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ELECTRON TRANSFER—II

ACCUMULATION OF 5-ETHYL-3-METHYLLUMIFLAVIN RADICAL BY SPONTANEOUS CONVERSIONS OF 5-ETHYL-3-METHYLLUMIFLAVINIUM SALTS

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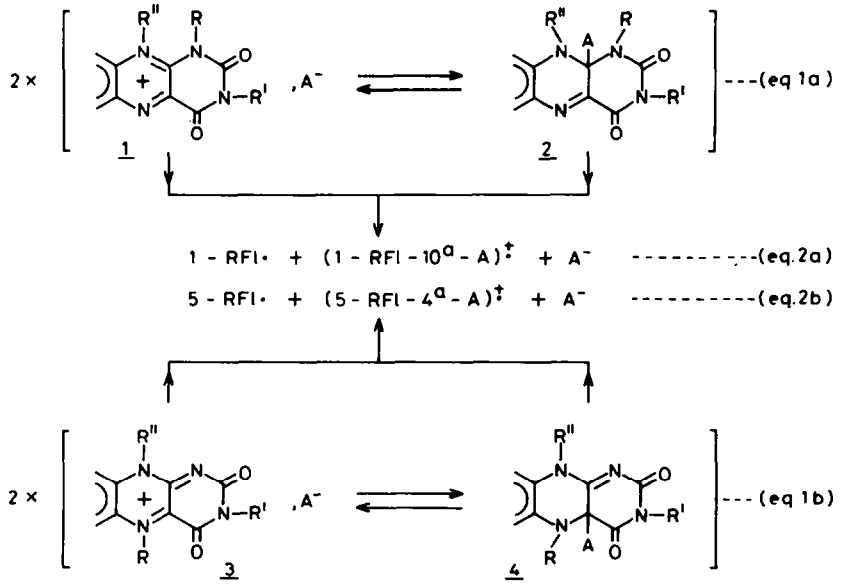
Abstract—5-Ethyl-3-methyllumiflavinium salts **3** (Scheme 1; 5-EtFl$_{ox}^{+}$, A^{-} in Scheme 2) may arise *in situ* on adding an acid (HA) to solutions of the 4^{a}-flavin adducts **5** in low polar solvents. The acidified solutions were kept under N_2 at 25° in the dark to give spontaneous accumulations of the 5-ethyl-3-methyllumiflavin radical **6** (5-EtFl· and/or 5-EtFlH‡) and of some 3-methyllumiflavin **10** (Scheme 3) in dependence on the nature of the solvent and, on the nature and the concentration of the acid.

The use of TFA; TCA; AcOH; α-ketoglutaric and salicyclic acid (Table 1) gave **6** and **10** in yields of 60–90% and 6–21%, respectively. The anaerobic production of **10** limits the formation of **6** to a theoretical yield of 66.7%. On suppressing the limiting pathway (eqn 3) the *formation* of **6** is increased which, however, will not always be revealed by an increased *accumulation* of **6**. In a radical termination, **6** could react with another radical to give a 4^{a}-flavin adduct. The use of TCA in MeCN gave a decrease of **10** coupled with the increased occurrence of Cl_3CCOO· and Cl_3C· radicals as appeared from the spontaneous generation of CO_2 (eqns 3 + 7). 5-EtFl· was probably trapped by Cl_3C· to give 5-ethyl-3-methyl-4^{a}-trichloromethyllumiflavin (eqn 8). In contrast, the use of HCOOH promised the achievements of quantitative accumulations of **6** which was indeed realized (Table 2; Figs 2 and 3).

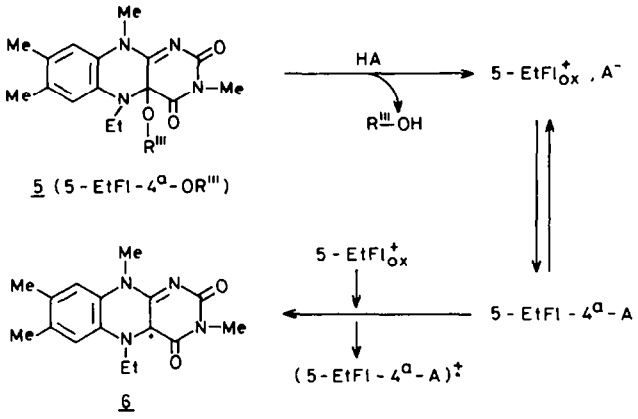
In previous papers[1,2] we concluded that radicals may arise spontaneously in solutions of N^{1}- and N^{5}-alkylflavinium salts **1** and **3** (Scheme 1)—abbreviated as 1-RFl$_{ox}^{+}$, A^{-} and 5-RFl$_{ox}^{+}$, A^{-}, respectively—at room temp in the dark.

The first studies were performed with N^{1}-alkylflavinium salts **1**. The spontaneous transformations are proposed to start with the formation of a covalent adduct **2** (eqn 1a) in dependence on the nature of the acid anion and the polarity of the medium. The 1,10^{a}-dihydroflavin ester **2** (1-RFl-10^{a}-A) is considered to be a key intermediate in the production of radicals. Though not excluding the possibility of an unimolecular homolysis of **2** or **4** (RFl-A → RFl· + A·), we think that the dihydroflavin esters are more important as one-electron donors (eqns 2a and 2b). They reduce the flavinium cation to the flavin radical leaving (1-RFl-10^{a}-A)‡ or (5-RFl-4^{a}-A)‡ as the counter-radical cations.

