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Isolation and structure elucidation of the major photodegradation products of seratrodast

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ABSTRACT

Photodegradation of seratrodast ([±]-7-(3,5,6-trimethyl-1,4-benzoquinone-2-yl)-7-phenyl-heptanoic acid, SD), a *p*-benzoquinone anti-asthmatic drug, has been investigated in different solutions. HPLC analysis showed that SD was degradated under UV irradiation at 254 nm to afford three major products (SD1, SD2 and SD3) in methanol and in acetonitrile/H_2O solutions, while only two of them (SD2 and SD3) could be detected in acetonitrile solution. Furthermore, SD1 was unstable even when the samples were protected from light, so the acetylated SD1 (Ace-SD1) together with SD2 and SD3 were isolated by semi-preparative reversed-phase HPLC. The structures were elucidated as 6-(2,5-diacetoxy-3,4,6-trimethyl-phenyl)-7-phenyl-hept-6-enoic acid (Ace-SD1), 7-phenyl-6-(2,4,5-trimethyl-3,6-dioxo-cyclohexa-1,4-dienyl)-hept-6-enoic acid (SD2) and 5-(5-hydroxy-4,6,7-trimethyl-2-phenyl-benzofuran-3-yl)-pentanoic acid (SD3) based on the spectral data of MS, UV spectrum, IR spectrum and NMR spectrum. Meanwhile, a possible route for the formation of these photoproducts was proposed, which might explain the generation procedure of SD1, SD2 and SD3 during UV irradiation.

1. Introduction

Drugs photostability represents a significant problem in pharmaceutical research because the photochemical decomposition of drugs may lead to a decrease in their therapeutic effectiveness or even to the appearance of toxic products [1–5].

Quinones are cofactors in photosynthetic reaction centers of photosystems I and II and act as electron carriers through the cell membrane [6]. The photochemistry of 1,4-benzoquinone (BQ) and derivatives, such as 1,4-naphthoquinone and 9,10-anthraquinone, is the subject of various investigations [7–11]. Quinones in the presence of alcohols are photoreduced by intermolecular H-atom transfer leading to the semiquinone radical and eventually into hydroquinones (QH_2). In contrast to quinones without a side chain, the photoreactions of BQs with a β-H-atom in the side chain are strikingly different, photoinduced intramolecular H-atom transfer is possible for phylloquinones, e.g. Vitamin K1 [12,13], and other appropriate quinones [14–21], in particular for 2-methyl-5-isopropyl-1,4-benzoquinone (thymoquinone, TQ). The isopropyl group in 5-position can act as an intramolecular H-atom donor [14–17].

Seratrodast ([±]-7-(3,5,6-trimethyl-1,4-benzoquinone-2-yl)-7-phenylheptanoic acid, SD) (see structure in Fig. 1) is a quinone derivative that has been shown to inhibit bronchoconstriction by the competitive inhibition of specific binding of thromboxane A_2 (TXA_2) with its receptor [22,23]. With a feature common to all compounds of *p*-benzoquinone class, SD underwent degradation in aqueous solutions when exposed to light [24]; however, only little information has been reported and specific, confirmatory studies have not been performed. Thus, the lack of detailed studies on the photostability of SD focused our attention. In order to investigate photochemical properties of SD, we isolated and elucidated the major photodegradation products (SD1, SD2 and SD3) occurring in different solutions. Furthermore, a possible photodegradation pathway of SD was proposed.

2. Experimental

2.1. Materials and general methods

Seratrodast substance (labeled with the batch number: 061027, 061031, 061223) was obtained from Jiangsu Chia-tai Tianqing

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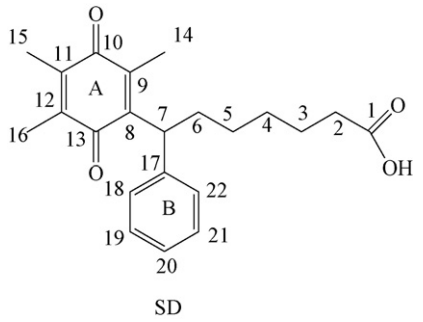


SD

Fig. 1. Chemical structure of seratrodast (SD).

Pharmaceutical Co., Ltd. (Jiangsu, China). All chemicals used in this study were of analytical grade or higher.

