Reduction of Vicinal Tricarbonyl Compounds by Reduced Nicotinamide Adenine Dinucleotide Model and Electron-Transport Systems

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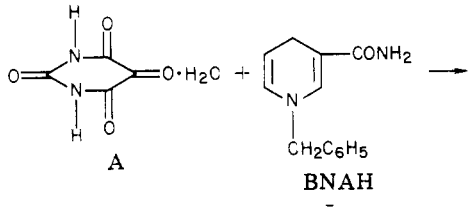
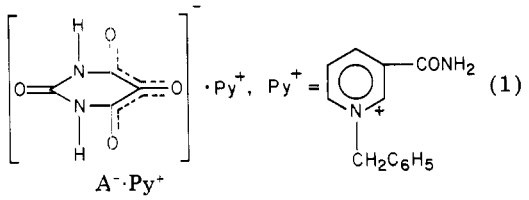
The reduction of vicinal tricarbonyl compounds such as alloxan (A) and ninhydrin (NY) with 1-benzyl-1,4-dihydronicotinamide (BNAH; NADH model) was investigated. In the reduction of A with BNAH, the radical anion ($A^{-}\cdot Py^{+}$) of A as the 1-benzyl-3-carbamoylpyridinium salt and the dialuric acid (D) was obtained by one-electron and two-electron reduction, respectively. Similarly, hydrindantin (HDT) and 2-hydroxy-1,3-indandione (HID) were also afforded in the reduction of NY with BNAH. Further, the reductions of alloxantin (AT) and HDT with BNAH were performed to give D and HID, respectively. The reduction of lipoic acid (LA) and viologens (V^{2+}) with BNAH, which could not be reduced without A or NY, proceeded smoothly in their presence, and they proved to be useful as mediators for catalytic reduction of LA and V^{2+}.

The reactions of 1-alkyl-1,4-dihydronicotinamide derivatives as a model for the reduced nicotinamide adenine dinucleotide (NADH) have been widely investigated because of their biochemical significance. 1-Benzyl-1,4-dihydronicotinamide (BNAH), a model of NADH, can reduce thiobenzophenone[1,2] and activated carbonyl compounds such as hexachloroacetone[3] and 2,2,2-trifluoroacetophenone,[4-7] but benzophenone and other alkyl or aryl ketones and aldehydes are insensitive to the reduction by BNAH.

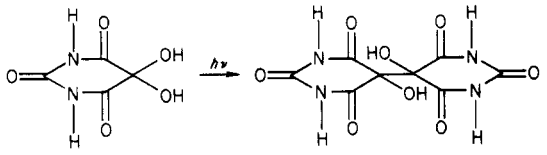
We have reported in a previous paper[8] that the radical anion ($A^{-}\cdot Py^{+}$) of alloxan can be isolated as a 1-benzyl-3-carbamoylpyridinium salt by one-electron reduction of alloxan hydrate (A) with BNAH (eq 1).





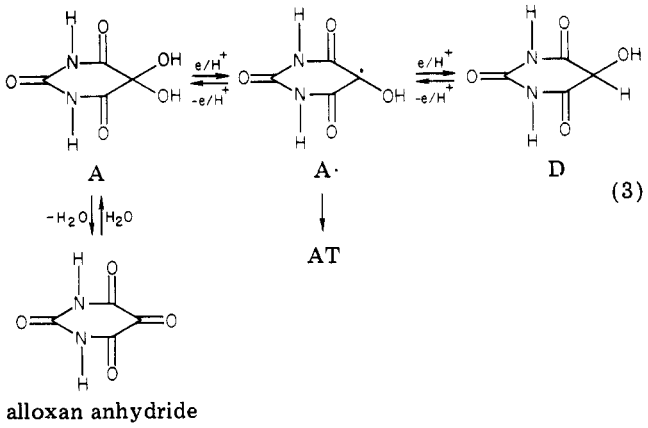
(1)

Alloxan is known as one of the reductones which play an important role in oxidation–reduction systems as illustrated by ascorbic acid. The central carbonyl group of alloxan activated by two adjacent carbonyl groups is so reactive that it easily combines with 1 mol of water to form an alloxan hydrate (A), and it has been reported[9,10] that alloxantin (AT) is obtained by photoirradiation of A in good yield (eq 2).



(2)

We have also investigated the redox system mediated by alloxan radical (A·) or dialuric acid (D) produced from A photochemically or chemically[8,11,12] (eq 3).