Since unprotonated 1-alkylflavosemiquinones (1-



Scheme 1. Spontaneous conversions of N^{1}- and N^{5}-alkylflavinium salts **1** and **3**.



Scheme 2. Formation and conversion of 5-ethyl-3-methyllumiflavinium salts (5-EtFl$_{ox}^{+}$, A^{-}) starting from the 4^{a}-flavin adducts **5** (R''' = H; Me).

RFl·) are unstable, their formation was not directly observed but revealed by sequential reactions like a N^{10}-dealkylation occurring in competition with the reaction with molecular oxygen.[1,2]

Unprotonated 5-alkylflavin radicals (5-RFl·) are known to be considerably more stable. Assuming that the N^{1}- and N^{5}-alkylflavinium salts **1** and **3** would behave similarly (Scheme 1) we expected that 5-RFl· radicals could be accumulated in a spontaneous process (eqns 1b + 2b). This expectation has appeared to be correct.

$$\text{5-RFl}_{ox}^{+}, A^{-} \rightleftarrows \text{5-RFlH-4}^{a}\text{-A} \quad (1b)$$

$$\text{5-RFl}_{ox}^{+} + \text{5-RFlH-4}^{a}\text{-A} \rightarrow \text{5-RFl}\cdot + (\text{5-RFlH-4}^{a}\text{-A})^{\ddagger} \quad (2b)$$

$$(\text{5-RFlH-4}^{a}\text{-A})^{\ddagger} \rightarrow \text{5-RFl}_{ox}^{+} + A\cdot \quad (3)$$

$$(\text{5-RFlH-4}^{a}\text{-A})^{\ddagger} \rightarrow \text{5-RFl}\cdot + A^{+} \quad (4)$$

$$(\text{5-RFlH-4}^{a}\text{-A})^{\ddagger} + X \rightarrow \text{5-RFlH-4}^{a}\text{-A} + X^{\ddagger} \quad (5)$$

$$2(\text{5-RFlH-4}^{a}\text{-A})^{\ddagger} \rightarrow \text{5-RFlH-4}^{a}\text{-A} + (\text{5-RFlH-4}^{a}\text{-A})^{++}. \quad (6)$$

Apart from the direct evidence which has now been provided for the radical formation, we wanted to establish the yields of the 5-RFl· accumulations, by which the behaviour of the counter-radicals would be betrayed.

A counter-radical cation may decompose (eqns 3 or 4), it may become an electron acceptor in a secondary electron transfer (eqn 5) or it may undergo a disproportionation (eqn 6).

A decomposition could give free acyloxy radicals (eqn 3) which may show a decarboxylation. In a secondary electron transfer (eqn 5) the solvent, the acid or a product formed might act as the electron donor X. A disproportionation (eqn 6) gives the adduct and, formally, a di-cation (5-RF-4^{a}-A)$^{++}$. The formulation of the di-cation also covers the formation of products on the same level of oxidation (Scheme 3) with a theoretical yield of 33.3% (eqns 1b + 2b + 6).

The theoretical yield of 5-RFl· is 50% on the basis of the sum of eqns 1b + 2b. It is calculated to be 100% based on the sum of eqns 1b + 2b + 3; 1b + 2b + 4; 1b + 2b + 5, while it is only 66.7% based on the sum of eqns 1b + 2b + 6.

Since the unprotonated 5-ethyl-3-methyllumiflavosemiquinone **6** (Scheme 2) is well characterized by its absorption spectrum,[3] we have focused our attention on the formation and the conversion of 5-ethyl-3-methyllumiflavinium salts **3** (Scheme 1; R = Et; R' = R'' = Me).

RESULTS AND DISCUSSION

In a general procedure as illustrated in Fig. 1, 5-ethyl-3-methyllumiflavinium salts (5-EtFl$_{ox}^{+}$, A^{-} in Scheme 2) were prepared *in situ* by adding an acid to a solution of the 4^{a}-hydroxy- or 4^{a}-methoxy-adduct **5** (R''' = H; Me) in a low polar solvent in an atmosphere of purified N_2. Curve *a* (Fig. 1) shows the molar absorbance of the 4^{a}-methoxy adduct *freshly* dissolved in dried $CHCl_3$. The addition of TFA to a final concentration of 1.3×10^{-1} M (1 volume %) immediately gave the flavinium cation in a yield of 100% (curve *b*; expt. 1, Table 1). The stability of 5-EtFl$_{ox}^{+}$ in a particular solvent/acid mixture is dependent on the concentration of the acid. On the addition of TFA to a lower final concentration of 5.0×10^{-4} M, a spectrum *c* was immediately obtained. Curve *c* was clearly composed of the absorbances of several components: 5-EtFl·, 5-EtFl$_{ox}^{+}$ and one or two 4^{a}-flavin adducts. Rapid spectral changes followed to give the rather stable curve *d* within a short time (t_{max} = 11 min). Curve *d* reflects the final formation of 5-EtFl· in a high yield (69%; see expt. 2, Table 1). Both a higher and a lower TFA concentration gave higher t_{max}-values. At higher acid concentration the protonated 5-ethyl-3-methyllumiflavin radical (5-EtFlH‡) is formed as the main product as is illustrated by the slow change of curve *b*, finally giving curve *e* (yield of 5-EtFlH‡ = 89%; see expt. 1, Table 1).

