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Relationships between Chemical Structure and Inhibition of Human Placental Choline Acetyltransferase by Keto Analogs of Acetylcholine

ARVIND K. CHATURVEDI, PETER P. ROWELL, and B. V. RAMA SASTRY ˣ

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Abstract □ Seven keto analogs of acetylcholine were synthesized and evaluated as inhibitors of human placental choline acetyltransferase. Their potencies for inhibition of horse serum cholinesterase and stimulation of cholinergic receptors in the longitudinal ileal muscle of the guinea pig were investigated. The most potent and selective inhibitor of choline acetyltransferase was (2-benzoylethyl)trimethylammonium chloride with an I_{50} of 3×10^{-6} *M*. It exhibited considerably low activities at muscarinic and nicotinic receptors and cholinesterases. Its high potency for inhibiting choline acetyltransferase was attributed to: (*a*) its cationic terminal, a site for an electron acceptor interaction; (*b*) an aryl moiety for hydrophobic and electron donor contributions; and (*c*) a positive charge on the carbon atom adjacent to the benzene ring due to the presence of the carbonyl group, which interacts with the nucleophilic residue on the enzyme.

Keyphrases □ Acetylcholine analogs, various—synthesized, evaluated as inhibitors of choline acetyltransferase □ Choline acetyltransferase activity—various acetylcholine analogs evaluated as inhibitors □ Enzyme activity—various acetylcholine analogs evaluated as inhibitors of choline acetyltransferase □ Structure–activity relationships—various acetylcholine analogs evaluated as inhibitors of choline acetyltransferase

Acetylcholine is synthesized *in vivo via* a coupled system involving an acetate-activating enzyme (acetyl coenzyme A synthetase) and an enzyme, choline acetyltransferase, that couples the activated acetate to choline. Therefore, an agent that inhibits the final step in the acetylcholine synthetic pathway is valuable for studying cholinergic mechanisms.

The lack of a suitable strong inhibitor has hampered study of cholinergic mechanisms related to this enzyme. Recently, 4-(1-naphthylvinyl)pyridine ($I_{50} = \sim 10^{-6}$ *M*) and related compounds were introduced (1–4) as inhibitors of choline acetyltransferase. These *trans*-isomers photoisomerize readily in solution to *cis*-isomers, which are poor inhibitors of choline acetyltransferase (4). During exposure to daylight, their use is limited for obtaining reliable data in *in situ* and *in vivo* pharmacological experiments.

Monohaloacetylcholines were synthesized, and their pharmacological activities were studied (5–10). Among haloacetylcholines, chloro-, bromo-, and iodoacetylcholines ($I_{50} = \sim 10^{-7}$–10^{-6} *M*) were strong specific inhibitors of choline acetyltransferase (11–15). However, they were hydrolyzed by cholinesterases (7, 8). The tertiary analogs of haloacetylcholines were less potent inhibitors of choline acetyltransferase than the corresponding quaternary ammonium compounds (15, 16). Persson (17, 18) prepared chloro-, bromo-, and iodoacetonyltrimethylammonium halides that inhibited choline acetyltransferase ($I_{50} = \sim 10^{-5}$–10^{-4} *M*). They were not chemically stable, and their specificity for inhibiting choline acetyltransferase is not known.

Haubrich and Wang (19) demonstrated that 5-hydroxy-1,4-naphthoquinone (juglone from the extract of walnut hulls) inhibits choline acetyltransferase. The selectivity of this inhibitor is not established. Several naphthoquinones were shown to inhibit acetylcholine formation in coupled acetylcholine synthesis using multienzyme systems (20). Recently, it was demonstrated (21, 22) that several thiol reagents inhibit choline acetyltransferase. However, these reagents inhibit all sulfhydryl-containing enzymes and are not specific for choline acetyltransferase.

