Photochemistry of Ommochromes and Related Compounds

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The photochemistry of some synthetic ommochromes, the 1-methoxy-11-(β-aspartoyl-*N*-acetyl-methyl ester)-5*H*-pyrido[3,2-*a*]phenoxazin-5-one (**2**), the 1-methyl-1-{1'-[11-(β-aspartoyl-methyl ester-imino)]ethenyl}-ketal-1*H*,5*H*-pyrido[3,2-*a*]phenoxazin-5-one (**3**) and the 1,5-dimethoxy-11-(β-aspartoyl-*N*-acetyl-methyl ester)-pyrido[3,2-*a*]phenoxazine (**4**), obtained from the oxidation of the 3-hydroxykynurenine (**1**), has been examined. In an acidic methanol solution of **2**, a reversible solvent photoaddition was observed, associated with a yellow-red bathochromic shift. The mixture, stored in the dark, quantitatively records the starting absorption spectrum. In an acidic enviroment, **3** and **4**, affording **2**, show the same photochemical behaviour. A plausible mechanism, for explanation of the process is suggested.

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Introduction.

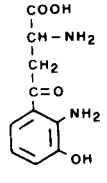
A photolabile ommochrome pigment, present in fly eyes, the structure of which could not be identified because of its small amount, was irradiated by visible light in acidic methanol, and undergoes a chromatic change from a yellow form to a red form [1].

Some synthetic ommochromes, obtained from the oxidation reaction of the 3-hydroxykynurenine (**1**) [2], the 1-methoxy-11-(β-aspartoyl-*N*-acetyl-methyl ester)-5*H*-pyrido-[3,2-*a*]phenoxazin-5-one (**2**), the 1-methyl-1-{1'-[11-(β-aspartoyl-methyl ester-imino)]ethenyl}ketal-1*H*,5*H*-pyrido[3,2-*a*]-phenoxazin-5-one (**3**) and the 1,5-dimethoxy-11(β-aspartoyl-*N*-acetyl-methyl ester)pyrido[3,2-*a*]phenoxazine (**4**), showed the same photochemical reactivity of the natural house fly pigment under irradiation by visible light in acidic methanol. Therefore we examined the photochemical behaviour of **2**, **3** and **4**.

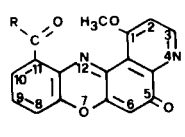
On the basis of the reported results, the natural pigment photochemistry seems to be due to a transformation of an oxidized form to a reduced form of the natural product [3,4] owing to a solvent photoaddition such as takes place in synthetic models **2**, **3** and **4**.

Results.

A 6 $10^{-5}M$ solution of **2** in a methanol-0.5*N* hydrochloric acid mixture (λ max at 458 and 373 nm) has been irradiated with visible light. After 2 hours of uninterrupted irradiation, the solution is photostable and its uv spectrum showed two maxima at 480 and 376 nm and an increase in the 600-500 nm range (the absorption curves are reported in Figure 1).



1



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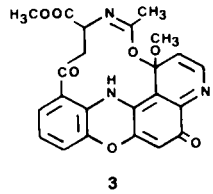

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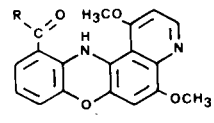

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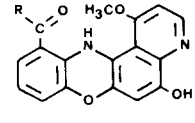

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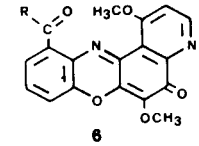

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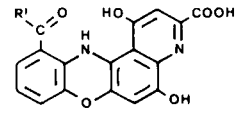
R = $CH_2CH(NHCOCH_3)COOCH_3$



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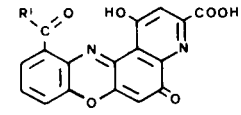

