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Gas chromatography–mass spectrometry identification of photoproducts of hexahydroquinoline derivatives: Potential calcium channel antagonists

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Abstract

Photodegradation products of hexahydroquinoline derivatives (HHQ) have been analysed with gas chromatography–mass spectrometry (GC–MS). The photodegradation was carried out under the conditions recommended in the first version of the document issued by the International Conference on Harmonization (ICH), currently in force in the studies of photochemical stability of drugs and therapeutic substances. The study was performed on the compounds having two chlorine atoms at different positions of the phenyl ring. Photodegradation of dichlorophenyl derivatives of HHQ resulted in formation of one or three photoproducts. The main product of their decomposition was aromatic compound formed as a result of dehydrogenation of the dihydropyridine ring. The most often observed fragmentation pathway of the photoproducts formed was elimination of methyl and methoxy radicals from the ester groups. The fragmentation of the photoproducts containing one chlorine atom at the *ortho*-position of the phenyl ring occurred through elimination of chlorine radical.

Keywords: Hexahydroquinoline derivatives; Dihydropyridine derivatives; Calcium channel blockers; Mass spectrometry; Photodegradation

1. Introduction

Photochemical decomposition of drugs may lead to a decrease in their therapeutic effectiveness or even to the appearance of toxic products. Sometimes intake of photochemically changed drugs may induce hypersensitivity to light resulting possibly in phototoxic and photoallergic effects [1,2]. The long list of photosensitive drugs includes, e.g. calcium channel antagonists from the group of dihydropyridine (DHP) derivatives [3,4]. According to literature data, photodegradation of DHP derivatives leads to disappearance of their pharmacological activity. One of the methods to decrease the photosensitivity of these substances was their modification leading to new groups of potential calcium channel blockers activity, like e.g. hexahydroquinoline (HHQ) derivatives [5–8]. These compounds were synthesised according to the modified Hantzsch synthesis by the Safak and coworkers [9,10]. Detailed analysis of their pharmacological properties confirmed the compounds capability of producing the effects of contraction and expansion of the blood vessels.

The HHQ derivatives are structurally similar to DHP derivatives used for many years in medical therapy, e.g. their common element is the presence of the dihydropyridine ring that on illumination is easily oxidised to the aromatic pyridine ring [11–14]. To the best of our knowledge no report on the photochemical properties of the compounds of the hexahydroquinoline group has been published.

The aim of this study was determination of the photochemical properties of the compounds from this group, with the emphasis on finding the number of stable photoproducts and their identification.

2. Experimental

2.1. Chemicals and reagents

2,6,6-Trimethyl-3-carbomethoxy-4-(dichlorophenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline derivatives (HHQ),

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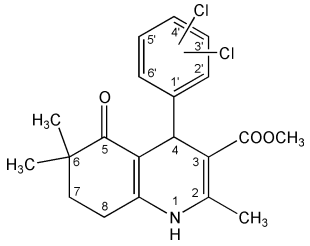

Fig. 1. Formula of 2,6,6-trimethyl-3-carbomethoxy-4-(*ortho*- or *meta*- or *para*-dichlorophenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline derivatives (HHQ).

$C_{20}H_{21}O_3NCl_2$ (m.w. = 393.3) were synthesised by the modified Hantzsch synthesis by the Safak's group from the Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Ankara, Turkey. The compounds contained two chlorine atoms at different positions of the phenyl ring were marked as (Fig. 1)

2′,3′-Dichloro HHQ	*ortho* and *meta*
2′,4′-Dichloro HHQ	*ortho* and *para*
2′,6′-Dichloro HHQ	*ortho* and *ortho*
3′,4′-Dichloro HHQ	*meta* and *para*

Samples were dissolved in methanol for liquid chromatography.

2.2. Sample preparation and condition of photodegradation

Methanol solutions of the HHQ derivatives at a concentration of 6.2×10^{-5} mol/l, were subjected to photodegradation according to the requirements of the first version of the Document issued by International Chemical on Harmonization (ICH), currently in force in photochemical studies of drugs and therapeutic substances [15,16]. The compounds were placed in a quartz cell of 2.8 ml in capacity, and irradiated with a high-pressure mercury lamp with a mercury burner HBO-50 from a distance of 3.5 cm, using a cutoff filter (λ_{exe} = 365 nm).

After specific time intervals, portions of 1.4 ml of the solution were collected, concentrated to dryness in nitrogen atmosphere and dissolved in 10 μl of methanol. The samples were concentrated in conical vials that were eventually capped.

