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Cytotoxic and aromatic constituents from *Salvia miltiorrhiza*

Ming-Jaw Don [a], Chien-Chang Shen [a,b], Wan-Jr Syu [c], Yi-Huei Ding [a], Chang-Ming Sun [a,b,*]

[a] *National Research Institute of Chinese Medicine, No 155-1, Section 2, Li-Nong Street, Shih-Pai, Taipei 112, Taiwan*
[b] *Department of Biochemistry, National Yang-Ming University, Taipei 112, Taiwan*
[c] *Institute of Microbiology and Immunology, National Yang-Ming University, Taipei 112, Taiwan*

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Abstract

As part of an ongoing study of traditional Chinese medicinal plants, the root tissue of *Salvia miltiorrhiza* was further investigated for its chemical constituents. Five naturally occurring products along with 13 known constituents were isolated from an ethyl acetate-soluble portion of its ethanol extract. Their structures were elucidated by means of spectroscopic methods. Some selected compounds were also evaluated for biological activity.

Keywords: *Salvia miltiorrhiza*; Labiatae; Danshen; Cytotoxicity

1. Introduction

The traditional Chinese medicine *Salvia miltiorrhiza* Bunge (Labiatae) has drawn attention by natural product chemists and medicinal clinicians as it has been used for treatment of menstrual disorder, menostasis, menorrhalgia, insomnia, arthritis, and coronary heart diseases, particularly angina pectoris and myocardial infarction (Jiangsu New Medical College, 1988; Chen, 1984; Chang and But, 2001). Numerous diterpenoid tanshinones have also been isolated from *S. miltiorrhiza* (Kakisawa et al., 1969; Chang et al., 1990) and many of them were shown to have various biological activities including antitumor (Chang and But, 2001; Ryu et al., 1997b; Yang et al., 1981; Wu et al., 1991) and antimicrobial (Honda et al., 1988; Gao et al., 1979) activities. Previously, we reported a novel compound with antitumor activity from *S. miltiorrhiza* (Wang et al., 2004). In the present paper, we describe the isolation and structural determination of several natural products from the EtOAc fraction of the ethanolic extract of this plant. Some selected compounds have also been evaluated for their biological activity.

2. Results and discussion

The further chemical investigation of *S. miltiorrhiza* was focused on the ethyl acetate-soluble portion of an ethanolic extract of the dried roots. Further fractionation by repeated column chromatography of the EtOAc extract resulted in the isolation of 18 components including five new naturally occurring products (**1**–**5**) along with 13 known compounds.

Compound **1** was obtained as a yellow oil with a molecular formula of $C_{18}H_{20}O_4$ as determined by HREIMS. The ^{1}H NMR spectrum of **1** showed five aromatic protons at δ 8.33, 7.45, 7.40, 7.67, and 7.39, one aliphatic methine signal, two methylene groups (one oxygenated), and three methyl protons at δ 2.65, 2.02, and 1.07 (Table 1). Furthermore, one OH group signal with intramolecular hydrogen bonding was observed at δ 14.00. The ^{13}C NMR and DEPT spectra of **1** indicated the presence of 18 carbon

* Corresponding author. Tel.: +886 2 28201999; fax: +886 2 28264276.
E-mail address: cmsun@nricm.edu.tw (C.-M. Sun).

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Table 1
^{1}H NMR (500 MHz) spectroscopic data of **1**–**5** in $CDCl_3$

