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Electrochemical Reduction of 3-Cyano-1-methylpyridinium Iodide, a Nicotinamide Adenine Dinucleotide Model Compound

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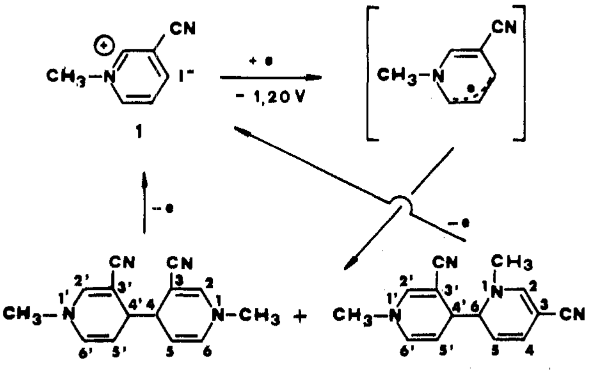
3-Cyano-1-methylpyridinium iodide (**1**) exhibits one polarographic wave at pH values below 11 ($E_{1/2} = -0.87$ V vs. SCE). Electrolysis at the plateau potential of this wave, involving a one-electron uptake, leads to the formation of a mixture of four dimeric products, unambiguously identified as two diastereoisomer pairs: 3,3′-dicyano-1,1′-dimethyl-1,1′,4,4′-tetrahydro-4,4′-bipyridines **2** and **3**, and 3,3′-dicyano-1,1′-dimethyl-1,1′,6,4′-tetrahydro-6,4′-bipyridines **4** and **5**.

Dimeric products are known to arise from the one-electron reduction of nicotinamide adenine dinucleotide (NAD$^+$), and related compounds, by chemical,[1] electrochemical,[2] and photochemical[3] methods. While in a few cases these products could be isolated, their formation has been generally postulated on the ground of process stoichiometry and spectroscopic evidence. However, the literature on this subject lacks detailed evidence regarding the structure to be assigned to these dimeric compounds, and further research to obtain a deeper knowledge on the subject appears highly desirable. Accordingly, the electrochemical reduction of 3-cyano-1-methylpyridinium iodide (**1**), a NAD$^+$ model compound, was performed obtaining a mixture of four reduction products (Scheme I), that were isolated and unambiguously identified.

Scheme I



Results and Discussion

A. Polarographic Behavior. The reduction polarogram of **1**, recorded in the Britton–Robinson buffer solutions, exhibits one wave (A) from pH 2 up to pH 11. Another wave (B) appears at more negative potentials, at pH values greater than 11. Wave A is diffusion controlled (as ascertained from the limiting current variations with the mercury head height and temperature) over the investigated pH range. Its height is proportional to the concentration of **1**. The diffusion current constant, I, is 2.10 ± 0.05 μA s$^{1/2}$ (mM)$^{-1}$ mg$^{-2/3}$ for concentrations of **1** ranging from 0.05 to 5.0 mM, and this value corresponds to a one-electron Faradaic process. The half-wave potential, $E_{1/2}$, is pH independent for wave A (average value −0.87 V). The addition of surface active tetraethylammonium ions Et_4N^+ to a pH 9.5 buffered solution shifts $E_{1/2}$ toward more negative potentials (e.g., 25 mV when the concentration of Et_4N^+ was 0.1 M).

For the same pH 9.5 buffer, an increase of the ionic strength from 0.1 to 2.0 M, obtained by addition of KCl, causes $E_{1/2}$ to shift 40 mV toward more negative potentials. $E_{1/2}$ shifts also with the concentration of **1**: it is −0.86 V at 0.1 mM and -0.87_5 V at 1 mM.

From its slope over the pH range investigated, wave A seems related with a totally irreversible process ($E_{1/4} - E_{3/4}$ falls between 77 and 86 mV). The log $i/(i_d - i)$ vs. E plot is not always strictly linear over the whole rising portion of wave A: such deviations are probably due to the chemical reaction which follows the one-electron uptake (see subsequent discussion) and to the adsorption of the depolarizer and/or the one-electron reduction products.

