Tetrahedron Vol 33, pp 981 to 989 Pergamon Press 1977 Printed in Great Britain

ACTIVATION AND TRANSFER OF OXYGEN—XII

ALKOXY ADDUCTS DERIVED FROM 1,3,10-TRIMETHYLALLOXAZINIUM (1,3-DIMETHYLFLAVINIUM) CATIONS

H. I. X. MAGER*
Biochemical and Biophysical Laboratory of the University of Technology, 67 Julianalaan, Delft, The Netherlands

(*Received in UK 8 November 1976; Accepted for publication 14 December 1976*)

Abstract—Monoalkoxy adducts, derived from an alloxazinium cation and an alcohol in the presence of a base, are readily converted into dialkoxy adducts. Two types of dialkoxy adducts are formed. In the reaction with a monohydric alcohol C_4 is preferred for the second *intermolecular* nucleophilic attack to give the 4,10a-dialkoxy adduct, which rearranges into a hexahydroimidazo[4,5-b]quinoxaline derivative **5c**. In the reaction with a dihydric alcohol C_{4a} is the second reaction site in an *intramolecular* nucleophilic ringclosure to give a 4a,10a-dialkoxy adduct **6c** which can be isolated as such. Both types of dialkoxy adducts can be reconverted into the alloxazinium cation.

The structures of the dialkoxy adducts give every reason to reject the conclusion in the literature that C_{9a} is the primary addition site.

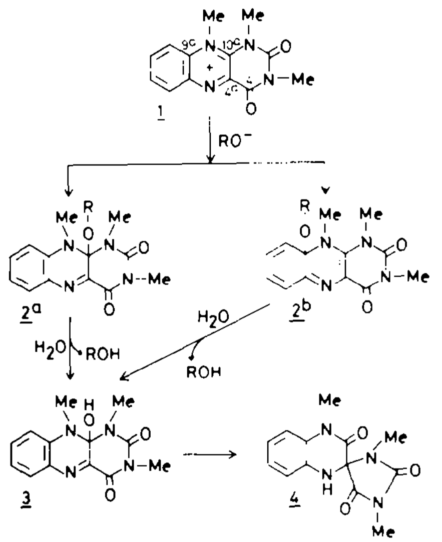
Starting from **5c** several reactions have been effected: (a) conversions into tetra- and di-hydroimidazo[4,5-b]quinoxalinium cations **7** and **9** and ring opening of **9** into a ureido-dihydroquinoxaline **10** (Scheme 4); (b) a rearrangement of a transient hexahydroimidazo[4,5-b]quinoxaline **5d** into spirohydantoin **4** and a rearrangement of cation **7** into the alloxazinium cation **1** (Scheme 5); (c) preparation of acetylated tetra- and hexa-hydroimidazo[4,5-b]quinoxalines **11** and **12**. Conversions of cation **11** into **9** and **1** and conversions of **12** into a benzimidazolinium cation **14** (Schemes 6 and 7). Degradation of **14** affords 1,2-dimethylbenzimidazole (**15**, Scheme 6).

There is little doubt that dihydroflavin-oxygen adducts (H_2Fl-O_2) are active participants in flavoprotein-catalyzed hydroxylations.[1] Speculations on the perhydroxy structure (HFl-OOH) are based on the comparison of absorption and fluorescence spectra with those of alkylated model alloxazines or flavins (R-Fl-X) substituted in a reactive position by a nucleophile X less reactive than HOO^-. However, the correct structures of some of these R-Fl-X models are still matters of controversy.

In spectrophotometric studies Müller[2] used MeO^- as a model nucleophile X in converting an alkylated flavinium cation (1-RFl^+_{ox}) into an adduct (1-RFl-?-OMe; λ_{max} = 410–430 nm), for which he initially claimed a 10a-structure in line with our proposals (**2a**, Scheme 1). Subsequently, he claimed[3] and rejected[4] the 6- and 8-methoxy structure and recently proposed[5] the 9a-methoxy structure **2b**. Hemmerich *et al.*[6] closely follow these proposals for the structure of the O_2-adduct and now assume that the biological relevant HFlOOH is a 9a-perhydroxydihydroflavin.[1,7]

Müller's recent NMR studies[5] have no bearing on a N_1-methylated alloxazinium cation like **1**, but on a 2-OMe isomer. The addition of methoxide or hydride ions causes changes of the NMR spectrum which were interpreted in terms of 9a-adduct formation. However, his final conclusion remains unjustified by the PMR spectrum of the hydride ion adduct, which does not show the coupling that can be expected from the adjacent C_9 proton. Therefore, we contradict the recent opinion[5,6] that Müller *et al.*[5] presented conclusive evidence for the 9a adduct structure and that this conclusion can be extended to the adducts from other derivatives like the 1,3,10-trimethylalloxazinium cation **1**.

So far, monoalkoxy adducts from **1** have never been isolated due to the high sensitivity to water and to conversions brought about by the presence of a base, which will be dealt with in the present paper. Water gives a rapid elimination of the alkoxy group and a linking of HO^- at C_{10a} which is followed by the well-known rearrangement of the transient 10a-hydroxy pseudobase **3** to the spirohydantoin **4**. This spirohydantoin formation gives no indication of the 9a-position as being a *primary* reaction site.



Scheme 1. Proposals on 10a- and 9a-alkoxy adducts vs the structure of spirohydantoin **4**.

Evidence for C_{10a} as an electrophilic addition site is not only obtained from investigations on alloxazinium species. In an early paper[8] from this laboratory it was already reported that alkaline hydrolysis of 10-methylisoalloxazine led to the isolation of 4 - methyl - 3 - oxo - 1,2,3,4 - tetrahydroquinoxaline - 2 - spiro - 5′ - hydantoin from which we concluded that C_{10a} had been attacked by HO^-. Although not referring to our conclusion Smith and Bruice[9] recently gained the same insight.

The studies on the alkaline hydrolysis of an isoalloxazine substituted at N_{10} by the 2',6'-dimethylphenyl group are of further interest. Smith and Bruice[9] found that this steric blocking of the 10a-position results in a hydrolytic scission between positions 3 and 4 which proves a preferential HO^- attack at C_4.

From recent studies on autoxidative conversions of partially reduced alloxazines and pteridines[10,11] we have obtained evidence that in neutral and basic media C_4 can be a reaction site in combination with C_{4a} or $C_{10a(8a)}$. It is supposed that migration of HOO^- or the cleavage of one C–O bond in cyclic peroxy transients could lead to intermediates having two and, finally, one oxygen linked only at C_{10a}, C_{4a} or C_4 (apart from the C_4 carbonyl oxygen).

The linkage of two or one additional O atom(s) at C_4 implies the occurrence of reactive transients in which the original C_4 may become a carboperoxy- or a carboxy-group, respectively. In oxidation models this can result in irreversible rearrangements of the heterocyclic ring system or in degradation of the pyrimidine ring with the formation of carbon dioxide.[11,12]

1,3,10-Trimethylalloxazinium cation (**1**, Scheme 1) is an important participant in *nonenzymic* hydroxylation systems.[13] Hydrogen peroxide may add to the cation to give a dihydroalloxazine hydroperoxide and derived hydroxylating species. Since alcohol adducts have been considered as less reactive models for peroxy intermediates, it is now questioned whether a thorough investigation of the alcohol adduct formation from **1** would reveal the reactivity not only of the 10a and 4a bridge positions but of C_4 also. As the alcohol adduct formation is reported[2] to be independent of the methyl substituents in positions 7 and 8, the results given by the trimethylalloxazinium cation **1** are representative for the behaviour of 1-alkylated flavinium cations (1-RFl_{ox}^+).

RESULTS

(I) *Mono- and di-alkoxy adduct formation*

Starting from the alloxazinium cation **1** (curve **a**, Fig. 1) monoalkoxy adduct formation is shown in a variety of alcohols. It is exemplified by the spectrum of the monomethoxy adduct (curve **b**) and of the hydroxyethyleneoxy adduct (curve **c**). The equilibrium: $\text{1-R'Fl}_{ox}^+ + \text{ROH} \rightleftarrows \text{1-R'Fl-OR} + H^+$ is shifted by acid or base. In this connection it is noted that spectral changes can be caused by the acid or alkaline impurities of reagent grade alcohols. The assumption[2] that dissociation of the monomethoxy adduct into the flavinium cation and methanol would occur on diluting a methanolic solution of the adduct with methanol has to be considered as erroneous. In our hands such a cation formation was only effected when acid impurities were present in the methanol.

