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ISOLATION OF RADICAL ANION OF ALLOXAN DERIVED FROM THE REACTIONS OF ALLOXAN OR ALLOXANTIN WITH 1-BENZYL-1,4-DIHYDRONICOTINAMIDE

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Radical anion of alloxan could be isolated as 1-benzyl-3-carbamoylpyridinium salt (III) by the reactions of 1-benzyl-1,4-dihydronicotinamide with alloxan or alloxantin (II). III was also prepared by the reactions of II with 1-benzyl-3-carbamoylpyridinium chloride.

The reductions by use of 1-alkyl-1,4-dihydronicotinamide derivatives as a model for reduced nicotinamide-adenine dinucleotide have been widely investigated because of their biochemical significance. For instance, the reductions of activated carbonyl compounds such as hexachloroacetone1) or 2,2,2-trifluoroacetophenone2) by 1-benzyl-1,4-dihydronicotinamide(BNAH) have been reported, and also a mechanism on the reduction of thiobenzophenone by BNAH has been discussed.3)

We, in this communication, wish to report the preparation of radical anion of alloxan (III) by the reactions of BNAH with alloxan (I) or alloxantin (II), and of 1-benzyl-3-carbamoylpyridinium chloride (BNA^+Cl^-) with II.

It was observed in preliminary experiments by UV spectrum that the amount of BNAH(λ_{max}; 356 nm) decreased in methanol by adding alloxan hydrate (I)4) at room temperature, whereas the oxidized form (BNA^+)(λ_{max}; 260 nm) of BNAH increased. These observations suggest that BNAH can easily reduce I. Thus, the reaction of BNAH with I was performed in the preparative scale. A solution of 0.8g(5 mmol) of I and 1g(5 mmol) of BNAH in 50 ml of methanol was allowed to stand in the atmosphere of nitrogen at room temperature to produce a precipitate immediately. After stirring for 2 hr, the precipitate was filtered off and dried in vacuo to give 0.75g of a yellow powder (mp. 195-196°C) which is soluble in water, but insoluble in common organic solvents. Further, alloxantin dihydrate (II)5) (0.25g, mp. 229-231°C) was isolated by bubbling air into a filtrate.

The structure of the precipitate was studied by UV, ESR, NMR, and elemental analyses. The precipitate showed to be the 1:1 salt (III) of BNA^+ and alloxan anion ($I^{\bullet -}$) by elemental analyses. Found(Calcd. for $C_{17}H_{15}O_5N_4$)(%): C 57.30(57.25);

H 4.53(4.52); N 15.72(15.46). The UV spectrum in methanol has an absorption at 260 nm showing the presence of BNA^+. The NMR spectrum (in D_2O) of III corresponding completely to BNA^+ was obtained, as indicated in Fig. 1. The ESR spectrum in bulk showed the broad singlet line at 25°C, as shown in Fig. 2. Treating the precipitate with aq. hydrochloric acid, alloxantin (II) and BNA^+Cl^- were obtained quantitatively.

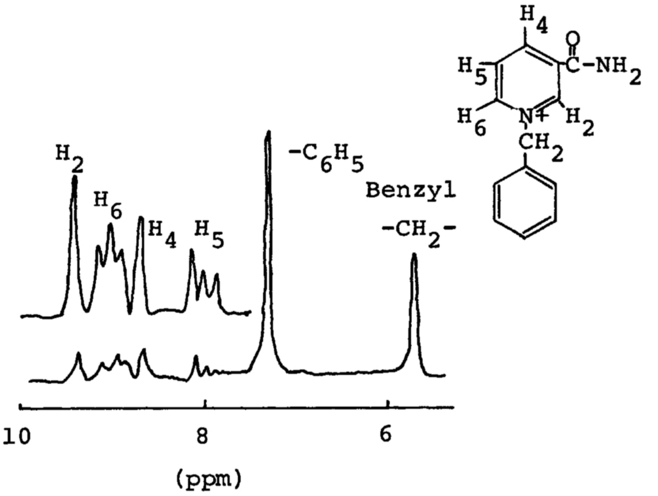


Fig.1. NMR spectrum(D_2O) of the precipitate (III)