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R' = $CH_2CH(NH_2)COOH$

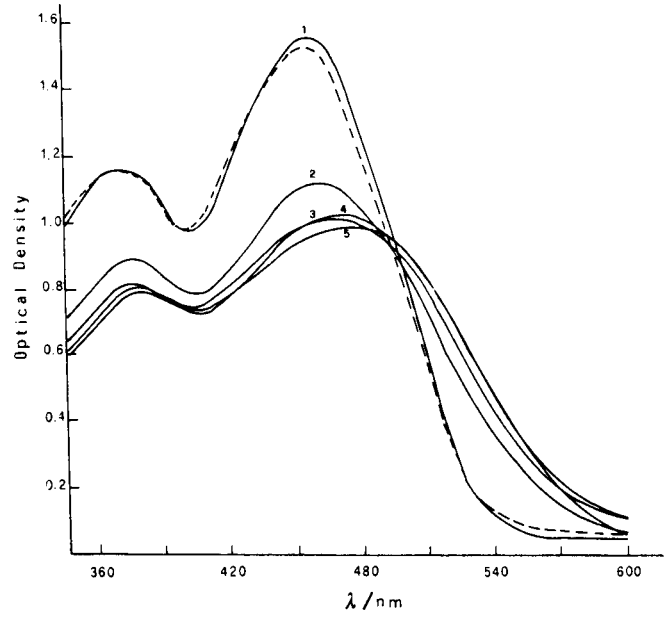


Figure 1. Spectral changes of **2** in aerated acidic methanol caused by irradiation with visible light. Curve 1: first spectrum; curve 5: after 3 hours of irradiation; dashed curve: solution of **2** kept in a cold and dark place (standard sample) for 3 hours.

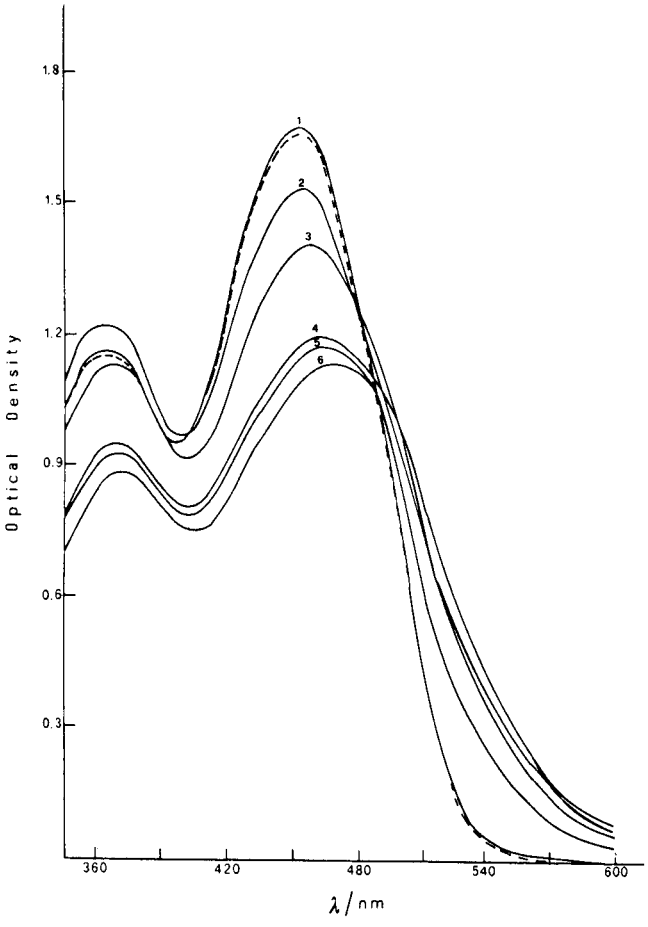


Figure 2. Spectral changes of the irradiated solution of **2**, kept in a cold and dark place overnight, in aerated acidic methanol caused by visible light irradiation. Curve 1: first spectrum; curve 6: after 3 hours of irradiation; dashed curve: standard sample.

The irradiated solution has been cooled in the dark overnight and its spectrum successively recorded. After this treatment it showed the same maxima of an unphotoirradiated solution of **2** (Figure 2).

After the photoirradiated mixture has been neutralized with sodium acetate, extracted with chloroform and analysed by tlc chromatography, it afforded three coloured products: a yellow compound, identified by comparison of its spectral (ir, mass, nmr, uv) and chromatographic properties with an authentic sample of **2**, a red compound, the 1-methoxy-5-hydroxy-11-(β-aspartoyl-*N*-acetyl-methyl ester)pyrido[3,2-*a*]phenoxazine (**5**), and the yellow-orange compound **6**.