Melting points (mp) were determined on a digital display binocular microscope X-4 (Beijing Tech Instrument Co., Ltd., Beijing, China). IR spectra were recorded on a Shimadzu 8400-S spectrometer. UV spectra were acquired by the photodiode array (PDA) detector (190–350 nm) as the peaks eluted off the analytical column. NMR spectra were recorded on a Bruker Avance II 300 spectrometer (^{1}H, 300 MHz; ^{13}C, 75 MHz) in $CDCl_3$. The 2D NMR experiments, COSY, DEPT, HSQC and HMBC were performed using standard Bruker pulse sequences. Analytical HPLC was performed on a Shimadzu LC-10A system equipped with a UV detector (monitoring at 260 nm) and a PDA detector. The column was Lichrospher ODS (5 μm particle size, 150 mm × 6 mm i.d.; Jiangsu Hanbon Science & Technology Co., Ltd., Jiangsu, China). Mobile phase was methanol–H_2O–HAc (80:20:0.02, v/v/v) and flow rate was kept at 1 ml/min. The column temperature was 35 °C. Aliquots (20 μl) of the sample solution were injected onto the HPLC column. Liquid chromatography/mass spectrometry (LC/MS) data were obtained with a quadrupole mass spectrometer equipped with electrospray ionization (ESI) source coupled to an Agilent 1100 LC system. And MS spectra were obtained on the same MS spectrometer. HPLC condition was the same as analytical HPLC condition described above. The scanned *m*/*z* range was 100–750 Da. Semi-preparative HPLC was performed on an Agilent 1200 LC system equipped with a UV detector (monitoring at 260 nm). The column was Kromasil ODS (5 μm particle size, 250 mm × 10 mm i.d., 100 A; Shanghai Anpel Instrument Co., Ltd., Shanghai, China).

2.2. Photodegradation procedure

Photodegradation of SD solutions was conducted in Pyrex glass cells (50 mm × 50 mm × 80 mm) of 200 ml capacity. Reaction mixture inside the cell, consisting of 100 ml of SD solution, was kept at room temperature and stirred all the time by a magnetic stirrer during the experiment. The initial concentration of SD in all experiments was 100 mg l^{-1} (2.8×10^{-4} mol l^{-1}). For photoconversion the 254 nm line of a low pressure Hg lamp (20 W, Shanghai Hailian Co., Ltd., Shanghai, China) was applied, and the working sample was placed in front of it at a distance of 45 cm. After irradiation all samples were immediately protected from light with aluminum foil. To follow SD photodegradation rate and pathway, the photoexposed solutions were then subjected to HPLC analysis. These analyses were also carried out on dark control samples, i.e. 100 ml SD solution in a Pyrex glass cell wrapped in aluminum foil during irradiation. The methanol samples used for the isolation were irradiated for a fixed period of time: 90 min (sample A), 150 min (sample B) and 450 min (sample C), respectively.

2.3. Isolation of photodegradation products

2.3.1. Acetylated SD1 (Ace-SD1)

After 1000 ml of SD solution was irradiated under UV light for 90 min, solvent of sample A was distilled immediately at low pressure. The residue was reconstituted in 10 ml of acetic anhydride, and 0.1 ml of pyridine was added. The Reaction mixture was stirred on a

Table 1
Spectral data for SD and photodegradation products

Parameter	Compound				
	SD	Ace-SD1	SD1	SD2	SD3
Molecular formula	$C_{22}H_{26}O_4$	$C_{26}H_{30}O_6$	$C_{22}H_{26}O_4$	$C_{22}H_{24}O_4$	$C_{22}H_{24}O_4$
Molecular ion $[M-H]^-$	353	437	353	351	351
UV (λ_{max}, nm)	202	204	204	204	206
	265	244	249	257	258
IR (ν_{max})	3600–2400 (–OH)	3600–2400 (–OH)	–	3600–2400 (–OH)	3600–2400 (–OH)
	1703 (–C=O)	1747 (–C=O)		1707 (–C=O)	3377 (–OH)
	1641 (–C=O)	1699 (–C=O)		1645 (–C=O)	1705 (–C=O)

Table 2
^{1}H NMR data for SD and photodegradation products

Position	SD			Ace-SD1			SD2			SD3		
	^{1}H	δ (ppm)	J (Hz)	^{1}H	δ (ppm)	J (Hz)	^{1}H	δ (ppm)	J (Hz)	^{1}H	δ (ppm)	J (Hz)
2	2	2.322–2.371	t, 14.7	2	2.081–2.130	t, 14.7	2	2.232–2.280	t, 14.4	2	2.039–2.087	t, 14.4
3	2	1.593–1.666	m	2	1.381–1.479	m	2	1.513–1.610	m	2	1.353–1.452	m
4	2	1.280–1.329	brm	2	1.221–1.295	m	2	1.365–1.461	m	2	1.452–1.552	m
5	2	1.397–1.434	m	2	1.686–1.753	m	2	1.862–1.910	m	2	2.379–2.426	t, 14.1
6	2	2.119–2.238	brm	–	–	–	–	–	–	–	–	–
7	1	4.281–4.332	t, 15.3	1	5.988–6.035	t, 14.1	1	6.081–6.130	t, 14.7	–	–	–
14	3	2.075	s	3	1.925	s	3	2.005	s	3	2.054	s
15	3	1.980	s	3	1.753	s	3	1.862	s	3	1.712	s
16	3	2.013	s	3	1.839	s	3	1.942	s	3	2.212	s
18–22	5	7.171–7.294	m	5	6.973–7.061	m	5	7.135–7.197	m	5	7.303–7.430	m
24	–	–	–	3	1.812	s	–	–	–	–	–	–
26	–	–	–	3	2.155	s	–	–	–	–	–	–

