Photochemistry of 6-Formylpterin in Alkaline Medium

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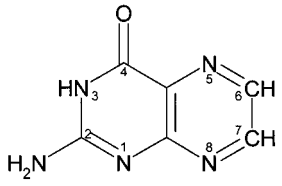
Dedicated to Professor Dr. *André M. Braun* on the occasion of his 60th birthday

6-formylpterin solutions at pH 11 were photolyzed at 350 nm at room temperature. The photochemical reactions were followed by UV/VIS spectrophotometry, thin layer chromatography (TLC), and high-performance liquid chromatography (HPLC). *In the presence of oxygen*, 6-carboxypterin is the only photoproduct detected by the analytical techniques mentioned. *In the absence of oxygen*, a new compound showing an absorbance maximum at 480 nm is observed. The latter compound is thermally oxidized very fast in the presence of oxygen to 6-carboxypterin. The quantum yields of substrate disappearance and of photoproduct formation are reported

Introduction. – Pterins are a very important group of natural pigments [1][2]. Although they are present in small amounts in living systems, they play an important biological role [3]. Some aspects of their photochemistry and photophysics is not completely described and understood [4]. Pterin is the simplest form of this family of compounds.



Pterin and its derivatives participate in a series of processes that range from the metabolic pathway in the biochemistry of nucleic acids to their potential capacity to act as a natural antenna for blue light [5]. Some compounds, such as pterin itself, are also able to induce photoinduced damage in genetic material [6][7].

Several pterins of biological interest present different substituents at C(6) of the molecule. Among them, 6-formylpterin, 6-carboxypterin, biopterin, xanthopterin, *etc.* are well-known derivatives. Most of these compounds are photosensitive to light in the UV-A region (320–400 nm) [8][9], and are also able to chelate metal ions in aqueous solution [10–12]. Their photoreactivity is strongly dependent on the nature of the substituent at C(6). The excited states of these compounds are able to quench dioxygen, leading singlet oxygen [13].

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We have recently studied the photochemistry, in aqueous solution, of 6-carboxypterin and folic acid [14–16]. The latter compound can be considered a derivative of pterin, where the substituent is the *para*-aminobenzoylglutamic acid. When solutions of folic acid are photolyzed in acid media, 6-formylpterin is obtained with a quantum yield of $\Phi = 2.5 \times 10^{-2}$. 6-Formylpterin is further oxidized to 6-carboxypterin and pterin. The quantum yields of these processes have been reported, and the effect of oxygen and singlet oxygen on folic acid has also been discussed in the literature [16].

The photochemistry of this family of compounds is strongly dependent on pH. In the present papter, we report and discuss the photochemistry of 6-formylpterin in alkaline medium in order to obtain more detailed information about this kind of compounds and about the effect of the pH of the fate of the photoexcited species.

Experimental. – 6-Formylpterin, 6-carboxypterin as well as other pterins, were provided by *Schircks Laboratories* (Switzerland) and used without further purification. Other chemicals from *Sigma-Aldrich* were used as received. The pH of solns. was adjusted to 11 by adding drops of conc. solns. of NaOH. The ionic strength was *ca.* 10^{-3} M in all the experiments.

UV/VIS Spectra were performed on a *CARY 3 (VARIAN)* spectrophotometer, with a program for smoothing and averaging signals. Measurements were made using quartz cells of 1-cm optical length and double cells, one of them with an optical path of 1 cm for irradiation purposes, whereas the second one was of 0.2 cm for absorbance measurements.

The continuous photolysis of 6-formylpterin solns. were carried out in the presence and absence of air. Deaerated solns. were obtained by bubbling with O_2-free N_2 for 20 min. *Rayonet RPR 3500 Å* lamps (*Southern N.E. Ultraviolet Co.*) were employed.

Products were identified by analyzing the absorbance change during irradiation, as well as by TLC and HPLC.

Absorption spectra of the solns. were recorded at regular intervals during the irradiation time. As explained in [14][16], experimental-difference (ED) spectra were obtained by subtracting the spectrum at time $t = 0$ from the subsequent spectra recorded at different times. Each ED spectrum was normalized relative to the maximum absolute value of the absorbance difference, yielding the normalized experimental difference (NED) spectrum.

Reference-difference (RD) and normalized reference-difference (NRD) spectra were obtained from aq. solns. of commercial standards. The RD spectra were calculated as follows: the absorption spectrum of the reactant in a given experiment was subtracted from the spectrum of standard solns. of the commercial compounds assumed to be possible products of the reaction. These spectra were recorded using solns. of equal concentrations of both compounds, at concentrations similar to those used in the photolysis experiments. NRD Spectra were obtained by normalizing RD spectra as described above.

The procedures described previously were carried out for all experimental conditions reported in this paper. The comparison between NED and NRD spectra allows us to characterize the major photolysis products.

TLC Experiments were performed using *DEAE*-cellulose plates (100 μm) and aq. NH_4Cl 0.3% (*p*/*v*) soln. as eluent. Irradiated 6-formylpterin (1.5×10^{-4} M) and aq. solns. of pteridin-derivative standards were developed by exposing them to 350 nm light. Under the present conditions, pterin derivatives show blue fluorescent spots. R_f values have already been reported in [16].

