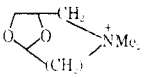
mation of cholinomimetic molecules. Superimposition of molecular (Dreiding) models of *cis*-2-methyl-4-dimethylaminomethyl-1,3-dioxolane methiodide (VI), in the extended configuration, and *exo*-3-methyl-7-methyl-7-aza-2,4-dioxa-*cis*-bicyclo[3.3.0]octane methiodide (IIId) initially at the quaternary groups results in further superimposition of one ring oxygen and, most importantly, the C-methyl groups (Figure 2). The C-methyl group of the *endo* isomer (IIIc) is not superimposable on the C-methyl of VI but is, however, superimposable on the C-methyl of *trans*-2-methyl-4-dimethylaminomethyl-1,3-dioxolane methiodide. The tenfold difference in activity between IIIc and IIId is approximately that observed between the *cis* and *trans* isomers of VI.[5]

It is perhaps pertinent also to note that the MO calculations on muscarine also suggest[18] the biological significance of the conformation in which the quaternary head is maximally extended away from the tetrahydrofuran ring. Further discussion of the implications of this conformation in the general interpretation of the structure–activity relationship of muscarinic agents will be presented in a subsequent publication.

(18) L. B. Kier, *Mol. Pharmacol.*, **3**, 487 (1967).

TABLE VII

INTERATOMIC DISTANCES IN SOME MUSCARINIC AGENTS

Compd	Distances, Å: $(CH_3)_3{}^+N \rightarrow O_1$	$(CH_3)_3{}^+N \rightarrow O_2$
VI	3.6	4.6[a]
IIIa–d	3.2	3.2 (min)
	3.5	3.5 (max)
		
$n = 1$	2.5	3.3[b]
	2.8	2.8[c]
$n = 2$	2.5	3.9[d]
	2.85	3.5[e]

[a] Measured for conformation of VI in which ^+N is at maximum distances from both oxygen atoms. Measurements also apply to IVc and IVd. [b] Morpholine ring in boat conformation. [c] Morpholine ring in chair conformation. [d] 1,4-Oxaazacycloheptane ring in boat conformation. [e] 1,4-Oxaazacycloheptane ring in chair conformation.

Choline Acetyltransferase Inhibitors. Configurational and Electronic Features of Styrylpyridine Analogs

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A variety of molecular modifications were made of styrylpyridine prototype choline acetylase (choline acetyltransferase) inhibitors. Among these, enzyme inhibitory activity is favored by the presence of an aromatic ring system conjugated to a pyrido ring through an exocyclic unsaturated bond in such a manner as to provide an over-all coplanar molecule with minimal third dimensional structure. Optimum size appears to be provided by linkage of fused bicyclic and of monocyclic ring systems through either a double or triple bond. One of the cyclic structures should contain at least a weakly basic moiety; quaternization generally increases choline acetylase inhibitory potency. Acetylcholinesterase inhibitory activities of most of these compounds are relatively low and bear no relationship to the activities against choline acetylase.

A review on choline acetylase (ChA) (choline acetyltransferase, acetyl CoA, choline O-acetyltransferase, EC 2.3.1.6), published 5 years ago, concludes with the statement,[1] "A really potent and specific inhibitor of ChA has not been found as yet. Such a compound obviously would be of great interest." Recently, potent and selective inhibitors (reversible, noncompetitive) of this enzyme system have been discovered among some congeners of styrylpyridine.[2] The present report describes a variety of molecular modifications designed to provide further insight as to the steric and electronic features of this type of compound which are conducive to choline acetylase inhibitory properties. Inhibitory activities against acetylcholinesterase (AChE) (acetylcholine acetylhydrolase, EC 3.1.1.7) also were determined in order to assess specificities.

