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Identification of Photoproducts of Hexahydroquinoline Derivatives by GC-EI-MS and HPLC-ESI-MS

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ABSTRACT Photoproducts of hexahydroquinoline derivatives have been analyzed with gas chromatography electro ionization-mass spectrometry (GC-EI-MS) and high performance chromatography electrospray ionization-mass spectrometry (HPLC-ESI-MS). The study was performed on four HHQ derivatives: 2,6,6-trimethyl-3-carbomethoxy-5-oxo-4-(R-phenyl)-1,4,5,6,7,8-hexahydroquinoline; R=2′-Me, 3′-Me, 2′-MeO, and 3′-MeO. The photochemical degradation of each of the HHQ derivatives led to the appearance of one product. The photoproducts were identified as the corresponding tetrahydroquinoline analogues, which were formed by dehydrogenation of dihydropyridine moiety. In GC-mass spectra, the most frequent way of fragmentation was elimination of CH_3^* or CH_3O^* radical of the ester group. In the photoproducts substituted at 2′-position of the phenyl ring, elimination of isobutene (C_4H_8) was observed. In the photoproducts with 3′-position substituents, elimination of $COOCH_3^*$ radical was noted.

KEYWORDS Hexahydroquinoline derivatives, Calcium channel blockers, Dihydropyridine derivatives, Photodegradation, HPLC-MS, GC-MS

INTRODUCTION

Calcium channel blockers belonging to the group of 1,4-dihydropyridine (DHP) derivatives have been used for the last 30 years in therapy for essential hypertension, angina pectoris, and other cardiovascular diseases. These drugs have been widely used in the treatment of arterial hypertension (Hibbard, 2002), neurology (Jacobson et al., 2000), gynaecology (Belfort et al., 2003; Brown et al., 2002; King et al., 2003; Magee, 2001; Papatsonis et al., 2001), nephrology (Bakris & Shaikh, 1996), gastroenterology (Iashikawa et al., 2000; Prat et al., 2002), and oncology (Hahn et al., 1997). A new group of calcium channel antagonists, hexahydroquinoline (HHQ) derivatives structurally related to DHP derivatives, has been synthesized by C. Safak's group. The common structural elements of these two groups are the dihydropyridine ring, substituted phenyl ring, and ester substituent in the C_3 position of the dihydropyridine ring (Altas et al., 1999; Safak et al., 1990, 1993, 1995; Simsek

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et al., 2000, 2001). The pharmacological activity of HHQ derivatives depends on the type and position of the substituent in the phenyl ring.

From the pharmaceutical point of view, a particularly troublesome disadvantage of HHQ and DHP derivatives is their high sensitivity to light. The chemical changes in the DHP derivatives under light are irreversible and lead to a weakening or vanishing of the therapeutic effect (Beijersbergen van Henegouwen, 1997). Low photochemical stability of the dihydropyridine ring has already been established, e.g., in the studies of photostability of DHP derivatives. The photochemical sensitivity of nifedipine (Dankers et al., 1998) and second generation DHP derivatives such as nitrendipine (Tipre & Vavia, 2000), nimodipine (Zanocco et al., 1992), furnidipine (Nuñez-Vergara et al., 1994), nisoldipine (Marinkovic et al., 2000), amlodipine (Ragno et al., 2002), nilvadipine (Mielcarek et al., 2000), and felodipine (Eriksson et al., 1991) has been analyzed by many authors. It has been shown that upon irradiation of these DHP derivatives, the dihydropyridine ring undergoes oxidation and leads to the formation of photoproducts of aromatic properties.

Assessment of photostability of pharmaceutical substances and drugs is a complex problem. The problem of the photostability of drugs has been of great interest in recent years, and regulatory guidelines by the International Conference on Harmonization (ICH) have been introduced. Photostability protocols varied greatly regarding the preparation of samples for drug substances and drug products, types of photolysis sources, spectral range of exposure, exposure times, and other parameters. These variations in design made it difficult to correlate photostability results between different research groups. At present, according to the ICH Document two procedures for determination of photostability have been recommended (Drew, 1998).

