Coenzyme Models. Part 52.† Diastereo-differentiating Hydrogen Transfer in 5-Deazaflavin Oxidation of Alcohols and Amines

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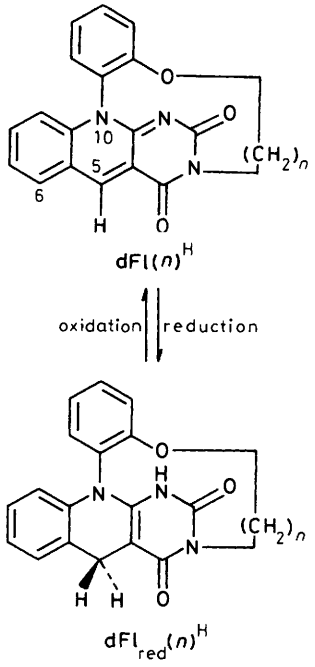
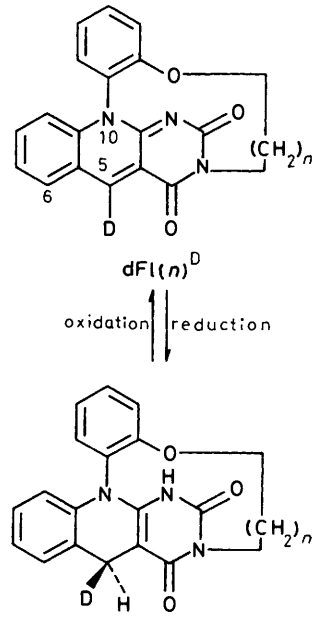
5-Deuteriated 5-deazaflavinophanes (dFl(n)^D) were synthesized in which N-3 in the isoalloxazine group and O-2′ in the 10-(2-hydroxyphenyl) group were linked by a $-(CH_2)_n-$ chain (n = 8 and 10). ^{1}H NMR studies established that $NaBH_4$ reduction of dFl(8)^D to the reduced form dFl$_{red}$(8)^D, in which ring inversion is inhibited because of a short $(CH_2)_8$ strap, gives an axial C-5 proton (H_{ax}). This indicates that hydrogen is exclusively incorporated into the axial C-5 position. In contrast, $NaBH_4$ reduction of dFl(10)^D to dFl$_{red}$(10)^D, in which ring inversion is allowed because of a long $(CH_2)_{10}$ strap, gave an axial and an equatorial proton (H_{ax} and H_{eq}) in the ratio 1:1. This indicates that hydrogen is primarily incorporated into the axial C-5 position but is rapidly exchanged through ring inversion. Oxidation of alcohols and amines by dFl(n)^D gave ^{1}H NMR spectra similar to those obtained after $NaBH_4$ reduction. Therefore we conclude that hydrogen from these substrates is also transferred to the axial C-5 position. The 'axial preference', which is generally observed for 5-deazaflavin-mediated redox reactions, was rationalized in terms of the stereoelectronic effect.

In NAD(P)H the two protons at C-4 of the 1,4-dihydronicotinamide entity occupy diastereotopic positions. We can discriminate between two protons by the difference in their ^{1}H NMR chemical shift, but the difference (if any) becomes quite slight in the free coenzymes.[1,2] In an NAD(P)H model system these two protons usually give the same chemical shift, but Rob *et al.*[3] and de Kok *et al.*[4] demonstrated that they give quite different chemical shifts when the nicotinamide skeleton is included in a ring structure. This is because the ring inhibits or suppresses the inversion of the boat-shaped 1,4-dihydronicotinamide. In tracer experiments using these NAD(P)H model compounds they established that hydrogen exchange occurs exclusively *via* the axial C-4 position.[3,4] In contrast, the hydrogen exchange mechanism of flavin coenzymes is still controversial. The difficulty is related to the fact that the redox reaction occurs at the weakly basic N-5 position and therefore the 'labelled' 5-H proton is exchangeable with solvent protons.[5,6] Thus, one cannot apply the tracer experiments to flavin coenzymes. 5-Deazaflavin is known to be an essential skeleton in cofactor F_{420}.[5-8] Since the 5-nitrogen in flavin has been replaced by a carbon and the 5-H proton is not exchangeable, 5-deazaflavin is well suited for tracer studies of the hydrogen transfer mechanism not only in cofactor F_{420} but also in flavin coenzymes. Previously, in order to obtain an insight into diastereotopic differentiation in 5-deazaflavin, we





