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ACETYL DANSHENXINKUN A FROM SALVIA MILTIORRHIZA

Yun-Lian Lin[1], Ching-Yang Liu[2], Tai-Hung Chen[2],
Mei-Yi Kang[2] and Ming-Jaw Don[1]

[1]National Research Institute of Chinese Medicine,
[2]Department of Chemistry, Chinese Culture University,
Taipei, Taiwan
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A new diterpenoid, acetyl danshenxinkun A (**1**), and five known tanshinones, tanshinone IIA (**2**), 1,2-dihydrotanshinone I (**3**), tanshinone I (**4**), cryptotanshinone (**5**), and 15,16-dihydrotanshinone I (**6**) were isolated from the roots of *Salvia miltiorrhiza*. Their structures were determined by spectroscopic methods.

Key words: *Salvia miltiorrhiza*, Acetyl danshenxinkun A, Tanshinone.

INTRODUCTION

The dried roots of *Salvia miltiorrhiza* under the name Danshen has been used in Chinese traditional medicine for treatment of hemorrhage, menstrual disorder, miscarriage, and swelling.[1] According to literature reports, a lot of tanshinones were isolated and identified from *S. miltiorrhiza* and many of them were also reported to exhibit diverse biological activities such as antitumor,[2] antioxidant,[3] antimicrobial,[4] antiplatelet aggregation,[5,6] and anti-allergic activities.[7] As a part of our interests in bioactive tanshinone constituents, the chemical investigation of *S. miltiorrhiza* was carried out. Herein we wish to report the isolation and identification of a new diterpenoid, acetyl danshenxinkun A (**1**), together with five known tanchinones (**2-6**).

RESULTS AND DISSCUSSION

The ethanolic extract of *S. miltiorrhiza* was partitioned between EtOAc and H_2O. The EtOAc layer was separated by silica gel column chromatography and preparative TLC to obtain a new diterpenoid, acetyl danshenxinkun A (**1**), and five known tanshinones including tanshinone IIA (**2**), 1,2-dihydrotanshinone I (**3**),

Correspondence to: Ming-Jaw Don; 155-1, Sec. 2, Li-Nung Street, Peitou, Taipei 112, Taiwan, Tel: 02-28201999 ext. 8411; E-mail: mjdon@cma23.nricm.edu.tw

tanshinone I (**4**), cryptotanshinone (**5**), and 15,16-dihydrotanshinone I (**6**).

Acetyl danshenxinkun A (**1**) was obtained as a red solid with a molecular formula of $C_{20}H_{18}O_5$ determined by HREIMS ($[M]^+$, *m/z* 338.1150). The IR spectrum showed absorptions for an aromatic ring (1589 cm^{-1}) and a 2-hydroxy-1,4-benzoquinone (3335, 1648, 1627, cm^{-1}) moiety.[8] The ^1H NMR spectrum ($CDCl_3$) of **1** revealed an ABX pattern for 1,2,3-aromatic protons at δ 9.42 (d, *J* = 8.5 Hz), 7.62, (dd, *J* = 8.5, 7.0 Hz) and 7.46 (d, *J* = 7.0 Hz), an AB pattern for *ortho*-aromatic protons at δ 8.42 (d, *J* = 9.0 Hz) and 8.26 (dd, *J* = 9.0 Hz), one oxygenated methylene proton at δ 4.39 (d, *J* = 7.5 Hz), a methine proton at δ 3.55-3.64 (m), one acetyl at δ 1.98 (s), one aromatic methyl at δ 2.73, an aliphatic methyl at δ 1.33 (d, *J* = 7.0 Hz), and one chelated hydroxyl proton at δ 7.89 without corresponding carbon in the HMQC spectrum. The signals in ^1H NMR spectrum of **1** were very similar to those of danshenxinkun A, except for an additional methyl signal in acetyl moiety. In the EIMS spectrum, 42 amu (an acetyl group) more than that of danshenxinkun A, a fragmentation peak at *m/z* 296 also indicated the possible presence of a danshenxinkun A moiety. Furthermore, the other molecular ion peaks at *m/z* 278 (prominent, $[M\text{-acetic acid}]^+$), 266, 250, and 235 showed the same fragmentation patterns as danshenxinkun A.[9] The location of 16-*O*-acetyl was assigned from the observation of the three-bond coupling of H-16 to carbonyl carbon of the acetyl group (Figure 1). The complete assignments of ^1H and ^{13}C NMR of **1** was shown in Table 1, based on the extensive COSY, HMQC, and HMBC data. Thus, **1** was identified as 16-*O*-acetyl danshenxinkun A.

