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Salvianolic acids T and U: A pair of atropisomeric trimeric caffeic acids derivatives from root of *Salvia miltiorrhiza*

Wei Li [a], Shui-Ping Zhou [a,*], Yuan-Peng Jin [a], Xue-Feng Huang [b,*], Wei Zhou [a], Min Han [a], Yun Yu [b], Kai-jing Yan [a], Shu-Ming Li [a], Xiao-Hui Ma [a], Zhi-Xin Guo [a], Yong-Hong Zhu [a], He Sun [a]

[a] *Tasly R&D Institute, Tasly Pharmaceutical Co., Ltd., Tianjin 300410, China*
[b] *Department of Natural Medicinal Chemistry, China Pharmaceutical University, Nanjing 20009, China*

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ABSTRACT

Two new trimeric caffeic acids, named salvianolic acids T and U (**1** and **2**), were isolated from the underground part of *Salvia miltiorrhiza*. Their structures, consisting of three caffeic acid units, were determined based on extensive 1D- and 2D-spectroscopic analyses and electronic circular dichroism (ECD) calculations.

1. Introduction

The dried root of *Salvia miltiorrhiza* (Danshen in Chinese) is one of the most popular traditional Chinese medicines, which was extensively used for prevention and treatment of coronary heart disease [1], chronic renal failure [2], atherosclerosis [3], ischemic myocardium [4], ischemic heart disease, myocardial infarction [5], liver fibrosis and cirrhosis [6], and showed an efficacy in relieving angina pectoris [7]. In Japan, Danshen products are acted as a medicine to promote circulation and improve blood flow. In our present research, two new trimeric caffeic acids, salvianolic acids T and U (**1** and **2**) (Fig. 1), were isolated from the roots of *S. miltiorrhiza*. Their structures, consisting of three caffeic acid units, were determined based on extensive 1D- and 2D-spectroscopic analyses and chemical reactions, and their absolute configurations were determined by electronic circular dichroism (ECD) calculation. In the present paper, we describe the isolation and structural determination of these two new products.

2. Experimental

2.1. General

Optical rotations were measured with a *Jasco* P-1020 polarimeter. CD spectra were obtained on a *Jasco* 810 spectrometer. UV spectra were performed on a Shimadzu UV-2450 spectrophotometer. IR spectra were obtained on a Shimadzu FTIR-8400S spectrophotometer. NMR spectra were recorded by Bruker Avance-III NMR instrument (^{1}H: 500 MHz, ^{13}C: 125 MHz) with TMS as internal standard. Chemical shifts were given in values of ppm and coupling constants in Hertz. Mass spectra were obtained on an MS Agilent 1100 Series LC/MSD iron-trap mass spectrometer (ESI-MS) and an Agilent 6520 B Q-TOF spectrometer (HR-ESI-MS), respectively. Semi-preparative HPLC was carried out on a Waters Delta prep 4000 instrument (flow rate set at 10 ml/min) using an Agilent ZORBAX XDB-C_{18} (21.2 × 150 mm, 5 μm). Silica gel (100–200, 200–300 mesh, Qingdao Haiyang Chemical Co. Ltd., China),

* Corresponding authors. Tel.: +86 25 52716876; fax: +86 25 85301528.
E-mail addresses: zhousp@tasly.com (S.-P. Zhou), hxf@cpu.edu.cn (X.-F. Huang).

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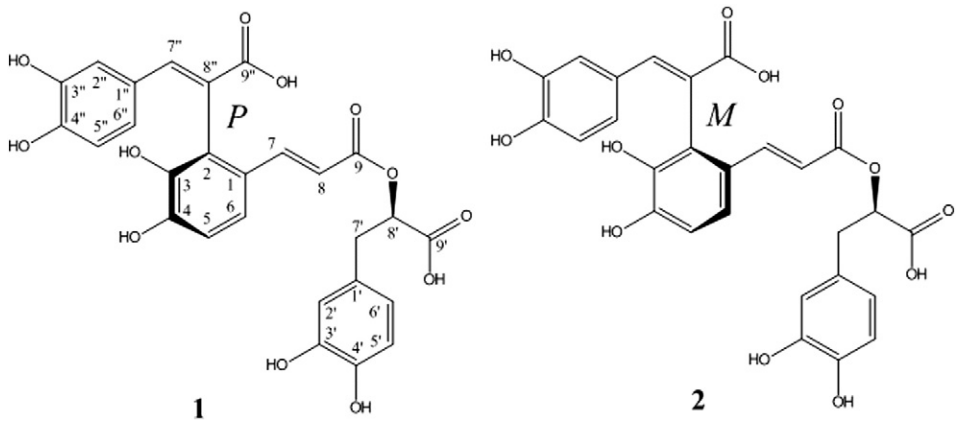

