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CHEMILUMINESCENCE—I

SYNTHESIS OF 7-HYDROXY-7,8,10-TRIMETHYL-6,7-DIHYDROISOALLOXAZINE

R. ADDINK and W. BERENDS*

Biochemical and Biophysical Laboratory of the Technological University, Julianalaan 67, Delft, The Netherlands

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Abstract—The condensation of dimeric diacetyl with 5-amino-6-(methylamino)pyrimi-2,4-dione at different pH values is described. Structures **4**, **5**, and **6** are assigned to the three products obtained in this reaction.

In the course of investigations on the autoxidation of reduced pteridines and flavins,[1] we were confronted with a chemiluminescence phenomenon. When an acid solution of 6,7,8-trimethylpteri-2,4-dione (**3**) was treated with hydrogen peroxide, a a flash of light could be observed. Extensive recrystallization of the compound decreased the amount of chemiluminescence considerably. Apparently a contaminating compound formed during the synthesis was responsible for the chemiluminescence.

The synthesis of **3**[2,3] was carried out by condensation of diacetyl with 5-amino-6-(methylamino)-pyrimi-2,4-dione (**1**). The diacetyl appeared to contain a small amount of its dimer (**2**). This dimer could be obtained on a preparative scale by carrying out an aldol condensation with diacetyl.[4,5]

The condensation of **2** with **1** has been described,[2,6] but no definite structures of the products were given.

Reinvestigation of this reaction, indicated a strong dependence on acidity. By carefully adjusting the pH to the values indicated in the Scheme, compounds **4**, **5** and **6** could be isolated.

To the "pH5" product we assigned structure **5** on account of:

1. Comparison of the NMR spectra of **5** and **3** showed that the 7-Me group was still present in **5**.

2. UV absorption spectra showed that **5** was converted quantitatively into **4** in alkaline medium. This further established the acid character of the 7-Me group, which was already known from deuterium exchange experiments.[7–9]

To the "pH6" product we assigned structure **4** on account of:

1. The compound **4** is converted quantitatively into lumiflavin (**7**) by refluxing in aqueous acid solution.

2. Mass spectra of **7** and **4** are identical with exception of the molecular ion peak of **4** (**7** $M^+ = 256$; **4** $M^+ = 272$).

3. Refluxing **5** in dilute dideutero sulfuric acid afforded **7** in which the aromatic proton on the 9-position, originating from the 7-Me group of **5**, was exchanged for deuterium.

4. Refluxing **4** in dilute dideutero sulfuric acid afforded **7** in which the aromatic protons were still present. If **4** bears a methylene moiety in the 9-position one should expect incorporation of deuterium. As this was not observed the methylene moiety of **4** is supposed to be in the 6-position.

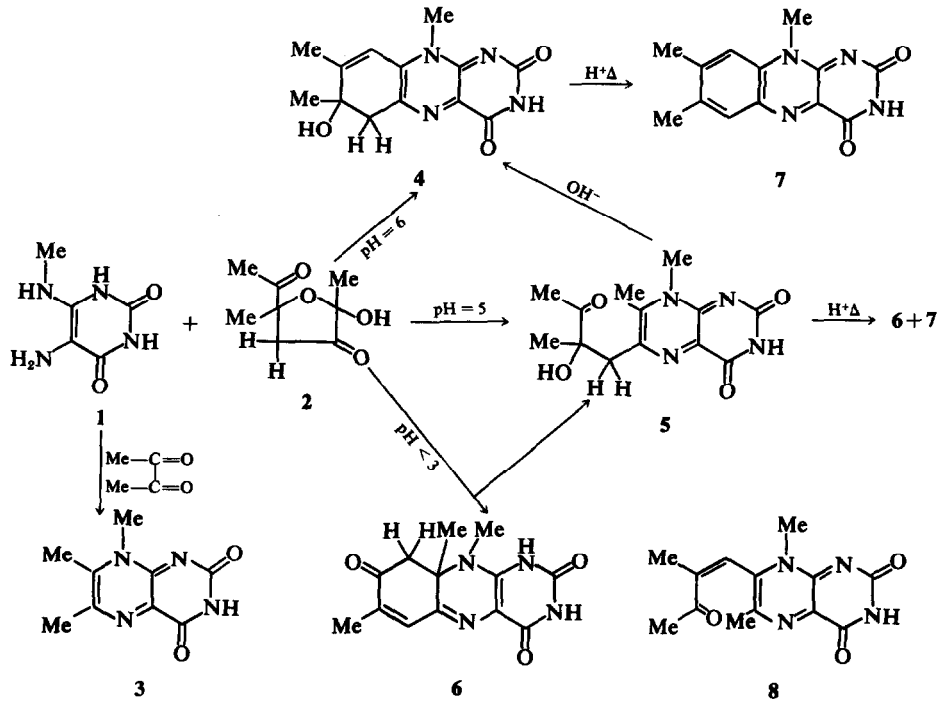
Carrying out the condensation of **1** with **2** at pH < 3 gave a mixture of **5** and **6**.

