Two New Fatty Diterpenoids from *Salvia miltiorrhiza*

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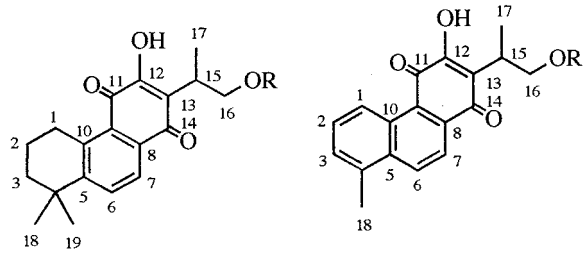
Two new fatty abietane diterpenoids, oleoyl neocryptotanshinone (**1**) and oleoyl danshenxinkun A (**2**), were isolated as minor components from the roots of *Salvia miltiorrhiza*. Their structures were established on the basis of spectral evidence. In preliminary tests, they selectively inhibited rabbit platelet aggregation induced by arachidonic acid.

Dan-Shen, the root of *Salvia miltiorrhiza* Bunge (Labiatae), is a traditional Chinese herb used in the treatment of cardiovascular diseases, particularly angina pectoris and myocardial infarction.[1] We previously reported that abietane tanshinones inhibited platelet aggregation[2,3] and showed significant cytotoxic activity against human cancer cell lines.[4−7] Recently, Wang et al. reported that three unsaturated fatty acids (linolenoic, linoleic, and oleic acids) isolated from Dan-Shen inhibited the amidolytic activity of soluble tissue factor/activated factor VII complex (TF/VIIa).[8] Due to our interest in exploring biologically active components from Dan-Shen, we have now investigated the nonpolar fraction of this plant and report herein the isolation and characterization of two new fatty diterpenoids, **1** and **2**.

The $CHCl_3$-soluble part of the EtOH extract of the root of *Salvia miltorrhiza* was subjected to silica gel chromatography and preparative TLC to isolate two fatty abietane tanshinones, oleoyl neocryptotanshinone (**1**) and oleoyl danshenxinkun A (**2**). This is the first report of the isolation of these fatty tanshinones.



1 R= Oleoyl
3 R= H

2 R= Oleoyl
4 R= H

Compound **1** was obtained as a yellowish oil with a molecular formula of $C_{37}H_{54}O_5$ (HREIMS), 264 amu more than that of neocryptotanshinone (**3**).[2] This additional molecular weight corresponds to an oleoyl group, as evidenced in the ^{1}H NMR spectrum of the oleoyl signals at δ 0.85 (t, J = 7.4 Hz, 2H), 1.20 (m, 4H), 1.50 (m, 2H), 1.98 (m, 4H), 2.20 (t, J = 7.5 Hz, 2H), and 5.31 (m, 2H). The presence of the oleoyl group was further supported by a COSY-45 spectrum and comparison of the NMR data with those of oleic acid. In the EIMS spectrum, a fragmentation peak at *m*/*z* 265, corresponding to $C_{17}H_{33}CO$, also indicated the presence of an oleoyl group. The base peak at *m*/*z* 296 ($C_{19}H_{20}O_3$, [M − oleic acid]$^+$) suggested the possible presence of a neocryptotanshinone moiety. The UV absorbances [248 (sh), 255, 278, 288 (sh), 355 nm] and the IR spectrum (1665, 1645, 1560 cm^{-1}) were similar to those of neocryptotanshinone.[2] In addition to the oleoyl signals, the ^{1}H NMR spectrum ($CDCl_3$) revealed an AB pattern for two *ortho*-aromatic proton signals at δ 7.74 (d, J = 8.1 Hz) and 7.98 (d, J = 8.1 Hz), a geminal dimethyl group at δ 1.30 (s, 6H), a methyl group (δ 1.26, d, J = 7.2 Hz), a methine proton at δ 3.54 (sextet, J = 7.2 Hz), and four methylene groups at δ 1.65 (m), 1.82 (m), 3.23 (t, J = 6.0 Hz), and 4.34 (d, J = 7.2 Hz, 2H), similar to those of neocryptotanshinone.[2] Analyses of COSY-45, HMQC, and HMBC data allowed for complete ^{1}H and ^{13}C NMR spectral assignments (Table 1) for the neocryptotanshinone moiety of **1**. The location of 16-*O*-oleoyl was made from the observation of the three-bond coupling of H-16 (δ 4.34, d) to C-1 (δ 173.70, s) of the oleoyl group (Figure 1). Hydrolysis of **1** with 1 N NaOH−EtOH afforded oleic acid and neocryptotanshinone, which were identified by comparison with authentic samples (TLC and EIMS). Thus, **1** is 16-*O*-oleoyl neocryptotanshinone.

