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Bioactive Constituents, Metabolites, and Functions

Water-assisted/water-accelerated photoreaction of trans-TSG from the roots of Polygonum multiflorum

Ni-Man Bao, Jin Dai, Ning-Lin Liao, Wen-Feng Ying, and Rong-Hua Zhao

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1 Water-assisted/water-accelerated photoreaction of *trans*-TSG from the roots of

2 Polygonum multiflorum

3 Ni-Man Bao^a, Jin Dai^a, Ning-Ling Liao^a, Wen-Feng Ying^a, Rong-Hua Zhao^{a,*}

4

⁵ ^a Yunnan University of Chinese Medicine, Kunming, 650500, Yunnan, China

7 ABSTRACT

8	The photoreaction of 2,3,4',5-tetrahydroxystilbene-2- O - β - D -glucoside (TSG) has
9	been investigated. Water-assisted/water-accelerated photodimerization of trans-TSG
10	favoured the formation of syn-HT (head to tail) [2+2] photocyclobutane under 365 nm
11	irradiation due to hydrophobic association and a fluorescent solute-solute aggregate
12	from their excited singlet states. In contrast, irradiation with 254 nm led to [2+2]
13	photocycloreversion. The two cyclobutane dimers were first obtained through
14	straightforward photoreaction, and identified as multiflorumiside A and
15	multiflorumiside C through the detailed analysis of HRESIMS, 1D/2D NMR.
16	Therefore, trans-TSG should be protected from light and water.

17

18 Keywords: [2+2] photocycloreversion-photocyclobutane; *trans*-TSG; *Polygonum*19 *multiflorum*

20 1. INTRODUCTION

21	<i>Trans</i> -2,3,4',5-tetrahydroxystilbene-2-O- β -D-glucoside (<i>trans</i> -TSG), a
22	photosensitive stilbenoid, was first isolated in 1975 by Hata et al. from the traditional
23	Chinese herb Polygonum multiflorum Thunb. (Polygonaceae). 1-2 It shows diverse
24	bioactivities, including antitumour effects, ³ antioxidant activity, ⁴ promotion of hair
25	growth, ⁵ antimelanogenic activity, ⁶ neuron protection, ⁷ amelioration of vascular
26	dysfunction, ⁸ antiaggregation, ⁹ antiatherosclerosis, ¹⁰ improvement of age-related
27	cognitive impairment, ¹¹ and anti-inflammatory effects. ¹²⁻¹³ Quantification of the
28	main active stilbenoid trans-TSG has been documented in Chinese Pharmacopeia as a
29	criterion for the quality control of Polygonum multiflorum Thunb. (in Chinese
30	Heshouwu). ¹⁴ The photosensitivity of <i>trans</i> -TSG and related bioactivities have
31	increasingly attracted the attention of both pharmaceutical and natural product
32	chemists, due to the hepatotoxicity and notable biological activities reported. Previous
33	reports are consistent with the conclusion that trans-TSG efficiently isomerizes into
34	cis-TSG through ultraviolet light or sunlight irradiation ¹⁵ , and cis-TSG can induce
35	immunological idiosyncratic hepatotoxicity in LPS-treated rats by suppressing
36	PPAR- γ ¹⁶ .

Purification of *trans*-TSG from the ethanol-extraction of *Polygonum multiflorum* Thunb. is performed through D-101 resin column chromatography, fllowed by recrystallization in aqueous solution to yield *trans*-TSG with purity less than 95%. Typically, either the purity or the colour of *trans*-TSG not only fails to make the grade but also decrease and darkens, respectively, through repeated recrystallization. Additionally, it is well known that colourless *trans*-TSG solid dissolved into an aqueous darkens, especially with prolonged exposure time. The phenomenon aroused our interest in determining whether or not *trans*-TSG kept its initial state in water. Based on the photochemistry of stilbene compounds, we conducted further investigation regarding the photoreactions of *trans*-TSG.