Assignments made on the basis of DEPT, COSY, HSQC and HMBC experiments. s, singlet; d, doublet; t, triplet; m, multiplet; brm, broad multiplet; J, coupling constant.

Table 3
^{13}C NMR data for SD and photodegradation products

Position	SD (δ (ppm))	Ace-SD1 (δ (ppm))	SD2 (δ (ppm))	SD3 (δ (ppm))
1	179.152	178.967	179.028	178.804
2	33.789	33.763	33.707	33.509
3	24.504	24.325	24.360	24.056
4	29.148	28.451	28.415	27.659
5	27.990	29.284	29.797	25.373
6	31.596	130.777	142.516	154.181
7	43.444	131.309	131.326	118.118
8	145.792	135.718	134.083	118.965
9	140.819	129.169	142.677	117.345
10	187.895	146.053	187.715	147.673
11	142.208	127.582	140.839	111.404
12	140.224	128.288	140.877	124.804
13	187.045	144.908	185.977	147.910
14	12.539	13.282	13.611	11.528
15	12.383	13.486	12.397	12.000
16	12.416	13.282	12.410	12.073
17	141.489	140.360	139.451	134.341
18, 19	127.887	128.288	128.549	130.725
20, 21	128.270	126.989	125.697	128.117
22	126.173	125.927	127.353	127.201
23	–	168.720	–	–
24	–	20.346	–	–
25	–	169.016	–	–
26	–	20.521	–	–

Assignments made on the basis of DEPT, COSY, HSQC and HMBC experiments.

magnetic stirrer at room temperature for 24 h and then was evaporated in vacuo. The residue was dissolved in 10 ml of mobile phase, methanol–H_2O–HAc (85:15:0.015, v/v/v). Aliquots (25 × 0.4 ml) of the sample solution were injected onto the semi-preparative HPLC column monitoring at UV 260 nm. Fractions containing the desired compound were collected and evaporated in vacuo. Finally, the product was purified by recrystallization with methanol to give Ace-SD1 (10 mg, 10%) as a colorless solid. mp: 146–147 °C. UV, IR and ESI-MS spectral data is listed in Table 1. ^{1}H NMR and ^{13}C NMR spectral data is listed in Tables 2 and 3, respectively.

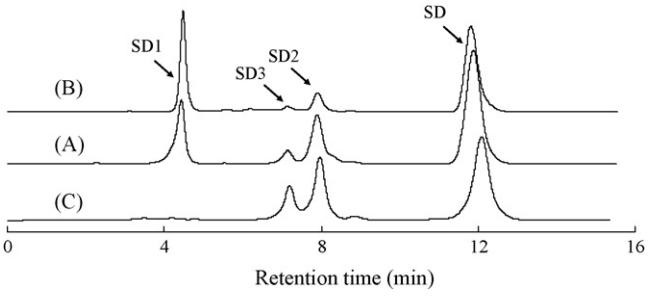


Fig. 2. UV chromatograms of photodegradation samples irradiated for 90 min in (A) methanol solution, (B) acetonitrile/H_2O (80:20) solution and (C) acetonitrile solution.

2.3.2. SD2

After irradiation, 1000 ml of sample B was put at dark place for 72 h and then was distilled at low pressure. The residue was reconstituted in 3 ml of methanol and 2 ml of purified water was dropped slowly into the solution, a yellowish precipitate appeared which was removed by filtration. The precipitate was dissolved in 10 ml of mobile phase, methanol–H_2O–HAc (80:20:0.02, v/v/v). Aliquots (10 × 1 ml) of the sample solution were injected onto the semi-preparative HPLC column monitoring at UV 260 nm. Fractions containing the desired compound were collected and evaporated in vacuo. Finally, the product was purified by recrystallization with methanol–H_2O (80:20, v/v) to give SD2 (20 mg, 20%) as a saffron yellow solid. mp: 111–112 °C. UV, IR and ESI-MS spectral data is listed in Table 1. ^{1}H NMR and ^{13}C NMR spectral data is listed in Tables 2 and 3, respectively.

