November, 1976] BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN, VOL. 49 (11), 3269—3273 (1976) 3269

# Photoinduced One-Electron Reduction of 1-Benzyl-3-carbamoylpyridinium Chloride and 3,5-Bis(ethoxycarbonyl)-2,6-dimethylpyridine[1]

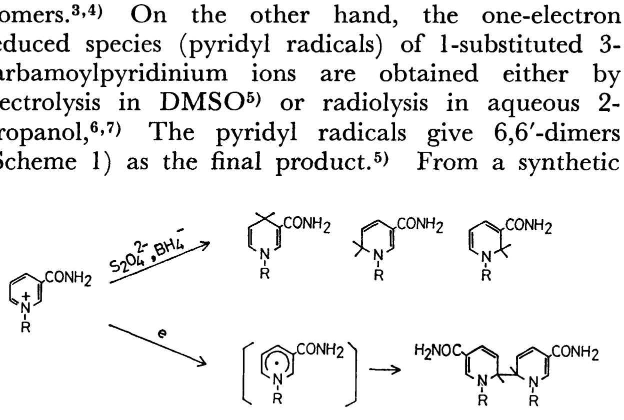
Koji KANO and Taku MATSUO*

*Department of Organic Synthesis, Faculty of Engineering, Kyushu University, Hakozaki, Higashi-ku, Fukuoka 812*

(Received May 7, 1976)

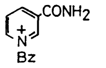
Photolysis of 1-benzyl-3-carbamoylpyridinium chloride ($\text{BNA}^+$) in aqueous solution in the presence of diethylamine and/or ethylenediaminetetraacetate gives the 6,6′-dimer (**1**), a coupled product of the pyridyl radicals generated by one-electron transfer from alkylamine to photoexcited $\text{BNA}^+$. Thermal one-electron reduction of $\text{BNA}^+$ either with zinc dust/copper sulfate or with magnesium powder in aqueous alkaline medium yields the 4,6′-dimer (**2**) together with a small amount of **1**. In the case of 3,5-bis(ethoxycarbonyl)-2,6-dimethylpyridine ($\text{HE}_{\text{ox}}$), the 2,2′-dimer (**3**) is formed as an initial photoreduction product, which is converted into the 4,4′-dimer (**4**) and 1,2-dihydropyridine (**5**) upon prolonged irradiation.

The non-enzymatic reduction of $\text{NAD}^+$ and its analogues is classified into one- and two-electron reductions.[2] Sodium dithionite and hydroborate are two-electron reducing agents, the former affording 1,4-dihydronicotinamides and the latter 1,2- and 1,6-isomers.[3,4] On the other hand, the one-electron reduced species (pyridyl radicals) of 1-substituted 3-carbamoylpyridinium ions are obtained either by electrolysis in DMSO[5] or radiolysis in aqueous 2-propanol,[6,7] The pyridyl radicals give 6,6′-dimers (Scheme 1) as the final product.[5] From a synthetic viewpoint, however, neither electrolysis nor radiolysis is a convenient method for preparing the reduced pyridines. In 1959, Wallenfels and Gellrich reported that the reduction of 1-benzyl- and 1-(2,6-dichlorobenzyl)-3-carbamoylpyridinium chlorides either with magnesium powder or with zinc dust/copper sulfate in aqueous alkaline solution gave the 6,6′-dimers in 20—26% yields.[8]

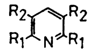


Scheme 1.

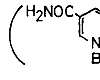
In the present paper, a new type of one-electron reduction of pyridinium and pyridine compounds is reported.[9] The compounds investigated are 1-benzyl-3-carbamoylpyridinium chloride ($\text{BNA}^+$) and 3,5-bis(ethoxycarbonyl)-2,6-dimethylpyridine ($\text{HE}_{\text{ox}}$), which are also useful as $\text{NAD}^+$ models. Diethylamine (DEA) and ethylenediaminetetraacetate (EDTA) were used as the reducing agents under photo-irradiation. The thermal reduction of $\text{BNA}^+$ was also carried out according to the method of Wallenfels and Gellrich. 4,6′-Dimer (**2**) was obtained as the main reduction product accompanied by a small amount of 6,6′-dimer (**1**). This result differs from that reported by Wallenfels and Gellrich.[8]