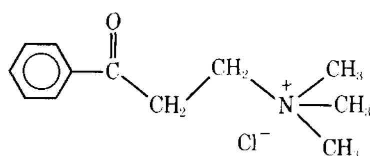
All of these observations indicate that a highly potent, selective, and stable inhibitor has yet to be synthesized. Therefore, (2-benzoylethyl)trimethylammonium chloride and related compounds were synthesized and tested for their inhibition of choline acetyltransferase and their activities at various cholinergic sites (muscarinic receptors, nicotinic receptors, and cholinesterases).

EXPERIMENTAL

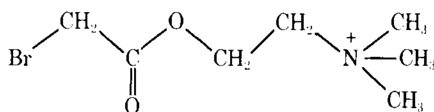
Materials—All compounds were keto analogs of acetylcholine and satisfied some requirements necessary for the inhibition of choline acetyltransferase. All were characterized by their sharp melting point, elemental analyses[1], and IR spectra[2]. Wherever necessary, they also were identified by UV absorption and NMR spectra. Their chemical structures and other characteristics are shown in Table I. Their interactions with various cholinergic sites are summarized in Table II.

4-Oxopentyltrimethylammonium Perchlorate (I)—Compound I was prepared by keeping an equimolar mixture (1.3 moles) of anhydrous trimethylamine and 5-chloro-2-pentanone in 150 ml of anhydrous ether in a pressure bottle at room temperature for 14 days. After this time, the separated solid (58.1 g, 24.8%) was filtered and dissolved in 100 ml of

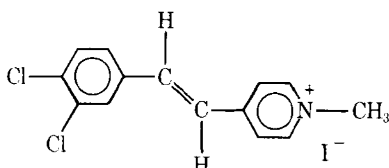
[1] Performed by Galbraith Laboratories, Knoxville, TN 37921.
[2] Obtained with a Perkin-Elmer model 257 grating IR spectrophotometer equipped with sodium chloride optics in potassium bromide films in the range of 625–4000 cm^{-1}.



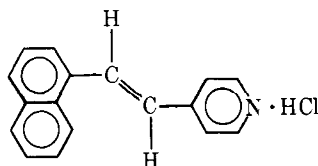
VII: (2-benzoylethyl)trimethylammonium chloride



VIII: bromoacetylcholine



IX: 3′,4′-dichloro-4-stilbazole methiodide



X: 4-(1-naphthylvinyl)pyridine

ice-cold absolute ethanol. To this solution, 32.5 g (46.4 ml) of 70% perchloric acid was added. A brown crystalline precipitate separated and was filtered and recrystallized from ethanol–ethyl acetate. The final product was obtained as colorless crystals.

4-[2-(1,3-Dioxolanyl)]pentyltrimethylammonium Chloride (II)—To an ice-cold solution of 50.0 g (0.304 mole) of 5-chloro-2-[2-(1,3-dioxolanyl)]pentane in 100 ml of dry toluene, 26.8 ml of anhydrous trimethylamine was added. The reaction mixture was kept in a pressure bottle for 7 days. The separated salt was filtered and recrystallized as a colorless product from acetonitrile–ethyl acetate.

1-Trimethylammonium-4-oxoheptyltrimethylammonium Diperchlorate (III)—An ice-cold solution of 1,7-dichloro-4-heptanone (40.0 g, 0.219 mole) in 100 ml of ether was treated with 38.5 ml of anhydrous trimethylamine. The reaction mixture thus obtained was kept in a pressure bottle at 40° for 7 days. The reaction product (43 g, 66%), a viscous mass, was dissolved in 75 ml of ice-cold absolute ethanol, and 20.7 ml of 70% perchloric acid was added. A brown crystalline precipitate (30 g) separated and was filtered and recrystallized from ethanol–water.

Ethyl 4-Trimethylammoniumbutyrate Chloride (IV)—Compound IV was prepared by keeping a mixture of ethyl 4-chlorobutyrate (50.0 g, 0.339 mole) and anhydrous trimethylamine (19.6 g, 0.339 mole) in 150 ml of dry toluene in a pressure bottle at room temperature for 15 days. The separated solid was filtered and recrystallized from acetonitrile as colorless crystals.

