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# ACTIVATION AND TRANSFER OF OXYGEN XV
# EVIDENCE FOR THE TRANSIENT OPENING OF THE PYRAZINE RING IN $N^1$- AND $N^5$- ALKYLFLAVIN MODELS. $4^A$- TO $10^A$- ADDUCT ISOMERIZATION AND PYRAZINE- AND PYRIMIDINE- RING CONTRACTIONS.

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*Summary:* The pyrazine ring opening is proven by rearrangements leading to benzimidazoles 4, 13 and 14. A $4^a$- to $10^a$-adduct isomerization (6 ⟶ 7) and the intermediacy of a carbonyl oxide 15 are indicated by pyrimidine ring contractions into 8 and 9, respectively.

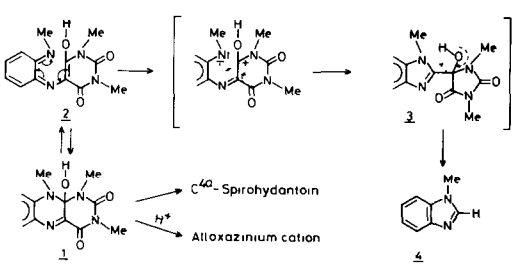
In the preceeding paper[1] it was shown that the $10^a$-hydroxy pseudobase 1 is in equilibrium with a blue intermediate for which structure 2 has been proposed.[2,3] In order to find evidence for the transient opening of the pyrazine ring, efforts had to be made to effect some kind of trapping reaction. An intramolecular attack at the original $C^{4a}$ might be pictured to be such a trapping reaction resulting in a benzimidazole 4 (Scheme 1).

Organic solutions of the blue intermediate 2 undergo a spontaneous bleaching to give the $C^{4a}$-spirohydantoin as the main product. However, the presence of traces of a benzimidazole derivative could indeed be demonstrated by extracting the bleached solutions with dilute hydrochloric acid. The acidic extracts showed a characteristic spectrum with absorption maxima at 262, 268 and 275 nm, identical with the spectrum of 1-methylbenzimidazole 4.

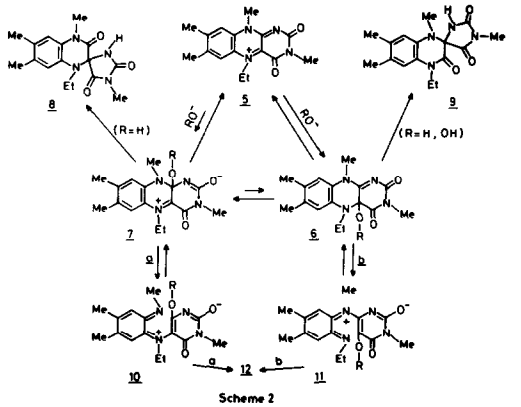
Attempts were made to increase the yield of the benzimidazole by increasing the electrophilicity of the $C^{4a}$ bridgehead carbon of the flavinium ring system by protonation and $N^5$-alkylation. Although the intramolecular rearrangement has to compete with the regeneration of the alloxazinium cation, a stimulation of the first by protonation has appeared to be undeniable. The effect of a $N^5$-alkyl group even proved to be dramatic.

Starting from the 5-ethyl-3-methyllumiflavinium cation 5 (Scheme 2), rather stable $4^a$-adducts 6 (R= H; OH; Me) are obtained as the results of competitive, intermolecular nucleophilic attacks at $C^{4a}$. Nevertheless, an irreversible ring contraction into a benzimidazole 12 may be expected to become a predominant reaction, since $4^a$-adducts are in equilibrium with the cation 5.

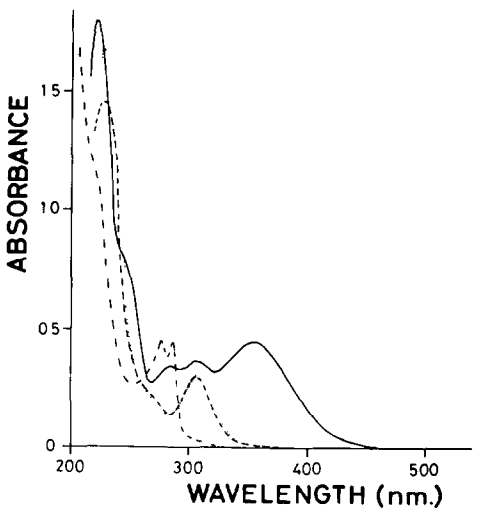
The $4^a$-hydroxy pseudobase 6 (R= H) was of particular interest. Only the reconversion into the cation 5 by acidification seems to have been well studied.[4] In attempts to recrystallize the pseudobase from both aqueous and organic solvents, we observed the occurrence of some transformations. The main product isolated in a yield of 60% from aqueous solutions was analyzed for a new isomer of the pseudobase, the $C^{4a}$-spirohydantoin 8, which was quite unexpected. By this remarkable transformation it has been substanciated for the first time that even the less electrophilic $10^a$-position of $N^5$-alkylflavinium cations can be preferably attacked by nucleo-