Fig. 1. Structures of compounds **1** and **2**.

macroporous resin D101 (Qingdao Haiyang Chemical Co. Ltd., China), Sephadex LH-20 (Pharmacia, U.S.A.), and RP-C_{18} (40–63 μm, YMC, Japan) were used for column chromatography. The pre-coated silica gel GF_{254} plates (Qingdao Marine Chemical Plant, China) were used for TLC. Spots were visualized by heating silica gel plates immersed in vanillin-H_2SO_4 in ethanol. All solvents used were of analytical grade (Jiangsu Hanbon Science and Technology Co. Ltd., China).

2.2. Plant material

The roots of *S. miltiorrhiza* (batch number: 20100301) were acquired in March 2010 from Shaanxi Tasly Plant Pharmaceutical Co., Ltd. (Shaanxi, China), which is Good Agricultural Practices (GAP) base for the agriculture and process of *S. miltiorrhiza* licensed by the State Food and Drug Administration, P. R. China (SFDA). A voucher specimen (No: 20100318) has been deposited in the Modern TCM Department, Tasly R&D Institute.

2.3. Extraction and isolation

Air-dried roots of the plant material (20 kg) were extracted with 95% EtOH under reflux for 3 h. The EtOH extract (2 kg) was suspended in water and partitioned with $CHCl_3$ (210 g), EtOAc (450 g) and *n*-BuOH (190 g). The EtOAc extract was chromatographed over a D101 macroporous resin column (15 × 60 cm), eluted successively with H_2O, 30%, 50%, 80%, and 95% EtOH in H_2O. The 80% EtOH fraction was chromatographed on silica gel column eluted with $CHCl_3$:MeOH (100: 0–60: 40, *v*/*v*) to obtain eight fractions (A–H). Fraction D (20 g) was applied to a silica gel column eluted with $CHCl_3$:MeOH:HCOOH (95:5:0–60:38:2, *v*/*v*/*v*) to give four fractions (D_A–D_D). Fraction D_C (2.5 g) was separated over silica gel column eluted with $CHCl_3$:MeOH:HCOOH (90:10:3–40:10:0.5, *v*/*v*/*v*) to afford three fractions (D_{CA}–D_{CC}). Fraction D_{CB} (200 mg) was separated over RP-C_{18} column eluted with MeOH:H_2O:HCOOH (60:40:0.3–90:10:0.5, *v*/*v*/*v*) to afford three fractions (D_{CBA}–D_{CBD}). Fraction D_{CBD} (60 mg) was purified by Sephadex LH-20 with $CHCl_3$:MeOH (50:50, *v*/*v*) and preparative HPLC to afford compounds **1** (11 mg, t_R = 16.5 min) and **2** (24 mg, t_R = 13.5 min) using MeOH:H_2O (90:10, *v*/*v*).

2.3.1. Salvianolic acid T (**1**)

Yellowish amorphous powder; $[\alpha]_D^{25}$ + 196.6 (c = 0.25, MeOH); UV (MeOH) λ_{max} nm: 220, 326; IR (KBr) ν_{max} cm^{-1}: 3150, 2878, 2817, 1693, 1600, 1519, 1445, 1360, 1250, 1115, 968, 865, 812; negative HR-ESI-MS $[M-H]^-$ *m*/*z*: 537.1027 (Calcd for $C_{27}H_{21}O_{12}^-$, 537.1038); ^1H and ^{13}C NMR data, see Table 1.