A variety of styrylpyridines have been prepared in the past, particularly for studies of physical–chemical characteristics of position and of *cis–trans* isomers. The most general synthetic route involves condensation of an arylaldehyde with a methylpyridine to yield the *trans*-stilbazole derivatives.[3–6] Additional literature sources may be derived from the cited references. Appropriate methylquinolines form analogous compounds.[7] Quaternary pyridinium or quinolinium derivatives may be formed by quaternizing the condensation product or, preferably, the heterocyclic base component is quaternized prior to condensation.[8–11] Most

(1) D. Nachmansohn in "Cholinesterases and Anticholinesterase Agents," G. B. Koelle, Ed., Springer-Verlag, Berlin, 1963, pp 40–54.

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

(3) J. L. R. Williams, R. E. Adel, J. M. Carlson, G. A. Reynolds, D. G. Borden, and J. A. Ford, Jr., *J. Org. Chem.*, **28**, 387 (1963).

(4) D. Jerchel and H. E. Heck, *Ann.*, **613**, 171 (1958).

(5) E. A. Wagstaff, *J. Chem. Soc.*, 276 (1934); B. D. Shaw and E. A. Wagstaff, *ibid.*, 77 (1933).

(6) G. M. Bennett and W. L. C. Pratt, *ibid.*, 1465 (1929).

(7) D. Sutherland and C. Compton, *J. Org. Chem.*, **17**, 1257 (1952); C. Compton and W. Bergmann, *ibid.*, **12**, 363 (1947).

(8) A. Kaufmann and L. G. Vallette, *Ber.*, **45**, 1736 (1912).

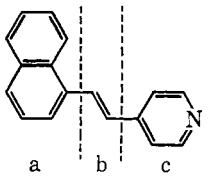
(9) W. H. Mills and R. Raper, *J. Chem. Soc.*, 2466 (1925).

(10) C. F. Koelsch, *J. Am. Chem. Soc.*, **66**, 2126 (1944).

(11) A. P. Gray, W. L. Archer, E. E. Spinner, and C. J. Cavallito, *ibid.*, **79**, 3805 (1957).

of the compounds described in the present study are new but were prepared by essentially conventional procedures.

Among an initial group of 4-styrylpyridine analogs evaluated,[2] 4-(1-naphthylvinyl)pyridine (I) was of particular biochemical interest. Although not the most potent inhibitor of ChA ($I_{50} = 2.5 \times 10^{-5}$ *M*)



I

within the series, I was essentially devoid of cholinesterase inhibitory activity thus showing a high level of specificity. The N-methyl quaternary derivative of I was about eight times as potent against ChA and demonstrated weak antiesterase activity. It also was shown that a single bond at b resulted in loss in activity and that naphthyl was superior to phenyl at a. From the limited analogs available, it was tentatively suggested that the *trans*-conjugated coplanar structure might favor receptor-bonding interactions involving van der Waals and hydrophobic bonding and possibly charge-transfer complexing. In the present study, model compounds were designed to further explore the influences on activity of electronic and structural features such as coplanar mass, position isomers, substituent effects, etc.

Results and Discussion

For molar I_{50} (or lower) values for these compounds measured against rat cerebral cortical ChA and AChE, see Table I. These results are generally reproducible within a range of 5% of the values reported. In the sections that follow, the discussion primarily will relate to influences of some steric and electronic variables on ChA inhibitory activities. Most of these compounds are poor inhibitors of AChE.