$\text{BNA}^+$



$\text{HE}_{\text{ox}}$



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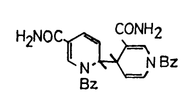

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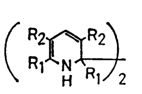

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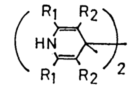


(4)

(5)

$R_1$ : Me
$R_2$: $CO_2Et$

Yellow solid materials adhered to the surface of the lamp upon irradiation of a solution of $\text{BNA}^+$ in water

TABLE 1. SPECTRAL AND PHYSICAL DATA OF DIMERS

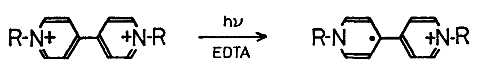
| Compound | Mp, °C | $\lambda_{\text{max}}$, nm (log $\varepsilon$) | $F_{\text{max}}$, nm[a] | IR, cm$^{-1}$ (KBr) |  | Mass | Anal[Found(Calcd)] |  |  |
|---|---|---|---|---|---|---|---|---|---|
|  |  |  |  | NH | C=O |  | C | H | N |
| 6,6′-Dimer (**1**) | 164—165 (dec) | 277 (3.44),<br>354.5 (3.89)<br>in MeOH |  | 3440, 3320<br>1640, 1630 | 1678 | 214, 213<br>(1/2M$^{\cdot+}$) | 72.76<br>(73.21) | 6.12<br>(6.14) | 13.03<br>(13.14) |
| 4,6′-Dimer (**2**) | 168.7—169.2 (dec) | 273 (4.17),<br>360 (3.96)<br>in MeOH |  | 3480, 3320<br>1660, 1650<br>1640, 1630 | 1679 | 214, 213<br>(1/2M$^{\cdot+}$) | 72.63<br>(73.21) | 6.16<br>(6.14) | 12.96<br>(13.14) |
| 2,2′-Dimer (**3**) | 175.8—176 (dec) | 387 (3.86)<br>in pyridine | 445 in pyridine | 3300 | 1695, 1686<br>1670, 1652 | 252<br>(1/2M$^{\cdot+}$) | 61.60<br>(61.89) | 7.12<br>(7.19) | 5.54<br>(5.55) |
| 4,4′-Dimer (**4**) | >200 (sublim.) | 240 (4.31),<br>275 (4.00)<br>360 (3.93)<br>in MeOH | 450 in MeCN | 3300 | 1692, 1655 | 252<br>(1/2M$^{\cdot+}$) | 61.84<br>(61.89) | 7.16<br>(7.19) | 5.61<br>(5.55) |

a) Fluorescence maximum.

containing DEA with a high-pressure mercury lamp. Based on spectroscopic data, the solid material was identified to be the 6,6′-dimer (**1**) as obtained by the coupling of the pyridyl radicals generated by the one-electron reduction of BNA$^+$. The yield of **1** was 50% as calculated on the basis of consumed BNA$^+$. 6,6′-Dimer (**1**) was synthesized by Wallenfels and Gellrich,[8] its UV and NMR spectroscopic data being reported.[8,10] The UV and NMR spectra of **1** obtained in the present experiment are in good agreement with those reported,[8,10] although the mp (164—165 °C (dec)) of **1** we obtained differs distinctly from that given by Wallenfels and Gellrich (mp 191—193 °C (dec)).[11] An attempt was made to prepare the 6,6′-dimer (**1**) by the receipe of Wallenfels and Gellrich.[8] Reduction was carried out either with zinc dust/copper sulfate or with magnesium powder in aqueous ammonia solution, the main product with mp 169 °C (dec) being obtained (yield, 6—20%) in both cases.[11] The UV and NMR spectra (Table 1) suggest that the thermal reduction product is not the 6,6′-dimer (**1**). By means of NMR spectroscopy, the compound was confirmed to be the 4,6′-dimer (**2**) (see Experimental). As in the case of 6,6′-dimer (**1**), 4,6′-dimer (**2**) was rapidly oxidized by methyl viologen in methanol. In the case of Zn/Cu system, a small amount of byproduct (mp 164—165.5 °C (dec), 0.3%) was also isolated together with **2** (20%). Both the spectroscopic data and mp (dec) proved that the byproduct is the same 6,6′-dimer (**1**) as obtained by photoreduction. In spite of the fact that experiment was carried out very carefully, **1** was obtained only in a low yield.