HPLC (*Konic Instruments 500 B*) was employed to monitor the reaction and identify photoproducts: with a *C18 Spherisorb S5 ODS2* (250 × 46 mm) column, eluent: 10% MeCN/H_2O 60:40, 90% aq. soln. of 20 mM K_3PO_4 (pH 5.5), and 2.5 mM EDTA (soln. 2). A *Lambda 1000 Bischoff* HPLC spectrophotometer was used as detection system. For irradiated 6-formylpterin solns., HPLC runs were monitored at 280 and 340 nm. The same eluent was used to identify 6-carboxypterin.

The quantum yields of 6-formylpterin disappearance and 6-carboxypterin formation were determined in experiments performed under different conditions. A chemical actinometer, ($[Co^{III}(NH_3)_5)Br]^{2+}$) was employed for the measurements of photon rates [17]. When possible, the initial concentrations of reactants were adjusted so that more than 99.99% of the incident radiation at 350 nm was absorbed. The evolution of the concentrations of reactants and photoproducts during the irradiation time was followed by HPLC. Aq. solns. of commercial standards were employed to obtain the corresponding calibration curves.

The O_2 consumption during the photolysis was measured with an O_2-selective electrode (*Orion*, model *37-08-99*). The exper. set-up for these measurements was described in [18].

Photolysis experiments were carried out in the presence of furfuryl alcohol (10 mM; *Riedel-de Haën*). The high reactivity of the latter compound towards singlet oxygen [19] greatly reduces its concentration during the photolysis. In this group of experiments, 6-formyl- and 6-carboxypterin concentrations as a function of irradiation time were determined by HPLC measurements. Results are compared with those obtained in the absence of the selective singlet-oxygen scavenger.

Results and Discussion. – Alkaline solutions of 6-formylpterin show very strong changes when they are irradiated at 350 nm. The effect of oxygen on the photochemical transformation is quite remarkable.

Photolysis in the Presence of Oxygen. Spectral changes on photolyzed solutions can be observed as shown in *Fig. 1,a*. ED Spectra were obtained as described under *Experimental*. Solutions were irradiated under steady conditions for almost 50 min. An isosbestic point at 274 nm can be observed during the whole irradiation time. For simplicity, only the behavior during the first 20 min of continuous photolysis is shown in *Fig. 1,b*.

No further changes are detected in irradiated solutions when stored in the dark. After 50 min of irradiation, 6-formylpterin is photolyzed to yield a product that accumulates in the solution. Upon further photolysis, this product itself suffers photochemical transformation.

In a previous study of the photochemistry of folic acid in acidic medium [16], 6-formylpterin is observed as one of the photoproducts. Under these pH conditions ($pH = 6$), this compound is photolyzed to yield 6-carboxypterin. This compound is also the main photoproduct at pH 11, as seen in *Fig. 2*, though the rate of photo-transformation of 6-formylpterin is slower than in acidic conditions. As already described in [14][16], a NED spectrum can be directly compared with a NRD spectrum obtained from standard solutions of 6-formylpterin and 6-carboxypterin. A direct comparison allows us to propose 6-carboxypterin as the main photoproduct under these experimental conditions.

Moreover, TLC analysis performed on the irradiated solutions shows only the presence of two fluorescent substances. The R_f values correspond to the standards of 6-forylpterin and 6-carboxypterin (*Fig. 3*).

In a similar way, HPLC experiments show the decrease of 6-formylpterin and the appearance of a photoproduct that shows a similar t_R value as that observed for solutions of 6-carboxypterin.

Finally, in *Fig. 4*, the spectrum observed for solutions after 50 min of irradiation is compared with that of 6-carboxypterin. These facts support strongly the conversion of 6-formyl- to 6-carboxypterin.

Linear behavior for the time evolution of the concentrations of 6-formylpterin and 6-carboxypterin as measured by HPLC is observed and depicted in *Fig. 5,a* and *b*.

The rate of degradation of 6-formylpterin is 3.7 ± 0.3 μM/min, whereas the rate of formation of 6-carboxypterin is 3.7 ± 0.2 μM/min. The agreement between the two values suggests that the photoproduct arises from the 6-formylpterin.

Actinometry measurements were performed as described under *Experimental*. The quantum yield obtained under the present conditions is 9.0×10^{-3}, corrected by the absorbance of the solution. This value is smaller than the value reported at pH 6 (*ca.* 3.8×10^{-2}).

