sensitive toward the synthesized compounds (III and VIII–X) than the *Torpedo* electric organ enzymes. However, the best *in vitro* choline acetyltransferase inhibitor was the charged compound (X), whereas the uncharged compounds (III, VIII, and IX) were relatively poor inhibitors (Table I).

Several choline analogues ($SCNCH_2CH_2\overset{+}{N}R^1R^2R^3X^-$) were previously tested (22) on rats as possible *in vivo* choline acetyltransferase inhibitors; the data revealed poor correlation between the K_i value and the insecticidal activity of such compounds when tested against several insect species. The most likely explanation was that since the molecule is charged, it would not readily penetrate the nerve cord and reach the cells in which the enzyme is located. The present work was thus directed toward *in vitro* inhibition studies using some uncharged phenethylamines (III, VIII, and IX) to avoid the problems of compound penetration to the target. The quaternary analogue (X) was also investigated.

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Excitatory Amino Acid Receptor Interactions of a Novel α-Phosphinic Acid Analogue of α-Methylaspartic Acid

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Abstract □ An α-phosphino analogue of α-methylaspartate has been synthesized. The compound may not interact with excitatory amino acid receptors directly, as assessed by direct *in vitro* radioreceptor binding methods; however, it possesses weak anticonvulsant activity and exhibits an excitant action *in vitro* that is apparently not mediated by a *N*-methyl-D-aspartate receptor.

Keyphrases □ Excitatory amino acid receptors—*N*-methyl-D-aspartate, glutamate, kainate □ Anticonvulsants—*in vitro*, rats, excitatory amino acids □ Phosphinic acids—excitatory amino acid receptors, anticonvulsant activity

Phosphorus analogues of amino acids have been investigated previously; however, most studies have involved phosphonic acid derivatives such as aminomethanephosphonic acid (I). Only in recent years have aminophosphinic acids with unsubstituted amino groups been prepared. The first, glycine analogue II, was synthesized in 1964 by a procedure which provides only limited types of products (1).

Phosphinic acids possess a hydrogen atom in lieu of one of the two hydroxyl groups found in phosphonic acids and, therefore, are monobasic with less bulk on the phosphorus atom. Phosphinic acids are the closest phosphorus analogues of carboxylic acids and this similarity has led to a limited number of investigations of aminophosphinic acids for biological activity. In 1961, Linfield *et al.* prepared phosphinic acids bearing substituted α-anilino and phenyl groups (III) by the addition of hypophosphorus acid to the appropriate

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