DEA could be replaced with EDTA. In this case, the solution should be made slightly alkaline since **1** decomposes to an unknown compound either in neutral or in acidic medium.

Benzyl and methyl viologens were analogously photoreduced to the corresponding cation radicals in the Ar-flushed aqueous solution (pH 7.9) containing EDTA:



R: $CH_2Ph$ $\lambda_{max}$ 371, 388, 400, 550, 560, 599, and 660 (sh) nm.
R: $CH_3$ $\lambda_{max}$ 367, 384, 395, 570 (sh), 600, and 660 nm.[12]

The pyridine ring itself could also be photoreduced by the above method devised for pyridinium ions. Upon irradiation of a mixture of $HE_{ox}$, EDTA, and potassium carbonate in 40% (v/v) aqueous acetonitrile, the 2,2′-dimer (**3**) precipitated as very pure, lemon yellow crystals, its yield being 51% as calculated on the basis of consumed $HE_{ox}$. The 2,2′-dimer (**3**) is hardly soluble in organic solvents, such as acetonitrile, dimethyl sulfoxide, carbon tetrachloride, chloroform, ethanol, methanol, acetone, and benzene, but easily soluble in basic solvents such as pyridine and DEA. Its structure was deduced from the following spectroscopic data. In the NMR spectrum of **3** in pyridine-$d_5$, the proton signals due to methyl groups at the 6,6′- and 2,2′-positions are observed at δ 2.11 and 2.58 ppm, respectively, and those due to the ethoxycarbonyl groups at 1.03 and 1.26 ppm. The proton at the 4-position can not be detected because the signal overlaps with that of non-deuterated pyridine (7.68 ppm) in the solvent. As to the methylene protons of the ethoxycarbonyl group, the four quartet signals were observed (Fig. 1). Since **3** contains two asymmetric carbon atoms, it is expected that *meso* and racemic isomers are present (Fig. 2) and that the chemical shift of the methylene protons of the ethoxycarbonyl groups in the two isomers differs. Judging from the distance between the asymmetric center and the protons in question, the difference in the chemical shift for the ethoxycarbonyl groups at 5- and 5′-positions should be less than that for the 3- and 3′-positions. Therefore, the two quartet signals at δ 4.12 and 4.13 ppm should be assigned to the methylene protons at 5(5′)-positions of the *meso* and racemic isomers. The ones at lower magnetic field (δ 4.32 and 4.36 ppm) are thus ascribed to the methylene protons at 3(3′)-positions of these isomers. In agreement with the assignment of the NMR spectra, four different carbonyl absorptions are observed for **3** (Table 1).

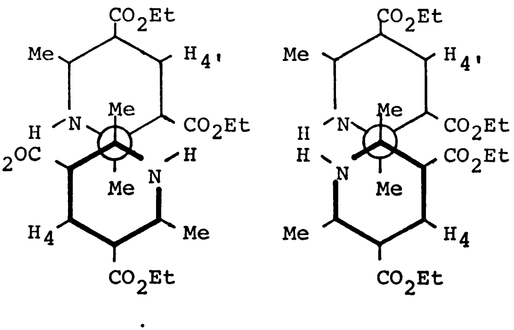


Fig. 1. Steric isomers of the 2,2′-dimer (**3**).

