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ACTIVATION AND TRANSFER OF OXYGEN—XI

AUTOXIDATIVE BEHAVIOUR OF SOME $N_{1,3,5}$-METHYLATED TETRAHYDROLUMAZINES AND DIHYDROALLOXAZINES

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Abstract—The *autoxidation* of $N_{1,3,5}$-methylated tetrahydrolumazines and dihydroalloxazines and the *covalent hydration* of the oxidized (6,7-dihydrolumazinium and alloxazinium) species are described. *Autoxidation* led to ring contractions in which either the original C_{4a}- or $C_{8a(10a)}$-atom became the spiro center. In *covalent hydration* of the $N_{1,3,5}$-methylated cationic species a bridge position was not appreciably attacked, in contrast with the $N_{1,3,8(10)}$-methylated series. Instead, the N_5-substituent was preferably attacked by HO^- to give N_5-hydroxymethyl transients decomposing into formaldehyde and N_5-dealkylated tetrahydrolumazines or dihydroalloxazines, respectively. 4a-Hydroxy derivatives were neither found as final products in the autoxidation nor in the covalent hydration.

The two types of ringcontraction suggest that in some peroxy intermediates both C_{4a} and $C_{8a(10a)}$ may be linked to oxygen. The ringcontractions and N_5-demethylation are conversions on the level of the pseudobase. They must be distinguished from two other new intramolecular nucleophilic rearrangements on *peroxide* level leading respectively to: (1) the conversion of the N_5-methyl into a N_5-formyl group, indicating that the peroxy group may migrate to the N_5-carbon; (2) a cleavage of the C_4–C_{4a} and N_3–C_4 bonds with the formation of CO_2 and a 2-oxo-3-ureido-tetrahydropyrazine derivative showing that the peroxy group may also be linked to C_4 to give other types of endoperoxides or cyclic peresters (peroxylactones).

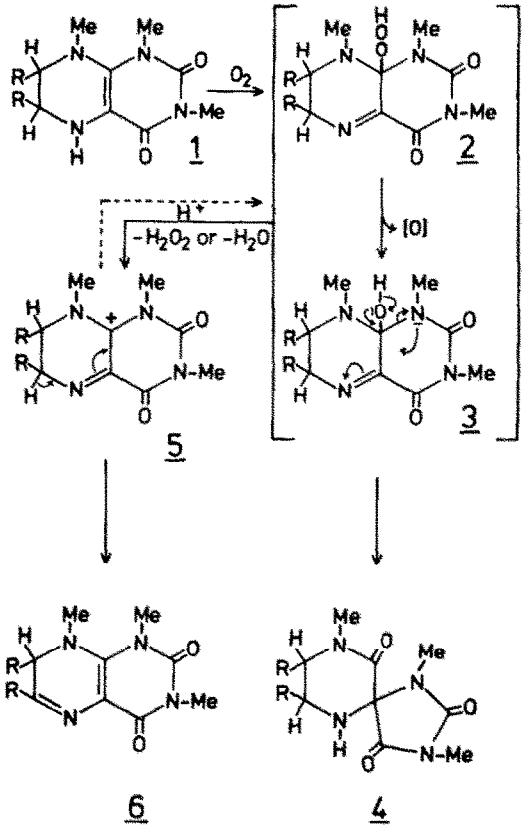
Nonenzymic hydroxylation of phenylalanine by the use of 1,3,5,7,8-pentamethyl-5,10-dihydroalloxazine was studied and the results compared with those obtained from a $N_{1,3,10}$-methylated derivative.

INTRODUCTION

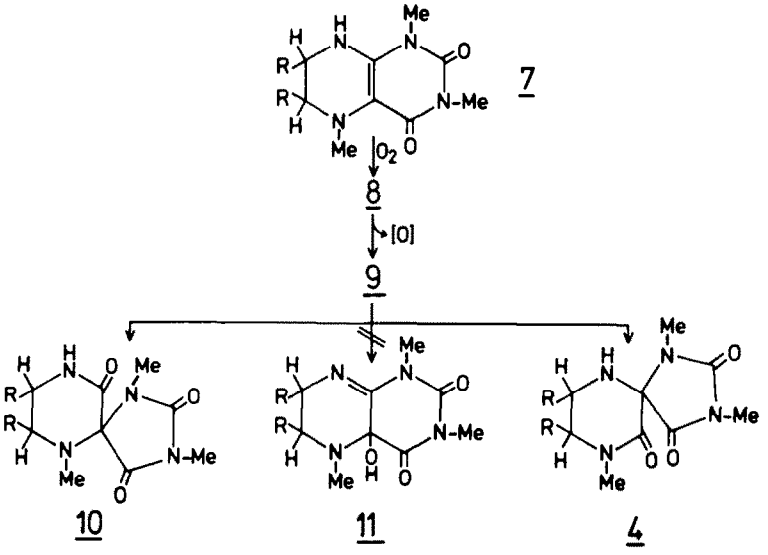
The structure of the reactive peroxy intermediates occurring in the spontaneous oxidation of partially reduced pteridines and alloxazines has been the subject of many speculations.[1-4] On studying derivatives substituted at N_1 and $N_{8(10)}$ some information was obtained as summarized in Scheme 1. Following the activation of O_2, the transfer of oxygen or the production of H_2O_2 may result in the formation of a transient pseudobase **3** carrying the HO-group at the 8a(10a)-position. Both the peroxy and monooxy intermediates appear to be in equilibrium with cationic species **5**, isolated in the alloxazinium series.[5] The presence of substituents at N_1 and $N_{8(10)}$ may also cause the process to end irreversibly in a ringcontracted product **4**. The oxygen introduced at the original $C_{8a(10a)}$ is derived from either the gasphase or the solvent,[6] while the original C_{4a}-atom becomes the spiro center in the piperazine- and tetrahydroquinoxaline-spiro-hydantoins found up to now.

When a Me group is substituted at N_5, the 4a-position in 6,7-dihydropteridinium ions is considered to be more electrophilic than the center 8a. Some authors have put forward[7] that the 4a,5-bond of these cationic species will be hydrated to give 4a-hydroxytetrahydropteridines analogous to the stable derivative isolated by Viscontini and Okada.[8] It has also been assumed[7] that such "hydrates" (cf. **11** in Scheme 2) could demethylate by splitting off methanol.

In extending our studies to N_5-methylated pteridines we tried to verify whether the presence of the N_5-Me group would really favour the formation of a 4a-hydroxy pseudobase like **11** either in a coupled oxidation or in a nucleophilic attack of the 4a-position. Our results are inconsistent with the assumptions mentioned above. In a previous paper[9] it has already been shown that the



Scheme 1. Autoxidation of $N_{1,3,5}$-methylated tetrahydrolumazines.



Scheme 2. Formation of piperazine-*spiro*-hydantoins from $N_{1,3,5}$-methylated tetrahydrolumazines.

product of Viscontini and Okada has been erroneously considered as a 4a-hydroxytetrahydropterin or a product on that level of oxidation. The present paper deals with some new rearrangements: (1) (C_{4a})-spirohydantoin formation in spite of the presence of a N_5-Me group; (2) ($C_{8a(10a)}$)-spirohydantoin formation which has not been found in the $N_{1,3,8(10)}$-methylated series; (3) N_5-demethylation with the production of formaldehyde; (4) some rearrangements on peroxide level. In addition the hydroxylating properties of a $N_{1,3,5}$-methylated dihydroalloxazine have been worked out in a comparative investigation.

RESULTS

(I) $N_{1,3,5}$-*methylated tetrahydrolumazines*

$N_{1,3,5}$-methylated tetrahydrolumazines **7** (R = -Me; -Ph) were prepared by catalytic hydrogenation of 1,3-dimethyllumazines **16** in the presence of formaldehyde. This principle to introduce a Me group at N_5 has already been applied in the pterin series.[10]

(1) (C_{4a})-*Spirohydantoin formation.* The pentamethyltetrahydrolumazine **7** (R = -Me) is considerably more stable towards O_2 than the N_8-Me isomer **1** (R = -Me). Nevertheless, autoxidation in water at room temperature and atmospheric pressure (cf. curve **a** in Fig. 1) slowly gave a product containing one additional O according to the mass spectrum and the elemental analysis. UV, IR and PMR spectra showed the characteristic features of a spirohydantoin. The compound was identified as the new spirohydantoin **10** (R = -Me). The structure was confirmed by X-ray analysis.[11] It can be well distinguished from the isomer **4** (R = -Me) by the PMR spectra (the new C=O group which arises on autoxidation specifically changes the chemical shift of the original $N_{5(8)}$-Me group to higher or lower field, respectively).

The rate of autoxidation can be increased on increasing the O_2-pressure. In combination with the decoupling effect of catalase,[1] autoxidation of **7** (R = -Me) in aqueous solution at an O_2-pressure of 100 atm in the presence of some catalase proved to be a convenient method for preparing **10**. At atmospheric pressure, autoxidation can be considerably accelerated by the use of catalytic amounts of Cu^{2+} (cf. Fig. 1).