Compounds **2**-**6** were all identified by spectral analysis and by comparison with their literature data.[4,5]

Table 1. NMR Data for 1 in $CDCl_3$

position	^1H	^{13}C
1	9.42 (d, *J* = 8.5 Hz)	125.4
2	7.62 (dd, *J* = 8.5, 7.0 Hz)	130.5
3	7.46 (d, *J* = 7.0 Hz)	129.3
4		135.2*
5		135.2
6	8.42 (d, *J* = 9.0 Hz)	132.6
7	8.26 (d, *J* = 9.0 Hz)	122.5
8		133.7
9		124.0
10		130.4*
11		183.6
12		153.8
13		120.8
14		184.9
15	3.55-3.64 (m)	29.7
16	4.39 (d, *J* = 7.5 Hz)	66.4
17	1.33 (d, *J* = 7.0 Hz)	15.0
18	2.73 (s)	19.9
CH_3CO	1.98 (s)	171.0
		21.0
OH	7.89 (s)	

*interchangeable

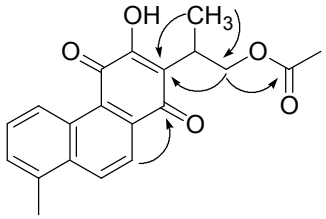
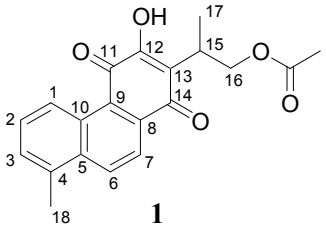
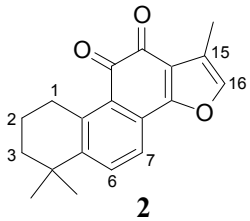
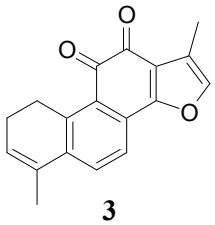
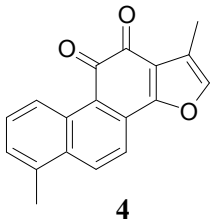


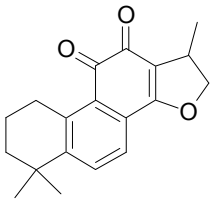
Fig. 1. Partial HMBC correlations for 1.

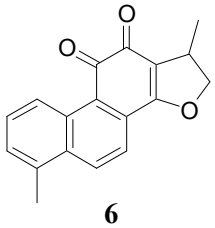












EXPERIMENTAL

General Experimental Procedures

Melting points were determined with a Yanaco micro-melting point apparatus and are uncorrected. Infrared spectra were obtained on a Nicolet Avatar 320 FTIR spectrophotometer. Nuclear magnetic resonance spectra were recorded on a Varian Unity Inova-500 spectrometer. Chemical shifts are reported in parts per million (δ) units relative to internal tetramethylsilane. The EIMS spectra were measured from a Finnigan GCQ GC/MS spectrometer at 30 eV. HREIMS was recorded on a Finnigan MAT 95S mass spectrometer. Column chromatography was performed with E. Merck 230-400 mesh silica gel.