47 2. MATERIALS AND METHODS

48 2.1 General experimental procedures

NMR spectra were recorded in CD₃OD using a Bruker AV600 MHz 49 50 spectrometer with TMS as an internal standard. ESIMS spectra were recorded using a Waters Xevo TQ-S ultra-high-pressure liquid chromatography triple quadrupole mass 51 spectrometer. HRESIMS data were obtained using an Agilent 1290 UPLC/6540 52 53 Q-TOF mass instrument. Both HPLC-UV analysis and semi-preparation were all performed on an Agilent 1260 HPLC system (Agilent, USA) consisting of a 54 quaternary solvent delivery system, an online degasser, an autosampler, a column 55 56 temperature controller and an ultraviolet detector. The chromatographic separation was performed on an Agilent Zorbax SB-C18 column (4.6×250 mm, 5 μ m) at a 57 solvent flow rate of 1 mL/min at 30 °C. Water and MeOH were used as mobile phases 58 A and B, respectively. The solvent isocratic adopted was as follows: 0-30 min, 30% B. 59 The detection wavelength was 220 nm, and the injection volume was 5 μ L. 60 Semi-preparation was carried out with same conditions described above, except 61 62 chromatographic separation was performed on an Agilent Zorbax SB-C18 column $(9.4 \text{ mm} \times 250 \text{ mm}, 5 \mu\text{m})$ at a solvent flow rate of 3 mL/min at 30 °C, and the injection 63

64	volume	was	100	μL
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65 2.2 Plant Materials

The roots of *P. multiflorum* were collected in Simao County of Yunnan Province,
People's Republic of China, in October of 2017. The plant material was identified by
Prof. Rong-Hua Zhao (Yunnan University of Chinese Medicine), and a voucher
specimen (No. 20171203) was deposited at the Institute.

70 2.3 Extraction and Isolation

71 The roots of *P. multiflorum* (25 kg) were dried, crushed, and extracted using 90% 72 EtOH under reflux (80 L, 2 h \times 3). A residue (2.2 kg) was obtained by removing the solvent under reduced pressure. The residue was sequentially partitioned into EtOAc 73 and water. The EtOAc fraction (1.0 kg) was chromatographed on a macroporous resin 74 75 (D101) column, eluting successively with H₂O, 20% EtOH, 50% EtOH, 70% EtOH, and 95% EtOH. The 20% EtOH portion (200 g) was recrystallized repeatedly to yield 76 trans-TSG at 95% purity, purified by semi-preparation HPLC. Trans-TSG was 77 78 dissolved into ethanol solution by stirring, followed by 365 nm irradiation for 1 h. Then, purification was carried out through chromatographic separation with 79 semi-preparation (with the same conditions as in 2.1) to yield cis-TSG, which 80 identified by ¹H and ¹³C-DEPT NMR spectra, and MS data (showed in supporting 81 information). 82

83 2.4 Photoreactions of *trans*-TSG and *cis*-TSG

84 *Both trans*-TSG and *cis*-TSG were dissolved fairly effectively in water and 85 MeOH individually to yield a water-solution (6 mg/mL) and MeOH-solution (6