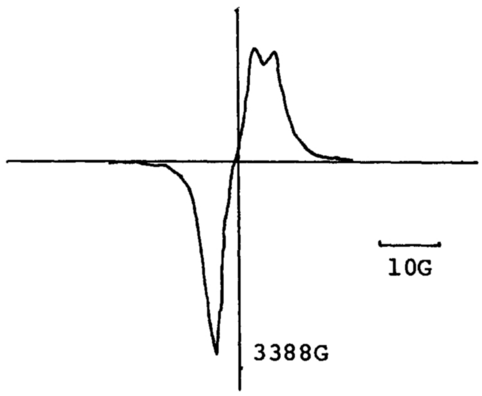
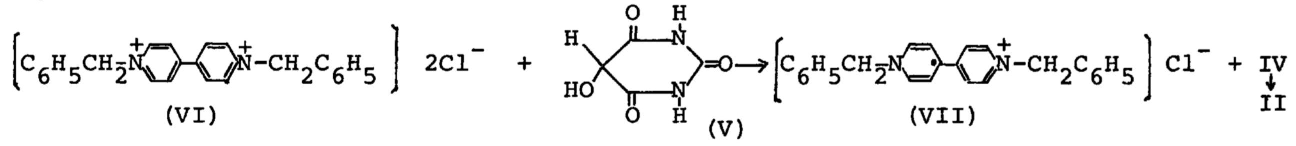


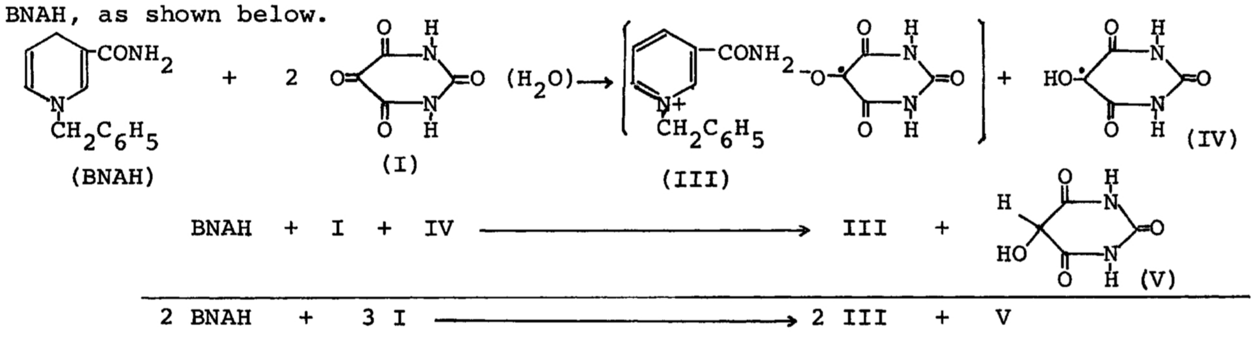
Fig.2. ESR spectrum(25°C, bulk) of the precipitate (III)

Based on the results described above, the reasonable structure of the precipitate is considered to be the salt (III) of BNA^+ and alloxan anion.

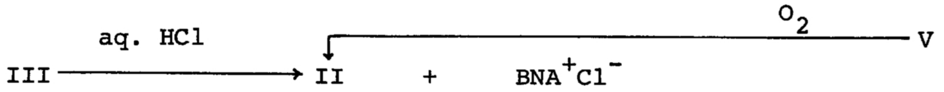
Alloxan anion and dialuric acid (V)[6] might be formed by one or two electron reduction of alloxan, respectively. The formation of the latter (V) was demonstrated actually in the reaction of BNAH and alloxan hydrate from the facts that both the authentic dialuric acid or the reaction mixture obtained above can easily reduce benzyl viologen (VI) to give blue colored radical cation (VII) (λ_{max}; 600 nm) and alloxantin, while BNAH or alloxantin itself has no ability to reduce benzyl viologen.



Thus, we can represent the reasonable reaction scheme for the reaction of I and BNAH, as shown below.

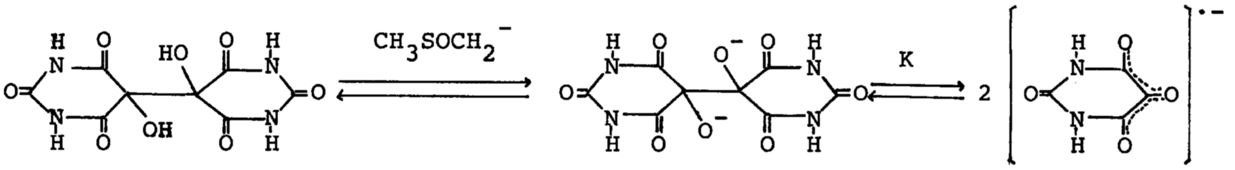


Then, acidic cleavage of III and air oxidation of the filtrate mentioned before should be described as follows.



