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REDUCTION POTENTIALS OF IMINE-SUBSTITUTED, BIOLOGICALLY ACTIVE PYRIDINES: POSSIBLE RELATION TO ACTIVITY

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Cyclic voltammetry data were obtained for a number of biologically active compounds which incorporate imine substitution on the pyridine nucleus. The reductions in acid (iminium ion formation) were for the most part reversible, and in the range of -0.5 to -0.7 V. The toxic effect of these drugs is thought to be caused by the generation of reactive oxygen radicals that arise *via* charge transfer, or by disruption of electron transport chains.

KEY WORDS: Myosmine, anabaseine, dipyridyls, terpyridyl, reduction potentials, electron transfer, biomechanism.

INTRODUCTION

An impressive array of research is rapidly accumulating, which points to an important role for electron transfer (ET) and oxidative stress in the mechanistic action of various physiologically active compounds.[1,2] Of the principal ET classes (quinones, metal complexes, nitro compounds, flavins, and iminium ions), the iminium category has received the least amount of systematic attention. We have applied[3] these concepts to a wide range of drugs and toxins, including carcinogens, anticancer agents, antimalarials, antibacterials, amebicides, antiprotozoan drugs, anthelmintics, CNS types, and antimycobacterials.[4] Also, proposals have been made for an ET role involving iminium in the chemistry of vision (retinal iminium)[5,6] and in the primary process of photosynthesis (protonated pheophytin enol).[7] In the medicinal area, the pyridine nucleus is found incorporated in drugs belonging to a broad variety of classes.[8] Of course, NAD plays a central role in enzymology.

Our aim was to determine the reduction potentials of biologically active pyridines containing the imine substituent. The imine type can be either aliphatic, as in myosmine and anabaseine, or aromatic, as in bi- and terpyridyls. Electrochemical properties are relevant to the possible mode of action by electron transfer (ET) *in vivo*.

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MATERIALS AND METHODS

The N-methyliminium hydrochloride salt of myosmine was obtained from Dr. Edward Leete (University of Minnesota, Minneapolis, MN). Myosmine[9] and anabaseine[10] were prepared by literature methods. The electrolyte used was tetraethylammonium perchlorate (0.1 M) (G.F. Smith Chemical Co., Columbus, OH). Absolute ethanol for solution preparation was purchased from U.S. Industrial (Tuscola, IL). Other chemicals used were purchased from Aldrich Chemical Co. (Milwaukee, WI). All compounds were investigated at a concentration of 0.5 mM.

The cyclic voltammetric measurements were performed at ambient temperature with a Princeton Applied Research Corp. model 174A polarographic analyzer associated with a Houston Instrument model 200 X-Y recorder. The operation of the instrument and the electrodes was checked against a benzil standard before each use. The scan rate generally ranged from 20 to 200 mV/s. Solutions were purged of oxygen for fifteen minutes with prepurified nitrogen. The working electrode consisted of a hanging mercury drop electrode (HMDE). A platinum wire was used as the counter and saturated calomel (SCE) was the reference electrode. Observed potentials were converted to the normal hydrogen electrode (NHE) reference by adding 0.24 V to the SCE values. The reported data are an average of two or more measurements involving freshly made solutions.

The following equations were used to calculate the half-wave potentials and current function: $E^{\circ\prime} = [(E_{pc} + E_{pa})/2]$, and $CF = i_p/[V^{\frac{1}{2}} \times C](A/(Vs)^{\frac{1}{2}}M)$.

