1242 (1971).
(11) K. Rezabek, M. Semonsky, and N. Kucharczyk, *Nature (London)*, **221**, 666 (1969).
(12) R. M. Macleod and J. E. Lehmeyer, *Endocrinology*, **94**, 1077 (1974).
(13) E. B. Smalstig, B. D. Sawyer, and J. A. Clemens, *Endocrinology*, **95**, 123 (1974).
(14) T. W. Stone, *Arch. Int. Pharmacodyn. Ther.*, **202**, 62 (1973).
(15) K. Fuxe, H. Corrodi, T. Hokfelt, P. Lidbrink, and U. Ungerstedt, *Med. Biol.*, **52**, 121 (1974).
(16) L. Pieri, M. Piere, and W. Haefely, *Nature (London)*, **252**, 586 (1974).
(17) N. J. Bach, D. A. Hall, and E. C. Kornfeld, *J. Med. Chem.*, **17**, 313 (1974).
(18) S. H. Snyder and C. R. Merril, *Proc. Natl. Acad. Sci. U.S.A.*, **54**, 258 (1965).
(19) S. Kang and J. P. Green, *Nature (London)*, **226**, 645 (1970).
(20) M. E. Speeter and W. C. Anthony, *J. Am. Chem. Soc.*, **76**, 6208 (1954).
(21) C. F. Barfknecht, D. E. Nichols, D. B. Rusterholz, J. P. Long, J. A. Engelbrecht, J. M. Beaton, R. J. Bradley, and D. C. Dyer, *J. Med. Chem.*, **16**, 804 (1973).
(22) P. E. Norris and F. F. Blicke, *J. Am. Pharm. Assoc.*, **41**, 637 (1952).
(23) J. G. Cannon, J. C. Kim, M. A. Aleem, and J. P. Long, *J. Med. Chem.*, **15**, 348 (1972).

Improved Delivery through Biological Membranes. 1. Synthesis and Properties of 1-Methyl-1,6-dihydropyridine-2-carbaldoxime, a Pro-Drug of *N*-Methylpyridinium-2-carbaldoxime Chloride†,1

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A dihydropyridine–pyridine type redox pro-drug system was developed for delivering quaternary pyridinium salts through biological membranes. As a first application, the dihydropyridine derivative of *N*-methylpyridinium-2-carbaldoxime chloride (2-PAM) was synthesized using a reduction–addition–elimination sequence. The dihydro-2-PAM obtained has all the required properties for an effective transport through lipoidal barriers and it reverts easily back to 2-PAM as a result of a chemical or enzymatic oxidation process.

The movement of molecules through biological barriers requires passage of the molecular species from one aqueous environment to another aqueous compartment via the cell membrane. It is generally accepted that the ability of substances to penetrate cell membranes can be correlated with their relative solubilities in aqueous and nonaqueous solvents. Molecules which have a high affinity for an aqueous environment find it difficult to enter the proposed lipid interior of the membrane. Therefore, ionic species encounter the most difficulty penetrating various biological membranes. The absorption of many weak organic acids and bases is explicable in terms of the diffusion of only the un-ionized form across the lipoidal barrier. However, the ionic quaternary pyridinium and/or ammonium salts, unless they are small, can be absorbed through a biological membrane, such as the gastrointestinal wall, only as a complex with some endogenous substance[2] or as a lipid-soluble ion pair.[3]

The boundary between the plasma and the central nervous system compared to the boundary between the plasma and other tissue organs is very impermeable to a wide variety of water-soluble, lipid-insoluble compounds. This barrier to highly polar species has been termed the blood–brain barrier (BBB). The endothelial cells in the brain capillaries appear to be joined by continuous, tight, intercellular junctions.[4] Therefore, the molecules which cross this barrier must pass through the cells rather than between them. There exist a few areas in the brain where the capillaries are not tightly linked which may provide a route for small amounts of a hydrophilic drug to enter the brain. It is generally accepted that the ability of the molecule to pass the choroidal cells or capillary endothelial cells is a function of the lipid–water partition ratio for the molecule as described for other biological membranes. If the chemical is lipid insoluble, it will not appreciably cross the cell membrane unless it is among a group of compounds which are actively transported into the CNS, such as certain sugars and amino acids.[5]

Although it is extremely difficult for a relatively hydrophilic quaternary pyridinium salt to penetrate biological barriers, such as the blood–brain barrier, in certain instances it is extremely important to efficiently deliver through these barriers quaternary ammonium and/or pyridinium salts. For example, overcoming serious poisoning with anticholinesterase agents, such as organophosphates, is a great and increasing problem.[6,7] While pyridinecarbaldoxime type quaternary salts are the best of the blocked acetylcholinesterase (AChE) reactivating agents, among which *N*-methylpyridinium-2-carbaldoxime salt (2-PAM) is the drug of choice, the quaternary pyridinium structure of 2-PAM is one of the most disadvantageous in terms of delivery across biological membranes. The order of magnitude for the protection against an organophosphate poison conferred by 2-PAM in vivo was only a small fraction of that expected from in vitro studies of reactivation of AChE.[8] This observation has been attributed largely to the physiological distribution of 2-PAM based on its polarity. This aspect is especially important since most organophosphate poisons are very lipid-soluble and readily penetrate the CNS. Consequently, these agents efficiently deactivate the CNS acetylcholinesterase. Although 2-PAM is useful in overcoming the effects of anticholinesterase agents, its utility suffers in terms of (1) its unfavorable distribution (almost exclusively in the plasma), (2) its poor retention in the body (fast elimination, short biological half-life), (3)

† Dedicated to the memory of Professor Edward E. Smissman.

its inability to appreciably cross the blood-brain barrier, and (4) its poor oral bioavailability.