86	mg/mL); both the water-solution and MeOH-solution were exposed to UV(365 nm $$				
87	and 254 nm) irradiation separately, followed by HPLC-UV analysis to investigate				
88	photoreactional adducts in varying solvent and time conditions. Photo-induced				
89	reactional products including a photoisomer and two dimers were purified by				
90	semi-preparative HPLC. Two photodimer aqueous solutions were irradiated				
91	separately with UV (254 nm) light and subjected to HPLC-UV analysis.				
92	2.5 Quantification analysis				
93 94	Table 1 Calibration Curve of <i>trans</i> -TSG, <i>cis</i> -TSG, multiflorumiside A and multiflorumiside C.				
	compounds regression equation Linearity (µg) r^2				
	multiflorumiside A $v = 1681 16 x + 32.77$ 0.15-6.00 0.9998				
	cis-TSG $v = 246358 x - 4421 022-880 0999990$				
	multiflorumiside C $v = 111143 \text{ x} - 8546 = 0.10-1.20 = 0.9992$				
	$trans-TSG$ $v = 1354\ 24\ x\ -240\ 73$ $1\ 40\ -14\ 00$ $0\ 9997$				
95	The calibration curve ($y = a + bx$) for each stilbenoid was constructed by plotting				
96	the peak area against the concentration, and the linearity was verified by correlation				
97	coefficients (r^2). The lower limit of detection (LOD) and lower limit of quantification				
98	(LOQ) were determined on the basis of the response at signal-to-noise ratios (S/N) of				
98 99	(LOQ) were determined on the basis of the response at signal-to-noise ratios (S/N) of 3 and 10, respectively. All calibration curves showed good linearity ($r^2 > 0.999$).				
98 99 100	 (LOQ) were determined on the basis of the response at signal-to-noise ratios (S/N) of 3 and 10, respectively. All calibration curves showed good linearity (r² > 0.999). 3. Results and Discussion 				

101 Compound **2** was isolated as an isomerization product from **4** (*trans*-TSG) in the 102 photoreaction, and it has been reported from *Polygonum multiflorum* Thunb. The 103 structures of **2** and **4** were identified by their NMR and MS data (Table 2 and Table 3), 104 which are consistent with the literature ¹⁷. Compounds **1** and **3** were purified as 105 dimerization products due to their molecular weights, all being 835 ($[M + Na]^+$ 106 ESI-MS m/z); the structures were deduced by comparing spectroscopic data with previous literature ¹⁸. All deduced structures were confirmed by the 2D NMR spectra;
the relative configurations of compounds 1 and 3 were determined by the major
ROESY correlations (Fig. 1), which were first reported here.

The trans-cis isomerization of TSG efficiently occurred in both aqueous and 110 methanol solutions under vis/uv lights (sunlight; 254 nm and 365 nm). In addition, 111 photodimerizations of [2+2] cyclobutane have only been observed in trans-TSG 112 aqueous solution within 3 h under 365 nm or sunlight irradiation, rather than in 113 methanol media or under 254 nm light irradiation. In contrast, 254 nm light irradiation 114 115 for more than 3 h of the trans-TSG aqueous solution resulted in lower yields of photodimers (Fig. 3). Investigation of 254 nm and 365 nm irradiation over 3 h both in 116 cis-TSG aqueous and methanol solutions separately, resulted only in inefficient 117 118 isomerization rather than dimerization. In addition, investigation of photoisomerization and photodimerization of the trans-TSG solution in varying 119 thermal conditions below 100 °C been carried out; neither dimerization nor 120 121 isomerization were observed (Fig. 2).

Since water played an important role in the TSG photoreaction, it may be attributed to the hydrophobic association of the stilbenoids in aqueous solution ¹⁹ facilitating molecular aggregation ²⁰. It is known that the degree of a hydrophobic interaction in aqueous solution is related to the entropy of the solution and of the solute $\triangle S$. Values of $\triangle S$ (cal K⁻¹.mol⁻¹) when gaseous cyclohexane is dissolved in various solvents at 298 K are as follows: -21.5 (hexane); -20.5 (benzene); -21.5 (acetonitrile); -29.3 (methanol); and -45.0 (water) ²¹. A large negative value of $\triangle S$ is

considered to reflect a strong solute-solute interaction ²². These data indicate that 129 strong solute aggregation occurs in water, resulting in the enhanced photodimerization 130 131 in the aqueous solvent. Isomerization of trans-TSG efficiently occurred both in methanol and aqueous solutions under uv/vis light irradiation, while the 132 photodimerization inefficiently occurred, except in the water with 365 nm or vis 133 irradiation. This seems to be consistent with a previous proposition 23-24 that 134 photodimerization of trans-TSG only occurred from the excited singlet states and that 135 trans-cis isomerization is known to occur from either the excited singlet or triplet 136 states. 137