Refluxing an aqueous acid solution of **5** afforded a mixture of **6** and **7**. In a similar experiment Cresswell and Wood[6] obtained a compound to which they tentatively assigned structure **8**. Although **6** contained one mole of water of crystallization instead of half a mole, comparison of UV absorption data and chromatographic behaviour showed we had isolated the same compound.

However we had to reject structure **8**. Our findings mentioned below are in favour of structure **6** for the "pH < 3" product:

1. In an NMR spectrum of **6** the following signals are present: two 3-proton singlets (N—Me shifted to higher field in relation to **4** and **5**, indicating a more saturated character, and a Me group resonating in the aliphatic region), one 3-proton doublet coupled with a one-proton quartet (allylic coupling), an AB system ($J = 15$ Hz, suggesting either an open chain or a ring), and two exchangeable protons (presumably present in the pyrimidine part of the molecule, which were confirmed by methylation experiments in which **6** took up two Me groups giving two isomeric dimethyl derivatives).

2. Hydrogenation of a dimethyl derivative of **6** resulted in the uptake of three moles of hydrogen



SCHEME 1

per mole of compound, ruling out the possibility of the above mentioned open chain.

3. 2,4-Dinitrophenylhydrazones were obtained from **6** as well as from its two dimethyl derivatives. This indicates the presence of a CO group, which was substantiated by IR data.

4. Refluxing **5** in dilute dideutero sulfuric acid afforded **6** in which the Me group resonating at highest field was deuterated. The protons of the methylene group were also exchanged partially for deuterium.

5. When **6** was dissolved in either dideutero sulfuric acid or sodium deuterooxide only exchange of the protons of the methylene group was observed.

From these data we conclude that the Me group resonating in the aliphatic region (C_{9a}—Me) originated from the 7-Me group of **5**. The deuteration of the methylene group was caused by the adjacent CO group.

In acid medium only compounds **4** and **5** produced light when treated with hydrogen peroxide. In alkaline medium chemiluminescence occurred with oxygen as a reactant. As compound **5** is converted into **4** under these conditions, compound **4** is chemiluminescing in alkaline medium. Details of the latter reaction will be described in the next part of these series.

EXPERIMENTAL

NMR spectra were recorded on a Varian A-60, or where indicated on a Varian XL-100 spectrometer, with TMS as an internal standard.

6,7,8-Trimethylpteri-2,4-dione (**3**).[2,3] This compound was prepared according to a method described; NMR (d_6–DMSO): $\delta = 2{\cdot}53$ (s, 3, C_6—Me); 2·64 (s, 3, C_7—Me); 3·99 (s, 3, N_8—Me).

5-Amino-6-(methylamino)pyrimi-2,4-dione hydrochloride (**1**). 6-Methylamino-5-nitropyrimi-2,4-dione was synthesized according to Cresswell and Wood.[6] Reduction of the nitro compound to the 5-formylamino derivative, and subsequent deformylation was carried out according to Pfleiderer and Nübel.[10]

2-Acetyl-5-hydroxy-2,5-dimethyl-4-oxotetrahydrofuran (**2**). This dimer of diacetyl was prepared according to Diels *et al.*[4] The product was purified by recrystallization from CCl_4. Two diastereo-isomers could be isolated: (a) m.p. 38·5–40·5°; NMR ($CDCl_3$): $\delta = 1{\cdot}52$(s, 3); 1·65 (s, 3); 2·35 (s, 3); 2·69 and 3·02 (AB, $J = 19{\cdot}5$ Hz, 2); 3·90 (s, 1). (b) m.p. 48–50°; NMR ($CDCl_3$): $\delta = 1{\cdot}48$ and 1·49 (2s, 6); 2·32 (s, 3); 2·22 and 3·35 (AB, $J = 19$ Hz, 2); 3·91–4·16 (1H). Both isomers gave the same products in reaction with **1**.