2.3.3. SD3

After irradiation, 1000 ml of solvent of sample B was distilled at low pressure. The residue was dissolved in 10 ml of mobile phase, methanol–H_2O–HAc (80:20:0.02, v/v/v). Aliquots (20 × 0.5 ml) of the sample solution were injected onto the semi-preparative HPLC column monitoring at UV 260 nm. Fractions containing the desired compound were collected and evaporated in vacuo. Finally, the product was purified by recrystallization with methanol–H_2O (60:40, v/v) to give SD3 (15 mg, 15%) as a light yellow solid. mp: 95–96 °C. UV, IR and ESI-MS spectral data is listed in Table 1. ^{1}H NMR and ^{13}C NMR spectral data is listed in Tables 2 and 3, respectively.

3. Results and discussion

3.1. Photochemical characteristics in various conditions

Photodegradation of SD in different solutions and in solid state was evaluated. The analytical UV chromatograms of photodegradation of SD in methanol, in acetonitrile and in acetonitrile/H_2O (80:20) solutions are shown in Fig. 2. Three major products (SD1, SD2 and SD3) were detected in methanol and in acetonitrile/H_2O (80:20) solutions during UV irradiation; two major products (SD2 and SD3) were observed in acetonitrile solution. However, SD was very stable in solid state under UV irradiation for a week; no other peak was detected by HPLC-PDA analysis except for that of SD. Therefore, we decided to elucidate the structures of SD1, SD2 and SD3 in different solutions.

Meanwhile, it was found that SD1 was unstable even when the samples were protected from light. As shown in Fig. 3, SD1 was converted into SD2 absolutely in 3 days when the irradiated samples were presented in dark. The absorbance of SD2 was higher than that of SD1 at 260 nm (Fig. 4), so a higher increase in peak area than the corresponding decrease in that of SD1 was observed in Fig. 3. According to LC/MS analysis (Table 1), the molecular weight of SD2

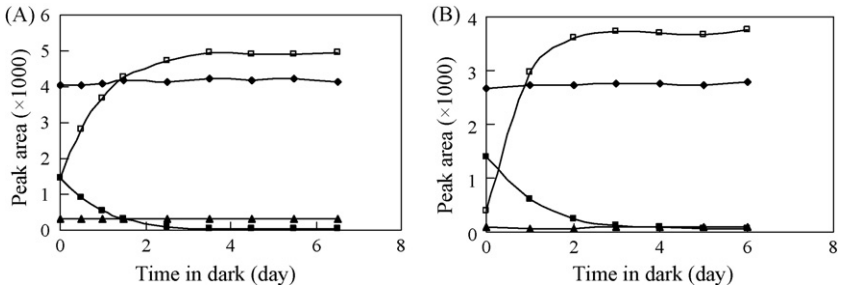


Fig. 3. Evolution of SD and the main photoproducts when the irradiated samples were presented in dark: (A) SD in methanol solution irradiated for 90 min, (B) SD in acetonitile/H_2O (80:20) solution irradiated for 90 min. (♦) SD; (■) SD1; (□) SD2; (▲) SD3.

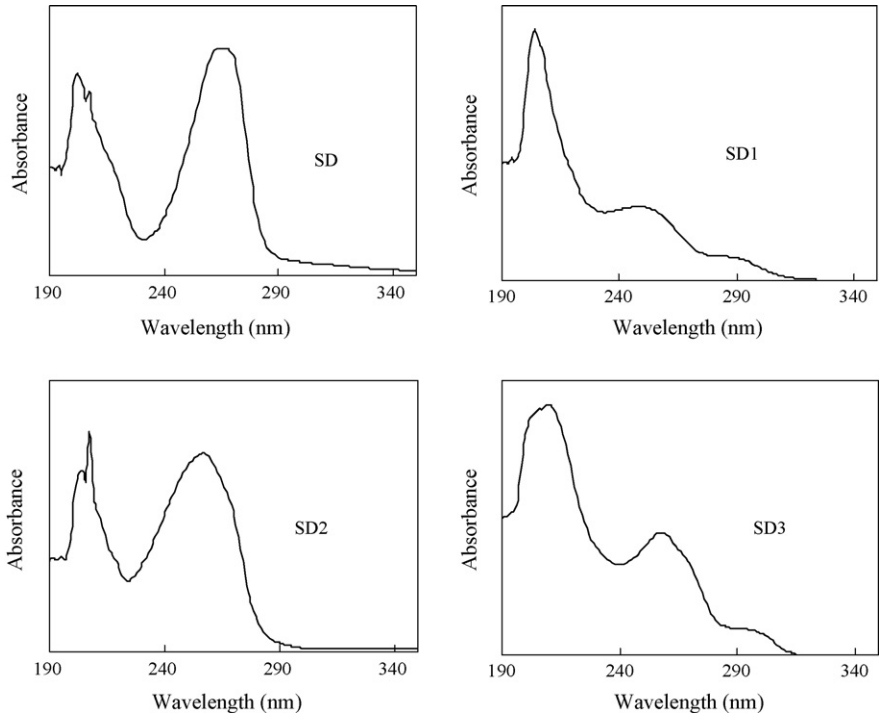


Fig. 4. UV spectra of SD and the three main photoproducts.