Position	$\delta(J)$[a]				
	1	**2**	**3**	**4**	**5**
1	8.33 *d* (7.5)	8.30 *m*	ax 1.54 *t* (11.5) eq 2.53 *m*		2.28 *s*
2	7.40 *t* (7.5)	7.45–7.46 *m*	5.17 *m*	2.90 *t* (7.0)	
3	7.45 *d* (7.5)	7.45–7.46 *m*	eq 1.86 *dd* (3.5, 13.5) ax 1.33 *t* (13.5)	2.03 *t* (7.0)	6.56 *d* (16.0)
4					7.19 *d* (16.0)
5			1.86 *dd* (3.5, 13.5)		
6	7.39 *d* (9.0)	7.78 *d* (9.0)	ax 2.58 *dd* (13.5, 18.0) eq 2.68 *dd* (3.5, 18.0)	7.43 *d* (9.0)	6.59 *d* (3.0)
7	7.67 *d* (9.0)	7.95 *d* (9.0)		7.95 *d* (9.0)	6.35 *d* (3.0)
9					4.61 *s*
11			6.71 *s*		
12	a 2.89 *dd* (8.0, 16.0) b 3.18 *dd* (6.0, 16.0)				
13	2.62 *m*	3.37 *m*			
14	a 4.01 *dd* (6.5, 11.0) b 4.08 *dd* (5.5, 11.0)	a 4.47 *dd* (1.0, 11.5) b 4.76 *dd* (3.5, 11.5)	7.90 *s*	7.25 *s*	
15			3.18 *m*	3.13 sept (7.0)	
16	2.02 *s*		1.20 *d* (7.0)[b]	1.27 *d* (7.0)	
17	1.07 *d* (6.5)	1.42 *d* (7.5)	1.21 *d* (7.0)[b]	1.27 *d* (7.0)	
18	2.65 *s*	2.62 *s*	0.97 *s*	1.42 *s*	
19			1.04 *s*	1.42 *s*	
20			1.26 *s*		
22			2.05 *s*		
Palmitate					
2				2.67 *t* (7.5)	
3				1.83 *m*	
16				0.86 *t* (7.0)	

[a] J = Coupling constant in Hertz.
[b] Interchangeable.

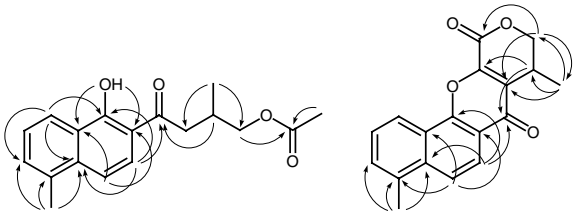
signals (Table 2), which were assigned to three methyl, two methylene (one oxygenated), one saturated methine, one ketone (δ 205.1), one ester (δ 171.0), and 10 aromatic (five protonated and five quaternary) carbons. The connectivities of the ^{1}H and ^{13}C signals were determined by analysis of its HMQC spectrum, whereas 2D COSY correlations (H-1/H-2; H-6/H-7) and HMBC correlations (H-1/C-3, C-5, C-9; H-18/C-3, C-4, C-5; H-6/C-8, C-10; H-7/C-5, C-9) (Fig. 1) revealed the presence of a naphthalene ring. In addition, 2D COSY correlations (H-13/H-12, H-14, H-17) and HMBC correlations (H-13/C-11; H-14/C-15; H-16/C-15) suggested the presence of a 4-acetoxy-3-methylbutanoyl moiety. The moiety was also supported from analysis of the EIMS data which showed a molecular ion peak at m/z 300 and major fragment peaks at m/z 240, 225 and 185, suggesting $[M-CH_3COOH]^+$, $[M-CH_3COOH-CH_3]^+$ and $[M-CH_3CO_2C_4H_8]^+$, respectively. Furthermore, the IR spectrum exhibited one carbonyl absorption of a saturated aliphatic ester at 1739 $cm^{-1}$and the other carbonyl group with intramolecular hydrogen bonding and conjugation to an aromatic ring at 1623 cm^{-1}. The HMBC correlations of the OH group (δ_H 14.00) with C-8 and C-10 and H-7 with C-11 (Fig. 1) indicated that the 4-acetoxy-3-methylbutanoyl and hydroxyl groups were located on C-8 and C-9, respectively. The structure of **1** was therefore assigned as 4-(1-hydroxy-5-methylnaphthalen-2-yl)-2-methyl-4-oxobutyl acetate and compound **1** was given the trivial name salvianonol.