In every instance in which both base (acid addition salt) and quaternized forms of a structural type were compared, the quaternary was a more potent inhibitor of both ChA and AChE. Whether this represents a true difference in affinity toward a particular (anionic) receptor group in each enzyme is uncertain since the net distribution to such a group also would be influenced by the relative molecular affinity of the quaternary and nonquaternary forms for other bonding functional groups in the prepared enzyme complex. The favorable influence of a cationic charge on ChA inhibitory activity, however, is supported not only by the greater activity of the quaternaries relative to the corresponding pyridine bases, but also by the essentially inactive nature of the nearly neutral N-oxide (XXXI *vs.* III) and of the anionic stilbenecarboxylic acid (XXVII). The significant ChA inhibitory activity of compounds as weakly basic as I (III is reported to have a pK_a of 5.73[12]) suggests that electrostatic interaction with a receptor anionic moiety would more likely involve a strongly acidic group such as phosphate rather than carboxyl. The presence of multiple cationic groups has diverse influences. In the earlier report,[2] it was shown that a second cationic (quaternary) group could have an enhancing or diminishing influence on ChA inhibitory activity relative to prototype II depending on the relationship to one another of the two cationic groups. Among the present compounds, the dipyridyl derivative XVII is less active than the monopyridyl analog III; the bis-substituted XXIII and XXIV each is less active than the monopyridyl III or IV. A hexamethylene-α,ω-bis-stilbazolium derivative (XXX), on the other hand, is considerably more active than the monoquaternary IV. It is of further interest that with stilbazole monoquaternization with N attachment of methyl, *n*-hexyl, or benzyl increases activity to a comparable degree against ChA, but the benzyl derivative is distinctly more potent than the other two as an AChE inhibitor. The nature of the N-attached group here seems to be a less critical structural feature for ChA than for AChE inhibitory activity. Among the diquaternaries both XXIV and XXX are moderately active inhibitors of AChE but only the flexible chain-linked XXX is active against ChA. The anion is negligibly if at all involved since iodide, bromide, and chloride salts all are active (iodide and certain pyridinium compounds can form charge-transfer complexes).

Interesting reference compounds are the compact tricyclic N-methylbenzoquinolinium derivatives XXVIII and XXIX which are essentially inactive against ChA but moderately active AChE inhibitors. Compound IV, with a molecular mass comparable to these, but with an exocyclic conjugated component and more extended configuration, shows a reversal of the order of activities against these enzyme systems and is primarily a ChA inhibitor.

The necessity for the unsaturated conjugated bond b in I and III in contributing to activity was shown earlier.[2] This has both electronic and steric influences. The interannular bond b provides a high order of electron delocalization and a coplanar structure for the whole molecule. The essentially inactive nature of the *cis* isomer I′ and of the presumably *trans*, completely conjugated X and XIV strongly supports the conclusion that the steric factor is critical with requirement of coplanarity, and that conjugation and electron delocalization are primarily important in stabilizing coplanarity among the active ChA inhibitors. In X, the ring systems probably are coplanar, but the 3,4,5-trimethoxy substituents project from the ring to provide noncoplanar moieties. In XIII and XIV, the biphenylyl system in the solution state presumably is not coplanar. Biphenyl itself is reported to be coplanar in the crystalline state but to show an angle of twist of the order of 20° in solution and 40° in the gaseous state.[13-15] An interesting modification is the inactive ferrocene derivative XV. This aromatic system could have one coplanar face, but the ferrocene "sandwich" provides a considerable third dimension to part of the molecule. The similar anti-ChA activities of the 5-indanyl and

(12) G. Cauzzo, G. Galiazzo, U. Mazzucato, and N. Mongiat, *Tetrahedron*, **22**, 589 (1966).

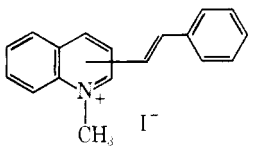
(13) H. Suzuki in "Electronic Absorption Spectra and Geometry of Organic Molecules," Academic Press, New York, N. Y., 1967, pp 269–271, and references therein; also *Bull. Chem. Soc. Japan*, **32**, 1340 (1959).

(14) G. B. Robertson, *Nature*, **191**, 593 (1961).

(15) O. Bastiansen, *Acta Chem. Scand.*, **4**, 926 (1950); A. Almenningen and O. Bastiansen, *Kgl. Norske Videnskab. Selskabs, Skrifter*, **No.4**, 1 (1958); *cf.* also ref 13 and *Chem. Abstr.*, **53**, 11917 (1959).

