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ACTIVATION AND TRANSFER OF OXYGEN XIII
EVIDENCE FOR C^{10A} AS THE PRIMARY NUCLEOPHILIC ADDITION SITE OF N^1-ALKYLFLAVINIUM CATIONS

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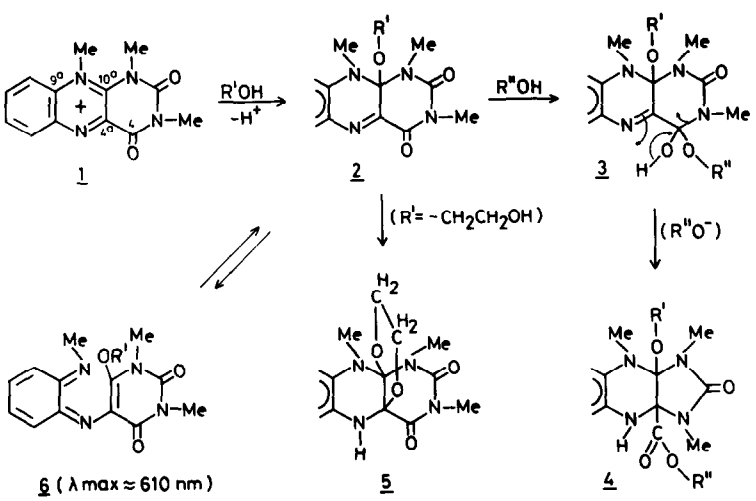
Summary: The formation of a primary adduct and its successive trapping were carried out with a deuteriated and non-deuteriated nucleophile. The addition sites were ascertained by NMR-spectra. The consequences for the structure of ESR-silent blue flavin transients are discussed.

In questioning the 1-alkyl-10^a-hydroperoxy-1,10^a-dihydroflavin structure 2 (R'= OH), alkoxy adducts 2 (R'= alkyl) from 1-alkylalloxazinium cations 1 have been used as less reactive models in comparative spectral studies.[1,2]



The alkoxy models have never been isolated, which has contributed to the many controversies on the primary nucleophilic addition site of 1-alkylflavinium cations. In preceding papers[1,2] of these series inter- and intra-molecular nucleophilic trapping of primary alkoxy adducts were described. Starting from a 1-alkylflavinium cation (1-RFl^+_{ox}) and monohydric alcohols the overall stoichiometry for the formation of the primary adduct (1-RFl-OR') and its successive trapping is given by Eqs (1) and (2).

$$1\text{-RFl}^+_{ox} + \text{R'OH} \rightleftharpoons 1\text{-RFl-OR'} + H^+ \qquad (1)$$

$$1\text{-RFl-OR'} + \text{R"OH} \longrightarrow 1\text{-RFlH}\begin{matrix}\text{OR'}\\ \text{OR"}\end{matrix} \qquad (2)$$

The final product from the 1,3,10-trimethylalloxazinium cation 1 and methanol was shown to be the hexahydroimidazoquinoxaline 4 (R'=R"= Me) proving the intermediacy of the $4,10^{a}$-dimethoxyalloxazine adduct 3 (R'=R"= Me) and indicating C^{10a} as the primary addition site This indication was amplified by the elucidation of the tetrahydroalloxazine structure 5 being the result from an *intramolecular* trapping, occurring at C^{4a} in this particular case.

Further studies have now been focussed on the primary addition site. The PMR spectrum a of 4 (R'=R"= Me) opens up the possibility to obtain definite evidence for this. The chemical shift of the methoxy group attached at the original C^{10a} (δ_{OCH_3} = 3.43 ppm) is well distinguished from the chemical shift of the methoxy group added at the original C^{4} (δ_{COOCH_3} = 3.84 ppm).