decreased to 352 Da compared with 354 Da of SD1; and referring the information from previous papers [14–21], a β-H-atom in the side chain of BQs can act as an intramolecular H-atom donor, it was inferred that SD1 might be a hydroquinone compound which was produced by intramolecular H-atom transfer of SD. So acetylation of SD1 with acetic anhydride and pyridine was performed to prevent it from transforming. The structure of SD1 was deduced from that of acetylated SD1 (Ace-SD1).

3.2. Structure elucidation of Ace-SD1

LC/MS data in Table 1 showed that the molecular weights of SD1 and Ace-SD1 were 354 and 438 Da, respectively; which illustrated that Ace-SD1 was the double acetylated product of SD1. It also could be interpreted from the IR spectral data of Ace-SD1 in Table 1. A characteristic absorption band was appeared at 1747 cm^{-1} for ⟩C═O stretching on the ester instead of 1641 cm^{-1} ⟩C═O stretching on the two ketone groups of *p*-benzoquinone of SD. Furthermore, compared the UV spectrum of SD1 with that of SD in Fig. 4, the intensity of the absorption region at 230–280 nm of SD1, which could be ascribed to $n \rightarrow \pi^*$ transitions, decreased dramatically with a blue shift of λ_{max}; it indicated that, during UV irradiation, the *p*-benzoquinone of SD was converted into dihydroxyhenzene of SD1 which was then acetylated. The ^{1}H NMR spectral data of Ace-SD1 in Table 2 showed that the pattern of side chain was different from that of SD. The resonances of H-5 and H-7 were shifted to lower field and that of H-6 was disappeared. The resonances of H-2, H-3, H-4 of the side chain, H-14, H-15, H-16 of benzene ring A, and ψ-H of benzene ring B were largely retained. In the ^{13}C NMR (Table 3) and DEPT spectra, 26 carbon signals (5 CH3, 4 CH2, 6 CH and 11 C) were identified. COSY and HSQC spectra confirmed the downfield shifts of H-5 (δ 1.397 → δ 1.710) and H-7 (δ 4.308 → δ 6.012), and showed a correlation peak of H-5 with H-7. In the HMBC spectrum, H-7 (δ 6.012) was correlated with C-5, C-6, C-8 and C-17; H-24 (δ 1.812) was correlated with C-23; and H-26 (δ 2.155) was correlated with C-25. Therefore, these data established the structure of Ace-SD1 as 6-(2,5-diacetoxy-3,4,6-trimethyl-phenyl)-7-phenylhept-6-enoic acid (Fig. 5, Ace-SD1). Thus, it is easier to deduce the structure of SD1 as 6-(2,5-dihydroxy-3,4,6-tri-methylphenyl)-7-phenylhept-6-enoic acid (Fig. 5, SD1).

3.3. Structure elucidation of SD2

MS analysis (Table 1) suggested that the molecular weight of SD2 was 352 Da, 2 Da less than that of SD. In the IR spectrum of SD2 (Table 1), a characteristic absorption band appeared at 1645 cm^{-1} for ⟩C═O stretching on the two ketone groups of *p*-benzoquinone. The UV spectrum of SD2 in Fig. 4 was very similar to that of SD, except for a slight blue shift of λ_{max}. And comparing the ^{1}H NMR and ^{13}C NMR spectral data of 3,5,6-trimethyl-1,4-benzoquinone

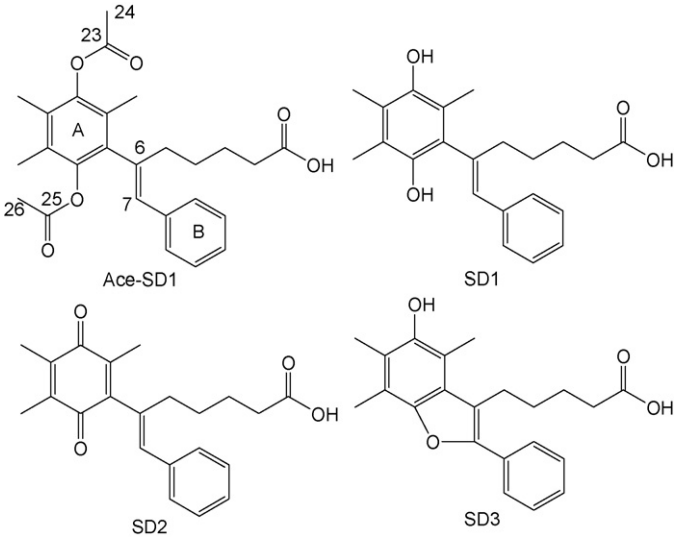


Fig. 5. Elucidated chemical structures of the three main photoproducts.