RESULTS AND DISCUSSION

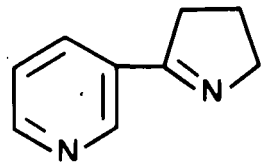
Myosmine and Iminium Derivatives

Myosmine **1**, a metabolic dehydrogenation product of nornicotine, is a tobacco alkaloid.[11] It is also formed after harvesting of the plants. In electrochemical studies, **1** underwent a one-electron reduction (CF ratio with benzil, 1.02) at -1.15 V in a reversible manner (Table I). The product was stable, with i_{pa}/i_{pc} of 0.98 at all scan rates

TABLE I
Cyclic Voltammetry of Myosmine, N-Methyl Iminium Derivative and Anabaseine

Compound	$E^{\circ\prime}$ (V)[a]	i_{pa}/i_{pc}	CF^{b}_{ratio}
1	−1.15	0.98	1.02
1·HCl	−0.74[c]		
1·2HCl	−0.51[d]		
2	−0.60[e]	0.88	0.91
2·HCl	−0.47		
6	−1.12[f]	0.97	1.01
6·HCl	−0.70[g]		
6·2HCl	−0.48		

[a] 100 mV/s, HMDE, 50% EtOH.
[b] $CF_{ratio} = CF/CF_{benzil}$; $CF_{benzil} = A/[(V/s)^{\frac{1}{2}}C] = 16.27$
[c] 1 equivalent $HClO_4$.
[d] 2 equivalents $HClO_4$.
[e] 1 equivalent $[OH^-]$ added to **2**·HCl.
[f] 2 equivalents $[OH^-]$ added to **6**·2HCl.
[g] 1 equivalent $[OH^-]$ added to **6**·2HCl.



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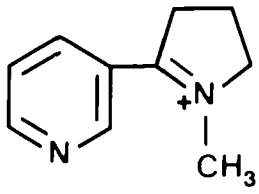
employed. The E_{pc} was constant, and ΔE_p was consistently 60–65 mV, in line with Nernstian behavior. When 1 equivalent of acid was added, the potential shifted cathodically to $E^{\circ\prime} = -0.74$ V. The addition of a second equivalent of $HClO_4$ produced a further shift to -0.51 V. The compound displays physiological activity by inducing DNA damage in *E. coli*, which could be repaired by the organism.[12] The action is conceivably related to its electrochemical properties. The suggestion had been advanced previously that **1** may exert its biological activity *via* conversion to the iminium form.[13]

The N-methyliminium derivative **2** of myosmine appears to be generated during conversion of nicotine to nornicotine in the plant.[11] Apparently, precursor **3** under-



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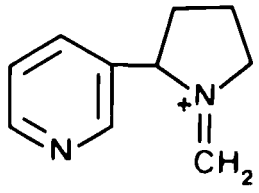

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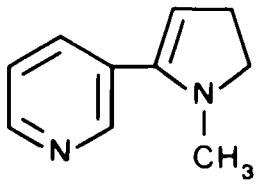

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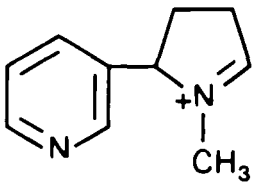
goes a tautomeric shift to **2**. Iminium **2**, which may also be derived from **4**, can exist in equilibrium with the open-chain keto-amine formed by hydrolysis. In cyclic voltammetry, **2** · HCl reduced with $E^{\circ\prime}$ of -0.47 V, similar to **1** · 2HCl. the reaction was 88% reversible ($i_{pa}/i_{pc} \times 100\%$) at 0.1 *V/s*, with ΔE_p ranging from 70–100 mV (Table I). When 1 equivalent of base was added to 2 · HCl, the potential shifted anodically to -0.60 V.



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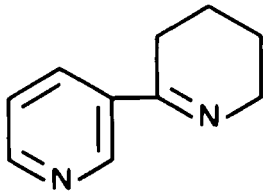
There is good evidence that **5** is also a product of oxidative metabolism.[11] Since this iminium is non-conjugated, the reduction potential[14] is quite negative, -0.82 V, even in acid, which makes it an unlikely candidate for ET reactions.