The photostability testing was monitored by using a chemical actinometer. The number of the quanta absorbed was measured by a chemical actinometer of Reinecke salt—*trans*-tetrathiocyandiamminechromate (III) potassium (I) obtained from the ammonium salt. A Reinecke salt solution was irradiated with the wavelength λ = 365 nm for 85 s. The number of quanta absorbed by the actinometer (I_R) was 1.42×10^{17} and was equal to the number of quanta falling on the HHQ solution studied in the time of 85 s.

2.3. Conditions of chromatographic separation of the photodegradation products

The products of the photochemical degradation were analysed on a gas chromatograph 5890II, equipped with a selective mass detector 5971A; Hewlett-Packard. The separation was conducted on a capillary silica column DB-5; J&W (USA), of the internal diameter 0.25 mm, length of 30 m and the film thickness of 0.25 μm. The analysis was made in the following temperature regime: the injection chamber temperature 250 °C, the initial temperature in the oven 140 °C kept for 2 min, the rate of temperature increase 5 °C/min up to 200 °C, and then 10 °C/min up to 300 °C, and the final temperature was maintained for 13 min. The carrier gas was divided between the column and the injection chamber at the ratio 1:2. The carrier gas was helium, purity = 99.99%, flow-rate 1 ml/min, pressure 5 bar.

Gas chromatograph was connected to the mass spectrometer by an AMD402 interface the temperature of which was 300 °C.

2.4. Low-resolution mass spectra of the photoproducts

The low-resolution mass spectra of the photodegradation products of HHQ derivatives were taken on a two-sector mass spectrometer (type B/E) AMD 402, in the Nier-Johnson geometry. The unit resolution was R = 1000. The ionisation was performed by a stream of electrons of the energy 70 eV applying the accelerating voltage of 8 kV. The temperature of the source of electrons was 200 °C; while the temperature of evaporation varied from 100 to 250 °C. The low-resolution mass spectra in the normalised form are shown in the range from 50 *m*/*z* to 400 *m*/*z*.

2.5. High-resolution mass spectra of the photoproducts

In order to identify the fragmentation pathway of the photodegradation products the method of peak superposition was applied and the elemental compositions of the fragment ions were determined using perfluoroxene as a standard. The measurements were made on a mass spectrometer JMS D 100, with a resolution of R = 10,000. The error in determination of the elemental composition of the ions did not exceed 5 Da, relative to the results of theoretical calculations.

3. Results and discussion

3.1. Photochemical studies

The quantum yield of HHQ derivatives photodegradation was determined using the Reinecke salt as a chemical actinometer [17]. The HHQ derivatives solutions were irradiated until about 60% conversion, determined from a difference in the absorbance measured before and after a given irradiation time.

Table 1
Real quantum yields of dichlorophenylhexahydroquinoline derivatives

HHQ derivatives	Real quantum yields $\phi \pm \Delta\phi$
2′,3′-Dichloro	$3.95 \times 10^{-5} \pm 1.11 \times 10^{-6}$
2′,4′-Dichloro	$4.33 \times 10^{-4} \pm 2.70 \times 10^{-5}$
2′,6′-Dichloro	$1.69 \times 10^{-4} \pm 1.26 \times 10^{-6}$
3′,4′-Dichloro	$2.73 \times 10^{-5} \pm 6.75 \times 10^{-6}$

The energy of a quantum of radiation (E_q) of $\lambda = 365$ nm was calculated from the formula:

$$E_q = h\frac{c}{\lambda} = 6.626 \times 10^{-34}\,\mathrm{J\,s}\frac{2.998 \times 10^{8}\,\mathrm{m\,s^{-1}}}{365 \times 10^{-9}\,\mathrm{m}} = 5.442 \times 10^{-19}\,\mathrm{J}.$$

The intensity of irradiation (P) absorbed by the actinometer was

$$P = \frac{E_q I_R}{t} = \frac{5.4423 \times 10^{-19}\,\mathrm{J} \times 1.42 \times 10^{17}}{85\,\mathrm{s}} = 9.092 \times 10^{-4}\,(\mathrm{J\,s^{-1}}) = 9.092 \times 10^{-4}\,(\mathrm{W})$$

I_R is the number of quanta absorbed by actinometer.

