 DOI: 10.17344/acsi.2014.1140

Scientific paper

Photodegradation of Methoxy Substituted Curcuminoids

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Received: 17-10-2014

Dedicated to the memory of Prof. Dr. Jurij V. Brenčič.

Abstract

Photodegradation of dimethoxy curcuminoids in acetonitrile solution was found to depend on the position of the methoxy group bonded to the phenyl ring. The rate of decomposition was expressed as the lifetime of the decomposing substrate, being the shortest in the case of the 3,5-dimethoxy and the longest for the 2,5-dimethoxy derivative. For the 3,5-dimethoxy curcuminoid, the major degradation products were 3,5-dimethoxybenzaldehyde, 3,5-dimethoxybenzoic acid and the *Z* and *E* isomers of dimethoxycinnamic acid, together forming about 90% of the reaction mixture. Minor products found were 4,5-bis(3,5-dimethoxyphenyl)hex-2-endionic acid, products with the molecular formula $C_{23}H_{24}O_6$ and $C_{23}H_{22}O_6$ attributed to the reaction of intramolecular [2 + 2] cycloaddition of the dimethoxy curcuminoid, and the dioxygenated bicyclopentadione derivative ($C_{23}H_{24}O_8$) derived from autoxidative transformation of the dimethoxy curcuminoid.

Keywords: Dimethoxy curcuminoids; photodegradation

1. Introduction

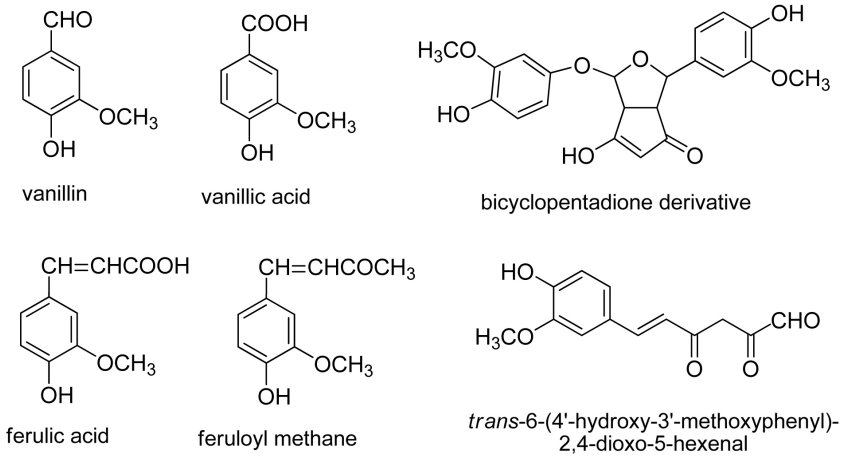
Curcumin is an antioxidant that inhibits lipid peroxidation, and a scavenger of reactive oxygen species which decreases the formation of inflammatory compounds such as prostaglandins and leukotrienes.[1–4] Its efficacy as a cancer chemopreventive agent is limited by its chemical and metabolic instability in buffers of neutral to alkaline pH.[5–8]

When curcumin was incubated in 0.1M phosphate buffer and serum-free medium at pH 7.2, the initial degradation products ferulic acid and feruloyl methane were formed after only a few minutes, and feruloyl methane was then transformed by hydrolysis to the degradation products vanillin and acetone, their amounts increasing with incubation time.[8] The formation of these products cannot explain the brownish-yellow colour observed when curcumin is decomposed in various buffer-systems (phosphate, carbonate) at pH>7 and in 1N NaOH.[10] It is well known that in alkaline solution aldehydes and ketones can form condensation products which are probably responsible for the colour of the solution.[5]

Curcumin is not stable to light, especially in solution. After photo-irradiation a cyclisation product, as well as decomposition products such as vanillic acid, vanillin and ferulic acid[11] are formed.

Curcumin is more stable in cell culture medium containing 10% foetal calf serum, and in human blood in which less than 20% of the compound decomposed within an hour while after 8 hours about 50% of curcumin still remained.[8] Trans-6-(4'-hydroxy-3'-methoxyphenyl)-2,4-dioxo-5-hexenal was predicted as the major degradation product, and vanillin, ferulic acid and feruloyl methane were identified as minor degradation products.