The compound **5** was very unstable and was rapidly oxidized by air into **2** from which **5** is regenerated by treatment with ascorbic acid.

The structure of **6** was identified as the 1,6-dimethoxy-11-(β-aspartoyl-*N*-acetyl-methyl ester)-5*H*-pyrido[3,2-*a*]-phenoxazin-5-one. The mass spectrum showed the molecular ion at m/e 479 in the ei mode. The uv spectrum showed, in methanol, a maximum at 435 nm characteristic of a phenoxazinone structure [5].

The ^{1}H nmr spectrum of **6**, in deuteriochloroform, showed the proton signals of the β-aspartoyl-*N*-acetyl-methyl ester chain at δ 7.2 for the NH near at the acetyl group, a doublet coupled with the multiplet of the methine proton at δ 4.96. This multiplet was also coupled with the multiplet at δ 3.7 of the methylene group. The protons at C-8 and C-9 appeared as an almost overlapping doublet and triplet at δ 7.3 and 7.35; the proton at C-10 appeared as a doublet at δ 7.4. The proton at C-2 and C-3 appeared as two coupled doublets at δ 6.7 and 8.5. The reported chemical shifts are consistent with a pyrido[3,2-*a*]phenoxazinone system. The methoxy group signals appeared at δ 3.65, 3.7 and 3.8.

The uv spectra of **6** and **2** are very similar in methanol, shifted to higher wavelenghts (474 and 458 nm respectively) in acidic methanol, owing to the protonation of the products, but the larger shift of **6** is due to the methoxy group mesomeric effect [5].

A 3 $10^{-5}M$ acidic methanol solution of the compound **3**, showing λ max at 461 and 390 nm, was irradiated under the reported conditions and showed, after 2 hours, two stable maxima at 480 and 380 nm.

As reported for **2** photochemical behaviour the irradiated solution of **3**, kept in the dark overnight, quantitatively recorded the starting spectrum of the unphotoirradiated compound **2**.

From the irradiated solution, neutralyzed with sodium acetate, extracted with chloroform and examined by tlc chromatography, the same compounds present in the photoirradiated mixture of **2** were isolated and identified.

A 4 $10^{-5}M$ acidic methanol solution of the compound **4** (λ max at 470 and 380 nm), irradiated under the reported conditions, after 2 hours of irradiation, became stable displaying two stable maxima at 487 and 380 nm.

As reported above, also this irradiated solution, kept in the dark overnight, quantitatively recorded the starting spectrum of the unphotoirradiated compound **2**. Moreover from the irradiated acidic solution, after the reported treatments, only compound **6** was isolated and identified. The described photochemical behaviour of the solutions of **2**, **3** and **4** were observed many times on the same solution.

Compounds **2**, **3** and **4** were also irradiated with visible light in methanol. Compound **2** (λ max 430-350 nm) was stable to irradiation in methanol.

Compound **3** (λ max 436-390 nm) showed degradation suggesting a phenoxazinonic ring opening by the solvent [6] preceding the cleavage of the Shiff base on the cyclized

β-aspartoyl chain. The mixture, evaporated *in vacuo*, yielded unidentified products. Compound **4** (λ max 460-375 nm), under the reported conditions, showed an uv spectum, after 5 hours, with a maximum at 375 nm, a shoulder at 436 nm and an isosbestic point at 415 nm and the reaction was not reversible in darkness overnight (Figure 3).