Table 2 ¹H (600 MHz) and ¹³C NMR (150 MHz) data of compounds 2 and 4 in methanol- d_4 (δ , ppm).

no.	4		no.	2		
	$\delta_{\rm C}$	$\delta_{ m H}$		$\delta_{ m C}$	$\delta_{ m H}$	
1	133.75, s		1	133.87, s		
2	137.92, s		2	137.94, s		
3	155.95, s		3	155.33, s		
4	103.57, d	6.27, (d, 2.8)	4	103.79, d	6.20, (d, 2.9)	
5	152.03, s		5	152.10, s		
6	102.68, d	6.64, (d, 2.8)	6	107.62, d	6.10, (d, 2.9)	
7	121.70, d	7.72, (d, 16.5)	7	125,54, d	6.69, (d, 12.2)	
8	130.06, d	6.92, (d, 16.5)	8	131.25, d	6.45, (d, 12.2)	
1′	130.84, s		1′	129.75, s		
2'	129.22, d	7.74, (d, 8.5)	2'	131.43, d	7.04, (d, 8.4)	
3'	116.43, d	6.78, (d, 8.5)	3'	115.89, d	6.59, (d, 8.5)	
4′	157.77, s		4′	157.77, s		
5'	116.43, d	6.78, (d, 8.5)	5'	115.89, d	6.59, (d, 8.5)	
6′	129.22, d	7.74, (d, 8.5)	6′	131.43, d	7.04, (d, 8.4)	
1″	108.21, d	4.52, (d, 7.8)	1″	107.94, d	4.55, (d, 7.8)	
2"	75.47, d	3.58, (overlap)	2″	75.35, d	3.45, (m)	
3″	77.93, d	3.45, (t-like, 9.1)	3″	77.92, d	3.38, (m)	
4″	70.76, d	3.55, (overlap)	4″	70.97, d	3.44, (m)	
5″	78.21, d	3.29, (m)	5″	78.26, d	3.17, (ddd, 9.4, 4.7, 2.5)	
6″	62.06, t	3.83, (dd, 11.8, 2.3)	6″	62.21, t	3.75, (dd, 11.9, 2.6)	
		3.78, (dd, 11.8, 4.0)			3.68, (dd, 11.9, 4.6)	