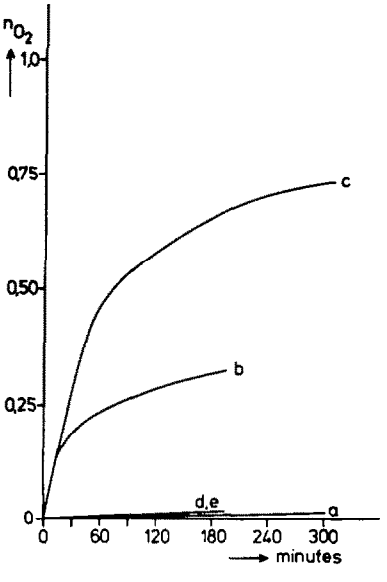
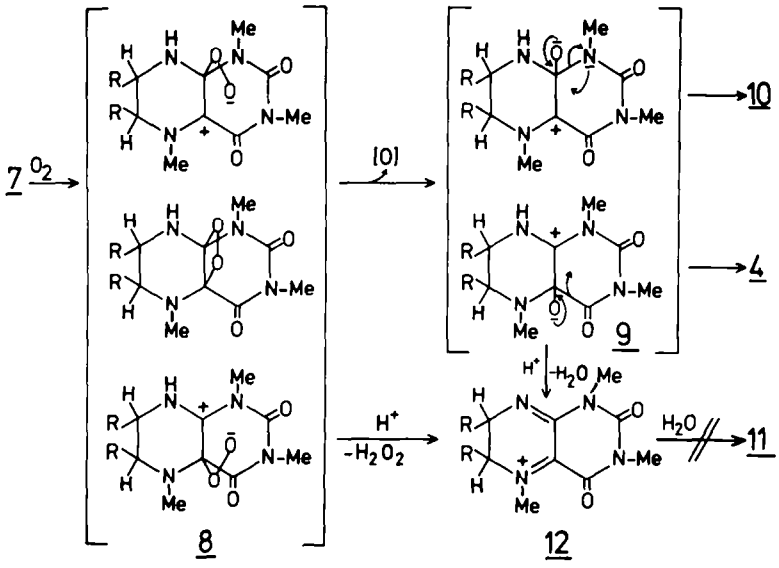


Fig. 1. (a) Autoxidation of **7** (R = -Me; 1 mmole) in water (50.0 ml) by 100% O_2 at 23° and atmospheric pressure, pH 6.0; (b) **a** + 0.05 mmole of $Cu(OAc)_2$, pH 6.0; (c) **a** + 0.1 mmole of $Cu(OAc)_2$, pH 6.0; (d) **a** + 0.1 mmole of $FeCl_3$, pH 2.9; (e) **a** + 0.1 mmole of $FeCl_3$, pH 6.0.

The autoxidative behaviour is also influenced by the substituents at the positions 6 and 7 as is indicated by the greater reactivity of the 6,7-diphenyltetrahydrolumazine **7** (R = -Ph). Unexpectedly, this water insoluble derivative is also more reactive towards O_2 than the N_8-Me isomer **1** (R = -Ph). Autoxidation in aqueous ethanol afforded a product identified as the (C_{4a})-spirohydantoin **10** (R = -Ph).

In conclusion, a 4a-hydroxy-tetrahydrolumazine **11** (Scheme 2) was not found in spite of the presence of a Me substituent at N_5. It has now appeared that a rearrangement on pseudobase level (**9** → **10**, Scheme 3) can take place, analogous to the one found in the N_5-unsubstituted series (Scheme 1).



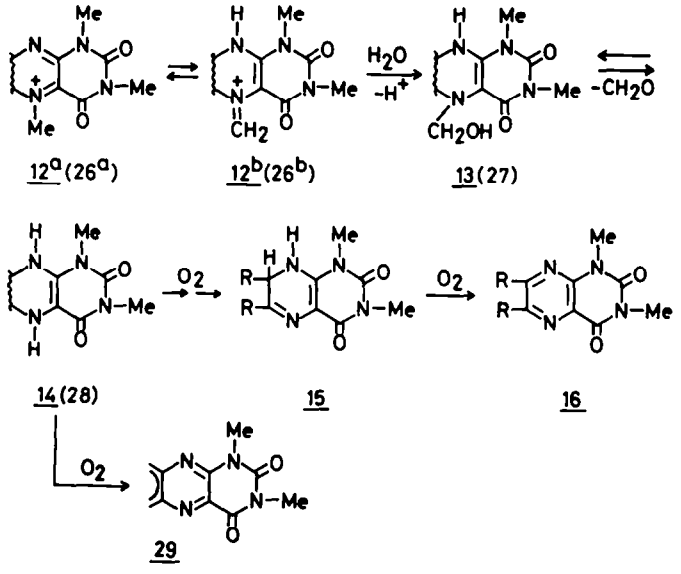
Schemes 3. Autoxidative spirohydantoin formations, conversions on pseudobase level.

(2) (C_{8a})-*Spirohydantoin formation.* The solvent effect on the ringcontraction is illustrated by performing the autoxidation of **7** (R = -Ph) in, e.g. anhydrous acetonitrile in which O_2 was taken up at a remarkably high rate. Surprisingly, the main crystalline product appeared to be the (C_{8a})-spirohydantoin **4** (R = -Ph). The same compound was also obtained in this laboratory by an unambigous synthesis.[12]

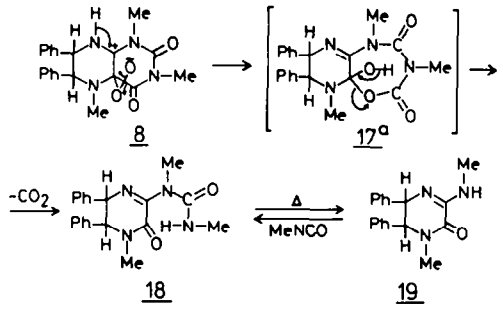
The above formation of spirohydantoin **4** represents an alternative rearrangement on the level of the monooxy transient. In this conversion the C_4–C_{4a} bond is broken, followed by a linking of C_4 and C_{8a} (**9**→**4**, Scheme 3).

(3) N_5-*Demethylation.* On varying the medium, N_5-demethylation may compete with the formation of spirohydantoins. In analogy with the hydrolytic conversion of the $N_{1,3,5}$-methylated alloxazinium cation **26** (Scheme 8), the initial reaction is considered to be a nucleophilic attack of HO^- at a 6,7-dihydropteridinium cation **12** (Scheme 3), *not at the* 4^a*-position, but at the* N_5*-substituent converting the latter into a hydroxymethyl group* (**13**, Scheme 4). Decomposition into the tetrahydrolumazine **14** and formaldehyde (determined by the use of dimedone) is followed by a spontaneous oxidation of the first to the 7,8-dihydrolumazine **15**, and subsequently to the corresponding lumazine **16**. For example, in non-buffered aqueous solution at an O_2-pressure of 1 atm, the ratio of the formation of spirohydantoin **10** (R = -Me) and the dealkylation was more than 10:1. In contrast, lumazine **16** and formaldehyde predominated in 0.1 N acetic acid, while the 7,8-dihydrolumazine **15** and formaldehyde were the main products in acetonitrile.

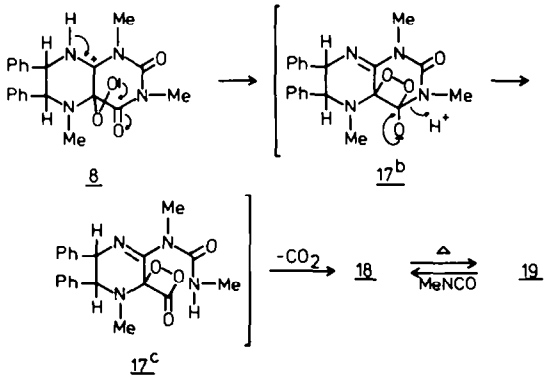
(4) *Rearrangements on peroxide level.* In acetonitrile, the ratio of the formation of spirohydantoin **4** (R = -Ph) and the dealkylation to the 7,8-dihydrolumazine **15** (R = -Ph) was about 3:1 with a combined yield of 60%. In addition, careful treatment of the mother liquor provided



Scheme 4. N_5-demethylation, a conversion on pseudobase level.



Scheme 5. Ringopening on peroxide level.



Scheme 6. Ringopening on peroxide level, an alternative reaction mechanism.

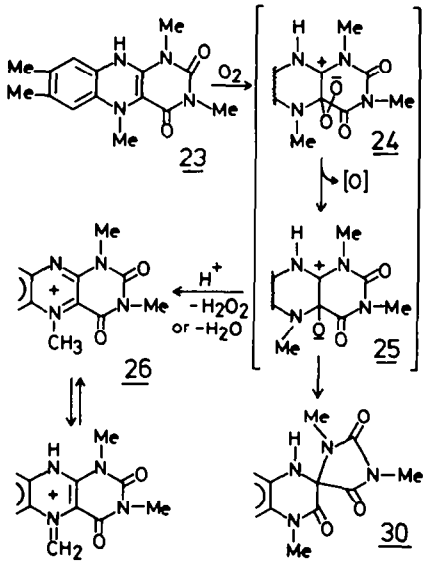
a crystalline side product of limited stability in a yield of 15%. It was identified as 1 - methyl - 3 - (N,N' - dimethylureido) - 2 - oxo - 5,6 - diphenyl - 1,2,5,6 - tetrahydropyrazine **18** (Scheme 5). In boiling chloroform, benzene, cyclohexane, etc. methylisocyanate was split off giving quantitatively 1-methyl-3-methylamino-2-oxo-5,6-diphenyl-1,2,5,6-tetrahydropyrazine **19**. The reverse reaction appeared to occur at room temperature on adding an excess of methylisocyanate to **19**.

The autoxidative conversion of the tetrahydrolumazine **7** into **18** and CO_2 is the result of an intramolecular nucleophilic rearrangement on peroxide level with a breaking of the N_3–C_4 and the C_4–C_{4a} bonds. This rearrangement might be either one of a "Criegee"-like type (**8** → **17**^{a} → **18**, Scheme 5) or one proceeding via a 4,4^a-endoperoxide **17**^{b} (Scheme 6) and a labile cyclic perester (α-peroxylacetone) **17**^{c}.

