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PIGMENTS FROM *SALVIA MILTIORRHIZA*

HOU-WEI LUO, BAO-JING WU, MEI-YU WU,* ZHONG-GEN YONG,* MASATAKE NIWA†‡ and YOSHIMASA HIRATA†

Nanjing College of Pharmacy, Nanjing, China; *Institute of Jiangsu Industrial Chemical Research, Nanjing, China; †Faculty of Pharmacy, Meijo University, Tempaku, Nagoya 468, Japan

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Key Word Index—*Salvia miltiorrhiza*; Labiatae; pigment; diterpene; *o*-naphthaquinone; tanshindiol A; tanshindiol B; tanshindiol C; nortanshinone; 3α-hydroxytanshinone IIA.

Abstract—Five new *o*-naphthaquinone diterpenes, tanshindiol A, tanshindiol B, tanshindiol C, nortanshinone and 3α-hydroxytanshinone IIA, have been isolated from the roots of *Salvia miltiorrhiza* as minor components. Their relative stereochemistries have been established on the basis of spectral and chemical evidence.

INTRODUCTION

'Dan-Shen', the dried roots of *Salvia miltiorrhiza* Bunge, is a clinically important Chinese drug in the treatment of heart disease. Many chemists have studied the physiologically active constituents of this drug and have isolated more than 16 orange-red crystalline pigments, abietanoids [1, 2 and refs. therein]. Most abietanoids have either a furano-*ortho*-naphthaquinone or a furano-*para*-naphthaquinone skeleton and are classified biogenetically as diterpenes. In the course of our search for physiologically active substances in Chinese drugs [3 and refs. therein], we have isolated five new pigments, tanshindiol A (**1**), tanshindiol B (**2**), tanshindiol C (**3**), nortanshinone (**4**) and 3α-hydroxytanshinone IIA (**5**), from Dan-Shen as minor components. This paper describes the isolation and characterization of these pigments.

RESULTS AND DISCUSSION

Repeated column chromatography of the ethanolic extract of Dan-Shen on silica gel afforded five new abietanoids (**1–5**), in addition to many known abietanoids which are described in the Experimental.

Tanshindiol A (**1**), orange-red needles, $C_{18}H_{16}O_5$, gave rise to spectra which were similar to those of tanshinone IIA (**6**) [4, 5]. However, notable differences between **1** and **6** were seen with regard to the following points. The ^{1}H NMR spectrum of **6** had a singlet due to a geminal dimethyl group at δ1.30 [δ31.9 (*q* + *q*) and 34.9 (*s*) in the ^{13}C NMR spectrum (Table 1)] and the IR spectrum of **6** showed no hydroxyl band. On the other hand, **1** had two hydroxyl groups instead of a geminal dimethyl group. One of them was a primary alcohol attached to a tetrasubstituted carbon [δ3.60 (2H, *s*); *m/z* 281 [M − 31]$^+$] and the other was a tertiary alcohol attached to the same carbon. This was further confirmed by acetylation of **1** with acetic anhydride–pyridine to give the corresponding monoacetate (**7**) [ν3500 and 1725 cm^{-1}; δ2.14 (3H, *s*), and 4.22 (2H, *s*); δ69.6 (*t*) and 71.4 (*s*)]. From the above results, the structure of tanshindiol A can be represented as **1**.

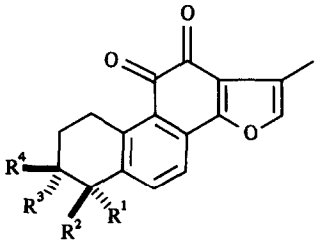
Tanshindiols B (**2**) and C (**3**) had the same molecular formula, $C_{18}H_{16}O_5$ (*m/z* 312 [M]$^+$), and their spectral data were quite similar to each other, indicating that they must be stereoisomers. Thus, in addition to each tertiary hydroxyl group, **2** had a secondary axial hydroxyl group [δ3.98 (1H, *dd*, *J* = 4.8, 2.9 Hz)] and **3** had a secondary equatorial one [δ3.96 (1H, *dd*, *J* = 4.0, 12 Hz)]. In fact, oxidation of either **2** or **3** with sodium periodate gave the same product (**8**). **8** had an acetophenone-type methyl ketone moiety [δ2.61 (3H, *s*)] and a phenylpropionaldehyde moiety. The presence of the latter moiety (Ar–CH_2–CH_2–CHO) in **8** was established by the ^{1}H NMR spectrum with the aid of double resonance