Given the area of the cell surface equal to 2.26 cm^2, the energy of irradiation falling onto area of 1 m^2 in 1 h (E_s) was calculated (W h m^{-2}).

$$E_s = 0.4023\,(\mathrm{W\,m^{-2}\,s}) = 1448.25\,(\mathrm{W\,h\,m^{-2}}).$$

The Reinecke salt used as an actinometer has many advantageous features: it enables measurements of the absolute number of quanta, has a wide spectral range 320–750 nm and gives highly repetitive results. The quantum yield for a given percent of conversion was calculated from the formula:

$$\Phi = \frac{\Delta c N_A}{I_{abs} t}$$

where is the $\Delta c N_A$ is the difference in the number of the HHQ derivative molecules in the solution before and after the irradiation, I_{abs} is the intensity of radiation absorbed by the sample, t is time (s).

Experimentally determined quantum yields for particular irradiation times were extrapolated to the initial HHQ derivative concentration (0% conversion) to obtain the real quantum yield. The data of the real quantum yield are given in Table 1.

The quantum yield values determined indicate differences in the photodegradation rate of the compounds analysed. The photochemical sensitivity of the HHQ derivatives studied decreased in the sequence:

2′, 4′-dichloro > 2′, 6′-dichloro > 2′, 3′-dichloro > 3′, 4′-dichloro.

The relations obtained prove that the rate of photochemical degradation depends on the position of chlorine atoms in the phenyl rings.

The values obtained were in range 10^{-4} to 10^{-5}, which indicate on different speed of decomposition. The most photosensitive to light was HHQ derivative, which contained chlorine atom at *ortho*- and *para*-position in the phenyl ring. More stable was the HHQ derivative with chlorine atom at the positions *meta* and *para* of phenyl ring.

Results of our earlier studies and literature data have revealed that similar dependencies have been observed for calcium antagonists from the group of 1,4-dihydropyridine. Summing up the results of studies on the photochemical stability of the DHP derivatives, it can be concluded that the rate of their degradation is different and depends on the type and position of a substituent in the phenyl ring. The results suggest that particularly photoliable are DHP derivatives containing a nitro group in the *ortho*-position of the phenyl ring. Under the same irradiation conditions, the *meta*-isomer appeared to be more stable. The isomer with a nitro group at the *meta*-position was significantly more photostable [18].

3.2. Photoproduct identification

At the next stage an attempt was made at identification of the photoproducts. The method of gas chromatography was employed, which after appropriate optimisation permitted separation of products of photochemical degradation. Analysis of the chromatograms obtained indicated exposition of different HHQ derivatives to light leads to formation of a different number of photoproducts.

The results of the study have shown that the photodegradation of the HHQ derivatives studied leads to formation of:

- three photoproducts—on irradiation of 2′,3′-dichloro and 2′,4′-dichloro HHQ
- one photoproduct—on irradiation of 2′,6′-dichloro and 3′,4′-dichloro HHQ derivatives.

Results of the GC analysis are illustrated on the example of two chromatograms for solutions of 2′,3′-dichloro and 2′,6′-dichloro HHQ, subjected to total photodegradation, Figs. 2 and 3. As shown in Fig. 2, the chromatogram

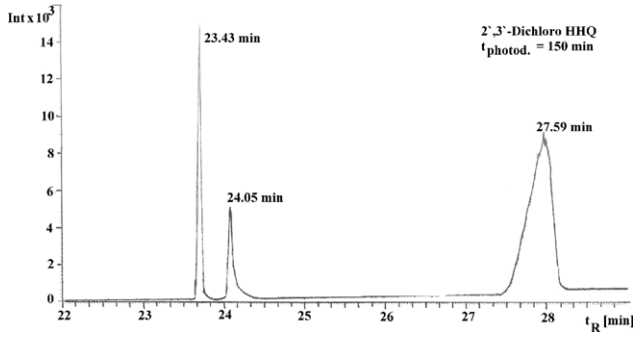

Fig. 2. GC chromatogram 2′,3′-dichloro HHQ derivative after all photodegradation (time photodegradation = 150 min); retention time of photoproducts: t_R = 23.43 min, t_R = 24.05 min, t_R = 27.59 min.