Compound **2** was obtained as orange crystals with a molecular formula of $C_{18}H_{14}O_4$ as determined by HRE-IMS. The ^{1}H NMR spectrum exhibited five aromatic protons at δ 8.30, 7.95, 7.78, and 7.45–7.46, one aliphatic methylene group at δ 4.47 and 4.76, one aliphatic methine proton at δ 3.37, and two methyl signals at δ 2.62 and 1.42 (Table 1). The ^{13}C NMR and DEPT spectra of **2** showed 18 carbon signals (Table 2), which were assigned to two methyl, one oxygenated methylene, one saturated methine, five protonated aromatic, and nine sp^2 quaternary carbons. The connectivities of the ^{1}H and ^{13}C signals were determined by analysis of its HMQC spectrum. Analysis of the COSY, HMQC and HMBC data indicated that **2** was partially similar to **1** having a naphthalene ring. The COSY spectrum showed that the methine proton (H-13) was correlated with the methylene group (H-14) and the methyl protons (H-17). In the HMBC spectrum, the correlations (H-17/C-12, C-14; H-13/C-11, C-16; H-14/C-12, C-15; H-7/C-9, C-11) were observed (Fig. 1), which suggested that a 5,6-dihydropyran-2-one moiety was fused to the bezochromen-4-one ring. In addition, the IR spectrum of **2**

Table 2
^{13}C NMR spectroscopic data of **1**–**8** in $CDCl_3$

Position	1	2	3	4	5	6	7	8
1	122.5	120.8	42.8	205.0	28.1	125.6	125.4	24.9
2	125.7	127.1	68.7	36.5	198.4	130.1	130.3	22.5
3	130.9	130.8	46.2	35.4	124.3	129.0	129.1	128.6
4	134.0	134.7	34.6	36.2	129.7	134.8	135.1[a]	131.0
5	136.4	135.0	48.9	157.6	150.9	135.6	135.0[a]	139.1
6	114.7	122.2	35.6	122.9	117.0	131.5	132.3	128.2
7	123.9	119.5	198.4	137.5	110.6	121.7	122.5	120.7
8	112.8	119.6	123.5	132.2	157.3	133.6	133.8	127.3
9	163.0	153.6	155.0	121.4	57.7	126.3	124.0	126.2
10	125.5	123.8	39.3	127.7		130.7	130.3	144.5
11	205.1	175.9	109.5	145.1		181.2	184.1	184.4
12	42.2	131.1	159.5	138.1		160.9	153.0	176.3
13	29.7	26.3	133.7	141.7		124.9	125.5	120.2
14	68.6	73.1	126.9	117.3		182.7	185.2	161.7
15	171.0	158.6	26.7	28.0		35.6	24.5	121.2
16	20.8	144.2	22.1[a]	22.8		80.4	20.0	141.3
17	17.1	16.5	22.4[a]	22.8		18.9	20.0	8.8
18	19.5	19.4	32.4	29.6		20.0	19.9	19.8
19			22.0	29.6				
20			23.9					
21			171.1					
22			21.4					
Palmitate								
1				171.9				
2				34.2				
3				25.1				
16				14.1				

[a] Interchangeable.



1 **2**

Fig. 1. Selected HMBC correlations of **1** and **2**.

showed the presence of a lactone (1745 cm^{-1}) and a conjugated ketone (1644 cm^{-1}) absorptions. The structure of **2** was therefore assigned as 4,8-dimethyl-8,9-dihydro-10, 12-dioxa-benzo[*a*]anthracene-7,11-dione and compound **2** was given the trivial name salviamone.

Compound **3** was obtained as an orange oil with a molecular formula of $C_{22}H_{30}O_4$ as determined by HREIMS. The ^{13}C NMR and DEPT spectra of **3** exhibited 22 carbon signals (Table 2), which were assigned to six methyl, three methylene, three aliphatic methine, two aliphatic quaternary, two carbonyl (δ 171.1 and 198.4), and six aromatic (two protonated and four quaternary) carbons. The ^{1}H NMR spectrum exhibited two aromatic protons (δ 6.71 and 7.90), two doublets of two methyl signals (δ 1.20 and 1.21), four singlets of four methyl groups, three aliphatic methine protons, and three methylene signals, which was partially similar to those of 2α-hydroxysugiol (Gonzàlez et al., 1988). The HMBC spectrum showed correlations of the carbonyl group (δ_C 171.1) with the methine (H-2, δ_H 5.17) and methyl groups (Me-22, δ_H 2.05), which indicated that an acetoxyl group was located on C-2. The relative stereochemistry of the acetoxyl group was determined on the basis of a NOESY experiment, which showed correlations of H-2 with Me-19 and Me-20 and suggested that the acetoxyl group was located in an equatorial orientation at C-2. In addition, the IR spectrum showed the presence of a hydroxyl group at 3286 cm^{-1} and the carbonyl groups of saturated aliphatic ester and conjugated ketone at 1733 and 1652 cm^{-1}, respectively. Thus, compound **3** was established as 2α-acetoxysugiol.