From the same acetonitrile reaction mixture another crystalline side product was isolated in 2–3% yield, identified as 5-formyl-1,3-dimethyl-6,7-diphenyl-5,6,7,8-tetrahydrolumazine **22** (Scheme 7). It is formulated as the result of an intramolecular nucleophilic rearrangement of a N_5-perhydroxymethyl derivative **20**, which arises either from an intramolecular migration of the peroxy group of transient **8** or from the addition of the nucleophile HOO^- to the cationic species **12**^{b}.

(II) 1,3,5,7,8-*Pentamethyl*-5,10-*Dihydroalloxazine*

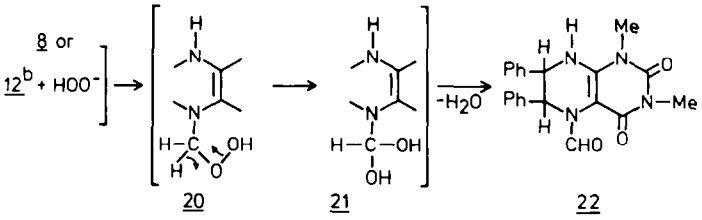
For comparison, the preparation and properties of some $N_{1,3,5}$-methylated-5,10-dihydroalloxazines were studied. Catalytic hydrogenation in the presence of formaldehyde also proved to be a convenient method for converting 1,3-dimethylalloxazines, e.g. 1,3-dimethyllumichrome into the 5 - methyl - 5,10 - dihydroalloxazine derivative **23** (Scheme 8).



Scheme 8. Autoxidation of 1,3,5,7,8-pentamethyl-5,10-dihydroalloxazine.

5-Methyl-5,10-dihydroalloxazines are considerably more reactive towards O_2 than 5-methyl-tetrahydrolumazines. Autoxidative formation of 4^a-hydroxy derivatives was not found. Dealkylation appeared to be the main process on autoxidation of **23** in non-acid aqueous media. Its mechanism has been extablished beyond doubt. In acid solution the cationic species **26** are quite stable and can be isolated on adding perchloric acid. Neutralizing an aqueous solution of this alloxazinium perchlorate immediately gives rise to the production of formaldehyde and 1,3-dimethyl-5,10-dihydrolumichrome **28** (Scheme 4). In the presence of O_2 the dealkylated species are rapidly oxidized to 1,3-dimethyl-lumichrome **29**, a process that has already been put forward in this paper as a model for the transformations of **12** → **13** → **14** → **15** in the pteridine series. The yield of formaldehyde may be decreased by secondary reactions like a H_2-generating peroxidation comparable or identical with the reaction of CH_2O and H_2O_2.[13]

The equilibrium between the N_5-hydroxymethyl and the N_5-demethylated intermediates (**27** ⇄ **28**) can be shifted to the right by trapping formaldehyde with dimedone. Consequently, the O_2-uptake occurring on neutralizing the



Scheme 7. Autoxidative formation of a N_5-formyl substituent, a conversion on peroxide level.

perchlorate of **26** in the presence of O_2 was considerably increased by the additional presence of dimedone (Fig. 2).

Nucleophilic attack of **26** by HO^- did not give a 4ª-hydroxy-dihydroalloxazine as was also observed by Ghisla and Hemmerich.[14] Further studies demonstrated that the dealkylation pathway can be displaced on changing the medium. For example, autoxidation of **23** in acetonitrile afforded spirohydantoin **30** (Scheme 8) in a yield of 50%, identical with the product obtained from the $N_{1,3,10}$-methylated isomer. However, in the present case it represents a new rearrangement as the C_4–C_{4a} bond is broken and C_4 and C_{10a} are linked transforming C_{10a} into the new spiro center.

(III) *Nonenzymic hydroxylation of phenylalanine*

Oxidative dealkylation of **26**→**29** (Scheme 4) in the presence of phenylalanine in the pH range of 5–7 gave aromatic hydroxylation in low yields, comparable with the results provided by other nonenzymic alloxazine (flavin) systems under the same conditions.[15] It was also reported[15] that efficient hydroxylations could be effected in acid media using 1,3,10-trimethyl-5,10-dihydroalloxazine. A comparative study has now been carried out on the hydroxylating ability of the HCl salt of **23** (Scheme 8) under aerobic and anaerobic conditions. The results are represented in Table 1. Summarizing it is concluded that in the system of **23**, HCl + 6 N H_2SO_4 aromatic hydroxylation can not well compete with coupled oxidation of **23** or the oxidation of Cl^- by the oxygenating radicals,[5,15,16] but that considerable improvements up to 80% of the theoretical yield can be obtained on decreasing the acid strength of the medium. Although the hydroxylating ability of 1,3,10-trimethyl-5,10-dihydroalloxazine could not be matched, these studies on the stoichiometry of the hydroxylation and on the distribution of the hydroxyphenylalanine isomers have shown that aerobic and anaerobic hydroxylations were accomplished by **23** by the same radical mechanisms.[2,15]

CONCLUSIONS

Extension of our studies to tetrahydrolumazines and dihydroalloxazines methylated at the $N_{1,3,5}$-positions has given new knowledge of autoxidative conversions on pseudobase- and peroxide-levels. It is concluded that the structures of the reactive transients may vary under influences of substituents and the nature of the medium, apart from the question which position has been primarily attacked by O_2.

In contrast with some assumptions put forward, in the literature[8,7] no 4ª-hydroxy derivatives were obtained, but ringcontractions as represented in Schemes 2, 3 and 8. A breaking of the $N_1 - C_{8a(10a)}$ bond and a linking of N_1 and C_{4a} was already found before,[1,2,16], but was unexpected in the $N_{1,3,5}$-methylated series in view of the presence of the N_5-Me group (**7**→**10**, Scheme 2). The breaking of the C_4–C_{4a} bond and the linking of C_4 and $C_{8a(10a)}$ is an alternative rearrangement, which is quite new and remarkable (**7**→**4** in Scheme 2; **23**→**30** in Scheme 8). The two types of ringcontraction suggest that in some peroxy intermediates both the C_{4a} and $C_{8a(10a)}$ bridge positions may be linked to oxygen.

Table 1. Hydroxylation of phenylalanine (4 mmoles) by **23**, HCl (1.0 mmole) in various acid media (50.0 ml) at 23°, stirred at 1200–1500 rpm for 4 hr (different conditions are indicated by an asterisk (*)). Excpt numbers preceeded by the Roman numeral IX refer to comparative experiments performed with 1,3,10-trimethyl-5,10-dihydroalloxazine as described in part IX of these series[15]

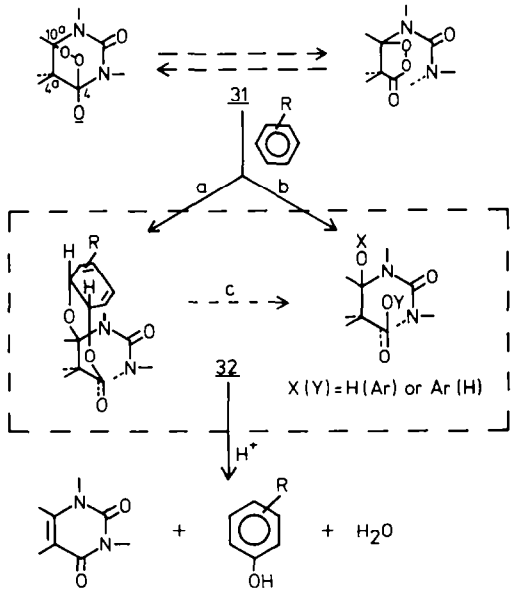
Expt No.	n_{O_2} (Δ_g)	$n_{H_2O_2}$	$n_{[O]_{calc}}$	$n_{[O]_{t+d}}$	$n_{[O]_t}$	o:m:p distr.	R_O^- value
Series 1: In 6N H_2SO_4 + air:							
1	0·65	0·23	0·07	0·03	0·03	43:31:26	0·17
2*	0·51	0	0·02	0·03	0·03	42:33:25	0·04
IX-3	0·67	0	0·34	0·32	0·31	44:32:24	0·51
Series 2: In 0·5N H_2SO_4 + air:							
3	0·83	0·58	0·08	0·11	0·10	44:33:23	0·32
4*	0·73	0·10	0·36	0·34	0·33	45:32:23	0·57
IX-40	0·86	0·41	0·31	0·32	0·31	44:30:26	0·69
Series 3: In 0·2N H_2SO_4 + air:							
5*	0·79	0·05	0·53	0·26	0·25	44:33:23	0·72
IX-53*	0·89	0·10	0·68	0·52	0·48	41:35:24	0·86
Series 4: In 0·2N $HClO_4$ + air:							
6*	0·78	0·24	0·32	0·26	0·26	42:36:22	0·59
7*	0·63	0·03	0·23	0·32	0·32	43:35:22	0·38
IX-58*	0·76	0·06	0·46	0·41	0·40	40:35:25	0·66
Series 5: In 6N H_2SO_4 + argon + addn of H_2O_2 (2 mmoles):							
8	(-0·03)	-1·11	0·05	0·06	0·06	45:32:23	0·05
IX-8	0	-1·58	0·58	0·59	0·58	45:31:24	0·37
Series 6: In 0·5N H_2SO_4 + argon + addn of H_2O_2 (15–16 mmoles):							
9	(-0·03)	-2·36	1·30	1·13	1·10	44:34:22	0·57
IX-47	0	-3·10	2·10	1·66	1·62	40:30:26	0·68
Series 7: In 0·2N $HClO_4$ + argon + addn of H_2O_2 (10–11 mmoles):							
10	(-0·07)	-2·68	1·54	1·60	1·52	45:33:22	0·61
11*	(-0·08)	-2·56	1·40	1·35	1·31	44:33:23	0·58
IX-61*	(-0·13)	-3·88	2·62	2·61	2·13	45:32:23	0·72

The structures of the reactive peroxides are probably not limited to the hydroperoxides and the dioxetane represented in Scheme 3. Autoxidations in neutral and basic media indicate that C_4 is another position which might be linked to the peroxy group. For example, the formation of **18** + CO_2 is the result of a cleavage of the N_3–C_4 and the C_4–C_{4a} bonds (Schemes 5 and 6), in the latter scheme formulated as an irreversible rearrangement of a 4–4[a] endoperoxide **17**[b] and a cyclic perester (α-peroxy lactone) **17**[c]. In the $N_{1,3,10}$-methylated alloxazinium series, C_4 has already appeared to be a reaction site besides C_{10a}.[15] The formation of the (C_{10a})-spirohydantoin could be suppressed by addition of H_2O_2 leading to a degradation of the alloxazine ringsystem with the generation of CO_2. In extension of Scheme 6, we now propose a possible occurrence of 4–8[a](10[a]) endoperoxides and β-peroxy lactones (cf. **31**, Scheme 9).