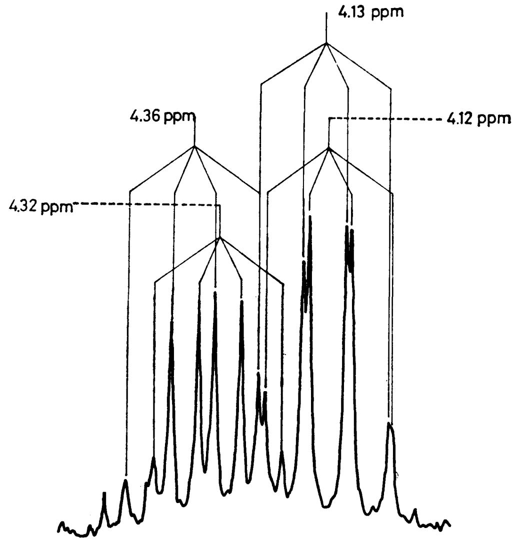


Fig. 2. NMR spectrum of $CO_2CH_2CH_3$ of the 2,2′-dimer(**3**) in DMSO-$d_6$ (90 MHz).

2,2′-Dimer (**3**) could not be isolated by the photolysis of $HE_{ox}$ in aqueous acetonitrile containing large

TABLE 2. EFFECTS OF IRRADIATION TIME ON YIELDS OF **4** AND **5**[a)]

| Run No. | Irradiation time, h | **4**, %[b)] | **5**, %[b)] |
|---|---|---|---|
| 1 | 3 | 14 | 12 |
| 2 | 6 | 19 | 13 |
| 3 | 6.25 | 19 | 17 |
| 4 | 14 | 3 | 27 |

a) A mixture of $HE_{ox}$ (1.0 g) and DEA (20 ml) in 50% (v/v) aqueous acetonitrile (200 ml) was irradiated with a 30W low-pressure mercury lamp under nitrogen atmosphere. b) Except for Run No. 3, the yields of **4** and **5** were determined by NMR spectra of the mixture of **4** and **5** which were obtained by the evaporation of acetonitrile from the photolyzed solution. In Run No. 3, the yields were calculated from the weight of **4** and **5** isolated.

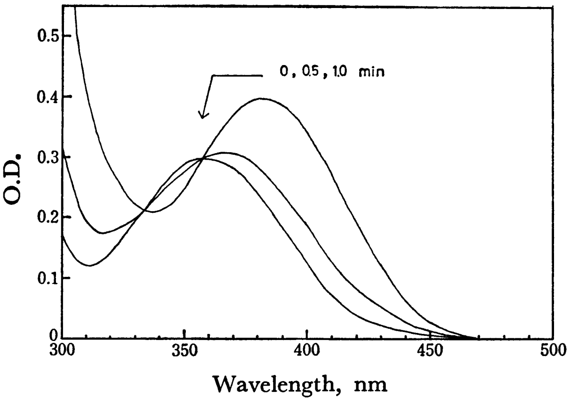


Fig. 3. Photoisomerization of the 2,2′-dimer (**3**) to the 4,4′-dimer (**4**). The 2,2′-dimer (**3**) in methanol was flushed with argon gas and irradiated with beams of light (>420 nm, a Toshiba V-Y42 filter) obtained from a Ushio 500 W super high-pressure mercury lamp.

excess of DEA. Instead, the 4,4′-dimer (**4**) and 3,5-bis(ethoxycarbonyl)-2,6-dimethyl-1,2-dihydropyridine (**5**) were obtained in 19 and 17% yields, respectively. It should be noted that dihydropyridine (**5**) is a two-electron reduced product. The effect of irradiation time on the yield of **4** and **5** is given in Table 2. It is seen that prolonged irradiation increases the yield of **5** at the expense of **4**. This suggests that **5** is formed *via* **4**. 4,4′-Dimer (**4**) ($\lambda_{max}$ 360 nm) was verified to be almost quantitatively converted to **5** ($\lambda_{max}$ 373 nm) upon irradiation of the aqueous acetonitrile solution in the presence of DEA, although **5** was not obtained in the absence of DEA.

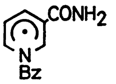
2,2′-Dimer (**3**) precipitated during irradiation (17%) when the photoreaction of $HE_{ox}$ was carried out in the presence of low concentration of DEA. It is suggested that **3** is an intermediate of the photochemical reactions. Figure 3 shows the spectral change along the photolysis of **3** in methanol. Upon irradiation (>420 nm, Ar), the absorption maximum at 382 nm due to **3** shifts to 358 nm which is ascribed to **4**. This is a 1,3-sigmatropic rearrangement, which is considered to proceed quantitatively since isosbestic points are observed at 257, 333, and 358 nm. The scheme of the photoreduction of $HE_{ox}$ in the presence of DEA can be summarized as follows (Scheme 2):

$$HE_{ox} \xrightarrow[\text{DEA}]{h\nu} [HE_{ox}H\cdot] \longrightarrow \mathbf{3} \xrightarrow{h\nu} \mathbf{4} \xrightarrow[\text{DEA}]{h\nu} \mathbf{5}$$

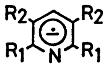
Scheme 2.