Compound **4** was obtained as a yellow oil with a molecular formula of $C_{35}H_{52}O_4$ as determined by HREIMS. The EIMS of **4** gave a molecular ion peak at *m*/*z* 536 and a major fragment peak at *m*/*z* 298, suggesting a [M−palmitoyl moiety + H]$^+$. In the ^{1}H NMR spectrum, the palmitoyl signals were observed at δ 0.86, 1.42, 1.83, and 2.67. The presence of the palmitoyl moiety was further supported by analysis of the ^{13}C NMR spectrum and comparison of the NMR spectroscopic data with those of methyl palmitate (Vandevoorde et al., 2003). In additional to the palmitoyl signals, the ^{1}H NMR spectroscopic data (Table 1) showed an AB pattern for two *ortho*-aromatic protons at δ 7.43 and 7.95, one aromatic signal as a singlet at δ 7.25, a geminal dimethyl group at δ 1.42 (6H), two methylene groups at δ 2.03 and 2.90, and an isopropyl group at δ 1.27 (6H) and 3.13 (1H), which were similar to those of arucadiol (Majetich et al., 1997). The IR spectrum of **4** indicated presence of a carbonyl stretch of an ester at higher frequency at 1762 cm^{-1}, suggesting the presence of aromatic group conjugation with an alcohol (OH) moiety. In addition, a carbonyl absorption was observed at lower frequency at 1642 cm^{-1}, due to the effects of conjugation with the aromatic ring and intramolecular hydrogen bonding. Furthermore, an OH signal with intramolecular hydrogen bonding (δ_H 10.55) suggested that an hydroxyl group was located on C-11, which was confirmed by its HMBC correlations with C-9, C-11 and C-12. Thus, compound **4** was established as palmitoyl arucadiol.

Compound **5** was obtained as a yellow oil with a molecular formula of $C_9H_{10}O_3$ as determined by HREIMS. The EIMS of **5** gave a molecular ion peak at *m*/*z* 166 and major fragment peaks at *m*/*z* 135, suggesting [M−CH_3O]$^+$. The ^{1}H NMR spectrum exhibited two *trans*-olefinic protons at δ 7.19 and 6.56, two aromatic signals at δ 6.59 and 6.35, one oxygenated methylene group as a singlet at δ 4.61, and one methyl singlet at δ 2.28 (Table 1). The ^{13}C NMR and DEPT spectra of **5** showed nine carbon signals (Table 2), which were assigned to one methyl, one oxygenated methylene, four sp^2 methine, and two oxygenated quaternary sp^2 carbons. The connectivities of H and C were determined by analysis of an HMQC experiment. The HMBC data revealed the correlations of H-4 with C-2, -3, -5, and -6 and H-9 with C-7 and -8, indicating that **5** is a 2,5 disubstituted furan. The structure of **5** was therefore

assigned as (*E*)-4-[5-(hydroxymethyl)furan-2-yl]but-3-en-2-one.