(3-Thenoylpropyl)trimethylammonium Chloride (V)—This compound was synthesized in the same manner as IV from 50.0 g (0.264 mole) of γ-chloro-2-butyrothienone and 23.3 ml of anhydrous trimethylamine. The product was recrystallized as light-brown prisms from isopropyl alcohol.

(3-Benzoylpropyl)trimethylammonium Chloride (VI)—Compound VI was prepared according to the procedure described for IV from 50 g (0.273 mole) of γ-chloro-2-butyrophenone and 24.1 ml of anhydrous trimethylamine. Upon recrystallization from ethanol–ethyl acetate, colorless needles were obtained.

(2-Benzoylethyl)trimethylammonium Chloride (VII)—This compound was synthesized by the procedures described for IV from 26.1 ml of anhydrous trimethylamine and 50 g (0.296 mole) of β-chloropropionophenone. The reaction product was recrystallized from isopropyl alcohol as colorless crystals.

Choline Acetyltransferase—Full-term human placentas were used as a source of enzyme. The enzyme was prepared and partially purified according to the methods described by Sastry and Henderson (23).

Inhibition of Choline Acetyltransferase—Modifications of previous methods (23, 24) were used for measuring choline acetyltransferase activity. The assay was performed in 75 × 10-mm test tubes, and the incubation medium consisted of 0.6 ml of potassium phosphate buffer (50 mM, pH 7.4) containing 300 mM NaCl, 0.2 mM physostigmine sulfate, 20 mM $MgSO_4$, 0.5 mM choline iodide, 3.36×10^{-5} M acetyl coenzyme A with 0.33 μCi of 1-^{14}C-acetyl coenzyme A, 1 mg of freeze-dried choline acetyltransferase/ml, 6.67 mM NaCN, 1.67 mM edetate disodium, and variable amounts of inhibitors.

After 10 min of incubation at 37°, 0.6 ml of ice-cold water was added to stop the reaction. The tubes were chilled in ice, and a 0.2-ml aliquot of the incubation mixture was placed immediately on a column (12 × 1 cm) of an anion-exchange resin[3]. The column was washed with 0.5 ml of deionized water four times. The effluent was collected in a scintillation vial, 15 ml of counting solution[4] was added, and the vial was cooled to 4° for 24 hr prior to being placed in a refrigerated liquid scintillation counter[5]. The radioactivity in the vial was proportional to the ^{14}C-acetylcholine formed.

Cholinesterase—This enzyme was prepared commercially[6] by a modified literature procedure (25) from horse serum and supplied as a stable lyophilized powder containing about 5 units/mg. One unit was equal to 1 μmole of acetylcholine hydrolyzed/min.

Inhibition of Cholinesterase—The ability of I–VII to inhibit cholinesterase activity was determined by the method of Ellman *et al.* (26). In this method, acetylthiocholine is hydrolyzed to thiocholine and acetic acid. Thiocholine reacts with 5,5-dithiobis-2-nitrobenzoate[7] to give the yellow 5-thio-2-nitrobenzoic anion. The reaction mixture consisted of 3 ml of sodium phosphate buffer (pH 8.0, 10 mM) containing 5,5-dithiobis-2-nitrobenzoate (0.32 mM), cholinesterase (0.01 mg/ml), acetylthiocholine (0.6 mM), and appropriate amounts of inhibitors. The absorbance was measured[8] every 2 min for 15 min at 412 nm, corresponding to the amount of acetylthiocholine hydrolyzed.