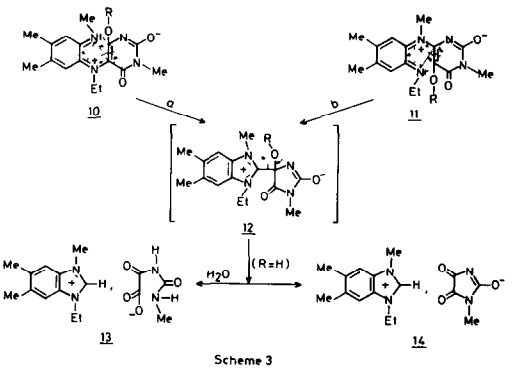



Scheme 1



Scheme 2



Scheme 3

Fig. UV spectra of $10^{-5}$ M solutions in 96% EtOH of: 6 (R=H, —— $\lambda_{max}$, nm (ε): 222 (36,000), 286 (6900), 308 (7200), 355 (8800)); 8 (····· $\lambda_{max}$, cf. example 1); 9 (----- $\lambda_{max}$, cf. example 2); 14 (-·-·-·- $\lambda_{max}$, nm (ε): 279 (9100), 287 (8900)).

philes to give a $10^a$-adduct 7. It illustrates, that in spite of a stablizing $N^5$-substituent, a $4^a$-adduct can act as a storage isomer of a transient $10^a$-adduct.

Example 1: A mixture of 6 (R= H; 0.50 g), $H_2O$ (10 ml) and MeCN (5 ml) was refluxed until the blue colouration had changed into a yellow one (2.5-4 h). The starting compound was then completely converted according to TLC ($CHCl_3$-AcOEt, 4:1) on silicagel. The colourless crystalline product 8 was filtered off and recrystallized from aqueous ethanol, yield 0.30 g (60%) m.p. 236-236.5° ($C_{16}H_{20}N_4O_3$ (316.37) Calcd: C, 60.74; H, 6.37; N, 17.71 Found: C, 60.9; H, 6.4; N, 17.7). Mass spectrum, m/e (%): 316 ($M^+$, 100); 287 (43); 258 (6); 230 (36); 203 (52); 175 (31). PMR ($CDCl_3$): δ= 1.22 (3, t, J= 7 Hz, C-Me); 2.23 (6, s, C-Me); 3.08 (3, s, N-Me, overlapping $-CH_2$); 3.41 (3, s, N-Me); 6.03 (1, s, NH); 6.65 (1, s, Ar-H); 6.82 (1, s, Ar-H). IR (KBr), $cm^{-1}$: 3250; 1783; 1733; 1640. UV (96% EtOH), $\lambda_{max}$ (ε): 227.5 nm (33,500); 307.5 nm (6100).

From the mother liquor, the $C^{10a}$-spirohydantoin 9 was obtained in low yield (5%), representing the result of a ring contraction of the $4^a$-hydroxy pseudobase. The yields of the

spirohydantoins varied in dependence on the nature of the medium and the experimental conditions. (Spirohydantoin 9 was more readily obtained by the spontaneous decomposition of the $4^a$-hydroperoxydihydroflavin, cf. example 2 and Scheme 4).

Most rewarding were the attempts to find a close material balance. Besides the two spirohydantoins 8 and 9, two crystalline organic salts 13 and 14 (Scheme 3) were obtained, both from the 1-ethyl-3-methylbenzimidazolinium cation. Displacements of the organic anions by $ClO_4^-$ gave N-methyloxaluric acid and N-methylparabanic acid. The salts 13 and 14 were formed as the final products in aqueous and anhydrous media, respectively.

The reactions leading to the $C^{4a}$-spirohydantoin 8 and to the benzimidazolinium salts are competitive processes. This was demonstrated on varying the water content of the medium. The benzimidazolinium salt 13 was obtained in a yield of 15% from the mother liquor in example 1. On diminishing the water content of the reaction mixture, the yield of the spirohydantoin 8 decreased to a few percents, while the yield of 13 increased to 60%. From anhydrous solutions, e.g. acetonitrile, the benzimidazolinium salt 14 was isolated in a yield of 60%. The UV spectra of the $C_{16}H_{20}N_4O_3$-isomers 8, 9 and 14 formed from 6 (R= H) by the various ring contractions are given in the Figure.