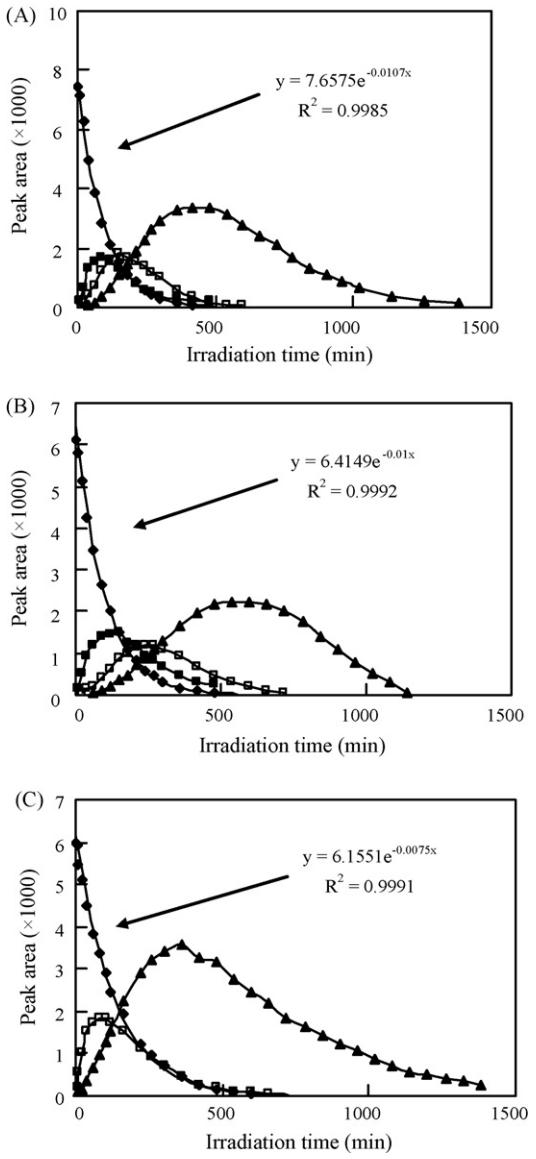


Fig. 6. Trends of SD and the main photoproducts as a function of irradiation time in (A) methanol solution, (B) acetonitile/H_2O (80:20) solution and (C) acetonitrile solution. (♦) SD; (■) SD1; (□) SD2; (▲) SD3.

substructure of SD with those of SD2 in Tables 2 and 3, resonances of H-14, H-15, H-16 and carbon signals from C-9 to C-16 were also very close to each other. All of these indicated the presence of *p*-benzoquinone substructure of SD2. In the ^{1}H NMR spectrum of SD2 (Table 2), the pattern of side chain was also different from that of SD, just the same as that of Ace-SD1. In the ^{13}C NMR (Table 3) and DEPT spectra, 22 carbon signals (3 CH3, 4 CH2, 6 CH and 9 C) were identified. COSY and HSQC spectra confirmed the downfield shifts of H-5 (δ 1.397 → δ 1.885) and H-7 (δ 4.308 → δ 6.106), and showed a correlation peak of H-5 with H-7. In the HMBC spectrum, H-7 (δ 6.012) was correlated with C-5, C-6 and C-17. Thus, the ^{1}H and ^{13}C NMR signals of the side chain, benzene ring A and benzene ring B of SD2 were easily assigned by comparison with those of SD and Ace-SD1 in Tables 2 and 3. The structure of SD2 was established as 7-phenyl-6-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dienyl)-hept-6-enoic acid (Fig. 5, SD2).