(3)

In this paper, we report on the reduction of vicinal tricarbonyl compounds such as A or NY by BNAH and on electron-transport systems mediated by A or NY in the reduction of lipoic acid (LA) or viologens (V^{2+}) with BNAH.

Results and Discussion

Reduction of Vicinal Tricarbonyl Compounds with 1-Benzyl-1,4-nicotinamide (BNAH). It was observed in preliminary experiments by UV spectroscopy that the amount of BNAH (λ_{max} 356 nm) decreased in ethanol when A or NY was added at room temperature, whereas the oxidized form (BNA^{+}, λ_{max} 260 nm) of BNAH increased. These observations suggest that BNAH can easily reduce A and NY. For the estimation of the reduction rates of A and NY by BNAH in ethanol, the apparent first-order rate constants (k) were measured from the decreased amounts of BNAH. Values of k for A and NY at 21 °C = 2.49×10^{-2} and 1.92×10^{-3} min^{-1}, respectively, which show that A can be reduced over 10 times faster than NY (relative rate; A/NY ratio of 14.3).

(1) R. H. Abeles, R. F. Htton, and F. H. Westtheimer, *J. Am. Chem. Soc.*, **79**, 712 (1957).
(2) A. Ohno and N. Kito, *Chem. Lett.*, 391 (1977).
(3) D. C. Dittmer and R. A. Fouty, *J. Am. Chem. Soc.*, **86**, 91 (1964).
(4) J. J. Steffens and D. M. Chipman, *J. Am. Chem. Soc.*, **93**, 6694 (1971).
(5) D. J. Creighton and D. S. Sigman, *J. Am. Chem. Soc.*, **93**, 6314 (1971).
(6) Y. Ohnishi, T. Numakunai, and A. Ohno, *Tetrahedron Lett.*, 3813 (1975).
(7) Y. Ohnishi, T. Numakunai, T. Kimura, and A. Ohno, *Tetrahedron Lett.*, 2699 (1976).
(8) T. Endo and M. Okawara, *Chem. Lett.*, 1487 (1977).
(9) T. Matsuura, R. Sugae, R. Nakashima, and K. Omura, *Tetrahedron*, **24**, 6149 (1968).
(10) Y. Otsuji, S. Wake, and E. Imoto, *Tetrahedron*, **26**, 4139 (1970).
(11) G. A. Russel and M. C. Young, *J. Am. Chem. Soc.*, **88**, 2007 (1966).
(12) T. Endo and M. Okawara, *Tetrahedron Lett.*, 3301 (1979).

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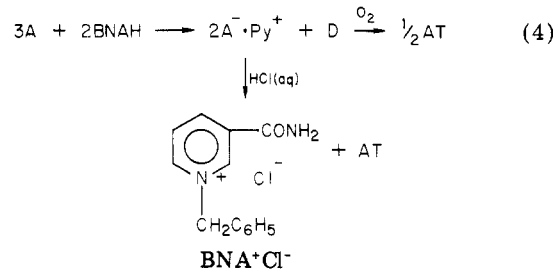
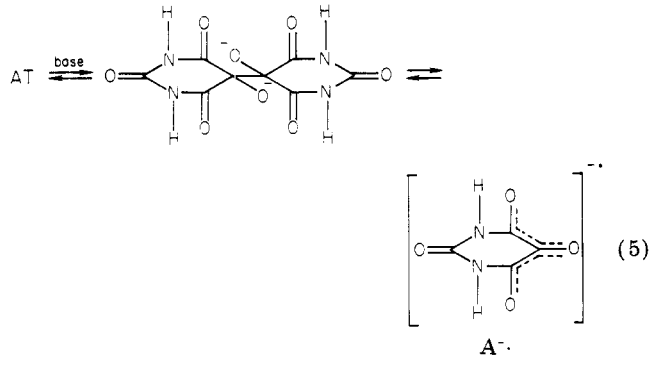
The reactions of A or NY with BNAH were carried out on a preparative scale under a nitrogen atmosphere at room temperature. First, the reaction of A and BNAH was attempted in ethanol and gave a yellow precipitate and dialuric acid (D).[8] The structure of the precipitate has been confirmed as the radical anion salt of alloxan ($A^{-}\cdot Py^{+}$) by UV, ESR, NMR, and elemental analyses. D obtained by a two-electron reduction is easily oxidized by air to afford AT. Treating $A^{-}\cdot Py^{+}$ with aqueous hydrochloric acid resulted in AT and 1-benzyl-3-carbamoylpyridinium chloride ($BNA^{+}Cl^{-}$) being obtained almost quantitatively (eq 4). It has been reported[11] that AT is dissociated



