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One-electron Reduction of 1-Benzyl-3-carbamoylpyridinium as a NAD$^+$ Model

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The pyridyl radical generated chemically or electrochemically by the 1e-reduction of 1-benzyl-3-carbamoylpyridinium undergoes dimerization to give mainly four dimers, diastereomers of 4,4′- and 4,6′-linked dimers. Isomerization between the dimers proceeds readily.

A pyridyl redical NAD· is recognized as a significant intermediate[1] in biological interconversion between NAD$^+$ and NADH, this being supported by results from oxidation and transhydrogenation in model systems.[2] Pyridyl radicals having carbamoyl group on C_3 can be readily generated by chemical,[3] photochemical,[4] electrochemical,[5–7] or radiochemical[8,9] one-electron reduction of the corresponding cations. The transient radicals react with each other to give dimerized products. However, there seems to be confusion concerning the products, particularly in the structures of the dimers.[3–7,10] Recently, Moracci *et al.* reported that 1e-reduction of 1-benzyl-3-carbamoylpyridinium (BzNA$^+$) gives a diastereomeric pair of 4,4′-linked dimer.[7] Formation of 4,6′- and 6,6′-dimers has also been postulated by other investigaters. Furthermore, Kosower *et al.* predicted the formation of a dihydronicotinamide by the dispropotionation of the 3-substituted pyridyl radical.[11] The formation of a dihydronicotinamide was also demonstrated in the electrolysis of the corresponding 1-alkyl-3-carbamoylpyridinium at a potential on the polarographic first wave.[12]

We have studied biomimetic conversion from a pyridinium into the corresponding dihydropyridine and found that a pyridyl radical, BzNA·, arising in the 1e-reduction of BzNA$^+$, gives none of 1-benzyldihydronicotinamides (BzNAH); it mainly gives two pairs of 4,4′- and 4,6′-linked dimers in analogy with the dimerization of 1-methyl-3-cyanopyridyl radical.[13] Isomerization between the dimers of BzNA· was also observed. In this article we wish to describe the details.

Results and Disscusion

Chemical 1e-reduction of the chloride of BzNA$^+$ was carried out using zinc powder–copper sulfate in methanol–aqueous ammonia.[3,4] Electrochemical 1e-reduction was performed with an Hg-pool electrode at −1.30 V, where the reversible 1e-reduction occurs[14] at pH 7.4 with McIlvaine's buffer. As shown in Fig. 1, many products were found in both chemical and electrochemical reaction mixtures by means of high pressure liquid chromatography (HPLC). We have confirmed the formation of 1,2-, 1,4-, or 1,6-BzNAH with HPLC. However, none of the products was found in the reaction mixtures. This result shows that disproportionation of BzNA·'s did not take place.[11,12] We have attempted the isolation and identification of four major products, **b**, **c**, **d**, and **e**. They could be isolated and purified by careful fractional recrystallization and column chromatography. In order to obtain unequivocal information on coupling positions of the dimers, the reduction of BzNA$^+$ deuterated on the C_4 of the nicotinamide ring was also carried out chemically.

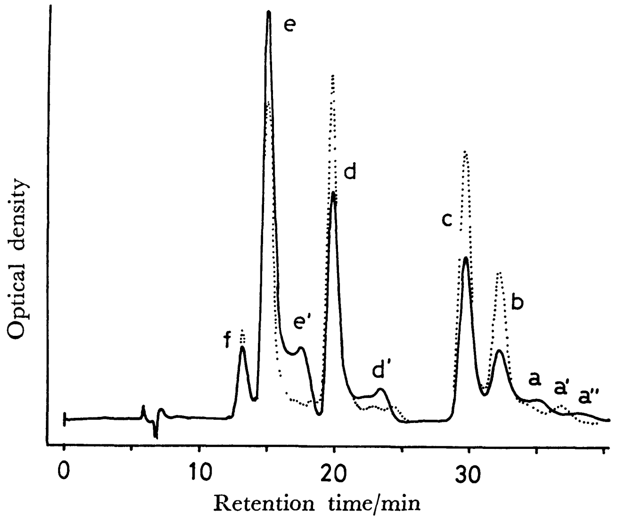
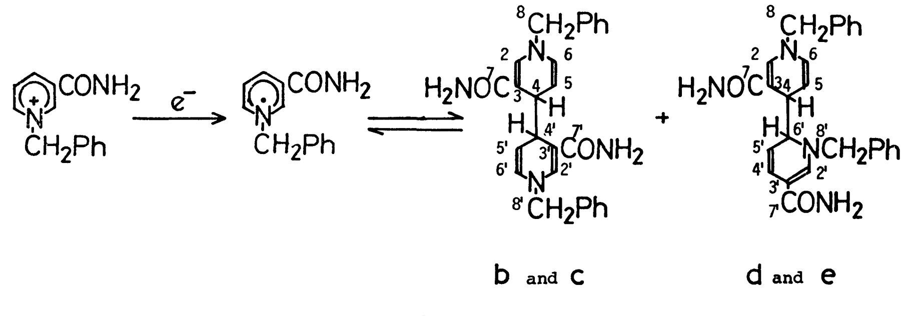