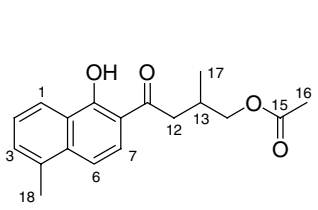
Thirteen known compounds were also identified as dihydroisotanshinone I (**6**) (Kong and Liu, 1984), danshenxinkun B (**7**) (Luo et al., 1994), 1,2-dihydrotanshinone I (**8**) (Feng and Li, 1980), tanshinone I (Ryu et al., 1997a), tanshinone IIA (Ryu et al., 1997a), methylenetanshinquinone (Luo et al., 1994), 15,16-dihydrotanshinone I (Ikeshiro et al., 1991), cryptotanshinone (An et al., 2002), ferruginol (Harrison and Asakawa, 1987), sugiol (Gao and Han, 1997), norsavioxide (Li et al., 1991), and a mixture of danshenspiroketallactone and its *epi*-isomer (Asari et al., 1990) by analysis of their MS, 1D and 2D NMR spectroscopic data and by comparison with those in the literature; however, ^{13}C NMR spectroscopic data of compounds **6**–**8** have not been previously reported and are reported herein.

Compounds **1**, **2**, and **6**–**8** might be biosynthesized from abietic acid (**9**) (Scheme 1). Abietic acid may firstly lead to **7** and further to danshenxinkun A (**10**) via several steps of oxidation. Dehydration of **10** potentially affords **6** and dihydrotanshinone I (**11**), of which the latter might lead to **8** by hydrogenation and dehydrogenation. After Baeyer–Villiger oxidation, compound **10** is envisaged to produce a seven-membered lactone intermediate **12**, which is then hydrolyzed, followed by esterification and dehydration with ring closure to result in compound **2**. Alternatively, the lactone intermediate **12** can be followed in turn by hydrogenation, dehydration, retro-aldol reaction, hydration, hydrolysis and acetylation to afford compound **1**.

Compounds **1**–**4** and **7** were evaluated for their cytotoxicity against selected cancer cell lines by using MTT method and cisplatin as positive control. Among the tested compounds, **4** was the most potent compound with CD_{50} values of 3.2 and 4.1 μg/ml against the HeLa and OVCAR-3 cells, respectively, which had slightly lower CD_{50} values than cisplatin (Table 3). The cytotoxicities of compounds **1**–**4** and **7** against the above cancer cell lines have not been reported so far. In addition, compounds **1**–**4** were subjected to evaluation of antibacterial activity against Gram-positive *Staphylococcus aureus* and *Enterococcus faecalis*, and Gram-negative *Escherichia coli*, using paper disk methods. However, all tested compounds were considered inactive (inhibition zone <10 mm/100 μg/disk).

3. Conclusions

So far about 50 abietanoids and diterpenoid tanshinones have been identified from the root of *S. miltiorrhiza*. The occurrence of tanshinones, however, can be used as a taxonomic characteristic for the genus *Salvia* (Patudin et al., 1974). Some *Salvia* species such as *Salvia yunnanensis* (Qian



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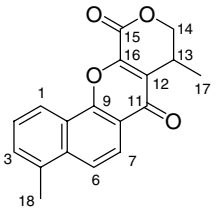

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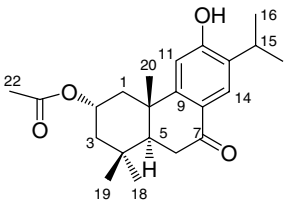

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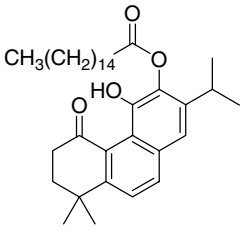

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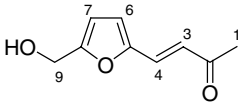

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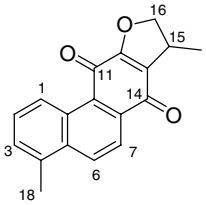

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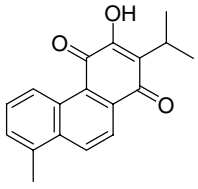

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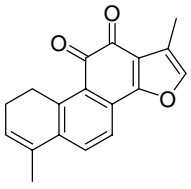