The addition of water to neutral or basic solutions of monoalkoxy adducts leads to the formation of the spirohydantoin **4** (curve **d**) which was confirmed by analyzing the product isolated.

The presence of an excess of a base, in general the corresponding alkoxide ion or an amine like triethylamine, in the alcoholic solutions gives rise to products not identified before. In the absence of water the spectra **b** and **c** of the monoalkoxy adducts changed into the spectra **e** and **f** (Fig. 2), which are very similar to the spectrum **d'** of the spirohydantoin **4**.

Further investigations showed that in spite of the similar UV spectra **e** and **f** two types of dialkoxy adduct formation can be distinguished:

(1) *4,10a-Dialkoxy adduct* (**5b**, Scheme 2) *formation with the transformation of the alloxazine (flavin) ring-system into the 1H-imidazo[4,5-b]quinoxaline ring-system.* Starting from the monomethoxy adduct **2a** (R = –Me) a new compound with spectrum **e** was isolated in a yield of 88%. It was analyzed for a dimethoxy adduct $\text{1-R'Fl-10a-OR} + \text{ROH} \xrightarrow{(RO^-)} \text{1-R'FlH(OR)}_2$) or a product derived from it. X-ray analysis[14] established structure **5c** (R = –Me) as the correct one. Its chemical reactivity is described under Section 2.

(2) *4a,10a-Dialkoxy adduct formation-isolation of 4a,5,10,10a-tetrahydroalloxazine (1,4a,5,10a-tetrahydroflavin) derivatives* (**6c**, Scheme 3). The structure of **5c** (R = –Me) raised the question whether the same type of conversion is shown by the hydroxyethyleneoxy adduct **2a** (R = $-CH_2CH_2OH$; identical with **6a** in Scheme 3) either in an *intermolecular* attack at C_4 by a $HOCH_2CH_2O^-$ anion and a subsequent rearrangement of the 4,10a-di(hydroxyethyleneoxy) adduct **5b** to **5c** (R = $-CH_2CH_2OH$) or in an *intramolecular* addition at C_4 and a rearrangement to **6d** (Scheme 3).

The compound with spectrum **f** was isolated in a yield of 94%. Analysis proved it to be a product from an *intramolecular addition*. However, the ringcontracted ethylenedioxy bridged structure **6d** was doubted on account of the different chemical reactivity (Section 2) and the mass spectrum showing no peak for an imidazoquinoxalinium fragment as found in the mass spectrum of **5c** (R = –Me). X-ray analysis[15] established the ethylenedioxy bridged 4a,5,10,10a-tetrahydroalloxazine (1,4a,5,10a-tetrahydroflavin) structure **6c** as the correct one.

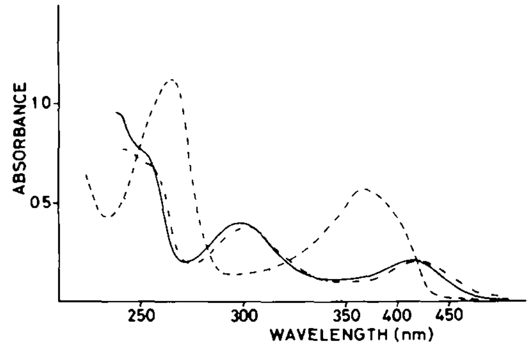


Fig. 1. UV spectra of 5×10^{-5} M solutions in MeOH of: **1** (--- a); **2a** (— b); **4** (··· d); **6a** in $HOCH_2CH_2OH$ (–·–·– c).

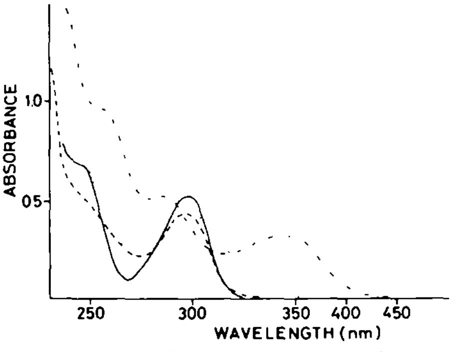
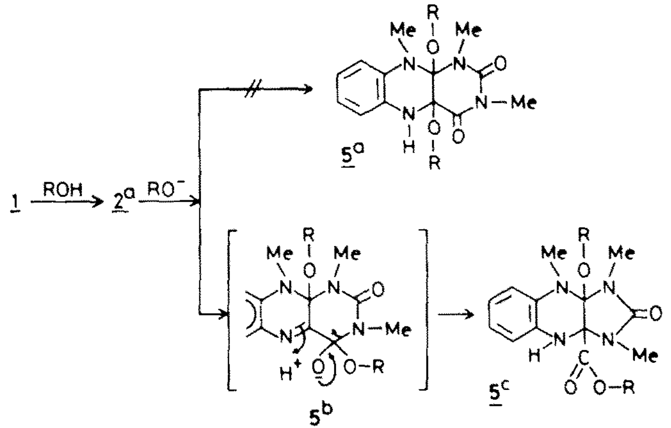
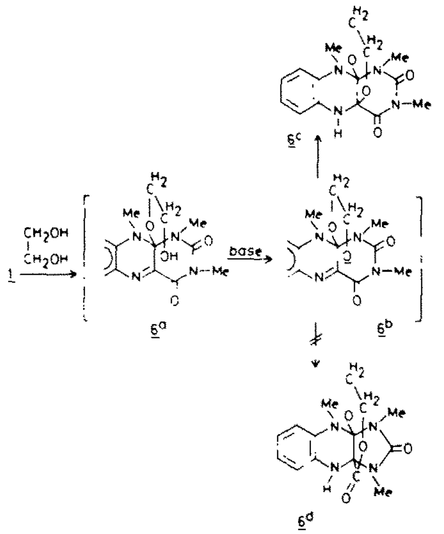


Fig. 2. UV spectra of 10^{-4} M solutions in MeOH of: **4** (··· d'); **5c** (--- e); **6c** (— f); **7** in 0.6 N methanolic H_2SO_4 (–··–·· g).



Scheme 2. 4,10a-Dialkoxy adduct (**5b**) formation and ring-contraction into a 1H-imidazo[4,5-b]quinoxaline derivative **5c**.



Scheme 3. Formation of a 4a,10a-ethylenedioxy bridged adduct **6c**.

(2) Characteristic conversions of the dialkoxy adducts

(1) *Conversion of* **5c** (R = -Me) *into the tetrahydroimidazo*[4,5-*b*]*quinoxalinium cation* **7**; *formation of the dihydroimidazo*[4,5-*b*]*quinoxalinium cation* **9** *and ringopening into the ureido-dihydroquinoxaline* **10** (Scheme 4). On acidifying a solution of **5c** (R = -Me) spectrum **e** changed into a new spectrum **g** (Fig. 2). Spectrum **e** was restored on adding a base. The product with spectrum **g** was isolated as its perchlorate salt and identified as the 3a-methoxycarbonyl-tetrahydroimidazoquinoxalinium cation **7** (R = -Me; Scheme 4). Reconversion to the methoxy adduct **5c** (R = -Me) was accomplished on a preparative scale.

The PMR spectrum of **7** (R = -Me) in CF_3COOH showed the occurrence of a rapid transesterification. This was followed by decarboxylation and autoxidation leading to an acid stable product with the UV spectrum **h** (Fig. 3). The compound was isolated as its perchlorate salt and identified as the dihydroimidazoquinoxalinium cation **9**.

Neutralizing an acidic solution of **9** gives ringopening to 1 - methyl - 2 - oxo - 3 - (N,N' - dimethylureido) - 1,2 - dihydroquinoxaline (**10**, curve **i**, Fig. 3).