The second wave (B) has a partially kinetic character, as ascertained from the limiting current variations with the mercury head height and the temperature. Its $E_{1/2}$ is virtually pH independent and has a value of −1.60 V in a pH 11.5 buffer with a 1.0 mM concentration of **1**, and its height is about ⅗ of the value of wave A. In the same buffer, addition of KCl, producing an increase in the ionic strength from 0.1 to 2.0 M, causes $E_{1/2}$ to shift 70 mV toward less negative potentials. The

Table I. NMR Data for Dimers 2, 3, 4, and 5

2			3		4			5	
δ, ppm	*J*, Hz	Protons	δ, ppm	*J*, Hz	δ, ppm	*J*, Hz	Protons	δ, ppm	*J*, Hz
6.62 (d)	1.8	$H_2 + H_{2'}$	6.64 (d)	1.8	6.81 (s broad)		H_2[a]	6.86 (s broad)	
5.89 (dd)	1.8	$H_6 + H_{6'}$	5.86 (dd)	1.8	6.65 (d)	1.8	$H_{2'}$	6.63 (d)	1.8
	8.0			8.0	6.08 (d broad)	10.0	H_4[a]	6.05 (d broad)	10.0
4.64 (dd)	4.0	$H_5 + H_{5'}$	4.72 (dd)	4.0	5.90 (dd broad)	8.0	$H_{6'}$	5.91 (dd broad)	8.0
	8.0			8.0		1.8			1.8
3.21 (d)	4.0	$H_4 + H_{4'}$	3.34 (d)	4.0	5.10 (dd)	5.0	H_5	4.95 (dd)	5.0
2.98 (s)		CH_3-1 CH_3-1′	2.96 (s)			10.0			10.0
					4.69 (dd)	4.0	$H_{5'}$	4.66 (dd)	4.5
						8.0			8.0
					4.12 (dd)	5.0	H_6	4.08 (dd)	5.0
						4.0			3.5
					3.54 (dd)	4.0	$H_{4'}$	3.36 (dd)	4.5
						4.0			3.5
					3.02 (s)		CH_3-1	3.12 (s)	
					2.96 (s)		CH_3-1′	2.99 (s)	

[a] H_2 and H_4 are coupled with $J = 1.5$ Hz.

slope of wave B suggests the occurrence of an irreversible electronic transfer.

B. Coulometric Behavior. Solutions of 1 in buffered media have been electrolyzed using potential values in the range corresponding to the plateau of wave A. The concentration of 1 varies from 0.2 to 20 mM; the pH of the buffer from 2 to 11.5. The Faradaic *n* value shows that the process corresponding to wave A is monoelectronic. The polarograms of the electrolyzed solutions do not show any reduction wave, indicating that the ultimate product of the reduction process at potential of wave A does not represent an intermediate in the formation of wave B and is not reduced further to different products. Furthermore, the polarograms show for pH ≥6 an anodic wave very close to the background anodic discharge; for example, after the electrolysis of a 2.0 mM solution of 1 at pH 9.0 the polarogram shows an anodic wave with $E_{1/2} = -0.27_5$ V and limiting current (i_l) equal to 87% of the i_l value for the initial cathodic wave (A). The electrolytic reoxidation, performed at −0.20 V, consumed slightly less than one electron per molecule of 1. The solution so obtained shows a well-defined polarographic wave, with an $E_{1/2}$ value in good agreement with that of 1, and with the height consistent with the Faradaic *n* value just mentioned. The height of the anodic wave may be lower than that of the cathodic wave, owing to different diffusion coefficients between 1 and the reduction products; a 0.87 ratio for the anodic to cathodic wave heights corresponds to a 0.75 ratio for the diffusion coefficients.

In the uv spectra taken after electrolysis, the characteristic maximum of 1 disappears and a maximum appears at 345 nm for electrolyzed neutral or alkaline solutions and at 275 nm for acidic electrolyzed solutions. The same 275-nm maximum appears after acidification with 0.5 M HCl of the electrolyzed neutral or alkaline solutions. The uv spectra taken after reoxidation show the characteristic absorption band of 1.

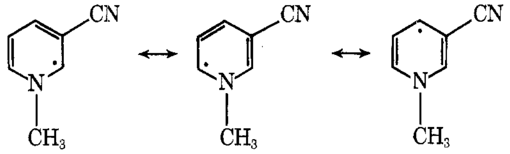
Therefore, the uv spectra and Faradaic properties of the solutions after electrolysis allow us to state that the nature of the electrolysis products is unaffected by variations in pH, concentration of 1, and potential within the range of values corresponding to the plateau of wave A.

C. Macroscale Electrolysis. Isolation and Characterization of the Dimers. After ascertaining that the reduction product of 1 does not depend on pH, potential, and depolarizer concentration, several electrolyses have been performed, at the potential values corresponding to the wave A plateau, on solutions whose concentration was suitable for the isolation and characterization of the reduction products. Ten samples of 1, ranging from 0.5 to 1.0 g, have been electrolyzed under the conditions described in the Experimental Section. During the electrolysis a yellow precipitate is formed. The reduction of 1 is practically completed within a period of about 3 h. Shortly after electrolysis completion, both solution and precipitate have been extracted with CH_2Cl_2 and the solvent evaporated to yield a gummy residue which solidifies upon water treatment (yield 65–88%). A chromatographic test (HPLC) allows us to detect four major compounds in the residue. All of them, on oxidation with an alcoholic I_2 solution, yield only salt 1.