(4)

effectively in basic media to afford alloxan radical anion ($A^{-}\cdot$, eq 5). $A^{-}\cdot Py^{+}$ was also obtained by treating $BNA^{+}Cl^{-}$



(5)

and AT with triethylamine in methanol at room temperature (eq 6). Similarly, the reaction of BNAH and NY

$$BNA^{+}Cl^{-} + [{}^{1}/_{2}AT \rightleftarrows A\cdot] \xrightarrow[MeOH]{Et_3N} A\cdot Py^{+} + Et_3N\cdot HCl \quad (6)$$

was carried out in methanol to afford hydrindantin (HDT) and 2-hydroxy-1,3-indandione (HID) without isolating the radical anion salt ($NY^{-}\cdot Py^{+}$) of NY (Scheme I). HID could be isolated as 2-acetoxy-1,3-indandione (AID) by treating it with acetic anhydride, because HID was sensitive to air (O_2). $BNA^{+}OH^{-}$ was also isolated as $BNA^{+}Cl^{-}$ by treating it with aqueous hydrochloric acid.

Reductions of AT or HDT with BNAH. In our preliminary experiment with UV spectroscopy, we found that AT and HDT could be reduced easily by BNAH as for the case of A or NY. The apparent first-order rate constants (k) of AT and HDT were measured at 21 °C in ethanol from the decreased amounts of BNAH in the presence of excess AT or HDT (AT/BNAH and HDT/BNAH ratios of 24), respectively. Values of k for AT and HDT were estimated as 5.0×10^{-2} and 3.85×10^{-3} min^{-1}, respectively, which indicated that AT could be reduced 13 times faster than HDT.

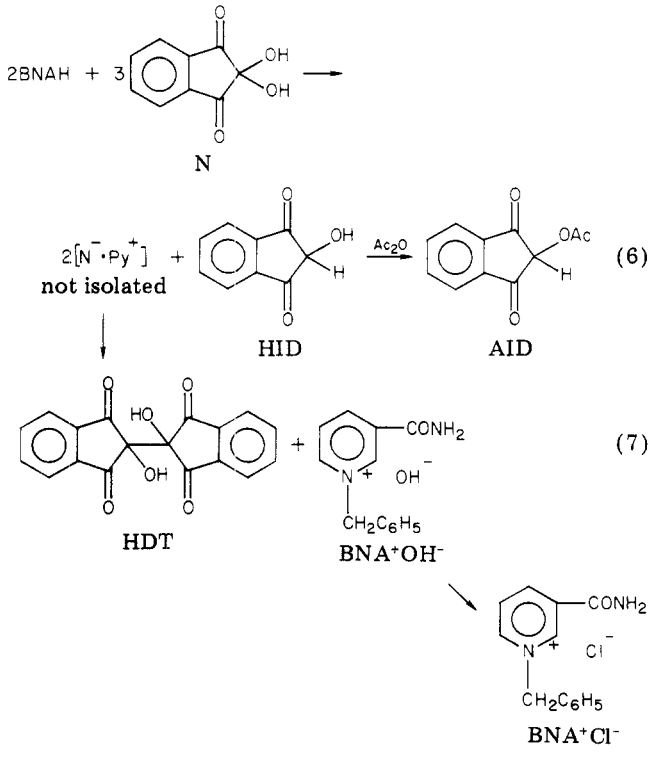
The reactions of AT or HDT with BNAH in methanol were performed on a preparative scale. In the reaction of AT and BNAH at room temperature (eq 7) a yellow precipitate and D were obtained as in the case of the reaction of A and BNAH. The precipitate corresponded completely to the $A^{-}\cdot Py^{+}$ obtained by the reaction of A and BNAH. Further, the reaction of HDT and BNAH was carried out at room temperature to give HID and $BNA^{+}OH^{-}$ in good yields. HID was isolated as AID by treating it with acetic anhydride, and $BNA^{+}OH^{-}$ was also obtained as $BNA^{+}Cl^{-}$ by adding aqueous hydrochloric acid to the reaction mixture (eq 8).