3.4. Structure elucidation of SD3

MS analysis (Table 1) suggested that the molecular weight of SD3 was 352 Da, the same as that of SD2. In the UV spectrum of SD3 (Fig. 4), significant decrease of the intensity of the absorption region at 230–280 nm indicated the disappearance of the two ketone groups of *p*-benzoquinone. Meanwhile, it also could be deduced from the IR spectral data of SD3 in Table 1, a characteristic absorption band appeared at 3377 cm^{-1} for –OH stretching on the phenolic group, with the characteristic absorption band at 1645 cm^{-1} 〉C═O stretching on the two ketone groups of *p*-benzoquinone disappeared. Compared the ^{1}H NMR spectrum of SD3 with that of SD (Table 2), the resonances of H-6 and H-7 of the side chain were disappeared; the resonance of H-5 was shifted to the much lower field, and the resonance of H-16 was shifted to the lower field, too. These data suggested that a benzofuran substructure was present in the structure of SD3. In the ^{13}C NMR (Table 3) and DEPT spectra, 22 carbon signals (3 CH3, 4 CH2, 5 CH and 10 C) were identified. COSY and HSQC spectra confirmed the downfield shifts of H-5 (δ 1.397 → δ 2.623) and H-16 (δ 2.013 → δ 2.212). In the HMBC spectrum, H-5 (δ 2.623) was correlated with C-3, C-4, C-6 and C-7; ψ-H (δ 7.303–7.430) was correlated with C-7 and C-17; H-2 (δ 2.063) was correlated with C-1, C-3 and C-4. Therefore, these data established the structure of SD3 as 5-(5-hydroxy-4,6,7-trimethyl-2-phenyl-benzofuran-3-yl)-pentanoic acid (Fig. 5, SD3).

3.5. Proposed photodegradation pathway

Sample irradiation led to a variation in the proportion of each product with the exposure time; nevertheless, the photodegradation modes were very similar to each other in these three solutions. As can be seen in Fig. 6, the photodegradation of SD in different solutions all followed first-order kinetics, to give SD2, SD3 and/or SD1. The degradation of SD occurred with a half-life of $t_{1/2}$ = 64.8 ± 0.6 min (n = 3) in methanol solution, $t_{1/2}$ = 69.1 ± 0.3 min (n = 3) in acetonitrile/H_2O solution and $t_{1/2}$ = 92.5 ± 1.7 min (n = 3) in acetonitrile solution, respectively. And trends of SD1, SD2 and SD3 as a function of irradiation time showed that proportions of these three degradation products

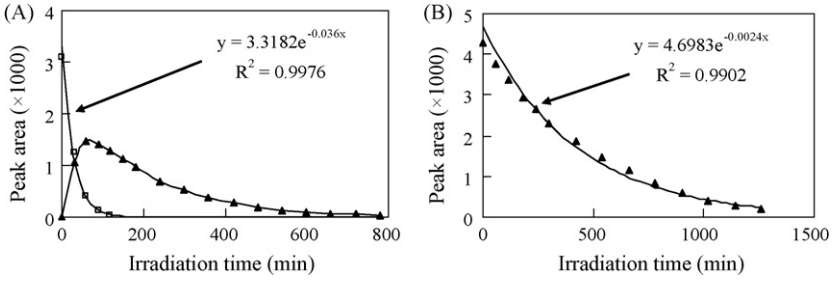
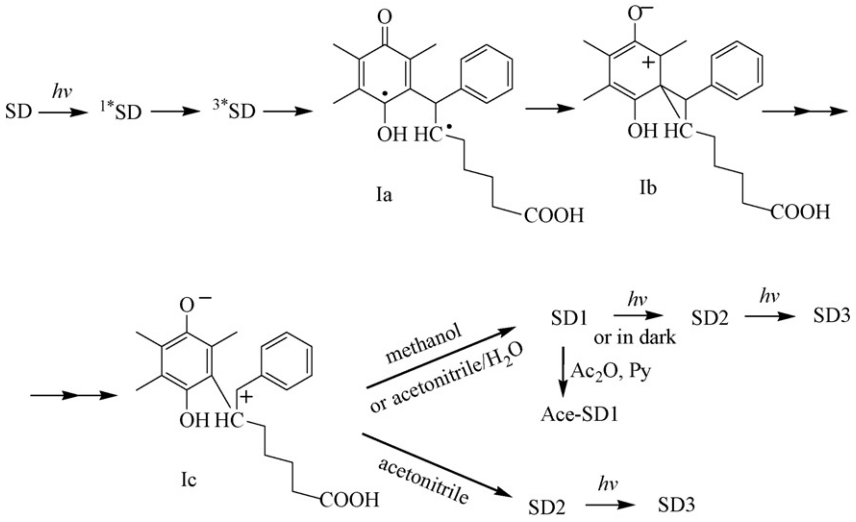


Fig. 7. Photodegradation of SD2 and SD3 in methanol solution, respectively: (A) trends of SD2 and the main photoproduct as a function of irradiation time, (B) trends of SD3 as a function of irradiation time. (□) SD2; (▲) SD3.