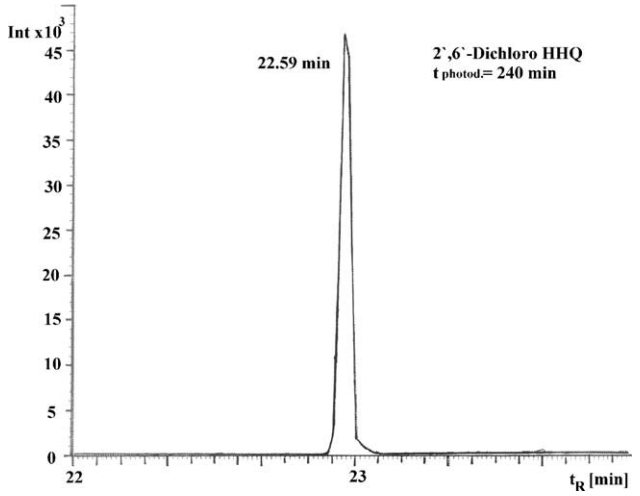

Fig. 3. GC chromatogram 2′,6′-dichloro HHQ derivative after all photodegradation (time photodegradation = 240 min); retention time of photoproduct: t_R = 22.59 min.

of 2′,3′-dichloro HHQ derivative indicates the presence of three photoproducts characterised by the retention times: (t_R) 23.43, 24.05 and 27.59 min. Photodegradation of 2′,6′-dichloro HHQ derivative (Fig. 3) leads to formation of only one photoproduct characterised by the retention time, t_R = 22.59 min. The retention times of the photoproducts appearing as a result of photodegradation of the other compounds analysed: 2′,4′-dichloro and 3′,4′-dichloro HHQ, are presented in the schemes of mass fragmentation in Figs. 7–9 and 11.

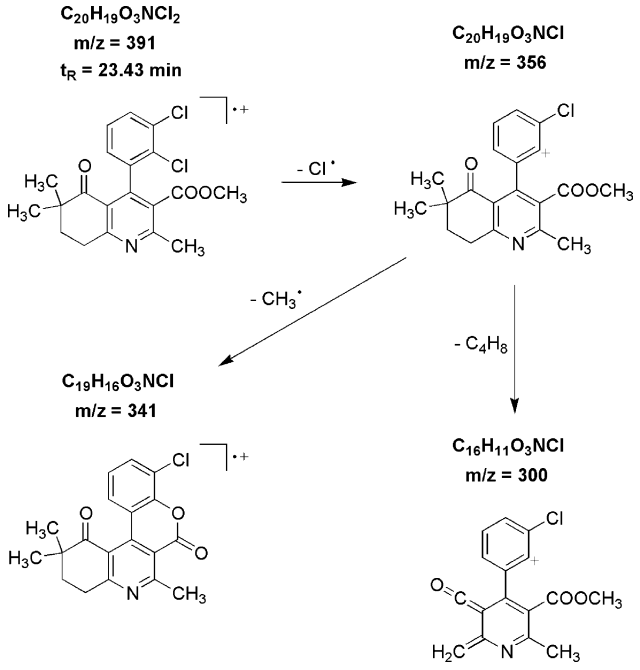

Fig. 4. Scheme of mass fragmentation of photoproduct (t_R = 23.43 min; Fig. 2) forming during photodegradation 2′,3′-dichloro HHQ derivative; identified photoproduct: 2,6,6-trimethyl-3-carbomethoxy-4-(2′,3′-dichlorophenyl)-5-oxo-5,6,7,8-tetrahydroquinoline.

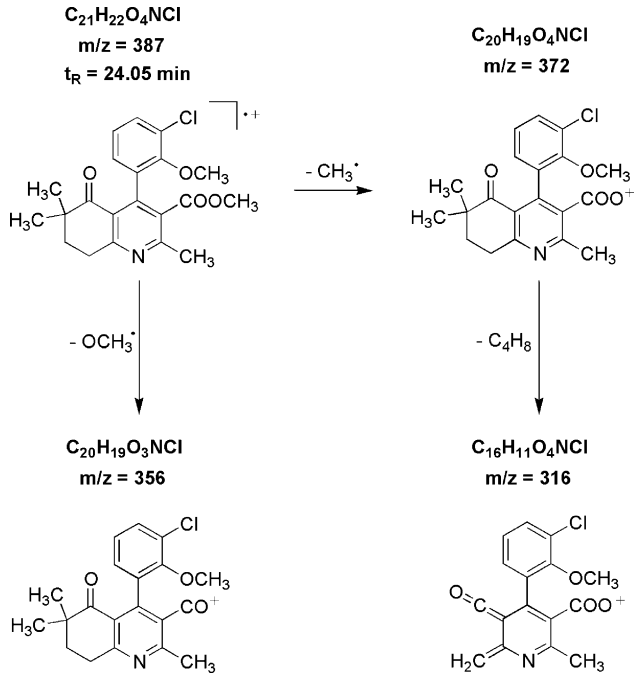

Fig. 5. Scheme of mass fragmentation of photoproduct (t_R = 24.05 min; Fig. 2) forming during photodegradation 2′,3′-dichloro HHQ derivative; identified photoproduct: 2,6,6-trimethyl-3-carbomethoxy-4-(2′-methoxy,3′-chlorophenyl)-5-oxo-5,6,7,8-tetrahydroquinoline.