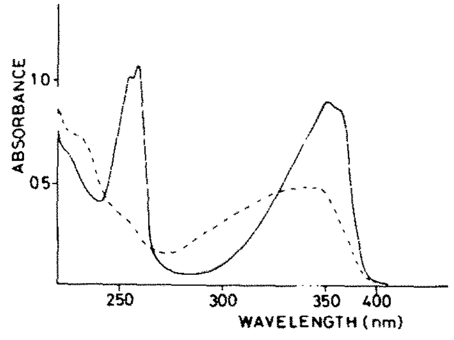
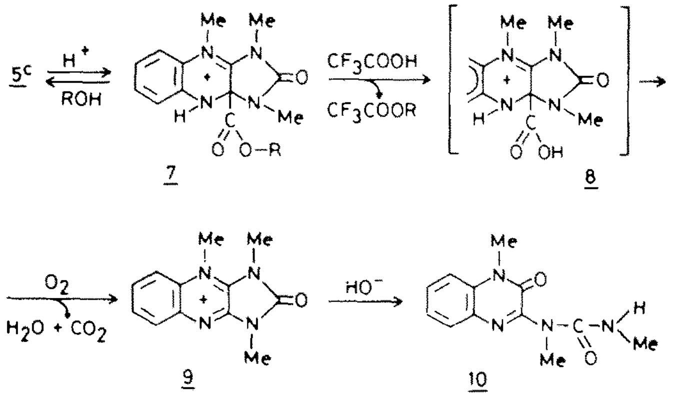


Fig. 3. UV spectra of 10^{-4} M solutions in 1N H_2SO_4 of: **9** (—— h); **10** (- - - i).

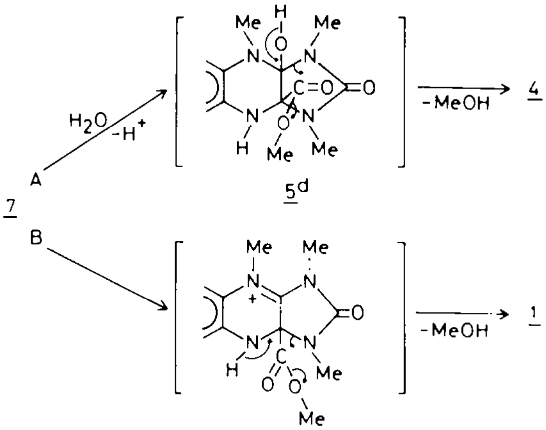


Scheme 4. Conversions of **7** in CF_3COOH.

(2) *Rearrangements of* **7** *under acidic conditions leading to the formation of spirohydantoin* **4** *or the repair of the alloxazine ring system.* The addition of water to acidified methanolic solutions of cation **7** (R = -Me) causes spectrum **g** (Fig. 2) to change rapidly into the spirohydantoin spectrum **d'** with isobestic points at 296 nm and 318 nm. The rate of the conversion is enhanced on increasing the amount of water and slowed down on increasing the acidity of the medium. The product was isolated and shown to be identical (Scheme 5) with the spirohydantoin **4** obtained by neutralizing an aqueous solution of the alloxazinium cation **1** (Scheme 1). The two types of spirohydantoin formation are distinguishable since the one mentioned in Scheme 1 does not occur in aqueous acid solution (e.g. 1N mineral acid).

In e.g. 70% aqueous $HClO_4$ (11.6 N), methanol-H_2SO_4 (1 : 1) and concentrated H_2SO_4 the imidazoquinoxalinium cation **7** rearranges differently according to the change of spectrum **g** into **a**. The alloxazinium ring system is then repaired (pathway B, Scheme 5) which was confirmed by the isolation of **1** as its perchlorate salt.

(3) N_4-*acetylated tetra- and hexa- hydroimidazo*[4,5-*b*]*quinoxaline derivatives. Heterolysis of the 9-9a-bond and formation of benzimidazole derivatives.* Acetylation of **7** (Re = -Me) readily furnished cation **11** (Scheme 6) isolated as its perchlorate salt (curve **k**, Fig. 4). On dissolving **11**, ClO_4^- in CF_3COOH transesterification, decarboxylation, autoxidation and deacetylation take place to provide the dihydroimidazoquinoxalinium cation **9** identical with the product obtained in the same way from **7** (Scheme 4). In 70% aqueous $HClO_4$ **11** is rapidly converted into the alloxazinium cation **1**, while **7** is the main product in concentrated HCl.



Scheme 5. Formation of spirohydantoin **4** and repair of the alloxazinium ring system in acidic solutions.

On adding a base to an aqueous or alcoholic solution of **11**, RO^- (R = H; Me) is added to the 9a-position to give the adducts **12** (Scheme 6). In contrast with the non-acetylated species **5d** and **5c** the acetylated adducts **12** show a ready heterolysis of the 9-9a-bond and consequently, no appreciable formation of the spirohydantoin or repair of the alloxazine ring system.

The primary adduct **12** (R = -H) was not isolated, but its ring opened isomer **13** was. On acidifying an aqueous or alcoholic solution of **13** (curve **n**, Fig. 4) ringclosure occurred to give the benzimidazolinium cation **14** which was isolated as its perchlorate salt. The rate-constant for the ringclosure in 0.08N acid was determined. Refluxing **14** in aqueous or alcoholic solution gives degradation to 1,2-dimethylbenzimidazole (**15**) isolated as the perchlorate and picrate salts and as the free base. The formation of benzimidazole derivatives is indicated by the appearance of the characteristic UV spectra **p**, **q** and **r** (Figs 4 and 5).

The adduct produced from **11** and methanol could be isolated and identified (**12**, Re = -Me; curve **m**, Fig. 4).

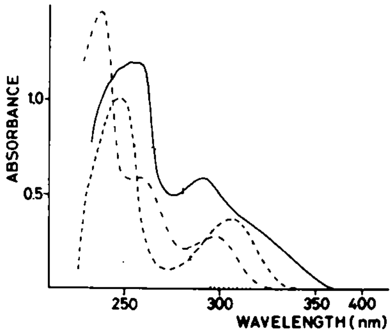
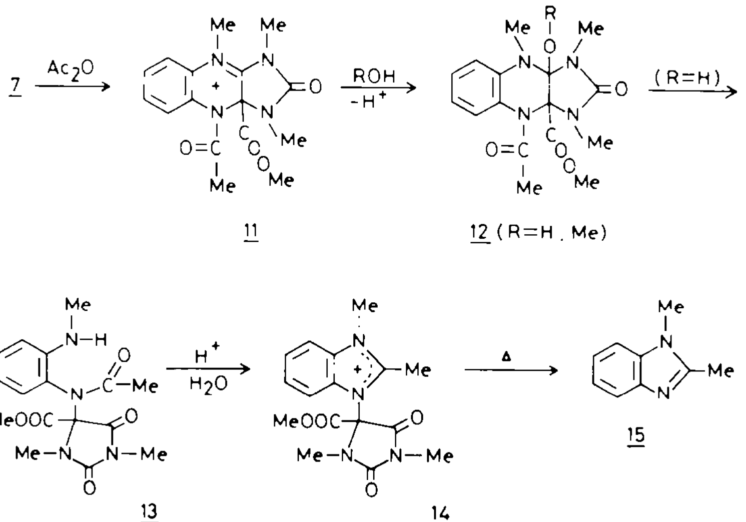


Fig. 4. UV spectra of 10^{-4} M solutions of: **11** in MeCN (— k); **12** (R = Me) in MeOH (-·- m); **13** in MeCN (--- n); **14** in H_2O (···· p).



Scheme 6. Acetylated hexahydroimidazo[4,5-b]quinoxalines. Hydrolytic ringopening and formation of benzimidazoles.

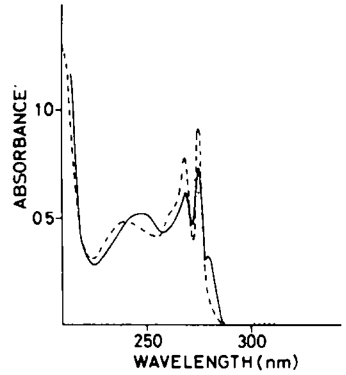


Fig. 5. UV spectra of 10^{-4} M solutions in H_2O of: **14** (··· p); **15**, $HClO_4$ (--- q); **15** (— r).

This adduct can be converted into **14**, even under conditions which seem less favourable for the formation of **13**. Under these conditions, a heterolysis of the 9–9a bond is assumed to take place in a rearrangement as depicted in Scheme 7.

The benzimidazolinium compound **14** was also obtained directly by heating **11** in aqueous or alcoholic solution or by suspending **11** in cold dilute acids.

(4) *Conversion of the 4a,10a-dialkoxy adduct* **6c** *into the alloxazinium cation* **1**. The conversion of **6c** into **1** occurs readily and quantitatively in media of different acidic strength. Trifluoroacetic acid and 1N aqueous mineral acid are suitable solvents to distinguish the 4a,10a-dialkoxy adduct from the ringcontracted 4,10a-dialkoxy adduct (cf. Schemes 4 and 5, pathway A).