$$BNAH + [HDT \rightleftarrows 2N\cdot] \longrightarrow 2HID + BNA^{+}OH^{-} \quad (8)$$

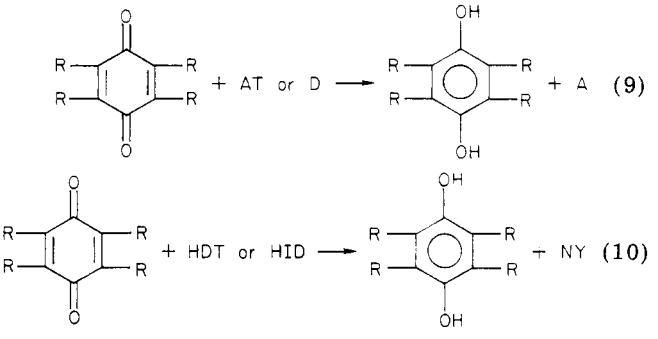
$$\xrightarrow{Ac_2O} AID \qquad \xrightarrow{HCl(aq)} BNA^{+}Cl^{-}$$

$$BNAH + [HDT \rightleftarrows 2N\cdot] \longrightarrow 2HID + BNA^{+}OH^{-} \quad (8)$$

Scheme I



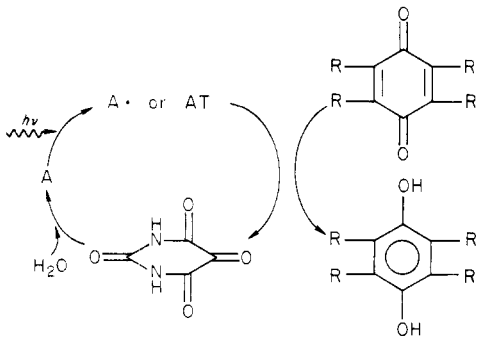
Reductions of Lipoic Acid (LA) and Viologens (V^{2+}) with BNAH in the Presence of Vicinal Tricarbonyl Compounds. Construction of Electron-Transport Systems. It has been reported in our previous paper that quinones can be reduced by AT or D, which are obtained by one-electron or two-electron reductions of A, to afford the corresponding hydroquinones and A[12] (eq 9 and 10).



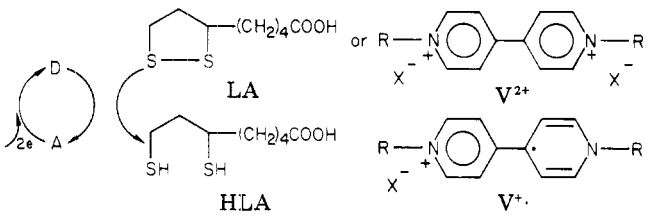
In addition, HDT and HID have the ability to reduce the quinones. We have also demonstrated that A or AT produced from A photochemically mediates the reduction of quinones with water[12] (Scheme II).

A and AT are capable of reducing some oxidants such as oxygen, DPPH, Tillman reagent (2,6-dichlorophenol-

Scheme II

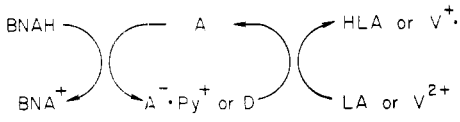


Scheme III[a]

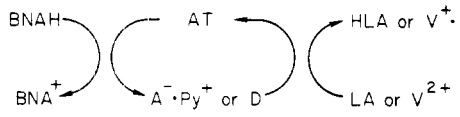


[a] R = CH_3 and $CH_2C_6H_5$, X = Cl and I.

Scheme IV



Scheme V



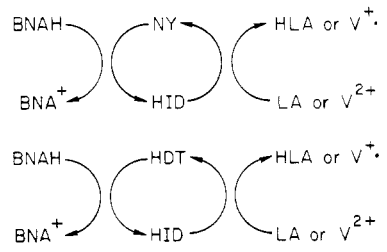
indophenol), and malachite green, but they have no ability to reduce lipoic acid (LA) and methyl or benzyl viologens (V^{2+}).

It has been found that D obtained by two-electron reduction of A can reduce LA and V^{2+} at room temperature to give dihydrolipoic acid (HLA) and viologen radical cation ($V^{+}\cdot$), respectively, in 70–80% yields (Scheme III), and A was recovered in 90% yield. HLA was isolated as diacetylated LA by treating HLA with acetic anhydride, because HLA was sensitive to air. Alloxan radical anion salt ($A^{-}\cdot Py^{+}$), which is obtained from A and BNAH, is also able to reduce quinones, LA, and V^{2+} in good yields.