The degradation of curcumin in aqueous buffer at physiological pH is an autoxidation reaction. A radical chain reaction leads to incorporation of oxygen into curcumin, resulting in dioxygenated bicyclopentadione products.[9,12,13] Oxidation was also catalysed by recombinant cyclooxygenase-2 and the rate was increased ~10-fold by the addition of H_2O_2.[9] An enzymatic or autoxidative mechanism proposing hydrogen abstraction from a phenolic hydroxyl resulting in a quinone methide



and a delocalized radical in the heptadiene chain that then undergoes 5-exo cyclisation and oxygenation.[12] Hydration of the quinone methide and rearrangement with loss of water gives the final dioxygenated bicyclopentadione product.

In the present work, the influence of methoxy groups bonded to the phenyl ring of curcumin on its photostability was studied. It is known that electron donating groups (such as methoxy group) have quite a different effect on the π-delocalization of a conjugate system when bonded at ortho and para as compared to meta positions of the phenyl ring.

2. Experimental

All methoxy curcuminoids were prepared by procedure described in the literature.[14]

Acetonitrile used in this work purchased from Sigma-Aldrich was of spectrophotometric grade (≥ 99.9%) and used without further purification.

Photochemical reactions were performed in Photochemical Reactors LTD MLU18, equipped with blacklight blue lamps (FL15BLB, Sankyo Denki, Japan) emitting at 360 nm (photonflux; UV-A). A 20 mL glass cuvette contained dilute acetonitrile solution of sample was inserted into the reactor with four lamps; each lamp has the intensity of light 15 W. A fiber optic probe was dipped into the solution and coupled with a Varian Cary 50 UV-Vis spectrophotometer. The probe enabled *in situ* monitoring of absorption spectra. Data were collected in time intervals of 3–20 minutes (for 3,5- and 2,3-dimethoxy curcuminoids every 3 minutes, for 2,6-dimethoxy every 4 minutes, every 5 minutes in the case of 3,4-dimethoxy, and for 2,4-dimethoxy, or 2,5-dimethoxy and 2,4,6-trimethoxy, every 8.3 and 20 minutes, respectively) in the range of 700–225 nm.

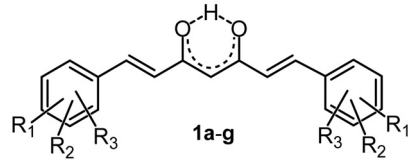
NMR Spectra were recorded at 302 K with a Bruker Avance III 500 MHz spectrometer using $Si(CH_3)_4$ as internal standard.

A time-of-flight (TOF) mass spectrometer equipped with a double orthogonal electrospray source at atmospheric pressure ionization (ESI) was used for recording HRMS spectra.

3. Results and Discussion

All six isomers of dimethoxycurcumin and one example of a trimethoxy derivative were synthetized;[14] see Figure 1.

Then 1.5×10^{-5} M acetonitrile solutions of these methoxy substituted curcuminoids were irradiated with light



$R_1 = R_2 = OCH_3$; $R_3 = H$		$R_1 = R_2 = R_3 = OCH_3$
1a (2,3-diOCH_3)	**1d** (2,6-diOCH_3)	**1g** (2,4,6-triOCH_3)
1b (2,4-diOCH_3)	**1e** (3,4-diOCH_3)	
1c (2,5-diOCH_3)	**1f** (3,5-diOCH_3)	

Figure 1. Di- and trimethoxy curcuminoids.

of wavelength 360 nm and their degradation monitored by the loss of absorbance at the wavelength of their absorption maximum (λ_{max}) (depending on the substrate) in the region of 394–431 nm using a UV-Vis spectrophotometer. Repetitive scanning from 700–225 nm confirmed that the chromophore at 394–431 nm was lost during the transformation, indicating that the conjugated double bond system between the phenyl rings of the curcuminoid was disrupted and products of smaller molecular weight and lower degrees of conjugation were formed, Figure 2.

From time-dependent measurements and the exponential decay of the absorbance of each compound at its