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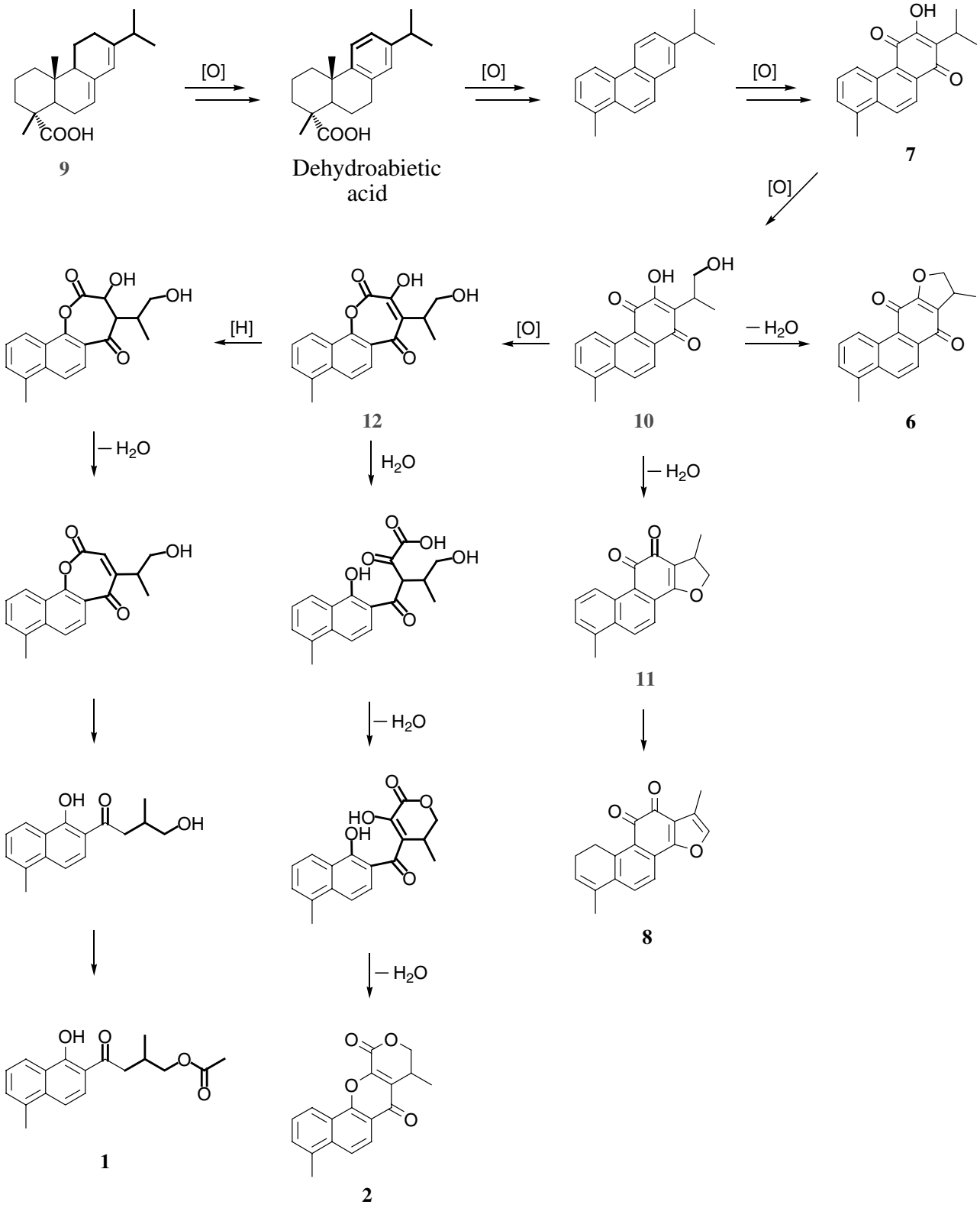

Scheme 1. Proposed biosynthetic pathways for **1**, **2**, **6**–**8** of *S. miltiorrhiza*.

et al., 2002; Yang et al., 1996), *Salvia przewalskii* (Li et al., 1991), and *Salvia paramiltiorrhiza* f. *purpureorubra* (Wang, 1981), which have been used as substitutes in Chinese folk medicine for Danshen, were also reported to contain the pharmacologically active tanshinones. We report here that five new natural products, one furan derivative and four