In aromatic hydroxylations the intermediacy of unstable alloxazine-oxy-substrate adducts has been observed.[2] Hydrolysis of these adducts gave rise to alloxazine (flavin) and hydroxylated substrate species. It is now questioned whether peroxides like **17**[b,c] (Scheme 6) and **31** (Scheme 9), *with either an unimpaired or broken* N_3–C_4 *bond*, are operative in the oxygen transfer. A homolytic or heterolytic O–O cleavage could lead to the intermediacy of "*di-adducts*". These are represented by the general formulae **32** (Scheme 9) in which *the* N_3–C_4 *bond could also be unimpaired or broken.* Intermediates with broken N_3–C_4 bonds could as well fit in with a flavin model, when hydrolysis involves a repair of the N_3–C_4 bond. Model adducts showing such a remarkable repair of the alloxazine (flavin) ringsystem have recently been isolated in this laboratory. The repair mechanism has been elucidated as a re-transformation of an imidazoloquinoxaline ringsystem, which will be dealt with in a subsequent paper.

The above hypothesis must be distinguished from the interesting suggestion[4] that flavoenzyme-catalyzed hydroxylations could be effected by α-carbonyl carbonyl oxide species, formed from a 4[a]-hydroperoxy flavin by cleavage of the C_{4a}–N_5 bond.

Another suggestion, that the hydroxylating reagent is a flavin oxaziridine[18], is inconsistent with our quantitative and mechanistic studies on autoxidations and aromatic hydroxylations.



Scheme 9. Aromatic hydroxylation, a proposal for the oxygen transfer by 4–10[a](4[a]) endoperoxides or $\beta(\alpha)$-peroxy lactones leading to the intermediacy of alloxazine(flavin)-oxy-substrate adducts.

EXPERIMENTAL

^{1}H NMR 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. Analytical hydrogenations and oxidations were carried out at atmospheric pressure and at an average temp. of 23° in all-glass manometric apparatus allowing continuous corrections for pressure and temp. changes. High pressure experiments were performed in vertical stainless autoclaves (of 250 ml or 1 L) in which the reaction mixtures were magnetically agitated.

Preparation of 1,3,5,6,7 - *pentamethyl* - 5,6,7,8 - *tetrahydro lumazine* (7, R = –Me). 1,3,6,7 - Tetramethyllumazine[17] (11.00 g, 50.00 mmol) was hydrogenated over Pt (500 mg PtO_2) in a mixture of 96% EtOH (135 ml), 35% aq CH_2O (15 ml) and conc HCl (6 ml) at room temp. and a starting H_2 pressure of 100 atm. for 2 hr. The catalyst was removed and the filtrate was evaporated to dryness *in vacuo*. Water (75–100 ml) was added, followed by $NaHCO_3$ and aq NaOH to adjust the pH to 7.4–7.5. A ppt was formed which was filtered off and discarded. The filtrate was extracted with $CHCl_3$ (12 × 25 ml). The extract was dried ($MgSO_4$) and evaporated to dryness *in vacuo*. Triturating the residue with AcOEt (10–15 ml) and filtrering gave a crystalline product. It was purified by continuous extraction with boiling cyclohexane (50 ml). Colourless crystals appearred from the extract, yield 10.1 g (85%) m.p. 170–171°. ($C_{11}H_{18}N_4O_2$ (238.30) Calcd: C, 55.44; H, 7.61; N, 23.51. Found: C, 55.6; H, 7.8; N, 23.4). Mass spectrum, *m/e* (%): 238 (M^+, 100); 223 (65); 208 (18); 195 (5); 193 (5); 181 (7); 166 (6); 153 (7); 138 (12). PMR ($CDCl_3$): δ = 0.83 (3, d, J = 7 Hz, C–Me); 1.28 (3, d, J = 7 Hz, C–Me); 2.63 (3, s, N_5-Me); 2.78 (1, m, C–H); 3.34 (1, s, N–Me); 3.47 (1, s, N–Me, overlapping 1 C–H); 5.14 (1, s, N–H). IR (KBr), cm^{-1}: 3360, 3310 (N–H); 1695, 1620 (C=O); 1610 (C=C). UV (1 N HCl), λ_{max} (ϵ): 264 nm (17,100). TLC (acetone), 10 cm: R_f = 0.55.

Preparation of 1,3,5 - *trimethyl* - 6,7 - *diphenyl* - 5,6,7,8 - *tetra hydrolumazine* (7, R = –Ph). 1,3 - Dimethyl - 6,7 - dipheny llumazine[17] (41.32 g, 120.0 mmol) was hydrogenated over Pt (2.0 g PtO_2) in a mixture of 96% EtOH (540 ml), 35% aq CH_2O (60 ml) and conc. HCl (40 ml) at room temp. and a starting H_2 pressure of 100 atm for 24 hr. The catalyst was removed and the filtrate was evaporated to dryness *in vacuo*. The residue was suspended in H_2O (800 ml) and filtered off. Conc. NH_4OH was added to the filtrate to adjust the pH to 7.1. The ppt formed was filtered off and washed thoroughly with AcOEt, yield 29.7 g (68%). It was purified by continuous extraction with boiling AcOEt (250 ml); colourless crystals appeared from the extract, yield 25.3 g (58%) m.p. 277–278°. ($C_{21}H_{22}N_4O_2$ (362.43) Calcd: C, 69.59; H, 6.12; N, 15.46. Found: C, 69.3; H, 5.9; N, 15.2). Mass spectrum, *m/e* (%): 362 (M^+, 100); 360 (5); 347 (3); 283 (10); 271 (17); 269 (3); 256 (9); 242 (3); 180 (83); 165 (11). PMR ($CDCl_3$): δ = 2.96 (3, s, N_5-Me); 3.34 (3, s, N–Me); 3.43 (3, s, N–Me); 4.02 (1, d, J = 3.5 Hz, C–H); 4.58 (1, d, J = 3.5 Hz, C–H); 4.75 (1, s, N–H, exchangeable); 6.41–6.62 (2, m, Ar–H); 6.92–7.35 (6, m, Ar–H). IR (KBr), cm^{-1}: 3260 (N–H); 1690, 1625 (C=O); 1605 (C=C). TLC ($CHCl_3$–AcOEt, 1 : 1), 10 cm: R_f = 0.45.

Preparation of 1,3,6,7-*tetramethyl*-7,8-*dihydrolumazine* (**15**, R = –Me) *by autoxidation of* **14** (R = –Me). 1,3,6,7-Tetramethyllumazine[17] (880 mg, 4.0 mmol) was hydrogenated over Pt (100 mg PtO_2) in H_2O (100 ml) at room temp. and atmospheric pressure. After 8 mmoles of H_2 were consumed the reaction mixture was evaporated to dryness *in vacuo*. MeCN (100 ml) was added, the catalyst was filtered off and the filtrate was stirred overnight under air in a manometric apparatus to oxidize **14** (R = –Me) giving both **15** (R = –Me) and **16** (R = –Me) according to

TLC. **16** (R = –Me) remained in soln while **15** (R = –Me) crystallized, yield 378 mg (43%) m.p. 223–224°. It was identical with the product obtained by autoxidative dealkylation of **7** (R = –Me) as described in the present paper. ($C_{10}H_{14}N_4O_2$ (222.25) Calcd: C, 54.04; H, 6.35: N, 25.21. Found: C, 54.1; H, 6.4; N, 25.2). Mass spectrum, *m/e* (%): 222 (M⁺, 51); 220 (36); 207 (100); 194 (7); 191 (5); 101 (5); 164 (4); 150 (13); 135 (10); 122 (15); 108 (6). PMR (d_6-DMSO): δ = 1.16 (3, d, J = 7 Hz, C_7–Me); 2.00 (3, s, C_6–Me); 3.15 (3, s, N–Me); 3.27 (3, s, N–Me); 4.15 (1, q, J = 7 Hz, C–H): 7.45 (1, s, N–H, exchangeable). IR (KBr), cm^{-1}: 3240 (N–H); 1690, 1625 (C=O). TLC (acetone), 10 cm; R_f = 0.20; for comparison: TLC of **16** (R = –Me) with $CHCl_3$–AcOEt (4:1), 10 cm: R_f = 0.43.