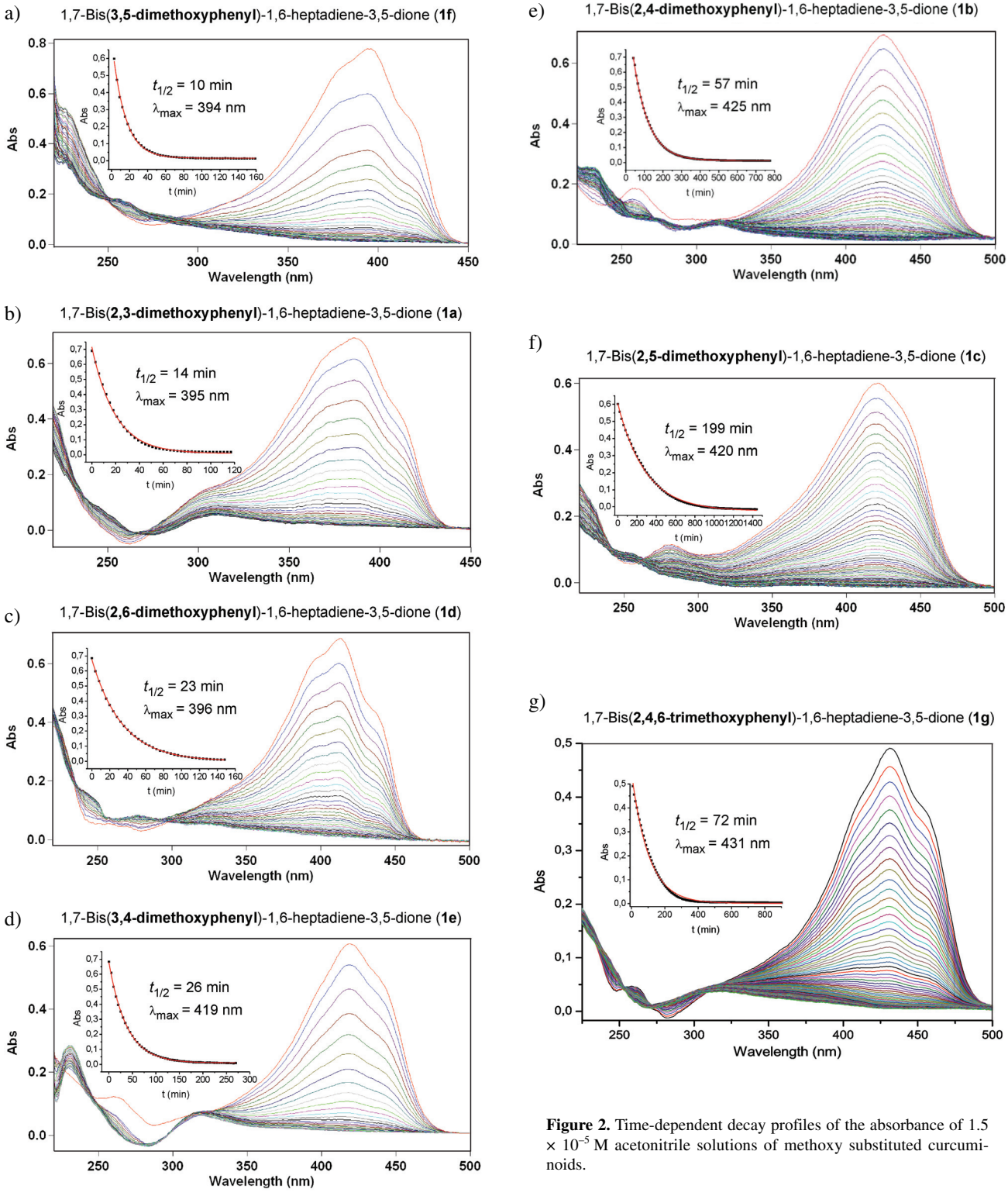


Figure 2. Time-dependent decay profiles of the absorbance of 1.5 × 10^{-5} M acetonitrile solutions of methoxy substituted curcuminoids.

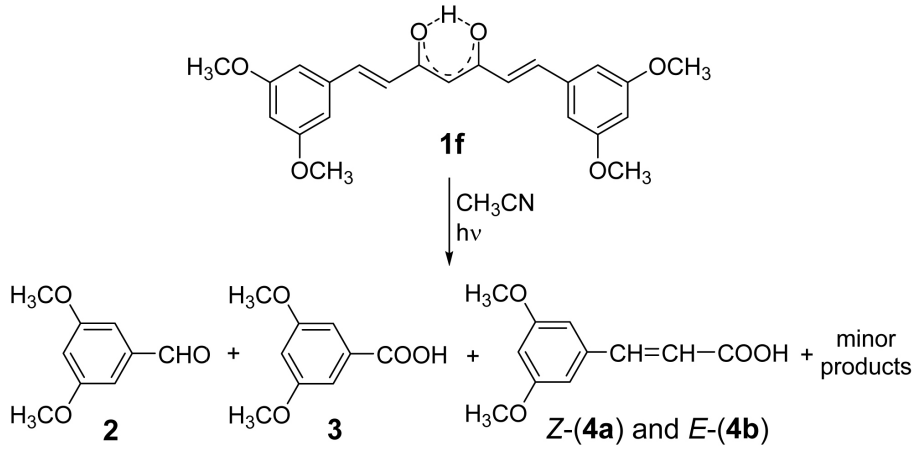
maximum (λ_{max}) its half-life ($t_{1/2}$), i.e. the period of time taken for the absorbance of the substance undergoing decay to decrease to half of its initial value, was determined using the following formulas:

$$F(t) = \mathrm{A}\exp(-t/\tau) \quad (1)$$

$$t_{1/2} = \tau\,\mathrm{In}(2) \quad (2)$$

where $F(t)$ is the quantity of the substance that still remains at time t, A is the initial quantity (at time $t = 0$), and τ is the mean lifetime of the decaying compound (also called the exponential time constant). The results obtai-

ned clearly show that $t_{1/2}$ strongly depends on the position and number of methoxy groups bonded to the curcumin molecule. The half-life of the 3,5-dimethoxy derivative (**1f**), with methoxy groups on both meta positions, is the shortest ($t_{1/2}$ = 10 min), while $t_{1/2}$ for 2,4-dimethoxy (**1b**), with methoxy groups on ortho and para positions is much longer ($t_{1/2}$ = 57 min). In the case of 2,5-dimethoxy (**1c**) with methoxy groups on ortho and meta positions it is surprisingly long ($t_{1/2}$ = 199 min). To determine how the number of methoxy groups influence the photostability, the 2,4,6-trimethoxy curcuminoid (**1g**) with two ortho and a para bonded methoxy groups was tested. The half-life of 2,4,6-trimethoxy is longer ($t_{1/2}$ = 72 min) in comparison with that of 2,4-dimethoxy, meaning that the ad-



Scheme 1. Photodegradation of 3,5-dimethoxy curcuminoid **1f**.

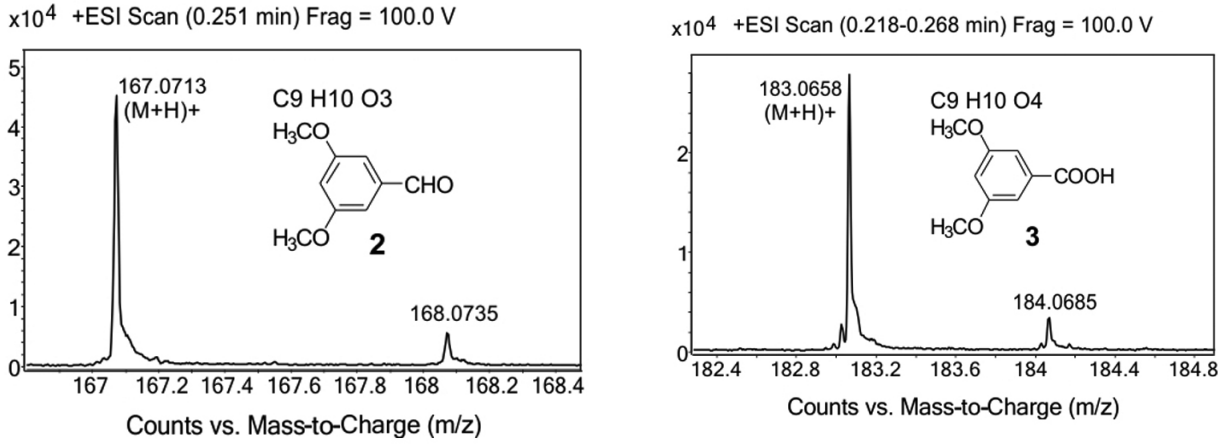


Figure 3. HRMS-ESI spectrum of the residue of **1f** after irradiation showing characteristic signals of the main degradation products **2** and **3**.

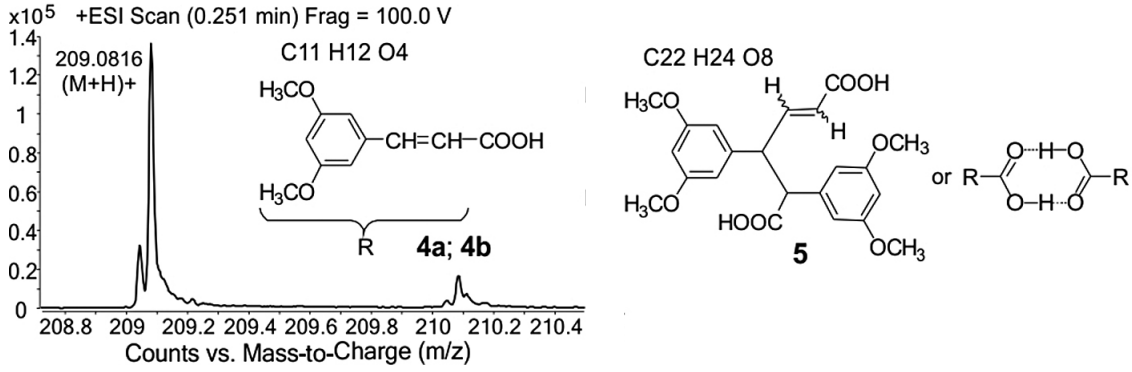


Figure 4. HRMS-ESI spectrum of the residue of **1f** after irradiation showing characteristic signals of products **4a**, **4b** and **5**.

ditional methoxy group at ortho position increases the photostability.

