The condensations were also performed at room temperature, in which case the concentrations of aldehyde and amine were one-third to one-half of those above.

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The Meerwein Reaction with *p*-Nitrostyrene and 2-Vinylpyridine

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Since the unsaturated linkages in *p*-nitrostyrene[2] and 2-vinylpyridine[3] resemble simple unsaturated carbonyl compounds in their electrophilic character, it was of interest to investigate the Meerwein reaction with these compounds. Brunner and Kustatscher[4] have reported that styrene and α,β-dimethylstyrene react with diazotized aniline, *p*-methoxyaniline, *p*-chloroaniline and *p*-nitroaniline to yield the corresponding stilbenes in low yield (13 to 41%).

In the present research the following series of reactions was performed with *p*-nitrostyrene.

$$p\text{-}O_2NC_6H_4CH{=}CH_2 + p\text{-}YC_6H_4N_2Cl \longrightarrow p\text{-}O_2NC_6H_4CHClCH_2C_6H_4Y\text{-}p \xrightarrow{KOH,\ CH_3OH} p\text{-}O_2NC_6H_4CH{=}CHC_6H_4Y\text{-}p$$

A similar series was investigated for 2-vinylpyridine. The method of Brunner and Kustatscher[4] was generally used for the Meerwein reactions. The products obtained could not be distilled *in vacuo* without decomposition and hence were purified by successive recrystallizations, which generally resulted in low yields. The purified materials contained the elements of hydrogen chloride and proved to be α-chlorobibenzyls and 1-chloro-1-(2-pyridyl)-2-phenylethanes. These were then converted to stilbenes or stilbazoles by dehydrohalogenation.

Experimental

***p*-Nitrostyrene.**—This material was prepared in 65% yield by the method of Strassburg, Gregg and Walling.[5]

α,4′-Dichloro-4-nitrobibenzyl.—The method used was that of Brunner and Kustatscher.[4] After coupling, the reaction solution was steam distilled to remove the acetone and dichloroacetone after which a dark viscous oil remained. The oil was extracted with ether and the extract was washed with water and dried. After removal of the solvent the residue was purified by repeated recrystallizations from alcohol. From 14.9 g. (0.10 mole) of *p*-nitrostyrene, 1.3 g. (4%) of fine white needles were obtained; m.p. 94.5–95° dec.

Anal. Calcd. for $C_{14}H_{11}NO_2Cl_2$: C, 56.77; H, 3.75. Found: C, 56.68; H, 3.93.

4-Chloro-4′-nitrostilbene.—The α,4′-dichloro-4-nitrobibenzyl (1.4 g., 0.005 mole) was dehydrohalogenated using potassium hydroxide (0.85 g.) in methanol (13 ml.). The product was recrystallized from alcohol; yield 0.4 g. (33%), m.p. 184–185° dec. (lit.[6] 186–187°).

The dibromide, α,α'-dibromo-4′-chloro-4-nitrobibenzyl was obtained by the method of Buckles, Hausman and Wheeler[7] and recrystallized from ethanol; yield 13%, m.p. 195–206° dec.

Anal. Calcd. for $C_{14}H_{10}NO_2Br_2Cl$: C, 40.08; H, 2.40. Found: C, 40.14; H, 2.69.

α-Chloro-4′-methyl-4-nitrobibenzyl.—The procedure of Koelsch[8] was used for the reaction of *p*-methylbenzenediazonium chloride with *p*-nitrostyrene. The coupling product was fractionated using a mercury vapor pump. The fraction (150–180°, oil-bath temperature), obtained by distillation with an alembic type flask, was redistilled using a Hickman vacuum still to give 5.0 g. (9%) of an orange viscous liquid which boiled at 122–123° (1 mm.) (oil-bath temperature).

Anal. Calcd. for $C_{15}H_{14}NO_2Cl$: C, 65.33; H, 5.12. Found: C, 65.28; H, 4.78.

α-Chloro-4,4′-dinitrobibenzyl.—Using the procedure of Brunner and Kustatscher,[4] 14.9 g. (0.1 mole) of *p*-nitrostyrene was caused to react with a solution of *p*-nitrobenzenediazonium chloride. The product was a dark brown oil. Repeated recrystallizations from alcohol yielded white needles which melted at 133–134° dec., yield 1.2 g. (4%).

Anal. Calcd. for $C_{14}H_{11}N_2O_4Cl$: C, 54.82; H, 3.62. Found: C, 55.12; H, 4.01.

4,4′-Dinitrostilbene.—Dehydrohalogenation of α-chloro-4,4′-dinitrobibenzyl was carried out with methanolic potassium hydroxide as described above. After recrystallization from dioxane, the product melted at 291–292° dec. (lit.[6] 294–295°), yield 59%.

