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The visible light irradiated solutions of the 1-methyl-1-[1'-(β -aspartoyl-methyl ester-imino)]ethenyl]ketal-1*H*,5*H*-pyrido[3,2-*a*]phenoxazin-5-one (**1**) and the 1,5-dimethoxy-11-(β -aspartoyl-*N*-acetyl-methyl ester)pyrido[3,2-*a*]phenoxazine (**2**) [1], in methanol and acidic methanol, are examined. Both methanolic solutions undergo light induced transformation according to an opening of the phenoxazinone and phenoxazine systems, not reversible in darkness. On the contrary, **1** and **2** in methanol-acid solutions, under visible light irradiation, yield a solvent photoaddition, reversible in darkness.

Some phototransformation products are examined and a plausible mechanism, for the reactions explanation, is suggested.

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Introduction.

The photochemical behaviour of a synthetic ommochrome, obtained from the oxidation of the 3-hydroxykynurenine (**3**) [1], the 1-methoxy-11-(β -aspartoyl-*N*-acetyl-methyl ester)-5*H*-pyrido[3,2-*a*]phenoxazin-5-one (**4**), was described in a preceding paper [2]. The phototransformation products, the 1-methoxy-11-(β -aspartoyl-*N*-acetyl-methyl ester)pyrido[3,2-*a*]phenoxazine (**5**) and the 1,6-dimethoxy-11-(β -aspartoyl-*N*-acetyl-methyl ester)-5*H*-pyrido[3,2-*a*]phenoxazin-5-one (**6**), present in the reaction mixture, were identified [2].

Two other synthetic ommochromes, obtained from the oxidation mixture of **3** [1], the 1-methyl-1-[1'-(β -aspartoyl-methyl esterimino)]ethenyl]ketal-1*H*,5*H*-pyrido[3,2-*a*]phenoxazin-5-one (**1**) and the 1,5-dimethoxy-11-(β -aspartoyl-*N*-acetyl-methyl ester)pyrido[3,2-*a*]phenoxazine (**2**) [1], were, at present, examined. The photochemical behaviours of **1** and **2**, in methanol and acidic methanol, were reported.

Results.

The compound **1** [1], dissolved in methanol, showed an uv spectrum with an absorption maximum at 436 nm (O. D. 0.424) and a shoulder at 390 nm (O. D. 0.330).

During the photoirradiation, the 436 nm optical density decreased so much that it reached an optical density of 390 nm. After 4 hours of uninterrupted irradiation, the uv spectrum showed two absorption maxima at 436 and 390 nm with the same optical density (O. D. 0.185), reduced to half compared with the starting spectrum (the absorption curves are reported in Figure 1). The uv spectrum of the irradiated solution, cooled in darkness overnight, did not display any variation the day after and therefore the reaction was not reversible. The uv spectrum of a standard non-photoirradiated sample was stable during the same period of time.

The photoirradiated mixture, evaporated *in vacuo*, af-

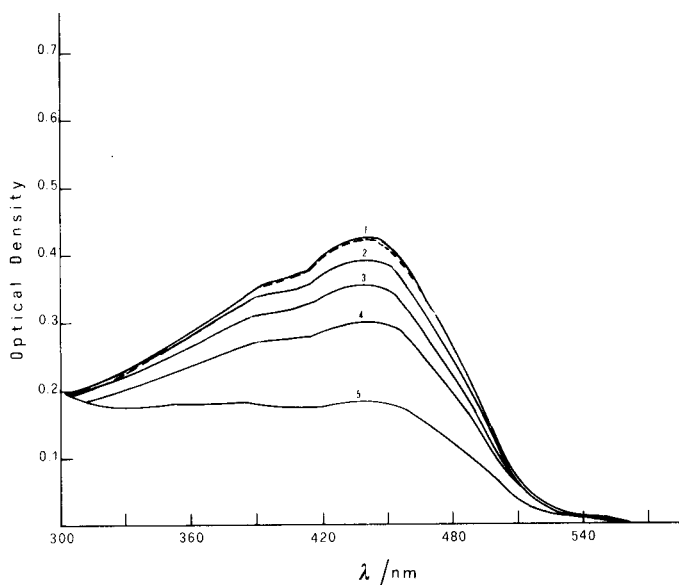


Figure 1. Spectral changes of **1**, in aerated methanol, caused by visible light irradiation; curve 1: first spectrum; curve 5: after ca. 4 hours of irradiation; dashed curve: solution of **1** kept in a cool, dark place for 4 hours.

forded two colored products: a red and a yellow compound which were difficult to analyze because of their small amounts.