7-Hydroxy-7,8,10-trimethyl-6,7-dihydroisoalloxazine (**4**). Compound **1** (3·85 g) was suspended in water (100 ml). The pH of the suspension was brought to 6·0 with 50% NaOH, and then **2** (3·5 g) was added. After stirring and readjusting the pH to 6·0 the deep orange soln was placed in the refrigerator. The soln soon became dark green and dark green crystals separated. After several days the solid was filtered off and washed with water, yield 3·90 g. Drying in a vacuum desiccator over P_2O_5 resulted in the loss of one mole of water of crystallization, which was taken up again upon exposure to air. Recrystallization from water (200 ml) yielded 3·06 g (52%) m.p. > 300°. ($C_{13}H_{14}N_4O_3 . H_2O$ (292·30) Calcd: C, 53·42; H, 5·52; N, 19·17; Found: C, 53·3; H, 5·7; N, 19·0%); NMR, 100 MHz (d_6-DMSO): $\delta = 1{\cdot}24$ (s, 3, C_7—Me);

2·19 (d, $J = 1·5$ Hz, 3, C_8—Me); 3·00 and 3·15 (AB, $J =$ 16 Hz, 2, C_6—H_2); 3·90 (s, 3, N—Me); 5·37 (s, 1, OH); 6·79 (q, $J = 1·5$ Hz, 1, C_9—H); UV (H_2O), λ_{max} (ϵ): 237 (15,800); 298 (14,360); 446 (18,400); λ_{min}: 269 (6,000); 354 (540); λ_{sh}: 254 (11,700).

6-(2′-*Hydroxy*-2′-*methyl*-3′-*oxo*)*butyl*-7,8-*dimethylpteri*-2,4-*dione* (**5**). Compound **1** (1·92 g) was suspended in water (25 ml). The pH was brought to 5·0 with 50% NaOH and then **2** (1·9 g) was added. After readjusting the pH to 5·0 the soln was placed in the refrigerator. The pH had to be checked every few hours. From the orange soln a yellow solid separated slowly. After 5 days it was filtered off, washed with water and dried in a vacuum desiccator over P_2O_5, yield 2·03 g (69%) m.p. 299° (dec), Lit.[2] m.p. 346–348°. ($C_{13}H_{16}N_4O_4$ (292·30) Calcd: C, 53·42; H, 5·52; N, 19·17; Found: C, 53·2; H, 5·6; N, 19·0%); NMR (d_6-DMSO): $\delta = 1·27$ (s, 3, Me—C—OH); 2·32 (s, 3, Me—C=O); 2·66 (s, 3, C_7—Me); 3·09 and 3·34 (AB, $J = 15$ Hz, 2); 3·90 (s, 3, N—Me); 5·13 (s, 1, OH); 11·0 (s, 1, NH); UV (H_2O), λ_{max} (ϵ): 258 (14,460); 276 (11,240); 403 (11,400); λ_{min}: 227 (6,400); 270 (11,000); 302–330 (1,000).

Compound **5** (0·503 g) was refluxed in 0·1 N HCl (25 ml). After 30 min the heating was stopped, and the suspension was allowed to cool to room temp. The solid was filtered off, washed with water and dried. The yield was so low, that the mother liquor was evaporated to the starting volume and again refluxed for 30 min. This procedure was repeated several times until no further ppt was formed, total yield 0·39 g mixture of **6** and **7**.

This mixture was heated to boiling in water (150 ml). Undissolved material was filtered off while hot. The residue (0·16 g) appeared to be lumiflavin (**7**). The soln was diluted with water (300 ml) and introduced on to a cellulose column (5 cm in diam, 1·2 kg cellulose powder Whatman CF11) with water as an eluent. First **6** was eluted from the column. The eluate was evaporated to dryness and recrystallized from water (135 ml), yield 0·13 g (26%). **7** was obtained in a yield of 0·04 g, bringing the total yield of lumiflavin to 0·20 g (45%).

7,9a,10-*Trimethyl*-8-*oxo*-1,9-*dihydroalloxazine* (**6**). Compound **1** (3·85 g) was suspended in water (50 ml). After addition of **2** (3·5 g) and stirring the clear deep orange soln of pH 1·5 was placed in the refrigerator. After 3 weeks a solid was filtered off, washed with water and dried in a vacuum desiccator over P_2O_5, yield 3·90 g as a mixture of **5** and **6**.