The four compounds **2**, **3**, **4**, and **5** could be separated owing to their different solubilities in ethanol. Their elemental analysis, together with molecular weight (238, from mass spectrum), suggest the same molecular formula $C_{14}H_{14}N_4$. The NMR spectrum of **2** (Table I) shows a signal at δ 2.98 ppm corresponding to two equivalent methyl groups, a signal at δ 3.21 ppm corresponding to two equivalent tertiary protons (H_4 + $H_{4'}$), three signals, each corresponding to two equivalent vinyl protons ($H_5 + H_{5'}$ at δ 4.64; $H_6 + H_{6'}$ at δ 5.89; $H_2 + H_{2'}$ at δ 6.62 ppm).

The NMR spectrum of **3** is almost the same as that of **2** with only minor changes of the chemical shifts of the various protons. Taking into account the molecular weight, these NMR data provide reasonable evidence for a symmetrical dimeric structure, namely 3,3′-dicyano-1,1′-dimethyl-1,1′,4,4′-tetrahydro-4,4′-bipyridine, for both products **2** and **3**, which are therefore a diastereoisomeric pair with respect to the C_4–$C_{4'}$ stereochemistry. The ir and uv spectra of both **2** and **3** are consistent with these structures since they show the same typical absorption bands reported[4] for 3-cyano-1-methyl-1,4-dihydropyridine. The NMR spectrum of **4** shows two methyl signals (δ 2.96 and 3.02 ppm), a tertiary proton signal (H_4, at δ 3.54 ppm), a signal corresponding to a tertiary proton adjacent to the N atom (H_6 at δ 4.12 ppm), six signals corresponding to six vinyl protons ($H_{5'}$ at δ 4.69; H_5 at δ 5.10; $H_{6'}$ at δ 5.90; H_4 at δ 6.08; $H_{2'}$ at δ 6.65; H_2 at δ 6.81 ppm). The NMR spectrum of **5** is almost identical with that of **4**, except for small differences in the chemical shifts for the various protons. These NMR data, together with the molecular weight, provide reasonable evidence for a dimeric asymmetric structure, namely 3,3′-dicyano-1,1′-dimethyl-1,1′,6,4′-tetrahydro-6,4′-bipyridine, for both products **4** and **5**, which are therefore a diastereoisomeric pair with respect to the C_6–$C_{4'}$ stereochemistry. It should be noted that also in this case the ir and uv spectra of both **4** and **5** show the typical absorption bands reported[4] for 3-cyano-1-methyl-1,4- and -1,6-dihydropyridines.

D. Reaction Pattern. It is well known that the first step in the one-electron reduction of pyridinium salts yields pyridinyl radical species. The electron release to pyridinium ion should yield a mesomeric radical, stabilized in particular by resonance of the forms A, B, and C. The structure of the dimers isolated, where only 4,4′, and 6,4′ binding occur, supports the severe steric hindrance to dimerization through position 2, when positions 1 and 3 are substituted. In fact no 2,2′-linked dimers have been reported.[1-3,5] Furthermore, in our case, the absence of 6,6′-linked dimers as well can be traced to steric hindrance as is readily apparent from the molecular models.



A B C

Experimental Section

Compound 1 was prepared according to ref 4, mp 197–198 °C, uv max (H_2O) 268 nm. The melting points were taken on a Kofler apparatus, and are uncorrected.

The same apparatus described in a previous paper[6] was used for electrochemical measurements. Britton–Robinson and NH_3–NH_4Cl buffers were used; the solutions were deoxygenated with 99.99% pure nitrogen or argon, and the temperature was kept constant within ±0.1 °C. The potentials were measured against a saturated calomel electrode. The capillary characteristic was $m^{2/3}\,t^{1/6} = 1.57$ mg$^{2/3}$ s$^{-1/2}$ when measured at $E = -1.25$ V and $T = 21.5$ °C under a mercury head 80.2 cm in height in a pH 6.8 buffer solution containing 1 at 0.5 mM concentration. The ^1H NMR spectra were recorded in $CDCl_3$ on a Varian XL-100-15 spectrometer; the chemical shifts are reported as δ units relative to Me_4Si (δ 0) as internal standard. The *m/e* values were measured with an AEI MS-12 70 eV low-resolution mass spectrometer. The ir spectra were recorded on a Perkin-Elmer 257 grating spectrophotometer, as Nujol mulls, and the uv spectra on a Perkin-Elmer 402 spectrophotometer. The chromatographic analyses were performed on a Du Pont 830 liquid chromatograph, using a 1 m × 2.1 mm i.d. stainless steel column packed with 1% BOP coated on Zipax; the eluent at 1400 psi was a mixture of isooctane–chloroform–ethanol (95:5:0.2 v/v). The column effluent was monitored at 247 nm using a Du Pont 837 spectrophotometer.