The intramolecular rearrangement into a benzimidazolinium cation does prove the opening of the pyrazine ring, but it does not provide unambigous evidence for either a 10,$10^a$- or a $4^a$,5-ring cleavage (Scheme 3). In order to answer this important question, studies on the formation of benzimidazoles from $^{13}C$-enriched flavin models are in progress.

During the transformations mentioned in Schemes 2 and 3 a blue intermediate was produced, which could be extracted from aqueous reaction mixtures. The absorption maxima were dependent on the nature of the solvent giving values of 502 and 580 nm ($H_2O$), 608 and 656 nm (benzene) and 603 and 641 nm ($CHCl_3$), identical with the spectra of the neutral 5-ethyl-3-methyllumiflavin radical.

A $N^5$-dealkylated dihydroflavin formed by solvolysis of 5 may act as a reducing agent for a 5-ethyl-3-methyllumiflavinium cation. However, in the preparative experiments on the ring transformations shown in Scheme 2, only small amounts of 3-methyllumiflavin were found. This implies that solvolysis of 5 had not been important.

The question whether a $4^a$-hydroperoxy-dihydroflavin can also functionate as a storage compound for a $10^a$-hydroperoxy isomer is being studied. However, in the $N^5$-alkylflavin model series, the $4^a$-hydroperoxide 6 (R= OH) is too labile to undergo a considerable transformation into a benzimidazolinium salt. Suspensions or solutions of the hydroperoxide in aqueous or organic media spontaneously gave rise to an evolution of oxygen with the formation of 9.

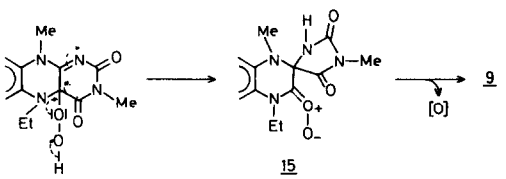
Example 2: In a manometric apparatus, a suspension of 6 (R= OH; 332 mg) in $H_2O$ (50 ml) was stirred at room temperature until $O_2$-evolution ceased. Spirohydantoin 9 was filtered off and recrystallized from aqueous ethanol, yield 0.25 g (79%) m.p. 205-205.5° ($C_{16}H_{20}N_4O_3$ (316.37) Calcd: C, 60.74; H, 6.37; N, 17.71 Found: C, 60.5; H, 6.4; N, 17.6). Mass spectrum, m/e (%): 316 ($M^+$, 100); 287 (6); 258 (11); 231 (75); 203 (60); 175 (46). PMR ($CDCl_3$): δ= 1.26 (3, t, J= 7 Hz, C-Me); 2.22 (6, s, C-Me); 2.80 (3, s, N-Me); 3.05 (3, s, N-Me); 4.01 (2, q, J= 7 Hz, $CH_2$); 6.27 (1, s, NH); 6.65 (1, s, Ar-H); 6.82 (1, s, Ar-H). IR (KBr), $cm^{-1}$: 3290; 1790; 1735; 1647. UV (96% EtOH), $\lambda_{max}$ (ε): 225.5 nm (35,000); 305 nm (6000).

These conditions were harmless to the $4^a$-hydroxy pseudobase as was shown by its quantitative recovery in blank experiments. (The pseudobase can be converted into 9 in boiling neutral solvents, requiring long refluxing times and giving considerably lower yields).

From these experiments it has appeared that the generation or transfer of oxygen by a non-radical decomposition of 6 (R=OH) is connected with a $4,4^a$-bond cleavage, which justifies[5] the formulation of a transient carbonyl oxide 15 (Scheme 4). A substituent at $N^5$ is not expected to decrease the chance for a $4^a,5$-ring opening. Nevertheless, no indication has been found for the intermediacy of a carbonyl oxide type derived from such a ring cleavage.

During autoxidation of 5-ethyl-3-methyl-dihydrolumiflavin, the pathway given in Scheme 1 is easily displaced by one-electron reductions of the $4^a$-hydroperoxy-dihydroflavin, resulting in hydroxylating radicals and the $4^a$-hydroxy pseudobase as the main products.[5]

Neither the rate and the yield of the $O_2$-production nor the yield of 9 (Scheme 4) were affected by the additional presence of an aldehyde. Apparently, the chemiluminescence[6] shown by a $4^a$-hydroperoxy-dihydroflavin and an aldehyde is a minor pathway.




6 (OR=OOH)

Scheme 4

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