No.		1	No.		3
	$\delta_{ m C}$	$\delta_{ m H}$	_	$\delta_{ m C}$	$\delta_{ m H}$
1a	137.63, s		1a	136.61, s	
2a	138.74, s		2a	137.11, s	
3a	151.08, s		3a	151.58, s	
4a	102.37, d	6.08, (d, 2.8)	4a	102.37, d	6.09, (d, 2.8)
5a	155.30, s		5a	155.63, s	
6a	106.99, d	6.45, (d, 2.8)	6a	108.25, d	6.50, (d, 2.8)
7a	42.45, d	4.91, (overlap)	7a	41.72, d	4.93, (overlap)
8a	46.08, d	4.41, (overlap)	8a	47.95, d	4.12, (t, overlap)
9a	133.86, s		9a	132.97, s	
10a	130.33, d	7.04, (d, 8.2)	10a	130.98, d	7.01, (d, 8.2)
11a	115.38, d	6.57, (d, 8.3)	11a	115.62, d	6.56, (d, 8.3)
12a	156.08, s		12a	156.39, s	
13a	115.38, d	6.56, (d, 8.3)	13a	115.62, d	6.56, (d, 8.3)
14a	130.33, d	7.04, (d, 8.2)	14a	130.98, d	7.01, (d, 8.2)
1b	137.59, s		1b	135.90, s	
2b	138.96, s		2b	138.15, s	
3b	151.20, s		3b	151.50, s	
4b	102.37, d	6.04, (d, 2.8)	4b	102.93, d	6.10, (d, 2.8)
5b	155.19, s		5b	155.34, s	
6b	107.34, d	6.32, (d, 2.8)	6b	107.58, d	6.49, (d, 2.8)
7b	42.62, d	5.16, (t, 9.1)	7b	41.34, d	5.04, (t, 9.1)
8b	48.11, d	4.12, (dd, 10.2, 7.2)	8b	48.19, d	4.29, (dd, 10.4, 6.7)
9b	133.59,s		9b	130.98,s	
10b	130.76,d	7.07, (d, 8.4)	10b	130.76,d	7.00, (overlap)
11b	115.45,d	6.52, (d, 8.3)	11b	115.56,d	6.55, (overlap)
12b	156.01,s		12b	156.18,s	
13b	115.45,d	6.52, (d, 8.3)	13b	115.56,d	6.55, (overlap)
14b	130.76,d	7.07, (d, 8.4)	14b	130.76,d	7.00, (overlap)
1′	108.22,d	4.34, (d, 8.0)	1'	108.12,d	4.38, (d, 7.9)
2′	75.55,d	3.55, (overlap)	2'	74.66,d	3.56, (m)
3′	78.14,d	3.42, (m)	3'	77.12,d	3.44, (t, 9.2)
4′	70.71,d	3.57, (overlap)	4'	70.77,d	3.53, (overlap)
5'	78.24,d	3.36, (m)	5'	78.11,d	3.09, (dt, 9.7, 3.0)
6′	62.18,t	3.88, (dd, 12.0, 4.1)	6'	61.84,t	3.68, (dd, 12.0, 4.1)
		3.96, (dd, 12.0, 2.4)			3.67, (dd, 12.0, 2.4)
1″	107.98,d	4.41, (overlap)	1″	107.49,d	4.38, (d, 7.9)
2″	75.49,d	3.56, (overlap)	2‴	75.53,d	3.56, (m)
3″	78.07,d	3.44, (m)	3‴	77.40,d	3.66, (m)
4″	70.64,d	3.57, (overlap)	4‴	70.62,d	3.69, (overlap)
5″	78.33,d	3.44, (m)	5‴	78.25,d	3.13, (ddd, 9.7, 4.3, 2.4)
6″	61.93,t	3.88, (dd, 12.0, 4.1)	6''	61.84,t	3.74, (dd, 12.2, 2.4)
		3.96, (dd, 12.0, 2.4)			3.69, (overlap)

141 Table 3 ¹H (600 MHz) and ¹³C NMR (150 MHz) data of compounds 1 and 3 in 142 methanol- d_4 (δ , ppm).

Water-assisted photodimerization prefers form trans-syn-HT 143 to (multiflorumiside A) products (Fig. 5) due to not only the "Breslow hydrophobic 144 effect" and the "hydrogen-bonding effect" but also the polarity effect 25. 145 Photocycloaddition of *trans*-TSG is a photo-allowed and thermo-forbidden reaction 146 that corresponds to the conservation of orbital symmetry rules and the frontier orbitals 147 theory by Woodward, Hoffmann, and Fukui for a 4π electron-system ²⁶⁻²⁸. Since no 148 other reagent would be more sustainable than light and no other medium greener than 149 water, we believe that the [2+2] photocyclobutane described here has a momentous 150 role as a synthetic tool to construct cyclobutane in the future. 151









177

178

Fig. 2 Molecular structures concerning photoreaction of the trans-TSG including photocycloreversion-photocyclobutane and photoisomerization.