A number of compounds related to 2-PAM have been synthesized in an attempt to overcome the disadvantages of 2-PAM. In order to improve the lipid solubility of 2-PAM, its dodecyl analog, the dodecylpyridinium-2-carbaldoxime iodide (2-PAD) was synthesized.[9] Although this derivative was much more soluble in chloroform than 2-PAM, its activity in vitro was found to be only one-third that of 2-PAM. In vivo studies have confirmed that 2-PAD is significantly less active than 2-PAM. The tetrahydropyridine derivatives of 2-, 3-, and 4-PAM were also synthesized.[10] It was the hypothesis that these tertiary amine derivatives would penetrate the CNS without difficulty and, after penetration, the nitrogen would mainly be in the protonated form at physiological pH. Therefore, the molecule would possess the positively charged center apparently required for reactivation capability. However, the pK_a of the oxime function in these compounds was raised to approximately 11. This pK_a value results in essentially no ionization of the oxime function in physiological conditions. The overall effect is that all of these derivatives essentially lack the protective ability against, for example, TEPP intoxication.

A recent work[11] reported that in vitro transport of 2-PAM across the small intestine of the rat was significantly increased by sodium dodecylsulfate. This observation was apparently due to some ion-pair formation. However, this approach would not effect the transport of the drug into the CNS.

In our search for a better cholinesterase reactivator, we did not look for a new drug but considered the problem as a specific delivery problem of 2-PAM itself. Therefore, we intended to synthesize a pro-drug[12] of 2-PAM which does not contain a positively charged nitrogen atom and as such can pass the various biological membranes such as the BBB and the gastrointestinal wall. Knowing the ease with which dihydropyridine derivatives are oxidized to the pyridine,[13] we have considered the dihydropyridine derivative of 2-PAM as the most desirable pro-drug form. The NAD–NADH coenzyme system is only one example of this well-known type of in vivo redox system.

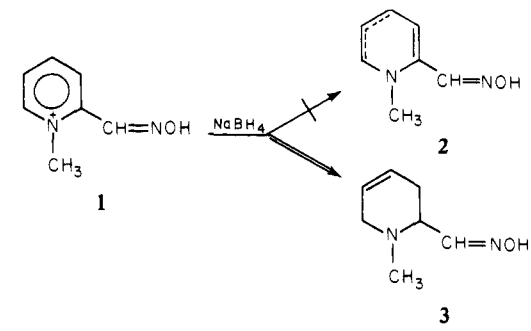
Results and Discussion

There are two basic synthetic approaches which can be utilized to prepare the dihydropyridine ring system. The first one is based on the original Hantzsch[14] synthesis. Following this procedure, the total synthesis of the dihydropyridine involves the reaction between an aldehyde, an active methylene compound, and a primary amine. A 1,4-dihydropyridine derivative is usually the product of this synthetic procedure. The second approach to the preparation of the dihydropyridine system involves the direct reduction of the corresponding pyridine or pyridinium compound using complex metal hydrides[13,15] or sodium dithionite.[16] We have considered that the direct reduction of the pyridinium system using sodium borohydride would be the most attractive and suitable procedure for our purpose.

Based on these considerations, the reduction of 2-PAM (1) with sodium borohydride was investigated as a route to the 1-methyldihydropyridine-2-carbaldoxime (2) using a modification of the procedure described by Kinoshita[17] (Scheme I).

Following this procedure, the product of the reaction was invariably the 1-methyl-1,2,3,6-tetrahydropyridine-2-carbaldoxime (3). The identity of the reaction product as a tetrahydro derivative of 1 was confirmed by comparison with the tetrahydro reduction product of 1 using an excess

Scheme I



of sodium borohydride. The structure of the reaction product as 3 was established on the basis of its ir, uv, and NMR spectra. The position of the carbon-to-carbon double bond as Δ^4 was assigned primarily on the basis of its NMR spectrum. The absence of the β-CH_2 proton signals characteristic for Δ^3-tetrahydropyridines at approximately 2.1 ppm suggested the reaction product to be the Δ^4-tetrahydropyridine derivative 3. The dihydropyridine derivative 2 could not be obtained even when the reduction of 1 was conducted using a significantly greater concentration of base.

In an alternative approach to the preparation of 2, the 2-pyridinemethanal diethyl acetal methiodide (4) was prepared and subsequently reduced with sodium borohydride. The dihydropyridine derivative obtained was identified as the 1,6-dihydro reduction product 5. 5 was a relatively stable product although it could be easily oxidized to its pyridinium precursor 4 by hydrogen peroxide, iodine, silver nitrate, or other suitable oxidizing agents (Scheme II).

