Diastereo-differentiating Hydrogen Transfer in 5-Deazaflavins

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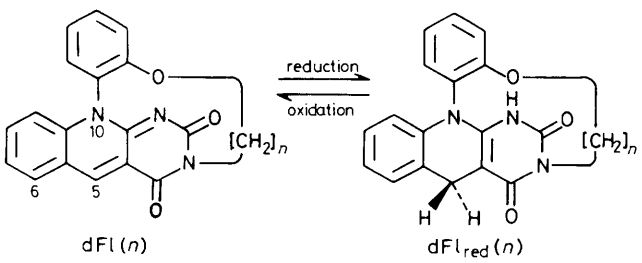
Reduction of 5-deazaisoalloxazinophanes dFl(n) in which N(3) and O(2′α) in the 10-(2-hydroxy)phenyl group were linked by a $(CH_2)_n$ chain (n = 6,8) resulted in the boat-shaped 1,5-dihydro forms $dFl_{red}(n)$; hydrogen transfer to dFl(n) and from $dFl_{red}(n)$ occurred exclusively at the axial C(5) position.

In NAD(P)H, the two protons at C(4) of the 1,4-dihydronicotinamide moiety occupy diastereotopic positions. Discrimination between these two protons is possible from their ^{1}H n.m.r. chemical shifts, but the difference (if any) becomes minimal in the free coenzymes.[1] In an NAD(P)H model system Rob *et al.*[2] and de Kok *et al.*[3] demonstrated that these protons show quite different chemical shifts when the nicotinamide skeleton is included in a ring structure. By using these NAD(P)H model compounds they showed that hydrogen exchange occurs exclusively at the axial C(4) position.[2,3] 5-Deazaflavin is known to serve as an essential skeleton in cofactor F_{420}.[4] Since the nitrogen at the 5-position in flavin has been replaced by a carbon in this compound, it is well suited for studies of the hydrogen exchange mechanism not only in cofactor F_{420} but also in flavin coenzymes. In order to investigate this problem we synthesised 5-deazaisoalloxazinophanes dFl(n),[5] the reduced forms [$dFl_{red}(n)$] of which were expected to give the different chemical shift for the two C(5) protons, and studied how hydrogen is transferred to and from the C(5) position (Scheme 1).

When dFl(n = 6,8,10) were reduced with $NaBH_4$ in CD_3OD, the ^{1}H n.m.r. spectrum (400 MHz; JEOL GX-400 spectrometer) gave a pair of doublets for the two C(5) protons [Figure 1(a)]. The result indicates that the central ring in $dFl_{red}(n)$ adopts a boat-shaped conformation affording magnetically non-equivalent protons, H_{ax} and H_{eq}. Here, we applied the nuclear Overhauser effect (n.O.e.) in ^{1}H n.m.r. to the assignment of H_{ax} and H_{eq}. The result [measurements with respect to 6-H in $dFl_{red}(n = 6)$; CD_3OD, 20 °C] established that H_{eq} and H_{ax} can be assigned to the lower and the higher magnetic field, respectively.

First, we reduced dFl(n) by three different methods: (i) $NaBD_4$ (98% isotope purity) in CD_3OD; (ii) $Na_2S_2O_4$ in D_2O (100% isotope purity)–CD_3OD (99.5% isotope purity) (1 : 4 v/v); and (iii) 4,4-dideuterio-1-benzyl-1,4-dihydronicotinamide (BNAD; 96% isotope purity) in D_2O–CD_3OD (1 : 4 v/v). In the ^{1}H n.m.r. spectra of $dFl_{red}(n = 6)$ (Figure 1) and $dFl_{red}(n = 8)$, the H_{eq} (integral intensity 1H) was still present as a single line, while the H_{ax} peak had almost completely disappeared. This indicates that the incorporated hydrogen occupies almost exclusively (>95% judging from the accuracy of ^{1}H n.m.r.) an axial position.

In contrast, $dFl_{red}(n = 10)$ gave both H_{eq} and H_{ax} signals (integral intensity 0.5 H each) in 1 : 1 intensity ratio. We previously found that dFl(n) (n = 6,8,10) with planar chirality are optically stable at room temperature, but dFl_{red} (n = 10) is rapidly racemised while $dFl_{red}(n = 6)$ and $dFl_{red}(n = 8)$ are not (Scheme 2).[5] It is known that the 5-deazaflavin ring has a 'tense' planar structure, while the 1,5-dihydro-5-deazaflavin ring has a 'relaxed' structure folded through C(5) and N(10) like butterfly wings,[5,6] similar to that of 1,5-dihydroflavins.[7] Thus, sterically-relaxed $dFl_{red}(n = 10)$ can racemise in a 'rope-skipping' manner. In $dFl_{red}(n = 6)$ and $dFl_{red}(n = 8)$, on the other hand, the 'rope' is too short to 'skip'. Conceivably, hydrogen is incorporated primarily into the axial C(5) position of $dFl_{red}(n = 10)$, but is rapidly exchanged through the 'rope-skipping' interconversion.