Compound **2** was obtained as reddish oil with a molecular formula of $C_{36}H_{48}O_5$ (HREIMS), 264 amu (an oleoyl group) more than that of danshenxinkun A (**4**).[9] The signals for the oleoyl moiety in ^{1}H NMR are the same as those of **1**. In the EIMS spectrum, a fragmentation peak at *m*/*z* 265, corresponding to $C_{17}H_{33}CO$, indicated the presence of an oleoyl moiety, while a prominent peak at *m*/*z* 278 ($C_{18}H_{14}O_3$, [M − oleic acid]$^+$) suggested the presence of the danshenxinkun A moiety of **2**. The UV spectrum [285 (sh), 290, 335, 377 nm] and the IR spectrum (1665, 1646, 1584 cm^{-1}) were similar to those of danshenxinkun A.[9] Except for the oleoyl signals, the ^{1}H NMR spectrum ($CDCl_3$) of **2** revealed an ABX pattern for 1,2,3-aromatic protons at δ 9.41 (d, J = 9.0 Hz), 7.60 (dd, J = 9.0, 7.2 Hz), and 7.45 (d, J = 7.2 Hz), an AB pattern for *ortho*-aromatic protons at δ 8.25 (d, J = 8.6 Hz) and 8.41 (d, J = 8.6 Hz), a methyl group (δ 2.73, s), a methine proton at δ 3.60 (sextet, J = 7.2 Hz), and a methylene group at δ 4.38 (dd, J = 16.5, 7.2 Hz) and 4.40 (dd, J = 16.5, 7.2 Hz), similar to those of danshenxinkun A.[9] The full spectral assignments of ^{1}H and ^{13}C NMR of the danshexinkun A moiety of **2** were made by analyzing the COSY-45, HMQC, and HMBC data (Table 1). The location of 16-*O*-oleoyl was made from the observation of the three-bond coupling of H-16 [δ 4.38 (dd); 4.40 (dd)] to C-1 (δ 173.71, s) of the oleoyl group (Figure 1). Thus, **2** was identified as 16-*O*-oleoyl danshenxinkun A.

In preliminary tests, **1** and **2** selectively inhibit platelet aggregation (rabbit platelets) induced by arachidonic acid,

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Table 1. NMR Data for **1** and **2** in $CDCl_3$