Compound **1**, dissolved in mixture of methanol 0.5 *N* hydrochloric acid, showed two absorption maxima at 461 nm (O. D. 0.220) and 390 nm (O. D. 0.185). After 2 hours of uninterrupted photoirradiation, the uv spectrum showed maxima at 480 nm (O. D. 0.150) and 380 nm (O. D. 0.135). The solution, cooled in darkness overnight, showed the same uv spectrum of a non-photoirradiated solution of **1** kept in the dark for the same period of time, in fact the spectrum initially showed two maxima at 461 and 390 nm that, after irradiation, shifted to 480 and 380 nm. The described photochemical behaviour was observed many times on the same solution.

From the irradiated mixture, neutralized with sodium acetate, extracted with chloroform and examined by thin layer chromatography, were isolated and identified three colored products: a yellow compound, the 1-methoxy-11-(β -aspartoyl-*N*-acetyl-methyl ester)-5*H*-pyrido[3,2-*a*]phenoxazin-5-one (**4**), a red compound, the 1-methoxy-11-(β -aspartoyl-*N*-acetyl-methyl ester)pyrido[3,2-*a*]phenoxazine (**5**) and a yellow-orange compound, the 1,6-dimethoxy-11-(β -aspartoyl-*N*-acetyl-methyl ester)-5*H*-pyrido[3,2-*a*]phenoxazin-5-one (**6**). Compounds **4** and **6** were identified by comparison of their chromatographic and spectral (ir, nmr, uv) properties with the authentic samples of **4** and **6**; compound **5** was very unstable and was rapidly oxidized by air into **4**.

Compound **2**, dissolved in methanol, showed an uv spectrum with an absorption maximum at 460 nm (O. D. 0.825) and a shoulder at 375 nm (O. D. 0.675). After 6 hours of uninterrupted photoirradiation, the uv spectrum showed a stable absorption maximum at 375 nm (O. D. 0.675), an isosbestic point at 415 nm (O. D. 0.460) and a shoulder at 436 nm (O. D. 0.430), while the standard unphotoirradiated sample was stable in darkness during the same period of time. This transformation was not reversible in darkness overnight (the absorption curves are reported in Figure 2).

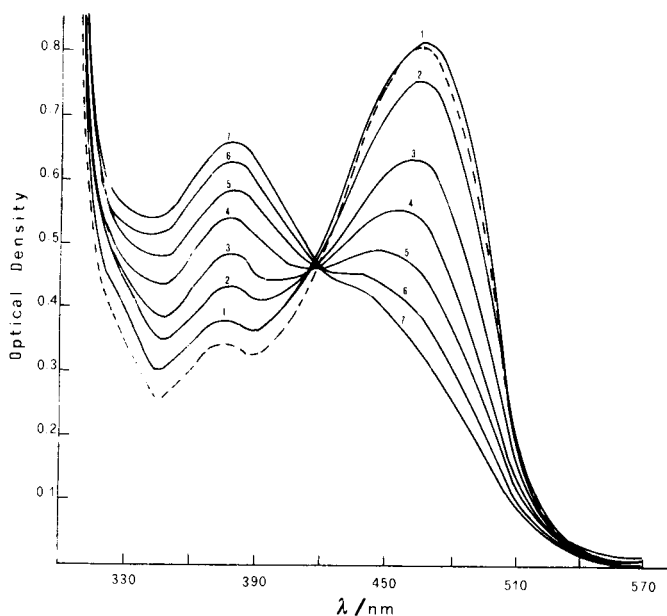
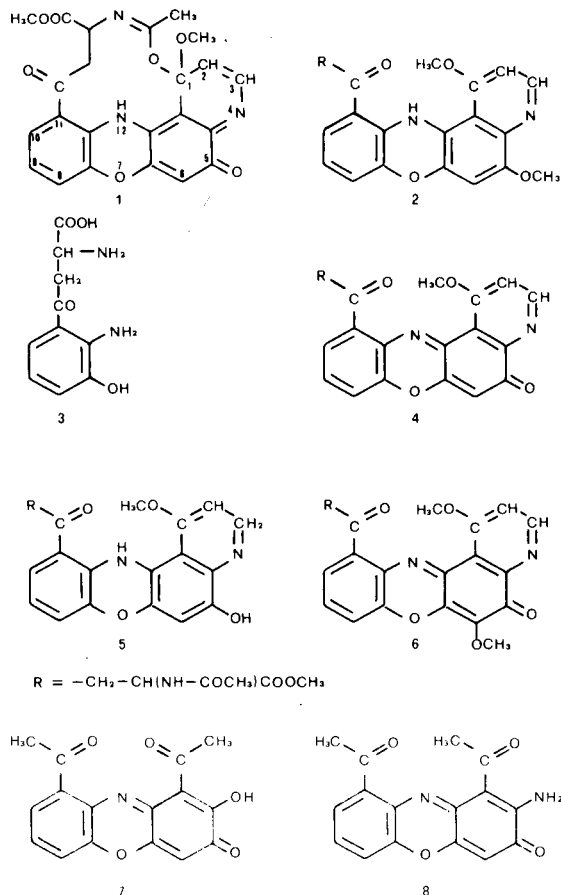


