On Mechanistic Aspects of 5-Deazaflavin Dependent Dehydrogenation of Alcohol

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Received July 2, 1986

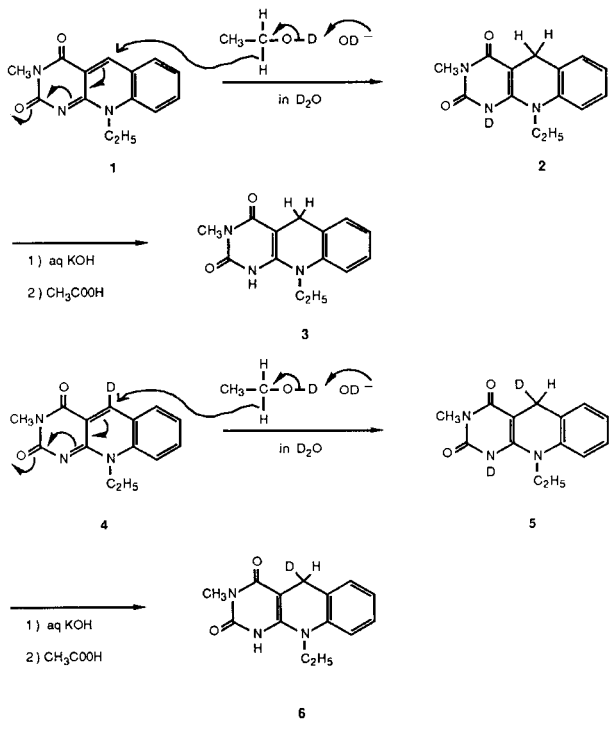
The reaction of 5-deazaflavins with alcoholates was investigated and the direct hydride equivalent transfer from C_1 of alcoholates to C_5 of 5-deazaflavins was confirmed by chemical methods. 5-Alkoxy-10-butyl-3-methyl-5-deazaflavins were synthesized by treatment of 10-butyl-5-chloro-3-methyl-5-deazaflavin with the corresponding alcoholates. The 5-alkoxy-5-deazaflavins were reduced by sodium borodeuteride or sodium hydrosulfite in deuterium oxide or monodeuteriomethanol to give 10-butyl-3-methyl-1,5-dihydro-5-deazaflavin-5,5-D_2 exclusively. 3,10-Dimethyl-5-deazaflavin radical anion was detected by esr technique on treatment of 3,10-dimethyl-5-deazaflavin with potassium in DMF. From the above reactions, a mechanism of 5-deazaflavin dependent dehydrogenation of alcoholate was proposed.

J. Heterocyclic Chem., **24**, 251 (1987).

For the past decade 5-deazaflavins (5-deazaisoalloxazines) have extensively been studied in both enzymatic [1,2] and model [3] systems to provide mechanistic insight into flavin catalyzed reactions. Particularly, the use of 5-deazaflavin has allowed direct demonstration of hydride equivalent transfer from NADH to C_5 of the 5-deazaflavin, which was illustrated for a direct hydride equivalent transfer in the NADH-flavin oxidoreductases [4].

In the meantime, we have found that 5-deazaflavin is able to abstract hydride equivalent directly from alcoholates to give the corresponding carbonyl compounds, while 5-deazaflavin itself is reduced to 1,5-dihydro-5-deazaflavin [5,6]. This paper concerns the mechanism of this hydride equivalent transfer from alcoholates to 5-deazaflavins.