The separated photoproducts were identified with the use of GC–MS, on ionisation by a stream of electrons (EI). The low- and high-resolution mass spectra permitted a determination of the *m*/*z* values and the elemental composition of molecular and fragmentation ions.

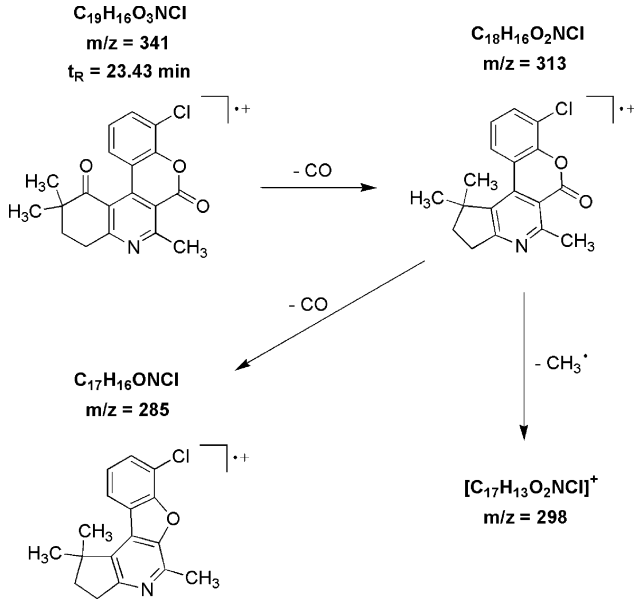

Fig. 6. Scheme of mass fragmentation of photoproduct (t_R = 27.59 min; Fig. 2) forming during photodegradation 2′,3′-dichloro HHQ derivative; identified photoproduct: 3-chloro-7,11,11-trimethyl-10,11-dihydro-9*H*-5-oxa-8-aza-benzo[c]fenantren-6,12-dion.

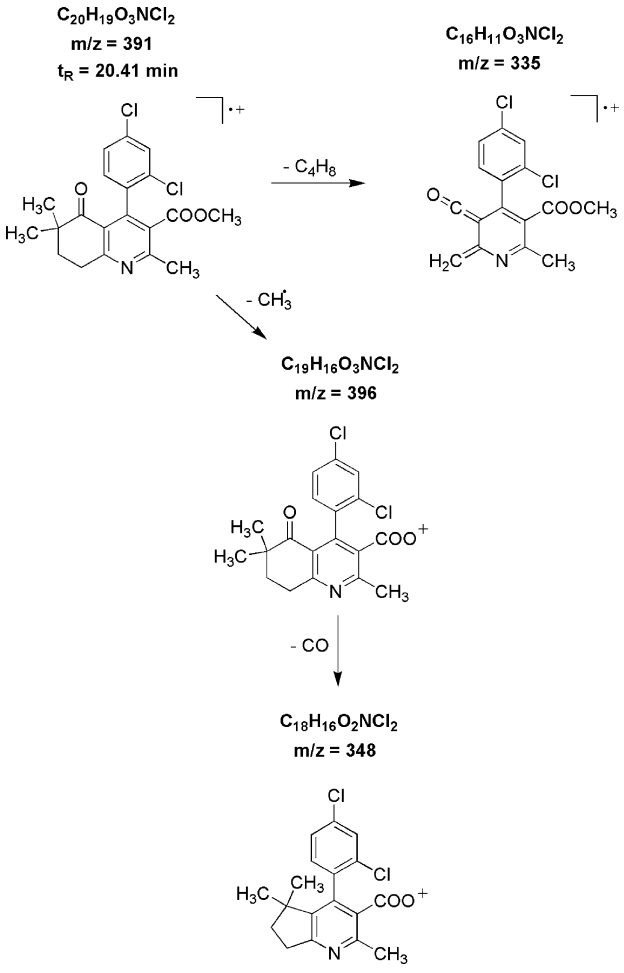

Fig. 7. Scheme of mass fragmentation of photoproduct (t_R = 20.41 min), forming during photodegradation 2′,4′-dichloro HHQ derivative; identified photoproduct as: 2,6,6-trimethyl-3-carbomethoxy-4-(2′,4′-dichlorophenyl)-5-oxo-5,6,7,8-tetrahydroquinoline.