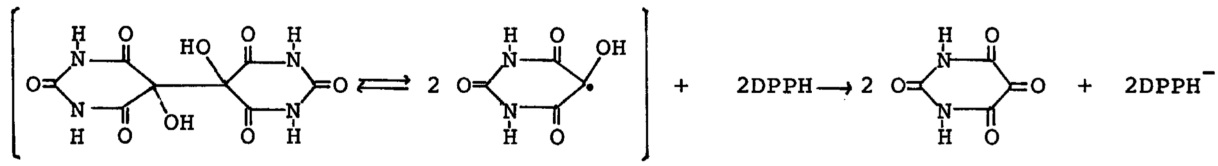
$$\mathrm{III} \xrightarrow{\text{aq. HCl}} \mathrm{II} + \mathrm{BNA^{+}Cl^{-}} \qquad \mathrm{V} \xrightarrow{O_2} \mathrm{II}$$

The formation of alloxan and ninhydrin anions has been observed by the reduction of alloxan or ninhydrin with sodium dithionite in water or with methylsulfinylmethanide ($CH_3SOCH_2^-$) in dimethyl sulfoxide (DMSO), and the ESR was analyzed in detail. Further, alloxan anion was effectively produced by the dissociation of alloxantin in DMSO and the value of equilibrium constant (K) of 2-3 x 10^{-4} mol/l was estimated.7)



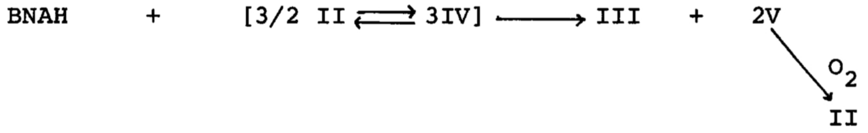
(II)

Furthermore, it was found that II could be reduced by BNAH in analogy with I. A solution of 0.5g(5 mmol) of BNAH in 20 ml of methanol was added to a solution of 2.4g(7.5 mmol) of alloxantin dihydrate (II)5) in 30 ml of methanol at room temperature, and the mixture was allowed to stand for 2 hr in the atmosphere of nitrogen. The precipitate(1.4g) in the form of a yellow powder was obtained. Air was bubbled into a filtrate to recover 0.8g of II. The spectra(UV, IR, ESR, and NMR), elemental analyses, and melting point (195-196°C) of the precipitate obtained above agreed doubtlessly with those of III produced by the reaction of BNAH and I. In another experiment, 1,1-diphenyl-2-picrylhydrazyl (DPPH) was discolored with alloxantin in methanol at room temperature, accompanied by the formation of I quantitatively. This fact suggests that the equilibrium is in a reversible process leading to the formation of the radical (IV) from II, which suffers one electron oxidation with DPPH.



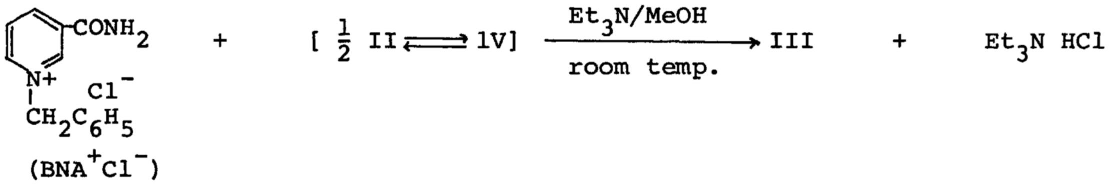
(II) (IV) (I)

Therefore, the reduction of (IV) with BNAH should be expected in the reaction of II and BNAH, as shown below.



$$\mathrm{BNAH} + [3/2\ \mathrm{II} \rightleftarrows 3\mathrm{IV}] \longrightarrow \mathrm{III} + 2\mathrm{V} \xrightarrow{O_2} \mathrm{II}$$

In addition, the reaction of BNA^+Cl^- with II was tried to prepare III obtained by the reactions of BNAH with I or II. A solution of 0.5g(5 mmol) of triethylamine in 20 ml of methanol was added dropwise to a solution of BNA^+Cl^- (1.3g, 5.2 mmol) and II (0.8g, 2.5 mmol) in 50 ml of methanol in the atmosphere of nitrogen at room temperature to afford 1.2g of a yellow precipitate and 2.2g of triethylamine hydrochloride from a filtrate. The spectra (UV, IR, NMR, and ESR), melting point (195-196°C), and elemental analyses of the precipitate obtained above agreed completely with those of III.



References and Notes

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2) J. J. Steffens and D. M. Chipman, J. Amer. Chem. Soc., 93, 6694(1971).
3) A. Ohno and N. Kito, Chem. Lett., 1977, 391.
4) While the various forms (tetra, tri, monohydrate and anhydrous) of alloxan have been reported, monohydrate used in this study is known as gem-diol structure.
5) Alloxantin dihydrate (mp. 230-231°C) used in this study was prepared by the reduction of alloxan with hydrogen sulfide; S. Tipson, Org. Synth., Coll. Vol.IV, 25(1963).
6) Dialuric acid has also been prepared by the reduction of alloxan with hydrogen sulfide (R. S. Tipson and L. H. Cretcher, J. Org. Chem., 16, 1091(1951).), and is easily oxidized by air to give alloxantin.
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