	1		**2**	
position	^{13}C (mult.)[a]	^{1}H mult (*J* Hz)	^{13}C (mult.)[a]	^{1}H mult (*J* Hz)
1	29.94 t	3.23 t (6.0)	125.38 d	9.41 d (9.0)
2	19.08 t	1.82 m	130.50 d	7.60 dd (9.0, 7.2)
3	37.67 t	1.65 m	129.30 d	7.45 d (7.2)
4	34.80 s		135.20 s	
5	152.70 s		123.90 s	
6	133.50 d	7.74 d (8.1)	132.50 d	8.41 d (8.6)
7	125.00 d	7.98 d (8.1)	122.50 d	8.25 d (8.6)
8	132.50 s		133.60 s	
9	126.30 s		135.15 s	
10	140.90 s		130.40 s	
11	182.86 s		183.56 s	
12	153.90 s		153.80 s	
13	121.49 s		120.92 s	
14	184.20 s		184.84 s	
15	29.75 d	3.54 sextet (7.2)	29.75 d	3.60 sextet (7.2)
16	66.12 t	4.34 d (7.2)	66.16 t	4.38 dd (16.5, 7.2)
				4.40 dd (16.5, 7.2)
17	14.82 q	1.26 d (7.2)	14.91 q	1.32 d (7.2)
18	31.70 q	1.30 s	19.84 q	2.73 s
19	31.70 q	1.30 s		
12-OH		7.79 s		7.88 s
oleoyl-1[b]	173.70 s		173.71 s	
2	34.32 t	2.20 t (7.5)	34.35 t	2.21 t (7.5)
3	24.95 t	1.50 m	24.99 t	1.50 m
8	27.20 t	1.98 m	27.20 t	1.98 m
9	129.74 d	5.31 m	129.74 d	5.30 m
10	129.95 d	5.31 m	129.95 d	5.30 m
11	27.20 t	1.98 m	27.20 t	1.98 m
16	31.91 t	1.20 m	31.91 t	1.20 m
17	22.67 t	1.20 m	22.67 t	1.20 m
18	14.10 q	0.85 t (7.4)	14.10 q	0.85 t (7.4)

[a] Multiplicities were obtained from DEPT experiment. [b] Signals for other oleoyl groups: ^{1}H NMR δ 1.17−1.27 (H-4−H-7, H-12−H-15); ^{13}C NMR δ 29.05−29.6 (C-4−C-7, C-12−C-15).

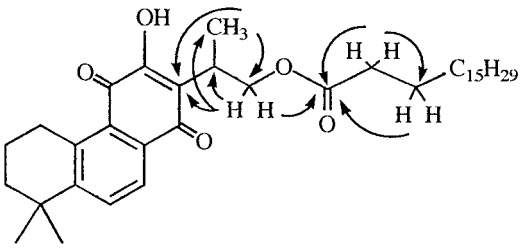

Figure 1. Partial HMBC correlations for **1**.

Table 2. Inhibitory Effects (IC_{50} (μM)) of **1** and **2** on Platelet Aggregation[a]

	stimulant		
compound	arachidonic acid (100 μM)	collagen (10 μg/mL)	thrombin (0.1 U/mL)
1	5.1 ± 0.8	50.4 ± 1.4	>100
2	25.5 ± 1.9	60.5 ± 2.6	>100
aspirin[b]	27.0 ± 1.1		

[a] Washed rabbit platelets were preincubated with **1** or **2** for 3 min, and then collagen (10 μg/mL), arachidonic acid (100 μM), or thrombin (0.1 U/mL) was added to trigger platelet aggregation. Values are presented as means ± SE (n = 3−5). [b] Aspirin was used as positive control.

with IC_{50} values of 5.1 and 25.5 μM, respectively. They are less active against collagen-induced platelet aggregation, with an IC_{50} of about 50−60 μM, and are inactive against thrombin-induced platelet aggregation (Table 2).

Experimental Section

General Experimental Procedures. Melting points are uncorrected. ^{1}H NMR (300 MHz, Bruker AM-300 NMR spectrometer), ^{13}C NMR (75 MHz, Bruker AM-300 NMR spectrometer), and 2D NMR (500 MHz, Bruker AM-500 NMR spectrometer): $CDCl_3$ using the solvent peak as internal standard. MS: direct inlet system. UV: Shimadzu UV-160, MeOH. IR: Perkin-Elmer 983 G, KBr disk.

Plant Material. "Dan-Shen" was supplied from Chien-Yuan Co., Taipei, and was identified by Prof. W. L. Wu of the National Defense Medical Center, where a voucher specimen was deposited.