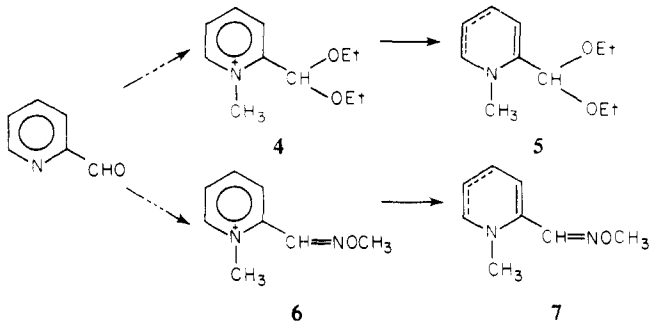
The reduction of O-protected oxime derivatives of 1 was also investigated. The O-methyl oxime derivative 6 was prepared and its reduction using sodium borohydride was examined. The reduction product isolated was a quasi-stable oil which was identified as the 1,6- or 1,4-dihydropyridine (7) based on interpretation of its ir, uv, and NMR spectra. Attempts to prepare 2 via the dihydro derivatives 5 and 7 were unsuccessful.

Using a modification of the procedure described by Fry,[18] 2 was successfully prepared. The method is based on the observation that the dihydropyridines initially formed by reduction with sodium borohydride are capable of reversible or irreversible nucleophilic additions to the double bond nearest to the nitrogen atom. This addition reaction is mechanistically analogous to the reduction of the dihydro system to the tetrahydro derivative. Thus, in the reduction of 3,5-lutidines[18] with a solution of sodium borohydride containing a large excess of cyanide ion, the cyanide ion was an effective competitor for the second active site in the initially formed dihydropyridine. Analogously, the hydrogen cyanide efficiently added to the dihydropyridine system formed after reduction of 1. More importantly, due to the reversible nature of this addition reaction, the tetrahydro product 8 could be considered a "masked" dihydropyridine. The appearance of the oxime methine proton signal as a singlet suggested the cyano group was introduced at C-2. Further, the complexity observed for the olefinic proton signals suggested that the product has a Δ^4 double bond (8) rather than a Δ^3 double bond (9).

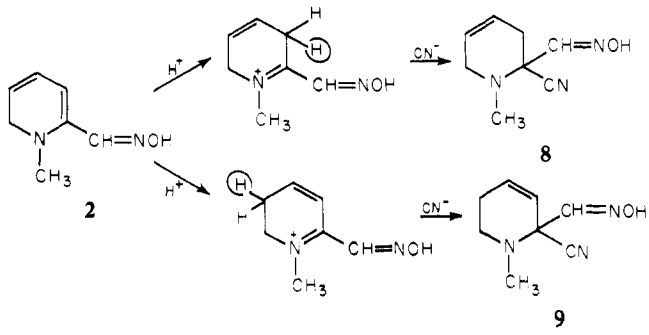
The position of the carbon-to-carbon double bond in the tetrahydrocyano product is essentially determined by the site of the nucleophilic addition of the cyanide ion (Scheme III).

Electrophilic additions of a proton to the dienamine

Scheme II

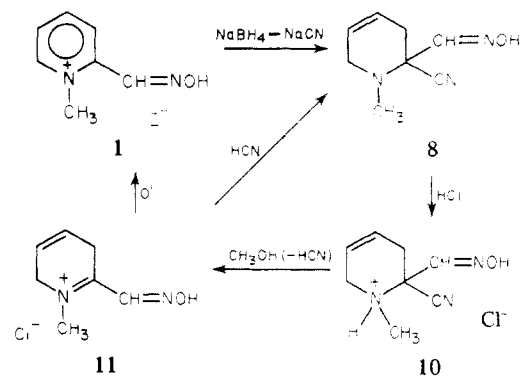


Scheme III



system of the initially formed dihydropyridine could occur at the terminal carbon atom of the central double bond (C-3) to give **8** or at the end carbon atom of the system (C-5) to give **9**. Lyle[19] has demonstrated that protonation of dienamines follows Ingold's rule;[20] using weak acids, protonation of the dienamine system gives the kinetically controlled product resulting from addition of the proton to the central carbon atom of the dienamine system. On the other hand, protonation of the dienamine system using strong acids allows equilibration of the kinetically formed product to the thermodynamically favored isomer. Therefore, the product obtained under these conditions is that resulting from addition of the proton to the terminal carbon atom. In order to effectively compete with the hydride reduction, the addition of the cyanide ion to the protonated dienamine system must be of comparable rate. On this basis, it is assumed that the addition of the cyanide ion to the protonated dienamine system must occur prior to any equilibration of the kinetically formed protonated product to the thermodynamic isomer. Therefore, addition of the proton was at C-3 and, consequently, the tetrahydrocyano derivative is **8**. Since the free base of **2** is too unstable to be isolated, **8** was transformed into a proton salt **10** in order to protect the system during and after elimination of hydrogen cyanide (HCN). While dihydro derivatives have been obtained[18] by elimination of HCN from the corresponding tetrahydrocyano adduct only under strongly alkaline or acidic conditions, we have found that use of the salt form of the corresponding tetrahydrocyano derivative allows elimination of HCN under essentially neutral conditions. While **10** has no absorption in the ultraviolet region (220–350 nm), a methanolic solution of **10** showed the appearance of an absorption band at 251 nm. This observation indicated the formation of a dihydro system via elimination of HCN from **10**. The progress and extent of the elimination reaction could be followed by monitoring the increase in this absorption maximum. Although the exact mechanism of the elimination is not entirely clear, it would appear that the elimination of HCN is simply the reverse reaction of the addition of HCN to