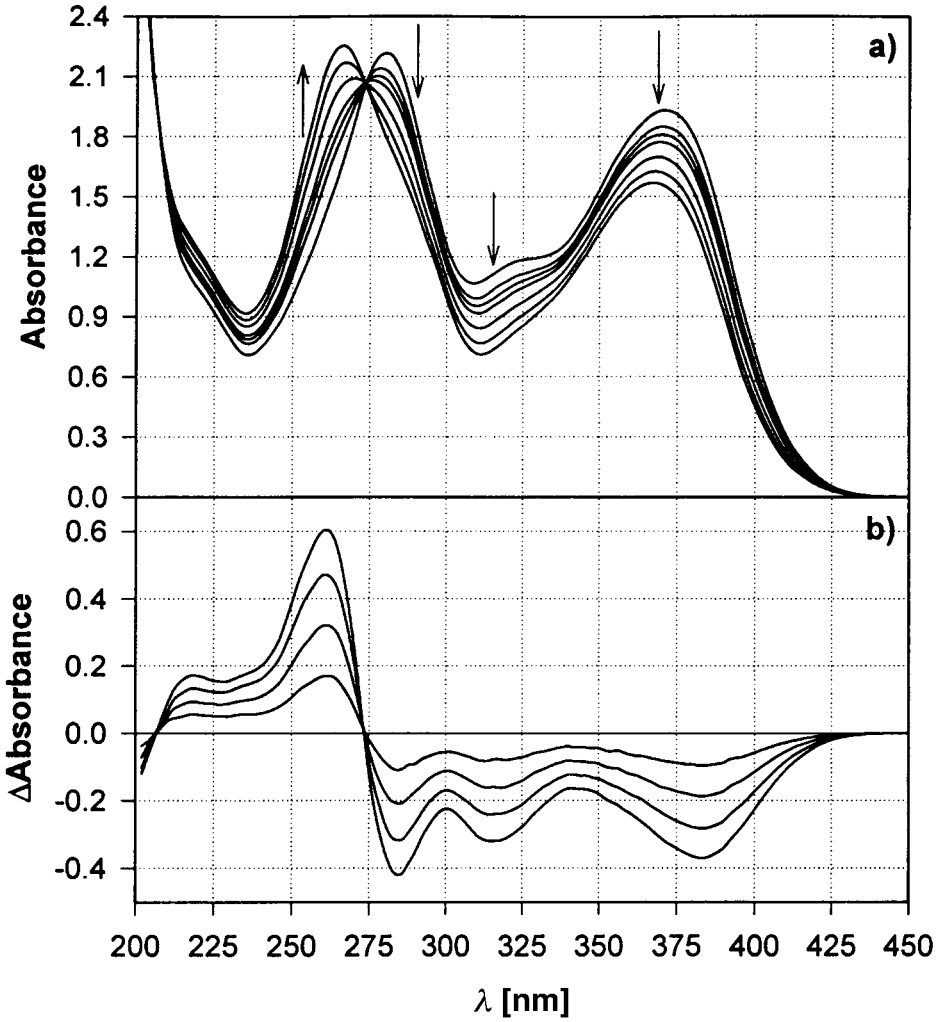


Fig. 1. a) *Time evolution of the absorption spectrum of a 150* μM *aqueous solution of 6-formylpterin at pH 11 and in the presence of oxygen.* Each spectrum was taken every 4 min, and the arrows show the direction of the recorded changes. Optical cell length 1 cm; b) *Experimental difference (ED) spectrum.*

Effect of Oxygen on Photolysis. The O_2 evolution during irradiation was monitored in a closed cell. Experiments were performed with an oxygen electrode as described under *Experimental*. As can be seen in *Fig. 6*, $[O_2]$ decreases steadily with the irradiation time.

To investigate whether singlet oxygen is involved in the reaction, some experiments were performed in the presence of furfuryl alcohol [20]. However, no conclusive evidence can be reached from these experiments because a strong photoinduced interaction was observed between the substrate and the singlet-oxygen scavenger. In fact, mixtures of 6-formylpterin and furfuryl alcohol are stable in the dark, but, when

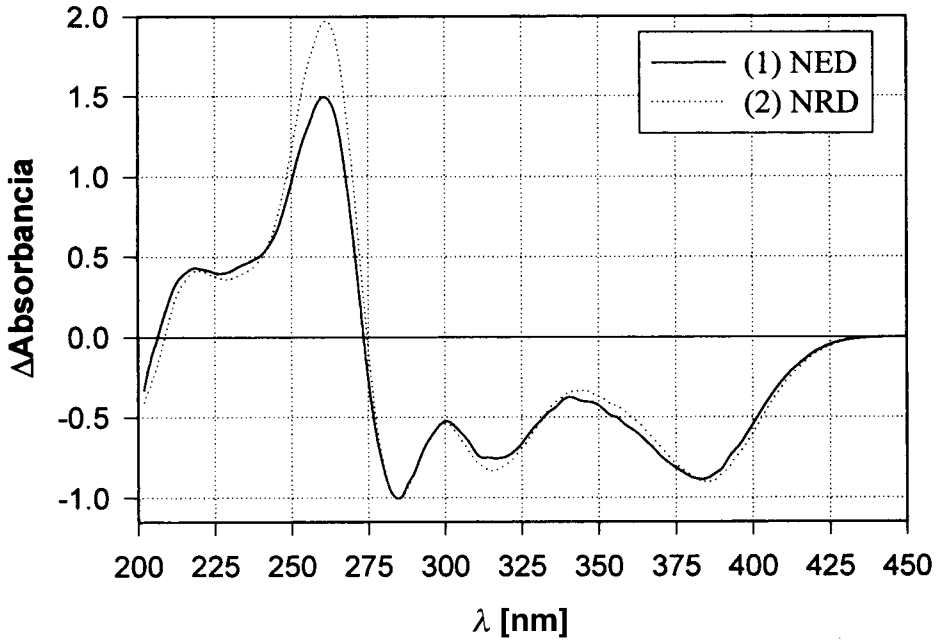


Fig. 2. *Difference experimental spectra (NDE) normalized at 280 nm.* In spectrum (*1*), a NDE obtained after 10 min of irradiation is shown. Spectrum (*2*) is a typical NRD obtained from 100 μM solutions, pH = 11, of 6-formylpterin and 6-carboxypterin.