CONCLUSIONS

The equilibrium between a 1,3,10-trimethylated alloxazinium (1,3-dimethylated flavinium) cation, an alcohol and the monoalkoxy adduct is shifted towards the side of the monoalkoxy adduct by adding a base. Moreover, the presence of a base gives rise to a subsequent conversion of the monoalkoxy- into a dialkoxy-adduct. This might be easily overlooked in spectrophotometric studies because of the appearance of spectra which are closely similar to the spectrum of the spirohydantoin.

The structures of two types of dialkoxy adducts have been established by X-ray analysis[14,15] (Schemes 2 and 3). More information was obtained by the elucidation of several rearrangements (Schemes 4–7).

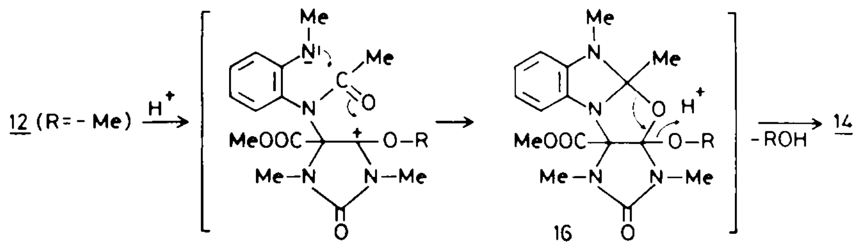
It has been ascertained that dialkoxy adducts are formed in a nucleophilic attack at position 10a followed by an *inter-* or *intramolecular* nucleophilic attack at C_4 or C_{4a}. In the reaction with a monohydric alcohol like methanol C_4 is preferred for the second *intermolecular* nucleophilic attack, but with a dihydric alcohol like ethyleneglycol C_{4a} is the second reaction site in an *intramolecular* nucleophilic ring closure. A 4,10a-dialkoxy adduct undergoes a ringcontraction, while a 4a,10a-dialkoxy adduct can be isolated as such.

The conclusion in the literature[1,5,7] that C_{9a} is the *primary* addition site, at first doubted by us because of the NMR spectra of the monoalkoxy adducts and the conversion of these adducts into the well-known (C_{4a})-spirohydantoin **4**, is now rejected in view of the structures of the dialkoxy adducts. Therefore, we continue to attribute spectra like **b** and **c** (Fig. 1) to 10a-adducts in contrast with Müller *et al.*[5] and Hemmerich *et al.*[1,7] This includes the similar spectra[5] for the primary adducts obtained from alloxazinium cations and peroxy nucleophiles. Attention should be paid to the 1,10-bridged "alloxazinium" cation prepared from 10-phenylisoalloxazine.[16,17] Scission of the 2–3 bond is reported to occur readily in methanolic solution. However, the results published do not support the suppositions on the formation of a primary 6-, 8- or 9a-adduct put forward in these papers also.

It is of interest to compare the mono- and di-alkoxy adduct formation described in this paper with the alkaline hydrolysis of (iso)alloxazines. Alkaline hydrolysis of 1,3-dimethylalloxazine leads to a scission of the 2,3-bond,[18] while the N-unsubstituted alloxazine was known to give a hydrate of which the structure was not specified.[8] Koziol and Hemmerich[19] recently established that hydration of N-unsubstituted alloxazines results in a 2,3-cleavage with formation of a carbaminate, which on acidification is stabilized to an oxazolo[4,5-b]quinoxalinecarboxamide. It is noted that under comparable conditions no appreciable 2,3-scission is shown by isoalloxazines and 1,3,10-trimethylated alloxazinium cations. Hydrolysis of these compounds gives an addition of HO^- at C_{10a} and a cleavage of the 1,10a-bond.[8,9,20] Steric blocking of the position 10a of an isoalloxazine results in a hydrolytic scission of the 3,4-bond.[9] Smith and Bruice[9] formulated the 3,4-cleavage product as an ureidoquinoxaline carboxylic acid. They strongly disfavoured the occurrence of a ringcontracted, oxo-imidazo[4,5-b]-quinoxaline carboxy transient closely similar to **8** (Scheme 4) which, in contrast, has appeared to be important species in our studies.

The conclusion from the present studies, that C_{10a}, C_{4a} and C_4 can be the reaction sites, is consistent with the conclusion from investigations on autoxidative conversions of partially reduced alloxazines and pteridines in neutral and basic media.[10,11] Consequently, the adduct formation from 3,5-dialkylated flavinium cations is now a subject of further studies, also in connection with the assumption[21] that HOO is added at C_{4a} for which no evidence has been presented yet.

The monoalkoxy adducts (R′–Fl–OR) derived from a monohydric alcohol (ROH) have been considered[1-7] as less reactive models for perhydroxydihydroflavins (R′–



Scheme 7. Heterolysis of the 9-9a-bond in **12** (R = Me) and formation of **14** without the intermediacy of **13**.

Fl–OOH). However, since cyclic peroxides and substrate-oxyflavin adducts have also been postulated as transients,[10,11,13] it occurs to us that monoalkoxy adducts (R′–Fl–O–X–OH) derived from dihydric alcohols (HO–X–OH) are complementary or more useful models.

The two types of dialkoxy adducts (Schemes 2 and 3) can be reconverted into the alloxazinium (flavinium) cation, which makes them of interest as possible models for transients in the activation and transfer of oxygen. In current investigations it is now questioned whether peroxy nucleophiles will add to one or both bridge positions (C_{10a}, C_{4a}), or to one of the bridge positions and to C_4. Dependent on influences of the substituents, of the specific nucleophile and of the experimental conditions different peroxide structures may be expected like a 4a- or 10a-hydroperoxide, a 4a,10a-dioxetane and 4,10a(4a)-endoperoxides or their rearranged forms. The attack of e.g. the 10a and 4 positions might give rise to ring-contracted carboperoxy species as exemplified in Scheme 8.

EXPERIMENTAL

Compound **1**, ClO_4^- was prepared as described previously.[20] It was recrystallized by continuous extraction with MeOH; the pure compound crystallizes from the extract. The UV spectrum of **1** in MeOH is given by curve **a** (Fig. 1), λ_{max} (ϵ): 265 nm (24,700); 365 nm (12,200). The addition of base (MeO^-, NEt_3) initially gives the spectrum of the monomethoxy adduct (curve **b**, Fig. 1) with λ_{max} (ϵ): 250 nm (sh; 16,500); 298 nm (8400); 413 nm (4300). The addition of water to neutral or basic solutions of monoalkoxy adducts leads to the formation of **4** (curve **d**), λ_{max} (ϵ): 255 nm (sh; 5600); 302 nm (4200). The formation of **4** was confirmed by comparing the compound isolated with authentic **4**. The UV spectrum of **6a** in $HOCH_2CH_2OH$ is given by curve c (Fig. 1), λ_{max} (ϵ): 252 nm (sh; 15,800); 302 nm (8300); 418 nm (4300).

All solvents used in this investigation were reagent grade, but treated before use. Acetonitrile was distilled over P_2O_5, MeOH was distilled over magnesium activated with iodine and ethyleneglycol was fractionated *in vacuo*. The usefulness of the fractions was determined by means of the UV spectra of samples in which some **1**, ClO_4^- had been dissolved. Before redistilling, reagent grade alcohols may give either the cation or the monoalkoxy adduct spectrum or a spectrum indicating the presence of both species. Alcohols dried over Union Carbide molecular sieves generally give the spectra of the monoalkoxy adducts without addition of base.

PMR spectra were recorded on a Varian A-60 with TMS as an internal standard. UV and IR spectra were recorded on a Perkin Elmer 402 and a Hilger Watts Infrascan, respectively. Mass spectral data were obtained with a Varian Mat SM 1 or a Varian Mat 311 A. M.ps were determined in evacuated capillary tubes in a Büchi apparatus. Analytical TLC was done on "Bakerflex" silica gel IB 2-F.