Table 1. ^{13}C NMR spectra of **4**, **6** and **7** (25 MHz, $CDCl_3$ + CD_3OD, TMS as internal standard)

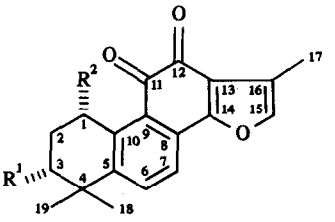
C	7	6	4
1	28.8 *t*	30.2 *t*	28.3 *t*
2	19.2 *t*	19.3 *t*	22.2 *t*
3	32.3 *t*	38.0 *t*	38.0 *t*
4	71.4 *s*	34.9 *s*	197.3 *s*
5	143.0 *s*	144.8 *s*	134.6 *s*
6	134.3 *d*	134.0 *d*	134.2 *d*
7	120.5 *d*	120.6 *d*	120.9 *d*
8	129.3 *s*	127.5 *s*	133.6 *s*
9	125.8 *s*	126.3 *s*	126.4 *s*
10	145.2 *s*	150.6 *s*	150.5 *s*
11	182.9 *s*	183.5 *s*	182.8 *s*
12	175.2 *s*	175.7 *s*	175.5 *s*
13	121.3 *s*†	121.3 *s*†	122.0 *s*†
14	161.2 *s*	162.2 *s*	162.7 *s*
15	142.1 *d*	141.9 *d*	143.2 *d*
16	120.5 *s*†	120.6 *s*†	120.9 *s*†
17	8.7 *q*	8.7 *q*	8.7 *q*
18	69.6 *t*	31.9 *q*	—
19	—	31.9 q	—
Me	20.8 *q*		
CO	182.9 *s*		

†Values can be interchanged.

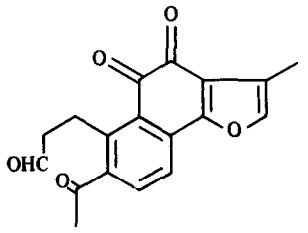
‡To whom correspondence should be addressed.



1 $R^1 = CH_2OH$, $R^2 = OH$, $R^3 = R^4 = H$
2 $R^1 = Me$, $R^2 = R^3 = OH$, $R^4 = H$
3 $R^1 = Me$, $R^2 = R^4 = OH$, $R^3 = H$
4 $R^1 = R^2 = O$, $R^3 = R^4 = H$
7 $R^1 = CH_2OAc$, $R^2 = OH$, $R^3 = R^4 = H$



5 $R^1 = OH$, $R^2 = H$
6 $R^1 = R^2 = H$
10 $R^1 = OAc$, $R^2 = OH$
11 $R^1 = H$, $R^2 = OH$



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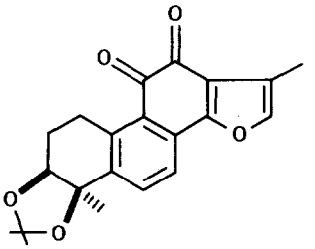

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experiments [δ3.32 (2H, *m*), 2.90 (2H, *m*) and 9.85 (1H, near *s*)]. Irradiation at δ2.90 caused, respectively, the multiplet at δ3.32 and the near singlet at δ9.85 to collapse to a singlet and a sharp singlet, whereas on irradiation at δ3.32 the multiplet at δ2.90 became a near singlet. The stereochemistries of the two vicinal hydroxyl groups in **2** and **3** were determined on the basis of the following chemical evidence. Treatment of **2** with acetone in the presence of anhydrous cupric sulphate resulted in the recovery of the starting material, but treatment of **3** under the same conditions readily afforded the corresponding acetonide (**9**). The structures of tanshindiols B and C can therefore be represented as **2** and **3**, respectively, including the stereochemistries of the two vicinal hydroxyl groups.