Scheme 1



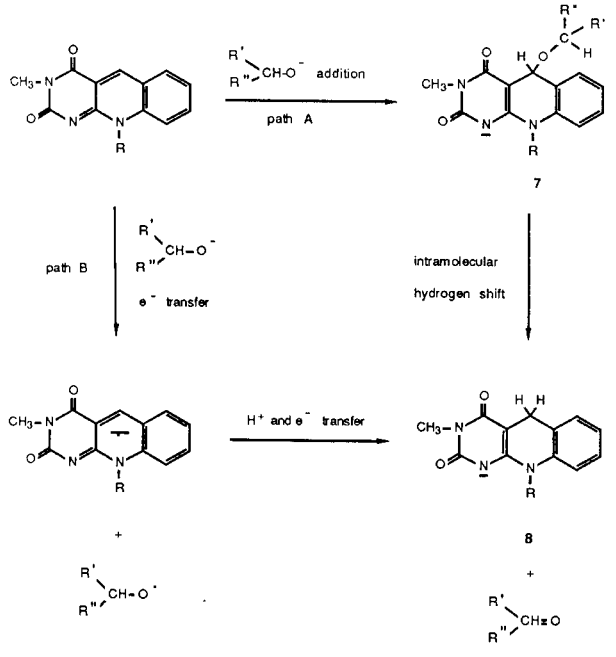
1 2 3 4 5 6

The direct hydride equivalent transfer was evidenced by the following two experiments. Treatment of 10-ethyl-3-methyl-5-deazaflavin (**1**) [7] with a mixture of monodeuterioethanol and sodium deuteroxide in deuterium oxide gave 10-ethyl-3-methyl-1,5-dihydro-5-deazaflavin-1-D (**2**) exclusively. Compound **2** was dissolved in aqueous potassium hydroxide, and the solution was acidified with acetic acid to cause the separation of 10-ethyl-3-methyl-1,5-dihydro-5-deazaflavin (**3**) [7] in high yield. Furthermore, treatment of 10-ethyl-3-methyl-5-deazaflavin-5-D (**4**) [7] with the same reagents as above gave exclusively 10-ethyl-3-methyl-1,5-dihydro-5-deazaflavin-1,5-D_2 (**5**), which was similarly converted to 10-ethyl-3-methyl-1,5-dihydro-5-deazaflavin-5-D (**6**) [7] in high yield.

For the hydride equivalent transfer from alcoholate to the 5-deazaflavin, at least two reaction pathways can be envisaged. One involves the initial addition of alcoholate on C_5 of 5-deazaflavin giving a covalent adduct as an intermediate **7** (path A), which slowly undergoes the intramolecular hydrogen equivalent shift within the adduct to give 1,5-dihydro-5-deazaflavin anion **8**, as shown in Scheme 2 [8]. The other involves the initial intermolecular one electron transfer from alcoholate to 5-deazaflavin without forming the adduct to give a transient 5-deazaflavin radical anion and an alkoxy radical (path B), followed by proton transfer and then one more electron transfer from the alkoxy radical, turning out to afford **8**.

In order to clarify the first mechanism, we have tried to isolate the intermediary covalent adduct **7** under several conditions but without success. Therefore the reactivity of

Scheme 2



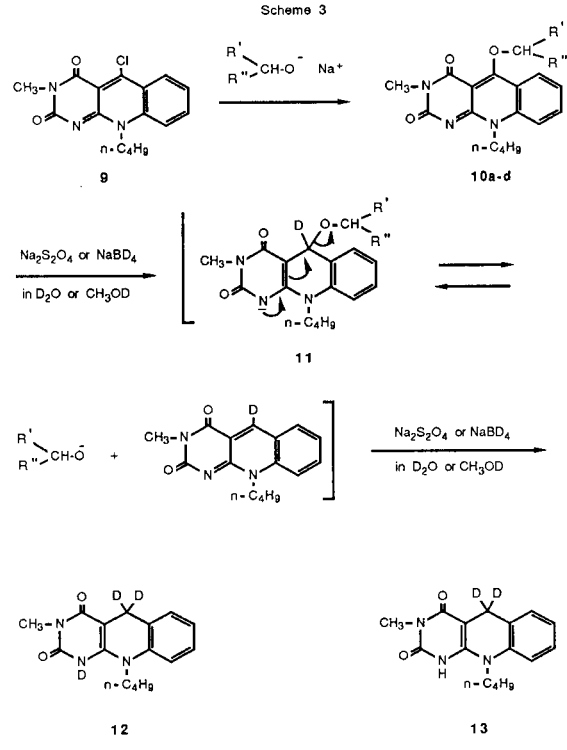
7 was indirectly investigated by means of the reduction of 5-alkoxy-5-deazaflavin with sodium hydrosulfite or sodium borohydride which would give the intermediate adduct **7**.

At the outset, several 5-alkoxy-10-butyl-3-methyl-5-deazaflavins (**10**) were synthesized by treatment of 10-butyl-5-chloro-3-methyl-5-deazaflavin (**9**) [9] with appropriate sodium alcoholates in the corresponding alcohols (Table I). The structures of **10** were established from satisfactory analytical and spectral data.

The reduction of **10** with sodium hydrosulfite or sodium borodeuteride in deuterium oxide or monodeuteriomethanol gave the corresponding 1,5-dihydro-5-deazaflavin-1,5,5-D_3 (**12**) [7] exclusively and in high yield, and the intermediary 5-alkoxy-1,5-dihydro-5-deazaflavin-5-D anion (**11**) could not be obtained under any conditions. Furthermore, no 1,5-dihydro-5-deazaflavin-5-D, which would be formed by intramolecular hydrogen shift within **11**, was obtained either. These results imply that the initially formed **11**, which corresponds to the alcoholate adduct to the 5-deazaflavin, is reversibly decomposed to the original 5-deazaflavin-5-D and alcoholate under these conditions and then further reduction of the 5-deazaflavin-5-D takes place to afford the final **12** exclusively.