We then performed a similar degradation reaction as described previously but on a larger scale and analysed the products formed using HRMS with electrospray ionization in the positive and negative modes i.e. $(ESI)^+$, $(ESI)^-$, and measured the ^{1}H NMR spectrum of the crude reaction mixture. The 3,5-dimethoxy curcuminoid (**1f**) was chosen as substrate due to its shortest half-life. Three milligrams of this compound was dissolved in 500 mL of CH_3CN and the solution irradiated in a UV reactor equipped with 360 nm lamps for 2 hours. The solvent was evaporated under reduced pressure and the ^{1}H NMR spectrum of the residue measured. From comparison of the ^{1}H NMR signals of authentic samples of known compounds with those of the residue we were able to determine the major degradation products amounting to approximately 90% of all formed products as 3,5-dimethoxybenzaldehyde (**2**); 3,5-dimethoxybenzoic acid (**3**) and *Z* and *E* isomers of 3-(3,5-dimethoxyphenyl)propenoic acid (dimethoxycinnamic acid) (**4a**, **4b**), Scheme 1. Ratio of the major and the minor degradation products was established from the ^{1}H NMR spectrum of the crude reaction mixture obtained after irradiation. The structures were also confirmed by the molecular signals in the MS-ESI^+ and MS-ESI^- spectra (Figure 3 and Figure 4).

Dimethoxybenzoic acid (**3**) was formed by photooxidation of dimethoxybenzaldehyde (**2**), while one isomer (or both) of dimethoxycinnamic acid were formed during degradation and were isomerized (or changed their ration) under reaction conditions, as verified by an independent experiment in which the *E* isomer of the mentioned acid (**4b**)[15] in CH_3CN solution was irradiated for 2h at 360 nm. From the ratio of the integrated signals of the *Z* and *E* isomers (Figure 5) we found that the mixture contained 80% of the *Z* and 15% of the *E* isomer. This ratio did not change after prolonged irradiation (24 hours), showing that the stationary state was present. Beside signals corresponding to both

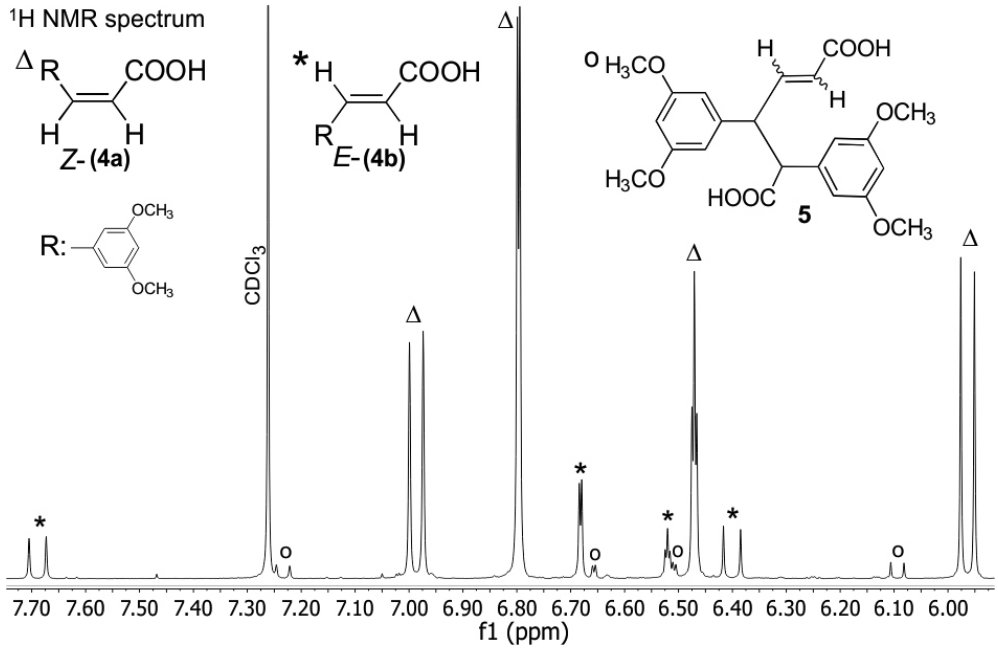
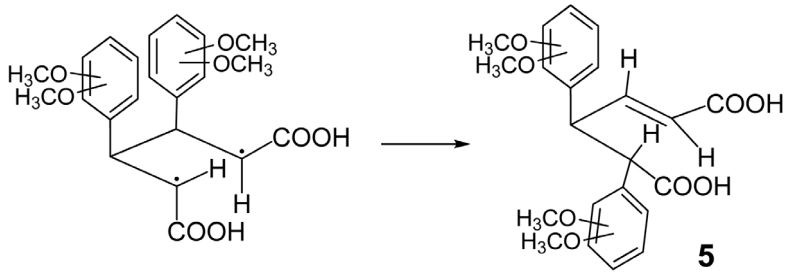