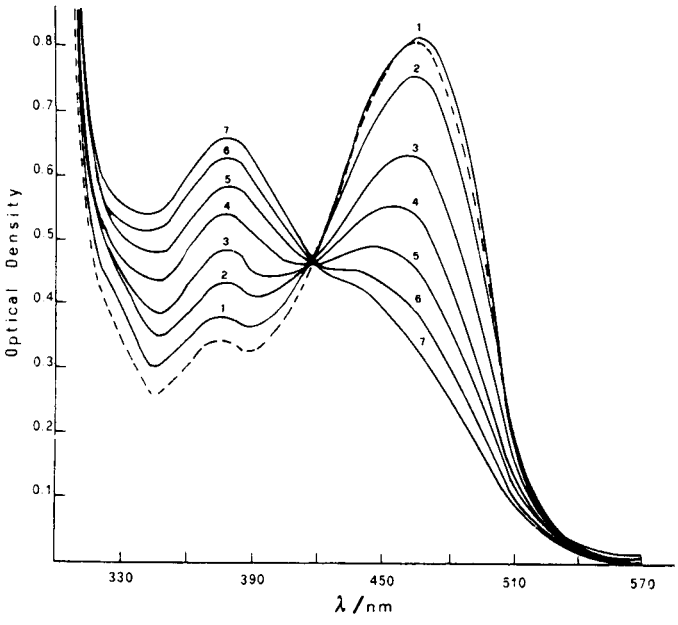


Figure 3. Spectral changes of **4**, in aerated methanol, caused by visible light irradiation. Curve 1: first spectrum; curve 7: after 6 hours of irradiation; dashed curve: standard sample.

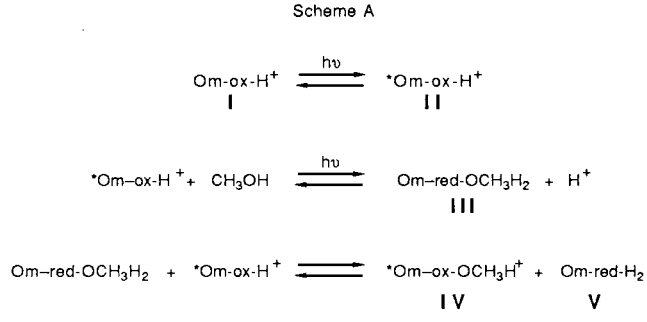
From this irradiated mixture, after evaporation *in vacuo*, **4** was recovered together with small amounts of **2** and **5**.

Discussion.

The hypsochromic and bathochromic shifts, during the irradiation of the solution of **2** in acidic methanol, seem due to the formation of a reduced form of **2** obtained from the solvent photoaddition on C-6, as is suggested by the isolation of **6** from the irradiated mixture [7]. In fact it is reported in literature that the phenoxazine structures, as dihydroxanthommatin (**7**), absorb at higher wave lengths (with lower ε values) than the corresponding phenoxazinone structures, as xanthommatin (**8**) [3,5].

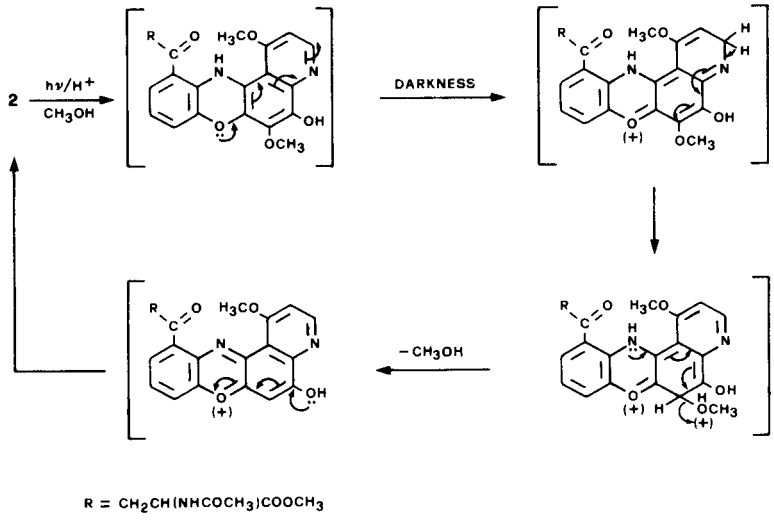
Therefore, on the basis of the isolated products, it is possible to hypothesize that, during the photochemical process, there is, at first, the formation of an excited form, *Om-ox-H$^+$, (**II**) of the protonated product **2**, Om-ox-H$^+$, (**I**), on which the solvent addition takes place. This process initially should give rise to the formation of **III** that, in the reaction mixture, is reoxidized to **IV** by **II** which is reduced to **V**.

