

CHEMILUMINESCENCE—II

THE AUTOXIDATION PRODUCT OF 7-HYDROXY-7,8,10-TRIMETHYL-6,7-DIHYDROISOALLOXAZINE

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Abstract—The emission spectrum of the chemiluminescent autoxidation of 7-hydroxy-7,8,10-trimethyl-6,7-dihydroisalloxazine(1) has been determined. The compound showing an identical fluorescence spectrum has been isolated. Structure 2 has been assigned to this emitting species. A number of derivatives are described.

In a preceding paper¹ we described the synthesis of 7-hydroxy-7,8,10-trimethyl-6,7-dihydroisalloxazine(1). We reported that the reaction of 1 with oxygen in aqueous alkaline solution is accompanied by chemiluminescence.

The chemiluminescence spectrum, represented in Fig 1a, is in close agreement with the fluorescence emission spectrum of the reaction mixture after chemiluminescence (Fig 1b). This fluorescence spectrum (Fig 1b) proved to be identical with the fluorescence spectrum given by a compound which was isolated and further studied.

In this paper evidence is provided, that structure 2 (Scheme 1) is correct for this compound, formed in the excited state in the chemiluminescent reaction.

Results and discussion (cf Scheme 2)

(1) Comparison of the NMR spectra of 2 and 1 shows that in 2 the C₆-Me group has disappeared.

(2) Catalytic hydrogenation of 2 appears to give a cyclohexanotetrahydropteridine(2a). The reaction mixture, after removal of catalyst, was autoxidised in alkaline medium. It is known^{2,3} that such autoxidation rapidly leads to a 7,8-dihydropteridine(2b). Further oxidation and dehydration in acid medium finally yielded 7,10-dimethylisalloxazine(9). The structure of 9 was confirmed by an independent synthesis.

The isolation of 9 is in agreement with the observation made under (1).

(3) Comparison of the NMR spectra of 2 and the methylated derivative 3 shows that two Me groups have been introduced in the latter. Elementary analysis of 3, as compared with 1, shows that an additional O atom is present.

In the IR spectra of 3, the dehydrated and demethylated derivative 4 and the dehydrated compound 5 two CO bands are present. One band represents the 4-CO group, the other represents the 2-CO and other conjugated CO groups. From the intensity differences in these spectra we conclude that 4 has three CO groups (2-CO + 8-CO, 4-CO), whereas 3 and 5 have two CO groups (2-CO or 8-CO, 4-CO). As 4 is formed from 3 or 5, this indicates the presence of a OMe group in 3 and 5 at the 2- or 8-position.