Scheme IV

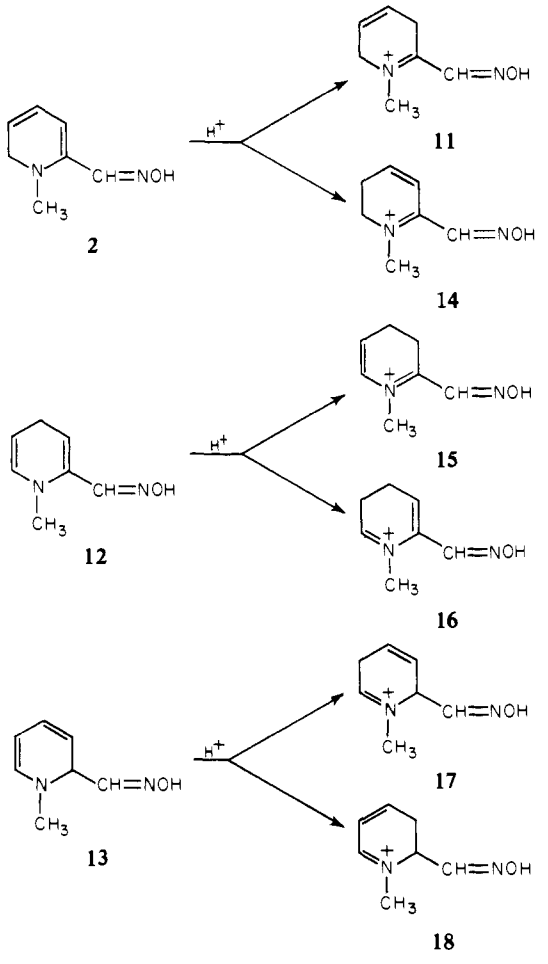


the initially formed dihydropyridine system. Further, since the addition of excess acid to a methanolic solution of **10** significantly decreases the elimination rate, this observation suggests that the elimination of HCN from **10** occurs via the free base **8**. In any case, the product **11** obtained is the hydrochloride salt of **2**. Support for the mechanism and the successive reactions proposed (Scheme IV) was obtained by reaction of **11** with HCN under the identical reaction conditions used to reduce **1**, with the exception that sodium borohydride was absent in the reaction medium. Following this procedure, **8** was isolated in essentially quantitative yield.

In order to establish the merits of the dihydro derivative as a useful pro-drug of 2-PAM, the physical and chemical properties of pro-2-PAM (**11**) were investigated. Initially, the pK_a of the tertiary amino group in **11** was determined by potentiometric titration methods. The pK_a value obtained for this group, 6.32 ± 0.06, was confirmed using an independent spectrophotometric method. The pK_a of the oxime function in **11** was also estimated to be between 10 and 11. **11** was indefinitely stable in the neat state and slightly acidic solutions of **11** exhibited moderate stability. In solutions of a pH equal to or greater than the pK_a of the tertiary amine, **11** underwent a rapid degradation. The reaction of **11** with a variety of oxidizing agents was also investigated. Invariably, oxidation of **11** resulted in the quantitative formation of 2-PAM (**1**). Based on the fact that at physiological pH **11** would exist as its free base, which is significantly more lipoidal than its salt or 2-PAM, and the fact that **11** can be quantitatively oxidized to 2-PAM, suggested the attractive and useful properties of **11** as a pro-drug of 2-PAM.

Since both the 1,2-dihydro and 1,4-dihydro derivatives of *N*-substituted dihydropyridines are known, our subsequent investigations were directed toward establishing the exact structure of the dihydro system of pro-2-PAM. Studies on the hydrogen-transfer reactions[21] and the equilibrium[22] between the 1,2- and 1,4-dihydropyridine isomers indicated that the 1,4-dihydro system was thermodynamically more stable than the corresponding 1,2-dihydro system. A recent quantitative study[23] demonstrated that *N*-methyl-1,4-dihydropyridine is more stable than *N*-methyl-1,2-dihydropyridine, $\Delta G^{91.6°} = 2.29$ kcal/mol. The increased stability was interpreted in terms of stabilization of the 1,4-dihydropyridine system by a "favorable electronic interaction" such as homoaromaticity and heteroconjugation. Substitutents α or β to the nitrogen in the dihydropyridine will obviously increase the number of possible dihydro isomers, the 1,2-, 1,4-, and 1,6-dihydro derivatives. On this basis, the reduction of **1** with sodium borohydride can produce three possible dihydropyridine isomers (Scheme V). These dihydro-