The procedure leading to the spontaneous accumulation of the 5-EtFl· and/or 5-EtFlH‡ radical (Fig. 1) was applied to a variety of acids and solvents. In general, three types of spectral changes were observed on adding the acid to the solution of the 4^{a}-hydroxy- or 4^{a}-methoxy-flavin adduct (column 5, Table 1):

(A) an immediate accumulation of 5-EtFl$_{ox}^{+}$ in a considerable amount (80–100%; see curve *b*, Fig. 1), in most cases followed by a slow conversion to 5-EtFlH‡ (expts 1; 5; 9). Sometimes, mixtures of 5-EtFl· and 5-EtFlH‡ were formed. The ratio may be influenced by the additional presence of other anions like ClO_4^{-} (expt. 13 vs 12; note k). The use of trichloroacetic acid in acetonitrile (expt. 10) did not lead to the accumulation of the flavin radical, but to the accumulation of a new 4^{a}-

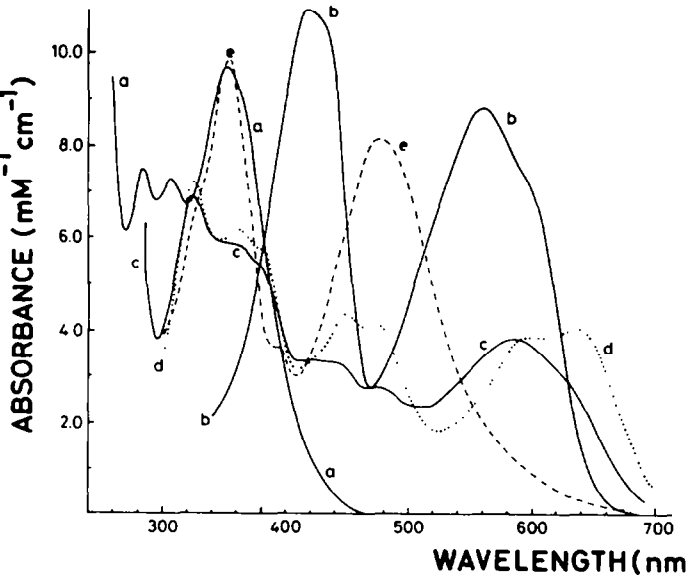


Fig. 1. (*a*) Molar absorbance of 5-EtFl-4ᵃ-OMe, freshly dissolved in dried $CHCl_3$ ($2.7 \sim 3.0 \times 10^{-5}$ M); (*b*; *c*) spectra of similar solutions showing the immediate effects of the addition of TFA to final concentrations of 1.3×10^{-1} M and 5.0×10^{-4} M, respectively; (*d*) the final spectrum at $t_{max} = 11$ min resulting from the rapid changes of curve *c* (cf. expt. 2, Table 1); (*e*) the final spectrum at $t_{max} \simeq 24$ hr resulting from the rather slow changes of curve *b* (see expt. 1, Table 1). (The spectral changes took place under N_2 at 25° in the dark.)

flavin adduct accompanied by a spontaneous generation of CO_2 (eqns 7+8; note i);

(B) an immediate formation of a mixture of 3 or 4 components (see curve *c*, Fig. 1), generally followed by a rapid accumulation of 5-EtFl· (expts 2–4; 6–8; 14; 18). Only in acetonitrile, a slow conversion took place (expts 17; 22);

(C) no appreciable accumulation of 5-EtFl^{+}_{ox}, but a direct appearance of 5-EtFl·, sometimes preceded by slight changes of the 4ᵃ-flavin adduct· spectrum indicating a primary transformation into a 4ᵃ-flavin ester. The accumulation of 5-EtFl· occurred slowly in expts 11; 15; 16; 19–21 (Table 1); 23; 26 (Table 2) and rapidly in expts 25; 29 (Table 2).

The type of the spectral change is dependent on the acid concentration and on the nature of the solvent (see note b). The rate of the conversion is considerably lower in acetonitrile than in chloroform or benzene (see note g). In particular, the pretreatment of chloroform can strongly influence the proceeding of the free radical reactions in flavin model systems as we have already observed in previous studies.[1,2]

Besides the final spectrum of the reaction mixture, two other criteria were used to judge the results of the process: (a) the content of 3-methyllumiflavin, established by absorbance and fluorescence measurements; (b) the overall recovery of 5-EtFl^{+}_{ox}, determined spectrophotometrically after reoxidation of 5-EtFl· and/or 5-EtFlH^{+} with sodium nitrite in the presence of a strong acid (see note a, Table 1).

For example, the reaction mixture represented by curve *d* (Fig. 1; expt. 2, Table 1) contained 3-methyllumiflavin in a yield of 13% at t_{max}, while 5-EtFl^{+}_{ox} was recovered in a yield of 70% in close agreement with the yield of the 5-EtFl· accumulation, giving an overall flavin material balance of 83%. There is no close agreement between the 5-EtFl^{+}_{ox} recovery and the 5-EtFl· accumulation in the case of an incomplete conversion (notes f; q) or an accumulation of a new 4ᵃ-flavin adduct (note i).

A reaction mixture as obtained under the anaerobic conditions described for curve *d*, appeared to be rather stable. After keeping it at room temp in the dark for about two weeks, the content of the 5-EtFl· radical had only decreased from 69 to 62%, while 3-methyllumiflavin increased from 13% to 18% (expt. 2, Table 1; see expts 11 and 18). On admitting air, the yield of 3-methyllumiflavin rapidly increased to 50% (expt. 2).

Although in the anaerobic experiments, traces of O_2 might have caused some N^5-dealkylations, the very low production of 3-methyllumiflavin in comparable experiments (Table 2) makes it very unlikely to assume that for instance in expt. 2, a yield of 13% at t_{max} would have been caused by O_2.