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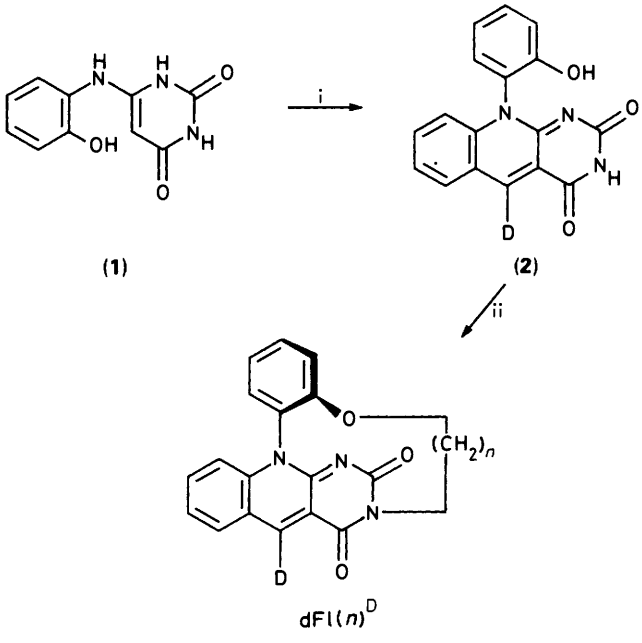
synthesized new cyclic 5-deazaflavins ($dFl(n)^H$).[9,10] ^1H NMR spectroscopy of the reduced forms ($dFl_{red}(n)^H$) gave a pair of doublets for the two C-5 protons, which were assigned respectively to axial and equatorial protons (H_{ax} and H_{eq})[10,11] indicating that cyclic $dFl(n)^H$ could be used to elucidate the diastereotopic hydrogen transfer in 5-deazaflavin-mediated redox reactions.

Yoneda and co-workers[12–15] found that under aerobic conditions 5-deazaflavin and its analogues act as recyclable oxidation catalysts for alcohols and amines. We were interested in establishing whether the hydrogen transfer mechanism is also subject to the diastereotopic differentiation. In these reactions substrates are used in great excess over 5-deazaflavins. We thus synthesized 5-deuteriated dFl(*n*) (*i.e.*, $dFl(n)^D$; n = 8 and 10) and carried out the tracer experiments.

Experimental

Materials.—$dFl(n)^D$ Was synthesized from *o*-chloro-α-deuteriobenzaldehyde according to Scheme 1. The synthetic methods were similar to those described previously,[9–11] so that only short comments on the methods are recorded in addition to their analytical data.



Scheme 1. i, *o*-Chloro-α-deuteriobenzaldehyde; ii, $Br(CH_2)_nBr$.

5-Deuterio-10-(2-hydroxyphenyl)-5-deazaisolloxazine (**2**).—This compound was synthesized from *o*-chloro-α-deuteriobenzaldehyde (deuterium purity 97%)[16] and 6-(2-hydroxyanilino)uracil (**1**). The reaction was carried out in DMF at 160–180 °C for 5 h.[9] The deuterium purity in product (**2**) decreased to 76–83%, suggesting that some H/D exchange (probably from trace amounts of water in DMF) takes place during the condensation reaction. According to the reaction mechanism proposed by Yoneda *et al.*,[17] in the intermediate the α-hydrogen of *o*-chlorobenzaldehyde is converted into an 'activated' hydrogen with which it is possible for solvent protons to exchange. We thus used carefully dehydrated DMF and the reaction was carried out for 3 h under a nitrogen stream. The yellow precipitate was recovered by filtration and recrystallized from ethanol, m.p. >310 °C, yield 52%, deuterium purity (determined by ^1H NMR spectroscopy) 91%. Since TLC analysis gave a single spot, we used this product for the next reaction without further purification.

Compound $dFl(8)^D$; m.p. 282–284 °C [m.p. for $dFl(8)^H$ 288–289 °C],[9] yield 29%, deuterium purity (determined by ^1H NMR spectroscopy) 91%. The data indicate that the H/D exchange does not take place in the cyclization step. The ^1H NMR spectrum ($CDCl_3$) was identical to that of $dFl(8)^H$ except for the weakened 5-H peak (8.99 ppm)[9] (Found: C, 71.9; H, 6.1; N, 9.8. $C_{25}H_{25}N_3O_3$ requires C, 72.3; H, 6.1; N, 10.0%).