TABLE I

CHOLINE ACETYLASE AND CHOLINESTERASE INHIBITORY ACTIVITIES[a]

No.			Cor mp, °C dec	% yield	ChA I_{50}, M[b,e]	AChE I_{50}, M[b,e]	Analyses[c]	ChA/ChE
			A. *trans*-RCH=CH–C$_6$H$_4$–N·R′X					
	R[a]	R′X						
I[d]	1-Np	HCl			2.5×10^{-5}	10^{-3}, I_5		>100
II[d]	1-Np	MeI			3.3×10^{-6}	2×10^{-4}		61
III[d]	Ph	HCl			6×10^{-4}	4.5×10^{-3}, I_1		>10
IV[d]	Ph	MeI			1.5×10^{-5}	1.8×10^{-3}		120
V	Ph	*n*-$C_6H_{13}Br$	224–228	68	6×10^{-6}	3.3×10^{-4}	C, H	55
VI	Ph	$PhCH_2Cl$	238–243	41	5×10^{-6}	8×10^{-6}	C, H	1.6
VII	2-Np	MeI	273–276	54	3.2×10^{-6}	3.8×10^{-4}	C, H	119
VIII	9-Phthr	MeI	300–305	25	10^{-5} S, I_{35}	10^{-5} S, I_0	C, H	...
IX	3,4-$(OH)_2$Ph	MeI	275	12	2.1×10^{-4}	5.4×10^{-4}	C, H	2.6
X	3,4,5-$(OCH_3)_3$Ph	MeI	214	34	10^{-4}, I_0	1.1×10^{-3}	C, H	...
XI	5-Indanyl	HCl	193–225	20[f]	10^{-4} S, I_{20}	10^{-4} S, I_{14}	C, H (base)	...
XII	5-Indanyl	MeI	245	58	3.4×10^{-6}	5×10^{-4}	C, H	147
XIII	4′-PhPh	HCl	>220	72[g]	5×10^{-5} S, I_0	5×10^{-5} S, I_{11}	C, H (base)	...
XIV	4′-PhPh	MeI	268	55	10^{-5} S, I_0	10^{-5} S, I_{10}	C, H	...
XV	Fer	MeI	256	61	10^{-4}, I_0	2.5×10^{-4}	C, H	...
XVI	2-OH-1-Np	MeI	250	15	1.2×10^{-5}	6×10^{-4}	C, H	50
XVII[h]	4-Py	2HCl			4×10^{-3}, I_{25}	2×10^{-3}		...
I′[i]	*cis*-1-Np	...			4×10^{-4}, I_{23}	...		
			B. $(R)_x$–C$_6$H$_3$N$^+$CH$_3$ I$^-$					
	$(R)_x$							
XVIII	3-Me, 4-styryl		249	44	5×10^{-6}	1×10^{-3}	C, H	200
XIX	2-(1-Np-vinyl)		250–265	10	1×10^{-5}	1.2×10^{-4}	C, H	12
XX	2,4-$(Styryl)_2$		258	43	10^{-5}, I_{40}	10^{-5} S, I_{12}	*j*	...
			C. 					
	Position							
XXI	2		248	14	1.3×10^{-5}	1.3×10^{-4}	C, H	10
XXII	4		254	27	3.5×10^{-6}	2×10^{-4}	C, H	57
			D. 1,3-(4-Py-CH=CH)$_2$Ph					
	Salt							
XXIII	2 HCl		297–307	26[k]	10^{-4} S, I_0	10^{-4} S, I_0	*j*	...
XXIV	2 MeI		320–327	20	4×10^{-4}, I_0	2.9×10^{-6}	*j*	<0.007
			E. RC≡C–C$_6$H$_4$–N·R′X					
	R	R′X						
XXV	1-Np	HCl	182–188	30[l]	2×10^{-4} S, I_{25}	2×10^{-4} S, I_0	*j*	...
XXVI	1-Np	MeI	248–251	33	1×10^{-5}	9×10^{-4}	*j*	900
			F. Miscellaneous					
	Compd							
XXVII	*trans*-Stilbene-4-carboxylic acid		244[m]		10^{-4}, I_0	2×10^{-4}, I_{10}		...
XXVIII	Benzo[*f*]quinoline MeBr		259[o]		4×10^{-4}, I_8	7.0×10^{-5}	Br	<0.17
XXIX	Phenanthridine MeBr		244–246[o]		4×10^{-4}, I_{12}	1.5×10^{-5}	Br	<0.04
XXX	$Stbz^+(CH_2)_6{}^+Stbz$ $2Br^-$		301–302	59	1.9×10^{-6}	1.3×10^{-5}	C, H	7
XXXI	*trans*-Stilbazole N-oxide		170–172[n,o]		4×10^{-3}, I_{32}	2×10^{-3}, I_{19}		...