The anaerobic formation of 3-methyllumiflavin does not always appear to be a step coming to an end as rapidly and completely as the 5-EtFl· formation step (e.g. expt. 19, Table 1; notes n; p).

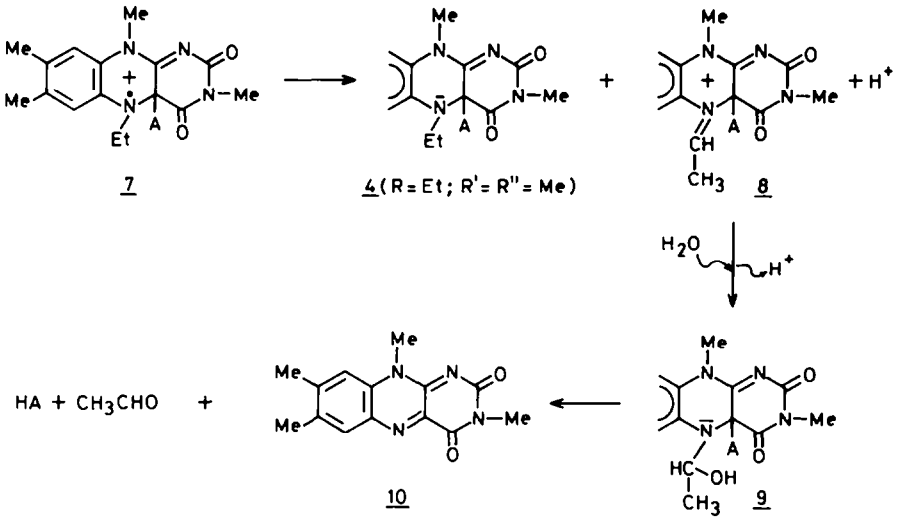
The anaerobic N^5-dealkylation seems to reflect in whole or in part a disproportionation (eqn 6) of $(5\text{-EtFl-4ᵃ-A})^{+}$ or, similarly, of $(5\text{-EtFl-4ᵃ-OR}''')^{+}$. (The latter is a product of an alternative electron transfer, see eqn 13.) More in particular, the anaerobic N^5-dealkylation probably represents a final result of a degradation of an intermediate, which is on the same level of oxidation as the di-cation $(5\text{-EtFl-4ᵃ-A})^{++}$ or $(5\text{-EtFl-4ᵃ-OR}''')^{++}$. The oxidation equivalent of $(5\text{-EtFl-4ᵃ-A})^{++}$ is proposed to be H^+ + intermediate **8** (Scheme 3). The subsequent reaction of **8** with H_2O is considered to be crucial for the formation of 3-methyllumiflavin **10**. (Karl-Fischer determinations on benzene, which was kept over Na wire, showed the presence of H_2O in the order of $\sim 10^{-3}$M which still is a 30–40 fold excess with reference to the flavin concentrations.)

Most experiments summarized in Table 1 gave an accumulation of the 5-ethyl-3-methyllumiflavin radical and of 3-methyllumiflavin in yields of 60–90% and 6–

Table 1. Spontaneous formation of 5-EtFl·/5-EtFlH$^+$ and 3-methyllumiflavin on adding an acid to 2.7 ~ 3.0 × 10^{-5} M solutions of **5** (R‴ = Me) under N_2 at 25° in the dark. The types of spectral changes A; B; C) are described in the discussion.

No.	ACID	SOLVENT	ACID CONC	TYPE OF SPECTRAL CHANGE	TIME OF MAXIMAL RADICAL FORMATION	POINT OF TIME OF ANALYSIS	RFl· (%)	RFlH$^+$ (%)	N^5-DEALK (%)	FLAVIN BALANCE FOUND (%)	NOTES a
1	TFA	$CHCl_3$	1.2×10^{-1}	A (100%)	~24 h	30 min		8			b
						24 h		86			c
						30 h		89			c
						50 h		89	15	104	c
2	"	"	5.0×10^{-4}	B	11 min	11 min	69		13	83	b
						4 days	63		16	79	c
						12 days	62		18	80	c
						12 days	(+ air)		50		c; d
3	"	Benzene	6.5×10^{-4}	B	6 min	6 min	63		15	78	
4	"	H_2O-satd. benzene	5.1×10^{-4}	B	8 min	8 min	55		13	73	e
5	"	CH_3CN	5.0×10^{-4}	A (99%)		12 days		60	7	86	a; b; f; g
6	TCA	CH_3Cl	5.1×10^{-4}	B	7 min	7 min	74		15	90	
7	"	Benzene	5.0×10^{-4}	B	3 min	3 min	64		13	78	
8	"	H_2O-satd. benzene	5.0×10^{-4}	B	3 min	3 min	62		13	75	e
9	"	Benzene	1.0×10^{-1}	A (~90%)	120 min	30 min		50			
						120 min		88	6	96	b; h
10	"	CH_3CN	4.9×10^{-4}	A (~100%)	~50 min	50 min	0	0		79	b; g; i
11	ACOH	$CHCl_3$	4.6×10^{-1}	(B) → C	130 min	130 min	81		15	96	j
						1 day	81		17	98	c
						3 days	77		17	94	c
						6 days	75		17	92	c
						14 days	73		18	91	c
12	"	AcOH/Ac_2O (7:3)	12.3	A (80%)	5~7 h	7 h	42	42	12	96	j
						1 day	39	39			
13	"	AcOH/Ac_2O (7:3)	12.3	A (100%)	>7 h	1 day	61	27	11	99	j; k
14	"	Benzene	6.1	B	12 min	12 min	80		21	101	j; l
15	"	Benzene	3.1×10^{-1}	C	140 min	140 min	75		18	93	b; m
16	"	Benzene	1.9×10^{-1}	C	>7 h	7 h	70		12	82	b; m
17	"	CH_3CN	1.8×10^{-1}	B		5 days	69		9		b; q
18	α-keto-glutaric acid	$CHCl_3$	9.1×10^{-4}	B	42 min	42 min	68		13	81	
						3 days	64		14	78	c
						11 days	62		15	77	c
19	Salicylic acid	$CHCl_3$	5.0×10^{-4}	C	(~10 h)	7.5 h	70		8	78	c; p
						21 h	73		9	81	c
						3 days	72		13	85	c; n
						6 days	70		15	85	c; n
						14 days	69		17	86	c; n
20	"	Benzene	5.0×10^{-4}	C	3.5 h	3.5 h	65		10	75	p
21	"	H_2O-satd. benzene	5.0×10^{-4}	C	3.5 h	3.5 h	63		11	74	e; p
22	"	CH_3CN	4.9×10^{-4}	A (20%) → B		3 days	50				
						6 days	49		9	69	a; b; g; q