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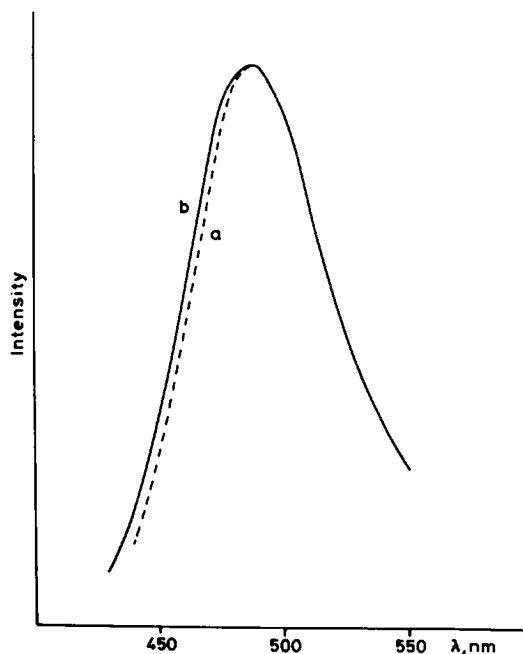
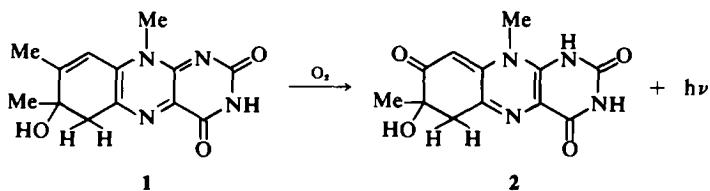
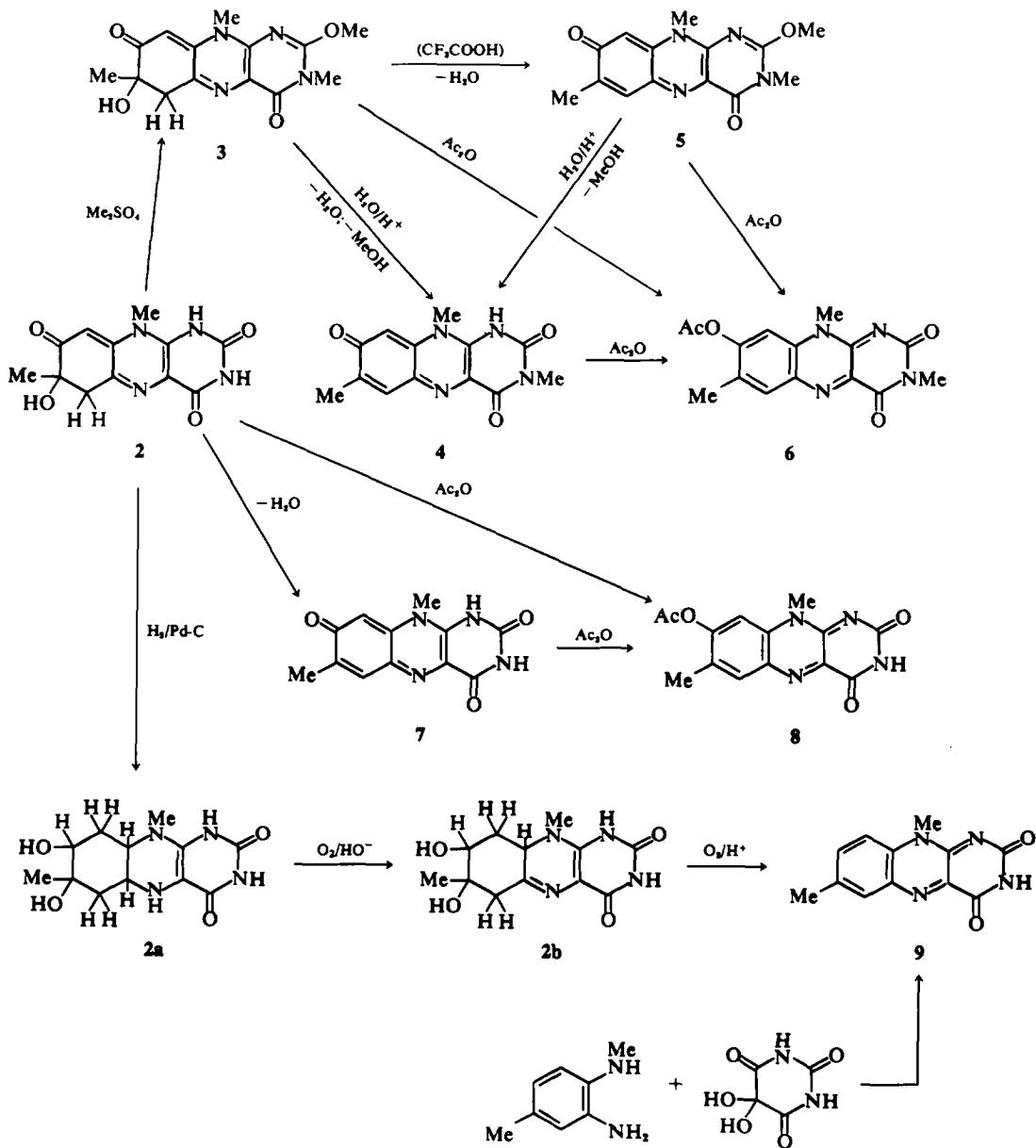


Fig 1. Corrected spectra of (a) Chemiluminescence of compound 1 (10^{-5} M, 0.05M NaHCO₃, pH = 8.7). (b) Fluorescence of compound 2 (10^{-5} M, 0.05M NaHCO₃, pH = 8.7), and of the reaction mixture after chemiluminescence.