Stimulation of Cholinergic Receptors by I–VII—The effects of the new compounds were studied on the isolated longitudinal ileal muscle of the guinea pig by measuring their muscarinic and nicotinic activities according to the general methods previously described (10, 27). Longitudinal ileal muscles (2 cm in length) were obtained from male guinea pigs of common strain (200–400 g), which were sacrificed by blows on their heads. The muscles were suspended in organ baths containing 20 ml of pH 7.4 Tyrode solution, which was oxygenated with a mixture of 95% oxygen and 5% carbon dioxide. The Tyrode solution contained 124.9 mM NaCl, 2.68 mM KCl, 1.80 mM $CaCl_2$, 1.05 mM $MgCl_2$, 23.80 mM $NaHCO_3$, 0.41 mM NaH_2PO_4, and 11.10 mM dextrose. Compounds were added to the organ baths in 5–100-μl volumes. Contractions were measured by an isometric transducer[9], and rectilinear contraction heights were recorded using a physiograph[10].

The contractions by I–VII represent stimulation of both muscarinic receptors on the ileal smooth muscle and the nicotinic receptors in the Auerbach plexus. To separate the nicotinic receptor stimulation from the muscarinic receptor stimulation, contractions induced by I–VII also were measured in the Tyrode solution containing hexamethonium (37×10^{-6} M), which blocked the nicotinic receptors. The details of these methods were described previously (10, 27).

RESULTS

Inhibition of Choline Acetyltransferase by I–VII—All compounds inhibited the activity of choline acetyltransferase at 1×10^{-3} M (Table II). The I_{50} values of I, II, IV, and V were higher than 1×10^{-3} M. Compound I has a keto group attached to a quaternary nitrogen with an acyclic three-carbon chain. Replacement of the methyl group adjacent to carbonyl carbon of I by ethoxy or thiophene led to no increase in inhibitory potency. Similar results were also observed with II. However, the bis quaternary analog (III) increased inhibition.

Replacement of the methyl group of I by phenyl gave an active compound, VI ($I_{50} = 9.2 \times 10^{-5}$ M). Shortening the methylene chain linking the quaternary nitrogen and the carbonyl carbon in VI from three to two (as in VII) increased the inhibitory potency by a factor of 30. Compound

[3] Biorad AG-1-X8, chloride form, 200–400 mesh.
[4] Phase Combining System (PCS), Amersham/Searle Corp.
[5] Nuclear Chicago model III.
[6] Worthington Biochemicals, Freehold, N.J.
[7] Calbiochem, Los Angeles, Calif.
[8] Beckman model 25 automatic recording spectrophotometer.
[9] Model F-50 microdisplacement myograph transducer, Narco Biosystems.
[10] Desk model DMP-4A, Narco Biosystems.

Table I—Physical Constants of Keto Analogs of Acetylcholine $(CH_3)_3\overset{+}{N}CH_2CH_2R$

Compound[a]	R	Melting Point	Yield, %	Molecular Formula		Analysis, % Calc.	Found
I	$CH_2C(=O)CH_3$	127–129°	79	$C_8H_{18}ClNO_5$	C	39.42	39.44
					H	7.39	7.42
					Cl	14.57	14.65
					N	5.74	5.65
II	CH_2–C(–O–CH_2CH_2–O–)–CH_3 (dioxolane)	172–174°	44	$C_{10}H_{22}ClNO_2$	C	53.69	53.40
					H	9.84	9.66
					N	6.26	6.24
III	$CH_2C(=O)CH_2CH_2CH_2\overset{+}{N}(CH_3)_3$	205–208°	49	$C_{13}H_{30}Cl_2N_2O_9$	C	36.36	36.51
					H	6.99	7.27
					Cl	16.55	16.51
					N	6.52	6.43
IV	$CH_2C(=O)OCH_2CH_3$	95–97°	55	$C_9H_{20}ClNO_2 \cdot H_2O$	C	47.47	47.82
					H	9.67	9.65
					N	6.55	6.28
V	$CH_2C(=O)$–2-thienyl	250–251°	31	$C_{11}H_{18}ClNOS$	C	53.33	53.41
					H	7.27	7.32
					N	5.65	5.78
					S	12.92	12.95
VI	$CH_2C(=O)$–phenyl	246–249°	36	$C_{13}H_{20}ClNO$	C	64.59	64.66
					H	8.28	8.42
					N	5.79	5.63
VII	$C(=O)$–phenyl	180°	74	$C_{12}H_{18}ClNO$	C	63.29	63.09
					H	7.91	7.91
					N	6.15	6.15

[a] All compounds are chlorides except I and III which are perchlorates.