Preparation of 1,3-*dimethyl*-6,7-*diphenyl*-7,8-*dihydrolumazine* (**15**, R = –Ph) *by autoxidation of* **14** (R = –Ph). 1,3-Dimethyl-6,7-diphenyllumazine[17] (1.72 g, 5.0 mmol) was hydrogenated over 10% Pd on charcoal (100 mg) in glacial AcOH (100 ml) at room temp. and atmospheric pressure. After 10 mmoles of H_2 was taken up the mixture was heated to the boil, the catalyst filtered off and washed, and the filtrate evaporated to dryness under reduced pressure. CH_3CN (50 ml) was added to the residue. The flask was attached to a manometric apparatus and the mixture was stirred under air overnight at room temp. to oxidize **14** to **15** (R = –Ph). The precipitate was filtered off and washed with CH_3CN, yield 1.30 g (75%). It was identical with the product, m.p. 282.5–283° (dec), obtained by autoxidative demethylation of **7** (R = –Ph) as described in the present paper. ($C_{20}H_{18}N_4O_2$ (346.39) Calcd: C, 69.35; H, 5.24; N, 16.17. Found: C, 69.3; H, 5.3; N, 16.3). Mass spectrum, *m/e* (%): 346 (M⁺, 100); 331 (4); 329 (7); 288 (12); 269 (54); 260 (7); 258 (9); 242 (7); 229 (50); 213 (14); 204 (10); 190 (7); 184 (17); 181 (10); 178 (18); 165 (8); 156 (6); 152 (5); 141 (8); 129 (11); 116 (14); 105 (28); 91 (13); 77 (23). PMR (CF_3COOD): d = 3.66 (3, s, N–Me); 3.75 (3, s, N–Me); 6.82 (1, s, C_7–H: it becomes a doublet when taken in CF_3COOH); 7.58–8.27 (10, m, Ar–H); 11.98 (1, s, exchanged N–H). IR (KBr), cm^{-1}: 3210 (N–H); 1700, 1625 (C=O). TLC (AcOEt–96% EtOH, 9:1), 10 cm: R_f = 0.80.

Preparation of 5-*formyl*-1,3-*dimethyl*-6,7-*diphenyl*-5,6,7,8-*tetrahydrolumazine* (**22**) *by formylation of* **14** (R = –Ph). In a 250 ml autoclave 1,3-dimethyl-6,7-diphenyllumazine[17] (5.00 g, 14.5 mmol) was hydrogenated over Pt (500 mg PtO_2) in a mixture of 96% EtOH (135 ml) and conc. HCl (10 ml) at room temp. and a starting H_2 pressure of 100 atm. After 24 hr the mixture was evaporated to dryness under reduced pressure. The residue was taken up in HCOOH (300 ml), the catalyst was filtered off, and Ac_2O (30 ml) was added to the filtrate. This mixture was stirred at room temp. for 0.5 hr and then evaporated to dryness under reduced pressure. Water (100 ml) and $CHCl_3$ (100 ml) were added to the residue, the liquid layers were separated and the aqueous layer was twice washed with 25 ml-portions of $CHCl_3$. The combined $CHCl_3$ extracts were dried ($MgSO_4$) and evaporated to dryness under reduced pressure. Triturating the residue with hot benzene (20 ml) and filtering gave the crude product (3.75 g). It was purified by continuous extraction with boiling benzene; colourless crystals separated from the extract with one mole of benzene of crystallization, yield 3.07 g (47%) m.p. between 155 and 165°, followed by solidification to the benzene-free product m.p. 241.5–242.5°. It was identical with the side-product **22** obtained in the autoxidation of **7** (R = –Ph) as described in the present paper. ($C_{21}H_{20}N_4O_3 \cdot C_6H_6$ (454.54) Calcd: C, 71.35; H, 5.77; N, 12.33. Found: C, 71.2; H, 5.8; N, 12.2). Mass spectrum, *m/e* (%): 376 (M⁺, 12); 348 (100); 271 (4); 269 (8); 257 (60); 242 (10); 200 (14); 180 (42); 165 (12); 91 (38); 77 (52). Dissolving the product in $CHCl_3$ and evaporating the solution to dryness resulted in the loss of the benzene of crystallization. PMR ($CDCl_3$): d = 3.40 (3, s, N–Me); 3.48 (3, s, N–Me); 4.80 (1, d, J = 4.5 Hz, C–H); 5.27 (1, s, N–H, exchangeable); 5.95 (1, d, J = 4.5 Hz, C–H); 6.58–7.40 (10, m, Ar–H); 8.90 (1, s, CHO). IR (KBr), cm^{-1}: 3280 (N–H); 1700, 1668, 1612 (C=O). TLC (AcOEt–96% EtOH, 9:1), 10 cm: R_f = 0.80.

Autoxidation of **7** (R = –Me) *under different conditions*

(a) *In aqueous solution at room temperature and atmospheric pressure. Isolation of* 1,5,6,1′,3′-*pentamethyl*-3-*oxopiperazine*-2-*spiro*-5′-*hydantoin* (**10**, R = –Me). A soln of **7** (R = –Me; 3.575 g, 15 mmol) in water (500 ml) was stirred under 100% O_2 at room temp. and atmospheric pressure. O_2 was taken up very slowly: n_{O_2} = 0.50 (7.5 mmoles of O_2 after 1.5 week), 0.75 (3 weeks) and 1.00 (6 weeks). Addition of dimedone to a sample from the reaction mixture showed the formation of some formaldehyde. Peroxide tests performed after the oxidation were negative ($n_{H_2O_2}$ = 0). The reaction mixture was concentrated *in vacuo* to a volume of about 25 ml and extracted with $CHCl_3$ (6 × 15 ml). The extract was dried ($MgSO_4$) and evaporated to dryness *in vacuo*. The residue (2.24 g) was dissolved in H_2O (2–3 ml) and the soln was kept at room temp. to give **10** (R = –Me) as colorless crystals, yield 1.64 g (43%) m.p. 151–152°. ($C_{11}H_{18}N_4O_3$ (254.29) Calcd: C, 51.95; H, 7.13; N, 22.03. Found: C, 52.0; H, 7.1; N, 22.1). Mass spectrum, *m/e* (%): 254 (M⁺, 100); 239 (3); 221 (3); 197 (8); 182 (7); 168 (15); 156 (27); 154 (24); 141 (8); 140 (8); 126 (23); 84 (11). PMR ($CDCl_3$): d = 1.10 (3, d, J = 6.5 Hz, C–Me); 1.19 (3, d, J = 6.5 Hz, C–Me); 2.21 (3, s, N–Me); 2.83 (3, s, N–Me); 3.03 (3, s, N–Me); 3.25–3.68 (1, m, C–H); 3.73–4.24 (1, m, C–H); 7.90 (1, s, broad, N–H, exchangeable). IR (KBr), cm⁻¹: 3200, 3070 (N–H); 1780, 1720, 1690 (C=O). UV: no maxima above 200 nm. TLC ($CHCl_3$–AcOEt, 4:1), 10 cm: R_f = 0.25. TLC (AcOEt), 10 cm: R_f = 0.60. TLC of **16** (R = –Me; $CHCl_3$–AcOEt, 4:1), 10 cm: R_f = 0.43. TLC of isomer **4** (R = –Me; AcOEt), 10 cm: R_f = 0.35.

The mother liquor remaining after the isolation of **10** (R = –Me) was evaporated to dryness *in vacuo*. Acetone (1 ml) was added and the soln was cooled at −20° to give some oxidized, dealkylated side-product, yield <0.1 g, identical with authentic **16** (R = –Me).

Note. Values of n_{O_2} = 1 and $n_{H_2O_2}$ = 0 imply that degradations or rearrangements on peroxide level had occurred to a considerable extent or that at least 50% of the starting compound has been oxidized in one or more secondary processes beyond the level of the 8ᵃ-hydroxy pseudobase. Then, products on the level of the monooxy transient like a spirohydantoin could be expected only in yields <50%.

(b) *In aqueous solution at room temperature and high* O_2-*pressure in the presence of catalase. Isolation of* **10** (R = –Me). **7** (R = –Me; 3.575 g, 15 mmol) was dissolved in H_2O (500 ml). Catalase (25 mg, 5000 Sigma units/mg) was added and oxidation was carried out at room temp. and a starting O_2-pressure of 100 atm for 24 hr. The conversion of the starting compound was established by TLC (96% EtOH). The reaction mixture was worked up as mentioned under (a). The yield of pure **10** (R = –Me) was 1.60 g (42%).

(c) *In aqueous solution at room temperature and high* O_2-*pressure in the absence of catalase. Isolation of* **10** (R = –Me). The experiments were carried out as mentioned under (b), but giving **10** (R = –Me) in considerably lower yields (14%).

(d) *In* 0.1 N *acetic acid at room temperature and high* O_2-*pressure. Oxidative dealkylation to* **16** (R = –Me). **7** (R = –Me; 1.190 g, 5.0 mmol) was dissolved in 0.1 N AcOH (75 ml) and oxidized at room temp. and a starting O_2-pressure of 100 atm for 24 hr. The excessive formation of CH_2O was shown by adding dimedone to a sample from the reaction mixture. TLC ($CHCl_3$–AcOEt, 4:1) demonstrated that **10** (R = –Me) had not been produced, but that **16** (R = –Me) was the main product. The reaction mixture was extracted with $CHCl_3$ (5 × 25 ml). The extract was dried ($MgSO_4$) and evaporated to dryness *in vacuo*. The residue was recrystallized from AcOEt to give **16** (R = –Me), yield 0.55 g (50%).

(e) *In aqueous solution at room temperature and atmospheric pressure catalyzed by* Cu^{2+}. *Isolation of* **10** (R = –Me). The results from analytical experiments (cf. Fig. 1) were worked out to a preparative procedure. A soln of **7** (R = –Me; 3.575 g, 15 mmol) and $Cu(OAc)_2 \cdot H_2O$ (90.0 mg, 0.45 mmol) in water (500 ml) was stirred under 100% O_2 at room temp. and atmospheric pressure until O_2-uptake came to an end (n_{O_2} = 0.73–0.75; $n_{H_2O_2}$ = 0). The reaction mixture was extracted with $CHCl_3$ (5 × 50 ml). The extract was dried ($MgSO_4$) and evaporated to dryness *in vacuo*. The residue (2.30 g) was dissolved in H_2O (2–3 ml) and this soln was kept at room temp. to give crystalline **10** (R = –Me), yield 1.30 g (34%).