Plant Material

The dried roots of *Salvia miltiorrhiza* were purchased from local herbal drug store in Taipei, and identified by Mr. Jun-Chih Ou, a research fellow of National Research Institute of Chinese Medicine (NRICM). A voucher specimen was already deposited in the Herbarium of NRICM.

Extraction and Isolation

Slices of the dried roots of *S. miltiorrhiza* (1 kg) were extracted with EtOH (3 L × 3) at room temperature. The combined EtOH extracts were concentrated in *vacuo*. The residue (265 g) was then partitioned between EtOAc and H_2O. The concentrated EtOAc extract (57 g) was subjected to chromatography over silica gel and eluted with *n*-hexane-EtOAc (4:1), *n*-hexane/EtOAc (1:1), and EtOAc, successively. The first fraction was rechromatographed on silica gel using mixtures of *n*-hexane/EtOAc under gradient condition (10:1→2:1) to yield **2** [250 mg, R_f = 0.57 (*n*-hexane/EtOAc; 4:1)], **3** [20 mg, R_f = 0.52 (*n*-hexane/EtOAc; 4:1)], **4** [96 mg, R_f = 0.43 (*n*-hexane/EtOAc; 4:1)], **5** [125 mg, R_f = 0.24 (*n*-hexane/EtOAc; 4:1)], and **6** [18 mg, R_f = 0.19 (*n*-hexane/EtOAc; 4:1)]. The subfraction between tanshinone I and cryptotanshinone was further purified by preparative TLC (Si gel F_{254}) using *n*-hexane-EtOAc (3:1) as the mobile phase to give **1** [8 mg, R_f = 0.36 (*n*-hexane/EtOAc; 4:1)].

Acetyl danshenxinkun A (1)

Red solid (EtOAc/n-hexane); mp 134-136 °C; $[\alpha]^{20}_D$ +18.4° (*c* 0.38, $CHCl_3$); IR (KBr) ν_{max} 3335, 2921, 1741, 1648, 1627, 1589, 1356, 1238, 1036, 784, 763 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$), see Table 1; EIMS *m/z* (%) 338 (5) [M^+], 296 (17), 278 (100), 266 (24), 250 (43), 235 (11); HREIMS *m/z* 338.1150 (calcd for $C_{20}H_{18}O_5$, 338.1149).

Tanshinone IIA (2)

Red solid (EtOAc/n-hexane); mp 203-205 °C (lit.[2] 205 °C); IR (KBr) ν_{max} 2954, 1692, 1670, 1583, 1536, 1285, 1192, 836 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.29 (6H, s, CH_3 x 2), 1.62-1.65 (2H, m, H-3), 1.75-1.80 (2H, m, H-2), 2.24 (3H, s, CH_3), 3.16 (2H, t, *J* = 6.5 Hz, H-1), 7.20 (1H, s, H-16), 7.52 (1H, d, *J* = 8.0 Hz, H-7), 7.61 (1H, d, *J* = 8.0 Hz, H-6); EI-MS *m/z* (%) 294 (100) [M^+], 279 (47), 261 (89), 251 (24), 233 (32).

1,2-Dihydrotanshinone I (3)

Red solid (EtOAc/n-hexane); mp 162-164 °C (lit.[2] 169 °C); IR (KBr) ν_{max} 1686, 1647, 1619, 1447, 1384, 1293, 1173, 792 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.05 (3H, d, *J* = 1.5 Hz, CH_3), 2.22-2.25 (2H, m, H-2), 2.26 (3H, d, *J* = 1.0 Hz, CH_3), 3.33 (2H, t, *J* = 8.0 Hz, H-1), 6.02-6.04 (1H, m, H-3), 7.21 (1H, q, *J* = 1.0 Hz, H-16), 7.41 (1H, d, *J* = 8.0 Hz, H-6), 7.56 (1H, d, *J* = 8.0 Hz, H-7); EI-MS *m/z* (%) 278 (100) [M^+], 263 (28), 235 (18), 222 (13), 179 (16).