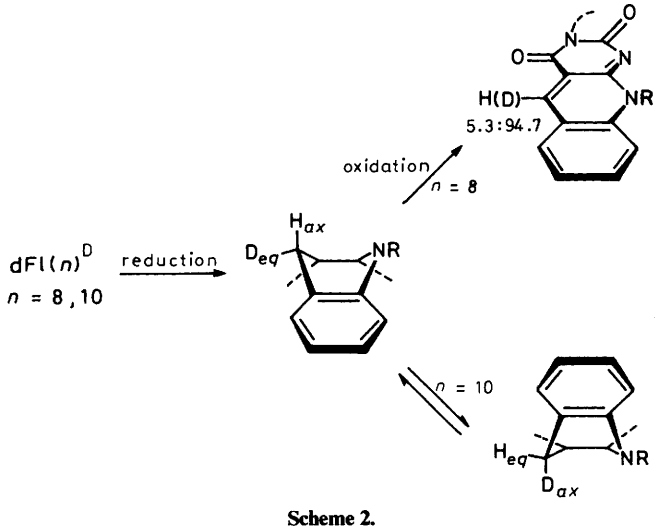
Compound $dFl(8)^D$; m.p. 252–253 °C [m.p. for $dFl(10)^H$ 260–261 °C],[9] yield 26%, deuterium purity (determined by ^1H NMR spectroscopy) 91%. The ^1H NMR spectrum ($CDCl_3$) was identical with that of $dFl(10)^H$ except for the weakened 5-H peak (9.00 ppm)[9] (Found: C, 73.0; H, 6.6; N, 9.4. $C_{27}H_{29}N_3O_3$ requires C, 73.1; H, 6.6; N, 9.5%).

Oxidation of Alcohols and Amines by $dFl(n)^D$.—The reactions were carried out either under a nitrogen stream (for one-cycle oxidation) or under aerobic conditions (for recycle oxidation). A typical run for anaerobic alcohol oxidation is as follows: $dFl(n)^D$ (1.0 mmol) and potassium hydroxide (5 mmol) were added to a mixture of alcohol (5 ml) and water (3 ml), and the mixture was heated under a nitrogen stream in the dark. The reaction mixture was diluted with water and neutralized with hydrochloric acid. The aqueous solution was extracted with chloroform, the chloroform layer being concentrated under reduced pressure. $dFl_{red}(n)^D$ was isolated by a TLC method [silica gel, MeOH–$CHCl_3$ (1:40, v/v)]. The yields of the carbonyl compounds were determined by GLC. In aerobic oxidation the amount of $dFl(n)^D$ was reduced to 0.2 mmol.

The method of amine oxidation is similar to that of alcohol oxidation except that potassium hydroxide was not used. The yields of the carbonyl compounds, which are formed by hydrolysis of imines,[13,14] were determined using 2,4-dinitrophenylhydrazone.[13]

Results and Discussion

In a previous study on $dFl(n)^H$ we found that (i) the oxidized forms do not racemize below 40 °C but those with a long strap ($n \geqslant 10$) can racemize slowly at high temperature (*ca* 100 °C), (ii) when they are reduced to the 1,5-dihydro forms [*i.e.*, $dFl_{red}(n)^H$], those with a long strap ($n \geqslant 10$) racemize even below 40 °C whereas those with a short strap ($n \leqslant 8$) do not



Scheme 2.

* This value was calculated on the basis of the following assumption, $k_{H_{ax}}/k_{D_{ax}} = k_{H_{eq}}/k_{D_{eq}}$ (=2.6).[11]

Table. Anaerobic oxidation of alcohols and amines.