Scheme A

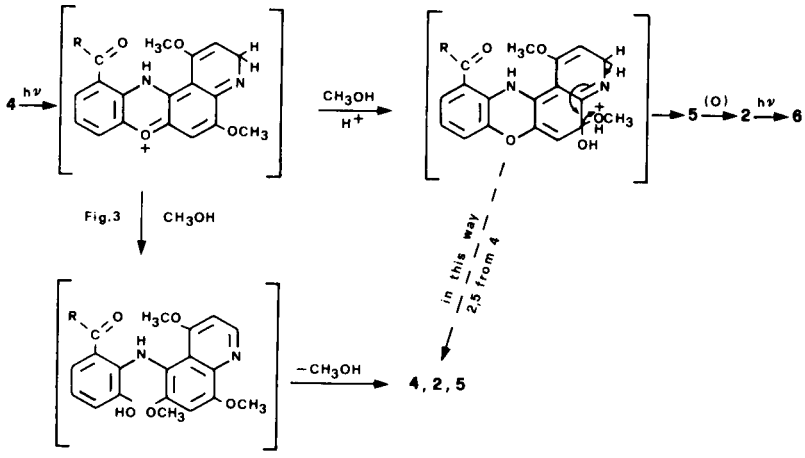


It is known that the reduction potential of quinones substituted with activating groups is lower than the one of unsubstituted quinones [8], so we hypothesize that the light enhances the above mentioned potential difference. Under the same reaction conditions, some riboflavins with

SCHEME B



SCHEME C



a chromophore similar to the one of the ommochromes yield reduced photoaddition products [9,10].

In the dark the excited forms **II** and **IV** disappear and a reverse reaction takes place with the concerted methoxy group taking off and return of the phenoxazinonic ring to the ground state (Scheme B).

Compound **3**, stable in acidic environment in the dark, irradiated under the reported conditions, is at first hydrolyzed into **2** and following shows the photochemical behaviour of **2**.

The isolation of **6** from the irradiated mixture of **4** demonstrated that the photoaddition velocity is larger than the one of the hydrolysis of the methoxy group on C-5 of **4**. The larger time of phototransformation is in agreement with the suggested first step of the reaction (Scheme C).

Compound **4**, stable in methanol in the dark, under visible light irradiation undergoes a solvent photoaddition on C-6a, as reported above for **3** in methanol, and consequently the opening of the phenoxazinone structure is observed and an isosbestic point emphasizes the one to one transformation. The elimination of the methoxy group on C-5, following the cyclization, during evaporation *in vacuo*, yields **5** and **2** together with the main product **4**.

The reported results suggest also that the natural fly-eye pigment photochemical behaviour is related to an oxidation-reduction transformation.

EXPERIMENTAL

The uv spectra were recorded with a Perkin-Elmer 550-S spectrophotometer. The ir spectra were detected in chloroform with a Perkin-Elmer 399 spectrophotometer. The ^{1}H nmr were recorded in deuteriochloroform with a Varian 200 spectrometer using tetramethylsilane as an internal reference, chemical shifts are given in δ (ppm), s = singlet, d = doublet, t = triplet, m = multiplet; signal attributions were confirmed with the homonuclear decoupling technique. Mass spectra were determined with a MS 30-AEI spectrometer in the ei mode. Melting points were determined with a Kofler apparatus and are uncorrected. The products were purified on 0.5 mm Whatman PK6F silica gel layers eluted with a benzene-methylene chloride-methanol 50:45:5 v/v mixture (mix A). The chromatographic purity and Rf were checked on 0.25 mm Whatman PK6F silica gel analytic layers eluted with mix A.

General Procedure for the Photoirradiation.

The solution was exposed to a 650 W OSRAM lamp (10 cm of distance) at a temperature of 5° and was spectrophotometrically controlled every half an 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 irradiated again the next day.

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

Two hundred of a 6 $10^{-5}M$ solution of **2**, in a methanol-0.5*N* hydrochloric acid 80:20 v/v mixture, showed two electronic absorption maxima at 458 and 373 nm. After 3 hours of irradiation the uv spectrum showed two stable maxima at 480 and 376 nm. This transformation was reversible in darkness overnight, in fact the uv spectrum initially showed two absorption maxima at 458 and 373 nm and, after irradiation, two maxima at 480 and 376 nm. This behaviour was observed many times on the same compound. The standard sample was stable with time.

After irradiation the mixture was neutralized with sodium acetate and extracted three times with 50 ml of chloroform. The chloroform solution was concentrated, analysed on silica gel, and the layers eluted with mix A affording three products: **2** (Rf 0.26, 1.3 mg), **5** (Rf 0.22, 2.1 mg), and **6** (2.3 mg). Compound **2** yielded **5** after treatment with ascorbic acid. Compound **5** was oxidized by air to **2**.