2.3.2. Salvianolic acid U (**2**)

Yellowish amorphous powder; $[\alpha]_D^{25}$ − 157.6 (c = 0.16, MeOH); UV (MeOH) λ_{max} nm: 225, 325; IR (KBr) ν_{max} cm^{-1}: 3306, 2881, 2822, 1687, 1602, 1519, 1445, 1360, 1250, 1113, 969, 865, 812; negative HR-ESI-MS $[M-H]^-$ *m*/*z*: 537.1034 (Calcd for $C_{27}H_{21}O_{12}^-$, 537.1038); ^1H and ^{13}C NMR data, see Table 1.

2.4. Hydrolysis reaction

Compounds **1** and **2** (10 mg) are respectively stirred in hydrochloric acid (5 ml, 6 M), at water bath (40 °C). The reaction is stirred until completion (high performance liquid chromatography, 12 h) then quenched with water, extracted 3 times with an equal volume of ethyl acetate, the combined organic phases, concentration in rotary evaporator is done at 30 °C.

Chromatographic conditions: use Agilent Eclipse XDB-C18 (4.6 mm × 250 mm, 5 μm) as the stationary phase and 0.2% formic acid-acetonitrile as mobile phase as specified in the following table gradient elution; flow rate: 1 ml/min; The wave length of the detection is 280 nm and the column temperature is 25 °C; injection volume 20 μl.

2.5. Preparation of fragment I by hydrolysis degradation

To determine the hydrolysis reaction mixture, major peaks in the chromatogram attribution were scanned by mass spectrometry. Centralized collection of degraded fragment I (m/z = 197) eluent, and extracted three times with an equal

Table 1
1D and 2D data of compounds **1** and **2** (data measured in DMSO at 500 MHz (^{1}H) and 125 MHz (^{13}C), *J* in Hz).

No.	δ_H		δc		COSY		HMBC	
	1	2	1	2	1	2	1	2
1	–	–	123.7	123.8			H-5, H-8	H-5, H-8
2	–	–	126.4	126.3			H-6, H-7, H-7″	H-6, H-7, H-7″
3	–	–	142.9	142.9			H-5	H-5
4	–	–	147.7	147.7			H-5, H-6	H-5, H-6
5	6.85 (1H, *d*, 8.5)	6.85 (1H, *d*, 8.5)	115.0	115.0	H-6	H-6		
6	7.31 (1H, *d*, 8.5)	7.29 (1H, *d*, 8.5)	118.4	118.4	H-5	H-5	H-7	H-7
7	7.41 (1H, *d*, 15.5)	7.41 (1H, *d*, 15.5)	143.7	143.7	H-8	H-8	H-6	H-6
8	6.27 (1H, *d*, 15.5)	6.27 (1H, *d*, 15.5)	113.9	114.0	H-7	H-7	H-7	H-7
9	–	–	166.0	165.9			H-7, H-8, H-8′	H-7, H-8, H-8′
1′	–	–	127.1	127.2			H-2′, H-5′, H-8′, H-7′	H-2′, H-5′, H-8′, H-7′
2′	6.62 (1H, *d*, 2.0)	6.62 (1H, *d*, 2.0)	116.5	116.5	H-6′	H-6′	H-6′	H-6′, H-7′
3′	–	–	143.9	143.9			H-2′, H-5′	H-2′, H-5′, H-6′
4′	–	–	144.8	144.9			H-2′, H-5′, H-6′	H-2′, H-5′
5′	6.63 (1H, *d*, 8.0)	6.63 (1H, *d*, 8.0)	115.5	115.5	H-6′	H-6′	H-6′	
6′	6.47 (1H, *dd*, 8.0, 2.0)	6.45 (1H, *dd*, 8.0, 2.0)	120.0	120.1	H-2′, 5′	H-2′, 5′	H-2′, H-5′	H-2′, H-5′, H-7′
7′	2.85 (1H, *d*AB, 14.0, 8.0)	2.83 (1H, *d*AB, 14.0, 8.0)	36.0	36.1	H-8′	H-8′	H-2′, H-5′, H-6′, H-8′	H-2′, H-5′, H-6′, H-8′
	2.91 (1H, *d*AB, 14.0, 4.5)	2.91 (1H, *d*AB, 14.0, 4.5)						
8′	4.93 (1H, *dd*, 8.0, 4.5)	4.92 (1H, *dd*, 8.0, 4.5)	72.8	72.9	H-7′	H-7′	H-7′	H-7′
9′	–	–	170.6	170.6			H-7′, H-8′	H-7′, H-8′
1″	–	–	126.0	126.0			H-2″	H-5″
2″	6.44 (1H, *d*, 2.0)	6.43 (1H, *d*, 2.0)	117.3	117.3	H-6″	H-6″	H-6″, H-7″	H-6″, H-7″
3″	–	–	144.8	144.8			H-2″, H-5″	H-2″, H-5″
4″	–	–	147.2	147.2			H-2″, H-5″, H-6″	H-2″, H-5″, H-6″
5″	6.55 (1H, *d*, 8.5)	6.55 (1H, *d*, 9.0)	115.3	115.3	H-6″	H-6″		
6″	6.43 (1H, *dd*, 8.5, 2.0)	6.43 (1H, *dd*, 8.5, 2.0)	122.9	122.9	H-2″, 5″	H-2″, 5″	H-2″, H-7″	H-2″, H-7″
7″	7.69 (1H, *s*)	7.69 (1H, *s*)	141.1	141.1			H-6″	H-2″, H-6″
8″	–	–	123.4	123.3			H-7″	
9″	–	–	168.4	168.4			H-7″	H-7″
−OH	8.63–9.93 (6-OH)	8.64–9.91 (6-OH)						
−COOH	12.53 (2-COOH)	12.46 (2-COOH)						