irradiated, deep changes in the absorbance spectrum can be detected with a very small production of 6-carboxypterin. These changes are shown in *Fig. 7.*

Photoexcited 6-formylpterin is quenched by O_2 to yield singlet oxygen [13][21]. Therefore, the results shown in *Fig. 7* suggest that furfuryl alcohol, after reacting with singlet oxygen, produces intermediates that react with 6-formylpterin, leading to its degradation by an alternative pathway. This behavior is quite different from that reported for the photolysis of folic acid in acidic medium in the presence of this singlet-oxygen scavenger.

Moreover, the depletion rate of 6-formylpterin is faster in the presence of furfuryl alcohol, *i.e.*, after 5 min of irradiation, the degradation rate of the substrate reaches 25.4 μM/min, that is almost 7 times higher. The amount of 6-carboxypterin determined by HPLC is very small, thus indicating that it is not the main photoproduct (see *Fig. 8*). Therefore, in the presence of furfuryl alcohol, the formation of 6-carboxypterin is partially inhibited.

Photolysis in the Absence of Oxygen. To evaluate the role of oxygen in the photochemistry of 6-formylpterin, solutions previously bubbled with ultrapure dinitrogen were irradiated. Under these conditions, visible changes in the color of the solutions can be observed. The originally yellowish solutions developed an orange-red color after irradiating during more than 50 min. This compound will be referred to as 'red intermediate or compound'. These changes are seen in *Fig. 9*, where the spectrum shows a new band around 480 nm (*Fig. 9,b*).

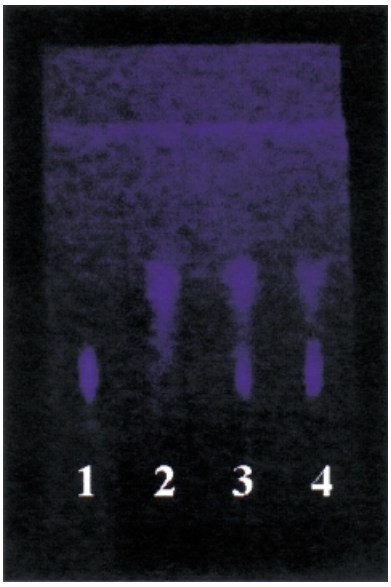


Fig. 3. *TLC Runs.* These lanes were obtained after dropping solutions of standards and irradiated solutions: *1:* 6-carboxypterin (150 μM); *2:* formylpterin (200 μM); *3* and *4:* irradiated 150 μM of 6-formylpterin solutions at pH 11.0 after 10 and 20 min of continuous irradiation, respectively.

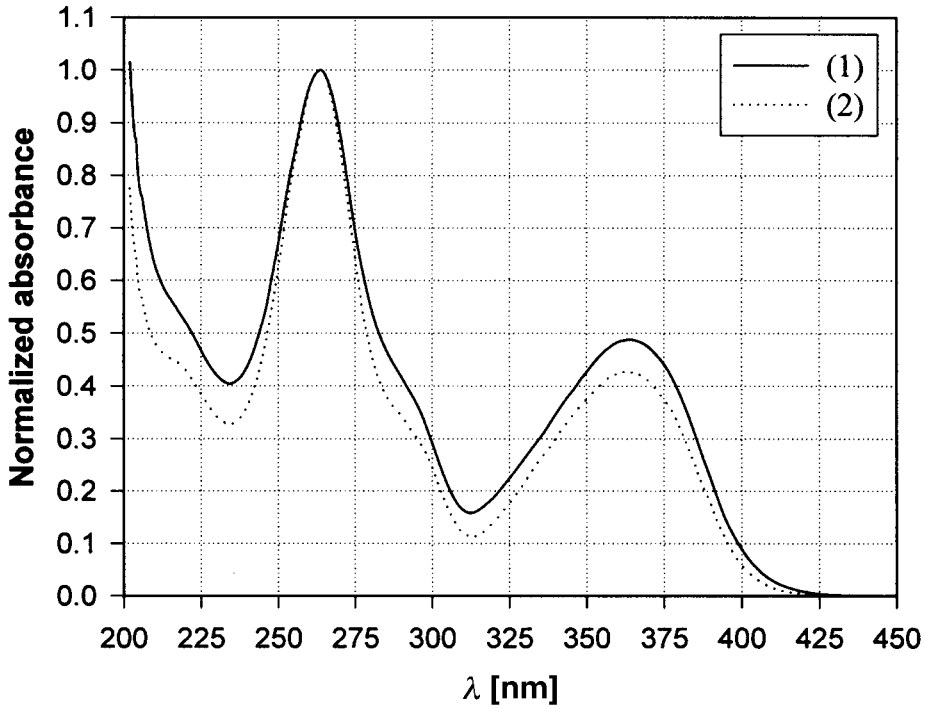


Fig. 4. 1) *Normalized spectrum of a solution of 6-formylpterin after 50 min of continuous irradiation.* 2) *Normalized spectrum of a solution of 6-carboxypterin* (100 μM, pH 11.0).