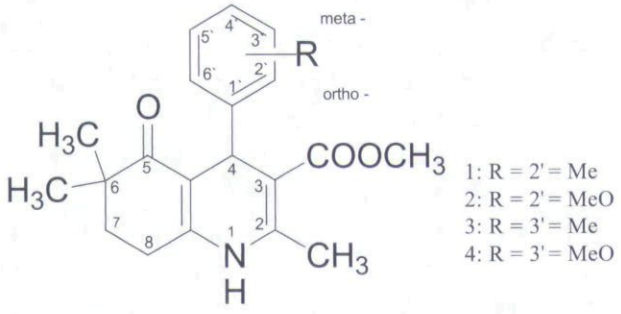

FIGURE 1 Formula of 2,6,6-Trimethyl-3-Carbomethoxy-4-(R-Phenyl)-5-Oxo-5,6,7,8-Hexahydroquinoline; R = Substituent in Different Positions of the Phenyl Ring: Orto-2'-Me or 2'-MeO; Meta-3'-Me or 3'-MeO.

This paper presents the results of a study into the photochemical properties of four HHQ derivatives (1–4) differing in the position of the methyl or methoxyl substituent at the phenyl ring (Fig. 1), identification of their photoproducts, and determination of their structure by the analytical techniques GC-EI-MS and HPLC-ESI-MS.

MATERIALS AND METHODS

Materials

Four hexahydroquinoline derivatives (HHQ)—2,6,6-trimethyl-3-carbomethoxy-5-oxo-4-(2'-methylphenyl)-1,4,5,6,7,8-hexahydroquinoline (**1**), 2,6,6-trimethyl-3-carbomethoxy-4-(2'-methoxyphenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline (**2**), 2,6,6-trimethyl-3-carbomethoxy-5-oxo-4-(3'-methylphenyl)-1,4,5,6,7,8-hexahydroquinoline (**3**), and 2,6,6-trimethyl-3-carbomethoxy-4-(3'-methoxyphenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline (**4**)—were synthesized by the Safak research group from the Faculty of Pharmacy, at the Hacettepe University, Ankara, Turkey (Fig. 1).

Methanol and acetonitrile for liquid chromatography were purchased from Merck. The water used was doubly distilled.

Photodegradation Conditions

The methanol solutions of the HHQ derivatives in a concentration of $6.2 \cdot 10^{-5}$ mol l^{-1} were subjected to photodegradation according to the requirements of the first version of the ICH Document (Drew, 1998). The compounds were placed in a quartz cell of 2.8 mL with a capacity, and irradiated with a high-pressure mercury lamp with a mercury burner HBO-50, using a cut-off filter (λ_{exe}=365 nm) for 180 min. After specific time intervals, 1.4 mL portions 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 capped.

The number of the quanta absorbed was measured by a chemical actinometer of Reinecke salt—trans tetrathiocyandiamminechromate (III) potassium (I) (Dankers et al., 1998). A Reinecke salt solution was