Table 3
Cytotoxicity of **1**–**4** and **7** against human cancer cell lines

Compound	CD_{50} (μg/ml)		
	HeLa	HepG2	OVCAR-3
1	17.4	37.5	>100
2	>100	>100	>100
3	25.5	37.5	30.2
4	3.2	25.1	4.1
7	40.5	34.5	33.5
Cisplatin	7.2	7.1	9.0

abietanoids, and 13 known diterpenoid tanshinones were isolated from an ethyl acetate-soluble portion of ethanol extract of *S. miltiorrhiza*. These new components could provide a support of the chemotaxonomic significance for the species of *S. miltiorrhiza*.

4. Experimental

4.1. General experimental procedures

Melting points were measured using a Yanaco MP-S9 micro-melting point apparatus and uncorrected. UV spectra were performed on a Hitachi U-3310 spectrophotometer. IR spectra were recorded on a Nicolet Avatar 320 FT-IR spectrometer. The ^1H and ^{13}C NMR spectra were recorded on a Varian Unity Inova 500 spectrometer in $CDCl_3$ with tetramethylsilane (TMS) as an internal standard. COSY, HMQC, HMBC, and DEPT spectra were obtained using standard Varian pulse sequences. EIMS spectra were measured with a direct insertion probe on a Finnigan GCQ spectrometer at 30 eV. HREIMS data were taken on a Finnigan MAT 95XL mass spectrometer. Silica gel (Kieselgel 60, 70–230 mesh, Macherey-Nagel) was used for column chromatography. TLC was carried out on aluminum sheets precoated with silica gel 60 F_{254} (layer thickness 0.2 mm, Merck Art. 5554). The chromatograms were visualized under UV light (254 or 365 nm) or by spraying with 5% phosphomolybdic acid in 5% H_2SO_4 containing a trace of ceric sulfate, followed by heating on a hot plate (120 °C).

4.2. Plant material

The dried and sliced roots of *S. miltiorrhiza* (5 kg) were purchased from the Cherng-Chi Chinese herbal shop in Taipei in April 2004. A voucher specimen (NRICM 04024) was deposited in the herbarium of National Research Institute of Chinese Medicine, Taipei.

4.3. Extraction and isolation

The root of *S. miltiorrhiza* was extracted with EtOH (30 l) three times at 60 °C for 24 h. The EtOH extracts were combined and concentrated in vacuo to 1 l. The concentrated extract was suspended in H_2O (4 l) and partitioned successively with EtOAc. After concentration of the EtOAc extract, the concentrate was mixed with 700 g of silica gel (230–400 mesh). The air-dried mixture was subjected to silica gel column chromatography (cc) using a mixture of hexane-EtOAc of increasing polarity as eluents. Fractions were collected, with similar fractions (monitored by TLC) combined to give 5 fractions (F-1 to F-5). F-1 was further applied to silica gel cc to give danshenxinkun B (**7**) (12 mg) and ferruginol (153 mg). F-2 was resubjected to silica gel cc to give tanshinone IIA (1.2 g), 1,2-dihydrodanshinone I (**8**) (35 mg), palmitoyl arucadiol (**4**) (10 mg), methylenetanshinquinone (63 mg), and tanshinone I (480 mg), respectively. F-3 was separated by further silica gel cc to yield dihydroisotanshinone I (**6**) (12 mg), sugiol (75 mg), norsalvioxide (18 mg), and a mixture of danshenspiroketallactone and *epi*-danshenspiroketallactone (30 mg). F-4 was reapplied to silica gel cc to afford salvianonol (**1**) (28 mg), cryptotanshinone (700 mg), 2α-acetoxysugiol (**3**) (41 mg), and 15,16-dihydrotanshinone I (175 mg). F-5 was also subjected to silica gel cc to give salviamone (**2**) (15 mg) and (*E*)-4-[5-(hydroxymethyl)furan-2-yl]but-3-en-2-one (**5**) (43 mg).