No photoreduction products of $HE_{ox}$ (**3**, **4**, and **5**) in methanol are oxidized by methyl viologen.

The photolysis of either $BNA^+$ or $HE_{ox}$ in aqueous media containing DEA and/or EDTA afforded the corresponding dimers of one-electron reduced species. In the case of $BNA^+$, the one-electron transfer from alkylamine to photoexcited $BNA^+$ should yield a radical **6**. On the basis of both quantum mechanical calculation[13)] and stereochemical consideration, it seems that coupling of the radical takes place at the 6-position.



(6)



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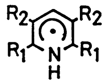


(8)

In the case of $HE_{ox}$, on the other hand, a pyridyl radical anion **7** should be a primary photoproduct. According to Brühlmann and Hayon, the $pK_a$-values for radical anions of nicotinamide analogues are generally larger than 13.[7)] The radical anion **7** can be immediately protonated to yield a free radical **8** under the present experimental conditions. The free radical **8** will afford the dimer **3** as the final product. The pyridyl radicals are thus proposed as the common intermediates in the photoreduction of both $BNA^+$ and $HE_{ox}$. The presence of the pyridyl radicals has also been verified by the kinetic study of the reduction of the Hemin pyridine complex (one-electron acceptor) coupled with the photoreduction of $BNA^+$, $HE_{ox}$, and other related compounds in the presence of *N,N,N′,N′*-tetramethylethylenediamine.[14)]

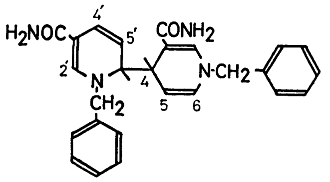
In connection with the redox property of the pyridine derivatives, it should be mentioned that the Hemin complex was also reduced by all the above-described dimers (**1**, **2**, **3**, and **4**) although the rates were somewhat lower than those with the pyridyl radicals. On the other hand, the Hemin complex was hardly reduced by two-electron reduced $NAD^+$-model compounds such as 1-benzyl-1,4-dihydronicotinamide[15)] and 3,5-bis(ethoxycarbonyl)-2,6-dimethyl-1,4-dihydropyridine (Hantzsch ester). This difference may be useful in elucidating the mechanism of redox-systems where pyridine derivatives play important roles.

## Experimental

Melting points were determined on a hot-stage apparatus and are corrected. Spectroscopic measurements were made with Shimadzu UV-200 and Hitachi 124 recording spectrophotometers, Varian A-60 and Bruker WH-90 FT NMR spectrometers, a Hitachi RML-6E mass spectrometer, and a JASCO DS-403 G grating IR spectrophotometer. A Shimadzu Baush & Lomb Spectronic 505 recording spectrophotometer was modified for the fluorescence measurements. $BNA^+$[16] and $HE_{ox}$[17] were prepared according to the procedures given in the literature. Reagent grade DEA (Wako Pure Chem.) and disodium ethylenediaminetetraacetate dihydrate (Dohzin Pharm. Co.), DMSO-$d_6$ (Stohler Isotope Chem., 99.5%), and pyridine-$d_5$ (Merck, 99.5%) were used. Water was distilled and deionized with a column of ion-exchange resin. The photolysis in a preparative scale was carried out by the use of Riko immersion-type 100W high-pressure and 30W low-pressure mercury lamps.