On the basis of the fragmentation pathways analysis, the mass fragmentation schemes were proposed, Figs. 4–11.

A comparison of the data presented in Fig. 4 (2′,3′-dichloro), Fig. 7 (2′,4′-dichloro), Fig. 10 (2′,6′-dichloro), Fig. 11 (3′,4′-dichloro) has shown that the photodegradation of all HHQ derivatives analysed leads to the formation of pyridine derivatives as the main products.

The photodegradation of each HHQ derivative gives the product which on mass fragmentation gives molecular ions of *m*/*z* = 391. Therefore, these compounds are formed as a result of dehydrogenation of the dihydropyridine ring leading to the appearance of the aromatic pyridine ring.

Low photochemical stability of the dihydropyridine ring has already been established, e.g. in the studies of photostability of DHP derivatives used as calcium channel blockers. The photochemical sensitivity of nifedipine [19] and second generation DHP derivatives such as nitrendipine [20], nimodipine [21], furnidipine [22] nisoldipine [23], amlodipine [24], isradipine [25], nilvadipine [11], felodipine [12] has

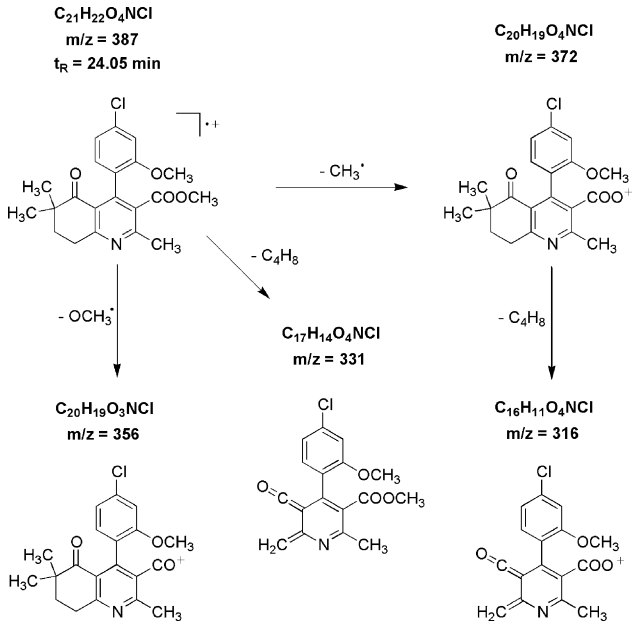

Fig. 8. Scheme of mass fragmentation of photoproduct (t_R = 24.05 min), forming during photodegradation 2′,4′-dichloro HHQ derivative; identified photoproduct as: 2,6,6-trimethyl-3-carbomethoxy-4-(2′-methoxy,4′-chlorophenyl)-5-oxo-5,6,7,8-tetrahydroquinoline.

been analysed by many authors. It has been shown that upon irradiation, the dihydropyridine ring in these DHP derivatives undergoes oxidation leading to formation of photoproducts of aromatic properties.

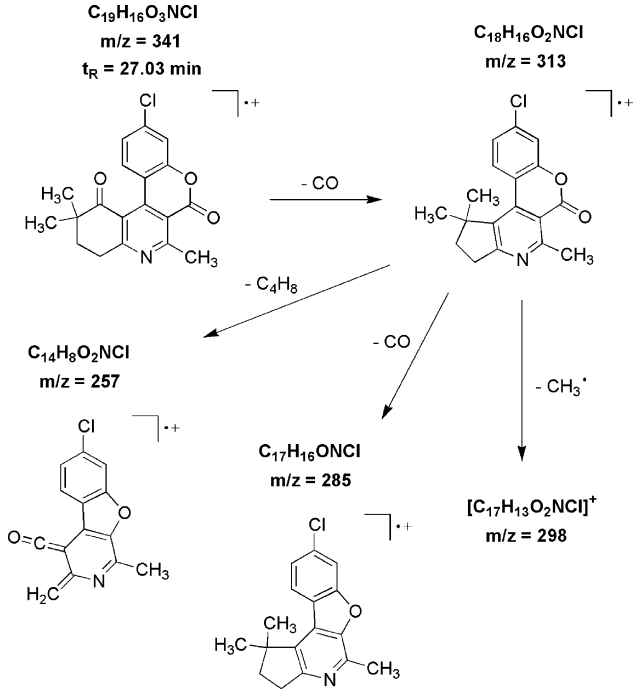

Fig. 9. Scheme of mass fragmentation of photoproduct (t_R = 27.03 min), forming during photodegradation 2′,4′-dichloro HHQ derivative; identified photoproduct as: 4-chloro-7,11,11-trimethyl-10,11-dihydro-9*H*-5-oxabenzo[c]fenantren-6,12-dion.