Notes: a the flavin material balance covers the contents of 5-EtFl· + 5-EtFlH$^+$ + 3-MeLuFl + non-converted 5-EtFl$^+_{ox}$ + non-converted or new 4^a-flavin adducts; *b* the type of the spectral changes is dependent on the concentration of the acid (expts 1 vs 2; 9 vs 7; 15 and 16 vs 14) and on the nature of the solvent (expts 5 vs 2–4; 10 vs 6–8; 17 vs 16; 22 vs 19–21); *c* analyzed after keeping the reaction mixt in the closed apparatus for the time indicated at room temp in the dark; *d* increased N^5-dealkylation, due to a deliberate admission of air; *e* expts in H_2O-saturated benzene gave no remarkable differences (expts 4 vs 3; 8 vs 7; 21 vs 20); *f* incomplete conversion of 5-EtFl$^+_{ox}$; *g* t_{max} is considerably higher in MeCN than in $CHCl_3$ or benzene (expts 5 vs 2–4; 10 vs 6–8; 17 vs 16; 22 vs 19–21; *h* t_{max} is considerably lower in 10^{-1} M TCA than in 10^{-1} M TFA (expt. 9 vs 1); *i* in contrast with expts 6–9, a new 4^a-flavin adduct was accumulated accompanied by a generation of CO_2 (eqns 7 + 8). The new adduct was reoxidized to 5-EtFl$^+_{ox}$ by $NaNO_2$; *j* in order to diminish the content of H_2O, glacial AcOH (99%) was first mixed with Ac_2O in a ratio of 7 : 3, refluxed for 10 min and cooled off to room temp before use; *k* in contrast with the other expts, 5-EtFl$^+_{ox}$, ClO_4^- was used as the starting compound in expt. 13. The additional presence of the ClO_4^- anion (~3×10^{-5} M; expt. 13 vs 12) caused an increase of the t_{max} and influenced the ratio of 5-EtFl·/5-EtFlH$^+$; *l* in a dilution of the AcOH/Ac_2O mixt with benzene (1 : 1), the t_{max} was considerably lower, while the ratio of 5-EtFl·/5-EtFlH$^+$ was completely changed (expt. 14 vs 12); *m* the t_{max} increased on further lowering the conc of AcOH in benzene (expts 15; 16); *n* a relatively slow, anaerobic formation of 3-MeLuFl. It did not parallel an appreciable change of the 5-EtFl· content, but it improved the overall flavin material balance; *p* in rather slow processes of type C (expts 11; 15; 16; 19–21), anaerobic N^5-dealkylation might reflect a disproportionation of either (5-EtFl-4^a-A)$^+$ or (5-EtFl-4^a-OR‴)$^+$; *q* incomplete conversion of a 4^a-flavin adduct.



Scheme 3. Proposal for the anaerobic N^5-dealkylation of the counter-radical cation 7.

21%, respectively. In view of the theoretical yields outlined in the introduction, the experimental results indicate the simultaneous occurrence of a disproportionation (eqn 6) and one or more sequential conversions of the counter-radical cation as represented by eqns 3–5. Consequently, on suppressing such a disproportionation (eqn 6), one may expect to advance the occurrence of the other sequential reactions (eqns 3–5) and, therefore, to accomplish an increase of the *formation* of 5-EtFl·. However, this will not always be revealed by an increased *accumulation* of the free radicals.

The formation of 3-methyllumiflavin became unimportant by the use of TCA in MeCN (expt. 10) which, in contrast with the comparable expts 6, 7 and 8, showed a spontaneous and vigorous generation of CO_2. This indicates the increased decomposition of the counter-radical cation by eqn 3 giving the labile, free trichloroacetyloxy radical ($A\cdot = Cl_3CCOO\cdot$), followed by its decarboxylation (eqn 7). However, also in contrast with expts 6, 7 and 8, expt. 10 did not give any accumulation of 5-EtFl·. Instead, a new 4^a-flavin adduct was accumulated, probably 5-ethyl-3-methyl-4^a-trichloromethyllumiflavin as a product of a radical termination (eqn 8).