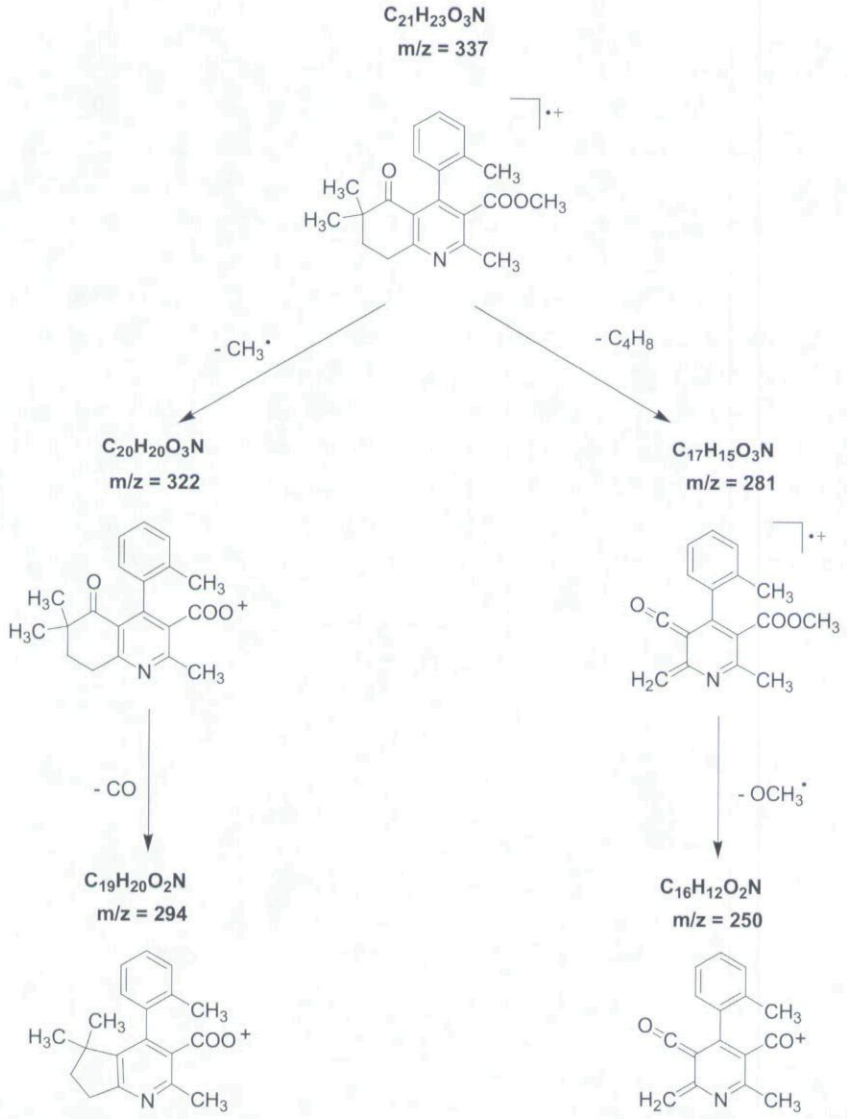

FIGURE 2 Scheme of Mass Fragmentation of Photoproduct (t_R=21.18 min) Forming During Photodegradation 2′-Me HHQ Derivative; Identified Photoproduct as: 2,6,6-Trimethyl-3-Carbomethoxy-4-(*o*-Tolyl)-5-Oxo-5,6,7,8-Tetrahydroquinoline.

irradiated with the wavelength $\lambda = 365$ nm for 85 sec. The number of quanta absorbed by the actinometer (I_R) was 1.42 10^{17}.

The energy of a quantum of radiation (E_q) of λ=365 nm was calculated using the formula:

$$E_q = h \cdot \frac{c}{\lambda} = 6.626 \cdot 10^{-34} Js \frac{2.998 \cdot 10^8\ ms^{-1}}{365 \cdot 10^{-9}\ m}$$
$$= 5.4423 \cdot 10^{-19} J$$

The intensity of irradiation (P) absorbed by the actinometer was [Watt=Js^{-1}]:

$$P = \frac{E_q \cdot I_R}{t} = \frac{5.4423 \cdot 10^{-19} \cdot 1.42 \cdot 10^{17}}{85}$$
$$= 9.092 \cdot 10^{-4} [Js^{-1}] = 9.0918 \cdot 10^{-4} [W]$$

I_R equals the number of quanta absorbed by the actinometer.

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

$$E_s = 0.4023\ [W\ m^{-2}s] = 1448.25\ [W\ h\ m^{-2}]$$

Gas Chromatography–Electro Ionization–Mass Spectrometry (GC-EI-MS)

The products of the photochemical degradation were analyzed on a gas chromatograph 5890II, equipped with a selective mass detector 5971A, Hewlett-Packard

(Palo Alto, CA). The separation was conducted on a capillary silica column DB-5, J&W (USA), with an 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 maintained for 2 min, the rate of temperature increase 5°C min^{-1} up to 200°C, and then 10°C min^{-1} 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 in a ratio of 1:2. The carrier gas was helium, purity = 99.99%, flow-rate 1 mL min^{-1}, pressure 5 bar.

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 ionization was performed by a stream of electrons with an energy of 70 eV, applying an accelerating voltage of 8 kV. The temperature of the source of electrons was 200°C, while the temperature of evaporation varied from 100°C to 250°C. The low-resolution mass spectra in the normalized form are shown in the range from 50 m/z to 400 m/z.