The time course for the photolysis of trans-TSG or cis-TSG in water was 179 monitored by High Pressure Liquid Chromatography (HPLC) (Fig. 3). The results 180 indicated that the dimers multiflorumiside A and multiflorumiside C were formed 181

from the excitation of the trans-TSG rather than from excitation of the cis-TSG. The 182 time course for the photocycloreversion of dimers multiflorumiside A and 183 multiflorumiside C in water was monitored by HPLC (Fig. 4). This suggests that the 184 photocycloreversion of the two photodimers originally disassembled into *trans*-TSG, 185 followed by isomerization to form cis-TSG. Therefore, the results indicate that 254 186 nm irradiation switches on the photocycloreversion of the two main photodimers 187 within 0.5 h due to efficient trans/cis isomerization, which are related to 188 injury. Heshouwu-induced liver With regards to trans-TSG aggravating 189 190 cis-TSG-induced liver injury, purification of trans-TSG should be carried out folowing the photocycloreversion of multiflorumiside A and C. 191



Fig. 3 The contents of photoreaction products (compounds 1-3) of trans-TSG in 199 aqueous solution for varying irradiation times (3, 4, 5, 24 and 25 h). (a is compound 1; 200 b is compound 2; c is compound 3; d is compound 4; 245 nm and 365 nm are the 201 wavelengths of fluorescent light used for irradiation); (i is the phtocyclodimerization 202 ii photoisomerization is the of *trans*-TSG.) 203 and



Fig. 4 The contents of photocycloreversion products (2, 4) of two photodimers (1, 3)
in aqueous media for varying irradiation times (0.5, 1.5, 2.5, 4, 5, 24 and 25 h) under
254 nm light. (a is compound 1; b is compound 2; c is compound 3; d is compound 4).
(iii is the photocycloreversion of the *syn*-HT product multiflorumiside A and iv is the
photocycloreversion of the *anti*-HT product multiflorumiside C)



Fig. 5 v. the yields of photoreaction products of the photocycloreversion -photocyclobutane of *trans*-TSG, irradiated separately for 3 and 21 h by 254 and 365 nm light; vi. HPLC-UV chromatogram of *trans*-TSG in aqueous media by 365 nm light irradiation. (1 is *syn*-HT product multiflorumiside A; 2 is isomerization product *cis*-TSG, 3 is *anti*-HT product multiflorumiside C; 4 is *trans*-TSG).

In conclusion, *trans*-TSG should be protected from light and aqueous media. This suggests that the detection of photodimerization or photoisomerization should be

conducted by HPLC or nuclear magnetic resonance (NMR) for cyclobutane signals 226 $[\delta_{\rm C} 42.45(d); 46.08(d); 42.62(d); 48.11(d)]$ in the quality control of *trans*-TSG. 227 The usage of a fluorescence photoswitch for the photocycloreversion of the two main 228 photodimers by 254 nm light irradiation in order to maintain high quality control of 229 trans-TSG should be finished within 0.5 h (Fig. 5), due to the efficient trans/cis 230 isomerization, which is related to Heshouwu-induced liver injury. Owing to the 231 efficient fluorescence photoswitching [2+2] photocycloreversion- photocyclobutane 232 of TSG through 254 nm and 365 nm light irradiation in aqueous media, trans-TSG 233 234 was not only used for disease therapies but also used as a photoswitchable fluorescent materials. 235 supporting information includes 1D/2D NMR, ESI-MSHRESI-MS The 236

spectrums of compounds 1-4, and quantitative HPLC-UV analysis of 1-4 in thephotoreaction.

- 239 ASSOCIATED CONTENT
- 240 Supporting Information is available free of charge on the ACS Publications website.
- 241 AUTHOR INFORMATION
- 242 * Corresponding author
- 243 Tel: +86 13888074508.
- 244 E-mail: <u>kmzhaoronghua@hotmail.com</u>. (Rong-Hua Zhao)
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Water-assisted/water-accelerated photoreaction of trans-TSG from the roots of

Polygonum multiflorum

Ni-Man Bao^a, Jin Dai^a, Ning-Ling Liao^a, Wen-Feng Ying^a, Rong-Hua Zhao^{a,*}

^a Yunnan University of Chinese Medicine, Kunming, 650500, Yunnan

Province, China

* Corresponding author: Rong-Hua Zhao; <u>kmzhaoronghua@hotmail.com</u>;

Tel: +86 13888074508.

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