$$Cl_3CCOO\cdot \rightarrow Cl_3C\cdot + CO_2 \qquad (7)$$

$$5\text{-EtFl}\cdot + Cl_3C\cdot \rightarrow 5\text{-EtFl-}4^a\text{-}CCl_3 \qquad (8)$$

$$5\text{-EtFl}\cdot + H\cdot \rightarrow 5\text{-EtFlH} \qquad (9)$$

$$2\ 5\text{-EtFl}\cdot + H^+ \underset{b}{\overset{a}{\rightleftarrows}} 5\text{-EtFlH} + 5\text{-EtFl}^+_{ox}. \qquad (10a;\ 10b)$$

In efforts to suppress the disproportionation of the radical cations, much attention was further given to formic acid. Apart from its role in the formation of a dihydroflavin ester (eqn 1b), HCOOH may also be expected to advance the occurrence of sequential reactions by providing a more reactive intermediate to give a decomposition (eqn 3; $A\cdot = HCOO\cdot$) or by being or providing a secondary electron donor X in eqn 5. Moreover, a radical termination (eqn 9) would not produce a relatively, stabilized adduct as with TCA (eqn 8), but the (4^a-unsubstituted) dihydroflavin (5-EtFlH). This would also lead to 5-EtFl· in a comproportionation (eqn 10b). Therefore, the use of HCOOH promises the accomplishments of quantitative accumulations of 5-EtFl· which was indeed fulfilled.

In a procedure, similar to the one which already led to the results of Fig. 1 and Table 1, the addition of HCOOH gave the remarkable results summarized in Table 2 and illustrated in Fig. 2. These are distinguished by the high radical extinctions, the very low N^5-dealkylations (< 1%) and the almost quantitative recoveries of 5-EtFl^+_{ox} (97–100%). The spectra obtained at t_{max} are assumed to represent the absorbances of practically pure 5-EtFl· (Table 2; Fig. 2), which implies that in these experiments the disproportionation of 5-EtFl· (eqn 10a) would have been insignificant. The molar absorbance of 5-EtFlH^+ in solutions containing 1.3×10^{-1} M TFA are given in Fig. 3. The new molecular extinction coefficients for 5-EtFl· and 5-EtFlH^+ (Table 2; Fig. 3), which are higher than the ones mentioned in the literature,[3] were used to calculate the results of Table 1.

The spectra of 5-EtFl· (Fig. 2) were the final results of pseudo first order changes. The details of the kinetical studies will be discussed in a separate paper. Evidence will also be provided that the formation of the flavin-formate ester is the rate-limiting step (eqn 11).

$$5\text{-EtFl-}4^a\text{-}OR''' + HCOOH \rightarrow 5\text{-EtFl-}4^a\text{-}OOCH + R'''OH. \qquad (11)$$

We have always started from *fresh* stock solutions of the 4^a-hydroxy- or 4^a-methoxy-flavin adduct **5** (R''' = H; Me) in MeCN. These stock solutions were *practically* stable on the time scale of most experiments of Tables 1 and 2, but they were definitely unstable from an absolute point of view. This is consistent with an early assumption[2] that the reactions shown by dihydroflavin esters are not unique, but that the properties are more or less shared with other types of adducts like the hydroxy-, alkoxy- and hydroperoxy-flavins having the same dihydroflavin structure. This implies the possible occurrence of such an adduct as an

Table 2. Spontaneous formation of 5-EtFl· obtained on adding HCOOH to 2.7 ~ 3.0 × 10^{-5} M solutions of **5** (R''' = Me). The spectral changes were of type C with the exception of the one of type B shown by expt. 30. The anaerobic N^5-dealkylations were < 1%; 5-EtFl_{ox}^{+} was almost quantitatively recovered (97–100%)

No.	Solvent	HCOOH Conc.	Time of Maximal Radical Formation	λ_{max} in nm (ε)
**	Benzene	--	--	655 (4700); 608 (4250); 486 (1950); 451 (2050); 389 (5600); 326 (6900).
23*	"	1.3×10^{-3}	3 h	652 (5200); 606 (4800); 480 (2300); 446 (2500); 389 (6400); 325 (7100).
24	"	6.0×10^{-3}	23 min	639 (5200); 598 (4800); 484 (2500); 450 (2600); 385 (6400); 324 (7000).
25	"	1.3×10^{-2}	7 min	639 (5200); 599 (4800); 486 (2600); 451 (2500); 386 (6300); 324 (6700).
**	$CHCl_3$	--	--	642 (4400); 603 (4000); 490 (2350); 460 (2000); 385 (5900); 326 (7200).
26*	"	6.0×10^{-4}	4.5 h	642 (6100); 599 (5500); 484 (3400); 452 (3400); 383 (7500); 325 (8900).
27	"	1.3×10^{-3}	40 min	641 (6000); 599 (5500); 488 (3200); 455 (3100); 383 (7400); 325 (8300).
28	"	4.9×10^{-3}	10 min	639 (6000); 599 (5500); 488 (3300); 456 (3200); 382 (7500); 325 (8600).
29	"	1.3×10^{-2}	3 min	636 (5700); 598 (5400); 488 (3300); 460 (3100); 382 (7100); 325 (7800).
30*	MeCN	1.3×10^{-2}	40 min	631 (6000); 600 (5700); 486 (3500); 456 (3400); 379 (7700); 324 (8600).