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

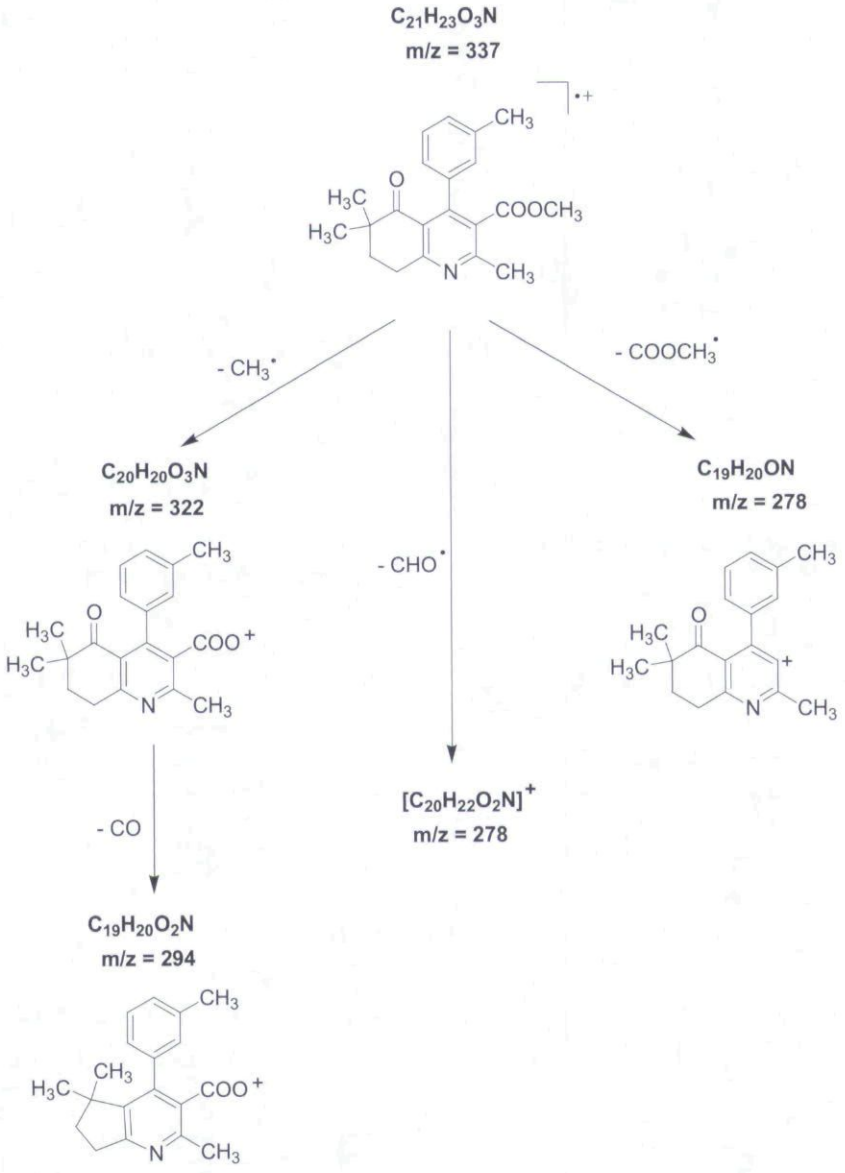

FIGURE 3 Scheme of Mass Fragmentation of Photoproduct (t_R=21.45 min) Forming During Photodegradation 3′-Me HHQ Derivative; Identified Photoproduct as: 2,6,6-Trimethyl-3-Carbomethoxy-4-(*m*-Tolyl)-5-Oxo-5,6,7,8-Tetrahydroquinoline.

made on a mass spectrometer JMS D 100, with a resolution of R = 10000. Error in determinating of the elemental composition of the ions did not exceed 12 ppm, relative to the results of theoretical calculations.

