Disproportionation in Hydrolysis of Pyrimido[4,5-*b*]quinoline-2(3*H*),-4(10*H*)-diones (5-Deazaflavins)

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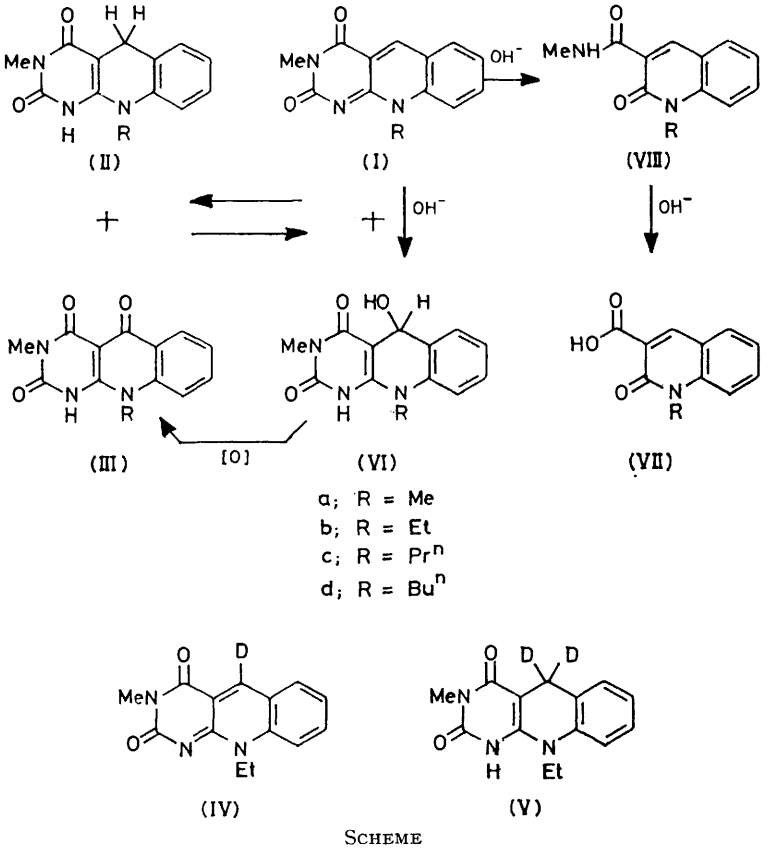
Treatment of 5-deazaflavins with concentrated aqueous potassium hydroxide led to the exclusive formation of 1,5-dihydro-5-deazaflavins and 1,5-dihydro-5-deazaflavin-5-ones *via* intermolecular oxidation–reduction between initially formed 5-hydroxy-1,5-dihydro-5-deazaflavins and unchanged 5-deazaflavins; under dilute alkaline conditions the reverse oxidation–reduction between 1,5-dihydro-5-deazaflavins and 1,5-dihydro-5-deazaflavin-5-ones occurred to form the original 5-deazaflavins and 5-hydroxy-1,5-dihydro-5-deazaflavins, which were oxidized to 1,5-dihydro-5-deazaflavin-5-ones by air. When hydrolysis was carried out with dilute alkaline solution, the corresponding 2-oxoquinoline-3-carboxylic acids were obtained besides the disproportionation products 1,5-dihydro-5-deazaflavins and 1,5-dihydro-5-deazaflavin-5-ones. This disproportionation and hydrolytic scission at the 2-position compete with each other. Higher concentrations of hydroxide ion favoured the formation of the reduced 5-deazaflavins and 5-ketones by disproportionation and reduced the proportion of 2-quinolones formed by hydrolytic scission.

SEVERAL chemical and biological analogies between 5-deazaflavin {pyrimido[4,5-*b*]quinoline-2(3*H*),4(10*H*)-dione}[1] and flavin have been pointed out.[2] In particular, 5-deazaflavins serve as co-factors for some flavin-dependent enzymatic reactions.[3] Also, 5-deazaflavin can be considered chemically as well as structurally as a model for nicotinamide nucleotide protected by annelation. For example, 5-deazaflavin oxidizes simple alcohols under alkaline conditions to yield the corresponding carbonyl compounds, while itself being hydrogenated to 1,5-dihydro-5-deazaflavin.[4] On the contrary, 1,5-dihydro-5-deazaflavin reduced carbonyl compounds under acidic conditions to yield the corresponding alcohols and 1,5-dihydro-5-deazaflavin was reoxidized to 5-deazaflavin.[5] This paper describes the hydrolysis of 5-deazaflavins,[6] which is in contrast with that of flavins reported previously.[7]



SCHEME

Stirring 10-ethyl-3-methyl-5-deazaflavin (Ib)[8] in 60% aqueous potassium hydroxide at 90 °C for 4 h gave 10-ethyl-3-methyl-1,5-dihydro-5-deazaflavin (IIb) and 10-ethyl-1,5-dihydro-5-deazaflavin-5-one (IIIb). Other 5-deazaflavins (Ia, c and d) similarly gave the correspond-

ing reduced 5-deazaflavins (IIa, c, and d) and 5-ketones (IIIa, c and d) exclusively (Table 1). Compounds (II) were identical in all respects with the authentic samples obtained by sodium dithionite reduction of (I). The structures of (III) were determined by elemental analyses and molecular weight determination by mass spectrometry as well as n.m.r. spectra (disappearance of the 5-H signal).