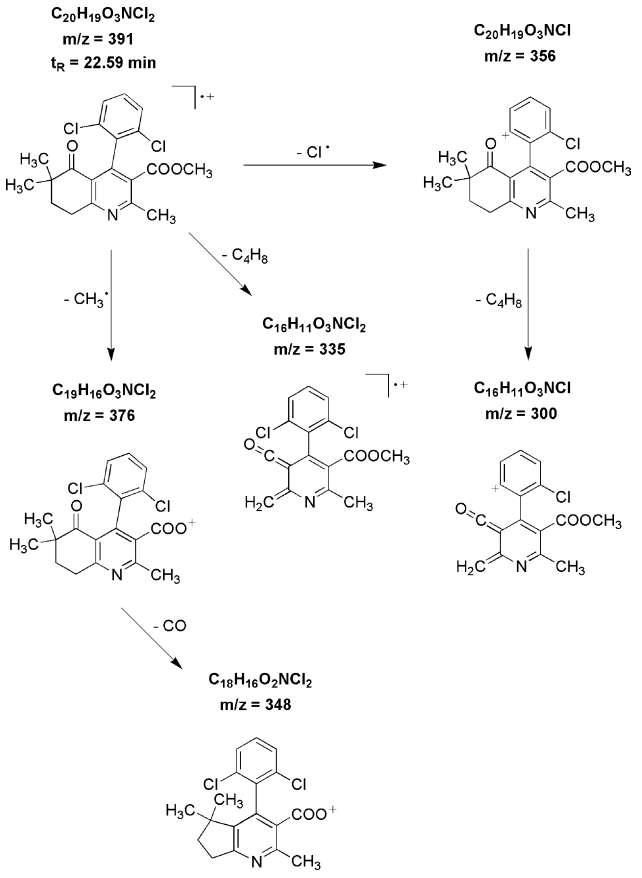

Fig. 10. Scheme of mass fragmentation of photoproduct (t_R = 22.59 min; Fig. 3) forming during photodegradation 2′,6′-dichloro HHQ derivative; identified photoproduct as: 2,6,6-trimethyl-3-carbomethoxy-4-(2′,6′-dichlorophenyl)-5-oxo-5,6,7,8-tetrahydroquinoline.