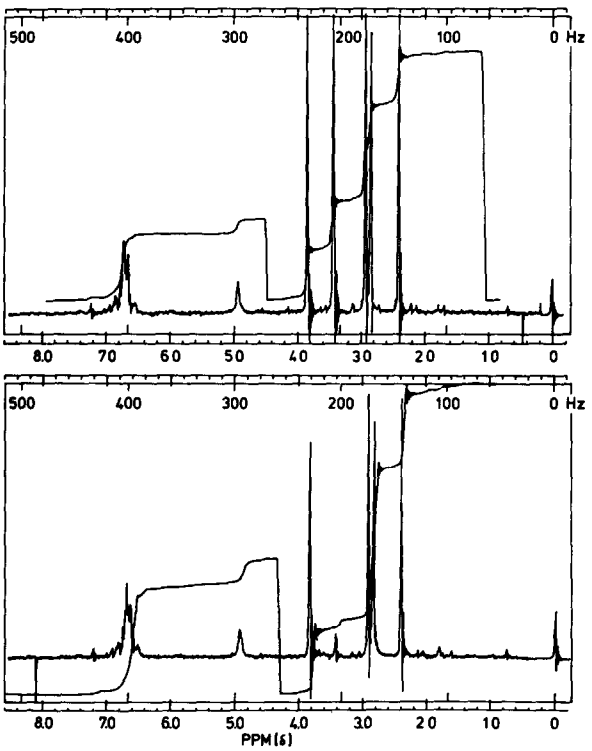


Fig. PMR spectra in $CDCl_3$. Top curve a for 4 (R'=R"= CH_3). The bottom curve b for the product 4 obtained from 1 by successive additions of CD_3OD and CH_3OH.

Experimental conditions have now been found to separate the two reaction steps (1) and (2). The primary adduct formation was carried out with deuteriomethanol and triethylamine, shifting equilibrium (1) to the right. The nucleophilic trapping (eq 2) was performed with an excess of non-deuteriated methanol and sodium methoxide:

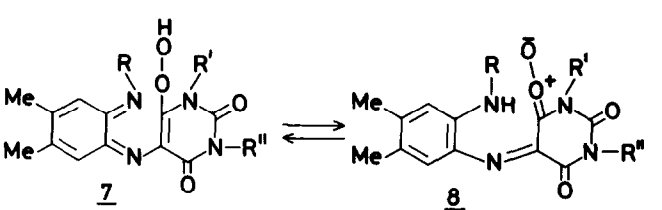
A mixture of 1,3,10-trimethylalloxazinium perchlorate (1.07 g= 3.00 mmol), deuteriomethanol (3 ml) and triethylamine (1.5 ml) was stirred at room temperature to give a

clear solution of the primary deuteriomethoxy adduct within 12 minutes. After 30 minutes a solution of sodium (0.60 g) in non-deuteriated methanol (15 ml) was added giving rise to crystallization within half a minute. Stirring was continued for another 30 minutes, after which the product was filtered off and washed with methanol. Yield 0.68 g (70%). For the spectral studies the compound was thrice recrystallized from non-deuteriated methanol; m.p. 195° (decomp). Mass spectrum, m/e (%): 326 (M^+, 24); 323 (M^+, 100); 320 (M^+, 9); 308 (8); 305 (32); 289 (2); 273 (5); 264 (46); 246 (10); 229 (97); 215 (16); 207 (39); 203 (40); 191 (5); 188 (19); 185 (33); 172 (69); 161 (52). Compared with the mass spectrum of the non-deuteriated compound[1], m/e (%): 320 (M^+, 100); 305 (26); 289 (4); 272 (21); 261 (30); 246 (6); 229 (53); 215 (8); 204 (16); 200 (39); 191 (4); 188 (5); 185 (24); 172 (27); 161 (25).

From the comparison of the mass spectrum of the deuteriated product with the mass spectrum of the non-deuteriated compound[1] it is concluded that the main component of the first contained one deuteriomethoxy group (m/e 323, M^+, 100%). The PMR spectrum b clearly shows that the deuteriomethoxy group is added at the original C^{10a} (δ= 3.43 ppm) and that no exchange of importance has taken place during the trapping and recrystallization processes (cf. the mass spectra, m/e 320). This unequivocally confirms that the primary nucleophilic attack has taken place at the 10^a-position and the secondary addition at C^4.

The arguments put forward by Hemmerich[3] in favour of a primary C^{9a}-adduct formation as well as the conclusive proof for a primary attack at C^{9a} as claimed to have been given by Müller[4], are all invalid (note).[5] Since the NMR spectra demonstrate that any speculation on an alkoxy exchange and migration from C^{9a} to C^{10a} must be rejected, there are also no reasons to assume exchange and migration of other nucleophiles like HO^- and HOO^- from C^{9a} to C^{10a} as postulated by Hemmerich[3] and as Müller's view[4] implies. Therefore, the hydroperoxy transients occurring in the autoxidation of 1-alkyl-dihydroflavins, showing UV spectra with absorption maxima in the 410-425 nm region similar to those of 1-alkyl-10^a-alkoxy-1,10^a-dihydroflavin models, should be formulated as 10^a-hydroperoxy-1,10^a-dihydroflavins[1,2] (cf. 2, R'= OH) and definitely not as 9^a-hydroperoxy-1,9^a-dihydroflavins[3,4] 9.