(f) *In anhydrous acetonitrile at room temperature and atmospheric pressure. Oxidative dealkylation to* **15** (R = –Me). A soln of **7** (R = –Me; 1.190 g, 5.0 mmol) in anhyd CH_3CN (50 ml) was stirred under 100% O_2 at room temp. and atmospheric pressure until

O_2-uptake stopped. The O_2-consumption was fast but incomplete ($n_{O_2} = 0.35$–0.40; $n_{H_2O_2} = 0$). TLC (acetone) showed the presence of the starting compound ($R_f = 0.55$) and the dealkylated **15** (R = –Me; $R_f = 0.20$). The reaction mixture was evaporated to dryness *in vacuo* and AcOEt (3 ml) was added to the residue. A crystalline product was separated from the AcOEt soln, yield 165 mg (15%). It proved to be identical with authentic **15** (R = –Me) prepared by autoxidation of **14** (R = –Me). The AcOEt filtrate was evaporated to dryness *in vacuo* and the residue was dissolved in 96% EtOH (10 ml). 70% $HClO_4$(0.5 ml) was added to give the crystalline perchlorate of the starting compound **7** (R = –Me), yield 660 mg (39%).

Note 1. the incomplete O_2-uptake is probably due to the inhibitory effect of H_2O arising during the autoxidation. In similar experiments, the initial, rapid O_2-uptake occurring in CH_3CN solns could be immediately stopped at the start by introducing small amounts (0.5–1%) of water. *Note* 2. blanks proved that **7** (R = –Me) could be recovered from 96% EtOH soln as its perchlorate salt with 97% yield.

Autoxidation of **7** (R = –Ph) *under different conditions*

(a) *In aqueous ethanol at room temperature and atmospheric pressure catalyzed by* Cu^{2+}. *Isolation of* 1,3-*dimethyl*-6,7-*diphenyl*-7,8-*dihydrolumazine* (**15**, R = –Ph) *and* 1,1',3'-*trimethyl*-3-*oxo*-5,6-*diphenylpiperazine*-2-*spiro*-5'-*hydantoin* (**10**, R = –Ph). A soln of **7** (R = –Ph; 3.624 g, 10 mmol) and $Cu(OAc)_2 \cdot H_2O$ (99.8 mg, 0.5 mmol) in a mixture of water (250 ml) and 96% EtOH (250 ml) was stirred under 100% O_2 at room temp. and atmospheric pressure. The rapid O_2-consumption came to an end at a final uptake of 10 mmoles, while peroxide tests after the reaction were negative ($n_{O_2} = 1$ and $n_{H_2O_2} = 0$ imply that products on pseudobase level could be present only in yields lower than 50%). The reaction mixture was evaporated to dryness *in vacuo*. The residue was triturated with $CHCl_3$ (50 ml) and the insoluble, **15** (R = –Ph) was filtered off, yield 65 mg (2%). The $CHCl_3$ filtrate was evaporated to dryness *in vacuo*. The residue was dissolved in CH_3CN (2–5 ml). Cooling at –20° afforded crystalline **10** (R = –Ph), yield 1.03 g (27%)m.p. 250.5–251°. ($C_{21}H_{22}N_4O_3$ (378.44) Calcd: C, 66.65; H, 5.86; N, 14.81. Found: C, 66.8; H, 6.0; N, 15.0). Mass spectrum, *m/e* (%): 378 (M^+, 46); 259 (100); 242 (3); 230 (7); 224 (17); 216 (32); 208 (9); 179 (26); 165 (15); 154 (42); 145 (14); 126 (29); 120 (85); 104 (16); 91 (16); 77 (24). PMR ($CDCl_3$): $\delta = 2.06$ (3, s, N–Me); 3.01 (3, s, N–Me); 3.04 (3, s, N–Me); 4.80 (1, m, C–H); 5.32 (1, d, J = 5 Hz, C–H); 6.67–7.30 (11, m, 10 Ar–H overlapping 1 N–H). IR (KBr), cm^{-1}: 3250 (N–H); 1775, 1710, 1685 (C=O). TLC (AcOEt–$CHCl_3$, 1:1), 10 cm: $R_f = 0.75$.

(b) *In anhydrous acetonitrile at room temperature and atmospheric pressure. Isolation of*: 1,3 - *dimethyl* - 6,7 - *diphenyl* - 7,8 - *dihydrolumazine* (**15**, R = –Ph); 1,1',3' - *trimethyl* - 2 - *oxo* - 5,6 - *diphenylpiperazine* - 3 - *spiro* - 5' - *hydantoin* (**4**, R = –Ph); 1 - *methyl* - 3 - (N,N' - *dimethylureido*) - 2 - *oxo* - 5,6 - *diphenyl* - 1,2,5,6 - *tetrahydropyrazine* (**18**); *and* 5 - *formyl* - 1,3 - *dimethyl* - 6,7 - *diphenyl* - 5,6,7,8 - *tetrahydrolumazine* (**22**). A soln of **7** (R = –Ph; 7.248 g, 20.0 mmol) in anhyd CH_3CN (400 ml) was stirred under 100% O_2 at room temp. and atmospheric pressure. The rapid O_2-consumption ceased at a final uptake of 14.5 mmoles ($n_{O_2} = 0.73$), while peroxide tests after the reaction were negative ($n_{H_2O_2} = 0$). A crystalline compound could be filtered off and washed with acetonitrile, yield 1.20 g (17%). It proved to be pure **15** (R = –Ph) identical with the authentic compound obtained by autoxidation of **14** (R = –Ph).

The CH_3CN filtrate was evaporated to dryness under reduced pressure. Diethyl ether (250 ml) was added to the residue and the mixture was stirred at room temp. for 2 hr. Practically pure **4** (R = –Ph) could then be filtered off and washed with diethyl ether, yield 2.76 g (36%). (Note: higher amounts of product (3.0–3.1 g) were sometimes filtered off, but in those cases some **18** proved to be included). **4** (R = –Ph) was purified by continuous extraction with boiling cyclohexane, crystals appearing from the extract, m.p. 228–229°. ($C_{21}H_{22}N_4O_3$ (378.44) Calcd: C, 66.65; H, 5.86; N, 14.81. Found: C, 66.6; H, 6.1; N, 15.1). Mass spectrum, *m/e* (%): 378 (M^+, 56); 273 (75); 243 (5); 230 (7); 216 (100); 180 (70); 165 (7); 154 (13); 145 (4); 126 (6); 120 (21); 104 (9); 91 (13); 77 (8). PMR ($CDCl_3$): $\delta = 2.52$ (1, N–H, exchangeable); 2.96 (3, s, N–Me); 3.09 (3, s, N–Me); 3.13 (3, s, N–Me); 4.52 (1, d, J = 4.5 Hz, C–H); 5.06 (1, d, J = 4.5 Hz, C–H); 6.67–7.27 (10, m, Ar–H). IR (KBr), cm^{-1}: 3340 (N–H); 1785, 1723, 1660 (C=O). TLC ($CHCl_3$–AcOEt, 1:1), 10 cm: $R_f = 0.48$.

The diethyl ether filtrate obtained after the isolation of **4** (R = –Ph) was cooled off at –20° to give fractional crystallization of crude **18**, yield 0.94 g (13%), m.p. 126–127°. Careful recrystallization from CH_3CN (heating must be limited to short moments as **18** is easily converted into **19**) gave an analytically pure product, m.p. 130–130.5°, containing 0.5 mole of CH_3CN of crystallization. ($C_{20}H_{22}N_4O_2 \cdot \frac{1}{2}\,CH_3CN$ (370.96) Calcd: C, 68.00; H, 6.39; N, 17.00. Found: C, 67.8; H, 6.4; N, 17.0). Mass spectrum, *m/e* (%): 350 (M^+, 0.1); 293 (100); 278 (3); 216 (3); 202 (12); 193 (4); 188 (4); 178 (10); 167 (6) 165 (7); 152 (2); 146 (20); 131 (49); 120 (99); 118 (20); 104 (19); 91 (8); 89 (9); 77 (21). PMR ($CDCl_3$): $\delta = 2.03$ (1.5, s, 0.5 mole of CH_3CN); 2.88 (3, d, J = 5 Hz, N–Me); 3.18 (3, s, N–Me); 3.53 (3, s, N–Me); 4.72 (1, d, J = 6 Hz, C–H); 5.53 (1, d, J = 6 Hz, C–H); 6.75–7.00 (2, m, Ar–H); 7.00–7.58 (8, m, Ar–H). After evaporating the $CDCl_3$ soln to dryness under reduced pressure and dissolving the residue in $CDCl_3$ the same PMR spectrum was obtained but without $\delta = 2.03$. The addition of CF_3COOD transformed the N–Me doublet at $\delta = 2.88$ into a singlet. One exchanged H (N–H) was obtained in CF_3COOD. IR (KBr), cm^{-1}: 3320 (N–H); 1680, 1655, 1628 (C=O; C=N). TLC (AcOEt–$CHCl_3$, 1:1): $R_f = 0.20$.

Prolonged cooling of the diethyl ether filtrate at –20° provided another fraction with a higher m.p. (0.22 g, m.p. about 200°). This fraction which consisted of 3 components (TLC with $CHCl_3$–AcOEt, 1:1) was not subjected to further investigation. The diethyl ether filtrate was evaporated to dryness under reduced pressure. The residue was dissolved in benzene (5 ml) from which crystals appeared at room temp., yield 0.16 g (2%), identical with authentic **22** (containing 1 mole of C_6H_6 of crystallization) as obtained by reductive formylation of **14** (R = –Ph).