*Reduction of 1-Benzyl-3-carbamoylpyridinium Chloride with Zinc Dust-Copper Sulfate in Aqueous Ammonia.*[8] A solution of 2.5 g copper sulfate pentahydrate in 20 ml of water was gradually poured into water containg 6 g of zinc dust under vigorous stirring at room tempreature. A mixture of 10 ml of 28% aqueous ammonia and 50 ml of methanol was added to it and then 5 g (20 mmol) of $BNA^+$ in 20 ml of water was poured into the solution. At this time, the reaction mixture turned yellow. After stirring was continued for 20 min at room temperature, the yellow precipitates and inorganic materials were collected by filtration. The residue was extracted with 20 ml of boiling methanol in nitrogen atmosphere and the insoluble matter was filtered. The residue was further extracted with 30 ml of boiling methanol. The former extract was kept in a refrigerator (*ca.* −5 °C) overnight to give lemon yellow crystals (0.85 g), which was identified to be 4,6′-dimer (**2**) (yield, 20%); mp 168.5−169 °C (dec); NMR (DMSO-$d_6$): $\delta$ 7.38 (s, aromatic ring), 7.18 (s, 2,2′H), 6.78 and 6.38 (broad s, $CONH_2$), 6.36 (d, 4′H or 6H, $J_{4',5'}$ or $J_{5,6}$ 8.0 Hz), 6.20 (d, 6H or 4′H, $J_{5,6}$ or $J_{4',5'}$ 8.0 Hz), 4.79 (q, 5′H, $J_{5',6'}$ 5.0 Hz), 4.66 (q, 5H, $J_{4,5}$ 4.4 Hz), 4.42 (s, $NCH_2$), 4.00 (d, 4H), and 3.28 ppm (d, 6′H). The NMR spectrum (5 mg/1 ml of DMSO-$d_6$) was obtained after 100 scans with the Bruker WH-90 FT NMR spectrometer.

When nitrogen was bubbled into a methanolic solution of the latter extract, 13 mg (0.3%) of the 6,6′-dimer (**1**) separated out of the solution; mp 164—165.5 °C (dec). The analytical date are given in Table 1 and in the photochemical section (*vide infra*).



*Reduction of 1-Benzyl-3-carbamoylpyridinium Chloride with Magnesium Powder in Aqueous Ammonia.*[8] Magnesium powder (3 g) was gradually added into a 1.1% aqueous ammonia solution (100 ml) of $BNA^+$ (5 g, 20 mmol) and ammonium chloride (9 g) under vigorous stirring at 48—50 °C, about 1.5 h being required for the addition. Stirring was continued for additional 30 min at 48—50 °C after all of magnesium powder had been consumed. When the reaction was completed, the reaction mixture was cooled with an ice-bath and the yellow precipitates and colorless inorganic solid were collected by filtration. The residue was dissolved into 100 ml of methanol in order to remove inorganic matter and the methanolic layer was concentrated to *ca.* 50 ml. The residue was gradually poured into 200 ml of 2.8% aqueous ammonia under cooling with an ice-bath. The pale yellow precipitates (3 g) were filtered and recrystallized from acetonitrile to give crude 4,6′-dimer (**2**) (mp 144—145.5 °C (dec)). Further recrystallization from methanol afforded the pure 4,6′-dimer (**2**) (0.27 g, 6%); mp 168.7—169 °C (dec).

*Photoreduction of 1-Benzyl-3-carbamoylpyridinium Chloride in the Presence of Diethylamine.* A solution of $BNA^+$ (2 g, 8.0 mmol) and DEA (10 ml, *ca.* 96 mmol) in 140 ml of water was irradiated with a 100W high-pressure immersion lamp under nitrogen bubbling for 2 h. Orange yellow precipiates (119 mg) were collected by filtration. The filtrate was further irradiated for 2 h after the solid covering the lamp wall had been removed. The second precipitates (212 mg) were collected and combined with the former. The NMR spectrum indicated that the precipitates were mainly 6,6′-dimer (**1**) (19%). The crude **1** (67 mg) was recrystallized from methanol and the pale yellow plates obtained were dried at 50 °C overnight to give 42 mg of pure **1**; mp 164—165 °C (dec); NMR (DMSO-$d_6$): $\delta$ 7.29 (s, aromatic ring), 7.18 (s, 2H), 6.97 (broad s, $CONH_2$), 6.04 (d, 4H, $J_{4,5}$ 7.6 Hz), 4.34 (s, $NCH_2$), 4.33 (q, 5H), and 3.18 ppm (d, 6H, $J_{5,6}$ 5.0 Hz). The NMR spectrum was measured with the Bruker WH-90 FT NMR spectrometer. After the second photolysis, unreacted $BNA^+$ was recovered (1.23 g, 61%) from the filtrate.