[a] Abbreviations: Np = naphthyl, Phthr = phenanthryl, 4′-PhPh = 4′-biphenyl, Fer = ferrocenyl, Py = pyridyl, Stbz = stilbazole. [b] Rat brain enzyme source. [c] Where figures are not given (in Experimental Section), found analyses conform to within 0.3% of the calculated values for the elements indicated. [d] Compounds I–IV, reported earlier,[2] are included to facilitate comparisons. [e] Percentages of less than 50% inhibition as molar I values are identified by the letter S when the assays were carried out with essentially saturated solutions of inhibitor. Where an I_{50} could not be determined, a lower I value is given as an approximation. I_0 represents no inhibition at the concentration cited. [f] Of free base, mp 82–84°. [g] Of free base, mp 220–222°. [h] Base from Aldrich Chemical Co., Inc. [i] Kindly provided by Dr. David Whitten, Department of Chemistry, University of North Carolina, Chapel Hill, N. C. [j] See Experimental Section. [k] Of free base, mp 205°. [l] Of free base, mp 54°. [m] G. A. R. Kon, *J. Chem. Soc.*, 224 (1948), also reports 244°. [n] Reference 4 also reports 171–172°. [o] No decomposition.

the naphthyl derivatives (XII and II) suggests that deviation from coplanarity by a few H atoms is not sufficient to reduce activity among the quaternary derivatives; however, the nonquaternary indanyl compound XI is less active than its naphthyl analog I. Similarly, in the pyrido ring 3-substituted methyl derivative XVIII, activity is comparable to that of unsubstituted IV. A requirement of coplanarity and minimal axial third dimension or "thickness" of the molecule suggests that bonding at the ChA receptor occurs at a planar interface or that intercalation occurs in a relatively narrow groove of a receptor structure. The coplanar requirement for anti-ChA activity appears to be limited to the molecular components other than those comprising pyrido N-attached quaternizing moieties.

One of the variables of interest in influencing activity is that of optimum dimensional area of the coplanar mass in congeners of I. It already had been shown[2] that a phenyl group (III, IV) was less effective than naphthyl (I, II) in position a of I in contributing to activity. It now is shown that the phenanthryl moiety (VIII) is less effective than naphthyl. Whether this is directly a steric limitation to receptor interaction or an indirect influence from molecular associating or other bonding loss cannot be stated. It is interesting, however, that within certain limits, the shape of the molecule relative to its cationic center is not critical. Small differences such as 1- *vs.* 2-naphthyl substitution in position a (II *vs.* VII) produce no change in ChA inhibitory activity. The naphthylvinylpyridinium (II) and 4-phenylvinylquinolinium (XXII) isomers have similar enzyme inhibitory properties. The corresponding pair of 2-substituted isomers (XIX and XXI) also are comparable to one another in ChA inhibitory potency. The somewhat lower ChA inhibitory activity of the 2- than the 4-substituted isomer sets may result from greater steric hindrance of the cationic center of the former to approach to an anionic site. As mentioned previously, the essentially inert nature of the metho quaternaries of phenanthridine (XXIX) and benzo[*f*]quinoline (XXVIII) against ChA do show that a conjugated fused ring system of mass comparable to IV is of itself not sufficient. The interannular unsaturated bond may be an important feature *per se* and is being further investigated. The presence of a second styryl substituent (XX) shows remarkably little influence on activity compared to the monoderivative (IV); however, oppositely acting factors may be self-negating in over-all influence.