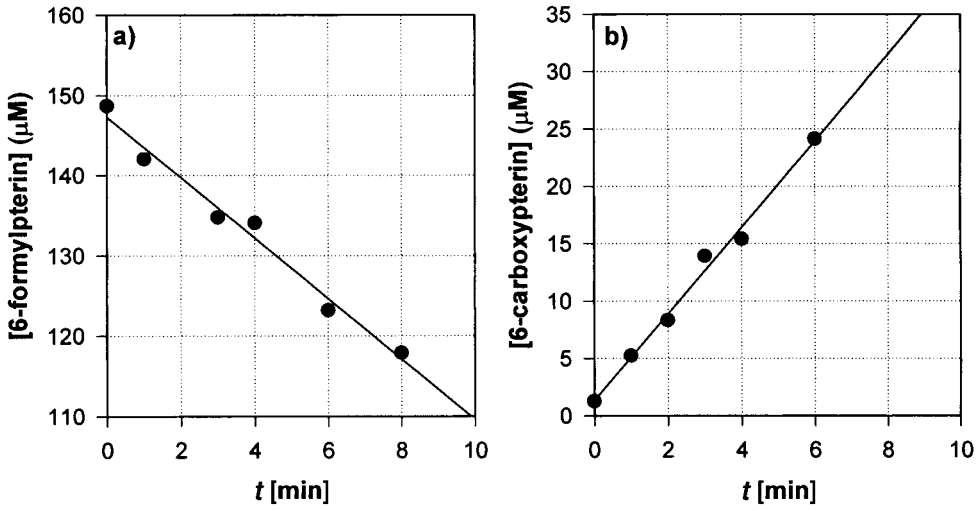


Fig. 5. *Time* vs. *concentration profiles of* a) *6-formylpterin* (initial concentration 150 μM) *photolyzed at pH 11 and* b) *6-carboxypterin.* Calibration curves were previously obtained in HPLC experiments as described under *Experimental.*

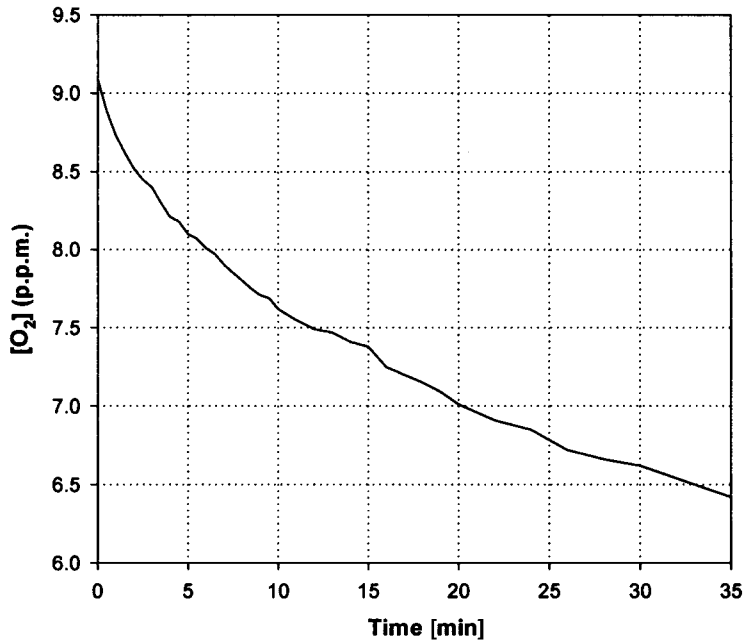


Fig. 6. *Time evolution of the O_2 concentration in irradiated solution of 6-formylpterin* (150 μM and pH 11.0)