LA and V^{2+} are scarcely reduced with BNAH under similar conditions.[13] However, the addition of catalytic amount of A induces the reduction of LA and V^{2+} to afford HLA and $V^{+}\cdot$, respectively, in 60–70% yields, suggesting the reaction shown in Scheme IV. In addition, it was confirmed that LA and V^{2+} could be reduced smoothly with BNAH by the addition of a catalytic amount of AT to afford HLA and $V^{+}\cdot$ in good yields, respectively (Scheme V). Furthermore, it was also found that the reduction of LA and V^{2+} with BNAH proceeded by the addition of a catalytic amount of NY or HDT to give LAH and $V^{+}\cdot$ in 60–70% yields, respectively (Scheme VI).

Electron-transport systems play very important roles in biological energy conversion as shown in the case of photosynthesis. It may then be useful to organize a biomimetic electron-transport system as one step in the construction of man-made energy-conversion systems. It has been reported that BNAH can also reduce viologens or 2,3,5-triphenyltetrazolium chloride by addition of a catalytic amount of *N*-methylphenazinium salt to provide an electron bridge.[14] Our results suggest that vicinal tricarbonyl compounds such as A and NY have played the role of a catalyst for an electron-transfer reaction or have provided an electron bridge for the reduction of LA and V^{2+} by BNAH.

Scheme VI



Experimental Section

Materials. Alloxan monohydrate (A, mp 253 °C dec) was prepared by the oxidation of barbituric acid with chrominium trioxide.[15] Alloxantin (AT, mp 230–231 °C) used in this study was prepared by the reduction of A with hydrogen sulfide,[16] and dialuric acid (D, mp 212 °C) was synthesized by the reduction of A with hydrogen sulfide and oxidized easily by air to AT.[17] 1-Benzyl-1,4-dihydronicotinamide (BNAH) was prepared according to the method reported previously.[18] Ninhydrin (NY) and hydrindantin (HDT) were of commercial reagent grade.

Kinetics. The kinetic measurements were carried out at 21 °C in absolute ethanol. The progress of the reaction was followed spectrophotometrically by monitoring the decrease in the absorption of BNAH at 356 nm.

Reaction of A and BNAH. A solution of 0.8 g (5 mmol) of A and 1 g (5 mmol) of BNAH in 50 mL of methanol was allowed to stand under an atmosphere of nitrogen at room temperature and produced a precipitate immediately. After the mixture was stirred for 2 h, the precipitate was filtered off and dried in vacuo to give 0.75 g of alloxan anion salt ($A^{-}\cdot Py^{+}$, mp 195–196 °C[8]). AT (0.25 g, mp 229–231 °C) was isolated by bubbling air into the filtrate. Further, hydrochloric acid (2 mL, 37%) was added to the filtrate, and then the filtrate was evaporated to dryness. The residue was recrystallized from ethanol to give 0.5 g of $BNA^{+}Cl^{-}$.

Preparation of $A^{-}\cdot Py^{+}$ by the Reaction of $BNA^{+}Cl^{-}$ with AT. A solution of 0.5 g (5 mmol) of triethylamine in 20 mL of methanol was added dropwise to a solution of $BNA^{+}Cl^{-}$ (1.3 g, 5.2 mmol) and AT (0.8 g, 2.5 mmol) in 50 mL of methanol under an atmosphere of nitrogen at room temperature to afford 1.2 g of $A^{-}\cdot Py^{+}$, and 2.2 g of triethylamine hydrochloride was obtained from the filtrate.

Reaction of BNAH and NY. Preparation of 2-Hydroxy-1,3-indandione (HID). A solution of 0.89 g (5 mmol) of NY and 1.2 g (6 mmol) of BNAH in 50 mL of methanol was allowed to stand under an atmosphere of nitrogen at room temperature for 15 h and produced a dark purple solution. After methanol was removed in vacuo, 30 mL of acetic anhydride and 1 mL of pyridine were added to the residue. Unreacted acetic anhydride and pyridine were removed, and then the residue was recrystallized from ethyl acetate–petroleum ether to obtain 0.78 g (70%) of

(13) It has been reported previously that LA can be reduced by BNAH [C. H. Wang, *Experimentia*, **27**, 243 (1971)]. We reinvestigated the reduction of LA by BNAH, but it was found that LA could not be reduced by BNAH.