Treatment of 10-ethyl-3-methyl-5-deaza[5-^{2}H]flavin (IV) with 60% aqueous potassium hydroxide under the same condition gave the corresponding 1,5-dihydro-5-deaza[5,5-$^2H_2$]flavin (V) and (IIIb) in almost quantitative yields. Furthermore, treatment of 10-ethyl-3-methyl-5-deazaflavin (1b) with 40% sodium [^{2}H]-hydroxide in deuterium oxide gave (IIb) and (IIIb) without the introduction of a deuterium atom. Compound (IV) was prepared by the cyclization of 6-(*N*-ethylanilino)-3-methyluracil with a mixture of [$^2H_7$]-dimethylformamide and phosphoryl chloride by a known procedure.[8] Compound (V) was identical with the compound synthesized by treatment of (IV) with sodium dithionite in trideuterioammonia–deuterium oxide solution.

The reaction is, therefore, rationalized in terms of initial nucleophilic attack of hydroxide ion on the 5-position of one molecule of (I) giving 5-hydroxy-1,5-dihydro-5-deazaflavin (VI). Subsequent transfer of hydrogen from the 5-position of (VI) to the 5-position of another molecule of (I) affords the corresponding products (II) and (III). An analogous disproportionation has been reported for the reaction of pyrimido-[4,5-*b*]quinolinium salts with aqueous sodium hydroxide.[9]

When hydrolysis was carried out with 10% aqueous potassium hydroxide at 90 °C for 4 h, the corresponding 2-oxoquinoline-3-carboxylic acids (VIIa—d) and (VIIIc and d) were obtained besides the disproportionation products (II) and (III) (see Table 2). However, on prolonged hydrolysis (20 h) the yields of the 5-ketones (III) and 2-oxoquinoline-3-carboxylic acids (VII) increased significantly, with a corresponding decrease in the yields of the reduced 5-deazaflavins (II). When hydrolysis was carried out under anaerobic conditions, the reduced 5-deazaflavins (II) did not decrease. This phenomenon suggests that reverse oxidation–reduction of (II) and (III) into (I) and (VI) occurs under these conditions. Compounds (VI) could be converted into the 5-ketones (III) by air oxidation and (I) again underwent the usual disproportionation and hydrolytic scission at the 2-position to give the 2-oxoquinoline-3-carboxylic acid derivatives.

In fact, treatment of (IIb) and (IIIb) in 10% aqueous potassium hydroxide gave the 5-ketone (IIIb) and the 2-oxoquinoline-3-carboxylic acid (VIIb). No other products were detected. It should be noted here that treatment of (IIb) alone with 10% aqueous potassium hydroxide led to complete recovery of starting material.

The disproportionation and the hydrolytic scission at the 2-position have been shown to compete against each other from the results of the reactions carried out with various concentrations (5—60%) of aqueous potassium hydroxide. Thus the higher the concentration of hydroxide ion, the more 5-ketones (III) and reduced 5-deazaflavins (II) were formed by disproportionation and the less 2-oxoquinoline-3-carboxylic acids (VII) by hydrolytic scisson at the 2-position were obtained (Table 3).

TABLE 1

Disproportionation of the 5-deazaflavins (1a—d) with 60% aqueous potassium hydroxide

Starting deazaflavin	Yield (%) (II)	[M.p. (°C)]	(III)	[M.p. (°C)]	Total
(Ia)	44.9	[278]	46.3	[295]	91.2
(Ib)	46.8	[285]	47.4	[275]	94.2
(Ic)	46.6	[257]	48.2	[263]	94.8
(Id)	47.2	[214]	46.0	[229]	93.2

TABLE 2

Hydrolysis of 5-deazaflavins with 10% aqueous potassium hydroxide

Starting deaza-flavin	Conditions [a]	Yield (%) (VII)	(VIII)	(II)	(III)	Total
(Ia)	A	40.0	0	26.0	26.8	92.8
	B	50.0	0	0	37.7	87.7
(Ib)	A	37.2	0	26.5	27.1	90.8
	B	43.8	0	0	43.4	87.2
(Ic)	A	31.2	18.5	21.9	22.8	94.4
	B	44.2	13.6	0	34.0	91.8
(Id)	A	45.2	8.9	17.4	18.5	90.0
	B	51.2	4.9	9.0	24.0	89.1

[a] A, 90 °C, 4 h; B, 90 °C, 20 h.