Scheme 1. The possible transients and photodegradation pathways for SD in different solutions. ^{1}*SD, the single state of SD; ^{3}*SD, the triplet state of SD.

initially increased to the maximums, but after that their bands were decreased. This type of behavior indicated that the products were initially formed in the solutions but also underwent degradation processes during UV irradiation.

The photodegradation procedure of SD in methanol solution was qualitatively similar to that in acetonitrile/H_2O solution; however, one of the major photoproducts SD1 was not observed in acetonitrile solution (Fig. 2). This phenomenon was probably due to the lack of reactive hydrogen donor in acetonitrile solution. LC/MS analysis (Table 1) showed that the molecular weight of SD2 decreased to 352 Da compared with 354 Da of SD1. Furthermore, as shown in Fig. 3, SD1 was converted to SD2 absolutely in 3 days when the irradiation sample was kept at dark place. All of these indicated that, during UV irradiation of SD in protonic solution, SD1 was readily formed and stayed for a longer time in the system of enough hydrogen donors; however, SD2 was structurally more stable when compared with SD1, so SD1 was oxidized and transformed into SD2 eventually. During UV irradiation of SD in non-protonic solution, SD1 might be formed, but owing to the lack of any hydrogen donor in the system, even protons from SD1 was immediately taken away by the surrounding solvent, so only SD2 was eventually observed to be formed from SD.

As far as the photodegradation products could be detected in protonic solvent, SD1 appeared first followed by SD2, SD3 was the last; and the appearance of the maximal proportions of SD1, SD2 and SD3 also followed the same order (Fig. 6). Meanwhile, the first-order kinetic behavior of SD suggested that this form would be involved in the disappearance and formation of only one product present. These phenomenon indicated that SD1 was the first product formed from SD but also underwent a degradation process to give SD2 or/and SD3.

Comparing the time for SD1 to disappear in protonic solution, it is about 360 min under irradiation (Fig. 6) and 3 days in dark (Fig. 3), we concluded that the rate for SD1 to transform into SD2 or/and SD3 was accelerated by UV irradiation.

Since SD1 was unstable, the photodegradation properties of SD1 could not be determined directly. As shown in Fig. 3, SD1 was converted into SD2 absolutely in 3 days when the sample was protected from light; furthermore, SD2 was the second product observed during the photodegradation process of SD in protonic solution (Fig. 6), and the time when its proportion got to the maximum was also in the second order; so it was more possible for SD1 to convert into SD2 instead of SD3 under UV irradiation in protonic solution.

As to SD2 and SD3, we examined their photodegradation properties in methanol solution (Fig. 7). Both SD2 and SD3 were sensitive to UV light. The variation of SD2 with irradiation time followed first-order kinetics with a half-life of $t_{1/2} = 19.3 \pm 0.3$ min ($n = 3$), and SD3 was the main product. However, the variation of SD3 with irradiation time did not follow first-order kinetics. Many other minor products were formed from SD3, and those products were difficult to be isolated and elucidated.

Finally, many researches have been done in the former literatures on photochemistry of 1,4-benzoquinone with a β-H-atom in the side chain [14–21]. A spirocyclopropyl intermediate has been proposed and four photoproducts have been identified for TQ in methanol [16]. The properties of a zwitterion as first observable transient of TQ are outlined and the different results with 2,5-dimethyl-1,4-benzo-quinone (Me_2BQ) are discussed [20]. For 2,5-dibromo-3-methyl-6-isopropyl-1,4-benzoquinone (Br_2TQ) in ethanol 2,5-dibromo-3-allyl-6-methyl-1,4-hydro-benzo-quinone as the major photoproduct has been reported [19]. The properties of the triplet state and a zwitterion as first and second observable transient of Br_2TQ are outlined and the different results with respect to TQ are discussed. In contrast, no intermediate could be detected for 2-*tert*-butyl-1,4-benzoquinone (BuBQ) [21]. And in addition to the photoproducts analysis of SD, a zwitterion–diradical (Ia), a spirocyclopropyl intermediate (Ib) and a zwitterion prior to formation of relatively stable products (Scheme 1, Ia–Ic) could be postulated during UV irradiation of SD solution. As a result, the possible photodegradation pathway for the formation of SD1–SD3 could be summarized in Scheme 1.

4. Conclusion

Recent years, it is important to identify the impurities and degradation products of drugs from the viewpoints of the prediction for their toxicity. Although many researches have been conducted on the bioactivities of seratrodast [22,23], but little is known about its photoproducts and photodegradation pathways. We have firstly investigated the photoproducts of SD in different solutions and proposed the possible photodegradation pathway based on the photoproducts. The results of our study might contribute to the references of better formulation and manufacturing process of seratrodast.

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