1-Chloro-1-(2-pyridyl)-2-(4-chlorophenyl)-ethane.—*p*-Chlorobenzenediazonium chloride, prepared from 12.7 g. (0.1 mole) of *p*-chloroaniline, was caused to react as described above with 10.5 g. (0.1 mole) of freshly distilled 2-vinylpyridine. The dark brown oil which separated after steam distillation could not be obtained as a crystalline material. However, when the product was distilled under reduced pressure, 5.0 g. (20%) of a yellow viscous oil was obtained, b.p. 150–158° (1 mm.). Carbon and hydrogen values indicated that some dehydrohalogenation had occurred during the distillation.

4′-Chloro-2-stilbazole.—The above product was dehydrohalogenated as described earlier. White platelets were obtained on crystallization from alcohol; yield 61%, m.p. 82.5–83° (lit.[9] 83–84°).

1-Chloro-1-(2-pyridyl)-2-(*p*-tolyl)-ethane.—The product from the Meerwein coupling of *p*-methylbenzenediazonium chloride with 21.4 g. (0.2 mole) of 2-vinylpyridine was a dark oil and it was necessary to distil the product in a manner similar to that described for α-chloro-4′-methyl-4-nitrobibenzyl. The fraction obtained at 127–133° (1 mm.) (bath temperature), when redistilled in a Hickman still, yielded 24.8 g. (54%) of an orange liquid, b.p. 123–124° (1 mm.) (bath temperature). Carbon and hydrogen values indicated that some dehydrohalogenation had occurred during the distillation.

4′-Methyl-2-stilbazole.—A sample of the above 1-chloro-1-(2-pyridyl)-2-(*p*-tolyl)-ethane (1.6 g., 0.006 mole) was dehydrohalogenated with alcoholic potassium hydroxide. The 4′-methyl-2-stilbazole melted at 83–84° (lit.[10] 87°), yield 1.1 g. (82%).

1-Chloro-1-(2-pyridyl)-2-(4-nitrophenyl)-ethane.—When 10.5 g. (0.1 mole) of 2-vinylpyridine was caused to react with *p*-nitrobenzenediazonium chloride, a dark brown oil was isolated from the reaction mixture. Treatment with alcohol and recrystallization of the solid which separated gave 4.0 g. (15%) of white needles, m.p. 100.5–101° dec.

Anal. Calcd. for $C_{13}H_{11}N_2O_2Cl$: C, 59.43; H, 4.22. Found: C, 59.12; H, 4.32.

4′-Nitro-2-stilbazole.—1-Chloro-1-(2-pyridyl)-2-(4-nitrophenyl)-ethane (3.0 g., 0.011 mole) was dehydrohalogenated with alcoholic potassium hydroxide to give the correspond-

(1) Based on a thesis submitted August, 1952, by C. M. I. to the Graduate School of the University of Missouri in partial fulfillment of the requirements for the M.A. degree.

(2) H. B. Hass and M. L. Bender, THIS JOURNAL, **71**, 3482 (1951).

(3) W. E. Doering and R. A. N. Weil, *ibid.*, **69**, 2461 (1947).

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(7) R. E. Buckles, E. A. Hausman and N. G. Wheeler, THIS JOURNAL, **72**, 2494 (1950).

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ing stilbazole. After several recrystallizations from alcohol, 1.1 g. (45%) of the stilbazole was obtained which melted at 133–133.5° dec. (lit.[11] 136°).

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Nature of the Acetyl Cholinesterase Surface. II. The Ring Effect in Enzymatic Inhibitors of the Substituted Ethylenediamine Type[1]

BY S. L. FRIESS AND W. J. MCCARVILLE

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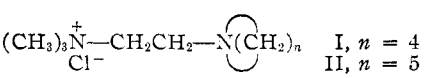
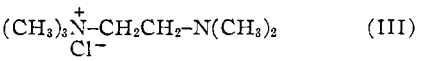
In a recent study[2] it was observed that compounds of the general type



$(CH_3)_3\overset{+}{N}(Cl^-)-CH_2CH_2-N(CH_2)_n$ I, $n = 4$; II, $n = 5$

are potent competitive inhibitors of the catalyzed hydrolysis of acetylcholine (AC) by the enzyme acetylcholinesterase (AChE). A general interpretation of the high order of activity of these inhibitors, comparable to or greater than that shown by the powerful anticholinesterase eserine, was based on the duality of catalytic enzymatic sites proposed by Nachmansohn and co-workers,[3] and the added working stipulation that the molecular region attracted to the so-called "esteratic" site on the enzyme is exclusively nucleophilic in its properties.[4]

The variation of enzyme–inhibitor dissociation constants with the size of the heterocyclic ring[2] in compounds I and II (K_I = 2.3 and 1.6 × 10^{-8}, respectively, at *p*H 7.4 and 25°) has now led to the study of the effectiveness of III as an AChE inhibitor.