Preparation of 3a - methoxy - 9a - methoxycarbonyl - 1,3,4 - trimethyl - 2 - oxo - 2,3,3a,4,9,9a - hexahydro - 1H - imidazo[4,5-b]quinoxaline (**5c**, R = Me)

Sodium (2 g) was dissolved in abs MeOH (100 ml). The soln was cooled to room temp. and **1**, ClO_4^- (10.00 g, 28.03 mmol) was added. The mixture was stirred to give a clear soln from which colourless crystals appeared. After 1 hr the product (6.63 g) was collected. The mother liquour was concentrated by evaporating *in vacuo* to give a second crop increasing the overall yield to 7.92 g (88%) m.p. 185–186°. This product was used for preparative purposes. For analytical purposes the compound was recrystallized from MeOH with 90% recovery, increasing the m.p. to 200.5–201.5° (decomp.) ($C_{15}H_{20}N_4O_4$ (320.36) Calc.: C, 56.24; H, 6.29; N, 17.49. Found: C, 56.3; H, 6.4; N, 17.7%). Mass spectrum, *m/e* (%): 320 (M^+, 100); 305(26); 289(4); 272(21); 261(30); 246(6); 232(3); 229(53); 220(2); 215(8); 204(16); 200(39); 191(4); 188(5); 185(24); 172(27); 161(25); 148(5); 131(12); 119(12); 100(25). PMR ($CDCl_3$): $\delta = 2.41$ (3, s, N–Me); 2.84 (3, s, N–Me); 2.93 (3, s, N–Me); 3.43 (3, s, O–Me); 3.84 (3, s, O–Me); 5.00 (1, s, N–H, exchangeable); 6.55–6.92 (4, m, Ar–H). IR (KBr), cm^{-1}: 3280 (N–H); 1745, 1700 (C=O). UV (MeOH), λ_{max} (ϵ): 245 nm (sh; 5000); 297 nm (4100). Acidification gives the UV spectrum of cation **7**. TLC ($CHCl_3$–AcOEt, 1:1), 10 cm: $R_f = 0.50$.

Preparation of 4a,10a-ethylenedioxy-1,3,10-trimethyl-4a,5,10,10a-tetrahydroalloxazine (**6c**)

Sodium (200 mg) was dissolved in anhyd ethyleneglycol (20 ml) and the soln was cooled off to room temp. **1**, ClO_4^- (1.07 g, 3.00 mmol) was added and the mixture was stirred for 30–60 min. A clear soln was obtained from which the colourless product crystallized. Aqueous 1 M NaH_2PO_4 soln (15 ml) and water (50 ml) were added. The product was filtered off and washed with water; yield 897 mg (94%). Hot MeOH (13 ml) was added and the mixture was rapidly refluxed to dissolve the solid, taking care that heating was not continued for more than 1 min (to prevent the occurrence of a conversion to be studied in more detail). Cooling afforded pure, colourless crystals, yield 770 mg (81%) m.p. 161–162°. ($C_{15}H_{18}N_4O_4$ (318.34) Calc.: C, 56.59; H, 5.70; N, 17.60. Found: C, 56.4; H, 5.7; N, 17.8%). Mass spectrum, *m/e* (%): 318 (M^+, 100); 273(5); 258(8); 246(28); 243(23); 233(8); 216(8); 203(11); 198(41); 188(4); 176(7); 170(9); 161(60); 159(18); 148(95); 142(17); 132(26); 129(10); 119(47); 114(9); 102(60). PMR ($CDCl_3$): $\delta = 2.93$ (3, s, N–Me); 3.03 (3, s, N–Me); 3.25 (3, s, N–Me); 3.72–3.82 (4, m, $2 \times CH_2$); 4.93 (1, s, N–H); 6.58–6.83 (4, m, Ar–H). IR (KBr), cm^{-1}: 3370 (N–H); 1720. 1668 (C=O). UV (MeOH), λ_{max} (ϵ): 243 nm (sh; 7100); 297 nm (5300). Acidification gives the UV spectrum of **1**. TLC (AcOEt–$CHCl_3$, 1:4), 10 cm: $R_f = 0.70$.

Conversion of **6c** *into* **1**, *in contrast with the conversion of* **5c** *into* **7**

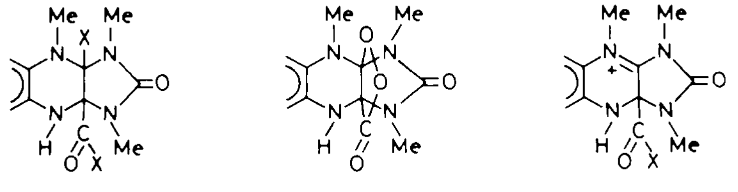
Compound **6c** (159.2 mg, 0.50 mmol) was dissolved in boiling MeOH (5 ml). The soln was cooled to room temp. after which a soln of 70% $HClO_4$ (0.050 ml) in MeOH (2 ml) was added with stirring to give a ppt of **1**, ClO_4^-, yield 169 mg (95%).

Conversion of **6c** *into* **1**, *in contrast with the conversion of* **5c** *into* **9**

Compound **6c** (318.3 mg, 1.00 mmol) was dissolved in CF_3COOH (5 ml) after which the soln was evaporated to dryness *in vacuo*. To the residue was added 2N alcoholic $HClO_4$ to give **1**, ClO_4^-, yield 338 mg (95%).

Preparation of 3a - methoxycarbonyl - 1,3,9 - trimethyl - 2 - oxo - 2,3,3a,4 - tetrahydro - 1H - imidazo[4,5-b]quinoxalinium perchlorate (**7**, ClO_4^-; R = –Me)

Crude **5c** (R = –Me; m.p. 185–186°; 4.00 g, 12.49 mmol) was dissolved in boiling MeOH (200 ml). After cooling off to room temp. 70% $HClO_4$ (16 ml) was added drop by drop to give



17 (X= -OOH; -OOR)   18   19 (X= -OOH; -OOR)

Scheme 8. Proposal on ringcontracted carboperoxy species.

crystalline **7**, ClO_4^- (R = Me) which was filtered off and washed with MeOH, yield 4.65 g. (96%) m.p. 222–223°. Recrystallization from anhyd MeCN did not raise the m.p. ($C_{14}H_{17}N_4O_3 \cdot ClO_4$ (388.78) Calc.: C, 43.25; H, 4.41; N, 14.41. Found: C, 43.3; H, 4.3; N, 14.3%). Mass spectrum, *m/e* (%): 289 (M^+, 3); 288 (17); 230 (52); 229 (100); 214 (60); 199 (7); 185 (16); 171 (5); 148 (3), 144 (4); 129 (17). PMR (CF_3COOD): $\delta = 3.19$ (3, s, N–Me); 3.79 (3, s, N–Me); 3.93 (3, s, N–Me); 4.20 (3, s, OMe); 7.27–7.55 (4, m, Ar–H); 11.28 (1, s, exchanged H). IR (KBr), cm^{-1}: 3280 (N–H); 1800, 1760, 1680 (C=O, $C=\underset{+}{N}-$). UV (0.6 N methanolic H_2SO_4), λ_{max} (ϵ): 255 nm (sh; 9100); 286 nm (4900); 347 nm (3000).

Reconversion of 7 (R = Me) *into* 5c (R = Me)

A soln of **7**, ClO_4^- (R = Me) in absolute MeOH gives an UV spectrum identical with the spectrum of **5c** (R = Me) indicating that the cation **7** does not exist in dilute alcoholic soln. A conversion on a preparative scale was performed as follows. Na (18 mg) was dissolved in MeOH (10 ml) after which **7**, ClO_4 (R = Me; 194.3 mg, 0.50 mmol) was added. The clear soln was evaporated to a small volume *in vacuo* to give a product identical with **5c** (R = Me), yield 120 mg (75%).

Conversion of 7 (R = Me) *into* 4

(a) The UV spectrum **g** (Fig. 2) in methanolic H_2SO_4 changed into spectrum **d'** of **4** in dependence on the amount of water added to the soln. From the decrease of the extinction at 347 nm in aqueous 1N H_2SO_4 the pseudo first-rate order-constant was calculated to be 1.27×10^{-3} sec^{-1} (23°).

(b) A mixture of **7**, ClO_4 (R = Me; 0.50 mmol) and water (5 ml) was heated to the boil. The soln was cooled to room temp. yielding **4** (95%), m.p. 192–193°.

(c) Similar expts carried out in 1N and 12N H_2SO_4 gave **4** in yields of 70% and 40%, respectively.

(d) **5c** (R = Me; 0.50 mmol) was dissolved in conc HCl (5 ml). The soln was diluted by adding water (55 ml), kept overnight at room temp. and then extracted with $CHCl_3$ (5 × 25 ml). The extract was dried ($MgSO_4$) and evaporated to dryness *in vacuo*. The residue was recrystallized from water (3 ml) to give **4**, yield 84%, m.p. 192–193°.