Therefore, in the alcohol dehydrogenation process by the 5-deazaflavin under alkaline condition, even if the alcoholate adds to C_5 of 5-deazaflavin, the formed adduct would only be in equilibrium with the original 5-deazaflavin and alcoholate. This means that the first mechanism through the alcholate addition followed by intramolecular hydrogen equivalent transfer is unlikely.

Scheme 3



Next, in order to give a support to the second mechanism including the stepwise transfers of electron, proton,

Table I

5-Alkoxy-10-butyl-3-methyl-5-deazaflavins

Compound	R	Yield %	Mp °C [a]	Formula	Analysis % Calcd. C	H	N	Found C	H	N
10a	CH_3	81	146	$C_{17}H_{19}N_3O_3$	65.16	6.11	13.41	65.30	6.08	13.12
10b	C_2H_5	68	148	$C_{18}H_{21}N_3O_3$	66.03	6.47	12.84	66.29	6.45	12.56
10c	n-C_3H_7	74	121	$C_{19}H_{23}N_3O_3$	66.84	6.79	12.31	66.61	6.48	12.09
10d	n-C_4H_9	68	113	$C_{20}H_{25}N_3O_3$	67.58	7.09	11.82	67.43	7.11	11.64

[a] All compounds were recrystallized from water and are pale yellow needles.

and electron, the ability of one electron acceptance of 5-deazaflavin was examined by means of esr technique. When metal potassium was added to a DMF solution of 3,10-dimethyl-5-deazaflavin (**14**) at −60° under anaerobic condition, the esr spectra of the corresponding radical anion was observed at g = 2.0027 although very short-lived. The radical showed hyperfine structure consisting of 8 lines with approximate intensity ratios 1:3:4:4:4:4:3:1 as depicted in Figure 1.

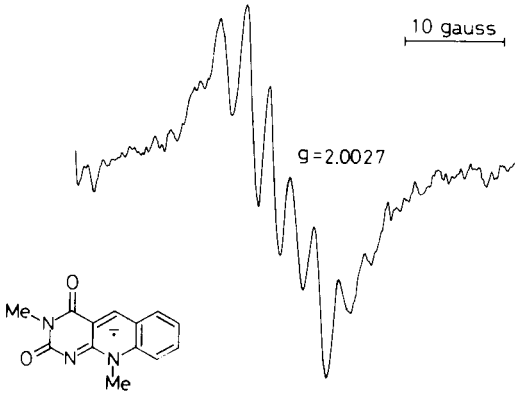


Figure 1. The esr spectra of 3,10-dimethyl-5-deazaflavin radical anion in DMF.