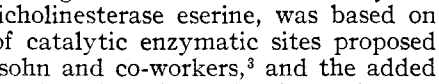
$(CH_3)_3\overset{+}{N}(Cl^-)-CH_2CH_2-N(CH_3)_2$ (III)

In compound III the constraint imposed on the valence angles about the ring N atom in I and II has been removed by cleavage of the ring, leaving a pair of methyl groups attached to this tertiary nitrogen center of high electron density.

The net result of this structural change is a marked diminution in the ability of III to compete with substrate for the catalytic sites on the enzyme, as compared with I and II. Under the standard conditions previously employed,[2] the value of the enzyme—inhibitor dissociation constant (K_I) deduced from the linear plot of the Wilson equation[5] was found to be (8.3 ± 0.6) × 10^{-8}. Using K_I values under comparable conditions as an index of the binding capacity of inhibitors for enzyme, this would imply that III is less effective than compounds I and II by factors of 3.5 and 5.2, respectively.

This decrease in inhibitory activity resulting from ring cleavage could arise from several structural features. First, the somewhat greater steric requirements of the $-\ddot{N}<$ function in III, as compared with I and II where the ring *o*-methylene groups are held back under greater restraint, might be approaching the limit of the effective dimensions of the cavitation available at the "esteratic" site. Secondly, it is possible that van der Waals interaction of the entire cyclic moiety of I and II with the site and its environs supplements the previously postulated electrostatic interaction of the localized high electron density function $-\ddot{N}<$ with the site to give increased strength of bonding over that shown by III. Both of these views assume direct interaction of the tertiary end of the inhibitor molecule with the surface of the site, and make no allowance for an intermediary spatial role of the Mg^{++} ion[2] at this point.

In the course of work on III, the chloro derivative of choline



$(CH_3)_3\overset{+}{N}(Cl^-)-CH_2CH_2Cl$ (IV)

came to hand as a synthesis intermediate. Since its primary chloro function also fulfills the condition of serving as a localized region of high electron density, this compound too was tested for its inhibitory power on AChE. It proved to be a surprisingly effective competitive inhibitor, with a K_I value (at *p*H 7.0 and 25.12°) of 12 × 10^{-8}. This makes it less potent than the series I, II, III and eserine, but more effective than the prominent anticholinesterase prostigmine (K_I = 16 × 10^{-8}).[3]

This relatively high level of activity of IV lends further support to the primary assumption that the non-quaternized end of the $Me_3\overset{+}{N}-CH_2CH_2$ chain need only possess a central locus of high electron density to complete the requirements for effective bonding at the esteratic site and produce a potent competitive inhibitor.

Experimental[6]

The known chloro derivative of choline chloride was prepared in standard fashion from crystalline choline chloride and Eastman thionyl chloride. The product was recrystallized repeatedly from methanol–ether mixture as the anhydrous salt, at the temperature of a Dry Ice–methanol-bath. *Anal.* Calcd. for $C_5H_{13}NCl_2$: N, 8.86. Found: N, 8.89.

Compound III was prepared by heating the chloro derivative above with approximately a fivefold excess of anhydrous dimethylamine, in a glass bomb at 100°, for a period of 24 hours. The salt remaining after evaporation of the excess amine was recrystallized repeatedly from a 1:1 methanol–ether mixture containing several drops of concd. hydrochloric acid per liter. It proved to be relatively hygroscopic; dec. above 235°. *Anal.* Calcd. for $C_7H_{19}N_2Cl\cdot HCl$: N, 13.79. Found: N, 13.97.

Enzymatic Rate Determinations.—The stock enzyme preparation used was the highly-purified one previously prepared[2] from electric eel tissue. Dilutions of the stock were made just prior to use in the kinetic determinations.

(1) Presented in part before the Division of Biological Chemistry, Meeting of the American Chemical Society, Kansas City, Mo., March, 1954. The opinions in this paper are those of the authors and do not necessarily reflect the views of the Navy Department.

(2) S. L. Friess and W. J. McCarville, THIS JOURNAL, **76**, 1363 (1954).

(3) See for example the review by D. Nachmansohn and I. B. Wilson, *Advances in Enzymol.*, **12**, 259 (1951).

(4) This constitutes a departure from the conclusion of I. B. Wilson that a basic group in the esteratic site is responsible for the nucleophilic binding to a relatively *positive* center (such as a carbonyl carbon) on a substrate or inhibitor. See I. B. Wilson, *J. Biol. Chem.*, **197**, 215 (1952).

(5) P. W. Wilson, "Respiratory Enzymes," H. A. Lardy, ed., Burgess, Minneapolis, Minn., 1949, p. 24.

(6) Analyses by courtesy of Dr. W. C. Alford, Microanalytical Laboratory, National Institute of Arthritis and Metabolic Diseases, Bethesda, Md.