$dFl(n)^D$	Substrate	Reaction		Yield (%)	$H_{ax}:H_{eq}$ [b] in $dFl_{red}(n)^D$
		Temp. (°C)	Time (min)		
$dFl(8)^D$	$PhCH_2OH$	60	30	90	90:10
$dFl(8)^D$	Cyclohexanol	90	60	82	88:12
$dFl(10)^D$	$PhCH_2OH$	60	30	86	50:50
$dFl(8)^D$	$PhCH_2NH_2$	100	240	100[a]	91:9
$dFl(8)^D$	Cyclohexylamine	100	240	92	90:10
$dFl(10)^D$	$PhCH_2NH_2$	100	240	100[a]	50:50

[a] The yields frequently exceeded 100% because of recycle oxidation mediated by a trace amount of O_2. [b] Since the deuterium purity of starting $dFl(n)^D$ is 91%, $H_{ax}:H_{eq}$ = 92:8 (= 100:9) indicates that hydrogen is totally incorporated into the axial C-5 position.

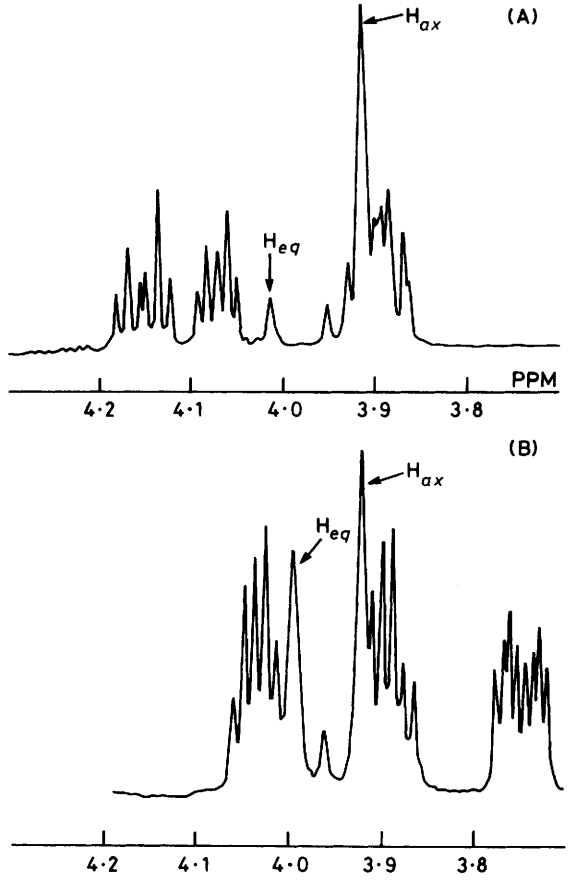


Figure. Partial ^1H NMR spectra (400 MHz) of the C-5 protons in $dFl_{red}(n)^D$ (reduced by $NaBH_4$ in [$^2H_4$]methanol at room temperature): (A) $dFl_{red}(8)^D$, (B) $dFl_{red}(10)^D$. Chemical shifts in these spectra are given relative to tetramethylsilane.

racemize, (iii) the hydrogen transfer to $dFl(n)^H$ and from $dFl_{red}(n)^H$ occurs exclusively at the 'axial' C-5 position, and (iv) the reactivity ratio of H_{ax} *vs.* H_{eq} is estimated to be 8.2:1.0[10,11,*] When $dFl(8)^D$ (deuterium purity 91%) was reduced by $NaBH_4$ in [$^2H_4$]methanol, the ^1H NMR spectrum (400 MHz) of the product $dFl_{red}(8)^D$ gave a strong singlet peak at 3.91 ppm and a weak singlet peak at 4.01 ppm, which can be assigned to H_{ax} and H_{eq} at the C-5 position, respectively [Figure, (A)].[10,11] The integral intensity ratio is *ca* 9:1. Since ring inversion racemization is disregarded for $dFl_{red}(8)^D$, the finding supports the view that hydrogen donated from $NaBH_4$ is exclusively incorporated at the 'axial' C-5 position. On the other hand, when $dFl(10)^D$ was reduced by $NaBH_4$ in [$^2H_4$]methanol, the ^1H NMR gave singlet H_{ax} and H_{eq} signals in the ratio 1:1 [Figure, (B)]. As described above, the ring inversion racemization is allowed for $dFl_{red}(10)^D$. Hence, hydrogen is incorporated primarily into the axial C-5 position but is rapidly exchanged through ring inversion. These stereochemical reaction processes are illustrated in Scheme 2.