Preparation of 1 - *methyl* - 3 - *methylamino* - 2 - *oxo* - 5,6 - *diphenyl* - 1,2,5,6 - *tetrahydropyrazine* (**19**) *from* **18** *and reconversion of* **19** *into* **18**.

(a). A soln of **18**, $\frac{1}{2}CH_3CN$ (371 mg, 1 mmol) in cyclohexane (10 ml) was refluxed for about 1.5 hr. TLC ($CHCl_3$–AcOEt, 1:1) showed the conversion of **18** ($R_f = 0.20$) into **19** ($R_f = 0.52$). The crystalline product was filtered off and washed with cyclohexane, yield 0.24 g (82%) m.p. 140–140.5°. Recrystallization from cyclohexane did not raise the m.p. ($C_{18}H_{19}N_3O$ (293.37) Calcd: C, 73.69; H, 6.53; N, 14.32. Found: C, 73.8; H, 6.7; N, 14.3). Mass spectrum, *m/e* (%): 293 (M^+, 64); 278 (2); 216 (6); 202 (8); 188 (2); 180 (3); 178 (6); 165 (4); 161 (4); 146 (24); 131 (54); 120 (100); 118 (23); 104 (14); 91 (10); 77 (25). PMR ($CDCl_3$): $\delta = 2.94$ (3, d, J = 5 Hz, N–Me); 3.00 (3, s, N–Me); 4.56 (1, d, J = 5.5 Hz, C–H); 5.35 (1, d, J = 5.5 Hz, C–H); 6.10 (1, N–H, exchangeable on addition of D_2O and transforming the N–Me doublet at $\delta = 2.94$ into a singlet); 6.48–6.63 (2, m, Ar–H); 6.97–7.32 (8, m, Ar–H). IR (KBr), cm^{-1}: 3380 (N–H); 1725, 1670, 1628 (C=O; C=N).

(b). *Reconversion of* **19** *into* **18**. To a soln of **19** (100 mg, 0.34 mmol) in acetonitrile (3 ml) was added an excess of methyl isocyanate (1.5 ml) and the mixture was kept at room temp. The reconversion into **18** was followed by TLC ($CHCl_3$–AcOEt, 1:1). After standing overnight the reaction mixture was evaporated to dryness under reduced pressure and the residue recrystallized from CH_3CN (1 ml) to give **18**, $\frac{1}{2}CH_3CN$, yield 113 mg (90%).

1,3-*dimethyllumichrome* (**29**). Lumichrome (28 g, 0.12 mol) was methylated at room temp. using DMF (400 ml), anhydrous K_2CO_3 (56 g) and dimethyl sulfate (60 ml) by the procedure described for 1,3-dimethylalloxazine.[5] Yield 30 g (96%) m.p. 249–251°. Recrystallization from AcOEt improved the m.p. to 251–251.5°.

Preparation of 1,3,5,7,8 - *pentamethyl* - 5,10 - *dihydroalloxazine hydrochloride* (**23**, HCl). *Isolation of* 1,3,5,7,8-*pentamethylalloxaziniumperchlorate* (**26**, ClO_4^-)

1,3-Dimethyllumichrome (1.08 g, 4.0 mmol) was dissolved in conc. HCl (8 ml). To this soln was added 96% EtOH (192 ml), 35%aq CH_2O (40 ml) and 10% Pd on charcoal (200 mg). This

mixture was stirred under H_2 at room temp. and atmospheric pressure until H_2-uptake ceased at a final value of about 7 mmoles. The mixture was filtered off. The ppt was dissolved in conc. HCl (25 ml), filtered over a glass filter and washed with conc. HCl (15 ml). The ethanolic and conc. HCl filtrates were combined and evaporated to dryness under reduced pressure. The residue was dissolved in conc. HCl (2 ml) from which **23**, HCl crystallized. It was filtered off, washed with conc. HCl (2 ml) and an excess of diethyl ether and kept in a vacuum desiccator over P_2O_5 and KOH; yield 850 mg. A second crop was obtained by evaporating the mother liquor to dryness under reduced pressure and dissolving the residue in conc. HCl (1 ml). Overall yield 920 mg (71%) m.p. 232–233° (dec). ($C_{15}H_{19}N_4O_2Cl$ (322.81) Calcd: C, 55.81; H, 5.93; N, 17.36. Found: C, 55.0; H, 5.9; N, 17.3). UV (6 N HCl), λ_{max} (ϵ): 222 nm (22,700); 250 nm (11,300); 285 nm (10,400); 306 nm (10,200).

The filtrate obtained after the isolation of the second crop of **23**, HCl was evaporated to dryness under reduced pressure. The residue was dissolved in conc. HCl (1 ml) giving a clear soln. Water (11 ml) was added and the green-colored precipitate was filtered off and washed with 1 N HCl (10–15 ml) and diethylether. This fraction (yield 0.12–0.24 g after drying) was discarded. The combined filtrates were evaporated to dryness under reduced pressure and the residue was dissolved in 0.1 N HCl (10 ml). 70% $HClO_4$ (0.12 ml) was added drop by drop to give **26**, ClO_4^- which was filtered off and washed with 0.1 N $HClO_4$ (5 ml) and diethylether, yield 90 mg (6%) m.p. 240–242° (dec). ($C_{13}H_{17}N_4O_6Cl$ (384.79) Calcd: C, 46.82; H, 4.45; N, 14.56. Found: C, 46.7; H, 4.4; N, 14.4). Mass Spectrum, *m/e* (%): 285 (M^+, 34); 270 (100); 241 (15); 227 (2); 213 (7); 200 (2); 198 (2); 185 (41); 170 (6); 158 (79); 143 (4); 131 (12). PMR (CF_3COOD): δ = 2.70 (3, s, C-Me); 2.75 (3, s, C-Me); 3.64 (3, s, N-Me); 3.98 (3, s, N-Me); 5.25 (3, s, N-Me); 8.17 (1, s, Ar-H); 8.26 (1, s, Ar-H). UV (6 N HCl), λ_{max} (ϵ): 220 nm (27,100); 270 nm (49,400); 402 nm (15,500); 460 nm (6100).

Note 1. The compound **23** is very liable to autoxidation depending on the O_2-pressure and the nature of the medium. The rate of the O_2-uptake increases considerably on decreasing the acid strength of the medium. Therefore, the yields of **23**, HCl and **26**, ClO_4^- will become lower and higher, respectively, when the above procedure is not carried out carefully and rapidly.

Note 2. On a preparative scale **26**, ClO_4^- was conveniently prepared by spontaneous oxidation of **23** in HCl in the ways as described under "analytical autoxidations".

Note 3. The purity of **23**, HCl was tested by: (a) determining the material balance in analytical autoxidations; (b) the UV spectrum in 6 N HCl and its change into the spectrum of **26** on aeration.

Autoxidation of 1,3,5,7,8-*pentamethyl*-5,10-*dihydroalloxazine* (**23**) *under different conditions*

(a) *In* 0.1 *and* 6 N HCl *as test for the purity of the compound. (Check of the material balance eq* $n_{[O]} = 2n_{O_2} - n_{H_2O_2} - 1$; *note*: 6 N HCl *is preferred as no* H_2O_2 *is present after the autoxidation*[16]). (a_1) A soln of **23**, HCl (322.8 mg, 1 mmol) in 0.1 N HCl (50.0 ml) was stirred under air at room temp. and atmospheric pressure. The rapid O_2-uptake came to an end after about 20 mins at a final value of $n_{O_2} = 0.77$, while H_2O_2 proved to be present. The reaction mixture was filtered off, any 1,3-dimethyllumichrome (0–20 mg) on the filter was washed with 0.1 N HCl (5 ml) and 70% $HClO_4$ (0.25 ml) was added to the filtrate. **26**, ClO_4^- was filtered off and washed with 0.1 N $HClO_4$ (10 ml) and diethylether, yield 285 mg (74%) m.p. 240–241° (dec). The acid filtrates were combined and subjected to the usual manometric peroxide determination[5] giving the value $n_{H_2O_2} = 0.53$ which is quite consistent with the value calculated from the material balance eq for $n_{[O]} = 0$.

(a_2) A soln of **23**, HCl (322.8 mg, 1 mmol) in 6 N HCl (50.0 ml) was stirred under 100% O_2 at room temp. and atmospheric pressure. The O_2-uptake came to an end after 3 hr at a final value of 0.5 mmole ($n_{O_2} = 0.50$), while no peroxide could be detected afterwards ($n_{H_2O_2} = 0$). (Similar experiments carried out under air proceeded considerably slower and had to be stirred overnight). The reaction mixture was evaporated to dryness under reduced pressure. The residue was dissolved in 0.1 N HCl (10 ml), any 1,3-dimethyllumichrome (0–20 mg) was filtered off and 70% $HClO_4$ (0.10–0.15 ml) was added drop by drop to give **26**, ClO_4^-, yield 335 mg (87%).

(b) *In aqueous solutions in dependence on the acid strength of the media*; *autoxidative* N_5-*dealkylation.* Autoxidation of **23**, HCl in aq media at pH 2→4 did not lead exclusively to the formation of **26**, but in addition to increasing amounts of 1,3-dimethyllumichrome and CH_2O. This autoxidative dealkylation is the main process at pH ≥ 5. The yield of CH_2O may be lowered by secondary processes. Example: A mixture of **23**, HCl (322.8 mg, 1 mmol) in aq 0.5 M sodium phosphate (50.0 ml, pH = 7.00) was stirred under 100% O_2 at room temp. and atmospheric pressure until the gas-volume did not change any more ($\Delta_g = 0.97$). Catalase (5 mg) was added to determine the amount of H_2O_2 ($n_{H_2O_2} = 0.41$). 1,3-Dimethyllumichrome was filtered off and washed with water (5 ml), yield 224 mg (83%) m.p. 250–251°. A soln of dimedone (0.88 g) in aq 0.5 M sodium phosphate (25 ml, pH = 6.50) was added to the mother liquor. Methylene bismethone was filtered off and washed with water (10 ml), yield 125 mg (43%) m.p. 190–190.5° (cf. the oxidative dealkylation of **26**, ClO_4^-).