VII was the most potent inhibitor of choline acetyltransferase in the series.

Inhibition of Cholinesterase by I–VII—The I_{50} values (Table II) of all compounds were within the range of 2×10^{-3}–3×10^{-4} *M*. Various structural modifications of I did not result in significant changes in I_{50} values. Compounds I, II, and IV were equally active. Similarly, III and V–VII were equally active, and they were slightly more active than I, II, and IV in inhibiting cholinesterase.

Compound VII was about 50 times less active in inhibiting cholinesterase than in inhibiting choline acetyltransferase. Compound VI was only about three times more active in inhibiting choline acetyltransferase than in inhibiting cholinesterase.

Stimulation of Cholinergic Receptors by I–VII—The responses elicited by I–VII were contractions of the longitudinal muscle of the guinea pig ileum (Table II). All compounds exhibited higher ED_{50} and lower maximal effects than those of acetylcholine on the ileal muscle in the presence of hexamethonium, which blocked nicotinic receptors in this tissue (10, 27). Doses of atropine higher than 10^{-6} *M* blocked the contractions induced by all compounds. These observations indicate that all of these compounds were considerably less active than acetylcholine for stimulating muscarinic receptors. Of the two potent choline acetyltransferase inhibitors, the muscarinic activity of VI was not significant even at a dose of 2×10^{-2} *M*, and VII was about 160 times less active in stimulating muscarinic receptors than in inhibiting choline acetyltransferase.

In the absence of hexamethonium, the ED_{50} doses for all compounds were considerably lower than the corresponding doses in the presence of hexamethonium. But maximal effects of all compounds were significantly higher in the absence of hexamethonium than in the presence of hexamethonium. The effects of all compounds were blocked by atropine ($>10^{-6}$ *M*) on the ileal muscle even in the absence of hexamethonium. These observations indicate that these compounds stimulate nicotinic receptors in the Auerbach plexus and stimulate the ileal muscle through release of acetylcholine from postganglionic cholinergic nerve terminals.

Compound VI did not exhibit any activity on the ileum even in the absence of hexamethonium. Compound VII was about 15–20 times less active in stimulating ileal muscle than in inhibiting choline acetyltransferase. Compound VII produced a maximal effect at higher doses, which was about half of that of acetylcholine.

DISCUSSION

In the present series, VII was the most potent, stable, and selective inhibitor of choline acetyltransferase. The chemical structure of VII is similar to the structures of known potent inhibitors of choline acetyltransferase (VIII–X); it is somewhere between the structures of the two groups of strong inhibitors (styrylpyridine analogs and halogenated keto analogs of acetylcholine) of choline acetyltransferase.

Table II—Interactions of Keto Analogs of Acetylcholine at Various Cholinergic Sites

Compound	Inhibition, I_{50}, Mean ± *SE* (4)[a] Enzyme A[b]	Enzyme B[c]	Contraction of Ileal Muscle in Presence of Hexamethonium, Mean ± *SE* (6)[a] $(ED_{50}) \times 10^6$ *A*	Maximal Effect[d]	Contraction of Ileal Muscle in Absence of Hexamethonium, Mean ± *SE* (6)[a] $(ED_{50}) \times 10^6$ *B*	Maximal Effect[d]	Index of Discrimination (*A/B*)[e]
I	$>10^{-3}$	$(2.1 \pm 0.7)\ 10^{-3}$	42 ± 3	0.92	8.6 ± 0.7	1.14	4.9
II	$>10^{-3}$	$(2.4 \pm 1.0)\ 10^{-3}$	302 ± 3	0.80	73 ± 9	1.08	4.1
III	$(1.3 \pm 0.3)\ 10^{-4}$	$(2.8 \pm 1.0)\ 10^{-4}$	12333 ± 667	0.81	3125 ± 381	0.79	3.9
IV	$>10^{-3}$	$(2.7 \pm 1.0)\ 10^{-4}$	2.33 ± 0.04	1.0	1.4 ± 0.3	1.26	1.7
V	$>10^{-3}$	$(3.5 \pm 1.2)\ 10^{-4}$	NS[f]	NS[f]	134 ± 4	0.58	—
VI	$(9.2 \pm 1.5)\ 10^{-5}$	$(3.2 \pm 1.7)\ 10^{-4}$	NS[f]	NS[f]	NS[f]	NS[f]	—
VII	$(3.1 \pm 0.8)\ 10^{-6}$	$(1.6 \pm 0.2)\ 10^{-4}$	485 ± 49	0.27	54 ± 4	0.48	9.0
Acetylcholine	—	—	0.29 ± 0.02	1.0	0.14 ± 0.01	1.0	2.0