This mixture was refluxed in 0·1 N HCl. Under these conditions **5** is converted into a mixture of **6** and **7**, which was purified on a cellulose column, as described above. No attention was given to the actual composition of the mixture; m.p. 237° (dec). ($C_{13}H_{14}N_4O_3 . H_2O$ (292·30) Calcd: C, 53·42; H, 5·52; N, 19·17; N—Me, 5·14; Found: C, 53·3; H, 5·6; N, 19·3; N—Me, 5·0%); NMR, 100 MHz (d_6-DMSO): $\delta = 1·10$ (s, 3, C_{9a}—Me); 1·88 (d, $J = 1·5$ Hz, 3, C_7—Me); 3·00 (s, 3, N—Me); 3·08 and 3·17 (AB, $J = 15$ Hz, 2, C_9—H_2); 7·00 (q, $J = 1·5$ Hz, 1, C_6—H); four exchangeable protons ($H_2O + 2$ NH); IR: 1705 cm^{-1} (4–CO); 1655 cm^{-1} (2–CO + 8–CO); UV (0·1 N HCl), λ_{max} (ϵ): 264 (19,100); 436 (12,100); λ_{min}: 224 (6,700); 342 (1,100); λ_{sh}: 298 (9,600). 0·1 N NaOH, λ_{max}: 226 (21,500); 272 (17,700); 456 (15,800); λ_{min}: 247 (7,700); 356 (2,000). The 2,4-dinitrophenylhydrazone m.p. 303° (dec): $C_{19}H_{18}N_8O_6$ Calcd: $M^+ = 454$; Found: $M^+ = 454$; IR: 1700 cm^{-1} (4–CO); 1660 cm^{-1} (2–CO, less intensive in relation to 4–CO than with **6**).

Methylation of **6**. Compound **6** (0·685 g) was methylated as described for the methylation of 5-acetyl-1,3-dimethyl-5,10-dihydroalloxazine.[1] The final products dissolved in a small volume of $CHCl_3$ were introduced on to a silicagel column (2·9 cm in diam, 100 g silicagel 0·05–0·20 mm in diam, product from E. Merck, A.G.). Elution of a yellow band was first effected with EtOAc. The eluate was evaporated to dryness and recrystallized from alcohol, yield 0·122 g (17%) 2,4-dimethoxy-7,9a,10-trimethyl-8-oxo-1,9-dihydrobenzo[*g*]pteridin m.p. 260·5–261·5°. ($C_{15}H_{18}N_4O_3$ (302·33) Calcd: C, 59·59; H, 6·00; N, 18·53; Found: C, 59·6; H, 6·1; N, 18·5%); NMR (CF_3-COOD): $\delta = 1·73$ (s, 3, C_{9a}—Me); 2·25 (d, $J = 1·5$ Hz, 3, C_7—Me); 3·43 and 3·63 (2s, 5, N—Me and C_9—H_2); 4·35 and 4·43 (2s, 6, C_2—OMe and C_4—OMe); 7·53 (q, $J = 1·5$ Hz, 1, C_6—H); IR: no 4–CO present, 1665 cm^{-1} (8–CO). The 2,4-dinitrophenylhydrazone m.p. 252° (dec). $C_{21}H_{22}N_8O_6$ Calcd.: $M^+ = 482$; Found: $M^+ = 482$; IR: no CO absorption present.

The second compound was then eluted with acetone. The eluate was evaporated to dryness and recrystallized from alcohol, yield 0·427 g (60%) either 4-methoxy-1,7,9a,10- or 4-methoxy-3,7,9a,10-tetramethyl-2,8-dioxo-1,9-dihydrobenzo[*g*]pteridin, m.p. 253·5–254°. ($C_{15}H_{18}N_4O_3$ (302·33) Calcd: C, 59·59; H, 6·00; N, 18·53; Found: C, 59·6; H, 6·1; N, 18·4%); NMR(CF_3COOD): $\delta = 1·67$ (s, 3, C_{9a}—Me); 2·27 (d, $J = 1·5$ Hz, 3, C_7—Me); 3·38 (s, 3, N_{10}—Me); 3·57 and 3·67 (2s, 5, N_1 or N_3—Me and C_9—H_2); 4·29 (s, 3, C_4—OMe) 7·63 (q, $J = 1·5$ Hz, 1, C_6—H); IR: no 4–CO present, 1655 cm^{-1} (2–CO + 8–CO).

This compound was hydrogenated in glacial AcOH (0·0754 g in 50 ml) with Pt as a catalyst. After 24 hr hydrogenation was complete. The compound had consumed 16·2 ml H_2 (0°, 760 mm; 2·9 mole H_2 per mole compound). The 2,4-dinitrophenylhydrazone m.p. 243° (dec): $C_{21}H_{22}N_8O_6$ Calcd: $M^+ = 482$; Found: $M^+ = 482$; IR: 1670 cm^{-1} (2–CO).

7,8,10-*Trimethylisoalloxazine* (**7**). Compound **4** (0·100 g) was refluxed in 0·1 N HCl (10 ml). After 30 min the heating was stopped and the suspension was allowed to cool to room temp. The solid was filtered off, washed with water and alcohol and dried, yield 0·0855 g (97·5%) m.p. 329–330°, lit.[6] m.p. 326°. ($C_{13}H_{12}N_4O_2$ (256·26) Calcd: C, 60·93; H, 4·72; N, 21·86; Found: C, 60·8; H, 4·8; N, 21·7%).

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