volume of ethyl acetate and the combined extracts after drying under N_2 at 40 °C, the residue was dissolved in ethanol, high speed centrifugation for 3 min (12000 r·min^{-1}), leaving the supernatant for analysis.

2.6. Analysis of chiral hydrolysis degradation fragments I

Preparation of reference solution: weigh *R*/*S*-salvianic acid A reference, dissolved with ethanol and diluted to the appropriate concentration. Chromatographic conditions: use Chiralcel OD-H (4.6 mm × 250 mm, 5 μm) as the stationary phase and hexane–ethanol–trifluoroacetic acid (88:12:0.5, *v*/*v*) as mobile phase; Flow rate: 0.5 ml·min^{-1}; The column temperature is 25 °C; accurately inject 10 μL of salvianic acid A *R*-reference, *R*/*S*-salvianic acid A reference and degraded fragment I solution.

3. Results and discussion

Salvianolic acid T (**1**) was isolated as yellowish amorphous powder and showed $[\alpha]_D^{25}$ + 196.6 (*c* = 0.25, MeOH), it was recognized as a phenolic acid from a positive test with ferric trichloride and bromocresol green. It possessed a molecular formula of $C_{27}H_{22}O_{12}$ as evidenced by its negative high resolution-electrospray ionization (HR-ESI)-MS at *m*/*z* 537.1027 $[M–H]^-$ (Calcd for $C_{27}H_{21}O_{12}^-$, 537.1038, errors in 2.0 ppm). The IR absorptions showed the presence of hydroxyl groups (3150 cm^{-1}) and aromatic rings (1600, 1519, and 1445 cm^{-1}), and UV maxima at 220, 326 nm suggested the presence of a highly conjugated system. The ^{1}H NMR spectrum of **1** showed the presence of two 1,2,4-trisubstituted aromatic rings [δ 6.62 (1H, *d*, *J* = 2.0 Hz), 6.47 (1H, *dd*, *J* = 8.0, 2.0 Hz), and 6.63 (1H, *d*, *J* = 8.0 Hz); δ 6.44 (1H, *d*, *J* = 2.0 Hz), 6.43 (1H, *dd*, *J* = 8.5, 2.0 Hz), and 6.55 (1H, *d*, *J* = 8.5 Hz)], one 1,2,3,4-tetrasubstituted aromatic ring [δ 6.85 (1H, *d*, *J* = 8.5 Hz), 7.31 (1H, *d*, *J* = 8.5 Hz)], one set of *trans* olefinic proton [δ 7.41 (1H, *d*, *J* = 15.5 Hz), 6.27 (1H, *d*, *J* = 15.5 Hz)], one set of trisubstituted olefinic proton [δ 7.69 (1H, *s*)], and one set of ABX resonance system [δ 2.85 (1H, *dAB*, *J* = 14.0, 8.0 Hz), 2.91 (1H, *dAB*, *J* = 14.0, 4.5 Hz), and 4.93 (1H, *dd*, *J* = 8.0, 4.5 Hz)].

