Studies in the Flavin Series. Part XVI.¹ Alkylation and Rearrangement Reactions of Dihydroalloxazines

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Alkylation of 5-acetyl-5,10-dihydro-1,3,7,8-tetramethylalloxazine (2) in dimethylformamide proceeded at the bridge position C(4a) or at N(10) to an extent dependent upon the alkylating agent. An increasing tendency for substitution at C(4a) in the order dimethyl sulphate < methyl iodide $\ll$ allyl bromide < benzyl bromide was found, and was interpreted as representing a trend from a hard to a soft electrophile. The relative reactivities indicated that C(4a) was a soft nucleophilic centre whereas N(10) was a hard centre. The bending of dihydro(iso)alloxazines about the N(5)–N(10) axis is considered to have an important influence on the reactivities at N(10) and C(4a). 5-Deacetylation resulted in migration of an allyl or benzyl substituent from N(10) to C(4a); the derivatives with a saturated alkyl group at N(10) did not show this migration.

THE chemistry of dihydro(iso)alloxazines[†] has remained largely unexplored, in spite of their biological significance as the fully reduced state of flavocoenzymes. However, the present knowledge of fully reduced flavins has recently been reviewed.² Flavin-dependent substrate dehydrogenations involve a two-electron transfer, which is thought to occur as a 'hydride transfer' [reaction (i)], but could alternatively be considered formally as a transfer of R^- , with subsequent protonation of a covalent $R-Fl$ (alkyl-flavin) intermediate [reaction (ii)]. In the case of reaction (i), the possibility

also exists that an interaction of FIH^-_{red} with R^+ may contribute significantly to the reaction path. Thus flavin-dependent dehydrogenations require an understanding of the nature of group transfer. This can be experimentally approached by the characterisation of reactive 'alkyl-flavin' intermediates. For this reason, our group has studied the alkylation reactions of dihydro(iso)alloxazines.

It was previously³ shown that alkylation of 5-acetyl-10-alkyl-1,5-dihydro-7,8-dimethylisoalloxazine (5-acetyl-flavohydroquinone) occurs at O(2) and N(3) or at O(2) and O(4), but never at N(1). This was attributed to steric overcrowding at N(1) by the *peri*-substituent at N(10). However, surprisingly, the alkylation of 5-acetyl-5,10-dihydro-1,3,7,8-tetramethylalloxazine (2) did produce a 1,10-dialkyl system.⁴ Recently¹ a direct photoalkylation of flavoquinone by 'activated' carb-

oxylic acids has been demonstrated in which benzyl and allyl residues have been substituted at either N(5) or C(4a). These residues prove to be very 'mobile' on the dihydro(iso)alloxazine nucleus, migrating from N(5) to C(4a) upon heating¹ and from C(4a) to N(5) upon illumination.⁵

RESULTS

When the alkylation of the 5-acetyl-dihydroalloxazine (2) was conducted in dimethylformamide-potassium carbonate as previously described,⁴ but with the more active alkylating agents benzyl and allyl bromide, the products consisted of mixtures of two monoalkyl isomers, which could be separated by fractional crystallisation. Three possible structures had to be considered, corresponding to alkylation at O(4), C(4), or N(10) (Scheme 1). Since both isomers showed three distinct C=O bands in the i.r. spectrum, O(4) was eliminated as a possible point of substitution. A further distinction was readily made by n.m.r. spectroscopy, since the isomers differed appreciably in the chemical shift of the methylene protons of either the benzyl or allyl residues. Thus one isomer was clearly derived from N(10) alkylation [(3; R = allyl) $\delta(CH_2)$ 4.15 p.p.m.; (3; R = benzyl) $\delta(CH_2)$ 4.17 p.p.m.], and the other was derived from C(4a) alkylation [(4; R = allyl) $\delta(CH_2)$ 2.34 p.p.m.; (4; R = benzyl) $\delta(CH_2)$ 2.97 p.p.m.]. The C(4)=O stretching frequency of the C(4a)-alkylated isomers (4) also exhibited a hypsochromic shift [21 cm^{-1} (R = allyl); 18 cm^{-1} (R = benzyl)] and a loss in intensity, compared to N(10)-alkylated isomers (3), which reflected the weaker conjugation of the C(4)=O system in structure (4) as compared to structure (3). When the alkylation of compound (2) was carried out under similar conditions with methyl iodide, compound (3; R = Me) was by far the major product, but

² P. Hemmerich, S. Ghisla, U. Hartmann, and F. Müller in 'Flavins and Flavoproteins,' ed. H. Kamin, University Park Press, Baltimore, in the press.