4.4. Salvianonol, 4-(1-hydroxy-5-methylnaphthalen-2-yl)-2-methyl-4-oxobutyl acetate (*1*)

Yellow oil; $[\alpha]_D^{25}$ + 30° ($CHCl_3$, *c* 0.1); UV (CH_3OH) λ_{max} (log ε) 215.0 (5.06), 256.8 (5.00), 371.4 (4.17) nm; IR (film) ν_{max} 2963, 2925, 2853, 1739, 1623, 1576, 1471, 1384, 1238, 1081, 1038, 796 cm^{-1}; for ^1H and ^{13}C NMR spectra, see Tables 1 and 2, respectively; HREIMS *m/z* 300.1363 (calcd. for $C_{18}H_{20}O_4$ 300.1356); EIMS *m/z* (rel. int.): 300 $[M]^+$ (80), 240 (67), 225 (100), 197 (8), 185 (33), 128 (10).

4.5. Salviamone, 4,8-dimethyl-8,9-dihydro-10,12-dioxabenzo[a]anthracene-7,11-dione (*2*)

Orange crystals, m.p. 203–204 °C, $[\alpha]_D^{25}$ − 35° ($CHCl_3$, *c* 0.2); UV (CH_3OH) λ_{max} (log ε) 228.8 (4.83), 275.0 (4.36), 360.4 (3.87) nm; IR (film) ν_{max} 2967, 2925, 1745, 1644, 1510, 1466, 1400, 1266, 1211, 1169, 1120, 772 cm^{-1}; For ^1H and ^{13}C NMR spectra, see Tables 1 and 2, respectively; HREIMS *m/z* 294.0887 (calcd. for $C_{18}H_{14}O_4$ 294.0887); EIMS *m/z* (rel. int.): 294 $[M]^+$ (100), 249 (72), 235 (12).

4.6. 2α-Acetoxysugiol, (3S,4aS,10aS)-1,2,3,4,4a,9,10,10a-octahydro-6-hydroxy-7-isopropyl-1,1,4a-trimethyl-9-oxophenanthren-3-yl acetate (*3*)

Orange oil; $[\alpha]_D^{25}$ − 16° ($CHCl_3$, *c* 1.0); UV (CH_3OH) λ_{max} (log ε) 205.2 (4.43), 232.2 (4.36), 282.2 (4.25) nm; IR (neat) ν_{max} 3286, 2963, 2925, 2870, 1733, 1652, 1596, 1464, 1366, 1268, 1179, 1129, 757 cm^{-1}; for ^1H and ^{13}C NMR spectra, see Tables 1 and 2, respectively; HREIMS *m/z* 358.2146 (calcd. for $C_{22}H_{30}O_4$ 358.2144); EIMS *m/z* (rel. int.): 358 $[M]^+$ (68), 298 (38), 283 (100), 241 (53).

4.7. Palmitoyl arucadiol, 1,2,3,4-tetrahydro-5-hydroxy-7-isopropyl-1,1-dimethyl-4-oxophenanthren-6-yl palmitate (**4**)

Yellow oil; UV (CH_3OH) λ_{max} (log ε) 216.8 (4.66), 271.6 (4.30) nm; IR (neat) ν_{max} 2959, 2925, 2854, 1762, 1642, 1594, 1465, 1412, 1282, 1136 cm^{-1}; for ^1H and ^{13}C NMR spectra, see Tables 1 and 2, respectively, ^{13}C NMR of palmitoyl moiety δ 14.11, 22.69, 25.11, 29.27, 29.34, 29.36, 29.52, 29.70, 31.92, 34.25, 171.90; HREIMS *m/z* 536.3870 (calcd. for $C_{35}H_{52}O_4$ 536.3866); EIMS *m/z* (rel. int.): 536 $[M]^+$ (3), 298 (100).

4.8. (*E*)-4-[5-(Hydroxymethyl)furan-2-yl]but-3-en-2-one (**5**)

Yellow oil; for ^1H and ^{13}C NMR spectra, see Tables 1 and 2, respectively; HREIMS *m/z* 166.0633 (calcd. for $C_9H_{10}O_3$ 166.0624); EIMS *m/z* (rel. int.): 166 $[M]^+$ (31), 151 (4), 135 (100), 67 (3).

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