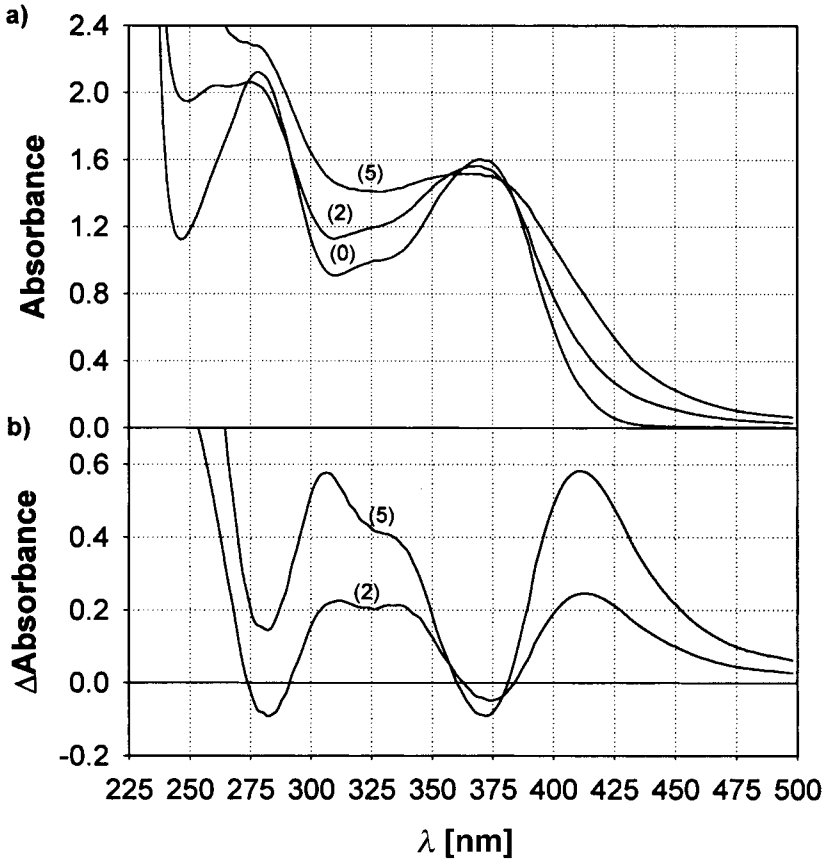


Fig. 7. a) *Spectral changes observed in solutions with initial concentrations of 125* μM *in 6-formylpterin and 10* mM *in furfuryl alcohol at pH 11, before irradiation, and after 2 and 5 min of continuous irradiation at 350 nm.* Experiments were performed in standard cells with an optical length of 1 cm. b) *Difference spectra obtained after subtracting the spectrum at* t = *0 from those recorded of irradiated solutions.*

The photoproduct obtained in oxygen-free solutions is stable in closed oxygen-free cells. However, it readily suffers a thermal reaction as soon as air or O_2 is admitted into the cell. The band around 480 nm disappears. Difference experimental spectra match the normalized theoretical spectra of the reference solution of 6-formylpterin and 6-carboxypterin. These observations show that 6-carboxypterin is the oxidation product of the 'red compound' (*Fig. 10*).

TLC Runs performed on the aerated solutions show a new fluorescent spot with an R_f value identical to that of 6-carboxypterin (not shown). The intensity of the spot of 6-formylpterin decreases with the irradiation time. No other spots or fluorescent products can be detected by this procedure.

HPLC Experiments confirm the same tendency as observed in TLC runs. The rate of degradation of 6-formylpterin is $4.7 \pm$ μM/min (quantum yield of 1.1×10^{-2}), whereas the rate of formation of 6-carboxypterin is 3.3 ± 0.1 μM/min (quantum yield of

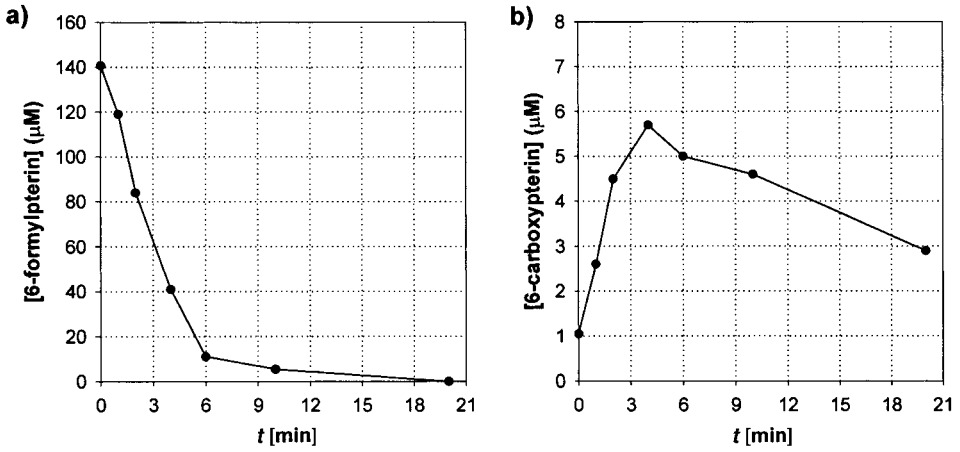


Fig. 8. a) *Time evolution of a photolyzed solution of 6-formylpterin* (140 μM at pH 11.0) *in the presence of furfuryl alcohol* (10 mM). b) *Time evolution of 6-carboxypterin in the same system.* Concentrations were determined by HPLC.

0.8×10^{-2}) (see *Fig. 11*). The quantum yield is of the same order of magnitude as that measured in the presence of O_2 (*i.e.*, 0.9×10^{-3}).

The 'red intermediate' detected in the absence of O_2 seems to be a relevant intermediate in the oxidation of 6-formyl- to 6-carboxypterin. The similar quantum yields measured in the presence and the absence of O_2 support this hypothesis. In air atmosphere, the 'red intermediate' oxidizes immediately to 6-carboxypterin, explaining why the 'red intermediate' can be detected only under anaerobic conditions. If this is the reaction pathway, singlet oxygen is not essential in the later stage of photooxidation of 6-formyl- to 6-carboxypterin, because this process takes place in the absence of light.

As reported in the literature, 6-carboxypterin suffers a slow photodegradation in acidic medium to yield pterin as a major photoproduct. However, at pH 11.0, pterin or other pteridinic compounds are not formed [14], the presence of oxygen being essential to observe photochemical changes. Moreover, 6-carboxypterin does not suffer any chemical modification as a consequence of the absorption of UV-A light in the absence of O_2.