Figure 2. Spectral changes of **2**, in aerated methanol, caused by visible light irradiation; curve 1: first spectrum; curve 7: after ca. 6 hours of irradiation; dashed curve: solution of **2** kept in a cool, dark place for 6 hours.

The photoirradiated mixture, evaporated *in vacuo*, afforded many coloured products: a red compound, the 1-methoxy-11-(β -aspartoyl-*N*-acetyl-methyl ester)pyrido[3,2-*a*]phenoxazine (**5**), a yellow compound, the 1-methoxy-11-(β -aspartoyl-*N*-acetyl-methyl ester)-5*H*-pyrido[3,2-*a*]-



phenoxazin-5-one (**4**), a yellow-orange compound, the 1,5-dimethoxy-11-(β -aspartoyl-*N*-acetyl-methyl ester)pyrido[3,2-*a*]phenoxazine (**2**), a violet compound and another yellow product.

Compound **5** was rapidly oxidized by air into **4**. Compounds **4** and **2** were identified by comparison of their chromatographic and spectral properties (ir, nmr, uv) with authentic samples of the products **4** and **2**. The violet and yellow compounds, that probably are products of further phototransformation of **2**, were not identified because of their small amounts.

The compound **2**, dissolved in a mixture of methanol 0.5*N* hydrochloric acid, showed an uv spectrum with two maxima at 470 nm (O. D. 0.790) and 380 nm (O. D. 0.550). After 2 hours of irradiation. The uv spectrum showed two stable maxima at 487 nm (O. D. 0.650) and 380 nm (O. D. 0.450). This transformation was reversible in darkness overnight, in fact the irradiated solution showed, the day after, the same spectrum of the non-photoirradiated solution of **2** kept in dark for the same time. The described photochemical behaviour was observed many times on the same solution.

From the photoirradiated mixture, neutralized with sodium acetate, extracted with chloroform and purified by tlc chromatography, one yellow-orange product was obtained identified as the 1,6-dimethoxy-11-(β -aspartoyl-*N*-

acetyl-methyl ester)-5*H*-pyrido[3,2-*a*]phenoxazin-5-one (**6**) by comparison of its spectral (ir, nmr, uv) and chromatographic properties with an authentic sample of **6**.

On the basis of the reported transformations, the photochemical behaviour of **1** and **2**, in methanol-acid, is the as that for compound **4**, as reported in a previous paper [2].

Discussion.

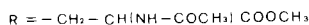
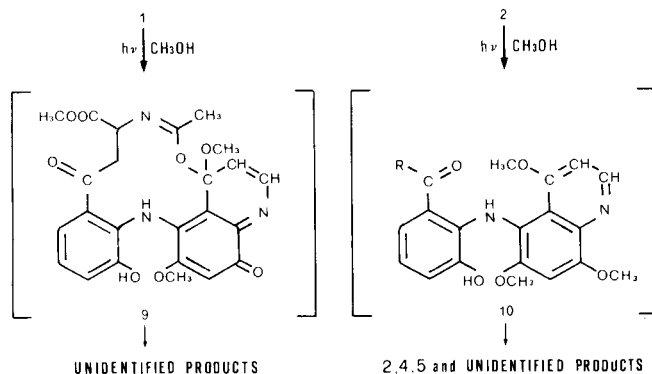
It has been reported in the literature [3] that the 1,9-diacetyl-2-hydroxy-3*H*-phenoxazin-3-one (**7**), in methanol, is in equilibrium with a form on which the solvent addition on C-4a takes place with the formation of an hemiketal, in equilibrium with an open structure. This transformation corresponds to an uv spectrum change from the maxima at 446-24 nm to 334 nm, according to the equilibrium reaction, and has been widely described [4,5]. The thin layer chromatography indicates the presence of only **7**.