Tanshinone I (4)

Reddish brown solid (EtOAc/n-hexane); mp 233-234 °C (lit.[2] 230 °C); IR (KBr) ν_{max} 1705, 1609, 1588, 1515, 1383, 1257, 1223, 1130, 1004, 747, 698 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.27 (3H, s, CH_3), 2.67 (3H, s, CH_3), 7.28 (1H, s, H-16), 7.33 (1H, d, *J* = 7.0 Hz, H-3), 7.53 (1H, dd, *J* = 9.0, 7.0 Hz, H-2), 7.80 (1H, d, *J* = 9.0 Hz, H-7), 8.28 (1H, d, *J* = 9.0 Hz, H-6), 9.23 (1H, d, *J* = 9.0 Hz, H-1); EI-MS *m/z* (%) 276 (49) [M^+], 248 (100), 191 (12).

Cryptotanshinone (5)

Orange solid (EtOAc/n-hexane); mp 193-194 °C (lit.[2] 191 °C); IR (KBr) ν_{max} 2957, 1654, 1625, 1560, 1465, 1403, 1195, 1169, 943 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.29 (6H, s, CH_3 x 2), 1.33 (3H, d, *J* = 7.0 Hz, CH_3), 1.62-1.65 (2H, m, H-3), 1.75-1.80 (2H, m, H-2), 3.19 (2H, t, *J* = 7.0 Hz, H-1), 3.54-3.61 (1H, m, H-15), 4.34 (1H, dd, *J* = 9.0, 6.0 Hz,

H-16a), 4.86 (1H, t, *J* = 9.0 Hz, H-16b), 7.47 (1H, d, *J* = 8.0 Hz, H-7), 7.61 (1H, d, *J* = 8.0 Hz, H-6); EI-MS *m/z* (%) 296 (76) [M^+], 281 (20), 268 (26), 253 (100), 235 (15), 171 (11).

15,16-Dihydrotanshinone I (6)

Reddish brown solid (EtOAc/n-hexane); mp 200-201 °C (lit.[2] 201 °C); IR (KBr) ν_{max} 1686, 1647, 1619, 1384, 1328, 1294, 1173, 792 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.40 (3H, d, *J* = 6.5 Hz, CH_3), 2.69 (3H, s, CH_3), 3.60-3.68 (1H, m, H-15), 4.42 (1H, dd, *J* = 9.5, 6.5 Hz, H-16a), 4.95 (1H, t, *J* = 9.5 Hz, H-16b), 7.39 (1H, d, *J* = 7.0 Hz, H-3), 7.56 (1H, dd, *J* = 8.5, 7.0 Hz, H-2), 7.75 (1H, d, *J* = 9.0 Hz, H-7), 8.30 (1H, d, *J* = 9.0 Hz, H-6), 9.29 (1H, d, *J* = 8.5 Hz, H-1); EI-MS *m/z* (%) 278 (18) [M^+], 250 (77), 235 (100), 207 (16), 179 (13).

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中藥丹參之丹參酮成分研究

林雲蓮[1]　劉清揚[2]　陳泰宏[1]　康美儀[2]　董明兆[1]

[1] 國立中國醫藥研究所

[2] 中國文化大學應用化學研究所

台北

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丹參為常用的中藥，用於治療心臟疾病、活血祛瘀、調經止痛和涼血消炎等。從丹參甲醇抽取物之乙酸乙酯層中分離出六個丹參酮化合物 acetyl danshenxinkun A (**1**)，tanshinone IIA (**2**)，1,2-dihydrotanshinone I (**3**)，tanshinone I (**4**)，cryptotanshinone (**5**) 及 15,16-dihydrotanshinone I (**6**)。其中 acetyl danshenxinkun A (**1**) 為新化合物。這些化合物皆由各種光譜分析確定其結構。

關鍵詞：丹參，丹參酮。

聯絡人：董明兆，台北市北投區立農街二段 155-1 號，Tel: 02-28201999 ext. 8411, Fax: 02-28264276。