Fig. 1. HPLC trace of the reaction mixtures of one-electron reduction of BzNA$^+$ chemically (——) and electrochemically (······).



Scheme.

Table 1. ^{1}H NMR data for dimers[a)]

	b	c	d	e
H_4	3.34(d, J_{45}=4.2)	3.22(d, J_{45}=3.8)	3.20(dq, J_{45}=4.4, $J_{46'}$=3.6)	3.80(dq, J_{45}=5.0, $J_{46'}$=4.2)
H_5	4.48(q, J_{45}=4.2, J_{56}=8.0)	4.35(q, J_{45}=3.8, J_{56}=7.8)	4.70(q, J_{45}=4.4, J_{56}=8.0)	4.63(q, J_{45}=5.0, J_{56}=8.0)
H_6	5.92(d, J_{56}=8.0)	6.04(d, J_{56}=7.8)	6.20(d, J_{56}=8.0)	6.18(d, J_{56}=8.0)
H_2	7.28(s)	7.24(s)	7.24(d, J=2.0)	7.26(d, J=2.0)
$H_{4'}$			6.37(d, $J_{4'5'}$=10.0)	6.39(d, $J_{4'5'}$=9.6)
$H_{5'}$			4.80(q, $J_{5'6'}$=5.2, $J_{4'5'}$=10.0)	4.72(q, $J_{4'5'}$=9.6, $J_{5'6'}$=4.8)
$H_{6'}$			4.03(bq, $J_{5'6'}$=5.2, $J_{46'}$=3.6)	4.04(bp, $J_{5'6'}$=4.8, $J_{46'}$=4.2)
$H_{2'}$			7.32(d, J=2.0)	7.34(d, J=1.0)
Amide	6.40(ds)	6.96(bs)	6.30(bs)	6.33(bs)
Amide			6.70(bs)	6.64(bs)
H_8	4.31(s)	4.36(s)	4.43(s)	4.38(s)
$H_{8'}$			4.42(ABq, J_{AB}=*ca.* 13, $\nu_0\delta_{AB}$=*ca.* 0)	4.38(ABq, J_{AB}=*ca.* 12 $\nu_0\delta_{AB}$=*ca.* 0)
Aromatic	7.3(m)	7.33(m)	7.40(m)	7.37(m)

a) The numbers represent chemical shifts, δ with ppm, from TMS in $(CD_3)_2SO$. Coupling constants, J with Hz, are shown in parentheses.

Table 2. ^{13}C NMR data for dimers[a)]

	d	e	1,4-BzNAH[b)]	1,6-BzNAH[b)]
C_7	169.5	169.3	169.0	167.5
C_2	138.0	138.5	137.8	144.9
C_6	130.7	131.0	129.5	47.0
C_5	101.4	100.9	101.8	109.1
C_3	100.2	100.1 or 100.2	100.3	99.2
C_8	56.2	55.9 or 56.1	55.9	58.5
C_4	41.4	37.5	22.3	122.5
$C_{7'}$	167.3	167.4		
$C_{2'}$	145.5	144.5		
$C_{6'}$	60.6	60.1		
$C_{5'}$	110.7	109.6		
$C_{3'}$	103.7	100.2 or 100.1		
$C_{8'}$	58.3	56.1 or 55.9		
$C_{4'}$	122.7	123.3		

a) The numbers present chemical shifts, δ with ppm, from TMS in $(CD_3)_2SO$. b) Moracci's data.[7)]