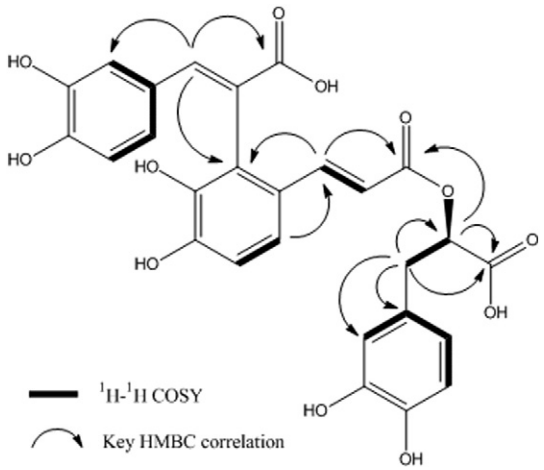

Fig. 2. Key ^{1}H–^{1}H COSY and HMBC correlations for compounds **1** and **2**.

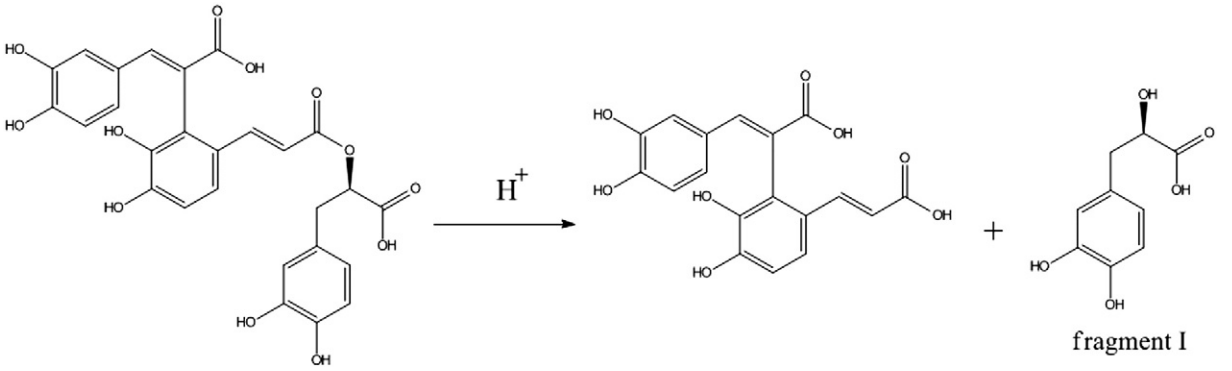

Fig. 3. The hydrolysis reaction of compounds **1** and **2**.

Above information suggested that compound **1** was a trimeric caffeic acid derivative. Further, HMBC experiment showed that the H-7″ (δ_H 7.69) was long range coupled to C-9″ (δ_C 168.4), thus showing that the carboxyl group was attached to C-8″. It has been reported that the olefinic proton signal in the ^{1}H NMR of carboxystilbene model compounds is more deshielded in the *Z*-configuration [8]. The ^{1}H–^{1}H COSY spectrum permitted the assignment of the aliphatic and aromatic protons and displayed the following connectivities: H-7′ (δ_H 2.91)/H-8′ (δ_H 4.93), and H-5 (δ_H 6.85)/H-6 (δ_H 7.31), and H-5′ (δ_H 6.63)/H-6′ (δ_H 6.47)/H-2′ (δ_H 6.62), and H-5″ (δ_H 6.55)/H-6″ (δ_H 6.43)/H-2″ (δ_H 6.44). The analysis of NMR data showed compound **1** was the same with salvianolic acid E excepted substituent of C_8″-COOH [9], thus the structural compound **1** was determined as shown in Fig. 2 and proton and carbon signals had been totally assigned on the basis of 2D NMR analysis (Table 1).