Scheme V



pyridine isomers have an enamine or a dienamine[24] structure. Therefore, protonation of these systems can occur at C-5 or at C-3.[25] In principle, each dihydropyridine base can form two isomeric proton salts. The 1,6-dihydro isomer **2** can form the 3,6- and the 5,6-dihydropyridinium salts **11** and **14**, respectively. On the other hand, the 1,4-dihydro isomer **12** can form the 3,4- and 4,5-dihydropyridinium salts **15** and **16**, respectively, while the 1,2-dihydropyridine **13** can form the 2,5- and 2,3-dihydropyridinium salts **17** and **18**, respectively. The method used to prepare the dihydropyridine system eliminates **13** and, therefore, its corresponding proton salts **17** and **18** as the isomer formed in the reduction of **1**. In addition, formation of **13** requires attack of the borohydride anion at C-2 which is sterically hindered by the 2-carbaldoxime group. Formation of the dihydropyridine system **12** would suggest the observation of two separate absorption bands in the ultraviolet spectrum due to the two cross-conjugated chromophores present: the 1,4-dihydropyridine and the conjugated oxime. Since the ultraviolet spectrum of pro-2-PAM in acidic solution is consistent with an α,β-unsaturated carbaldoxime,[26] while its ultraviolet spectrum at pH 9 is evident of only one conjugated system, **11** is the only dihydro isomer consistent with these observations. The molar absorptivity of the ultraviolet absorption spectrum of pro-2-PAM is also consistent with the dihydro isomer **11**. Analysis of the infrared and proton magnetic resonance spectra of pro-2-PAM further supports the dihydro isomer **11**. The presence of the absorption bands at 1660 and 1700 cm^{-1} and the lack of the ammonium absorption at 2500 cm^{-1} are consistent with the immonium structure of a 3,6-dihydropyridinium salt.[27] The proton magnetic resonance spectrum of pro-2-PAM in trifluoroacetic acid is consistent with the presence of a C=N^+ bond, as well as the presence of an isolated Δ^4 double bond by comparison with analogous systems.[28] When the NMR spectrum of pro-2-PAM was determined in a dilute solution of DCl in D_2O, the protons on the β-carbon were absent in the spectrum. The disappearance of this proton signal is a result of H–D exchange which supports our previous conclusion that the carbon atom β to the nitrogen was the site of protonation.

The 1,4-dihydro derivative of pyridine has been described[13] as being very unstable when exposed to oxygen or other oxidizing agents. The 1,2-dihydro derivative was even more reactive, preventing its isolation. Generally, the dihydropyridines are strong electron donors and this property may explain their ease of oxidation, as well as their ability to form stable π complexes with chromium.[29] Substitutents at C-3 or C-5 which are conjugated to the dienamine system of dihydropyridines lower[30] the energy of the HOMO of the dihydropyridine system. Therefore, their reactivity toward oxidation and complexation is decreased due to the more extensive delocalization of the π-electron density.

The instability of **2** and its reactions with various oxidizing agents has already been considered. Additional stability studies on pro-2-PAM (**11**) were conducted in order to assess the extent of degradation, if any, for aqueous solutions of pH 1 and 6 in the absence of oxygen or other oxidizing agents. The ultraviolet absorbance at 251 nm for a solution of **11** at pH 1 was reduced only 2% after 24 hr. It is known that enamines[31] and cyclic enamines[32] undergo addition of water in aqueous solution through the immonium form. It has also been demonstrated that the primary modification of NADH, a 1,4-dihydropyridine derivative, by acid involves the addition of water to one of the double bonds of the dihydropyridine.[33] Contrary to these observations, **11** was relatively stable in acidic solution. This behavior may be explained by the steric hinderance of the carbaldoxime group at C-2 which interferes with addition of water to the dihydropyridine. However, a rapid change in the ultraviolet spectrum of an aqueous solution of **11** at pH 6 was observed. Considering that the pK_a of the tertiary amine in **2** is 6.32 and the instability of **2**, this observation was expected.

Experimental Section

Melting points were taken in a Thomas-Hoover capillary melting point apparatus and were uncorrected. Elemental analyses were performed at the Department of Medicinal Chemistry, University of Kansas.

Infrared spectra were determined using a Beckman IR-33 instrument using KBr pellets for solid samples or, in the case of liquids, the neat material was compressed between two NaCl disks.

Proton magnetic resonance spectra were determined using a Varian T-60 spectrometer operating at 34°C probe temperature. All chemical shifts reported are in parts per million (ppm) relative to tetramethylsilane as the internal standard. In deuterium oxide the internal standard was sodium trimethylsilylpropanesulfonate.

Ultraviolet absorbance spectra were determined using Cary Model 14 or 15 spectrophotometers.

1-Methyl-1,2,3,6-tetrahydropyridine-2-carbaldoxime (3). To a solution of 10.6 g (0.04 mol) of 2-pyridinecarbaldoxime methiodide (2-PAM) in 200 ml of methanol, 7.6 g (0.2 mol) of sodium borohydride was introduced in small portions with stirring at a temperature of 20°. After addition of the sodium borohydride, the solution was stirred for an additional 45 min at 25°. Most of the methanol was then evaporated under vacuum, and the residue was dissolved in 100 ml of water. The aqueous solution was saturated with Na_2CO_3 and extracted with ether. The etheral

layer was dried over anhydrous Na_2SO_4 and the solvent was removed under vacuum. The remaining solid was recrystallized from a mixture of benzene-*n*-heptane: yield 4.2 g (75%); mp 105–107°; ir (KBr) 3170 (OH), 1650 (C=N), and 930 cm^{-1} (NO); NMR (acetone-d_6) δ 7.37 (d, 1, HC=N), 5.73 (m, 2, *HC=CH*), 2.95–3.33 (m, 5, CH_2, CH), and 2.30 (s, 3, CH_3N). Anal. ($C_7H_{12}N_2O$) C, H, N.