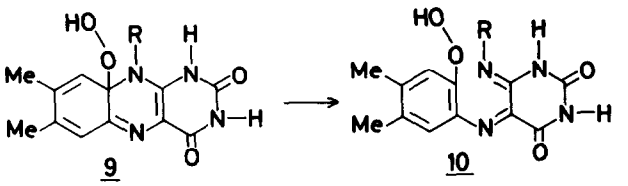
This conclusion implies that Hemmerich[3] has incorrectly assigned 10^a-substituted 1,10^a-dihydroalloxazine structures 2 to ESR-silent blue flavin transients absorbing at about 610 nm. Alternatively, we have assumed[2] that the stability of the 10,10^a-bond in a dihydroalloxazine 2 is dependent on the nature of the substituent at C^{10a}. The 10,10^a-ring opened structure 6 was postulated for these blue transients being in equilibrium with the 1,10^a-dihydroalloxazines 2. It is noted that the rapid ring contraction of a 10^a-hydroxy pseudobase 2 (R'= H) to a tetrahydroquinoxaline-spiro-hydantoin[6] is somewhat retarded in anhydrous, apolar solvents like benzene both in oxidative[6] and in non-oxidative processes.[2] Consequently, the accumulation



of a blue transient of the proposed structure 6 (R'= H) can then be observed. In oxidative processes the appearance of a blue colour could also be a direct indication for the presence of a 10^a-hydroperoxide 2 (R'= OH) through the ring opened isomer 6 (R'= OH).

It is now suggested that in the oxygen transfer by hydroperoxy-dihydroflavins the possible occurrence of a $10,10^a$-ring opened hydroperoxide 7 or its carbonyl oxide tautomer 8 should be considered (note).[7]

The above suggestion is in contrast with Hemmerich's preference[3] for a 9^a,10-ring opened hydroperoxide 10 which was put forward as the most powerful "oxene gun" in flavin biochemistry. Since evidence for a 9^a,10-ring opening has never been found or presented, this idea is rejected.



9 10

The formation of ring opened transients is not limited to N^1-alkylflavin models, but is also shown by N^5-alkylflavin derivatives[8] which seems to be relevant to the structure of the non-radical blue flavin transient arising in bacterial bioluminescence.[9] Evidence for the opening of the pyrazine ring will be presented in a subsequent paper of these series.

Acknowledgement— I am very much indebted to Mr. T. de Beer for his experimental assistance.

REFERENCES AND NOTES.

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4. F. Müller, H.J. Grande and T. Jarbandhan, in *Flavins and Flavoproteins* (T.P. Singer, ed.), pp. 38-50, Elsevier Scientific Publ. Co., Amsterdam, Oxford, New York, 1976.
5. Note: *Although not referring to our studies[1,2] showing the invalidity of Müller's proof[4] on the C^{9a}-adduct formation, C.G. van Schagen, H.J. Grande and F. Müller have recently gained the insight that C^{10a} is the correct nucleophilic addition site* (Rec. Trav. Chim. 97, 179-180 (1978)), *but no comments were made on the erroneous interpretations in their previous paper[4], which caused the 10^a-9^a adduct controversy.*
6. H.I.X. Mager, R. Addink and W. Berends, Rec. Trav. Chim. 86, 833-851 (1967).
7. Note: *G.A. Hamilton, in Progress in Bioorganic Chemistry (E.T. Kaiser and F.J. Kézdy, ed.), vol. 1, pp. 134-142 (1971)), first speculated on ring opened transients. The carbonyl oxide from a $10,10^a$-ring opening was considered to be less likely than the carbonyl oxide from a $4^a,5$-ring opening.*
8. H.I.X. Mager, (to be published).
9. R. Presswood and J.W. Hastings, Biochem. Biophys. Res. Comm. 82, 990-996 (1978).

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