(c) *In acetonitrile* +K_2CO_3: *isolation of* 3 - *oxo* - 4,6,7,1',3' - *pentamethyl* - 1,2,3,4 - *tetrahydroquinoxaline* - 2 - *spiro* - 5' - *hydantoin* (**30**). A mixture of **23**, HCl (3.228 g, 10 mmol), anhyd K_2CO_3 (10 g) and anhyd MeCN (250 ml) was stirred under 100% O_2 at room temp. and atmospheric pressure until the rapid O_2-uptake ceased. The reaction mixture was filtered off and washed with MeCN (20 ml). The filtrate was evaporated to dryness under reduced pressure. The residue (2.87 g) was dissolved in some $CHCl_3$ and the soln transferred to a silicagel column (4.5 cm in diameter; 300 g silicagel (Merck), 0.06–0.20 mm in diameter). Elution was performed with $CHCl_3$ and controlled by TLC with AcOEt–$CHCl_3$ (1:9) as a solvent. The spirohydantoin containing fraction was evaporated to dryness under reduced pressure and the residue triturated with AcOEt to give crystalline **30**, yield 1.24 g (41%) m.p. 205–206° ($C_{15}H_{18}N_4O_3$ (302.34) Calcd: C, 59.59; H, 6.00; N, 18.53. Found: C, 59.8; H, 6.1; N, 18.6) identical with the product obtained by neutralizing an aq soln of 1,3,7,8,10-pentamethyl-alloxazinium perchlorate.[19]

Oxidative dealkylation of **26**, ClO_4 (cf. Fig. 2)

(1) *In the absence of dimedone.* A mixture of **26**, ClO_4^- (385 mg, 1 mmol) in aq 0.5 M sodium phosphate buffer (50.0 ml, pH = 6.50) was stirred under 100% O_2 at room temp. and atmospheric pressure (curve 1). After stirring overnight the gas-volume did not change any more ($\Delta_g = 0.70$). The pH was adjusted to 7.00 and catalase (5 mg) was added to determine the amount of H_2O_2 ($n_{H_2O_2} = 0.49$). 1,3-Dimethyllumichrome was filtered off and washed with water (5 ml), yield 240 mg (89%) m.p. 250–251°. A soln of dimedone (0.88 g) in aq 0.5 M sodium phosphate buffer

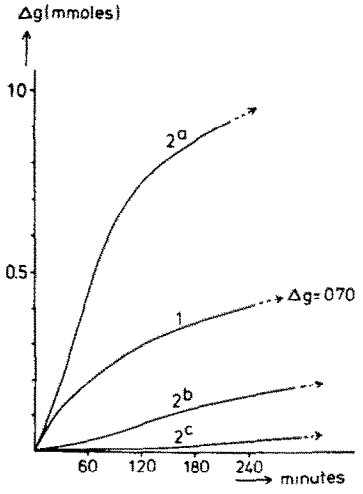


Fig. 2. Autoxidative demethylation of **26** in the absence (curve 1) and presence of dimedone (curve 2ª) and in comparison with the O_2-uptake by blanks of dimedone + H_2O_2 (curves 2ᵇ and 2ᶜ).

(25 ml, pH = 6.50) was added to the filtrate and the pH of the reaction mixture was adjusted to 6.50. Methylene bismethone was filtered off and washed with water (10 ml), yield 205 mg (70%) m.p. 188–189°, indicating that some CH_2O had disappeared in secondary reactions, but to a less extent than in the autoxidation of **23**, HCl under the same conditions.

(2*) *In the presence of an excess of dimedone.* **26**, ClO_4^- (385 mg, 1 mmol) was added to a soln of dimedone (1.75 g, 12.48 mmol) in aq 0.5 M sodium phosphate buffer (50.0 ml, pH = 6.50). The mixture was stirred under 100% O_2 at room temp. and atmospheric pressure (curve 2*) showing the effect of dimedone on the equilibrium **27**⇄**28** and therefore on the O_2-uptake. After stirring overnight the reaction mixture was worked up although the O_2-consumption had not yet ceased ($\Delta_g = 1.40$ after 24 hr, while addn of 5 mg of catalase gave $n_{H_2O_2} = 0.35$. The continued O_2-uptake and H_2O_2-consumption, however, were no longer connected with the oxidative dealkylation of **26** as appeared from the blanks mentioned below (cf. curves 2[b] and 2[c]). The precipitate consisting of 1,3-dimethyllumichrome and methylene bismethone was filtered off and washed with water (5 ml), yield 535 mg (95%). The components were separated by suspending the precipitate in aq 0.5 N NaOH (5 ml). The insoluble 1,3-dimethyllumichrome was filtered off and washed with water (10 ml), 0.1 N AcOH (10 ml) and water (10 ml), yield 235 mg (87%) m.p. 251–252°. The pH of the combined filtrates was adjusted to 5.0 by adding AcOH giving methylene bismethone, which was filtered off and washed with water (20 ml), yield 256 mg (88%) m.p. 188–189°. These results showed that CH_2O was not further converted under these conditions.

(2[b]) *Blank experiment (curve* 2[b]). 1,3-Dimethyllumichrome (270 mg, 1 mmol), CH_2O (1 mmol) and H_2O_2 (1 mmol) were added to a soln of dimedone (1.75 g, 12.48 mmol) in aq 0.5 M sodium phosphate buffer (50.0 ml, pH = 6.50) and stirred under 100% O_2 at room temp. and atmospheric pressure. Comparative expts showed that a reaction of dimedone and H_2O_2 may cause an O_2-uptake which is not connected with the actual oxidative dealkylation of **26** (curve 2[b], $\Delta_g = 0.38$ after 24 hr).

(2[c]) *Blank experiment (curve* 2[c]). H_2O_2 (1 mmol) was added to a soln of dimedone (1.75 g, 12.48 mmol) in aq 0.5 M sodium phosphate buffer (50.0 ml, pH = 6.50). The mixture was stirred under 100% O_2 at room temp. and atmospheric pressure: $\Delta_g = 0.25$ after 24 hr.

(3) *In the presence of phenylalanine.* **26**, ClO_4^- (385 mg, 1 mmol) was added to a saturated soln of phenylalanine in 0.5 M aq sodium phosphate (50.0 ml, pH = 5.00) changing the pH into 5.28. The mixture was stirred under air at room temp. and atmospheric pressure showing a slow oxidation. The gas-volume did not change any more after 4–5 days of stirring ($\Delta_g = 0.79$ and $n_{H_2O_2} = 0.02$). 1,3-Dimethyllumichrome was filtered off and washed with 1 N H_2SO_4 (10 ml) and water, yield 260 mg (96%). The mother liquor and the washings were not combined. Methylene bismethone was obtained from an aliquot of the mother liquor in the way as mentioned above, corrected yield 112 mg (38%). Another aliquot of the mother liquor was analyzed for mono- and dihydroxy-phenylalanines: $n_{[O]_I} = 0.05$ (o:m:p = 40:30:30) and $n_{[O]_{I-d}} = 0.06$. The low yield of aromatic hydroxylation in this pH-range has also been shown by other nonenzymic alloxazine (flavin) systems.[15]

Nonenzymic aromatic hydroxylations of phenylalanine effected by **23**, HCl *in 6–0.2 N acid media. Summary of the results (Table 1)*

The experiments were carried out as described in part IX of these series.[15] Different conditions for: (a) expts 2*, 4*, IX-53*, 6* and IX-58* (not stirred for 3 days); (b) expts 5* and 7* (not stirred for 10 days); (c) expts 11* and IX-61* (stirred overnight).

Series 1–4. *Aerobic hydroxylations* in 6 N H_2SO_4 (expt 1) gave low yields in contrast with the results given by expt IX-3, which are consistent with the theoretical values for a disproportionation of the transient hydroxy- or alloxazine-oxy- cyclohexadienyl radicals.[2,15] The results of expt 1 could not be improved by leaving the reaction mixture unstirred for 3 days (expt 2*). It is concluded that in the system **23**, HCl + 6 N H_2SO_4 aromatic hydroxylation can not well compete with the coupled oxidation of **23** or the oxidation of Cl^- by the oxygenating radicals.[5,15] As expected[15] better results were obtained on decreasing the acid strength of the medium (expt 3) and on leaving the reaction mixture unstirred for 3 days (expt 4*). No further improvements were observed in 0.2 N acid media (series 3 and 4) and on leaving the reaction mixtures unstirred for more than 3 days (expts 5* and 7*).

Series 5–7. *Anaerobic hydroxylation* was also inefficient in 6 N H_2SO_4 (cf. expts 8 and IX-8), but considerably improved on decreasing the acid strength of the medium (series 6 and 7). The oxygen transfer shown by expt 9 ($n_{[O]_{calc}} = 1.30$) was 65% of the theoretical value to be expected for a peroxidation of the hydroxy- or alloxazine-oxy- cyclohexadienyl radicals.[2,15] In dilute $HClO_4$ (expt 10) the yield was increased to 80%, which was not further improved on stirring the mixture overnight (expt 11*). In these cases the yields did not exceed the theoretical value as in expt IX-61*, due to some one-electron processes producing further oxygenating radicals from the excess of H_2O_2.[2,15]

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