Electrochemical Reduction of 3-Cyano-1-methylpyridinium Iodide (1). In a typical run, 1.0 g of 1 was dissolved in 50 ml of 0.1 M NH_3–0.1 M NH_4Cl aqueous solution. This solution was then electrolyzed at −1.20 V using a round mercury pool (area 20 cm^2) as a working electrode with a saturated calomel electrode as reference, and a platinum gauze cylinder as a counterelectrode. The solution was magnetically stirred and blanketed by a continuous nitrogen flux over its surface.

Usually the electrolysis took about 3 h to get to completion, as inferred from the complete disappearance of the reduction wave (A) of compound 1, and from the constant value of the current, equal to that obtained, at the same potential, on a solution containing only the supporting electrolyte.

After electrolysis, the mercury pool was covered by a yellow precipitate.

The whole content of the cell was repeatedly extracted with CH_2Cl_2. The gummy residue (0.5 g) recovered by evaporating the dried organic layer was washed with cold water till solidification and the solid (0.4 g) extracted three times with 10 ml of 95% ethanol at room temperature.

The residue from this extraction contained product 4 only. The slow evaporation of the ethanol, at room temperature, allowed compound 2 to precipitate together with its diastereoisomer 3 and 4 as impurities. The solution was treated under heating with small amounts of activated charcoal, filtered, and evaporated under vacuum. The residue, after recrystallization from ethanol yielded 3 while the solution contained practically only 5. All the four dimers could be further purified by repeated crystallizations from ethanol. The pure products exhibited the physicochemical properties reported below.

2: mp 146–148 °C; uv max (MeOH) 342 nm (ϵ 7400), 235 (sh); ir 2190 (CN) 1675, 1580 (C=C), 745 cm^{-1}; HPLC retention time 23.1 min. Anal. Calcd for $C_{14}H_{14}N_4$: C, 70.56; H, 5.92; N, 23.51; mol wt, 238.28. Found: C, 70.62; H, 5.95; N, 23.00; *m/e* 238 (parent peak).

3: mp 143–145 °C; uv max (MeOH) 342 nm (ϵ 7450), 235 (sh); ir 2185 (CN), 1670, 1590 (C=C), 815, 790 cm^{-1}; HPLC retention time 13.1 min. Anal. Calcd for $C_{14}H_{14}N_4$: C, 70.56; H, 5.92; N, 23.51; mol wt, 238.28. Found: C, 70.66; H, 6.04; N, 23.30; *m/e* 238 (parent peak).

4: mp 161–163 °C; uv max (MeOH) 349 nm (ϵ 6400), 247 (11 000); ir 2195 (CN), 1670, 1645, 1595, 1580 (C=C), 780, 730, 710 cm^{-1}; HPLC retention time 21.0 min. Anal. Calcd for $C_{14}H_{14}N_4$: C, 70.56; H, 5.92; N, 23.51; mol wt, 238.28. Found: C, 70.85; H, 5.92; N, 23.77; *m/e* 238 (parent peak).

5: mp 127–129 °C; uv max (MeOH) 349 nm (ϵ 6500), 247 (11 300); ir 2185 (CN), 1675, 1650, 1585, 1575 (C=C), 770, 725 cm^{-1}; HPLC retention time 32.0 min. Anal. Calcd for $C_{14}H_{14}N_4$: C, 70.56; H, 5.92; N, 23.51; mol wt, 238.28. Found: C, 70.72; H, 6.10; N, 23.18; *m/e* 238 (parent peak).

Chemical Oxidation of the Dimers. Both crude reduction mixture and pure compounds 2, 3, 4, and 5 on oxidation with iodine give the salt 1. As example, 70 mg of I_2 was added to a solution containing 50 mg of 2 in 25 ml of ethanol, warming at 40 °C for 30 min. The solution was allowed to cool, then $Na_2S_2O_3$ was added until the iodine color disappeared. The ethanol was removed, the residue was extracted with $CHCl_3$, and the chloroform solution was evaporated. The residue, crystallized from acetone, gave the salt 1.

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Registry No.—**1,** 1004-16-6; **2/3,** 60224-01-3; **3/2,** 60224-02-4; **4/5,** 60224-03-5; **5/4,** 60224-04-6.

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