Figure 5. ^{1}H NMR spectrum of the residue obtained after 2 hours UV-irradiation at 360 nm of 1.2×10^{-3} M acetonitrile solution of *E*-dimethoxycinnamic acid.



Scheme 2. The formation of dimeric product **5** from a biradical intermediate.

isomers of dimethoxycinnamic acid, some other signals of low intensity were also present. The doublets at chemical shifts of 7.23 ppm and 6.09 ppm with a coupling constant *J* = 12.3 Hz could belong to olefinic protons (–CH=CH–), while the doublets at 6.66 and 6.51 ppm with a coupling constant of 2.7 Hz correspond to meta protons of the aromatic ring. The molecular signal in the HRMS-ESI spectrum at 439.1359 belongs to $(2M+Na)^+$ of dimethoxycinnamic acid, showing that dimerization takes place (about 5% of the total). It has been known for a long time that cinnamic acid derivatives photodimerise to either cyclic or open-chain dimers.[16,17] The presence of olefinic protons clearly shows that in our case the dimer is not a cyclobutane derivative but 4,5-bis(3,5-dimethoxyphenyl)hex-2-endionic acid (**5**) formed from a biradical intermediate, followed by migration of the phenyl ring, Scheme 2. It should be mentioned that such a molecular signal was also present in the HRMS-ESI spectrum of the crude reaction mixture obtained after irradiation of a CH_3CN solution of the 3,5-dimethoxy curcuminoid.

The UV-Vis, ^1H NMR spectra and HRMS measurements of the crude mixture after photodegradation of 3,5-dimethoxy did not show the signals of the parent compound. For that reason, the molecular signal $C_{23}H_{24}O_6$ can be attributed to the product of intramolecular [2 + 2] cycloaddition **6**, which after photooxidation leads to a product with the molecular formula $C_{23}H_{22}O_6$ (**7**), Figure 6.

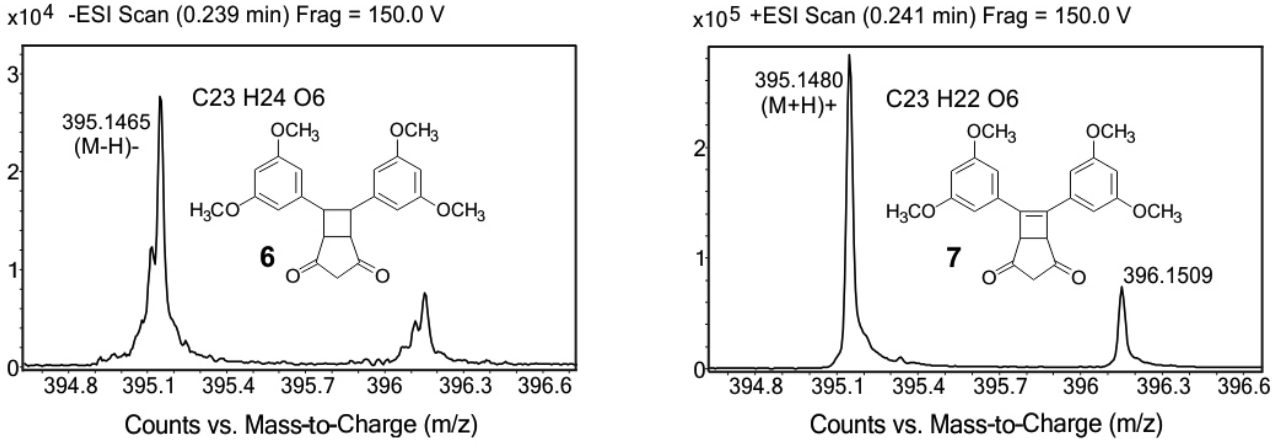
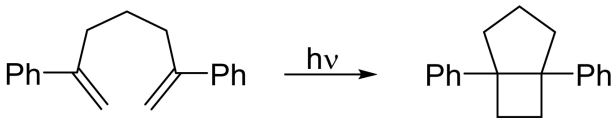