³ P. Hemmerich, B. Prijs, and H. Erlenmeyer, *Helv. Chim. Acta*, 1960, **43**, 372.

⁴ K. H. Dudley and P. Hemmerich, *Helv. Chim. Acta*, 1967, **50**, 355.

⁵ G. Blankenhorn and P. Hemmerich, unpublished data.

[†] Alloxazines and isoalloxazines are identical at the dihydro-level: 10-alkyl-1,5-dihydro-7,8-dimethylisoalloxazine $\equiv$ 10-alkyl-5,10-dihydro-7,8-dimethylalloxazine $\equiv$ flavohydroquinone.

¹ Part XV, W. H. Walker, P. Hemmerich, and V. Massey, *Helv. Chim. Acta*, 1967, **50**, 2269.

a small amount of the isomer (4; R = Me) was separated by careful fractional crystallisation. Again there was a clear distinction between the n.m.r. spectra of the N(10)- and the C(4a)-alkylation products: (3; R = Me) $\delta(10\text{-Me})$ 3.38 p.p.m.; (4; R = Me) $\delta(4a\text{-Me})$ 1.35 p.p.m. The C(4)=O stretching frequency of compound (4; R = Me)

SCHEME 1 Reagents: i, AcOH-Ac₂O-Zn; ii, RX-K₂CO₃-Me₂N-CHO; iii, HNO₂; iv, hv + O₂; v, hv + RCO₂H

also showed a hypsochromic shift (24 cm⁻¹) and loss of intensity as compared to compound (3; R = Me). When the methylation was conducted with dimethyl sulphate, compound (3; R = Me) was the only detected alkylation product.

In the Table the proportions of the isomeric products (3) and (4) are compared for the different alkylation reactions. The proportion of C(4a) alkylation decreases in the order PhCH₂Br > CH₂:CH-CH₂Br ≫ MeI > Me₂SO₄. Thus there is a clear distinction between reactions conducted with alkylating agents which are activated by π -orbitals and those in which the alkyl group is saturated.

The separated alkylation products (3) and (4) (R = PhCH₂) were not interconvertible or reconvertible into

starting material (2) under the conditions of alkylation; this implies a kinetic control of the products. Although the methyl group at N(1) may impart some steric restriction to reaction at N(10), the distinction between groups of similar sizes, *i.e.* ethyl and allyl or isopropyl and benzyl, indicated that the reaction course was not determined

Ratios of products isolated from alkylations of compound (2) in dimethylformamide-potassium carbonate

Alkylating agent (RX)	Product ratio (%) (4)	(3)
Me ₂ SO ₄	0	100
MeI	5-10	90-95
EtI, PrI		<i>ca.</i> 100 ^a
CH ₂ :CH-CH ₂ Br	25	75
PhCH ₂ Br	70	30

^a Ref. 4.

primarily by steric factors. Furthermore, alkylation with saturated alkyl halides would be expected to have less S_N1 character in the transition state than with allyl or benzyl bromide and would therefore cause the greater steric hindrance.

When alkylation of the 5-acetyl-dihydroisoxaloxazine (8) was conducted under the same conditions, with either methyl iodide³ or benzyl bromide, only one alkylation product could be isolated (Scheme 2). Again C(4a), N(1), O(2), and

SCHEME 2 Reagents: i, RX-K₂CO₃-Me₂N-CHO

O(4) must be considered as a possible site of attack. However, the respective products of methylation and benzylation showed only *two* carbonyl stretching bands [(R = Me) ν_{CO} 1670 and 1680 cm⁻¹, (R = benzyl) ν_{CO} 1665 and 1680 cm⁻¹]. The spectral properties of these products were closely similar to those of the O(2),N(3)-dialkyl analogue previously described.^{3,4} The chemical shifts shown by substituent methyl protons (δ 4.1 p.p.m.) and benzyl methylene protons (δ 5.48 p.p.m.) eliminated C(4a) as the position of substitution in each case. Thus both alkylation reactions must have occurred exclusively at O(2) to yield (9; R = Me or PhCH₂). This conclusion was supported by the results of acid hydrolysis, which removed the O(2) substituent, and of nitrous acid oxidation,⁴ which yielded the corresponding flavinium salt in each case.