Nortanshinone (**4**), $C_{17}H_{12}O_4$, gave rise to spectra similar to those of tanshinone IIA (**6**). Moreover, **4** had one arylic carbonyl group [δ197.3 (*s*)] instead of a geminal dimethyl group in addition to two *ortho*-quinone carbonyl groups [δ175.5 (*s*) and 182.8 (*s*)]. The location of the arylic carbonyl group was assigned by analysis of the ^{1}H NMR spectrum with the aid of double resonance experiments. On irradiation at δ2.12, the multiplets at δ2.71 and 3.46 were changed to sharp singlets. The signal at δ2.12 was changed to a triplet on irradiation at δ2.71 as well as at 3.46. Furthermore, the doublet at δ7.96 in **1** was shifted to δ8.37 in the ^{1}H NMR spectrum of **4**. This downfield shift seemed to be due to the anisotropic effect of the carbonyl group. From these results, the structure of nortanshinone can be represented as **4**. In addition, tanshindiol A (**1**) was subjected to oxidation using sodium periodate to afford a carbonyl compound which was identical to nortanshinone (**4**).

The last pigment, 3α-hydroxytanshinone IIA (**5**), $C_{19}H_{18}O_4$, had a geminal dimethyl group [δ1.33 (6H, *s*)] and a secondary hydroxyl group [δ3.67 (1H, *m*)], which was readily acetylated by acetic anhydride–pyridine to give the corresponding acetate (**10**) [δ2.05 (3H, *s*) and 5.00 (1H, *t*, J = 4.9 Hz)]. The presence of the partial structure

■–CH_2–CH_2–$\underset{\displaystyle OH}{CH}$–■

in **5** was established by the following experiments. On irradiation of **10** at δ5.00, the multiplet at δ2.03 changed to a triplet, and then the multiplet at δ3.32 as well as that at δ5.00 changed to a singlet on irradiation at δ2.03. Finally, the multiplet at δ2.03 changed to a doublet on irradiation at δ3.32. In 1968, hydroxytanshinone II (**11**) having a secondary hydroxyl group at C-1 was reported by Kakisawa *et al.* [5]. The physical data of **5** are clearly different from those of **11**. Therefore, the hydroxyl group in **5** must be located at C-3. This was also confirmed by the ^{1}H NMR spectra, which showed the signals due to the benzylic methylene protons at δ3.26 (2H) in **5** and at δ3.32 (2H) in **10**, respectively. From the above results, the structure of 3α-hydroxytanshinone IIA can be represented as **5**.

With the exception of **4**, we have not established the absolute configurations of the new pigments (**1**–**5**).

EXPERIMENTAL

Mps: uncorr; ^{1}H NMR (100 and 60 MHz) and ^{13}C NMR (25 MHz): $CDCl_3$ unless stated otherwise, TMS as internal standard; IR: KBr; MS: direct inlet system.

Extraction and separation. Dan-Shen (*ca* 120 kg) from the Shandong Province (China) was mechanically crushed and extracted with hot 95% EtOH. The conc. extract (1.2 kg) was treated with C_6H_6 to give a C_6H_6-soluble fraction (*ca* 800 g) and a C_6H_6-insoluble fraction (*ca* 400 g).

The former fraction was chromatographed on silica gel and

eluted successively with C_6H_6 and C_6H_6–$CHCl_3$–MeOH (9:1:0.5). Each fraction obtained was rechromatographed on silica gel using a gradient of $CHCl_3$–Me_2CO to afford five known pigments, tanshinone IIA (78 g), tanshinone I (6 g), methylene-tanshiquinone (2 g), cryptotanshinone (2 g) and dihydro-tanshinone I (1 g) as crystals, respectively. The mother liquor of tanshinone I from $CHCl_3$–MeOH was concd and further separated by CC on silica gel using $CHCl_3$ containing an increasing amount of Me_2CO to give nortanshinone (**4**) (101 mg).