Extraction and Isolation. The dried and powdered roots (45 Kg) of *Salvia miltiorrhiza* were extracted with 95% EtOH (150 L × 3) at room temperature. The combined extracts were concentrated in vacuo to yield a brown syrup (4.95 kg), which was partitioned between $CHCl_3$−H_2O (1:1). The concentrated $CHCl_3$ extract (1.13 kg) was subjected to chromatography over Si gel (70−230 mesh, 5 Kg) and eluted with *n*-hexane−$CHCl_3$ (1:1), $CHCl_3$, $CHCl_3$−Me_2CO (9:1), Me_2CO, and MeOH, successively. The first fraction was chromatographed over Si gel using *n*-hexane, *n*-hexane−$CHCl_3$ (9:1), *n*-hexane−$CHCl_3$ (4:1), and $CHCl_3$ as eluents. The subfraction between known danshexkun B and tanshinone I eluted with *n*-hexane−$CHCl_3$ (4:1) was rechromatographed over Si gel using *n*-hexane−$CHCl_3$ (4:1) as eluent to afford a yellow oil fraction (196 mg), which was further purified by preparative TLC (Si gel 60 F_{254}) using *n*-hexane−$CHCl_3$ (4:1) as the mobile phase to give oleoyl neocryptotanshinone (**1**, 26 mg; R_f = 0.20) and oleoyl danshenxinkun A (**2**, 20 mg; R_f = 0.26).

Oleoyl neocryptotanshinone (**1**): yellow oil ($CHCl_3$); $[\alpha]^{25}_D$ +14.3° (*c* 0.35, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 248 (sh, 4.15), 255 (4.20), 278 (4.06), 288 (sh, 4.03), 355 (3.47) nm; IR (KBr) ν_{max} 3340, 2965, 1665, 1645, 1560, 1390, 1320, 1280, 1270, 1140, 1090, 1080, 860, 760 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$), see Table 1; EIMS *m/z* 578 $[M]^+$ (0.4), 410 (2), 314 (10), 312 (15), 296 (100), 282 (20), 281 (40), 267 (40), 265 (10), 253 (70), 235 (15), 179 (10), 165 (15), 129 (15), 73 (20), 55 (25); HREIMS *m/z* 578.4011 (calcd for $C_{37}H_{54}O_5$, 578.3970).

Oleoyl danshenxinkun A (**2**): reddish oil; $[\alpha]^{25}_D$ −78.8° (*c* 0.25, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ): 285 (4.35, sh), 290 (4.40), 335 (3.78), 377 (3.50) nm; IR (KBr) ν_{max} 3360, 2918, 2851, 1737, 1665, 1646, 1584, 1462, 1462, 1354, 1319, 1196,

1173, 848, 786, 764 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$), see Table 1; EIMS *m*/*z* 560 $[M]^+$ (2), 296 (20), 278 (85), 265 (10), 250 (75), 235 (100), 222 (10), 207 (15), 179 (25), 169 (10), 139 (30), 129 (25), 97 (20), 73 (75), 69 (50); HREIMS *m*/*z* 560.3612 (calcd for $C_{36}H_{48}O_5$, 560.3501).

Hydrolysis of Oleoyl Neocryptotanshinone (1). 1 (5 mg) was added to a 0.5 mL solution of 1 N NaOH−EtOH and allowed to react at 70 °C for 10 min. The products were then acidified and separated by Si gel eluted with $CHCl_3$ to give oleic acid and neocryptotanshinone (**3**). The neocryptotanshinone (**3**) was analyzed with Si gel TLC using $CHCl_3$−Me_2CO (98:2) and was compared with an authentic sample (R_f 0.18). The EIMS spectrum of oleic acid is identical to that of an authentic sample.

Effects of 1 and 2 on Platelet Aggregation. The antiplatelet effects of **1** and **2** were determined in vitro using rabbit platelets as previously described.[10] The IC_{50} values of **1** and **2** against platelet aggregation induced by arachidonic acid (100 μM), collagen (10 μg/mL), or thrombin (0.1 U/mL) are shown in Table 2.

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