In contrast, 1,5-dihydro-3,7,8,10-tetramethylisoxaloxazine (12), underwent methylation at N(5) [product (10)], but benzylation and allylation at C(4a) [product (11)].³ Thus the presence of the 5-acetyl residue on the dihydroisoxaloxazine prevents a distinction between alkylating agents

and directs the reaction to the more electronegative O(2). The alkylation in dimethylformamide of 5,10-dihydro-1,3,7,8-tetramethylalloxazine [reduced form of (1)] by benzyl bromide occurred at C(4a), whereas dimethyl sulphate attacked only N(5).⁶ Again removal of the acetyl residue increased the distinction between alkylating agents (*cf.* Table).

FIGURE 1 ^1H N.m.r. spectrum of compound (5; R = benzyl) in $[\text{2H}]\text{chloroform}$

Hydrolysis of both compounds (3) and (4) (R = allyl or benzyl) in 6N-hydrochloric acid in the absence of air surprisingly yielded the same product, 4-alkyl-4a,5-dihydro-1,3,7,8-tetramethylalloxazine (5; R = allyl or benzyl) (Scheme 1). The n.m.r. spectra of compound (5; R = benzyl) (Figure 1) showed the benzyl methylene signal as an AB system centred at δ 3.06 p.p.m. (q , J_{AB} 12.5 Hz); compound (4; R = allyl) showed the methylene signal as an ABC system centred at δ 2.55 p.p.m. (J_{AB} 12 Hz). This multiplicity is to be expected from a methylene group linked to a centre of chirality. Similarly the methylene protons of the 4a-benzyl-3,7,8,10-tetramethyl analogue (11; R = benzyl) exhibited an AB system centred at δ 3.0 p.p.m. (J_{AB} 12–15 Hz).¹ Surprisingly the 5-acetyl derivatives (4; R = allyl or benzyl) and the 5-formyl derivative of (11) show unresolved methylene signals. This loss of resolution may be due to the presence of a carbonyl group in the vicinity of *both* methylene protons.

Deacetylation of compound (3; R = benzyl or allyl) appears to precede benzyl or allyl migration from N(10) towards C(4a). This was demonstrated in the following way. The deacetylation of compound (2; R = allyl) was conducted in the presence of nitrous acid to yield the expected 10-allylisoalloxazinium salt (6)⁴ (Figure 2). A solution of this in chloroform was then reduced with a supernatant

aqueous acidic tin(II) chloride solution. After the characteristic red colour of the flavosemiquinone cation had disappeared, the changes in the absorption of the chloroform layer were followed. In Figure 2 a progressive isosbestic change can be seen ($t_{\frac{1}{2}}$ *ca.* 20–30 min at 25°) from a spec-

FIGURE 2 Spectral course of the rearrangement (7) $\rightarrow$ (5) [the extinction of compound (6) has been reduced by a factor of 2]

trum which is characteristic of compound (7; R = allyl) (λ_{max} 335 and 280 nm), to that of compound (5; R = allyl) (λ_{max} 360 nm). This experiment could not be attempted with a benzyl analogue under the same conditions since the 10-benzylisoalloxazinium salt spontaneously loses the benzyl residue in aqueous solution through hydrolysis. In the case of compound (7; R = saturated alkyl) the saturated alkyl N(10) substituent did not migrate.

FIGURE 3 Spectral course of the photo-oxidation of compound (5) in methanol to form dimethyl-lumichrome (1)

A similar mobility of allyl and benzyl residues was found in photo-oxidation of 4a-alkyl-dihydro(iso)alloxazines (5) and (11).¹ Irradiation of compound (5; R = allyl or benzyl) with visible light resulted in a quantitative transfer of the 4a-alkyl group to molecular oxygen, yielding

⁶ C. R. Jefcoate, S. Ghisla, and P. Hemmerich, to be submitted to this Journal.