TABLE 3

Hydrolysis of 10-ethyl-3-methyl-5-deazaflavin (Ib) with various concentrations of aqueous potassium hydroxide [a]

Concentration of KOH in H_2O (%)	Yield (%) of products (VIIb)	(IIIb)	(IIb)	Total
5	52.6	11.9	10.5	75.0
10	34.9	20.8	20.0	75.7
15	37.2	22.6	22.0	81.8
20	30.2	32.1	29.0	91.3
25	18.6	36.8	33.0	88.4
30	10.5	40.0	39.5	90.0
40	1.4	45.8	46.2	93.4
60		46.8	47.4	94.2

[a] Conditions: (Ib) (0.5 g) and aqueous KOH (5 ml) at 90 °C for 4 h.

EXPERIMENTAL

M.p.s were obtained with a Yanagimoto microapparatus. N.m.r. spectra were determined with a JEOL JNM 3H-60 spectrometer (tetramethylsilane as internal standard) and i.r. spectra (Nujol mulls) with a JASCO IR-1A spectrophotometer. Analytical data are given in Supplementary Publication No. SUP 22649 (6 pp.).*

1,5-Dihydro-5-deazaflavins (IIa—d) *and* *1,5-Dihydro-5-deazaflavin-5-ones* (IIIa—d).—Stirring 5-deazaflavins (Ia—d) (0.002 mol) in 60% aqueous potassium hydroxide (5 ml) at 90 °C for 4 h, followed by neutralization with acetic acid, caused the separation of crystals, which were filtered off and washed with water. The crystals were extracted with

* For details of Supplementary Publications see Notice to Authors No. 7 in *J.C.S. Perkin I*, 1979, Index Issue.

5% aqueous potassium hydrogencarbonate (150 ml) and the residue was recrystallized from acetic acid to give 1,5-dihydro-5-deazaflavins (IIa—d). The aqueous extracts were neutralized with acetic acid and crystals separated. These were filtered off and washed with water. Recrystallization from acetic acid–water gave 1,5-dihydro-5-deazaflavin-5-ones (IIIa—d) (Table 1).

Reduction of (Ia—d) *with Sodium Dithionite to* (IIa—d).—To 10% aqueous ammonia (10 ml) was added a 5-deazaflavin (Ia—d) (4 mmol) and sodium dithionite (2.61 g, 15 mmol) and the mixture was heated at 90 °C for 1 h with stirring. After cooling, the mixture was neutralized with acetic acid and the precipitate was filtered off and washed with water. Recrystallization from acetic acid gave the respective 1,5-dihydro-5-deazaflavins (IIa—d) in high yields (Table 4).

TABLE 4

1,5-Dihydro-5-deazaflavin formation by the reduction of 5-deazaflavins with sodium dithionite

Compound	M.p. (°C)	Yield (%)
(IIa)	278	88
(IIb)	285	99
(IIc)	257	95
(IId)	214	92

Hydrolysis of 5-Deazaflavins with Aqueous Potassium Hydroxide. General Procedure.—Stirring of 5-deazaflavins (Ia—d) (0.002 mol) in aqueous potassium hydroxide (5 ml) at 90 °C for 4 h, followed by neutralization with acetic acid, caused the separation of crystals, which were filtered off and washed with water. The crystals were extracted with ethanol. The ethanol extracts were evaporated to dryness and the residue was extracted with 5% aqueous potassium carbonate (30 ml). The residue was recrystallized from acetic acid–water to give the 2-oxoquinoline-3-carboxamide derivatives (VIIIc and d) (Table 6). The aqueous extracts were neutralized with acetic acid to

TABLE 5

2-Oxoquinoline-3-carboxylic acids

Compound	M.p. (°C)
(VIIa)	228
(VIIb)	187
(VIIc)	163
(VIId)	159

separate crystals, which were filtered off and washed with water. Recrystallization from acetic acid–water gave the 2-oxoquinoline-3-carboxylic acid derivatives (VIIa—d) (Table 5). The first residue insoluble in ethanol was extracted with 5% aqueous potassium hydrogencarbonate (150 ml) and the residue was recrystallized from acetic acid to give 1,5-dihydro-5-deazaflavins (IIa—d). The aqueous extracts were neutralized with acetic acid to separate

TABLE 6

2-Oxoquinoline-3-carboxamides

Compound	M.p. (°C)
(VIIIc)	107
(VIIId)	108

crystals, which were filtered off and washed with water. Recrystallization from acetic acid–water gave the corresponding 1,5-dihydro-5-deazaflavin-5-ones (IIIa—d) (see Table 2).