* The results of expts 23, 26 and 30 at t_{max} are represented in Fig. 2 by the curves f_1; g_1 and h_1, respectively.
** For comparison: λ_{max} (ε)-data for 5-EtFl·, prepared by autoxidation of the corresponding dihydroflavin (5-EtFlH + O_2) as mentioned in ref. 3.

electron donor in the reduction of 5-EtFl_{ox}^{+} to 5-EtFl· leaving a radical cation (eqn 13) in analogy with eqn 2b.

$$5\text{-EtFl-4}^a\text{-OR}''' \rightleftarrows 5\text{-EtFl}_{ox}^{+} + \text{R}'''\text{O}^- \qquad (12)$$

$$5\text{-EtFl-4}^a\text{-OR}''' + 5\text{-EtFl}_{ox}^{+} \rightarrow (5\text{-EtFl-4}^a\text{-OR}''')^{\ddagger} + 5\text{-EtFl}\cdot \qquad (13)$$

The proceeding of the process (12)+(13) and the catalyzed accumulation of $(5\text{-EtFl-4}^a\text{-OR}''')^{\ddagger}$ will be dealt with in a subsequent paper of these series. In the light of this knowledge, the occurrence of $(5\text{-EtFl-4}^a\text{-OR}''')^{\ddagger}$ besides $(5\text{-EtFl-4}^a\text{-A})^{\ddagger}$ is considered to be possible in slow processes of type C (expts 11; 15; 16; 19–21; Table 1). Consequently, the relatively slow anaerobic formation of 3-methyllumiflavin in expt. 19 could have reflected the disproportionation of radical cations of more than one specific structure (see note p).

CONCLUSION

While only indirect evidence could be given for the stepwise conversions of 1-alkylflavinium salts into

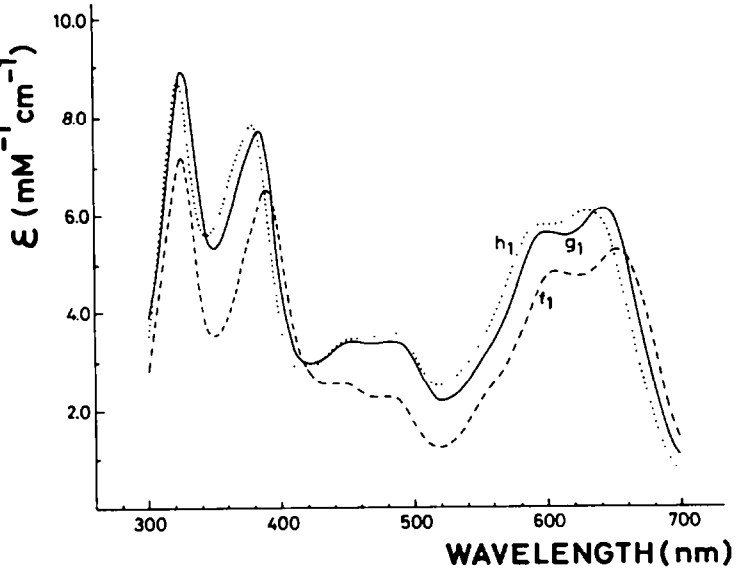


Fig. 2. Molar absorbance of 5-EtFl· in benzene (curve f_1), $CHCl_3$ (curve g_1) and MeCN (curve h_1), obtained by adding HCOOH to 2.7 ~ 3.0 × 10^{-5} M solutions of 5-EtFl-4^a-OMe in the solvents mentioned. These curves represent the results of expts 23, 26 and 30 at t_{max} (Table 2), which were carried out under N_2 at 25° in the dark.

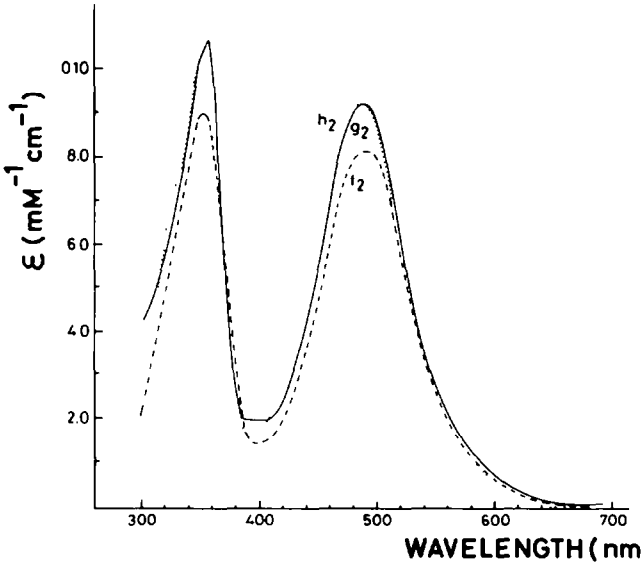


Fig. 3. Molar absorbance of 5-EtFlH$^{\cdot}$ in benzene (curve f_2), $CHCl_3$ (curve g_2) and MeCN (curve h_2), obtained by adding 1 volume% of TFA to the solutions of 5-EtFl$^{\cdot}$ (Fig. 2); the final concentration of TFA was 1.3×10^{-1} M. λ_{max} in nm (ε), *benzene*: 352 (9000); 490 (8200); $CHCl_3$: 356 (10,600); 489 (9200); MeCN: 353 (10,100); 486 (9200).

radicals,[1,2] it has now been directly proven that 5-alkylflavinium salts can do the same. Due to their greater stability, 5-RFl$^{\cdot}$ radicals could be accumulated. The overall yield of 5-RFl$^{\cdot}$ is dependent on the behaviour of the counter-radical cations. The competitive nature of some of the sequential reactions was shown. Experimental conditions can be found to suppress the pathway which limits the formation of 5-EtFl$^{\cdot}$ to 66.7%. This will also result in an increased accumulation of 5-EtFl$^{\cdot}$ provided that there is no concomitant production of trapping radicals like $Cl_3C^{\cdot}$ leading to a stabilized 4^a-flavin adduct.