1,3,7,8-tetramethylalloxazine isosbestically (Figure 3) and mainly the corresponding aldehydes.

DISCUSSION

In these alkylation reactions the dihydro(iso)alloxazines exhibit behaviour which is typical of ambident nucleophiles.⁷ Pearson⁸ has suggested a classification of bases into two classes: soft (valence electrons polarisable) and hard (valence electrons unpolarisable). Klopman⁹ has extended this concept by describing substitution reactions in terms of a combination of MO and charge interactions between the electrophile and nucleophile. In the case of alkylation of compound (2), C(4a) is the preferred reaction site for soft alkylating agents, whereas N(10) is preferred for harder alkylating agents (in increasing softness $\text{Me}_2\text{SO}_4 < \text{MeI} < \text{CH}_3\cdot\text{CH}\cdot\text{CH}_2\text{Br} < \text{PhCH}_2\text{Br}$). Vacant orbitals of low energy are provided by the systems of allyl and benzyl bromides; these are absent in saturated alkylating agents.

Knowledge of the conformation of the dihydro(iso)alloxazine is critical to an understanding of these reactions. Kierkegaard and his co-workers¹⁰ have recently demonstrated by X-ray crystallography that compound (3; R = Me, position 9 brominated) is bent about the N(5)–N(10) axis with a dihedral angle of 144.5° . The non-planar character of dihydro(iso)alloxazines has also been indicated by marked changes in the extinction coefficient of the weak absorption band at 400 nm.^{1,11} Since these changes depend on the nature of the 1-, 5-, and 10-substituents, it has been suggested that the 400 nm transition ($\pi\text{--}\pi^*$) becomes less allowed as the molecule is forced out of plane by steric interactions.¹¹

Calculations carried out on a planar model for the dihydroisoalloxazine nucleus¹² and e.p.r. spectra of planar flavosemiquinone radicals¹³ indicate high frontier orbital contributions (*i.e.* high polarisability) for planar dihydroisoalloxazines at positions N(5), N(10), and C(4a) [$\text{N}(5) > \text{N}(10) \approx \text{C}(4a)$]. The softness of N(5) and N(10) is reduced by non-planarity, since bending localises sp^3 electron pairs at these positions. Thus 5-acetylation increases the bending in compound (8) and consequently changes the reaction from frontier-controlled [as for compound (12)] to charge-controlled, *i.e.* C(4a) attack is diverted to O(2). The high tendency for C(4a) attack on compound (2) compared to compound (8) probably reflects the lower reactivity of O(4) in the former. This may arise from either a low charge density or steric hindrance. Furthermore, in the alkylation of the analogue of compound (2) lacking a 5-acetyl group we have recently found a similar competition between attack at C(4a) and N(5).⁶ However, it seems likely that compound (2), the analogue just mentioned, and compound (12) are all non-planar to some extent, perhaps sufficient to increase significantly the hardness of either N(10) or N(5) relative to C(4a) (*cf.* foregoing

prediction for planar dihydroisoalloxazines). The greater steric freedom at C(4a) may also cause a general increase in C(4a) availability relative to N(10) or N(5).

The instability of allyl and benzyl residues at N(10) in structure (7) must reflect a thermodynamic preference for the C(4a)-alkylated isomer, in the case of allyl or aryl substituents. This suggests a preference of 'soft' alkyl groups for the soft C(4a) position and of 'hard' alkyl groups or hydrogen for the 'harder' N(10) position. An alternative explanation is that allyl and aryl substituents but not saturated alkyl substituents provide sufficiently low activation energies for rearrangement. However, there is no evidence that hydrogen will bind at C(4a), suggesting that the distinction between substituents is thermodynamic rather than kinetic. A similar irreversible thermal rearrangement of compound (10; R = benzyl) to (11; R = benzyl) has been reported,⁴ in which the benzyl group migrated from N(5) to C(4a).