Conversion of 7 (R = Me) *into* 1

(a) A soln of **5c** (R = Me; 320.4 mg, 1.00 mmol) in 70% $HClO_4$ (10 ml) was kept at room temp. for 5 days. Spectra of diluted samples showed the formation of **1** in a yield of 65%. Water (25 ml) and solid $NaHCO_3$ (9.18 g, 109 mmol) were added to precipitate **1**, ClO_4^-, yield 165 mg (46%).

(b) A soln of **5c** (R = Me; 320.4 mg, 1.00 mmol) in a mixture of MeOH–conc H_2SO_4 (1:1; 6.4 ml) was heated in an oil-bath (70–90°) for 2 hr. Spectra of diluted samples showed the formation of **1** in a yield of 42%. To the reaction mixture were added water, solid $NaHCO_3$ (5.88 g, 70 mmol) and $NaClO_4$ (15 g) to a final volume of 50 ml giving a ppt of **1**, ClO_4^-, yield 82 mg (23%).

Preparation of 1,3,9 - *trimethyl* - 2 - *oxo* - 2,3 - *dihydro* - 1H - *imidazo*[4,5-*b*]*quinoxalinium perchlorate* (9, ClO_4^-)

A soln of **5c** (R = Me; 0.50 g, 1.56 mmol) in CF_3COOH (25 ml) was refluxed for 0.5 hr. After standing overnight at room temp the soln was evaporated to dryness *in vacuo*. The residue was dissolved in MeOH (5 ml) and 70% $HClO_4$ (0.2–0.3 ml) was added drop by drop to give **9**, ClO_4^-, yield 410 mg (80%) m.p. 301–302°. Recrystallization from MeCN did not raise the m.p. ($C_{12}H_{13}N_4O \cdot ClO_4$ (328.73) Calc.: C, 43.85; H, 3.99; N, 17.05. Found: C, 44.0; H, 4.1; N, 17.2%). Mass spectrum, *m/e* (%): 229 (M^+, 10); 214(50); 207(32); 199(3); 189(3); 185(13); 171(3); 155(5); 129(10). PMR (CF_3COOD): $\delta = 3.77$ (3, s, N–Me); 4.14 (3, s, N–Me); 4.74 (3, s, N-Me); 7.92–8.20 (4, m, Ar–H). IR(KBr), cm^{-1} 1785, 1777 (C=O, $C=\underset{+}{N}-$). UV (1N H_2SO_4), λ_{max} (ϵ): 225 nm (sh; 12,700); 255 nm (20,600); 260 nm (21,600); 352 nm (18,300); 360 nm (sh; 17,800).

Note. The PMR spectrum of **9** resembles the PMR spectrum of **1** in CF_3COOD: $\delta = 3.67$ (3, s, N–Me); 4.01 (3, s, N–Me); 4.62 (3, s, N–Me); 8.12–8.50 (4, m, Ar–H).

Preparation of 1 - *methyl* - 2 - *oxo* - 3 - (N,N' - *dimethylureido*) - 1,2 - *dihydroquinoxaline* (10)

To a mixture of **9**, ClO_4^- (164.4 mg, 0.50 mmol) and water (10 ml) 25% ammonia soln was added to give a clear orange soln. The colour turned into yellow accompanied by crystallization of **10**. The mixture was then extracted with $CHCl_3$ (5 × 5 ml). The extract was dried ($MgSO_4$) and evaporated to dryness *in vacuo*. The residue (124 mg) was recrystallized from AcOEt or water, yield 90–100 mg (73–81%) m.p. 155°. ($C_{12}H_{14}N_4O_2$ (246.28) Calc.: C, 58.52; H, 5.73; N, 22.75 Found: C, 58.4; H, 5.7; N, 23.0%). Mass spectrum, *m/e* (%): 246 (M^-, 40); 216(4); 189(100); 174(58); 161(50); 160(47); 148(49); 147(47); 132(47); 131(48); 119(50); 104(25); 102(12); 92(34); 90(29); 77(38). PMR ($CDCl_3$): $\delta = 2.88$ (3, d, J = 4.5 Hz, NHMe); 3.42 (3, s, N–Me); 3.67 (3, s, N–Me); 7.08–7.75 (5, m, 4 Ar–H overlapping 1 NH). Addition of D_2O showed one exchanged H (NH) at $\delta = 4.67$ and transformed the N–Me doublet at $\delta = 2.88$ into a singlet. IR (KBr), cm^{-1}: 3230 (N–H); 1675, 1645 (C=O); 1605 (C=N). UV (1N H_2SO_4), λ_{max} (ϵ): 229 nm (sh; 15,100); 252 nm (sh; 6700); 345 nm (9800). TLC (AcOEt–$CHCl_3$, 4:1), 10 cm: $R_f = 0.25$.

Preparation of 4 - *acetyl* - 3*a* - *methoxycarbonyl* - 1,3,9 - *trimethyl* - 2 - *oxo* - 2,3,3*a*,4 - *tetrahydro* - 1H - *imidazo*[4,5-*b*]*quinoxalinium perchlorate* (11, ClO_4^-)

Compound **7**, ClO_4 (R = Me; 388.8 mg, 1.00 mmol) was dissolved in boiling Ac_2O (4 ml). The soln was cooled to room temp. The colourless, crystalline product was filtered off and washed with Ac_2O (1 ml) and diethyl ether, yield 363 mg (84%) m.p. 189–189.5°. Addition of diethyl ether to the filtrate gave a second crop, yield 64 mg (15%, overall yield 99%) m.p. 188–188.5°, increased to m.p. 189–189.5° by recrystallization from MeCN. ($C_{16}H_{19}N_4O_4 \cdot ClO_4$ (430.82) Calc.: C, 44.61; H, 4.45; N, 13.01. Found: C, 44.4; H, 4.5; N, 12.8%). Mass spectrum, *m/e* (%): 330 (M^+–1, 15); 313(7); 288(35); 271(100); 263(18); 256(8); 243(17); 241(44); 229(100); 214(82); 199(34); 185(40); 171(27); 156(29); 144(25); 129(50); 114(45); PMR (CD_3CN): $\delta = 2.16$ (3, s, O=C–Me); 3.11 (3, s, N(3)–Me); 3.58 (3, 3, N(1)–Me); 3.77 (3, s, N(9)–Me); 4.08 (3, s, O–Me); 7.50–7.70 (4, m, Ar–H). IR (KBr), cm^{-1}: 1800, 1770, 1703, 1682 (C=O, $C=N^+-$). UV (MeCN), λ_{max}(ϵ): 256 nm (14,800); 292 nm (5600).

Conversion of 11 *into* 1 (*repair of the alloxazine ring system*).

A soln of **11**, ClO_4^- (430.8 mg, 1.00 mmol) in 70% $HClO_4$ (10 ml) was kept at room temp. for 2 days. Water (25 ml) and solid $NaHCO_3$ (9.0 g) were added to precipitate **1**. ClO_4–, yield 285 mg (80%).

Conversion of 11 *into* 7 (Re = Me)

Compound **11**, ClO_4^- (430.8 mg, 1.00 mmol) was dissolved in conc HCl (10 ml). Crystallization started within 10 min. After 5 hr **7**, ClO_4^- (R = Me) was filtered off and washed with MeOH, yield 245 mg (63%).

Note. **11** was converted into **14** in 1N H_2SO_4, water or MeOH (this paper).

Conversion of 11 *into* 9

A soln of **11**, ClO_4 (430.8 mg, 1.00 mmol) in CF_3COOH (25 ml) was kept at room temp for 5 days and evaporated to dryness *in vacuo*. The residue was dissolved in MeCN (3 ml). The soln was cooled to give crystalline **9**, ClO_4, yield 255 mg (78%).