Pyridine-2-methanal Diethyl Acetal (19). To 200 ml of absolute ethanol saturated with HCl, 107 g (1 mol) of 2-pyridinecarboxaldehyde and then 445 g (3 mol) of freshly distilled triethyl orthoformate were added. The solution was heated under reflux for 2 hr and then cooled and neutralized with 200 ml of 30% ethanolic KCl by filtration. The filtrate was distilled under vacuum (5 mmHg) and the fraction that distilled at 100–105° was collected: yield 130 g (71.8%); ir (neat) 1070 cm^{-1} (COC); NMR (CCl_4) δ 8.27, 7.63–6.80 (m, 4, pyridine ring protons), 5.23 (s, 1, CHO_2), 3.33 (m, 4, CH_2O), and 0.93 (t, 6, CH_3). Anal. ($C_{10}H_{15}NO_2$) C, H, N.

2-Pyridinemethanal Diethyl Acetal Methiodide (4). To 127 g (0.7 mol) of **19**, 199 g (1.4 mol) of methyl iodide was added. The mixture was heated under reflux for 1.5 hr and kept overnight at room temperature. Upon the addition of ether to the mixture a yellow solid precipitated. This was filtered, washed carefully with ether, and recrystallized from ethanol: yield 200 g (88.5%); mp 94–96°; uv max (H_2O) 263 nm; ir (KBr) 1050 cm^{-1} (OCO); NMR (D_2O) δ 8.93–8.07 (m, 4, pyridine ring protons), 5.97 (s, 1, CHO_2). Anal. ($C_{11}H_{18}INO_2$) C, H, N.

1-Methyl-1,6-dihydropyridine-2-methanal Diethyl Acetal (5). In 250 ml of absolute ethanol, 12.8 g (0.32 mol) of sodium hydroxide was dissolved. To this basic solution was added 3.2 g (0.09 mol) of sodium borohydride and the solution was cooled to 2–4°. To the solution, 51.7 g (0.16 mol) of 4 was added in one portion and the mixture was stirred for 1 hr at 2°. The ethanol was distilled at reduced pressure. The residue was mixed with 150 ml of ice–water and was extracted with three portions of 150 ml of freshly distilled ether. The combined ether layers were dried over anhydrous calcium chloride and the ether was evaporated in vacuo. Distillation under reduced pressure in a nitrogen atmosphere gave the product as a yellow liquid: yield 17.5 g (55.5%); bp 96–97° (4 mmHg); uv max (H_2O) 325 nm; ir (neat 1640, 1520 (C = C), and 1030 cm^{-1} (OCO).

Pyridine-2-carboxaldehyde Methiodide (20). Compound **20** was synthesized according to the method of Steinberg et al.[34] Pyridine-2-carboxaldehyde, 21.4 g (0.2 mol), was dissolved in 50 ml of dry acetone and 84.6 g (0.6 mol) of methyl iodide was added. The solution was placed in a pressure bottle and kept at 60° overnight. The product, **20**, precipitated as yellow crystals: yield 30 g (60%); mp 178–180° dec (lit.[34] 180–183° dec); ir (KBr) 1690 cm^{-1} (C=O); NMR (D_2O) δ 8.87–8.10 (m, 4, pyridine ring protons), 6.43 (s, 1, HC=O), and 4.47 (s, 3, CH_3N).

***O*-Methylpyridine-2-carbaldoxime Methiodide (6).** Methoxylamine hydrochloride, 5 g (0.06 mol), was dissolved in 50 ml of methanol, and the solution was neutralized to pH 6 with 1 *M* methanolic KOH. To the filtered solution was added 7.5 g (0.03 mol) of **20** and the mixture was heated under reflux for 4 hr. The methanol was evaporated and the solid obtained was recrystallized from methanol–ether: yield 7 g (85%); mp 144–145°; uv max (H_2O) 295 and 225 nm; ir (KBr) 1000 (NO) and 900 cm^{-1} (OCH_3); NMR (D_2O) δ 8.83–7.97 (m, 5, pyridine ring protons and CH=N), 4.37 (s, 3, CH_3N), and 4.13 (s, 3, CH_3O). Anal. ($C_8H_{11}IN_2O$) C, H, N.

1-Methyl-2-cyano-1,2,3,6-tetrahydropyridine-2-carbaldoxime (8). To a solution of 76 g (1.55 mol) of sodium cyanide in 300 ml of freshly distilled water saturated with nitrogen was added 114 ml of 18% hydrochloric acid. The aqueous solution was layered with 1 l. of freshly distilled ether and cooled to 15°. To the cold mixture was added 100 g (0.38 mol) of 2-pyridinecarbaldoxime methiodide. Then, 19 g (0.495 mol) of sodium borohydride was introduced and the solution was warmed to 25° and kept at that temperature for 5 hr with vigorous stirring. After separating the ether layer, the aqueous layer was filtered and the precipitate was extracted with ether. The combined ether solutions were evaporated. The product was purified by washing with a 30% mixture of ether in cyclohexane: yield 44.5 g (71%); mp 112–115° dec; ir (KBr) 3180 (OH), 2360 (C=N), 1660 cm^{-1} (C=N): NMR (acetone-d_6) δ 7.35 (s, 1, HC=N), 5.83 (m, 2, HC=CH), 2.50–3.05 (m, 4, CH_2, CH_2), and 2.30 (s, 3, CH_3N). Anal. ($C_8H_{11}N_3O$) C, H, N.