On the basis of above evidences, the structure of **1** was identified as (2*R*)-3-(3,4-dihydroxyphenyl)-2-[(*E*)-3-[(*E*)-1-carboxy-2-(3,4-dihydroxyphenyl)ethenyl-3,4-dihydroxyphenyl]prop-2-enoyl]oxypropanoic acid.

Salvianolic acid U (**2**) possessed a molecular formula of $C_{27}H_{22}O_{12}$, as evidenced by its negative high resolution-electrospray ionization (HR-ESI)-MS at *m/z* 537.1034 $[M-H]^-$ (Calcd for $C_{27}H_{21}O_{12}^-$, 537.1038, errors in 0.7 ppm). The data of ^{1}H and ^{13}C NMR were the same with compound **1**, further analysis of the 2D NMR spectroscopic data (^{1}H–^{1}H COSY, HMQC, and HMBC spectra) indicated that both compounds **1** and **2** had the same molecular structure (Table 1, Fig. 2). Nonetheless, the rotational direction and CD spectra of the two timers were different.

The hydrolysis reaction of salvianolic acid T and U indicated that the chiral center (C_8′) in degradation fragments of their hydrolysis products was identical to *R*-configuration (Fig. 3). But their experimental CD confused us for they are almost mirrored while they have the same chiral centers. The dilemma phenomena suggested that another chiral factor may existed.

The structure was rechecked and found that the chiral axis maybe a chiral factor. So we turn to the conformation search for help [10]. The dihedral angle scanning result indicated that two conformations existed (Table 2, Fig. **4**). Conformation A with dihedral angle C_7″–C_8″–C_2–C_1 being 65.9° was the most stable conformation, conformation C with C_7″–C_8″–C_2–C_1being −66.1° was the other stable conformation which has an energy difference of 0.53 KJ/mol to conformation A. With the scanning results, we found that there are rotational energy barrier of 121.39 and 130.24 KJ/mol from two sides. The rotational energy barriers arose from the steric bulk of substituents in the *o*-positions and the intramolecular hydrogen bonding, which were sufficiently high to prevent the interconversion of the two conformations at room temperature. Furthermore, intramolecular hydrogen bonding is another interaction to form the two conformations. As a result, conformations A and C could coexist stably at room temperature, which was in agreement with the hydrolyzed products. With this in hand, theoretical calculations of ECD spectra for conformations A and C were performed with the Gaussian 09 program package [11]. Geometry for them was optimized at the B3LYP/6-31++G (d, p) level of theory. The ECD calculations of compounds **1** and **2** were performed with DFT calculations at the BPV86/6-31++G (d, p) level of theory with UV correction. By comparing with the experimental ECD of **1** and **2** (Fig. 5), the absolute configurations of **1** and **2** were assigned as suggested by the structures of conformations A and C, respectively. At last, the structure of compound **1** was identified as (*P*, *R*)-3-(3,4-dihydroxyphenyl)-2-[(*E*)-3-[(*E*)-1-carboxy-2-(3,4-dihydroxyphenyl)ethenyl-3,4-ihydroxyphenyl]prop-2-enoyl]oxypropanoic acid, named as salvianolic acid T, and compound **2** was identified as (*M*, *R*)-3-(3,4-dihydroxyphenyl)-

Table 2
Energy of rotation around the C_2-C_8″ bond (conformations **A**–**D**).