SCHEME 1



SCHEME 2

According to the UV absorption spectrum, compound **5** has no isoalloxazine structure. Consequently the OMe group must be at the 2-position. This is consistent with the easy hydrolysis of this OMe group.

The formation of the acetylated compound **6**, with the UV absorption spectrum of an isoalloxazine, implies that the second Me group introduced by methylation of **2** must be present on N₃.

The structures **4**, **5** and **6** establish structure **3**.

(4) The dehydration of **3** to **5** shows that a tertiary OH has to be present. This indicates that **2** contains a similar OH group, which is consistent with the analogous formation of **7** from **2**.

(5) The oxidative demethylation of **1** implies the introduction of a CO group at the 8-position in **2**. This is confirmed by the structures of the methylated derivatives.

From these data we conclude the structure of **2**.

EXPERIMENTAL

¹H NMR spectra were recorded on a Varian A-60, or where indicated on a Varian XL-100 spectrometer, with TMS as an internal standard. UV spectra were recorded on a Perkin Elmer 402, and IR spectra in KBr on a Hilger Watts Infracan.

Chemiluminescence and fluorescence emission spectra were recorded on a spectrofluorimeter built in this laboratory.

The maximum of the chemiluminescence spectrum and the maxima of the fluorescence spectra coincide at 488 nm.

The spectra were measured with a bandpass of 30 nm. The relative spectral sensitivity was obtained using a calibrated tungsten lamp.

Fluorescence emission spectra were obtained by right angle excitation with 440 nm, isolated from a 1600 W Xenon arc with a double monochromator and a bandpass of 6.6 nm. From the fluorescence of **2** and the mixture after chemiluminescence, it was estimated that 40–50% **2** was present in the mixture.

The chemiluminescence spectrum was measured at a 2.10³ times higher sensitivity than used for the measurement of the fluorescence spectrum of the mixture.

7-Hydroxy-7,10-dimethyl-8-oxo-6,7,8,10-tetrahydroalloxazine (2). Compound **1** (14.0g) was added to a soln of NH₄HCO₃ (47.4g) and 25% NH₄OH in water (120 l) of pH = 8.7, saturated with O₂, and placed in the dark. The light produced could be observed easily; even after 5 days it was still visible to the dark-adapted eye. The soln was allowed to stand for another 5 days, and was then concentrated to a small volume (300 ml). The green solid which had separated was filtered off and washed with water, yield 5.66g. It contained at least five compounds according to chromatography. Only one of these compounds showed the blue-green fluorescence expected for the emitting species.

By further concentrating the mother liquor (100 ml) a second crop (2.34g) was obtained.

A small amount of formaldehyde (3% in relation to **1**) was demonstrated in the distillate, as the dimedone adduct.

The solid (5.66g) was dissolved in water of 80–90° (2 l). The soln was filtered, concentrated to a small volume

again and the ppt was collected. Repeating this procedure 3 times yielded 3.06g compound **2**. Ascending paper chromatography (Whatman 1), showed one spot under the 366 nm lamp with the following solvents: 3% NH₄Cl: R_f = 0.44; n-PrOH: 1% NH₄OH (2:1): R_f = 0.39; MeOH: R_f = 0.13; MeOH:n-BuOH:H₂O:C₆H₆ (2:1:1:1): R_f = 0.40; i-PrOH: 2% NH₄Ac (1:1): R_f = 0.45; n-BuOH: AcOH:H₂O (4:1:1): R_f = 0.11. Drying at 100° over P₂O₅ in vacuum resulted in the loss of three moles of water of crystallization, which was taken up again upon exposure to air. For calculations concerning compound **2** a molecular composition of C₁₃H₁₂N₄O₄·3H₂O was assumed, but no reproducible elementary analysis was obtained. NMR of the dried product at 100 MHz (d₆-DMSO): δ = 1.16(s, 3, C₇-Me); 2.88(s, 2, C₆-H₂); 3.38(s, 3, N-Me); 5.16(s, 1, C₇-H); three broad low absorptions at 4.8; 7.2 and 10.2 ppm (5H), which disappeared immediately upon addition of D₂O. The proton resonating at 5.16 ppm exchanged slowly with D₂O. UV (H₂O), λ_{max}(ε): 249(24,300); 265(16,600); 327(6,900); 338(7,300); 418(28,000); λ_{min}: 257(16,300); 282(4,300); 331(6,750); 350(4,500).