This hemiketal formation reaction, in methanol, does not occur on the 1,9-diacetyl-2-amino-3*H*-phenoxazin-3-one (**8**). Compound **8**, in methanol and acidic methanol, is also stable under visible light irradiation.

Indeed, it is reported in the present series [2], that compound **4** is stable in methanol in darkness and under visible light irradiation.

Compound **1**, on the contrary, is stable in methanol in darkness, but unstable under visible light irradiation. This phototransformation, on the basis of the recorded spectral data, arises from the solvent addition on C-6a and subsequent opening of the phenoxazinone system with formation of the structure **9** (Scheme). From the photoirradiated mixture of **1** the starting compound was not isolated, but two products of further transformation were obtained, arising from the unstable compound **9** under visible light irradiation.

Scheme



On the basis of the reported results, it could be hypothesized that the phenoxazinone system opening, in methanol in darkness and under visible light irradiation, subsequently at solvent addition, occurs on an *o*-quinone structure. The formation in compound **8** of an *o*-iminoquinone

is very difficult, in fact the simple *o*-iminoquinones are not described and nothing is known about their reactivity.

The photochemical behaviour of **1**, in methanol, where an *o*-iminoquinone structure is present, compared with the photochemical behaviour of **4**, under the same conditions, is in accordance with this hypothesis. The stability of **1** in methanol in darkness and the opening of **1** under visible light irradiation can be explained with the high stability related to the conjugation of the pyrido[3,2-*a*] moiety with the phenoxazinone system.

Compound **7**, also in acidic methanol in darkness, yields hemiketal formation reaction and subsequent ring opening, recorded by the uv spectrum with the typical maximum at 334 nm.

Compound **4**, irradiated in acidic methanol, undergoes a reversible solvent photoaddition on C-6, as reported in the previous paper [2].

Compound **1**, irradiated in acidic methanol with visible light, is easily hydrolyzed into **4** and, for this reason, has the same photochemical behaviour.

The compound **2** is stable in methanol in darkness but, under visible light irradiation, yields the solvent photoaddition on C-6a and subsequently opening of the phenoxazine structure **10**, in accordance with the described phenoxazine systems [4]. Compound **10** is more stable under the irradiation than **9**, in fact from the irradiated mixture of **2** isolated were isolated **2**, **4**, **5** and only small amount of two further transformation products. Compound **2** was obtained from the **10** closing, **5** was obtained from the hydrolysis of the methoxy group on C-5 and **4** was obtained from the light catalyzed oxidation of **5**, in fact preliminary studies show that the light is an oxidation catalyst [3,6].

Compound **2** is stable in acidic methanol in darkness but is transformed under visible light irradiation. On the basis of the reported results, it could be hypothesized the first oxidative hydrolysis of the methoxy group on C-5, catalyzed by light in an acidic environment, affords the formation of **4**. From the irradiated mixture of **2**, only **6** was obtained and, for this reason, it is possible to hypothesize that the hydrolysis of the methoxy group is slower than the subsequent oxidation.

EXPERIMENTAL

The uv spectra were recorded with a Perkin-Elmer 550-S spectrophotometer. The products were purified on a 0.5 mm Whatman PK6F silica gel layers eluted with a benzene-methylene chloride-methanol 50:45:5 v/v mixture (mixture A).

The chromatographic purity and R_f were checked on 0.25 mm Whatman PK6F silica gel analytic layers eluted with mixture A.

General Procedure for the Photoirradiation.

The solution was exposed to a 650 W OSRAM lamp, at 10 mm of distance, at a temperature of 5° and was spectrophotometrically controlled every hour. A small amount of solution was preserved in darkness at a temperature of 5° as the standard sample.

The irradiated solution was kept in darkness at a temperature of 5° overnight and was again irradiated the next day.

Photoirradiation of 1-Methyl-1-[1'-(11-(β -aspartoyl-methyl ester-imino)-ethenyl)]ketal-1*H*,5*H*-pyrido[3,2-*a*]phenoxazin-5-one (**1**) in methanol.