[a] The number in parentheses indicates the number of experiments. The I_{50} and ED_{50} values are given in moles per liter. [b] Partially purified choline acetyltransferase from human placenta. [c] Horse serum cholinesterase purified commercially by the Strelitz (25) procedure. [d] Relative intrinsic activity according to the nomenclature of Ariens (32). [e] Represents the ratio of ED_{50} doses in the presence of hexamethonium (C-6) and in the absence of hexamethonium. Contractions in the presence of hexamethonium indicate muscarinic activities, because hexamethonium blocks nicotinic receptors in this tissue. Contractions in the absence of hexamethonium indicate both combined muscarinic and nicotinic activities. The higher ratios (*A/B*) indicate higher nicotinic activities in relation to muscarinic activities. [f] No significant response at 2×10^{-2} *M*.

A structure–activity relationship study demonstrated three requirements for the inhibition of choline acetyltransferase by the alkylaminoethyl esters or their corresponding quaternary ammonium derivatives (15, 16): (*a*) a terminal cationic head on the amine end of the molecule, (*b*) the ability to delocalize (or stabilize) a partial negative charge on the acyl end, and (*c*) a leaving group on the α-carbon on the acyl end. Studies with other inhibitors indicated that only the first two requirements are essential for a strong inhibitor. For keto analogs of choline (17, 18), *e.g.*, 3-bromoacetonyltrimethylammonium bromide, a terminal cationic head and the ability to delocalize a partial negative charge are present.

Cavallito and collaborators (1, 3, 28) suggested that styrylpyridine analogs bind to the enzyme *via* hydrophobic and electron donor contributions of the aryl moiety and electron acceptor interaction of the pyridinium moiety. No specific binding contribution could be ascribed to the vinyl bridge other than transmission of electrons between the two rings and facilitation of coplanarity of the inhibitor. But Baker and Gibson (29–31) reported that the transmission of electrons from one ring to the other is of little consequence. They proposed that the double bond is polarized by a mesomeric interaction with the phenyl ring and the pyridyl ring, causing a partial positive charge on the carbon atom adjacent to the benzene ring and a partial negative charge on the carbon atom next to the pyridine ring. In this manner, a nucleophilic residue on the enzyme surface would have a strong interaction with the partial positive charge on the β-carbon to the pyridyl ring.

Compound VII has nearly all of the requirements necessary for the inhibition of choline acetyltransferase: (*a*) a cationic terminal, a site for an electron acceptor interaction; (*b*) an aryl moiety for hydrophobic and electron donor contributions; and (*c*) a positive carbon. There is no need for the C–C double bond which, with styrylpyridine analogs, helps to keep both the phenyl and pyridyl rings in one plane. Compound VII has only one phenyl ring attached to the quaternary nitrogen through single bonds. It can rotate with single bonds to acquire the necessary planarity to bind in a flat pocket of the enzyme. A partial positive charge on the carbon atom adjacent to the benzene ring due to the presence of keto oxygen interacts strongly with a nucleophilic residue on the enzyme.