We have described how the chemical behaviour of dihydro(iso)alloxazines can be altered by small changes in the molecular conformation. This is relevant to the chemistry of reduced flavocoenzymes since enzymes have a considerable potential to distort molecular shapes and it is becoming apparent that enzyme reactions are frequently controlled by such conformational changes. The rearrangement of activated alkyl groups which are attached to dihydroalloxazines will be further described in a forthcoming paper.⁶

EXPERIMENTAL

M.p.s were determined with a Kofler hot-stage apparatus. T.l.c. was carried out with MN-Silicagel S (Macherey, Nagel Co., Düren, Germany); benzene-di-isopropyl ether-ethyl alcohol (2:2:1 v/v) was used as solvent. Absorption spectra were measured with a Cary 14 spectrophotometer. I.r. spectra were taken with a Beckman IR 8 or a Perkin-Elmer 621 instrument, and ¹H n.m.r. spectra with a Varian A-60A instrument.

Benzylation of 5-Acetyl-5,10-dihydro-1,3,7,8-tetramethylalloxazine (2).—Compound (2) (3.5 g) was dissolved in dimethylformamide (75 ml) containing suspended potassium carbonate (6 g), and the mixture was heated to 80° under nitrogen. Benzyl bromide (2.0 ml) was added and the mixture was maintained at 80° for a further 2 h. T.l.c. indicated complete conversion of (2) into three products. Dimethylformamide was then distilled off under reduced pressure and chloroform (25 ml) was added to the residue. The mixture was filtered and the filtrate was evaporated to leave a yellow oil from which white crystals separated on addition of ethanol (10 ml). These were redissolved in ethanol (80 ml); white needles separated from the solution after 24 h at 0° (m.p. $242\text{--}245^\circ$). This product (A) (1.5 g) moved as one spot (R_F 0.9) on t.l.c. The remaining filtrate was evaporated to yield a pale yellow solid, which was redissolved in the minimum of methyl alcohol (15 ml). After 24 h at -15° cubic crystals separated (0.8 g) [product (B)]

¹¹ K. H. Dudley, A. Ehrenberg, P. Hemmerich, and F. Müller, *Helv. Chim. Acta*, 1964, **47**, 1354.

¹² T. K. Fox, S. P. Laberge, K. Nishimoto, and L. S. Forster, *Biochim. Biophys. Acta*, 1967, **136**, 544.

¹³ A. Ehrenberg, F. Müller, and P. Hemmerich, *European J. Biochem.*, 1967, **2**, 286.

⁷ N. Kornblum, R. A. Smiley, R. K. Blackwood, and D. C. Iffland, *J. Amer. Chem. Soc.*, 1955, **77**, 6269.

⁸ R. G. Pearson, *J. Amer. Chem. Soc.*, 1963, **85**, 3533.

⁹ G. Klopman, *J. Amer. Chem. Soc.*, 1968, **90**, 233.

¹⁰ P. Kierkegaard, in *ref. 2*.

which on t.l.c. showed a non-fluorescent main spot at R_F 0.6 and a small contamination of blue fluorescent 1,3,7,8-tetramethylalloxazine (1). The latter was removed by recrystallisation from methanol to yield pure product (B) (m.p. 197—200°). Product (A) was identified as 5-acetyl-4a-benzyl-4a,5-dihydro-1,3,7,8-tetramethylalloxazine (4; R = benzyl) (Found: C, 68.2; H, 5.9; N, 13.7; O, 12.3. $C_{23}H_{24}N_4O_3$ requires C, 68.3; H, 6.0; N, 13.8; O, 11.9%); $\nu_{\max.}$ (KBr) 1733 [C(4)=O], 1678 [C(2)=O], and 1621 (Ac) cm^{-1} ; $\lambda_{\max.}$ (MeOH) 307 and 237 nm ($\log \epsilon$ 3.73 and 5.08); δ (CDCl₃) 6.95 and 6.89 (9- and 6-H), 3.22 [N(1)Me], 3.06 [N(3)Me], 2.97 (2H, q, 4a-CH₂), 2.32 (7- and 8-Me), and 2.07 (5-Ac) p.p.m. Product (B) was identified as 5-acetyl-10-benzyl-5,10-dihydro-1,3,7,8-tetramethylalloxazine (3; R = benzyl) (Found: C, 68.3; H, 6.0; N, 13.8; O, 11.9. $C_{23}H_{24}N_4O_3$ requires C, 68.3; H, 6.1; N, 13.6; O, 12.2%); $\nu_{\max.}$ (KBr) 1715 [C(4)=O], 1684 [C(2)=O], and 1661 (Ac) cm^{-1} ; $\lambda_{\max.}$ (MeOH) 300 and 245sh nm ($\log \epsilon$ 3.49 and 3.82); δ (CDCl₃) 7.3 and 6.8 (6- and 9-H), 4.71 (10-CH₂), 3.61 [N(1)Me], 3.32 [N(3)Me], 2.22 (7- and 8-Me), and 1.56 (5-Ac) p.p.m.