High-Performance Liquid Chromatography–Electrospray Ionization–Mass Spectrometry (HPLC-ESI-MS)

LC-MS (Liquid Chromatography-Mass Spectrometry) was performed on Waters model 2690 HPLC instrument equipped with an ESI interface (Waters, Milford, MA, USA). The capillary voltage was 3 kV and the cone voltage was set at 30 V. The other ESI operating parameters were as follows: source temperature 120°C, desolvation temperature 300°C, nitrogen gas, desolvation gas 300 l h^{-1}, cone gas 100 l h^{-1}, and multiplier voltage 500 V. In these conditions, a full scan data acquisition was performed from 100–1000 m/z in centroid mode and with a cycle time of 0.1 min, consisting of 0.5 s scan time and 0.1 s interscan time.

The chromatographic separation was carried out in the isocratic mode on a column C-18 Nova-Pak (3.9 mm i.d. × 150 mm, Waters). The mobile phase is H_2O:CH_3OH:CH_3CN=38:42:20 (v/v/v) at the flow rate of 0.5 ml min^{-1}, with 10% formic acid (pH=4.0)

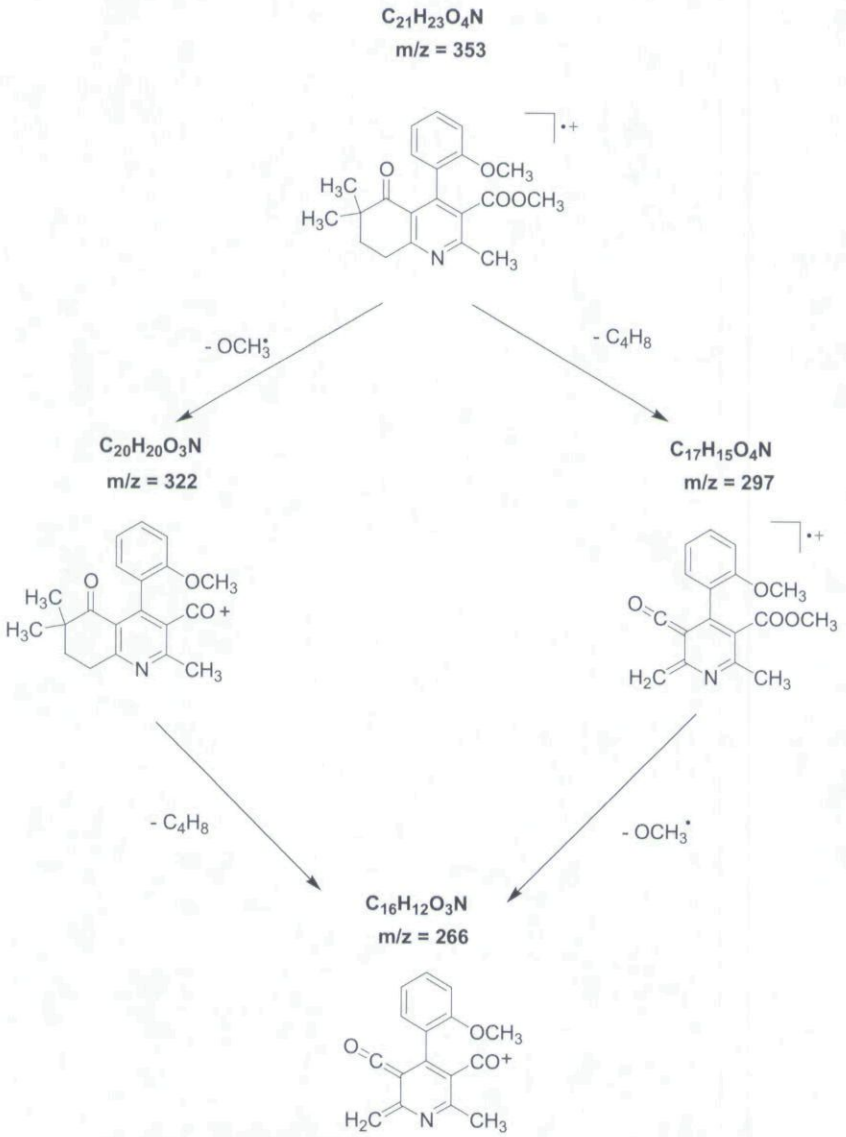

FIGURE 4 Scheme of Mass Fragmentation of Photoproduct (t_R= 21.21 min) Forming During Photodegradation 2′-MeO HHQ Derivative; Identified Photoproduct as: 2,6,6-Trimethyl-3-Carbomethoxy-4-(2′-Methoxyphenyl)-5-Oxo-5,6,7,8-Tetrahydroquinoline.

added. The eluent was directly introduced into the ESI source without a post column split.