Preparation of 4 - *acetyl* - 9*a* - *methoxy* - 3*a* - *methoxycarbonyl* - 1,3,9 - *trimethyl* - 2 - *oxo* - 2,3,3*a*,4,9,9*a* - *hexahydro* - 1H - *imidazo*[4,5-*b*]*quinoxaline* (12, R = –Me)

Sodium (120 mg) was dissolved in abs MeOH (8 ml) After cooling to room temp. **11**, ClO_4 (1.00 g, 2.32 mmol) was added with stirring to give a clear soln from which colourless crystals appeared. Stirring was continued for 10 min after which the mixture was cooled at −20°. The product was filtered off and washed with MeOH (5 ml), yield 610 mg, m.p. 201.5–202°. The mother liquor was concentrated by evaporating *in vacuo*. Addition of 0.4 M NaH_2PO_4 (15 ml) gave a second crop, which was filtered off and washed with water. Recrystallization from MeOH gave an overall yield of 745 mg (89%) m.p. 202–202.5°. ($C_{17}H_{22}N_4O_5$ (362.40) Calc.: C, 56.34; H, 6.12; N, 15.46. Found: C, 56.6; H, 5.9; N, 15.4%). Mass spectrum, *m*/*e* (%): 362 (M^+, 85); 347(17); 331(3), 320(2); 303(11); 287(3); 273(3); 246(12); 229(37); 217(31); 214(6); 204(15); 200(34); 185(31); 172(13); 163(25); 161(18); 157(63); 146(100); 131(8); 119(10). PMR (CD_3CN): $\delta = 2.13$ (3, s, O = C–Me); 2.46 (3, s, N(9)–Me); 2.93 (3, s, N–Me); 3.13 (3, s, N–Me); 3.27 (3, s, O–Me); 3.77 (3, s, COOMe); 7.05–7.40 (4, m, Ar–H). IR (KBr), cm^{-1}: 1755, 1710, 1625 (C=O). UV (MeOH), $\lambda_{max}(\epsilon)$: 257 nm (5900); 296 nm (2800). TLC (MeCN–$CHCl_3$, 1:1), 10 cm: $R_f = 0.78$.

Preparation of 4 - (*N* - *acetyl* - 2′ - *methylaminoanilino*) - 4 - *methoxycarbonyl* - 1,3 - *dimethyl* - 2,5 - *dioxo* - 2,3,4,5 - *tetrahydro* - 1H - *imidazole* (**13**)

Compound **11**, ClO_4^- (1.00 g, 2.32 mmol) was dissolved in MeCN (25 ml). The soln was diluted with water (50 ml) and the pH was adjusted to 7.0 by the gradual addition of 0.27 N NH_4OH giving a white ppt. MeCN was removed from the mixture by evaporating *in vacuo*. The product was filtered off, washed with water and recrystallized from cyclohexane, yield 571 mg (71%) m.p. 149.5–150°. ($C_{16}H_{20}N_4O_5$ (348.37) Calc.: C, 55.17; H, 5.79; N, 16.08. Found: C, 55.2; H, 5.9; N, 16.3%). Mass spectrum, *m*/*e* (%): 348 (M^+, 13); 190(2), 185(4); 163(100); 147(6); 131(3); 121(93); 119(8); 100(5); 94(20). PMR ($CDCl_3$): $\delta = 1.89$ (3, s, C–Me); 2.72 (3, s, N–Me); 2.93 (3, d, J = 3.5 Hz, NHMe); 3.22 (3, s, N–Me); 3.93 (3, s, O–Me); 5.10 (1, broad, N–H); 6.53–7.30 (4, m, Ar–H). Addition of D_2O transformed the N–Me doublet at $\delta = 2.93$ into a singlet. IR (KBr), cm^{-1}: 3400, 3380 (N–H); 1787, 1762, 1746, 1730, 1725^{sh} (C=O). UV (MeCN), $\lambda_{max}(\epsilon)$: 247 nm (10,400); 306 nm (3600). TLC (diethylether), 10 cm: $R_f = 0.85$.

Conversions into 1 - (4′ - *methoxycarbonyl* - 1′,3′ - *dimethyl* - 2′,5′ - *dioxo* - 2′,3′,4′,5′ - *tetrahydro* - 1H - *imidazol* - 4′ - *yl*) - 2,3 - *dimethylbenzimidazolinium perchlorate* (**14**, ClO_4^-).

(a_1) *Kinetics on the conversion of* **13** *into* **14**. The ringclosure of **13** to **14** was followed spectrophotometrically (cf curves **n** and **p**, Fig. 4) in 1:1 EtOH–H_2O solns of **13** with starting concentrations varying from 10^{-4} to 2.55×10^{-4} M. On adding 70% $HClO_4$ to a final acid strength of 0.08 N (t = 0) spectra changes were recorded giving isobestic points at 284 nm, 253 nm, 229 nm and 216 nm. From the decrease of the extinction at 306 nm the pseudo first-order rate-constant was calculated to be 1.20×10^{-3} sec^{-1} (23°).

(a_2) *Preparation of* **14**, ClO_4^- *from* **13** *in water.* Water (10 ml) and 70% $HClO_4$ (0.050 ml) were added to a soln of **13** (174.2 mg, 0.50 mmol) in MeCN (5 ml). The mixture was kept at room temp. for 4 hr and evaporated to dryness *in vacuo*. MeOH (2 ml) was added to the residue and the mixture was stirred for 15 min. The product was filtered off and washed with MeOH (2 ml), yield 190 mg (88%) m.p. 182–183°. For analytical purposes **14**, ClO_4^- was recrystallized from acetone or MeOH. ($C_{16}H_{19}N_4O_4 \cdot ClO_4$ (430.82) Calc.: C, 44.61; H, 4.45; N, 13.01. Found: C, 44.6; H, 4.5; N, 13.2%). Mass spectrum, *m*/*e* (%); 330 (M^-–1, 4); 271(23); 186(20); 146(100); 131(48); 118(4); 104(15); 100(5); 92(6); 90(5); 83(9); 77(25). PMR (CD_3CN): $\delta = 2.86$ (3, s, C–Me); 3.02 (3, s, N–Me); 3.13 (3, s, N–Me); 4.01 (3, s, N–Me); 4.03 (3, s, COOMe); 7.50–7.87 (4, m, Ar–H). IR (KBr), cm^{-1}: 1803, 1752, 1730 (C=O). UV (H_2O), λ_{max} (ϵ): 256 nm (7200); 263 nm (7200); 270.5 nm (7700); 278 nm (6300). TLC (MeCN–$CHCl_3$, 1:1), 10 cm: $R_f = 0.75$.

Note. **14** can be well characterized by adding an excess of base (MeO^-) to a methanolic soln. Apparently, a methoxy-adduct arises having a very strong absorption maximum at 333–334 nm. This adduct, which was not identified, is destroyed on acidifying the soln. UV (MeOH + 10^{-3} M NaOMe), $\lambda_{max}(\epsilon)$: 271 nm (5800); 276 nm (5700); 333.5 nm (42,400).

(a_3) *Preparation of* **14**, ClO_4^- *from* **13** *in methanol.* 70% $HClO_4$ (0.100 ml) was added to a soln of **13** (348.4 mg, 1.00 mmol) in MeOH (30 ml). The reaction was followed by subjecting samples from the mixture to spectrophotometric determinations and to TLC. The product started to crystallize after 45 min. After 5 hr the mixture was concentrated to a volume of 5 ml by evaporating *in vacuo*. **14**, ClO_4^- was filtered off and washed with MeOH, yield 366 mg (85%) m.p. 181–182°.

(b_1) *Preparation of* **14**, ClO_4^- *from* **12** (R = Me) *in methanol.* **12** (R = Me; 500 mg, 1.38 mmol) was dissolved in boiling MeOH (13 ml). The soln was cooled to room temp. 70% $HClO_4$ (0.125 ml, 1.45 mmol) was added and the mixture was kept at room temp. for 3 days. **14**, ClO_4^- was filtered off and washed with MeOH, yield 460 mg (77%) m.p. 181–182°.

(b_2) *Preparation of* **14**, ClO_4^- *from* **12** (R = Me) *in acidic solutions, in contrast with conversions of* **11** *under the same conditions.* UV spectra showed the occurrence of a very rapid conversion of **12** (R = Me) into **14** in various acidic solutions like 70% $HClO_4$; 70% $HClO_4$–Ac_2O (1:50); conc. H_2SO_4–Ac_2O (1:40); CF_3COOH; CF_3COOH–Ac_2O (9:1). In contrast, **14** did not arise from **11** dissolved in the same media. Example: **12** (R = Me; 362.4 mg, 1.00 mmol) was dissolved in CF_3COOH (5 ml) after which the soln was evaporated to dryness *in vacuo*. The residue was dissolved in MeOH (5 ml). Addition of 70% $HClO_4$ (0.100 ml, 1.16 mmol) gave a ppt of **14**, ClO_4^-, yield 400 mg (93%) m.p. 181–182°. Similar results were obtained when **12** (R = Me) was dissolved in mixtures of conc. acids and Ac_2O.