Conclusions. – Photo-oxidation of 6-formylpterin in alkaline medium show similar patterns to those observed in acidic medium. After absorption of a photon and without participation of dioxygen, a 'red intermediate' is generated, which shows spectral characteristics different than those observed for the substrate and 6-carboxypterin. 'Pterins' derivatives that show similar spectral characteristics than those observed in this paper have been reported in the photochemical studies on biopterin [22][23].

In a second stage, but corresponding to a themal oxidation processes, 6-carboxypterin is produced by reaction of this 'red intermediate' as seen in the *Scheme*.

As it was proposed in literature [22][23], the long-wavelength absorption at 480–500 nm of low intensity seems to be characteristic for 6-acyl-5,8-dihydropterins. These compounds suffer spontaneous dehydrogenation to corresponding 6-acylpterin upon

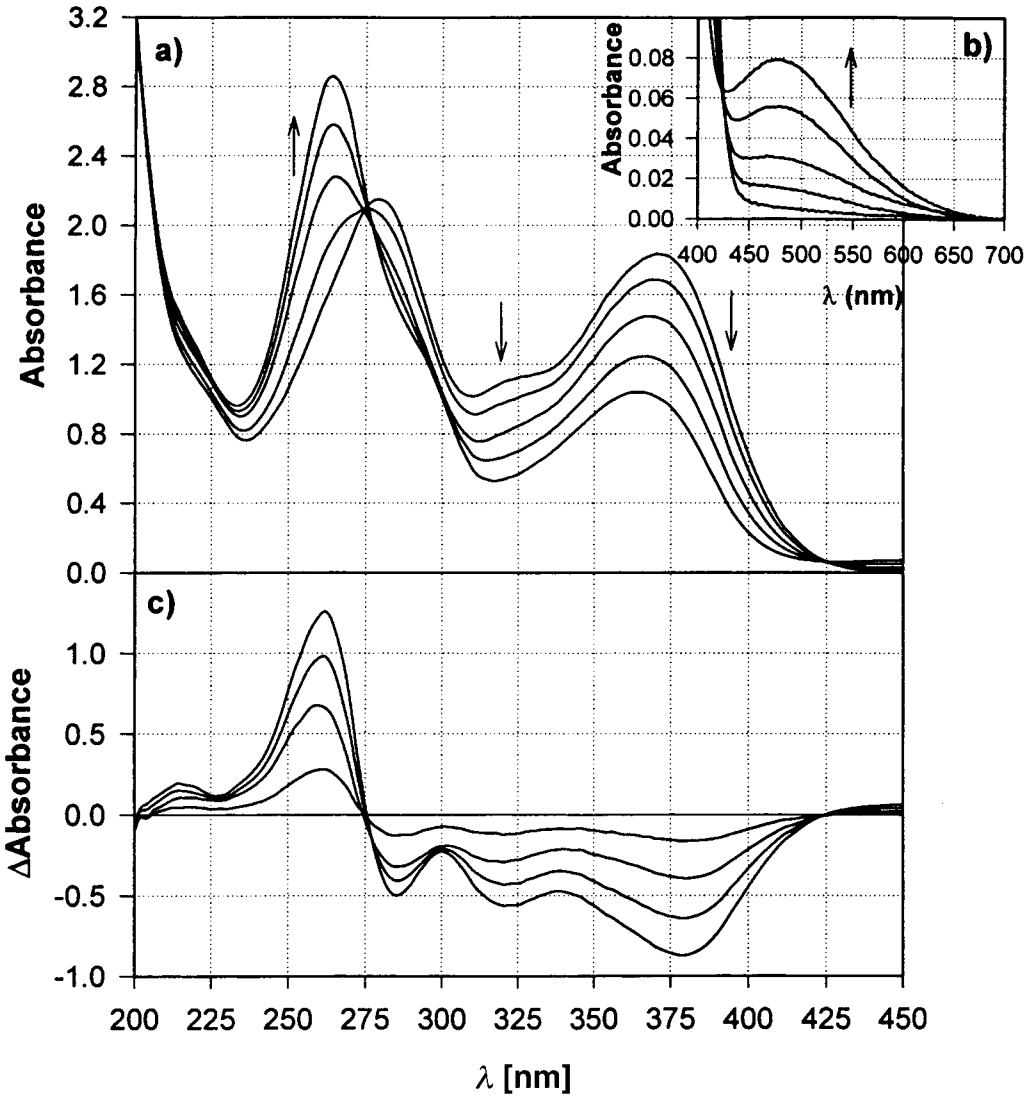


Fig. 9. a) *Time evolution of the absorption spectra of irradiated solutions of 6-formylpterin* (150 μM, pH 11.0) *in the absence of oxygen.* Spectra were recorded after irradiating these solutions 4, 9, 17, and 33 min in 1-cm-optical-length cells. Arrows shows the change direction recorded. The insert (*b*) show the recorded spectrum in the wavelength region 400–700 nm. *c*) *Difference spectra corresponding to those shown in* a.

exposure to O_2. Due to its spectral features and reactivity, the 'red intermediate' detected in our experiments could be 6-carboxy-5,8-dihydropterin. Further investigations are in progress to obtain additional evidence on the nature of the intermediate detected in this study.