The presence of hydroxyl groups on moiety a of IX and XVI results in reduction of ChA inhibitory activity relative to that of unsubstituted IV and II. There is insufficient basis for assigning this change to any one of the several influences of hydroxyl substitution.

One of the most interesting observations is the comparable order of potency of enzyme inhibitory activities of the acetylenic derivative XXVI and the ethylenic (*trans*) analog II. The acetylenic compound shows the greatest specificity or ChA/AChE ratio of I_{50} values among the compounds reported. The interannular acetylenic bond provides a linear rather than *trans* orientation of the planar ring systems relative to one another. Although rotation can occur about the triple bond, it is likely that a coplanar configuration is energetically favored as is the case for tolan (diphenylacetylene) in both the crystalline state and in solution.[16,17] There is a remarkably consistent correlation between ChA inhibitory activity and evidence of coplanarity among the various molecular prototypes illustrated in this report. Only with regard to the pyrido-N substituents is planarity not a critical feature.

Additional studies will include attempts to elucidate the nature of the chemical interaction between these compounds and binding sites within the ChA enzyme system.

Experimental Section

Preparative details are outlined only when an appropriate or adequate descriptive reference cannot be cited. The free bases generally are white crystalline compounds; the salts are yellow to orange in color, the ferrocene derivative is blue. Wherever possible, the compounds were dried *in vacuo* at 80°. Melting points were taken with a Fisher-Johns apparatus for temperatures up to 280° and with a Mel-Temp melting point apparatus at higher temperatures; reported values are corrected. Analyses were performed by either Spang Microanalytical Laboratory, Ann Arbor, Mich., or M-H-W Laboratories, Garden City, Mich.

Enzyme Inhibition Measurements.—Adult rat brains were homogenized in 9 vol of distilled water and the homogenate was used directly as a source of AChE. The assays were performed with a potentiometric titrimetric procedure (pH-Stat, Radiometer, Copenhagen), measurements being carried out at 37° with 3×10^{-3} *M* ACh as substrate in the presence of 0.5 *M* NaCl. The preparation was capable of hydrolyzing ACh at the rate of 20 mmoles/g of brain equivalent per hr. Forty milligrams of brain equivalent was used in each assay. The ChA assays employed a dilution of 100,000*g* supernatant of a 1:10 aqueous homogenate of rat brain using a modification of the method of McCaman and Hunt.[18] The preparation was capable of synthesizing ACh at the rate of 2 μmoles/g of brain equivalent per hr. Each assay contained 25 μg of brain equivalent. Where solubility permitted each inhibitor was run at five concentrations in triplicate and the I_{50} value was read from a graph of per cent inhibition *vs.* log of inhibitor concentration.

Among the nonquaternary salts, the hydrochlorides were used to facilitate initial solution; at the pH ranges of the test solutions (pH 7.7 for ChA, pH 7.4 for AChE) the bases are of limited solubility and only slightly ionized. The *cis* isomer I′ was dissolved as the free base in a very small volume of EtOH and diluted with the buffered medium. Many of these compounds are photosensitive and exposure to light should be minimized, particularly in dilute solutions.

Preparation of Arylethenylpyridines.—As a general procedure equimolar proportions of arylaldehyde[19] and a 2- or 4-methylpyridine in 1 to 2 molar proportions of Ac_2O were heated to reflux for from 6 to 16 hr. Some excess of the methylpyridine could be used if the aldehyde were in more limited supply. The reaction mixture was poured into excess 10% aqueous NaOH and allowed to stand for at least 3 hr in the cold and the crude product was separated and washed with cold H_2O (decantation or filtration). Purification could be carried out by either directly recrystallizing the crude bases from EtOH or *i*-PrOH or ligroin.