Anabaseine

Anabaseine **6**, a neurotoxin present in marine nemertine worms, is a paralyzing constituent of venom used by the carnivore to capture prey.[15,16] The compound also possesses insecticidal properties.[17] Anabaseine · 2HCl reduced reversibly ($i_{pa}/i_{pc} = 0.97$) with $E^{\circ\prime} = -0.48$ V (Table I). The potential shifted to a more negative value upon addition of base; 1 equivalent produced an anodic shift to $E^{\circ\prime} = -0.70$ V. A second equivalent gave an observed $E^{\circ\prime}$ of -1.12 V with 60 mV



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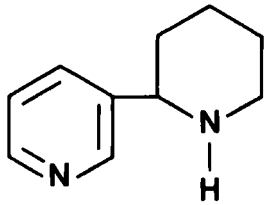
separating the peaks. The constant CF of 16.3 and linear plot with intercept of zero for the peak current *versus* the square root of the sweep rate indicated a diffusion controlled process. Since the structure is analogous to that of myosmine **1**, it is reasonable to speculate that the modes of action may be related. *In vivo* protonation would furnish conjugated iminium ions that should be able to participate in ET reactions. The electrochemical properties of **6** in acid correspond closely to those of **1** in salt form.

Anabasine **7**, is the major alkaloid of tree tobacco.[11] There is no definitive report for the presence of the more unstable **6**. From our viewpoint, it is quite significant that



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6 is metabolically derived from **7** in plant feeding experiments. Results with animals demonstrated unequivocally that **7** is tetratogenic.[18] Conceivably, the derived **6** in iminium form may be responsible for the observed effects *via* harmful ET reactions.

Di- and Terpyridyls

Recently, mutagenicities were reported[19] for the di- and terpyridyl compounds that are examined electrochemically in the present study. Significantly high responses were observed with 2,2′-, 3,3′-, 2,3′- and 2,4′- dipyridyl, as well as 2,2′2″-terpyridyl. The action of several dipyridinium ions, also found to be active, was attributed to reactive oxygen species generated *via* superoxide. Mutagenicity of paraquat in bacteria is apparently mediated by formation of superoxide. However, there is disagreement over the degree of activity; impurities may play an important role. The mutagenicity of several other pyridine derivatives was also determined.[20] If the free bases undergo protonation *in vivo*, then a common mechanistic theme would apply to all members of the group. The same types of pyridines, isolated from marine worms, were found to exhibit neurotoxic properties.[21] 2,3′-Dipyridyl, which also occurs in tobacco, is comparable to nicotine as a crustacean convulsant agent, but is less lethal to mice. It is known to cause DNA damage in *E. coli*, which, however, is not permanent.[12] Nemertelline, the first tetrapyridyl to be found in a living organism, resembles nicotelline.[21]

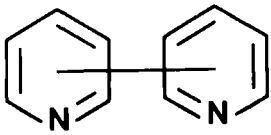
The bipyridyl compunds, **8a, 8b, 8c,** reduced at values ranging from -0.90 to -1.15 V (Table II), too negative to be involved in ET. Upon addition of 1 equivalent of acid, a diffusion controlled reaction was observed for each, in the range of -0.90 to -0.72 V, with **8a** being the most electro-positive, $E^{\circ\prime} = -0.72$ V. A second equivalent of $HClO_4$ produced a further cathodic shift, with **8a, 8b, 8c,** reducing at $E^{\circ\prime} = -0.51$, -0.63, and -0.72 V, respectively. Compound **8c** is reluctant to undergo diprotonation because the initial proton is coordinated to both nitrogens (pseudo 5-membered ring).

Compound **9** reduced in several steps, the first producing a reversible peak with

TABLE II
Cyclic Voltammetry of Di- and Terpyridyls

Compound	$E^{\circ\prime}$ (V)[a]	i_{pa}/i_{pc}	CF^{b}_{ratio}
8a	-0.91	0.97	1.02
	-0.72[c]		
	-0.51[d]		
8b	-1.04	0.97	1.01
	-0.82[c]		
	-0.63[d]		
8c	-1.15	0.93	1.04
	-0.90[c]		
	-0.72[d]		
9	-1.01, -1.61[e]		
	-0.83[c]		
	-0.64[d]		
	-0.64[f]		

[a] 100 mV/s, HMDE, 50% EtOH.
[b] $CF_{ratio} = CF/CF_{benzil}$; $CF_{benzil} = A/[(V/s)^{\frac{1}{2}}C] = 16.27$
[c] 1 equivalent $HClO_4$.
[d] 2 equivalents $HClO_4$.
[e] E_p.
[f] 3 equivalents $HClO_4$.