Synthesis of 10-Ethyl-3-methyl-5-deaza[5-^{2}H]*flavin* (IV).—A mixture of 6-(*N*-ethylanilino)-3-methyluracil (0.3 g, 0.012 mol) and [$^2H_7$]dimethylformamide (1.0 g, 0.014 mol) in phosphoryl chloride (50 ml) was heated at 90 °C for 1 h. After the solution was evaporated, the residue was diluted with water and neutralized with sodium hydrogencarbonate. The yellow crystals which separated were filtered off, washed with water, and dried. Recrystallization from acetic acid gave needles (2.5 g, 82%), m.p. 282 °C, M^+ 256, δ(CF_3-COOH): 1.80 (3 H, t, *J* 7 Hz, 10-CH_2CH_3), 3.63 (3 H, s, NMe), 5.01 (2 H, q, *J* 7, 10-CH_2CH_3), and 7.80—8.45 (4 H, m, Ar).

Hydrolysis of 10-Ethyl-3-methyl-5-deaza[5-^{2}H]*flavin* (IV).—Stirring of 10-ethyl-3-methyl-5-deaza[3-^{2}H]flavin (IV) (0.5 g, 0.002 mol) in 60% aqueous potassium hydroxide (5 ml) at 90 °C for 10 h, followed by neutralization with acetic acid caused the separation of crystals, which were filtered off and washed with water. The crystals were extracted with 5% aqueous potassium hydrogencarbonate (150 ml). The residue was recrystallized from acetic acid to give 10-ethyl-3-methyl-1,5-dihydro-5-deaza[5,5-$^2H_2$]flavin (V) (0.23 g, 47%), m.p. 285 °C, M^+ 259.

The aqueous extracts were neutralized with acetic acid to separate crystals, which were filtered off and washed with water. Recrystallization from acetic acid–water gave 10-ethyl-3-methyl-1,5-dihydro-5-deazaflavin-5-one (IIIb) (0.24 g, 45%).

Hydrolysis of 5-Deazaflavin in Deuterium Oxide.—A solution of 10-ethyl-3-methyl-5-deazaflavin (Ib) (0.5 g, 0.002 mol) in 40% NaOD in D_2O was stirred at 90 °C for 5 h. After cooling, the mixture was neutralized with acetic acid. The separated crystals were filtered off and washed with water. The crystals were dissolved in 10% aqueous sodium hydroxide (20 ml). The solution was neutralized with acetic acid to separate crystals, which were filtered off and washed with water. The crystals were extracted with 5% aqueous potassium hydrogencarbonate (150 ml) and the residue was recrystallized from acetic acid to give 10-ethyl-3-methyl-1,5-dihydro-5-deazaflavin (IIb) (0.23 g, 46%). The aqueous extracts were neutralized with acetic acid to separate crystals, which were filtered off, washed with water, and dried. Recrystallization from acetic acid–water gave 10-ethyl-3-methyl-1,5-dihydro-5-deazaflavin-5-one (IIIb) (0.24 g, 45%).

Alternative Synthesis of 10-Ethyl-3-methyl-1,5-dihydro-5-deaza[5,5-$^2H_2$]*flavin* (V).—A mixture of 10-ethyl-3-methyl-5-deaza[5-^{2}H]flavin (IV) (0.5 g, 0.002 mol), sodium dithionite (0.7 g, 0.004 mol), 20% trideuterioammonia–deuterium oxide solution (1 ml), and D_2O (4 ml) was refluxed for 30 min. After cooling, the separated crystals were filtered off, and washed with water. The crystals were dissolved in 10% aqueous potassium hydroxide (10 ml). The solution was neutralized with acetic acid to separate the crystals, which were filtered off, washed with water, and dried. Recrystallization from acetic acid gave a microcrystalline powder (0.4 g, 80%), m.p. 285 °C, M^+ 259.

Reverse Oxidation–Reduction of (II) *and* (III) *into* (I) *and* (VI).—Stirring of 10-ethyl-3-methyl-1,5-dihydro-5-deazaflavin (IIb) (0.26 g, 0.001 mol) and 10-ethyl-3-methyl-1,5-dihydro-5-deazaflavin-5-one (IIIb) (0.27 g, 0.001 mol) in 10% aqueous potassium hydroxide (5 ml) at 90 °C for 20 h, followed by cooling, separated crystals which were filtered off and neutralized with acetic acid to precipitate the 5-ketone (IIIb) (0.001 5 ml). Acidification of the filtrate with acetic acid caused the separation of the 2-quinolone (VIIb) (0.000 3 mol). No other products were detected.

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