The C_6H_6-insoluble fraction, which was soluble in a mixture of C_6H_6–MeOH (9:1), was subjected to dry CC on silica gel. The very polar fraction (*ca* 260 g) eluted with C_6H_6–MeOH–formamide (7:3:0.5) after elution with C_6H_6 was rechromatographed on polyamide powder using 95% EtOH to afford a mixture of reddish pigments (120 g). The mixture was separated further by CC on silica gel using CH_2Cl_2–EtOAc (3:1) to afford four crude pigments (**1**, **2**, **3** and **5**). These pigments were purified by prep. TLC on silica gel (CH_2Cl_2–EtOAc–MeOH, 15:5:1) followed by recrystalization from $CHCl_3$–MeOH to afford pure pigments, **1** (90 mg), **2** (90 mg), **3** (30 mg) and **5** (85 mg), respectively.

Tanshindiol A (**1**). Mp 222–223°; HRMS *m/z*: Found 312.0992 $[M]^+$ ($C_{18}H_{16}O_5$ requires 312.0996); IR ν cm^{-1}: 3530, 3400, 1655, 1570, 1530; ^1H NMR: δ1.81 (2H, *m*), 2.26 (3H, *d*, J = 1.8 Hz), 2.69 (2H, *m*), 3.21 (2H, *m*), 3.60 (2H, *s*), 7.39 (1H, *q*, J = 1.8 Hz), 7.64 (1H, *d*, J = 7 Hz), 7.91 (1H, *d*, J = 7 Hz).

Tanshindiol B (**2**). Mp 210–213°; HRMS *m/z*: Found 312.0987 $[M]^+$ ($C_{18}H_{16}O_5$ requires 312.0996); IR ν cm^{-1}: 3470, 1655, 1570, 1530; ^1H NMR: δ1.50 (3H, *s*), 2.14 (2H, *m*), 2.27 (3H, *d*, J = 1.5 Hz), 3.35 (2H, *m*), 3.98 (1H, *dd*, J = 4.4, 2.9 Hz), 7.26 (1H, *q*, J = 1.5 Hz), 7.66 (1H, *d*, J = 8 Hz), 8.03 (1H, *d*, J = 8 Hz).

Tanshindiol C (**3**). Mp 213–215°; HRMS *m/z*: Found 312.0988 $[M]^+$ ($C_{18}H_{16}O_5$ requires 312.0996); IR ν cm^{-1}: 3480, 1660, 1570, 1530; ^1H NMR: δ1.46 (3H, *s*), 1.55 (2H, *s*, OH, disappeared on addition of D_2O), 2.16 (2H, *m*), 2.27 (3H, *d*, J = 1.5 Hz), 3.36 (2H, *m*), 3.96 (1H, *dd* (*br*), J = 12, 4 Hz, changed to a sharp *dd* on addition of D_2O), 7.26 (1H, *q*, J = 1.5 Hz), 7.64 (1H, *d*, J = 8 Hz), 7.97 (1H, *d*, J = 8 Hz).

Nortanshinone (**4**). Mp 231–232°; HRMS *m/z*: Found 280.0711 $[M]^+$ ($C_{17}H_{12}O_4$ requires 280.0734); IR ν cm^{-1}: 3120, 1660, 1570, 1530; ^1H NMR: δ2.12 (2H, *m*), 2.30 (3H, *d*, J = 1.8 Hz), 2.70 (2H, *m*), 3.40 (2H, *m*), 7.31 (1H, *q*, J = 1.8 Hz), 7.77 (1H, *d*, J = 8 Hz), 8.33 (1H, *d*, J = 8 Hz).

3α-Hydroxytanshinone IIA (**5**). Mp 205–206°; HRMS *m/z*; Found 310.1183 $[M]^+$ ($C_{19}H_{18}O_4$ requires 310.1204); IR ν cm^{-1}: 3500, 1665, 1575, 1530; ^1H NMR: δ1.33 (3H, *s*), 1.35 (3H, *s*), 1.94 (2H, *m*), 2.26 (3H, near *s*), 3.31 (2H, *m*), 3.74 (1H, *dd*, J = 4, 8 Hz), 7.24 (1H, *s*), 7.56 (1H, *d*, J = 8 Hz), 7.68 (1H, *d*, J = 8 Hz).