7-Hydroxy-2-methoxy-3,7,10-trimethyl-4,8-dioxo-6,7,8,10-tetrahydrobenzo[g]pteridine (3). The crude product (11.0g) from an oxidation reaction was methylated according to the method described for the preparation of **5**-acetyl-1,3,10-trimethyl-5,10-dihydroalloxazine.² The methylated products were suspended in a small volume of CHCl₃ and filtered.

The residue (2.02g) was practically pure **3**. The filtrate was introduced on to a silicagel column (4.6 cm in diam, 400g silicagel, 0.05–0.20 mm in diam, product from E. Merck A.G.). Elution was effected with CHCl₃/acetone (1:1). The eluate was concentrated to a small volume and the ppt was filtered off, yield 1.57g. From this product 0.82g was recrystallized 3 times from MeOH, yield 0.123g m.p. > 320° (C₁₃H₁₂N₄O₄ (304.30) Calcd: C, 55.26; H, 5.30; N, 18.41; Found: C, 55.4; H, 5.3; N, 18.5%). Calcd: M⁺ = 304.1171; Found: M⁺ = 304.1163. NMR, 100 MHz (CDCl₃): δ = 1.30(d, 3, C₇-Me); 3.10 and 3.43(AB, J = 16Hz, 2, C₆-H₂); 3.50 and 3.54(2s, 6, N₃-Me and C₂-OMe); 4.15(s, 3, N₁₀-Me); 5.38(s, 1, C₇-H); 1.5–1.6(s, 1, C₇-OH). A long range coupling (J ≈ 1Hz) was observed between C₇-Me and one of the protons (δ = 3.10) of the AB system. Upon addition of D₂O the C₇-OH signal disappeared immediately, while the signal at 5.38 ppm disappeared only slowly. UV (H₂O), λ_{max}(ε): 245(19,100); 264(17,000); 346(14,700); 395(24,200); 407(23,900); λ_{min}: 253(14,300); 297(2,500); 355(12,100); 402(23,800); λ_{ab}: 333(10,300). IR: 1690 cm⁻¹(4-CO) and 1630 cm⁻¹(8-CO), of equal intensity.

3,7,10-Trimethyl-8-oxo-8,10-dihydroalloxazine (4). Compound **3** (0.100g) was refluxed during 30 min in 0.1N H₂SO₄ (375 ml). After cooling to room temp the ppt was filtered off, washed with water and dried in a vacuum desiccator over P₂O₅, yield 0.087g (91%). For analysis it was recrystallized 3 times from MeOH, m.p. > 320° (C₁₃H₁₂N₄O₃·H₂O (290.28) Calcd: C, 53.79; H, 4.86; N, 19.30; Found: C, 53.7; H, 5.0; N, 19.2%). NMR (CF₃COOD): δ = 2.63(s, 3, C₇-Me); 3.72(s, 3, N₃-Me); 4.45(s, 3, N₁₀-Me); 7.63(s, 1, C₇-H); 8.25(s, 1, C₆-H); three exchangeable protons (N₁-H and H₂O). UV (AcOH): λ_{max}(ε): 265(31,400); 440(21,900); λ_{min}: 310(1,640); λ_{ab}: 451(20,700). IR: 1695 cm⁻¹(4-CO) and 1650 cm⁻¹(2-CO and 8-CO, twice the intensity of 4-CO).