^{1}H and ^{13}C NMR data of these compounds are summarized in Tables 1 and 2. Spectra of both **b** and **c** provide reasonable evidence for a symmetric structure. The tracer experiment established the coupling positions in the dimers. ^{1}H NMR spectra of **b** and **c** derived from BzNA$^+$-4d_1 showed no methine signal at δ 3.34 and 3.22, respectively, indicating that they have only 4-substituted 1,4-dihydronicotinamide structure; namely **b** and **c** can be identified as 4,4′-dimers of BzNA· and are a diastereomeric pair with respect to the C_4–$C_{4'}$ stereochemistry.[15)]

The complexity of ^{1}H and ^{13}C NMR spectra of **d** where two types of amide-signals are present suggest unsymmetric structure. The ^{1}H NMR signals at δ 3.20 and 6.37, not present in the spectrum of **d** obtained in the tracer experiment, should be due to the methine proton on C_4 of the 4-substituted 1,4-dihydropyridine structure and the vinyl proton of the 1,2- or 1,6-dihydro structure, respectively. However, the signal at δ 4.80, which is assigned to the proton on C_5, has a large coupling constant, J=5.2 Hz. This excludes the 1,2-dihydropyridine structure for the dimer **d**.[17)] The ^{13}C NMR spectrum of **d** is composed of that of 1,4- and 1,6-BzNAH expect that signals for methylene carbons appear as signals for methine carbons. Since a similar agreement between ^{13}C NMR spectra of 1,4-BzNAH and **b** or **c** is recognized, this observation and ^{13}C NMR spectrum with the off-resonance proton decoupling technique in the dimer **d** strongly indicate the presence of 4-substituted 1,4-dihydro and 6-substituted 1,6-dihydronicotinamide structures. The ^{1}H and ^{13}C NMR spectral data are in line with the structure of 4,6′-linked dimer.[18)] The complex signals could be assigned as shown in Tables 1 and 2. By similar analyses of ^{1}H and ^{13}C NMR spectra of **e** and the corresponding deuterated one, it can be concluded that **e** is the other diastereomer of 4,6′-dimer **d**.

Thus, the structures of four products isolated in the 1e-reduction of BzNA$^+$ can be established as two pairs of diastereomeric isomers of 4,4′- and 4,6′-linked dimers. The ratios of the yields of compounds **b**, **c**, **d**, and **e** from the chemical reduction are 1.0 : 1.4 : 3.0 : 4.5 at 4 °C and 1.0 : 2.6 : 2.3 : 3.3 at 60 °C, respectively. The ratio in the electrolysis at ambient temperature is 1.0 : 1.8 : 1.7 : 1.4. Although the ratios are labile, the total yield of four major products is larger than 95% in all cases. We assume that the residue should contain 2,2′-, 2,4′-, 2,6′- and 6,6′-dimers.

Statistically dimerization of BzNA· may give 12 dimers; six pairs of diastereomeric isomers. However, taking into account the far larger spin densities on

C_4 and C_6 of pyridyl radicals than C_2,[19] three pairs, 4,4′-, 4,6′- and 6,6′-dimers, may predominate. Actually, it was proved that the dimers obtained mainly have 4,4′- or 4,6′-bonding, the formation of other dimers not being appreciable. A small or negligible amount, if any, of 6,6′-dimers results from steric hindrance for dimerization. Furthermore, it should be taken into account that pyridyl radical in our reaction systems was generated on and participated by a metal surface, Zn or Hg. The difference of the metal might contribute to the dissimilarity between chemical and electrochemical reductions with respect to the ratios of the yields of four major dimers.

We have found isomerization between dimerization products. Even upon being left to stand overnight at room temperature in the dark with exclusion of air, a solution of 4,6′-dimer **d** gave 4,4′-dimer **c** mainly together with minor amounts of **a**, **b**, **d**, **d′**, **e**, and **e′**. **e** in methanol kept at 82 °C for 2 h also isomerized into a mixture of **a**, **a′**, **b**, **c**, **d**, **d′**, **e**, and **e′**; **c** was predominant. A solution of the most stable dimer **c** gave a mixture of **a**, **a′**, **b**, and **c** by heating at 82 °C for 15 h. In this mixture, small amounts of **a″**, **e**, and **d** were also detected. It was observed that the rate of the isomerization of **d** is accelerated by exposing the solution to scattered light. The isomerization indicates that the 4,4′-dimers are thermally more stable than the 4,6′-dimers and that the C–C bond coupling two pyridyl moieties is so weak that the dimer dissociates reversibly into two BzNA·'s.[20] The latter seems reasonable because of steric crowd of the dimers and of the resonance-stabilized structure of the radical.