Acetylation of tanshindiol A (**1**). A mixture of **1** (10 mg), Ac_2O (0.3 ml) and C_5H_5N (0.3 ml) was stirred at room temp. for 3 hr. The residue obtained on removal of solvent was separated by CC on silica gel with $CHCl_3$–MeOH (5:1) to give **7** (10 mg) as orange-red crystals, mp 125–127°; MS *m/z*: 354 $[M]^+$ ($C_{20}H_{18}O_6$), 281 $[M-CH_2OCOMe]^+$; IR ν cm^{-1}: 3500, 1725, 1660, 1570, 1530; ^1H NMR: δ1.87 (2H, *m*), 2.14 (3H, *s*), 2.26 (3H, *d*, J = 1.8 Hz), 2.75 (2H, *m*), 3.20 (2H, *m*), 4.22 (2H, *s*), 7.25 (1H, *q*, J = 1.8 Hz), 7.54 (1H, *d*, J = 8 Hz), 7.91 (1H, *d*, J = 8 Hz).

Oxidation of tanshindiol B (**2**) *and tanshindiol C* (**3**). A mixture of **2** (11 mg) and $NaIO_4$ (26 mg) in 90% MeOH (10 ml) was stirred at room temp. for 5 hr. The mixture was concd under reduced pressure, diluted with H_2O, and extracted with $CHCl_3$. Evapn of solvent afforded **8** (7 mg), mp 182–184°; MS *m/z*: 310 $[M]^+$ ($C_{18}H_{14}O_5$); IR ν cm^{-1}: 2840, 2720, 1720, 1680, 1580, 1535; ^1H NMR: δ2.29 (3H, *d*, J = 1.5 Hz), 2.61 (3H, *s*), 2.90 (2H, *m*), 3.32 (2H, *m*), 7.33 (1H, *q*, J = 1.5 Hz), 7.73 (2H, *s*), 9.85 (1H, near *s*).

Under the same conditions, oxidations of **3** (4 mg) with $NaIO_4$ (10 mg) gave **8** (2.5 mg). Identification was made by spectral comparison (IR and ^1H NMR).

Reaction of **3** *with* Me_2CO. A mixture of **3** (4 mg), anhydrous $CuSO_4$ (5 mg) and Me_2CO (10 ml) was stirred at 40° for 5 hr, concd under reduced pressure, diluted with H_2O, and extracted with $CHCl_3$. Evapn of solvent afforded the acetonide (**9**) (2.5 mg), mp 117–119°; MS *m/z* 352 $[M]^+$ ($C_{21}H_{20}O_5$); ^1H NMR: δ1.54 (3H, *s*), 1.61 (6H, *s*), 1.99 (2H, *m*), 2.27 (3H, *d*, J = 1.2 Hz), 3.22 (2H, *m*), 4.15 (1H, *m*), 7.30 (1H, *q*, J = 1.2 Hz), 7.68 (1H, *d*, J = 8.4 Hz), 7.89 (1H, *d*, J = 8.4 Hz).

Oxidation of tanshindiol A (**1**). Oxidation of **1** (10 mg) with $NaIO_4$ (20 mg) in aq. MeOH gave **4** (7 mg), which was identical with the authentic sample.

Acetylation of 3α-hydroxytanshinone IIA (**5**). A mixture of **5** (6 mg), Ac_2O (0.2 ml) and C_5H_5N (0.5 ml) was stirred at room temp. for 5 hr. The residue, concd under reduced pressure, was chromatographed on silica gel with $CHCl_3$–MeOH (5:1) to afford **10** (5 mg), mp 200–201°; MS *m/z*: 352 $[M]^+$ ($C_{21}H_{20}O_5$); IR ν cm^{-1}: 1730, 1665, 1575, 1530; ^1H NMR: δ1.33 (6H, *s*), 2.03 (2H, *m*), 2.05 (3H, *s*), 2.26 (3H, *d*, J = 1.5 Hz), 3.32 (2H, *t*, J = 6.6 Hz), 5.00 (1H, *t*, J = 4.9 Hz), 7.24 (1H, *q*, J = 1.5 Hz), 7.62 (2H, *s*).

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