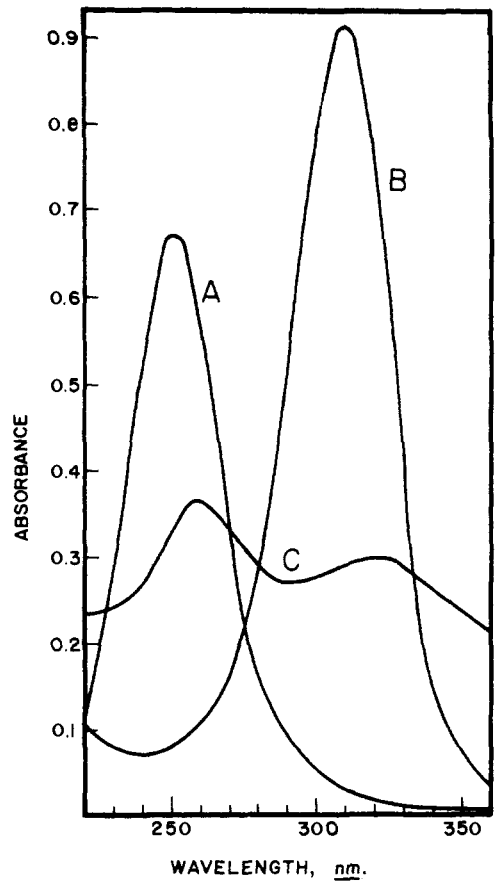


Figure 1. Ultraviolet spectra of 1-methyl-1,6-dihydropyridine-2-carbaldoxime (pro-2-PAM) in 0.1 *N* HCl (A), at pH 9 (B), and in 0.1 *N* NaOH (C).

1-Methyl-2-cyano-1,2,3,6-tetrahydropyridine-2-carbaldoxime Hydrochloride (10). Freshly distilled tetrahydrofuran (peroxide free), 200 ml, was saturated with hydrogen chloride gas. **8** (15 g) was added and the mixture was stirred for 30 min. After cooling with ice, the precipitate was collected, washed with ether, and dried under vacuum: yield 17.5 g (95%); mp 127–130° dec; ir (KBr) 2280 (C=N^+H), 1670 cm^{-1} (C=N). Anal. ($C_8H_{12}N_3OCl$) C, H, N.

1-Methyl-3,6-dihydropyridine-2-carbaldoxime Hydrochloride (11). To 200 ml of cooled freshly boiled methanol, 17 g (0.084 mol) of **10** was added. The suspension was stirred under a nitrogen atmosphere for 1.5 hr. During that time a solution was obtained and a new product precipitated. The product was filtered, washed with ether, and dried under vacuum: yield 11.7 g (80%); mp 179° dec; uv, see Figure 1; ir (KBr) 1700, 1660 cm^{-1} (C=N); NMR (TFA) δ 8.50 (s, 1, HC=N), 6.00 (s, 2, HC=CH), 4.57 [t, 2, $H_2C(6)$], 3.92 [m, 2, $H_2C(3)$], 3.85 (s, 3, H_3CN). Anal. ($C_7H_{11}N_2OCl$) C, H, N.

Oxidation of 11 by Hydrogen Peroxide. To 10 ml of 30% H_2O_2 was added 0.5 g of the dihydropyridine derivative **11**. The mixture was stirred and samples were taken to check the uv spectrum.

Oxidation by Silver Nitrate. To 10 ml of $AgNO_3$ solution was added 0.5 g of the dihydropyridine derivative **11**. A black precipitate of silver was formed after 1–2 min. The solution was centrifuged and an aliquot was taken to check the uv spectrum.

Oxidation by Iodine. In 10 ml of chloroform, 0.5 g of the dihydropyridine derivative **11** was added. A solution of 0.6 g of iodine in 20 ml of chloroform was added dropwise over 1 hr. The solution was extracted with six portions of 10 ml of hot water. The uv spectrum of the aqueous layer was taken.

Determination of the pK_a of 11 by Potentiometric Titration. **11** (86 mg, 5.04×10^{-2} mol) was dissolved in 50 ml of doubly distilled, freshly boilded cold water, saturated with de-

oxygenated nitrogen. The solution under a nitrogen atmosphere at 25° was then potentiometrically titrated with 0.1 *N* NaOH solution using a Radiometer Model 26 pH meter. The pK_a was then calculated according to the usual methods. A pK_a value of 6.32 ± 0.06 was obtained.

Confirmation of pK_a of 11 by Uv Spectrophotometry. In a uv cell, 0.01 ml of a solution of **11** in 0.1 *N* HCl was added to 3 ml of an appropriate deoxygenated buffer. The uv absorption at 251 nm was measured on a Cary Model 16 spectrophotometer. Knowing the absorptivities of the free base form and the salt form of **11**, the pK_a was calculated; using 0.01 *M* phosphate buffers of pH 6.0 and 7.0, a pK_a value of 6.25 was obtained.