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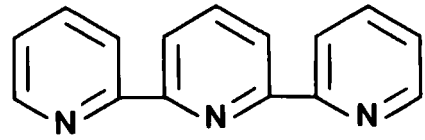
a) 4, 4'

b) 2, 4'

c) 2, 2'



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$E^{\circ\prime} = -1.01$ V (Table II). A second cathodic peak was observed at -1.61 V. Addition of acid (two equivalents) caused the first peak to shift to a more positive potential, $E^{\circ\prime} = -0.64$ V. The addition of a third equivalent produced no further shift.

It has been pointed out that 2,3′-dipyridyl is a weak base and, hence, would be largely unprotonated at physiological pH.[21] However, pH is known to vary in living organisms depending upon the site. For example, basic antimalarial drugs appear to concentrate in acidic vesicles of the parasite[22,23] and, perhaps, act by ET[24] and oxidative stress.[24,25] A similar situation pertains to pyridylacylhydrazones, veterinary anthelmintic agents, which are most effective in acidic compartments; an ET mechanism was invoked.[3] In addition, stereochemical effects *in vivo* can influence basicity. Theoretical calculations reveal that competition of imine and ammonia for proton (iminium-ammonium equilibrium) is importantly influenced, not only by inherent basicity, but also by geometrical considerations as would pertain at the active site in a biological system.[26] In an arrangement in which the lone pairs of the two bases point toward one another, the proton prefers the Schiff base. Alternatively, ET might be effected by metal (Cu or Fe) complexes of pyridine bases.

In the pyridine series, most attention in relation to physiological activity has centered on 2,2′-dipyridyl which acts as both an anticancer and antibacterial agent.[27] The ligand forms stable metal chelates that evidently are responsible for cytotoxicity by way of DNA strand cleavage. In the presence of oxygen and a reducing agent, the copper complex forms superoxide. There is a correlation between the degree of activity and reduction potential for the complexes of 2,2′-dipyridyl and o-phenanthroline. Another conceivable mechanistic route for copper 2,2′-dipyridyl entails dehydrogenase enzyme inhibition, apparently involving interference with energy yielding metabolism.[28] It is reasonable to hypothesize that agents which can participate in ET might interfere with electron transport chains essential for respiration. Since quinones are well-known redox cycling agents, a good example is the anthraquinone derivative rhein which also inhibits at the dehydrogenase coenzyme level *via* interference with ET.[29]

Since the diprotonated form of 4,4′-dipyridyl is structurally related to the herbicide paraquat (1,1′-dimethyl-4,4′-dipyridinium salt), similar electrochemical behavior in *vivo* might be expected. Paraquat possesses a reduction potential of about -0.31 V.[30] For the viologen series, there is a relationship between reduction potential and degree of herbicidal action.[31] Generation of active oxygen was positively correlated with ability to induce toxicity.[32] Also, in *E. coli* electron flow in the normal transport pathway was subverted resulting in increased amounts of superoxide.[33]

In our recent investigations, positive increases in reduction potential of as much as 0.5 to 0.9 V were found on conversion of imine to iminium in various conjugated systems.[3,4,34]

OTHER CONSIDERATIONS

Is it feasible that the drugs in this study evoke their biological activity by an ET mechanism? Most of the compounds in iminium form produced reduction in the range favorable (potential above -0.6 V) for biological activity.[35] All reductions of the iminium ions occurred reversibly, indicating the possibility of redox cycling *in vivo*. Several dipyridinium ions in previous studies have been found to produce their activity through reactive oxygen species generated by superoxide.[1] It may well be that the mechanism of action of these drugs is due to ET-oxy radical reactions, operating in concert with other effects.

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