Reaction of the Dihydroalloxazine (2) with Allyl Bromide.—The foregoing procedure was repeated with compound (2) (0.5 g), dimethylformamide (20 ml), potassium carbonate (1.0 g), and allyl bromide (1.0 ml). The reaction was again followed by t.l.c. and was complete after 3 h. Two products in addition to some 1,3,7,8-tetramethylalloxazine (1) were observed. The mixture yielded a yellow oil; crystallisation was induced by dropwise addition of methanol. The precipitate was filtered off and recrystallised 2—3 times from methanol to obtain the product (A) of high R_F , m.p. 179—182° (0.10 g). Product (B) was obtained by crystallising a highly concentrated solution of the combined methanol filtrates at -15°; m.p. 198—200° (0.25 g). Product (A) was identified as 5-acetyl-4a-allyl-4a,5-dihydro-1,3,7,8-tetramethylalloxazine (4; R = allyl) (Found: C, 64.4; H, 5.8. $C_{19}H_{22}N_4O_3$ requires C, 63.5; H, 5.8%); $\nu_{\max.}$ (KBr) 1727 [C(4)=O], 1672 [C(2)=O], and 1621 (Ac) cm^{-1} ; $\lambda_{\max.}$ (MeOH) 303, 240, and 216 nm ($\log \epsilon$ 3.59, 4.26, and 4.29); δ (CDCl₃) 7.13 and 7.19 (6- and 9-H), 5.0—5.35 (CH₂=CH), 3.47 [N(1)Me], 3.38 [N(3)Me], 2.34 (d, J 6 Hz, 4a-CH₂), 2.30 (7- and 8-Me), and 2.06 (5-Ac). Product (B) was identified as 5-acetyl-10-allyl-5,10-dihydro-1,3,7,8-tetramethylalloxazine (3; R = allyl) (Found: C, 64.4; H, 6.3; N, 15.8; O, 13.6. $C_{19}H_{22}N_4O_3$ requires C, 63.9; H, 6.1; N, 15.8; O, 13.9%); $\nu_{\max.}$ (KBr) 1706 [C(4)=O], 1675 [C(2)=O], and 1661 (Ac) cm^{-1} ; $\lambda_{\max.}$ (MeOH) 300 and 248 nm ($\log \epsilon$ 3.43 and 3.82); δ (CDCl₃) 7.35 and 6.9 (6- and 9-H), 4.95—6.61 (CH₂=CH-), 4.15 (d, J 6 Hz, 10-CH₂), 3.51 [N(1)Me], 3.38 [N(3)Me], 2.24 (7- and 8-Me), and 2.15 (5-Ac) p.p.m.

Acid Hydrolysis of Compounds (3) and (4) (R = benzyl).—A solution of compound (3; R = benzyl) (0.2 g) in ethyl alcohol (10 ml) was flushed with nitrogen for 20—30 min and then a solution of tin(II) chloride (0.05 g) in concentrated hydrochloric acid (10 ml) was slowly added while the mixture was maintained at 80° under nitrogen and in the dark. The reaction was followed by t.l.c. The product could be identified as a dark green fluorescent spot (R_F 0.9) which decomposed to yield a blue fluorescent spot upon u.v. irradiation. The reaction was complete after 1.5 h; the product was precipitated under nitrogen with 0.5M-borate buffer of pH 9 (100 ml) which had previously been flushed with nitrogen. Precipitation was completed at 0° during 2 h. The precipitate was rapidly filtered off, washed successively with 0.5M-phosphate buffer and water, and re-