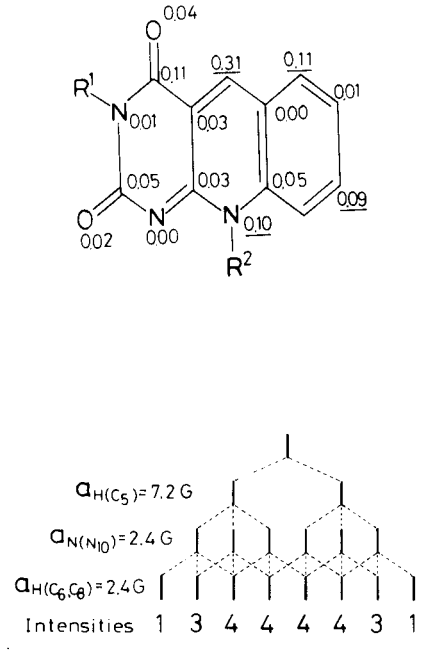
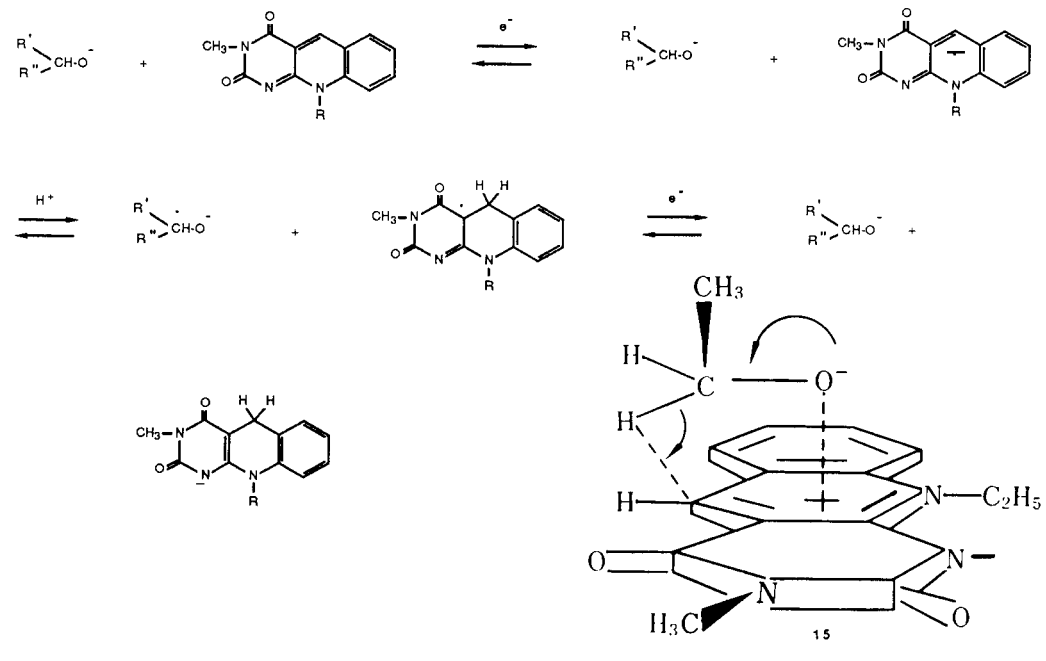


Figure 2. Spin densities of 5-deazaflavin radical anion and the coupling scheme based on the coupling constants.

Spin densities of the 5-deazaflavin radical anion were calculated by the Hückel MO method. The parameters of the coulomb and resonance integrals for substituent groups are as follows. For =N-, $a_x = 0.6$, $a_r = 0.1$, $I = 1$; for -N<, $a_x = 1$, $a_r = 0.1$, $I = 1$, for =O, $a_x = 2$, $a_r = 0.2$, $I = 1.4$. a_x is the coulomb integral of the substituent X: $\alpha_x = \alpha + a_x\beta$, a_r is the coulomb integral of the carbon atom adjacent to X: $\alpha_{adj} = \alpha + a_r\beta$. I is the resonance integral between that carbon and X: $\beta_{c\text{-}x} = I\beta$.

The esr spectra can be explained on the basis of the coupling scheme (Figure 2), in which the coupling to C_5-proton is three times larger than the nearly identical couplings to N_{10}, C_6- and C_8-protons. The Hückel LCAO-MO

Scheme 4



calculations on the radical anion of **14** give the spin densities indicated in Figure 2 and thus helps to clarify this interpretations. Much smaller coupling constants for other positions would be expected because of the much smaller spin densities at these positions. Therefore, their hyperfine couplings could not be observed under these conditions.

The generation of 5-deazaflavin radical anion by metal potassium would be irrelevant to the hydride transfer conditions. However, the ability of one electron acceptance to 5-deazaflavin has been confirmed at the least.

From the facts described above, it is concluded that the alcoholate addition to 5-deazaflavin does not lead to the dehydrogenation of alcoholate with the reduction of 5-deazaflavin. Although the possibility of one-step hydride transfer from alcoholate to 5-deazaflavin can not be ruled out at the moment, we consider that the stepwise mechanism by the initial one electron transfer within the charge transfer-like complex **15**, followed by proton and one more electron transfer, would be the most probable for the 5-deazaflavin dependent dehydrogenation of alcoholate (Scheme 4).

EXPERIMENTAL

Melting points were determined on a Yanagimoto hot-stage apparatus; they are uncorrected. The ^{1}H-nmr spectra were obtained in chloroform-D at 200 MHz on a JEOL FX 200 instrument using a tetramethylsilane internal standard. Mass spectra were taken on a JEOL JMX OISG-2 instrument by direct inlet at 70 eV.

ESR Measurement.

The esr measurement was made with a Varian E-4 X-band esr spectrometer. The sample was dissolved in dimethylformamide and to the solution was added metal K at −60° under anaerobic condition. The esr spectrum of the above mixture was measured immediately at −60°.

5-Alkoxy-10-butyl-3-methyl-5-deazaflavins (**10**). General Procedure.