The HPLC instrument was equipped with a photodiode array detector (DAD). Ultraviolet (UV) spectra were taken in the range of 200–500 nm.

RESULTS AND DISCUSSION

The process of photodegradation was assessed by gas (GC-MS) and liquid (HPLC-MS) chromatography coupled with mass spectrometry. The GC-MS measurements were performed with the electron ionization (EI) technique. The EI MS spectra are presented in the peaks illustrating the dependence of relative ion abundance (i_{wz}%) on molecular mass (m/z) of the fragment ions. On the basis of high-resolution mass spectra, it was possible to establish the elemental composition of the molecular ions and the fragment ions. Mass spectra of the photoproducts **5–8** revealed the presence of molecular ions at m/z=337, 337, 353, and 353, respectively (Fig. 1). The main pathways of mass fragmentation were analyzed and the schemes of decomposition proposed are presented in Figs. 2–5. The results have shown that a mass decomposition of **5–8** often occurs with elimination of the methyl (CH_3*) and methoxy (OCH_3*) radicals from ester chains. In photoproducts **5**, **7**, and **8**, the formation of the CH_3* radical was observed. However, elimination of the methoxy radical from the ester group was characteristic of

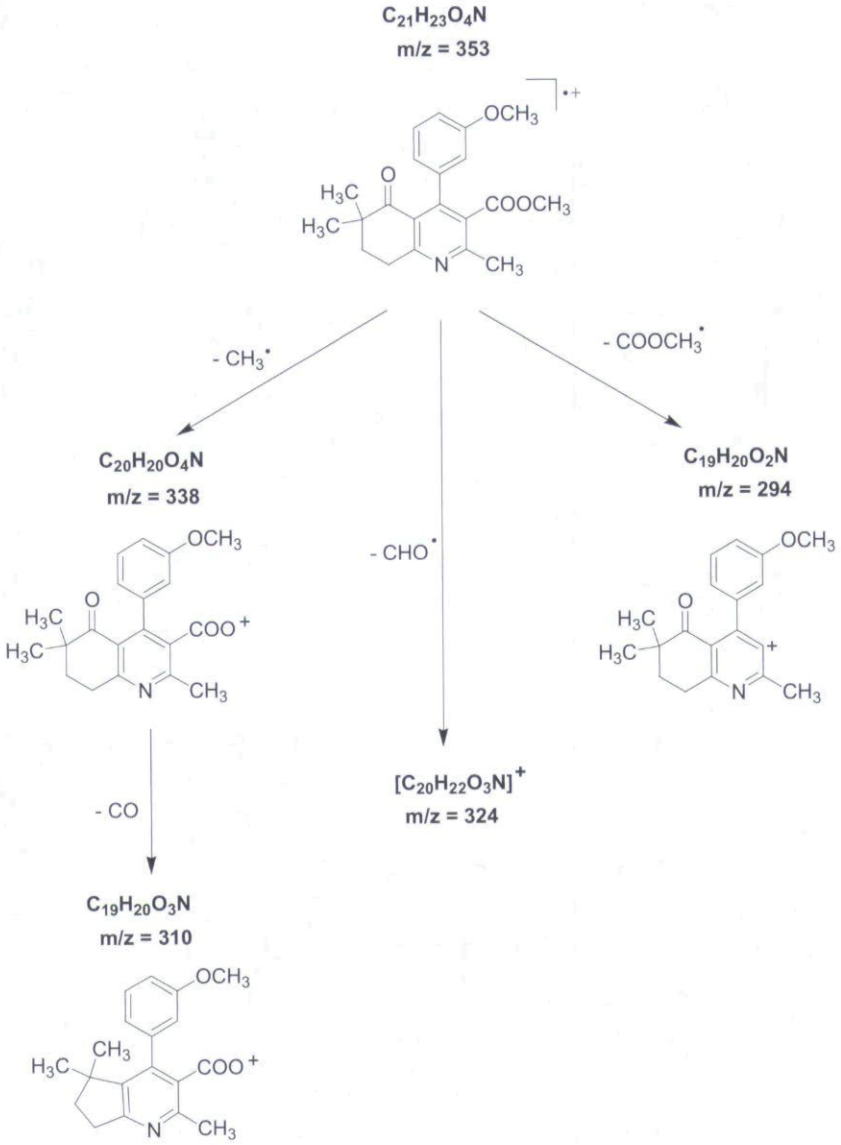

FIGURE 5 Scheme of Mass Fragmentation of Photoproduct (t_R=23.12 min) Forming During Photodegradation 3′-MeO HHQ Derivative; Identified Photoproduct as: 2,6,6-Trimethyl-3-Carbomethoxy-4-(3′-Methoxyphenyl)-5-Oxo-5,6,7,8-Tetrahydroquinoline.

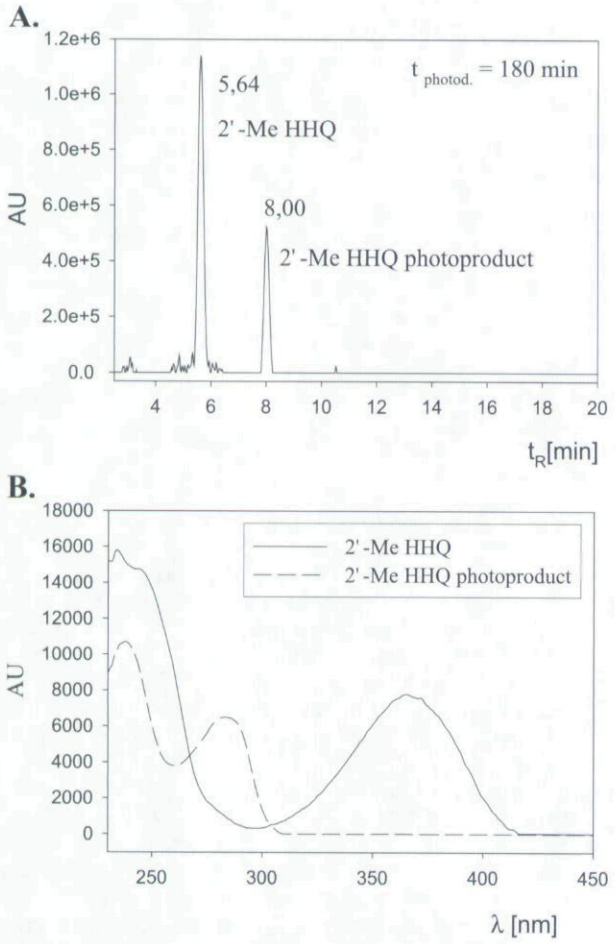

FIGURE 6 **A. HPLC-UV Chromatogram of 2'-Me HHQ Derivative and Photoproduct; B. UV Spectra of 2'-Me HHQ Derivative and Photoproduct; Time Photodegradation 180 min.**

photoproducts **5** and **7**. It is worth emphasizing that this fragmentation pathway was not observed for the isomeric photoproducts **6** and **8**. The crucial stage of fragmentation of the compounds studied was elimination of an ester group at the C_3 position within pyridine moiety, Figs. 3 and 5. In photoproducts **5**, **7**, and **8** (Fig. 4), elimination of the CO molecule was observed with simultaneous reorganization of moiety.

Analysis of the mass spectra of **5–8** has shown that photodegradation of the HHQ derivatives **1–4** led to formation of a single photoproduct **5–8**, respectively. Therefore, these compounds are formed as a result of dehydrogenation of the dihydropyridine moiety, leading to the appearance of the aromatic pyridine ring.