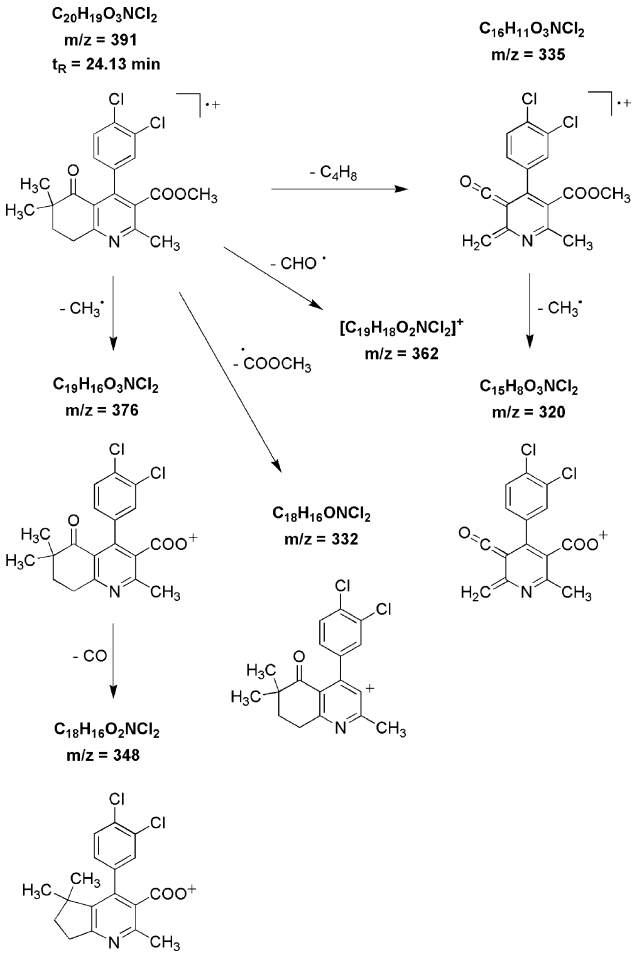

Fig. 11. Scheme of mass fragmentation of photoproduct (t_R = 24.13 min), forming during photodegradation 3′,4′-dichloro HHQ derivative; identified photoproduct as: 2,6,6-trimethyl-3-carbomethoxy-4-(3′,4′-dichlorophenyl)-5-oxo-5,6,7,8-tetrahydroquinoline.

The photoproducts Figs. 5 and 8 (m/z = 387), observed on degradation of the HHQ derivatives 2′,3′-dichloro HHQ and 2′,4′-dichloro HHQ, are characterised by identical mass spectra and elemental compositions of the fragment ions.

These compounds have been formed as a result of oxidation of the dihydropyridine ring and a simultaneous substitution of the chlorine atom by a methoxy substituent. The photodegradation leads to the excitation of the solute molecules, which induces the interactions between the solvent (methanol). The chemical reactions of the excited solute molecules with the solvent are a consequence of high photochemical activity of chlorine atoms in the phenyl ring and the solvent reactivity.

Photodegradation of the 2′,3′-dichloro and 2′,4′-dichloro HHQ derivatives, leads to the appearance of the third photoproduct containing the lactone ring and on mass fragmentation forms the molecular ion m/z = 341, as shown in Figs. 6 and 9. These compounds have occurred in low concentrations and have been secondary products of degradation of photoproducts Figs. 5 and 8 (m/z = 387).

As has been mentioned, the photodegradation of HHQ derivatives leads to formation of a different number of photoproducts, however, all of them have shown aromatic properties. Interestingly, for all these compounds no fragmentation involving breaking up of the C–C bond between the phenyl ring and the hexahydroquinoline ring (no ions at m/z = 248) has been noted. The favoured direction of mass fragmentation of all HHQ derivatives involves the breaking up of the C–C bond joining the hexahydroquinoline ring with the phenyl ring. This fact is understood taking into regard that dehydrogenation of the dihydropyridine ring and the related aromatisation lead to a significant increase in the system's stability as a result of the presence of resonance structures, which prevents breaking up of the bond between the two aromatic rings. Moreover, as follows from the spectra, the mass fragmentation of photoproducts Figs. 4–11, involved mainly the elimination of the methyl radical $CH_3^{\bullet}$ and the methoxy radical $OCH_3^{\bullet}$ from the ester group at the position C_3 of the pyridine ring. Moreover, the elimination of the ester radical $COOCH_3^{\bullet}$, the C_4H_8 molecule and extraction of the CO molecule accompanied by the skeleton regrouping have been observed.

The mass fragmentation of the photoproducts having chlorine atoms at the *ortho*-positions (2′,6′-dichloro HHQ) or

ortho- and *meta*-positions of the phenyl ring (2′,3′-dichloro HHQ), has been also observed to involve the elimination of $Cl^{\bullet}$ radical, Figs. 4 and 10. This pathway of fragmentation has not been observed, for the photoproducts containing a chlorine atom at the *para*-position of the phenyl ring.

4. Conclusions

The photodegradation of 2,6,6-trimethyl-3-carbomethoxy -4-(dichlorophenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline derivatives (HHQ) involves mainly the oxidation of dihydropyridine ring with formation of the aromatic 5,6,7,8-terahydroquinoline ring.

The products of photodegradation of the HHQ derivatives studied have been tentatively identified as the following compounds.

2,6,6-Trimethyl-3-carbomethoxy-4-(*ortho*- or *meta*- or *para*-dichlorophenyl)-5-oxo-5,6,7,8-tetrahydroquino- line —Figs. 4, 7, 10 and 11.

2,6,6-Trimethyl-3-carbometoxy-4-(*ortho*- or *meta*- or *para*-chloro-2′-methoxyphenyl)-5-oxo-5,6,7,8-tetrahydroquinoline—Figs. 5 and 8.

R-Chloro-7,11,11-trimethyl-10,11-dihydro-9*H*-5oxo-8aza-benzo[c]fenantren-6,12-dion—Figs. 6 and 9.

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