No.	Dihedral angle	Energy	KJ/mol	Boltzmann distribution
Conformation **A**	65.9	−1933.7373	0.00	0.56
Conformation **B**	−164.1	−1933.6911	121.39	0
Conformation **C**	−66.1	−1933.7371	0.53	0.44
Conformation **D**	17.9	−1933.6877	130.24	0

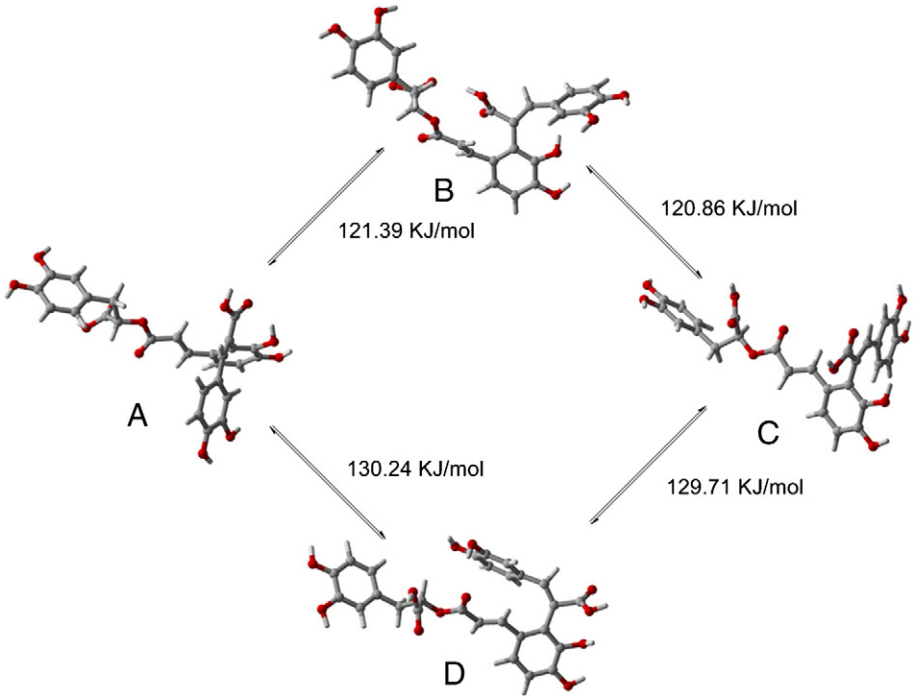

Fig. 4. Optimized geometric structures of rotation around the $C_2 - C_8''$ bond (A–D) and their rotational energy barriers (in KJ/mol).

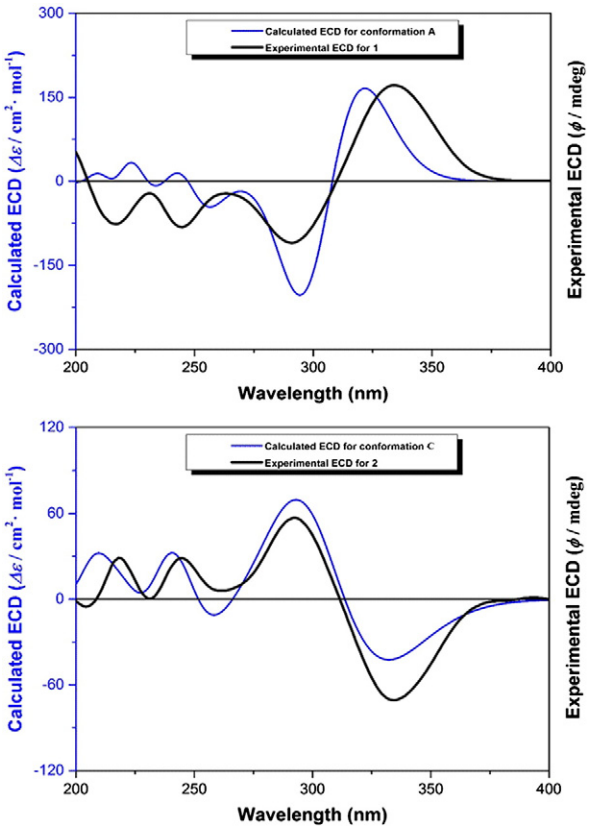

Fig. 5. Theoretical calculated (blue) ECD of conformation band experimentally observed (black) ECD of compounds **1** and **2**.