*Photoreduction of 1-Benzyl-3-carbamoylpyridinium Chloride in the Presence of Ethylenediaminetetraacetate.* A solution of $BNA^+$ (0.5 g, 2 mmol), disodium ethylenediaminetetraacetate dihydrate (1.1 g, 3 mmol), and potassium carbonate (2 g) in 200 ml of water was irradiated with a 30W low-pressure mercury lamp under argon bubbling for 10 h. The precipitates (63 mg, 15%) were filtered and recrystallized from methanol to give the 6,6′-dimer (**1**).

*Photoreduction of 3,5-Bis(ethoxycarbonyl)-2,6-dimethylpyridine in the Presence of Ethylenediaminetetraacetate.* A solution of $HE_{ox}$ (0.5 g, 2 mmol), disodium ethylenediaminetetraacetate dihydrate (2.25 g, 6 mmol), and potassium carbonate (2 g) in 40% (v/v) aqueous acetonitrile (250 ml) was irradiated with a 30W low-pressure mercury lamp under nitrogen bubbling and stirring for 9.5 h. The lemon yellow precipitates of the 2,2′-dimer (**3**) (171 mg, 34%) were collected by filtration; mp 178—179 °C (dec). $HE_{ox}$ (166 mg, 33%) was recovered by the evaporation of acetonitrile from the filtrate. 2,2′-Dimer (**3**) can be recrystallized from pyridine; mp 175.8—176 °C (dec); NMR (pyridine-$d_5$): $\delta$ 8.98 (braod s, NH), 7.68 (s, 4H?), 4.36 and 4.32 (q, 3,3′-$CO_2CH_2CH_3$), 4.13 and 4.12 (q, 5,5′-$CO_2CH_2CH_3$), 2.58 (s, 2,2′-$CH_3$), 2.11 (s, 6,6′-$CH_3$), 1.26 (t, 3,3′-$CO_2CH_2CH_3$, $J$ 7.0 Hz), and 1.03 ppm (t, 5,5′-$CO_2CH_2CH_3$, $J$ 7.0 Hz). The NMR spectrum (10 mg/0.6 ml of pyridine-$d_5$) was obtained after 35 scans with the Bruker WH-90 FT NMR spectrometer.

*Photoreduction of 3,5-Bis(ethoxycarbonyl)-2,6-dimethylpyridine in the Presence of Diethylamine.* a) A solution of $HE_{ox}$ (1 g, 4 mmol) and DEA (20 ml, 192 mmol) in 50%(v/v) aqueous acetonitrile (120 ml) was irradiated with a 30W low-pressure mercury lamp under nitrogen bubbling for 6.25 h. The reaction mixture was concentrated *in vacuo* to *ca.* 50 ml. Yellow precipitates were collected by filtration. Unreacted $HE_{ox}$ was recovered from the filtrate as brown oil, which was purified by silica gel column chromatography with ethyl acetate and recrystallized from acetonitrile (284 mg, 28%). The yellow precipitates were recrystallized from acetonitrile to give 193 mg of the 4,4′-dimer (**4**) (19%); mp>200 °C (sublim.). The filtrate was concentrated and the residue obtained was purified by silica gel column chromatography with ethyl acetate to give 172 mg of 3,5-bis(ethoxycarbonyl)-2,6-dimethyl-1,2-dihydropyridine (**5**) (17%); mp 149—153 °C (dec).

The 4,4′-dimer (**4**); NMR (DMSO-$d_6$): $\delta$ 8.35 (broad s, NH), 3.97 (s, 4,4′H), 3.97 (q, $CO_2CH_2CH_3$, $J$ 7.0 Hz), 2.05 (s, $CH_3$), and 1.18 ppm (t, $CO_2CH_2CH_3$).