Experimental

^1H and ^{13}C NMR spectra were taken on a Varian XL-100-15A. HPLC was effected with a Toyo Soda HLC-803 instrument and 30 cm LS-410K column under the following conditions. For BzNAHs, mobile phase, methanol–water (5:4 v/v); flow rate, 0.30 ml/min; detecter, UV-photometer at 350 nm; for the dimers, methanol–water (2:1 v/v); 0.54 ml/min, UV-photometer at 360 nm, respectively. Polarogram was recorded with a Yanaco Type P8-D Polarograph, macro scale electrolysis being performed with a potentiostat (Hokuto Denko Ltd.).

Reduction in Zinc Powder/Copper Sulfate System. To zinc powder (1.4 g) in 10 ml of water was added 0.5 g of copper sulfate pentahydrate in 20 ml of water under vigorous magnetic stirring, a mixture of 28% aqueous ammonia (10 ml) and methanol (50 ml) then being added. A solution of 0.1 g of $BzNA^+Cl^-$ in 20 ml of water was added to the above mixture at a definite temperature. After 20 min, the mixture was analyzed by HPLC (Fig. 1).

In preparative experiments, zinc powder (0.6 g)/H_2O (10 ml), $CuSO_4 \cdot 6H_2O$ (2.5 g)/H_2O (20 ml), 28% aqueous ammonia (10 ml)/methanol (50 ml) and 5.0 g of $BzNA^+Cl^-$/H_2O (20 ml) or 6.0 g of $BzNA^+$-4d_1 ClO_4^- in a mixture of H_2O (15 ml) and methanol (15 ml) were used, the reaction being carried out precipitate and inorganic materials were collected by filtration and were washed with 70 ml of hot methanol. Crystals of **c** obtained by cooling the methanol solution were separated by filtration. The filtrate was concentrated partially and cooled to give crystals of **d**. Further concentration and filtration gave crude crystals of **e** and the filtrate containing **b** predominantly. The filtrate was concentrated and chromatographed on a basic alumina eluting with a mixture of methanol and hexane (3:7 v/v). Several fractions containing only **b** were collected. Elimination of the solvents gave crystals of **b**. On the other hand, when the first aqueous filtrate was left to stand at room temperature for some time, yellow solids of **d** and **e** and orange viscous oil containing **b**, **d**, **e**, and **f** separated. After removal of the yellow solids and the oil by filtration and decantation, the solution was cooled in an ice bath to give crystals of **e** which were recrystallized from an aqueous methanol: mp (dec) 155 °C. Found: C, 72.99; H, 6.13; N, 13.19%. Calcd for $C_{26}H_{26}N_4O_2$: C, 73.22; H, 6.14; N, 13.14%. The yellow solids and the oil were treated similarly by fractional recrystallization to give pure dimers.

Absorption maxima of **b**, **c**, **d**, and **e** were observed at 355, 355, 359, and 359 nm, respectively, with a spectrometer (Shimadzu SPD-1) equipped with the HPCL instrument. In the treatments described above, HPLC was used effectively to estimate the composition of mixture and purity of isolated product.

Electrochemical Reduction. Electrolysis was carried out in a three-compartment cell, the cathode being a mercury pool (area, 12.6 cm^2), the anode a coil of Pt wire, and the reference electrode a saturated calomel electrode. The cathode was isolated from the anolyte with a glass filter and agar gel. Argon was blown continuously through the catholite before and during electrolysis. After preelectrolyzing the McIlvaine's buffer (pH 7.4, 30 ml), 102.5 mg of $BzNA^+Cl^-$ was added. Electrolysis was carried out at −1.30 V and stopped coulometrically at 25% conversion because of coating on the mercury surface with solid products. Dimethyl sulfoxide was then poured into the cathodic cell. After the mixture had been stirred until the mercury became clear, the solution was analyzed by HPLC.

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