As a result of the above process, a system of two connected aromatic rings was formed and stabilized with resonance structures. Interestingly, there is no fragmentation involving a breaking up of the C–C, bond between the phenyl ring and the hexahydroquinoline ring (no ions at m/z=248) in all these compounds. It should be emphasized that this type of mass degradation is characteristic of HHQ derivatives containing the dihydropyridine ring of boat conformation. The dihydropyridine ring of the active antagonists adopts a boat conformation. The ring planarity is associated with enhanced activity (Kendi et al., 1994). In these compounds, the four double-bonded carbons C_2, C_3, C_5, and C_6 make the plane of the ring with N and C_4 being above the plane.

In order to confirm the GC-MS results, the photoproducts of **5–8** were also separated and identified on a LC-MS with diode detection systems. The results for **1** are shown in Fig. 6. Figure 6A presents a chromatogram of an irradiated solution of **1** for 180 min, while Fig. 6B presents the UV spectra of separated substances. The retention time (t_R) and results of the wavelength maximum absorption (λ_{max}) for all compounds are given in Table 1. As follows from Fig. 6B, the UV spectra of the photoproducts are significantly different from those of the corresponding HHQ derivatives. In the spectra of all photoproducts there was no absorption band, related to the $\pi \rightarrow \pi^*$ electronic transitions in the diene system of the unsaturated DHP ring, but a new absorption band

TABLE 1 **Chromatographic Parameters HPLC-DAD-ESI-MS and Positive Molecular Ions of Hexahydroquinoline; Derivatives and Their Photoproducts and Chromatographic Parameters GC-ESI-MS and Photoproducts m/z**

	HPLC-DAD-ESI-MS						GC-EI-MS	
	HHQ derivative		Photoproduct		λ max [nm] of UV spectra		Photoproduct	
Method	t_R [min]	$[M+H]^+$	t_R [min]	$[M+H]^+$	HHQ derivative	photoproduct	t_R [min]	m/z
2'-Me	5.64	340	8.00	338	365	283	21.18	337
3'-Me	5.27	340	8.27	338	363	283	21.45	337
2'-MeO	6.39	356	20.83	354	366	284	22.21	353
3'-MeO	4.26	356	6.21	354	364	281	23.12	353

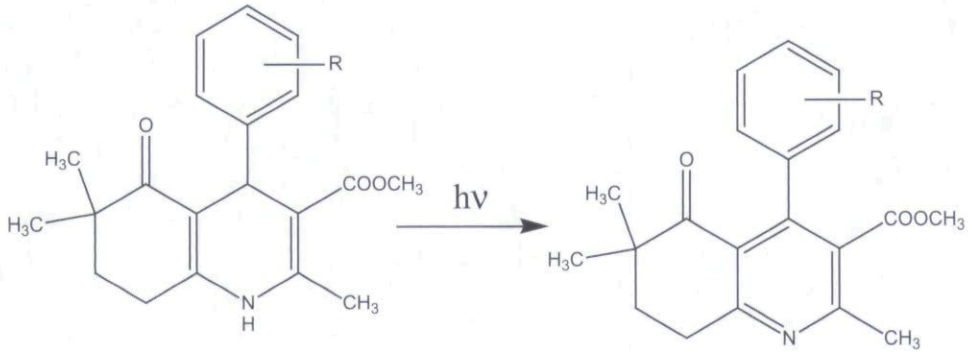

FIGURE 7 **Scheme of Photodegradation 2,6,6-Trimethyl-3-Carbomethoxy-4-(R-Phenyl)-5-Oxo-5,6,7,8-Hexahydroquinoline; R = Substituent in Different Position of the Phenyl Ring: Orto-2′-Me or 2′-MeO; Meta-3′-Me or 3′-MeO.**

appeared in the range 250–335 nm, with a maximum at about 280 nm.

The mass spectra of photoproducts of **5–8** revealed the presence of protonated molecular ions $[M+H]^+$ at m/z=338 for 2′-Me and 3′-Me as well as m/z=354 for 2′-Meo and 3′-MeO (see Table 1).

CONCLUSIONS

GC-EI-MS and HPLC-ESI-MS results revealed that the photodegradation of HHQ derivates **1–4** leads to the formation of corresponding tetrahydroquinoline analogues **5–8** by dehydrogenation of the dihydropyridine moiety (Fig. 7).

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