(14) N. Kito, Y. Ohnishi, M. Kagami, and A. Ohno, *Chem. Lett.*, 353 (1974).
(15) A. V. Holmgren and W. Wenner, "Organic Syntheses", Collect. Vol. IV, Wiley, New York, 1963, p 23.
(16) S. Tipson, "Organic Syntheses", Collect. Vol. IV, Wiley, New York, 1963, p 25.
(17) S. Tipson and L. H. Cretcher, *J. Org. Chem.*, **16**, 1019 (1951).
(18) D. Mauzerall and F. H. Westtheimer, *J. Am. Chem. Soc.*, **77**, 2261 (1955).

2-acetoxy-1,3-indandione [AID, mp 96 °C (lit.[19] mp 97 °C)].

Preparation of HDT. A solution of 1 g (1 mmol) of NY and 0.25 g (2.5 mmol) of BNAH in 30 mL of methanol was allowed to stand under an atmosphere of nitrogen at room temperature for 15 h, and then 5 mL of petroleum ether was added to the solution. The solution was cooled at 0 °C, and 0.5 g (60%) of HDT [mp 242 °C (lit.[9] mp 243 °C)] was obtained.

Reduction of AT by BNAH. A solution of 1.07 g (5 mmol) of BNAH and 1.61 g (5 mmol) of AT was allowed to stand under an atmosphere of nitrogen at room temperature for 4 h and produced a precipitate immediately. After the mixture was stirred for 4 h, the precipitate was filtered off and dried in vacuo to give 0.8 g of $A^{\cdot}$-Py^{+}, and 0.5 g of D [mp 213 °C (lit.[17] mp 212 °C)] was isolated from the filtrate.

Reduction of HDT by BNAH. A solution of 1.07 g (5 mmol) of BNAH and 1.61 g (5 mmol) of HDT was allowed to stand under an atmosphere of nitrogen at room temperature for 7 h and produced a dark purple solution. After the methanol was evaporated in vacuo, 30 mL of acetic anhydride and 1 mL of pyridine were added to the residue, and the reaction mixture was allowed to stand at room temperature for 10 h. After unreacted acetic anhydride and pyridine were removed under reduce pressure, the residue was washed with methylene chloride three times. From the organic layer 1.4 g (70%) of AID was obtained, and 1 g of $BNA^{+}Cl^{-}$ was isolated by dissolving the insoluble solid in aqueous hydrochloric acid.

Reduction of *p*-Benzoquinone by $A^{\cdot}$-Py^{+}. A solution of 3.3 g (10 mmol) of $A^{\cdot}$-Py^{+} and 0.5 g (5 mmol) of *p*-benzoquinone in 100 mL of ethanol and 2 mL of hydrochloric acid (37%) was allowed to stand under an atmosphere of nitrogen at room temperature for 7 h. After the ethanol was evaporated in vacuo, the reaction mixture was dissolved in water (150 mL), and the solution was extracted with ether five times. From the organic layer 0.5 g (90%) of hydroquinone was obtained, and 2.3 g (90%) of $BNA^{+}Cl^{-}$ and 1.1 g (70%) of A were isolated from the aqueous solution.

Reduction of Benzyl Viologen by $A^{\cdot}$-Py^{+}. A solution of 1.6 g (5 mmol) of $A^{\cdot}$-Py^{+} and 2 g (5 mmol) of benzyl viologen in 100 mL of ethanol and 2 mL of hydrochloric acid (37%) was allowed to stand under an atmosphere of nitrogen at room temperature and produced viologen cation radical (dark green solution, λ_{max} 605 nm in ethanol) immediately. The dark green solution was changed to a yellow solution by bubbling air into the reaction mixture. After the ethanol was removed in vacuo, the residue was recrystallized from ethanol to give 0.47 g (60%) of $BNA^{+}Cl^{-}$, and A (0.4 g, 60%) was isolated from the filtrate.