(c_1) *Preparation of* **14**, ClO_4 *from* **11** *in water.* Boiling water (25 ml) was added to **11**, ClO_4^- (1.00 g, 2.32 mmol). The mixture was rapidly heated until a clear soln was obtained within 1 min. The soln was then evaporated to dryness *in vacuo*. MeOH (5 ml) was added to the residue and crystalline **14**, ClO_4^- was filtered off and washed with MeOH (3 ml), yield 891 mg (89%) m.p. 181–182°.

(c_2) *Preparation of* **14**, ClO_4 *from* **11** *in methanol.* A soln of **11**, ClO_4^- (1.00 g, 2.32 mmol) in MeOH (50 ml) was refluxed for 1.5 hr. Cooling gave crystallization of **14**, ClO_4 , yield 568 mg (57%) m.p. 179–180°.

Note. On concentrating the mother liquor crystalline **15**, $HClO_4$ was obtained. On extending the refluxing time to 22 hr **15**, $HClO_4$ became the main product.

(c_3) *Preparation of* **14**, ClO_4^- *from* **11** *in* 1N H_2SO_4. A suspension of **11**, ClO_4^- (430.8 mg, 1.00 mmol) in 1N H_2SO_4 (10 ml) was stirred at room temp. for 4 hr. After standing overnight, **14**, ClO_4 was filtered off and washed with MeOH, yield 268 mg (62%) m.p. 182–182.5°.

Preparation of 1,2-*dimethylbenzimidazolinium perchlorate* (**15**, $HClO_4$)

A soln of **11**, ClO_4^- (1.00 g, 2.32 mmol) in water (15 ml) was refluxed for 2.5 hr and evaporated to dryness *in vacuo*. Addition of acetone (4 ml) and AcOEt (20 ml) gave a ppt., yield 450 mg, m.p. 183.5–184.5°. It was recrystallized by continuous extraction with acetone–AcOEt (1:4; 20 ml); **15**, $HClO_4$ crystallized from the extract, yield 354 mg (62%) m.p. 185°. ($C_9H_{10}N_2 \cdot HClO_4$ (246.66) Calc.: C, 43.83; H, 4.50; N, 11.36. Found: C, 43.9; H, 4.5; N, 11.1%). Mass spectrum, *m*/*e* (%): 146 (M^+, 100); 131(45); 118(3); 104(14); 92(6); 90(3); 83(4); 77(19). PMR (CD_3CN): $\delta = 2.81$ (3, s, C–Me); 3.90 (3, s, N–Me); 7.50–7.88 (4, m, Ar–H). UV (H_2O), $\lambda_{max}(\epsilon)$: 240 nm (4900); 263 nm (sh; 5400); 269 nm (7700); 275 nm (9100). TLC (MeCN–$CHCl_3$, 1:1), 10 cm: $R_f = 0.60$.

Note. **15**, $HClO_4$ can be distinguished from **14**, ClO_4^- by its UV spectrum in MeOH/MeONa, showing no absorption at 333–334 nm and, by its IR spectrum showing no absorptions in the C=O region.

Preparation of 1,2-*dimethylbenzimidazole* (**15**)

To a soln of **15**, $HClO_4$ (246.7 mg, 1.00 mmol) in water (25 ml) 0.1 N NH_4OH was added to adjust the pH to 7.0. The soln was extracted with $CHCl_3$ (5 × 25 ml). The extract was dried ($MgSO_4$) and evaporated to dryness *in vacuo* to give colourless crystalline **15**, yield 135 mg (92%) m.p. 110°. The product was pure according to TLC (MeCN–$CHCl_3$, 1:1), 10 cm: $R_f = 0.20$. It could be recrystallized from diethyl ether (−20°), m.p. 110°. ($C_9H_{10}N_2$ (146.20) Calc.: C, 73.94; H, 6.90; N, 19.16. Found: C, 74.0; H, 6.9; N, 19.3%). PMR (CD_3CN): $\delta = 2.48$ (3, s, C–Me); 3.65 (3, s, N–Me); 7.07–7.63 (4, m, Ar–H). UV (H_2O), $\lambda_{max}(\epsilon)$: 245–250 nm (5200); 269 nm (6400); 275 nm (7400); 280 nm (3500). UV (MeOH), $\lambda_{max}(\epsilon)$: 248 nm (sh; 6400); 251.5 nm (6600); 268 nm (sh; 4400); 275 nm (5900); 281.5 nm (6800).

Preparation of 1,2-*dimethylbenzimidazolinium picrate.*

Compound **15** (45 mg, 0.31 mmol) was dissolved in a saturated soln of picric acid in 96% EtOH (3 ml). The crystals separated on cooling were filtered off and recrystallized from 96% EtOH, yield 90 mg (77%) m.p. 242–243°; m.p. lit.[22] 243°. ($C_9H_{10}N_2$. $C_6H_3N_3O_7$ (375.31) Calc.: C, 48.00; H, 3.49; N, 18.66. Found: C, 48.2; H, 3.7; N, 18.4%).

Acknowledgements—I am very much indebted to Mr. T. de Beer for his experimental assistance, to Mr. H. M. A. Buurmans for carrying out the elemental analyses and to the Department of Organic Chemistry for taking the mass spectra.

REFERENCES

[1] V. Massey and P. Hemmerich, *Flavin and pteridine monooxygenases*. In *The Enzymes* (Edited by P. D. Boyer), Vol. XII, 3rd Edn, pp. 191–252. Academic Press, New York (1975).

[2] F. Müller, *On the reaction of flavins with alcohols*. In *Flavins and flavoproteins*. Proceedings of the Third International Symposium (Edited by H. Kamin), pp. 363–373. University Park Press, Baltimore. Butterworths, London (1971).

[3] F. Müller, T. Jarbandhan, R. Gast and H. J. Grande, *The structure of flavin-oxygen adducts*. In *Reactivity of flavins* (Edited by K. Yagi), pp. 51–70. University Park Press, Baltimore (1975).

[4] Cf. Ref. 1, p. 245.

[5] F. Müller, H. J. Grande and T. Jarbandhan, *On the interaction of flavins with oxygen ions and molecular oxygen*. In *Flavins and flavoproteins*. Proceedings of the Fifth International Symposium (Edited by T. P. Singer), pp. 38–50. Elsevier, Amsterdam, (1976).

[6] P. Hemmerich and F. Müller, *Ann. N.Y. Acad. Sci.* **212**, 13 (1973).

[7] P. Hemmerich and A. Wessiak, *The structural chemistry of flavin dependent O_2-activation*. In *Flavins and flavoproteins*. Proceedings of the Fifth International Symposium (Edited by T. P. Singer), pp. 9–20. Elsevier, Amsterdam (1976).

[8] H. I. X. Mager, R. Addink and W. Berends, *Rec. Trav. Chim.* **86**, 833 (1967).

[9] S. B. Smith and Th. C. Bruice, *J. Am. Chem. Soc.* **97**, 2875 (1975).

[10] H. I. X. Mager, *Autoxidative conversions of tetrahydropteridines and some related ringsystems*. In *Chemistry and Biology of pteridines* (Edited by W. Pfleiderer), pp. 753–773, Walter de Gruyter, Berlin (1976).

[11] H. I. X. Mager and W. Berends, *Tetrahedron* **32**, 2303 (1976).

[12] H. I. X. Mager and W. Berends, *Tetrahedron* **30**, 917 (1974).

[13] H. I. X. Mager, *Nonenzymic activation and transfer of oxygen by reduced alloxazines*. In *Flavins and flavoproteins*. Proceedings of the Fifth International Symposium (Edited by T. P. Singer), pp. 23–37. Elsevier, Amsterdam (1976).

[14] H. van Koningsveld, to be published.

[15] H. van Koningsveld, to be published.

[16] W. R. Knappe, *Chem. Ber.* **107**, 1614 (1974).

[17] W. R. Knappe, *Electron input at the N-1 position of the flavin system*. In *Flavins and flavoproteins*. Proceedings of the Fifth International Symposium (Edited by T. P. Singer), pp. 788–792. Elsevier, Amsterdam (1976).

[18] H. Bredereck and W. Pfleiderer, *Chem. Ber.* **86**, 1119 (1953).

[19] J. Koziol and P. Hemmerichs, *Liebigs Ann. Chem.* 1276 (1976).

[20] H. I. X. Mager and W. Berends, *Rec. Trav. Chim.* **91**, 611 (1972).

[21] C. Kemal and Th. C. Bruice, *Proc. Nat. Acad. Sci. U.S.A.* **73**, 955 (1976).

[22] F. E. King and R. M. Acheson, *J. Chem. Soc.* 1396 (1949).