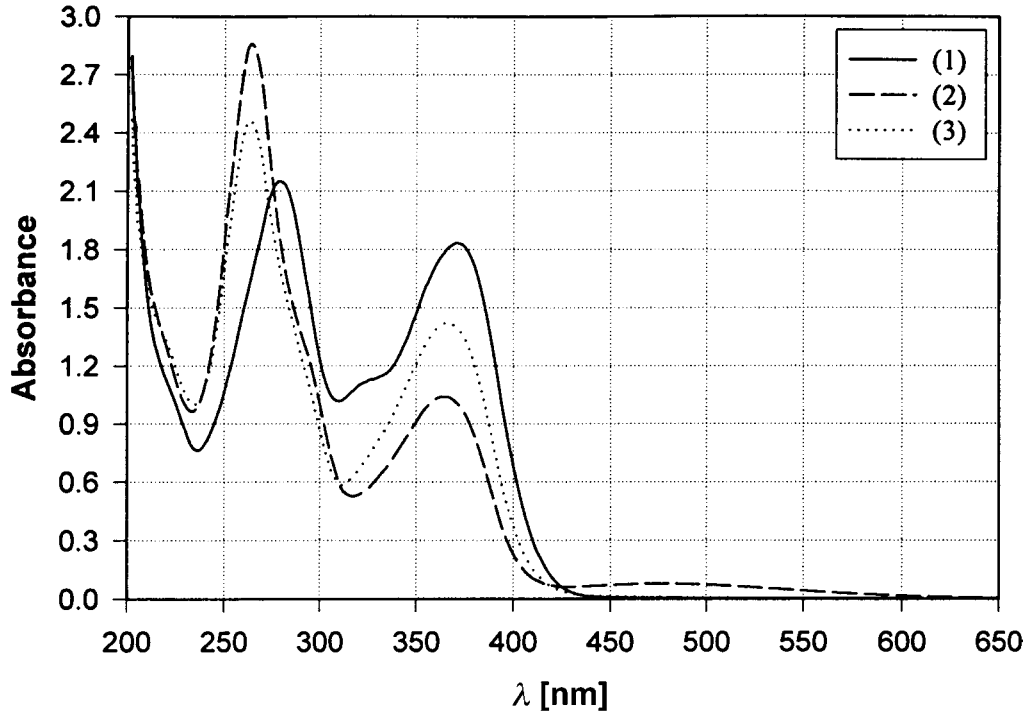


Fig. 10. *Photolysis of a oxygen-free solution of 6-formylpterin* (150 μM, pH 11.0). *1:* Spectrum of the started solution, *2:* Spectrum of the solution after 33 min of continuous irradiation. See the band between 400 to 600 nm. *3:* Same solution as that shown previously after aeration.

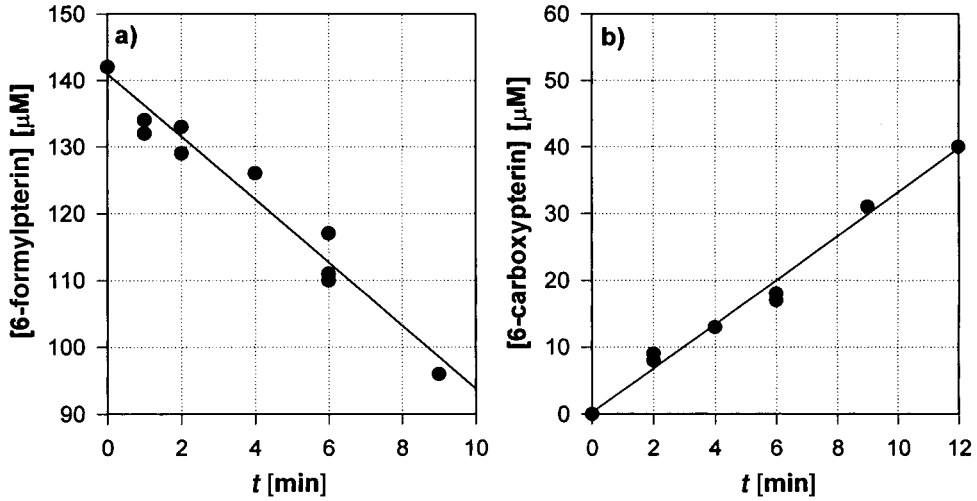
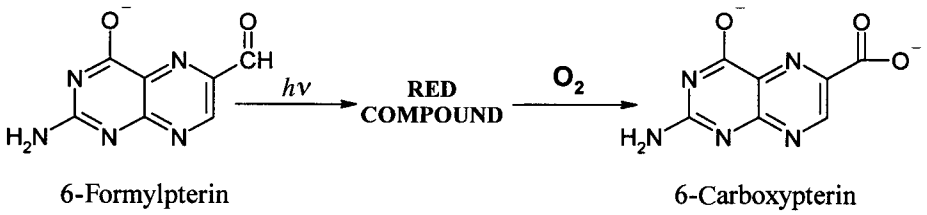


Fig. 11. *Time* vs. *concentration profiles of* a) *6-formylpterin photolyzed at pH 11 in deaerated solutions* (initial concentration 140 μM) *and* b) *5-carboxypterin*

Scheme



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