crystallised from acetone-water to yield a pale yellow product (0.09 g), m.p. 240—245° (decomp.). The foregoing conditions were also used for hydrolysis of compound (4), which gave 0.9 g. of recrystallised product, identical (m.p., i.r. and n.m.r. spectra, and t.l.c.) with the product from hydrolysis of compound (2), and identified as 4a-benzyl-4a,5-dihydro-1,3,7,8-tetramethylalloxazine (5; R = benzyl) (Found: C, 69.6; H, 6.1; N, 15.5; O, 8.8. $C_{21}H_{22}N_4O_2$ requires C, 68.6; H, 6.7; N, 15.0; O, 9.4%); $\nu_{\max.}$ (KBr) 3356 (N-H), 1730 [C(4)=O], and 1689 [C(2)=O] cm^{-1} ; $\lambda_{\max.}$ (MeOH) 360, 253, and 220 nm ($\log \epsilon$ 3.75, 4.34, and 4.38); $\lambda_{\max.}$ (6N-HCl) 395 nm ($\log \epsilon$ 3.58); δ (CDCl₃) 7.13 and 6.59 (6- and 9-H), 7.2—7.35 (Ph), 4.68 (5-H), 3.26 [N(1)Me], 3.06 (q, J_{AB} 12 Hz, 4a-CH₂), and 2.23 (7- and 8-Me) p.p.m.

Acid Hydrolysis of Compound (3; R = allyl).—The conditions were the same as for compound (3; R = benzyl). The product was precipitated with 0.5M-sodium phosphate buffer (pH 7) and washed once more with phosphate buffer and water. The precipitate was recrystallised from methanol [double m.p. 143—145 and 210—230° (decomp.)] and identified as 4a-allyl-4a,5-dihydro-1,3,7,8-tetramethylalloxazine (5; R = allyl) (Found: C, 65.4; H, 6.5; N, 17.9; O, 10.2. $C_{17}H_{20}N_4O_2$ requires C, 65.4; H, 6.6; N, 18.3; O, 10.6%); $\nu_{\max.}$ (KBr) 3360 (N-H), 1730 [C(4)=O], and 1672 [C(2)=O] cm^{-1} ; $\lambda_{\max.}$ (MeOH) 362, 225, and 220sh nm ($\log \epsilon$ 3.67, 4.32, and 4.45); $\lambda_{\max.}$ (6N-HCl) 395, 305, and 268 nm ($\log \epsilon$ 3.58, 3.83, and 4.19); δ (CDCl₃) 7.03 and 6.52 (6- and 9-H), 5.1—5.3 (CH₂=CH), 4.65 (5-H), 3.50 [N(3)Me], 3.32 [N(1)Me], 2.55 (m, J_{AX} 6 Hz, 4a-CH₂), and 2.20 (7- and 8-Me) p.p.m.

Oxidative Hydrolysis of Compound (3; R = allyl) to 10-Allyl-1,3,7,8-tetramethylflavinium Perchlorate (6).—Sodium nitrite (0.2 g) in m-perchloric acid (8 ml) was added to a solution of compound (3; R = allyl) (0.2 g) in ethyl alcohol (2 ml). Yellow crystals started to form rapidly and complete precipitation of the product had occurred after 4 h at 0°. The crystals were filtered off and recrystallised from ethyl alcohol-perchloric acid (2N) (1:1); yield 0.2 g, m.p. 198—199° (Found: C, 49.7; H, 4.7; N, 13.6. $C_{17}H_{19}ClN_4O_6$ requires C, 49.5; H, 4.7; N, 14.0%); $\nu_{\max.}$ (KBr) 1740 and 1705 cm^{-1} (C=O); $\lambda_{\max.}$ (MeOH) 395 nm ($\log \epsilon$ 4.30).