The same compound was obtained using **5** as a starting material.

2-Methoxy-3,7,10-trimethyl-4,8-dioxo-8,10-dihydrobenzo[g]pteridine (5). Compound **3** (0.103g) was

dissolved in trifluoroacetic acid (1 ml). After 3 hr at room temp dehydration was complete, as appeared from TLC, silicagel/MeOH, $R_f = 0.56$. The solvent was evaporated and the residue dried in a vacuum desiccator over NaOH pellets. Recrystallization was effected from MeOH (175 ml), yield 0.081g (84%). m.p. $> 320^\circ$ ($C_{14}H_{14}N_4O_2$, (286.29) Calcd: C, 58.74; H, 4.93; N, 19.57; Found: C, 58.8; H, 5.0; N, 19.6%). NMR (CF_3COOD): $\delta = 2.64$ (s, 3, C_7 -Me); 3.83(s, 3, N_3 -Me); 4.55 and 4.58(2s, 6, N_{10} -Me and C_7 -OMe); 7.64(s, 1, C_7 -H); 8.28(s, 1, C_6 -H). UV (AcOH), $\lambda_{max}(\epsilon): 252(27,100); 270(23,900); 305(11,500); 435(19,800); \lambda_{min}: 262(22,200); 287(7,100); 339(1,350); \lambda_{sh}: 311(11,100); 463(14,700)$. IR: 1690 cm^{-1} (4-CO) and 1630 cm^{-1} (8-CO), of equal intensity.

8 - Acetoxy - 3, 7, 10 - trimethylisalloxazine (6). Compound 4 (0.252g) was refluxed during 30 min in Ac_2O (10 ml). After cooling to room temp the ppt was collected and washed with MeOH. From the mother liquor a second crop was obtained. Together this was recrystallized from MeOH (250 ml), yield 0.193g (71%). m.p. $288^\circ(\text{dec})$ ($C_{15}H_{14}N_4O_4$ (314.30) Calcd: C, 57.32; H, 4.49; N, 17.83; Found: C, 57.4; H, 4.6; N, 17.7%). NMR (CF_3COOD): $\delta = 2.60$ and $2.63(2s, 6, C_7$ -Me and Me-C=O); 3.72(s, 3, N_3 -Me); 4.58(s, 3, N_{10} -Me); 8.31(s, 1, C_7 -H); 8.47(s, 1, C_6 -H). UV (AcOH), $\lambda_{max}(\epsilon): 269(42,200); 341(8,450); 441(12,500); \lambda_{min}: 299(2,500); 378(3,300)$.

The same compound was obtained using 3 or 5 as starting material.

7, 10 - Dimethyl - 8 - oxo - 8, 10 - dihydroalloxazine (7). Compound 2 (0.26g) was added to conc H_2SO_4 (1 ml). After soln was complete water (5 ml) was cautiously added and a yellow ppt was formed. This was filtered off, washed with water and recrystallized twice from 2N H_2SO_4 , yield 0.162g (75%). m.p. $> 320^\circ$ ($C_{12}H_{10}N_4O_2 \cdot H_2O$ (276.25) Calcd: C, 52.17; H, 4.38; N, 20.28; Found: C, 52.2; H, 4.3; N, 20.4%). NMR (CF_3COOD): $\delta = 2.65$ (s, 3, C_7 -Me); 4.49(s, 3, N-Me); 7.67(s, 1, C_7 -H); 8.29(s, 1, C_6 -H); two exchangeable protons (N_3 -H and N_3 -H). UV (AcOH), $\lambda_{max}(\epsilon): 263(26,500); 436(22,500); \lambda_{min}: 308(8,400); UV$ (EtOH/2% DMSO),* $\lambda_{max}(\epsilon): 255(32,500); 308(4,600); 463(26,000); \lambda_{min}: 299(4,300); 329(1,400); \lambda_{sh}: 264(26,000); 485(18,400)$.

Dehydration could also be effected by dissolving 2 (0.588g) in CF_3COOH (5 ml). After standing overnight, the soln was poured into water (25 ml), and the ppt was collected, yield 0.466g (95%) compound 7.