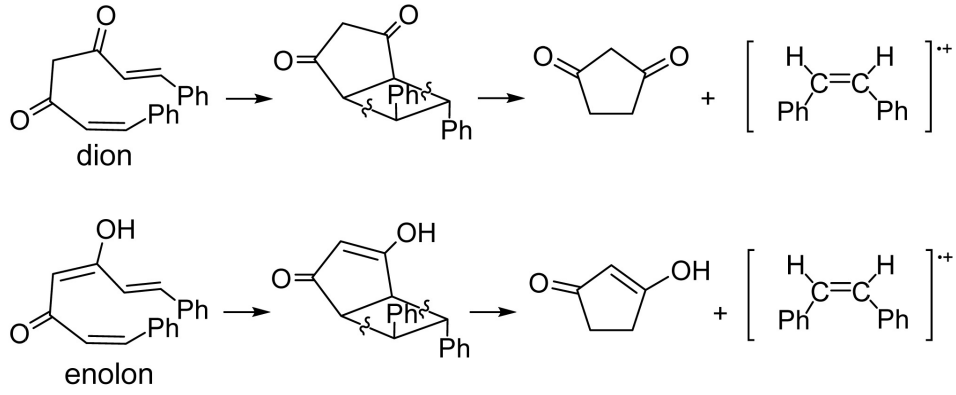
Figure 6. HRMS-ESI spectrum of the crude reaction mixture of **1f** after irradiation showing characteristic signals of the intramolecular [2 + 2] cycloadduct (**6**) and its oxidized product **7**.



Scheme 3. Photochemical conversion of 2,6-diphenylhepta-1,6-diene into 1,5-diphenylbicyclo[3.2.0] heptane.

Due to their very low content in the reaction mixture we were unable to characterize structures from the spectroscopic data, and therefore inferred the proposed structures from the HRMS analysis.

It is known from the literature[18] that irradiation of a benzene solution of 2,6-diphenylhepta-1,6-diene leads to



Scheme 4. Fragmentations of 1,7-diphenylhepta-1,6-diene-3,5-dione in the EI mass spectra.

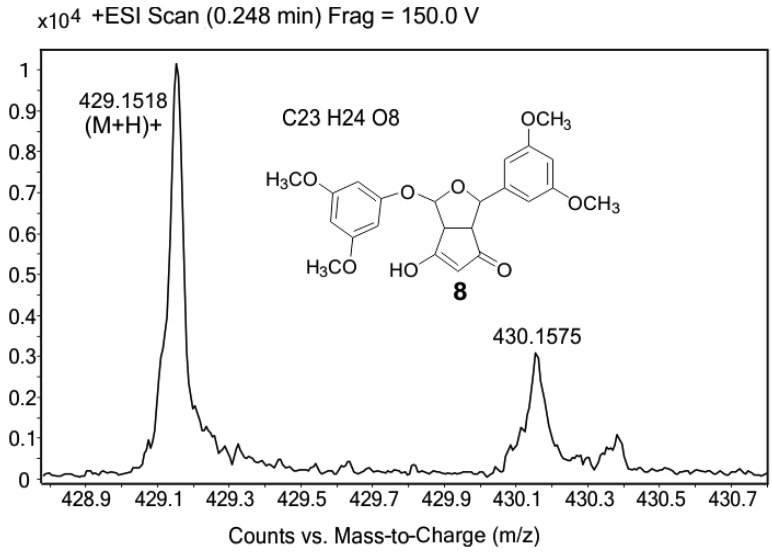


Figure 7. HRMS-ESI spectrum of the crude reaction mixture of **1f** after irradiation showing the characteristic signal of dioxygenated bicyclopentadione **8**.

the formation of 1,5-diphenylbicyclo[3.2.0] heptane through a synchronous or biradical mechanism, Scheme 3. Van Baar et al.[19] described the synthesis of 24 analogues of curcumin and confirmed their structures by ^{1}H NMR and electron ionization (EI) mass spectrometry. Most signals in the EI mass spectra can be attributed to commonly known fragmentations, except the 1,2-diphenylethene type radical cation formed by cleavage of the cyclobutane ring of the intramolecular [2 + 2] cycloadduct, Scheme 4.