References and Notes

(1) A preliminary account of some of this work has appeared: N. Bodor, E. Shek, and T. Higuchi, *Science*, **190**, 155 (1975). The expression "pro-drug" denotes a derivative of a known and proven drug, which derivative, due to its improved physiochemical properties, increases the bioavailability of the proven drug and which derivative is transformed by an enzymatic or chemical process into the proven drug, before reaching it and/or at the site(s) of action. See, for example, A. Albert, "Selective Toxicity", Methuen, London, 1968, p 91; A. A. Sinkula and S. H. Yalkowsky, *J. Pharm. Sci.*, **64**, 181 (1975).

(2) R. R. Levine and E. W. Pelkin, *J. Pharmacol. Exp. Ther.*, **131**, 319 (1961); R. R. Levine and A. F. Spencer, *Biochem. Pharmacol.*, **8**, 248 (1961).

(3) T. Higuchi in "Biopharmaceutics and Relevant Pharmacokinetics", J. G. Wagner, Ed., Drug Intelligence Publication, Hamilton, Ill., 1971, p 28.

(4) D. P. Rall, *Handb. Exp. Pharmakol.*, **27**, 1 (1971).

(5) R. A. Fishman, *Am. J. Physiol.*, **206**, 836 (1964).

(6) H. Behlback and W. A. Williams, *Arch. Environ. Health*, **30**, 49 (1975).

(7) T. H. Milby, *J. Am. Med. Assoc.*, **216**, 2131 (1971).

(8) H. Kewitz and I. B. Wilson, *Arch. Biochem. Biophys.*, **60**, 261 (1956).

(9) I. B. Wilson, *Biochim. Biophys. Acta*, **27**, 196 (1958).

(10) J. N. Wells, J. N. Davisson, I. Boime, D. R. Haubrich, and G. K. W. Yim, *J. Pharm. Sci.*, **56**, 1190 (1967).

(11) B. W. Masaki, E. J. Lien, and J. A. Biles, *Acta Pharm. Suecica*, **10**, 43 (1973).

(12) A. Albert, "Selective Toxicity", Methuen, London, 1968, p 91.

(13) U. Eisner and J. Kuthan, *Chem. Rev.*, **72**, 1 (1972).

(14) A. Hantzsch, *Justus Liebigs Ann. Chem.*, **215**, 1, (1882); D. Hofman, E. M. Kosower, and K. Wallenfels, *J. Am. Chem. Soc.*, **83**, 3314 (1961).

(15) R. E. Lyle and P. S. Anderson, *Adv. Heterocycl. Chem.*, **6**, 224 (1966).

(16) W. S. Caughey and K. A. Schellenberg, *J. Org. Chem.*, **31**, 1978 (1966).

(17) N. Kinoshita and T. Kawasaki, *J. Pharm. Soc. Jpn.*, **83**, 126 (1963).

(18) E. M. Fry, *J. Org. Chem.*, **29**, 1647 (1964).

(19) P. S. Anderson and R. E. Lyle, *Tetrahedron Lett.*, 153 (1964).

(20) C. K. Ingold, "Structure and Mechanism in Organic Chemistry", Cornell University Press, Ithaca, N.Y., 1953, p 565.

(21) P. T. Lansborg and J. O. Peterson, *J. Am. Chem. Soc.*, **85**, 2236 (1963).

(22) R. E. Lyle and G. J. Gauthier, *Tetrahedron Lett.*, 4615 (1965).

(23) F. W. Fowler, *J. Am. Chem. Soc.*, **94**, 5926 (1972).

(24) A. G. Cook, "Enamines: Synthesis, Structure and Reactions", Marcel Dekker, New York, N.Y., 1969.

(25) R. E. Lyle and D. A. Nelson, *J. Org. Chem.*, **28**, 169 (1963); A. C. Anderson and G. Berkelhammer, *J. Am. Chem. Soc.*, **80**, 4472 (1958).

(26) W. R. Benson and A. E. Pohland, *J. Org. Chem.*, **30**, 1126 (1965).

(27) B. Witkop, *J. Am. Chem. Soc.*, **78**, 2873 (1956); E. M. Fry, *J. Org. Chem.*, **29**, 1647 (1964).

(28) O. Cervinka, A. R. Katritzky, and F. J. Swinbourne, *Collect. Czech. Chem. Commun.*, **20**, 1736 (1965).

(29) N. C. Cook and J. E. Lyons, *J. Am. Chem. Soc.*, **87**, 3283 (1965).

(30) B. Pullman and A. Pullman, "Quantum Biochemistry", Wiley, New York, N.Y., 1963, Chapter XIII.

(31) W. Mass, M. J. Janssen, E. J. Stamhuis, and H. Wynberg, *J. Org. Chem.*, **32**, 1111 (1967).

(32) O. Cervinka, *Adv. Heterocycl. Chem.*, **6**, 147 (1966).

(33) C. S. Y. Kim and S. Chaykin, *Biochemistry*, **7**, 2339 (1968).

(34) G. M. Steinberg, E. J. Poziomek, and B. E. Hackley, *J. Org. Chem.*, **26**, 368 (1961).