

Two New Fatty Diterpenoids from *Salvia miltiorrhiza*

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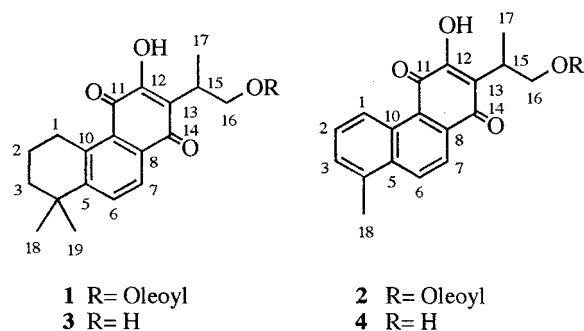
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Received July 10, 2000

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 (linolenic, 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 CHCl₃-soluble part of the EtOH extract of the root of *Salvia miltiorrhiza* 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.



Compound **1** was obtained as a yellowish oil with a molecular formula of C₃₇H₅₄O₅ (HREIMS), 264 amu more than that of neocryptotanshinone (**3**).² This additional molecular weight corresponds to an oleoyl group, as evidenced in the ¹H 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 C₁₇H₃₃CO, also indicated the presence of an oleoyl group. The base peak at *m/z* 296

(C₁₉H₂₀O₃, [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 ¹H NMR spectrum (CDCl₃) 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 ¹H and ¹³C 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 C₃₆H₄₈O₅ (HREIMS), 264 amu (an oleoyl group) more than that of danshenxinkun A (**4**).⁹ The signals for the oleoyl moiety in ¹H NMR are the same as those of **1**. In the EIMS spectrum, a fragmentation peak at *m/z* 265, corresponding to C₁₇H₃₃CO, indicated the presence of an oleoyl moiety, while a prominent peak at *m/z* 278 (C₁₈H₁₄O₃, [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 ¹H NMR spectrum (CDCl₃) 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 ¹H and ¹³C NMR of the danshenxinkun 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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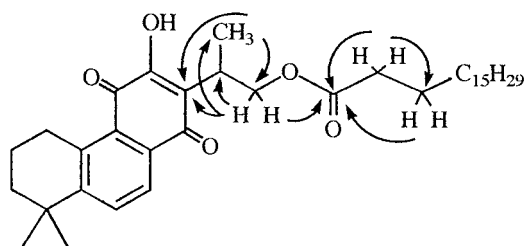
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Table 1. NMR Data for **1** and **2** in CDCl₃

position	1		2	
	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: ¹H NMR δ 1.17–1.27 (H-4–H-7, H-12–H-15); ¹³C 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 (IC₅₀ (μM)) of **1** and **2** on Platelet Aggregation^a

compound	stimulant		
	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 IC₅₀ values of 5.1 and 25.5 μM, respectively. They are less active against collagen-induced platelet aggregation, with an IC₅₀ of about 50–60 μM, and are inactive against thrombin-induced platelet aggregation (Table 2).

Experimental Section

General Experimental Procedures. Melting points are uncorrected. ¹H NMR (300 MHz, Bruker AM-300 NMR spectrometer), ¹³C NMR (75 MHz, Bruker AM-300 NMR spectrometer), and 2D NMR (500 MHz, Bruker AM-500 NMR spec-

trometer): CDCl₃ 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 CHCl₃–H₂O (1:1). The concentrated CHCl₃ extract (1.13 kg) was subjected to chromatography over Si gel (70–230 mesh, 5 Kg) and eluted with *n*-hexane–CHCl₃ (1:1), CHCl₃, CHCl₃–Me₂CO (9:1), Me₂CO, and MeOH, successively. The first fraction was chromatographed over Si gel using *n*-hexane, *n*-hexane–CHCl₃ (9:1), *n*-hexane–CHCl₃ (4:1), and CHCl₃ as eluents. The subfraction between known danshexkun B and tanshinone I eluted with *n*-hexane–CHCl₃ (4:1) was rechromatographed over Si gel using *n*-hexane–CHCl₃ (4:1) as eluent to afford a yellow oil fraction (196 mg), which was further purified by preparative TLC (Si gel 60 F₂₅₄) using *n*-hexane–CHCl₃ (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 (CHCl₃); [α]_D²⁵ +14.3° (*c* 0.35, CHCl₃); 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}; ¹H and ¹³C NMR (CDCl₃), 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 C₃₇H₅₄O₅, 578.3970).

Oleoyl danshenxinkun A (2): reddish oil; [α]_D²⁵ −78.8° (*c* 0.25, CHCl₃); 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 $[\text{M}]^+$ (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 $\text{C}_{36}\text{H}_{48}\text{O}_5$, 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 anti-platelet 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 $\mu\text{g}/\text{mL}$), or thrombin (0.1 U/mL) are shown in Table 2.

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NP000345V