Reduction of LA by $A^{\cdot}$-Py^{+}. A solution of 6.6 g (20 mmol) of $A^{\cdot}$-Py^{+} and LA in 100 mL of ethanol and 2 mL of hydrochloric acid (37%) was allowed to stand under an atmosphere of nitrogen at room temperature for 15 h. After the ethanol was removed in vacuo, 20 mL of acetic anhydride and 1 mL of pyridine were added to the reaction mixture, and the solution was allowed to stand at room temperature for 10 h. After unreacted acetic anhydride and pyridine were removed under reduced pressure, the residue was dissolved in water, and the solution was extracted with chloroform three times. After the chloroform was removed, the residue was distilled in vacuo to obtain 1.7 g (60%) of diacetylated lipoic acid: bp 202–205 °C (0.07 mmHg); IR (NaCl) 1680 ($C(O)CH_3$), 1740 cm^{-1} (COOH); NMR ($CDCl_3$) δ 1.5–1.8 (m, 8 H, $-(CH_2)_4-$), 1.5–1.8 (m, 2 H, CH_2), 2.33 (s, 6 H, $COCH_3$), 3.00 (t, SCH_2), 3.6 (m, 1 H, SCH), 10.7 (s, 1 H, OH). Anal. Calcd for $C_{12}H_{20}S_2O_4$: C, 49.31; H, 6.90; S, 21.89. Found: C, 49.20; H, 7.01; S, 21.80. $BNA^{+}Cl^{-}$ (2.4 g, 80%) and A (1.9 g, 60%) were also obtained from the aqueous solution.

Reduction of LA by BNAH in the Presence of Catalytic Amounts of Vicinal Tricarbonyl Compounds. Typical Procedure. To a solution of 2.2 g (10 mmol) of BNAH and 2.5 g (12 mmol) of LA in 100 mL of ethanol was added 0.16 g (1 mmol) of A. The reaction mixture was allowed to stand under an atmosphere of nitrogen at room temperature for 60 h. After the ethanol was removed in vacuo, 30 mL of acetic anhydride and 1 mL of pyridine were added to the residue, and the reaction mixture was allowed to stand at room temperature for 10 h. After unreacted acetic anhydride and pyridine were removed in vacuo, the residue was distilled to yield 1.5 g (70%) of diacetylated LA. $BNA^{+}Cl^{-}$ (1.5 g, 60%) was isolated from the residue. The reduction of LA by BNAH in the presence of catalytic amounts of AT, NY, or HDT was also carried out in a similar way. The reaction was stopped after the complete consumption of BNAH was checked spectrophotometrically.

Registry No. A, 50-71-5; BNAH, 952-92-1; $A^{\cdot}$-Py^{+}, 73636-25-6; $BNA^{+}Cl^{-}$, 5096-13-9; NY, 485-47-2; AID, 73636-26-7; HDT, 5103-42-4; AT, 76-24-4; *p*-benzoquinone, 106-51-4; benzyl viologen, 13096-46-3; LA, 62-46-4; diacetylated lipoic acid, 71288-69-2.

(19) M. Regitz and G. Heck, *Chem. Ber.*, 97, 1482 (1964).

Concurrent Methoxide Ion Attack at the 5- and 7-Carbons of 4-Nitrobenzofurazan and 4-Nitrobenzofuroxan. A Kinetic Study in Methanol

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In methanolic solution, methoxide ions attack both the 5- and 7-carbons of 4-nitrobenzofurazan (**5**) and 4-nitrobenzofuroxan (**8**) to give the Meisenheimer-type complexes **6**, **9** and **7**, **10**. The kinetics of the interactions have been studied by the stopped-flow method. The formation of the 5-methoxyl adducts **6** and **9** is found to always precede that of the thermodynamically more stable 7-methoxyl isomers **7** and **10**. The results indicate that the para-like 4-nitro group is more efficient than the furazan and the furoxan rings in delocalizing the negative charge in **7** and **10**. The kinetic and thermodynamic parameters $\Delta H°$, $\Delta S°$, $\Delta H^{\ddagger}$, and $\Delta S^{\ddagger}$ for the various reactions have been determined. It appears that only a large positive entropy change, $\Delta S°$ (about +80 J mol^{-1} K^{-1}), is responsible for both the formation and the greater stability of the 7-methoxyl complexes **7** and **10** as compared to that of their 5-methoxyl analogues. Stabilization of **7** and **10** by association of the 4-nitro group with the potassium counterion is suggested.

Nitrobenzofurazans and nitrobenzofuroxans have been shown to be potent in vitro inhibitors of nucleic acid synthesis in lymphocites.[2-5] It was suggested that a possible mode of action of these derivatives at the cellular

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