Sodium (0.023 g, 1 mg-atom) was dissolved in the respective alcohol (15-30 ml) and to the solution was added 10-butyl-5-chloro-3-methyl-5-deazaflavin (**9**) [9] (0.32 g, 1 mmole). The mixture was stirred at room temperature for 30 hours. The reaction mixture was evaporated *in vacuo* and the residue was treated with water to separate crystals, which were filtered off and recrystallized from water (Table I).

Reduction of 10-Ethyl-3-methyl-5-deazaflavin (**1**) with Monodeuterioethanol-Sodium Deuteroxide-Deuterium Oxide.

10-Ethyl-3-methyl-5-deazaflavin (**1**) (0.51 g, 2 mmoles) was added to a solution of monodeuterioethanol (7 ml), deuterium oxide (3 ml) and 40% sodium deuteroxide in deuterium oxide (1 ml including 0.41 g, 10 mmoles of sodium deuteroxide) and the mixture was heated at 80 °C for 1 hour. After cooling, the reaction mixture was neutralized with acetic acid to separate 10-ethyl-3-methyl-1,5-dihydro-5-deazaflavin-1-D (**2**) in almost quantitative yield. The 1,5-dihydro-5-deazaflavin-1-D was dissolved in 10% aqueous potassium hydroxide (6 ml), and the solution was acidified with acetic acid to cause the separation of 10-ethyl-3-methyl-1,5-dihydro-5-deazaflavin (**3**) (0.44 g, 85%). The nmr spectra showed the benzylic protons of C_5 at 3.77 ppm. M$^+$ 257.

Reduction of 10-Ethyl-3-methyl-5-deazaflavin-5-D (**4**) with Monodeuterioethanol-Sodium Deuteroxide-Deuterium Oxide.

10-Ethyl-3-methyl-5-deazaflavin-5-D (**4**) (0.51 g, 2 mmoles) was added to a mixture of monodeuterioethanol (7 ml), deuterium oxide (3 ml) and 40% sodium deuteroxide in deuterium oxide (1 ml including 0.41 g, 10 mmoles of sodium deuteroxide) and the mixture was treated in the same way as described above to give 10-ethyl-3-methyl-1,5-dihydro-5-deazaflavin-1,5-D_2 (**5**) in almost quantitative yield. The 1,5-dihydro-5-deazaflavin-1,5-D_2 was similarly treated as described above to give 10-ethyl-3-methyl-1,5-dihydro-5-deazaflavin-5-D (**6**). The nmr spectra showed one benzylic proton of C_5 at 3.77 ppm.

Reduction of 5-Alkoxy-10-butyl-3-methyl-5-deazaflavins (**10**) with Sodium Hydrosulfite in Deuterium Oxide. General Procedure.

Compounds **10** (0.4 mmole) and sodium hydrosulfite (0.26 g, 1.5 mmoles) were added to 15% potassium carbonate solution in deuterium oxide (2 ml) and the mixture was heated at 90° for 1 hour with stirring. After reaction, the reaction mixture was neutralized with acetic acid to separate 10-butyl-3-methyl-1,5-dihydro-5-deazaflavin-1,5,5-D_3 (**12**) in quantitative yield. This is dissolved in 10% aqueous potassium hydroxide (2 ml), and the solution was acidified with acetic acid to cause the separation of 10-butyl-3-methyl-1,5-dihydro-5-deazaflavin-5,5-D_2 (**13**) in almost quantitative yield. The nmr spectra of **13** did not show the benzylic protons of C_5 at 3.78 ppm. The mass spectra exhibited the corresponding mother peak (M$^+$ 287).

Reduction of 5-Butoxy-10-butyl-3-methyl-5-deazaflavin (**10d**) with Sodium Borodeuteride in Monodeuteriomethanol.

5-Butoxy-10-butyl-3-methyl-5-deazaflavin (**10d**) (10 ml, 0.028 mmole) was dissolved in monodeuteriomethanol (5 ml) and to the solution was added sodium borodeuteride (2 mg) under stirring at 0°. After stirring at room temperature for 5 minutes the reaction mixture was diluted with ice water and extracted with chloroform. The chloroform extracts were dried with anhydrous sodium sulfate and evaporated *in vacuo* to dryness giving exclusively 10-butyl-3-methyl-1,5-dihydro-5-deazaflavin-5,5-D_2 (**13**) in almost quantitative yield. The nmr spectra did not show the benzylic protons of C_5 at 3.78 ppm; M$^+$ 287.

Acknowledgements.

The authors thank the Hohansha Foundation for financial support. They are grateful to Professor M. Iwaizumi, Tohoku University, for measurement of esr spectra.

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