Alkylation of Compound (2) with Methyl Iodide.—Methyl iodide (6.4 ml, 14.4 g) in dimethylformamide (10 ml) was added slowly to a suspension of compound (2) (8.0 g) and potassium carbonate (16 g) in dimethylformamide (200 ml) at 80° and under nitrogen. The reaction was followed by t.l.c. After 4—5 h the mixture was poured into water (300 ml) and adjusted to pH 5 with acetic acid. The precipitate was filtered off, washed with water, and dried under vacuum. The product showed two non-fluorescent and one blue fluorescent components [identified as compound (1)] on t.l.c. The combined filtrates were extracted with chloroform. The extract was washed with 0.1N-sodium hydroxide, 0.1N-acetic acid, and water, dried (K₂CO₃), and evaporated. The combined residues were crystallised from chloroform-di-isopropyl ether to give pure product (A) (3.7 g), m.p. 235—241°. Fractional crystallisation of the material in the filtrate by addition of chloroform-di-isopropyl ether and then methanol-water produced a further 2.0 g of product (A) (total 5.7 g) and then 0.4 g of a second product (B), m.p. 203—205°. Product (A) was identical (m.p., $\nu_{\max.}$ and $\lambda_{\max.}$) with 5-acetyl-5,10-dihydro-1,3,7,8,10-pentamethyl alloxazine.³ Product (B) was identified as 5-acetyl-4a,5-dihydro-1,3,4a,7,8-pentamethyl alloxazine (4; R = Me) (Found: C, 61.9; H, 6.2;

N, 16.9. $C_{17}H_{20}N_4O_3$ requires C, 62.2; H, 6.1; N, 17.1%; $\nu_{\max.}$ (KBr) 1730, [C(4)=O], 1683 [C(2)=O], 1675 (Ac) cm^{-1} ; $\lambda_{\max.}$ (MeOH) 303 nm (log ϵ 4.18); $\lambda_{\max.}$ (6N-HCl) 305 nm (log ϵ 4.12); δ (CDCl₃) 7.20 and 7.10 (6- and 9-H), 3.48 [N(1)Me], 3.39 [N(3)Me], 2.30 (7- and 8-Me), 2.03 (5-Ac), and 1.35 (4a-Me) p.p.m.

Benzylation of 5-Acetyl-5,10-dihydro-3,7,8,10-tetramethylalloxazine (8).—The procedure for benzylation of compound (2) was repeated except that the temperature was kept at 20–25°. Only one product was detected by t.l.c.; it was isolated and identified as the 2-O-benzyl derivative (9; R = benzyl), m.p. 213–216° (from ethanol–water). No O(4) or C(4a) addition product could be detected (Found: C, 68.0; H, 5.8; N, 14.0. $C_{23}H_{24}N_4O_3$ requires C, 68.3; H, 6.0; N, 13.9%); $\nu_{\max.}$ (KBr) 1680 and 1670 cm^{-1} ; $\lambda_{\max.}$ (MeOH) 308 and 245 nm (log ϵ 3.87 and 4.41); δ (CDCl₃) 7.38 and 6.73 (6- and 9-H), 5.49 (2-O-CH₂), and 3.42 and 3.38 (10- and 3-Me) p.p.m.

Benzylation of 5-Acetyl-5,10-dihydro-7,8,10-trimethylalloxazine [(8) with no 3-Me].—The alkylation was conducted similarly at room temperature and gave two main products in addition to some lumiflavin. The isomers were separated by fractional crystallisation from chloroform–di-isopropyl ether and acetone–water; they were the 2-O-benzyl-3-benzyl derivative, m.p. 228–230° (Found: C, 72.6; H, 5.6; N, 12.1. $C_{29}H_{28}N_4O_3$ requires C, 72.5; H, 5.9; N, 11.7%); $\nu_{\max.}$ (KBr) 1680 and 1665 cm^{-1} ; $\lambda_{\max.}$ (MeOH) 315 and 248 nm (log ϵ 3.75 and 4.32); δ (CDCl₃) 5.41 (2-O-CH₂), 5.20 [N(3)-CH₂], and 3.33 [N(10)Me] p.p.m.; and the 2,4-di-O-benzyl derivative, m.p. 194–196° (Found: C, 72.2; H, 5.6; N, 11.8. $C_{29}H_{28}N_4O_3$ requires C, 72.5; H, 5.9; N, 11.7%); $\nu_{\max.}$ (KBr) 1630 cm^{-1} (Ac); $\lambda_{\max.}$ 297 and 212 (log ϵ 4.04 and 5.08); δ (CDCl₃) 5.48 (2-O-CH₂), 5.40 (4-O-CH₂), and 3.48 [N(10)Me] p.p.m.