Phenylethenylquinolines.—Benzaldehyde and 2- and 4-methylquinolines were condensed and the reaction products were isolated essentially as described previously.[20,21]

***m*-Phenylenebis(4-ethenylpyridine).**—A mixture of 6.7 g (0.05 mole) of isophthalaldehyde, 14 g (0.15 mole) of 4-methylpyridine, and 5 g (0.05 mole) of Ac_2O was heated to a reflux for 12 hr. The reaction mixture was poured into 10% NaOH and kept cold overnight. The crude product was filtered off, washed (cold H_2O), and recrystallized (hot EtOH–H_2O). The filtered, dried product was obtained in 26% (3.7 g) yield. *Anal.* ($C_{20}H_{16}N$) C, H. *Anal.* (dimethiodide XXIV, $C_{22}H_{22}N_2I_2$) C, H.

(16) J. M. Robertson and I. Woodward, *Proc. Roy. Soc.* (London), **A164**, 436 (1938).
(17) H. Suzuki, *Bull. Chem. Soc. Japan*, **33**, 389 (1960).
(18) R. E. McCaman and J. M. Hunt, *J. Neurochem.*, **12**, 253 (1965).
(19) The aldehydes were obtained from Aldrich Chemical Co., Inc.
(20) C. E. Kaslow and R. D. Stayner, *J. Am. Chem. Soc.*, **67**, 1716 (1945).
(21) A. D. Ainley and H. King, *Proc. Roy. Soc.* (London), **B125**, 60 (1938).

4-(1-Naphthylethynyl)pyridine.—The procedure is analogous to that reported for the preparation of 4-(1-phenylethynyl)-pyridine.[22] To a refluxing solution of 23.1 g (0.1 mole) of 4-(1-naphthylethenyl)pyridine in 150 ml of $CHCl_3$ was added dropwise with stirring, a solution of 21 g (0.13 mole) of Br_2 in 25 ml of $CHCl_3$. After refluxing for 3 hr, the solvent was evaporated and the dibromo adduct recrystallized (EtOH); mp 145–147° dec. A solution of 19 g (0.05 mole) of the dibromo compound was refluxed for 3 hr in 20% KOH in EtOH, then the solvent evaporated. The residue was extracted with low-boiling ligroin, treated with charcoal, filtered, and evaporated to yield an oil which solidified in the cold. Recrystallization from ligroin yielded 3.5 g of product. *Anal.* ($C_{17}H_{11}N$) C, H. *Anal.* (methiodide XXVI, $C_{18}H_{14}IN$) C, H.

Hydrochlorides.—Hydrochloride salts were prepared by solution of the base in an appropriate ether-miscible solvent to which was then added a solution of HCl in dry Et_2O. Excess Et_2O could be added if necessary to complete precipitation of the salts.

Monomethiodides. Method A.—The substituted pyridine base was dissolved in 3 or more molar equiv of MeI with initial cooling if necessary. The mixture was then refluxed, excess MeI was evaporated, and the crude product was recrystallized from an alcohol (usually *i*-PrOH). Compounds IV and XXVI were prepared in this manner.

Method B.—A solution of a methyl-substituted N-methylpyridinium or -quinolinium iodide (0.02 mole) and 0.02–0.03 mole of the appropriate aldehyde in 30 ± 10 ml of MeOH containing about 5 drops of piperidine was refluxed for 3–6 hr (VIII, IX, XVI, and XX required up to 20 hr). The reaction mixture was cooled and ether was added, if necessary, to complete precipitation of product which was then recrystallized from an alcohol or H_2O. Most of the methoquaternaries were prepared by this procedure.

N-Benzyl-4-(1-phenylethenyl)pyridinium Chloride (VI).—A solution of 3 g (0.017 mole) of 4-styrylpyridine and 3.2 g (0.025 mole) of benzyl chloride in 30 ml of dioxane was refluxed for 15 hr. The crude product which separated on cooling was filtered off and recrystallized several times (*i*-PrOH).