Consequently, efforts should further be made to suppress *all* sequential reactions in order to accomplish the accumulation of both 5-EtFl$^{\cdot}$ and the counter-radical cations.

EXPERIMENTAL

Materials and methods. The solvents were reagent grade, but treated before use. Acetonitrile was successively distilled over CaH_2 and P_2O_5. Special care was given to remove all traces of EtOH from the $CHCl_3$ by several washings with equal amounts of H_2O. Only freshly washed $CHCl_3$ was used after drying over $CaCl_2/K_2CO_3$. Benzene was dried over Na wire. The conc of the stock solns of **5** in MeCN was checked by measuring the E_{546} of a 100-fold dilution in 0.1 N HCl (ε = 9000).

UV spectra were recorded on a Perkin–Elmer 505. The cuvets (pathlengths of 10 mm) were thermostated at 25°.

The anaerobic expts were carried out in the way described earlier[2] in a Thunberg-like apparatus made in this laboratory. The solvent (~ 10 ml) and the acid were present in one of the compartments; ½ hr before closing the valves of the apparatus, a stock soln of **5** in MeCN (40 μl; $6.8–7.8 \times 10^{-3}$ M) was injected into the 2nd compartment. The valves were closed, the contents of the compartments were mixed and the spectra were recorded at 25°. The volumes of the mixts were derived from their nett weights.

The appearance of 3-methyllumiflavin was determined by fluorescence measurements of the same mixts using an adapted Eppendorf digital photometer 6115. Standard curves were determined for 3-methyllumiflavin in different solvents using the Hg-lamp, the 405 + 436 nm primary filter and, the 470–3000 nm secondary filter. The photometer was adjusted to 100% emission using the green fluorescent standard 106502.

5-EtFl$^+_{ox}$ was recovered by adding conc TFA (100 μl) and some grains of solid $NaNO_2$ to the mixts. The recovery values were calculated from the extinctions measured at λ_{max} in *benzene* (1.3×10^{-1} M TFA) at 564 nm (ε = 8200); in $CHCl_3$ (1.3×10^{-1} M TFA) at 560 nm (ε = 8850); in MeCN (1.3×10^{-1} M TFA) at 553 nm (ε = 9000).

Manometric expts (CO_2 generation; hydrogenation) were carried out at atmospheric pressure and at an average temp of 23° in an all-glass manometric apparatus allowing continuous corrections for pressure and temp changes. The mixts were magnetically stirred. The yields of CO_2 were derived from the changes of the gas-volumes and, afterwards, checked by absorption of the gas in 0.1 N NaOH/$BaCl_2$ (3%) and titration with 0.1 N HCl.

PMR and IR spectra were recorded on a Varian EM 360L NMR spectrometer 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.

5-Ethyl-3-methyl-1,5-dihydrolumiflavin (5-EtFlH). 3-Methyllumiflavin[4] (3.00 g; 11.1 mmol) was hydrogenated over 10% Pd-C (1 g) in a mixt of 96% EtOH (250 ml), H_2O (230 ml), conc HCl (20 ml) and acetaldehyde (20 ml) at room temp and at atmospheric pressure. The catalyst was filtered off to give a light yellow-red filtrate. Conc NH_4OH was added to a final conc of 1 N NH_4OH. Additions of small portions of solid $Na_2S_2O_4$ were required to prevent a blue coloration of the soln. The mixt was concentrated *in vacuo* to provide a yellow ppt of 5-ethyl-3-methyl-1,5-dihydrolumiflavin. The product was filtered off, washed with de-aerated H_2O and dried in a vacuum desiccator over P_2O_5, yield: 3.0–3.2 g (90–96%) m.p. 245–246°.

5-Ethyl-3-methyllumiflavinium perchlorate (5-EtFl$^+_{ox}$, ClO_4^-). 5-EtFlH was oxidized with $NaNO_2$ as described in the literature.[5]

5-Ethyl-4a-methoxy-3-methyl-4a,5-dihydrolumiflavin (**5**, R''' = Me). A soln of Na (325 mg) in MeOH (100 ml) was added to 5-$EtFl^{+}_{ox}$, ClO_4^- (5.00 g; 12.54 mmol). The mixt was stirred for 0.5 hr. The ppt was filtered off, washed with MeOH and recrystallized from MeOH. The product contained one molecule of MeOH of crystallization, yield 3.80 g (84%) m.p. 175°. ($C_{17}H_{22}N_4O_3 \cdot CH_3OH$ (362.44). (Found: C, 59.5; H, 7.2; N, 15.7. Calc. for C, 59.65; H, 7.23; N, 15.46%.) Mass spectrum, *m/e* (%): 330 (M^+, 15); 299 (35); 273 (21); 271 (35); 258 (47); 244 (23); 230 (8); 214 (21); 186 (11). PMR ($CDCl_3$): δ = 1.12 (3, t, J = 7 Hz, C—Me); 2.27 (6, s, Ar—Me); 3.07 (3, s, O—Me); 3.33 (3, s, N—Me); 3.54 (2, q, J = 7 Hz, CH_2); 3.70 (3, s, N—Me); 6.95 (2, s, Ar—H). IR (KBr), cm^{-1}: 1670; 1560 (C=O). UV (MeOH), λ_{max} (ε): 283 (7100); 309 (7800); 356 (9200).

5-Ethyl-4a-hydroxy-3-methyl-4a,5-dihydrolumiflavin (**5**, R''' = H). 5-$EtFl^{+}_{ox}$, ClO_4^- was converted as described in the literature.[5]

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