3,5-Bis(ethoxycarbonyl)-2,6-dimethyl-1,2-dihydropyridine (**5**); Mass: 253 ($M^+$); UV: $\lambda_{max}^{MeOH}$ 212, 283, and 373.5

nm; fluorescence: $F_{max}^{MeOH}$ 490 nm; NMR (DMSO-$d_6$): $\delta$ 7.70 (s, 4H), 6.93 (broad s, NH), 4.52 (q, 2H, $J_{2,Me}$ 6.5 Hz), 4.18 (q, $CO_2CH_2CH_3$, $J$ 7.0 Hz), 2.39 (s, $6CH_3$), 1.30 (t, $CO_2CH_2CH_3$), and 1.18 ppm (d, $2CH_3$). The NMR spectrum of **4** and **5** were measured with the Varian A-60 spectrometer.

b) A mixture of $HE_{ox}$ (0.5 g, 2 mmol), DEA (1 g, 14 mmol), and 100 ml of a phosphate buffer solution (1/15 M, pH 8.0) was disolved into 150 ml of 67%(v/v) aqueous acetonitrile. The $HE_{ox}$ solution was irradiated with a 30W low-pressure mercury lamp under nitrogen bubbling and stirring for 4 h. The lemon yellow precipitates were collected by filtration and washed with water and acetonitrile to give pure 2,2′-dimer (**3**) (84 mg, 17%); mp 176—176.5 °C (dec).

## References

1) Contribution No. 406 from the Department of Organic Synthesis, Faculty of Engineering, Kyushu University.

2) H. Sund, H. Diekmann, and K. Wallenfels, "Advances in Enzymology," Vol. 26, Interscience, New York, London (1964), p. 115.

3) K. Wallenfels and H. Schuly, *Angew. Chem.*, **67**, 617 (1955); **69**, 505 (1957).

4) K. Wallenfels and H. Schuly, *Justus Liebigs Ann. Chem.*, **621**, 106 (1959).

5) K. S. V. Santhanam and P. J. Elving, *J. Am. Chem. Soc.*, **95**, 5482 (1973).

6) R. L. Willson, *Chem. Commun.*, **1970**, 1005; *Trans. Faraday Soc.*, **67**, 3020 (1971).

7) U. Brühlmann and E. Hayon, *J. Am. Chem. Soc.*, **96**, 6169 (1974).

8) K. Wallenfels and M. Gellrich, *Chem. Ber.*, **92**, 1406 (1959).

9) A preliminary communication: K. Kano and T. Matsuo, *Tetrahedron Lett.*, **1975**, 1389.

10) H. Diekmann, G. Engert, and K. Wallenfels, *Tetrahedron*, **20**, 291 (1964).

11) The melting points (decomposition points) of **1** (164—165.5 °C) and **2** (169 °C) were reproducible as far as the measurement was made on a hot-stage apparatus. However, **1** and **2** decomposed at >187 °C and 180—182 °C, respectively, and turned into brown resins when the measurement was carried out with degassed samples in capillary tubes and a heating bath. Both resins gradually melted at 210—220 °C. **1** and **2** might have been oxidized by air during the course of mesurement on the hot-stage, the resulting oxidation products decomposing at 164—165.5 °C (**1**) and 169 °C (**2**). The difference in the mp (dec) of **1** between the present experiment and that by Wallenfels and Gellrich seems to be due to the difference in the method of measurement.

12) E. M. Kosower and J. L. Cotter, *J. Am. Chem., Soc.*, **86**, 5524 (1964).

13) B. Pullman and A. Pullman, *Proc. Natl. Acad. Sci. U.S.A.*, **45**, 136 (1972).

14) K. Kano, T. Shibata, M. Kajiwara, and T. Matsuo, *Tetrahedron Lett.*, **1975**, 3693.

15) A. Ohno, T. Kimura, S. Oka, Y. Ohnishi, and M. Kagami, *Tetrahedron Lett.*, **1975**, 2371.

16) P. Karrer and F. J. Stare, *Helv. Chim. Acta*, **20**, 418 (1937).

17) A. Singer and S. M. McElvain, *Org. Synth.*, Coll. Vol. II, 860 (1943).