REFERENCES

(1) C. J. Cavallito, H. S. Yan, J. C. Smith, and F. F. Foldes, *J. Med. Chem.*, **12**, 134 (1969).

(2) J. C. Smith, C. J. Cavallito, and F. F. Foldes, *Biochem. Pharmacol.*, **16**, 2438 (1967).

(3) C. J. Cavallito, H. S. Yan, T. Kapalau, J. C. Smith, and F. F. Foldes, *J. Med. Chem.*, **13**, 221 (1970).

(4) M. L. White and C. J. Cavallito, *Biochim. Biophys. Acta*, **206**, 242 (1970).

(5) C. Y. Chiou and B. V. R. Sastry, *Fed. Proc.*, **25**, 320 (1966).

(6) B. V. R. Sastry and C. Y. Chiou, *Chem. Ther.*, **2**, 164 (1967).

(7) C. Y. Chiou and B. V. R. Sastry, *Biochem. Pharmacol.*, **17**, 805 (1968).

(8) B. V. R. Sastry and C. Y. Chiou, *Biochim. Biophys. Acta*, **167**, 339 (1968).

(9) C. Y. Chiou and B. V. R. Sastry, *Arch. Int. Pharmacodyn. Ther.*, **181**, 94 (1969).

(10) C. Y. Chiou and B. V. R. Sastry, *J. Pharmacol. Exp. Ther.*, **172**, 351 (1970).

(11) D. Morris and D. S. Grewaal, *Life Sci.*, **8**, 511 (1969).

(12) G. I. Henderson and B. V. R. Sastry, *Fed. Proc.*, **31**, 561 (1972).

(13) B. V. R. Sastry and G. I. Henderson, *Toxicol. Appl. Pharmacol.*, **29**, 120 (1974).

(14) B. V. R. Sastry and G. I. Henderson, in "Drugs and Central Synaptic Transmission," P. B. Bradley and B. N. Dhawan, Eds., University Park Press, Baltimore, Md., 1976, pp. 89–98.

(15) P. P. Rowell and C. Y. Chiou, *Biochem. Pharmacol.*, **25**, 1093 (1976).

(16) P. P. Rowell and C. Y. Chiou, *Eur. J. Pharmacol.*, **40**, 83 (1976).

(17) B. O. Persson, *Acta Chem. Scand.*, **27**, 3307 (1973).

(18) B. O. Persson, *Acta Pharm. Suec.*, **11**, 77 (1974).

(19) D. R. Haubrich and P. F. L. Wang, *Biochem. Pharmacol.*, **25**, 669 (1976).

(20) D. Nachmansohn, *Handb. Exp. Pharmakol.*, **15**, 40 (1963).

(21) R. Roskoski, Jr., *J. Biol. Chem.*, **249**, 2156 (1974).

(22) R. Roskoski, Jr., C. T. Lim, and L. M. Roskoski, *Biochemistry*, **14**, 5105 (1975).

(23) B. V. R. Sastry and G. I. Henderson, *Biochem. Pharmacol.*, **21**, 787 (1972).

(24) B. K. Schrier and L. Schuster, *J. Neurochem.*, **14**, 977 (1967).

(25) F. Strelitz, *Biochem. J.*, **38**, 86 (1944).

(26) G. L. Ellman, D. Courtney, V. Andres, and R. M. Featherstone, *Biochem. Pharmacol.*, **7**, 75 (1961).

(27) B. V. R. Sastry and H. C. Cheng, *J. Pharmacol. Exp. Ther.*, **180**, 326 (1972).

(28) H. L. White and C. J. Cavallito, *Biochim. Biophys. Acta*, **206**, 343 (1970).

(29) B. R. Baker and R. E. Gibson, *J. Med. Chem.*, **14**, 315 (1971).

(30) *Ibid.*, **15**, 639 (1972).

(31) R. E. Gibson and B. R. Baker, *J. Med. Chem.*, **17**, 1290 (1974).

(32) E. J. Ariens, *Arch. Int. Pharmacodyn. Ther.*, **99**, 32 (1954).

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