Scheme 1

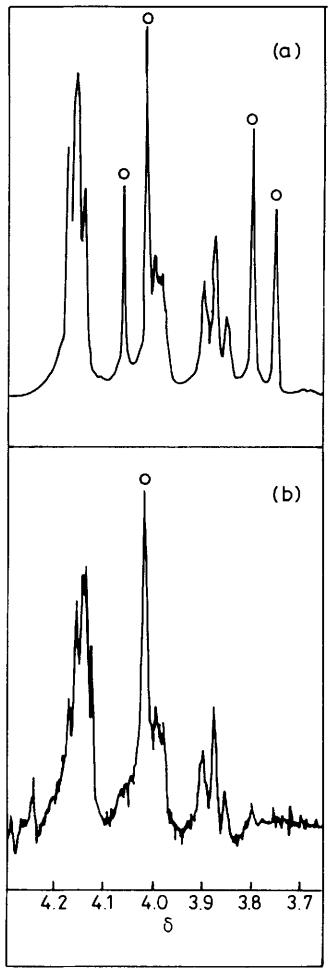


Figure 1. Partial ^{1}H n.m.r. spectra (400 MHz) of $dFl_{red}(n = 6)$ in CD_3OD at room temperature: (a) reduced by $NaBH_4$; (b) reduced by $NaBD_4$.

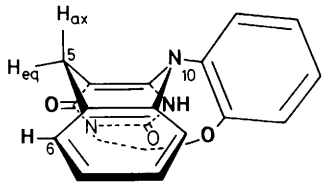
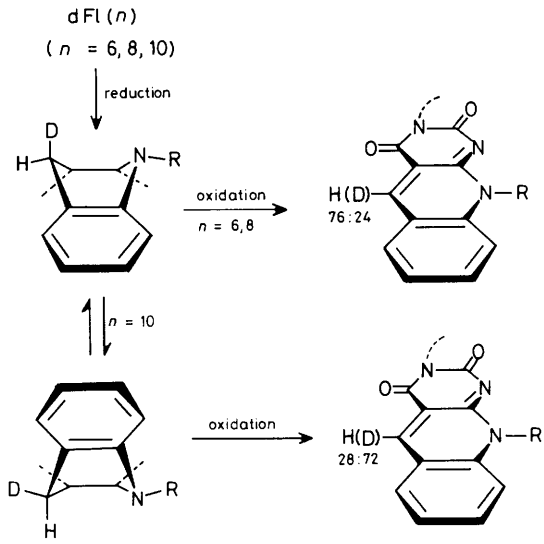


Table 1. Deuterium content (%) at the C(5) position in reoxidised dFl(*n*).

	dFl(*n*)		
Preparation method of $dFl_{red}(n)$	$n = 6$	$n = 8$	$n = 10$
$NaBD_4$	20	23	72
$Na_2S_2O_4$ in D_2O–CD_3OD	20	—	—
BNAD	28	—	—



Scheme 2

$dFl_{red}(n)$ in deuteriated solvents was mixed with *N*-methylacridinium iodide (1.2 equiv.) in water and stirred for 1 h at room temperature. Reoxidised dFl(*n*) was extracted with chloroform and purified by preparative t.l.c. The ^1H n.m.r. data of dFl(*n*) thus obtained (>90% recovery) are summarised in Table 1. Table 1 shows that dFl($n = 10$) contains 72% deuterium at the C(5) position. From this result one can estimate the primary isotope effect for $dFl_{red}(n = 10) \rightarrow dFl(n = 10)$ to be $k_H/k_D = 72/28 = 2.6$. On the other hand, the H_{ax}–H_{eq} exchange is impossible in $dFl_{red}(n = 6)$ and $dFl_{red}(n = 8)$,[5] which give products with almost constant deuterium content, 24 ± 4%. Taking the primary isotope effect into account, the reactivity ratio of H_{ax}:H_{eq} is calculated to be $(76/24) \times 2.6 = 8.2$. This corresponds to a diastereo-differentiation ability of dFl(*n*).

In conclusion, this study demonstrates that the hydrogen transfer to and from 5-deazaflavins occurs exclusively at the axial C(5) position. This finding may have important biochemical implications on diastereo-differentiation, not only in 5-deazaflavin-dependent cofactor F_{420}, but also in flavin-dependent enzymes.

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