$dFl(8)^D$ (Deuterium purity 76%) was reduced by $NaBD_4$ in a mixed solvent [DMF–water (8:2, v/v)]. After neutralization with 0.1M HCl, $dFl_{red}(8)^D$ was reoxidized at 80 °C by air. $dFl(8)^D$ was recovered by diluting the solution with water. The ^1H NMR spectrum indicated that the deuterium purity (80%) is scarcely improved. On the other hand, when $dFl(10)^D$ (deuterium purity 81%) was subject to the same redox treatment, the deuterium purity in recovered $dFl(10)^D$ was improved (93%). The results again support the view that in $dFl(8)^D$ the deuterium exchange occurs exclusively at the axial C-5 position, whereas in $dFl(10)^D$ deuterium may be first incorporated into the axial C-5 position but H_{eq} is translocated to the axial position through the ring inversion and eliminated by the oxidation.

The results of anaerobic oxidation of alcohols and amines by $dFl(n)^D$ (deuterium purity 91%) are summarized in the Table. The intensity ratio of $H_{ax}:H_{eq}$ was determined by ^1H NMR spectroscopy. It is seen from the Table that $dFl_{red}(8)^D$, in which the H_{ax}/H_{eq} exchange is inhibited, has an H_{eq} intensity comparable with the C-5 hydrogen concentration of starting $dFl(8)^D$. This indicates that hydrogen transferred from alcohols and amines is immobilized as the axial C-5 hydrogen. In contrast, $dFl_{red}(10)^D$ prepared by the oxidation of alcohols and amines has singlet H_{ax} and H_{eq} signals (intensity 0.5 H each) in the ratio 1:1. The result is also accounted for by the ring inversion which accompanies the H_{ax}/H_{eq} exchange. In conclusion, the stereochemistry of 5-deazaflavin oxidation of alcohols and amines is similar to that for the reaction with $NaBH_4$ (Scheme 2).

Aerobic oxidation of benzylamine by $dFl(8)^D$ was continued under an oxygen stream for 6 h at 100 °C. The yield of benzaldehyde, as determined by the 2,4-dinitrophenylhydrazone method, was 2 300%, indicating that $dFl(8)^D$ acts as recyclable oxidation catalyst under aerobic conditions. The deuterium purity of recovered $dFl(8)^D$ was 26%. Thus, 65% (= 91 − 26%) of the deuterium was eliminated through the 23 recycles. Provided that in the $dFl(8)^D \longrightarrow dFl_{red}(8)^D$ step hydrogen is incorporated into the axial C-5 position with 100% selectivity, the result allows the estimation of the ratio of elimination between H_{ax} *vs.* D_{eq} for one $dFl_{red}(8)^D \longrightarrow dFl(8)^D$ step as 94.7:5.3 ($k_{H_{ax}}/k_{D_{eq}}$ = 18) (see Scheme 2). We previously determined the primary isotope effect and the H_{ax} *vs.* H_{eq} reactivity ratio for the $dFl_{red}(n)^H \longrightarrow dFl(n)^H$ step to be 2.6 and 8.2, respectively.[11] The data suggest the value $k_{H_{ax}}/k_{D_{eq}}$ to be 21 (= 2.6 × 8.2). This value is in good accord with that determined from the present recycle oxidation system (*i.e.*, 18).

It is now clear that the hydrogen exchange occurs predominantly at the axial position not only in NADH model

compounds[3,4] but also in flavin model compounds.[10,11] This conclusion is quite general because it is hardly affected by reaction conditions and redox agents.[3,4,10,11] More recently, it was shown that in the reaction of dFl(*n*)^{H} and MeMgBr the methyl group is also incorporated at the axial C-5 position.[18] What is the origin of the *'axial preference'*? Two possible answers come to mind. The first answer is a steric effect: H_{eq} is surrounded by bulky groups, for example in 5-deazaflavin 3 C=O and 6-H, so that H_{eq} behaves as a less reactive 'buried hydride equivalent'. If this steric effect governs the stereochemical course, the selectivity should be affected by substrates and reagents. In the present system, however, the stereochemical course is unchanged by these factors. Therefore, this explanation seems unlikely. The second, more likely answer is a stereoelectronic effect.[3] It is possible that the axial transition state is more stable due to π-type overlap (hyperconjugation) between the partially broken (or partially formed) C(5)–H bond and the neighbouring benzene or pteridine π-system. We believe that the second answer is mainly responsible for the high degree of diastereo-differentiation.

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