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 (**3**) in Acidic Methanol.

Two hundred ml of a 3 $10^{-5}M$ solution of **3**, in a methanol–0.5*N* hydrochloric acid 80:20 v/v mixture, showed two absorption maxima at 461 and 390 nm. After 2 hours of irradiation the uv spectrum showed two stable maxima at 480 and 380 nm and an optical density increase in the 500-570 nm range, while the uv spectrum of the standard sample was stable in time. This transformation was reversible in the dark overnight, in fact the uv spectrum initially showed two maxima at 461-380 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 was concentrated, and analysed on silica gel, and the layers eluted with mix A, affording three products: **2** (1.2 mg), **5** (0.67 mg), and **6** (0.71 mg). Compound **2** was identified by comparison of its chromatographic and spectral (ir, nmr, uv) properties with an authentic sample.

Photoirradiation of 1,5-Dimethoxy-11-(β-aspartoyl-*N*-acetyl-methyl ester)-pyrido[3,2-*a*]phenoxazine (**4**) in Acidic Methanol.

Two hundred ml of 4 $10^{-5}M$ solution of **4**, in a methanol–0.5*N* hydrochloric acid 80:20 v/v mixture, showed two electronic absorption at 470 and 380 nm. After 2 hours of irradiation the uv spectrum showed two maxima at 487 and 380 nm, while the standard sample was stable with time. This transformation was reversible in the dark overnight, in fact the absorption maximum at 487 nm shifted to the starting 470 nm the next day.

The irradiated mixture, after neutralization with sodium acetate, extraction with chloroform and analysis on silica gel layers as above reported, afforded one product identified as **6** (3.2 mg).

Photoirradiation of 1,5-dimethoxy-11-(β-aspartoyl-*N*-acetyl-methyl ester)pyrido[3,2-*a*]phenoxazine (**4**) in Methanol.

One hundred ml of a 2 $10^{-4}M$ solution of **4** in methanol, with a maximum at 460 nm and a shoulder at 375 nm, were photoirradiated.

After 6 hours of irradiation the uv spectrum showed a maximum at 375 nm, an isosbestic point at 415 nm and a shoulder at 436 nm, while the standard sample showed the starting uv spectra.

This transformation was not reversible in the dark overnight.

The irradiated mixture, evaporated *in vacuo*, showed in methanol an uv spectrum with two maxima at 440 and 370 nm.

From the irradiated mixture, analysed on silica gel layers and eluted with mix A, three products were isolated and identified: **4** (7.0 mg), **2** (1.3 mg) and **5** (0.9 mg).

1,6-Dimethoxy-11-(β-aspartoyl-*N*-acetyl-methyl ester)-5*H*-pyrido[3,2-*a*]-phenoxazin-5-one (**6**).

From the irradiated mixture **6** was isolated as yellow-orange crystals of mp 260° and Rf 0.3 (mix A); ir (chloroform): ν cm^{-1} 3420-3300 (NH), 1740 ($COOCH_3$), 1670-1650 (CO); uv (methanol): λ max (log ϵ) 435 nm (3.8); uv (methanol–0.5*N* hydrochloric acid 80:20 v/v): λ max (log ϵ) 474 nm (3.5), 370 nm (3.7); ^{1}H nmr (deuteriochloroform): δ 2.1 (s, 3H, $COCH_3$), 3.65 (s, 3H, OCH_3), 3.7 (s, 3H, OCH_3), 3.7 (m, 2H, CH_2CO), 3.8 (s, 3H, OCH_3), 4.96 (m, 1H, C*H*NH), 6.7 (d, 1H, N*H*CH), 7.2 (d, 1H, C=CH), 7.5 (d, 1H, aromatic), 7.65 (t, 1H, aromatic), 7.75 (d, 1H, aromatic), 8.5 (d, 1H, CH=N); mass spectrum m/e: 479 (M^+).

Anal. Calcd. for $C_{24}H_{21}N_3O_8$: C, 60.12; H, 4.42; N, 8.77. Found: C, 60.20; H, 4.39; N, 8.63.

Acknowledgement.

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