One hundred ml of a $3 \cdot 10^{-5}M$ solution of **1** in methanol, with a λ max at 436 nm (O. D. 0.424) and a shoulder at 390 nm (O. D. 0.330), were photoirradiated. After 4 hours of irradiation, the uv spectrum showed two absorption maxima at 436 nm (O. D. 0.185) and 390 nm (O. D. 0.185). This transformation was not reversible in darkness overnight. The uv spectrum of the standard sample was stable with time. The irradiated mixture, evaporated *in vacuo* and analyzed on silica gel layers eluted with mixture A, afforded two products: a red compound (Rf 0.20) and a yellow compound (Rf 0.12) that required a more polar elution mixture such as benzene-methylene chloride-methanol 40:35:25 v/v.

Photoirradiation of 1-Methyl-1-[1'-(11-(β -aspartoyl-methyl esterimino)-ethenyl)]ketal-1*H*,5*H*-pyrido[3,2-*a*]phenoxazin-5-one (**1**) in Acidic Methanol.

One hundred ml of a $3 \cdot 10^{-5}M$ solution of **1**, in a methanol 0.5*N* hydrochloric acid 80:20 v/v mixture, showed two absorption maxima at 461 nm (O. D. 0.220) and 390 nm (O. D. 0.185). After 2 hours of irradiation, the uv spectrum showed two stable maxima at 480 nm (O. D. 0.150) and 380 nm (O. D. 0.135), and an optical density increase in the 500-570 nm range, while the standard sample uv spectrum was stable with time. This transformation was reversible in darkness overnight, in fact the uv spectrum initially showed two maxima at 461 and 390 nm that, after irradiation, shifted to 480 and 380 nm.

The irradiated mixture was neutralized with sodium acetate and extracted three times with 50 ml of chloroform. The chloroform solution, concentrated and analyzed on silica gel layers eluted with mixture A, afforded three products: **4** (Rf 0.26), **5** (Rf 0.22) and **6** (Rf 0.30). Compounds **4** and **6** were identified by comparison of their chromatographic and spectral properties with authentic samples of **4** and **6**.

Photoirradiation of 1,5-Dimethoxy-11-(β -aspartoyl-*N*-acetyl-methyl ester)-pyrido[3,2-*a*]phenoxazine (**2**) in Methanol.

One hundred ml of a $2 \cdot 10^{-4}M$ solution of **2** in methanol, with a λ max at 460 nm (O. D. 0.825) and a shoulder at 375 nm (O. D. 0.375), were photoirradiated. After 6 hours of irradiation, the uv spectrum showed a maximum at 375 nm (O. D. 0.675), an isosbestic point at 415 nm (O. D. 0.460) and a shoulder at 436 nm (O. D. 0.430), while the standard sample showed the starting uv spectrum. This transformation was not reversible

in darkness overnight. The irradiated mixture, evaporated *in vacuo*, showed, in methanol, an uv spectrum with two absorption maxima at 440 and 370 nm. From the irradiated mixture, analyzed on silica gel layers eluted with mixture A, several products were isolated: **2** (Rf 0.23), **4** (Rf 0.26), **5** (Rf 0.22), a violet compound (Rf 0.17) and yellow product (Rf 0.15). Compounds **2** and **4** were identified by comparison of their chromatographic and spectral properties with authentic samples of **2** and **4**. The yellow compound did not react with ascorbic acid on 0.25 mm thin layer chromatography.

Photoirradiation of 1,5-Dimethoxy-11-(β -aspartoyl-*N*-acetyl-methyl ester)-pyrido[3,2-*a*]phenoxazine (**2**) in Acidic Methanol.

One hundred ml of a $4 \cdot 10^{-5}M$ solution of **2**, in methanol 0.5*N* hydrochloric acid 80:20 v/v mixture, showed two electronic absorption maxima at 470 nm (O. D. 0.790) and 380 nm (O. D. 0.550). After 2 hours of irradiation, the uv spectrum showed two maxima at 487 nm (O. D. 0.650) and 380 nm (O. D. 0.450), while the standard sample was stable in time. This transformation was reversible the darkness overnight, in fact the absorption maximum at 487 nm shifted to the starting 470 nm. The irradiated mixture was neutralized with sodium acetate and extracted three times with chloroform. The chloroform solution, concentrated and analyzed on silica gel layers eluted with mixture A, afforded on product identified as **6** (Rf 0.30) by comparison of its chromatographic and spectral properties with an authentic sample of **6**.

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