The HRMS-ESI spectra of **1f** after photodegradation also displayed signals of molecular formula $C_{23}H_{24}O_8$ showing that incorporation of oxygen took place. The proposed structure of molecular formula $C_{23}H_{24}O_8$ is given in Figure 7. Such a dioxygenated bicyclopentadione derivative ($C_{21}H_{20}O_8$)[13] was isolated as the major product of the action of soybean lipoxygenase on curcumin ($C_{21}H_{20}O_6$) and the structure was elucidated by HPLC-APCI-M, tandem MS, as well 1D and 2D NMR techniques. Such a product was also isolated from the autoxidative transformation of curcumin and oxidation catalysed by recombinant cyclooxygenase-2.[9]

4. Conclusion

Photodegradation of acetonitrile solutions of dimethoxy substituted curcuminoids (**1a-f**) irradiated by light of 360 nm strongly depends on the position of the methoxy groups. 3,5-dimethoxy (**1f**) with methoxy groups on both meta positions is the most photosensitive, with a half-life of 10 min while 2,4-dimethoxy (**1b**) has a much longer degradation time ($t_{1/2}$ = 57 min). Introduction of an additional methoxy group increases the photostability; the 2,4,6-trimethoxy curcuminoid (**1g**) has a $t_{1/2}$ of 72 min.

The effect of methoxy groups depends on the position at which they are bonded to the phenyl ring, acting as an electron acceptor when bonded at meta position (σ = 0.10), and as an electron donor at para position (σ = –0.28), but the effect of an ortho bonded methoxy group is in many cases unpredictable as, beside electron effects, steric interactions are also important.

An experiment using photodegradation on a larger scale made feasible the determination of the products formed after degradation by ^{1}H NMR spectra and HRMS-ESI$^+$ and ESI$^-$ analysis: thus 3,5-dimethoxybenzaldehyde (**2**), 3,5-dimethoxybenzoic acid (**3**) and *Z* and *E* isomers of dimethoxycinnamic acid (**4a**, **4b**) were the major products, forming about 90% of the reaction mixture. Product **3** was formed by oxidation of **2**, while both *Z* and *E* isomers of cinnamic acid derivatives were the result of photoisomerization, as confirmed by an independent experiment. The minor product 4,5-bis(3,5-dimethoxyphenyl)hex-2-endionic acid (**5**) was derived from dimerization of dimethoxycinnamic acid, while products **6** and **7** with molecular formula $C_{23}H_{24}O_6$ and $C_{23}H_{22}O_6$ were attributed to the product of intramolecular [2+2] cycloaddition of the dimethoxy curcuminoid. The dioxygenated bicyclopentadione derivative ($C_{23}H_{24}O_8$) resulted from autoxidative transformation of the dimethoxy curcuminoid.

5. Acknowledgments

This work was supported by the Ministry of Higher Education, Science and Technology of the Republic of Slovenia and the Slovenian Research Agency (P1-0242). We also thank Damijana Urankar, Ph.D. (Faculty of Chemistry and Chemical Technology, University of Ljubljana) for HRMS measurements.

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Povzetek

Fotorazgradnja dimetoksi kurkuminoidov razredčenih acetonitrilnih raztopin pri valovni dolžini obsevanja 360 nm je predvsem odvisna od mesta vezanih metoksi skupin v fenilnem obroču. Hitrosti razgradnje substratov so podane z razpolovnimi časi, in sicer je ta najkrajši v primeru 3,5-dimetoksi in najdaljši pri 2,5-dimetoksi derivatu. Za 3,5-dimetoksi kurkuminoid so na osnovi ^{1}H NMR spektra, HRMS-ESI spektra in z neodvisnim eksperimentom fotolize 3,5-dimetoksicimetove kisline bili določeni glavni produkti fotorazgradnje: 3,5-dimetoksibenzaldehid, 3,5-dimetoksibenzojska kislina ter *Z* in *E* izomeri dimetoksicimetove kisline, ki predstavljajo več kot 90% vseh razgradnih produktov. Strukture preostalih produktov, ki so zastopani v sledovih, so podane na osnovi masnega spektra in primerjave dobljenih molekulskih formul s spojinami opisanih in predlaganih v literaturi. Ti so 4,5-di(3,5-dimetoksifenil)heks-2-endiojska kislina, spojini z molekulsko formulo $C_{23}H_{24}O_6$ in $C_{23}H_{22}O_6$, ki pripadata produktoma intramolekulske [2 + 2] cikloadicije dimetoksi kurkuminoida, ter biciklopentadionski derivat ($C_{23}H_{24}O_8$), produkt avtooksidativne transformacije kurkuminoida.