2-[(*E*)-3-[(*E*)-1-carboxy-2-(3,4-dihydrophenyl) ethenyl-3,4-ihydroxyphenyl]prop-2-enoyl]oxypropanoic acid, named as salvianolic acid U.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.fitote.2014.08.018.

References

[1] Lei XL, Chiou GC. Cardiovascular pharmacology of *Panax notoginseng* (Burk) F.H. Chen and *Salvia miltiorrhiza*. Am J Chin Med 1986;14:145–52.
[2] Lu Y, Foo LY. Polyphenolics of Salvia—a review. Phytochemistry 2002;59:117–40.
[3] Wu YJ, Hong CY, Lin SJ, Wu P, Shiao MS. Increase of vitamin E content in LDL and reduction of atherosclerosis in cholesterol-fed rabbits by a water-soluble antioxidant-rich fraction of *Salvia miltiorrhiza*. Arterioscler Thromb Vasc Biol 1998;18:481–6.
[4] Yagi A, Fujimoto K, Tanonaka K, Hirai K, Takeo S. Possible active components of tan-shen (*Salvia miltiorrhiza*) for protection of the myocardium against ischemia-induced derangements. Planta Med 1989;55:51–4.
[5] Sugiyama A, Zhu BM, Takahara A, Satoh Y, Hashimoto K. Cardiac effects of *Salvia miltiorrhiza*/*Dalbergia odorifera* mixture, an intravenously applicable Chinese medicine widely used for patients with ischemic heart disease in China. Circ J 2002;66:182–4.
[6] Nan JX, Park EJ, Kang HC, Park PH, Kim JY, Sohn DH. Anti-fibrotic effects of a hot-water extract from *Salvia miltiorrhiza* roots on liver fibrosis induced by biliary obstruction in rats. J Pharm Pharmacol 2001;53:197–204.
[7] Datta P, Dasgupta A. Effect of Chinese medicines Chan Su and Danshen on EMIT 2000 and Randox digoxin immunoassays: wide variation in digoxin-

like immunoreactivity and magnitude of interference in digoxin measurement by different brands of the same product. Ther Drug Monit 2002; 24:637–44.
[8] Li, S., Yuan, W., Ma, S., Highly regio- and stereoselective three-component nickel-catalyzed syn-hydrocarboxylation of alkynes with diethyl zinc and carbon dioxide, Angewandte Chemie (International ed. 50 2578–82
[9] Ai CB, Li LN. Salvianolic acids D and E: two new depsides from *Salvia miltiorrhiza*. Planta Med 1992;58:197–9.
[10] Berova N, Di Bari L, Pescitelli G. Application of electronic circular dichroism in configurational and conformational analysis of organic compounds. Chem Soc Rev 2007;36:914–31.
[11] GAUSSIAN. In: Frisch MJ TG, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Scalmani G, Barone V, Mennucci B, Petersson GA, Nakatsuji H, Caricato M, Li X, Hratchian HP, Izmaylov AF, Bloino J, Zheng G, Sonnenberg JL, Hada M, Ehara M, Toyota K, Fukuda R, Hasegawa J, Ishida M, Nakajima T, Honda Y, Kitao O, Nakai H, Vreven T, JAJr Montgomery, Peralta JE, Ogliaro F, Bearpark M, Heyd JJ, Brothers E, Kudin KN, Staroverov VN, Kobayashi R, Normand J, Raghavachari K, Rendell A, Burant JC, Iyengar SS, Tomasi J, Cossi M, Rega N, Millam JM, Klene M, Knox JE, Cross JB, Bakken V, Adamo C, Jaramillo J, Gomperts R, Stratmann RE, Yazyev O, Austin AJ, Cammi R, Pomelli C, Ochterski JW, Martin RL, Morokuma K, Zakrzewski VG, Voth GA, Salvador P, Dannenberg JJ, Dapprich S, Daniels AD, Farkas O, Foresman JB, Ortiz JV, Cioslowski J, Fox DJ, editors. 09. Wallingford CT: Gaussian Inc.; 2010.