8 - Acetoxy - 7, 10 - dimethylisalloxazine (8). Compound 7 (0.466g) was refluxed in a mixture of AcOH(400 ml) and Ac_2O (25 ml). After 30 min the heating was stopped and the soln was allowed to cool to room temp. The ppt was filtered off, washed with AcOH and dried in a vacuum desiccator over NaOH, yield 0.380g (75%). For analysis a small amount was subjected to continuous extraction with $CHCl_3$, m.p. $> 320^\circ$ ($C_{14}H_{12}N_4O_4$, (300.28) Calcd: C, 56.00; H, 4.03; N, 18.66; Found: C, 56.2; H, 4.2; N, 18.6%). NMR (CF_3COOD): $\delta = 2.62$ and $2.65(2s, 6, C_7$ -Me and Me-C=O); 4.60(s, 3, N-Me); 8.33(s, 1, C_7 -H); 8.49(s, 1, C_6 -H); one exchangeable proton(N_3 -H). UV (AcOH), $\lambda_{max}(\epsilon): 267(34,500); 343(7,300); 438(13,100); \lambda_{min}: 303(2,300); 378(4,300)$.

Acetylation of 2 (0.099g) in the same manner, yielded also compound 8 (0.072g, 80%).

Hydrogenation of 2. Compound 2 (0.1983g) was hydrogenated at atmospheric pressure and at room temp in water(100 ml) with 10% Pd/C (0.100g) as a catalyst. After 24 hr and an uptake of 2.91 moles of H_2 per mole of 2, hydrogenation was complete. The catalyst was filtered off and 0.3N NaOH(50 ml) was added to the filtrate. This soln was brought in an O_2 atmosphere. Under rapid stirring about one mole of O_2 per mole of 2 was taken up within 10 min. UV spectra of this solution, after diluting 80 times, indicated that a 7,8-dihydropteridine (2b) was present.^{2,3} Mixed probably with some already dehydrated or further oxidized material, as could be seen from the absorptions at the highest wavelengths. UV (0.1N NaOH), $\lambda_{max}(E): 236(1.20); 284(0.57); 327(\text{sh}, 0.25); 341(0.27); (0.1N H_2SO_4), \lambda_{max}: 216(0.38); 245(0.47); 275(0.66); 360(\text{sh}, 0.18); 394(0.22)$.

The oxidation mixture was acidified with conc HCl (4 ml) and refluxed for 1 hr. After cooling to room temp the yellow crystals were filtered off, and the filtrate was continuously extracted with $CHCl_3$, yield 0.115g (80%) 7,10-dimethylisalloxazine (9). m.p. $> 320^\circ$ ($C_{12}H_{10}N_4O_2$ (242.24) Calcd: C, 59.50; H, 4.16; N, 23.13; Found: C, 59.4; H, 4.3; N, 23.0%). NMR(CF_3COOD): $\delta = 2.82$ (s, 3, C_7 -Me); 4.68(s, 3, N-Me); 8.37 and 8.43(m, 3, Ar-H); one exchangeable proton(N_3 -H). UV (AcOH), $\lambda_{max}(\epsilon): 268(33,400); 340(6,900); 443(9,900); \lambda_{min}: 306(3,650); 378(3,050)$.

7, 10 - Dimethylisalloxazine (9). The reaction of 4 - chloro - 3 - nitrotoluene with methylamine gave 4 - methylamino - 3 - nitrotoluene, analogous to the preparation of 2 - nitro - methylaminobenzene.^{5,6} Catalytic reduction of this nitro derivative gave 3 - amino - 4 - methylaminotoluene, which upon condensation with alloxan⁷ yielded 9. m.p. $> 320^\circ$ ($C_{12}H_{10}N_4O_2$ (242.24) Calcd: C, 59.50; H, 4.16; N, 23.13; Found: C, 59.4; H, 4.3; N, 23.0%). According to NMR, UV and IR identical with the isalloxazine isolated at the hydrogenation of 2.

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