N-(*n*-Hexyl)-4-(1-phenylethenyl)pyridinium Bromide (V).—A mixture of 3 g (0.017 mole) of 4-styrylpyridine and 16.5 g (0.1 mole) of bromohexane was heated to reflux for 17 hr. To the cooled reaction mixture was added 60 ml of Et_2O and the precipitated product was filtered off. The crude preparation was recrystallized from warm *i*-PrOH to which Et_2O had been added to incipient precipitation, followed by cooling.

N-Methyl-2,4-bis(1-phenylethenyl)pyridinium Iodide (XX).—2,4-Lutidine methiodide was prepared by reaction of 2,4-lutidine in excess MeI; mp 128° (from *i*-PrOH). A solution of 12.5 g (0.05 mole) of the methiodide, 20 ml (0.2 mole) of PhCHO, and piperidine as catalyst in 40 ml of MeOH was refluxed overnight. The crude material which separated from the cooled reaction mixture was recrystallized (MeOH).

N-Methyl-3-methyl-4-(1-phenylethenyl)pyridinium Iodide (XVIII).—3,4-Lutidine methiodide was prepared by reaction of the lutidine with excess MeI; mp 124° (from *i*-PrOH). A solution of 12.5 g (0.05 mole) of the methiodide, 20 ml (0.2 mole) of PhCHO, and piperidine as catalyst was refluxed overnight. The crude material obtained was recrystallized from MeOH. The product obtained analyzed well as a monocondensation product. Although unequivocal evidence was not sought, the derivative would be expected to be that resulting from condensation with the 4-methyl group.

Hexamethylene-1,6-bis-N,N′-(1-phenylethenylpyridinium) Dibromide (XXX).—A solution of 5.4 g (0.03 mole) of 4-styrylpyridine, 2.4 g (0.01 mole) of 1,6-dibromohexane, and 40 ml of dioxane was refluxed overnight. The product which separated on cooling was filtered off and recrystallized (EtOH).

Methobromides of Phenanthridine and of Benzo[*f*]quinoline.—Excess MeBr was dissolved in 30 ml of dry dioxane containing 3 g of the aromatic base. After standing at room temperature overnight, the crystalline product was filtered off and recrystallized (hot *i*-PrOH).

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Role of Alkyl Substitution in 2,3-Disubstituted and 3-Substituted 4-Quinazolones on the Inhibition of Pyruvic Acid Oxidation[1]

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Several 2,3-disubstituted and 3-substituted 4-quinazolones have been synthesized to investigate structure–activity relationship of these quinazolones with respect to their ability to inhibit pyruvic acid oxidation by rat brain homogenate. 2-Methyl-3-(*o*-tolyl)-4-quinazolone was used for comparison. In general 2,3-disubstituted quinazolones exhibited greater inhibitory properties as compared to the coresponding 3-substituted quinazolones. Introduction of the alkyl substituent(s) on the phenyl nucleus, attached to the 3 position of the quinazolone molecule, significantly influenced the enzyme inhibitory properties of these quinazolones. In both series, maximum inhibition of the oxidation of pyruvic acid was observed with quinazolones synthesized from 2,4-dimethylaniline. Increase in the concentration of the quinazolones simultaneously increased the enzyme inhibition. Added NAD, responsible for the increase in the respiratory activity of brain homogenate during oxidation of pyruvic acid, reduced the inhibition produced by 2,3-disubstituted and 3-substituted 4-quinazolones.

The structure–activity relationship of various classes of drugs has been attributed to their interaction with receptor surfaces.[2] Such interactions are presumed to be greatly responsible for the inhibitory or stimulatory properties, toxicity, and metabolic fate of drugs where steric differences play an important role in determining their pharmacodynamic effects. Stereoisomeric effects of amphetamine and amphetamine derivatives have recently been reported on the activity of rat liver mito-

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