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Diterpenoids from *Salvia miltiorrhiza*

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Abstract

The abietane diterpenoid, neocryptotanshinone II, and the known 6,12-dihydroxyabieta-5,8,11,13-tetraen-7-one were isolated as minor components from the roots of *Salvia miltiorrhiza*. Their structures were established on the basis of spectral evidence.

Keywords: *Salvia miltiorrhiza*; Labiatae; Abietane diterpenoid; Neocryptotanshinone II; 6,12-Dihydroxyabieta-5,8,11,13-tetraen-7-one

1. Introduction

Dan-Shen, the root of *Salvia miltiorrhiza* Bunge (Labiatae), is a Chinese herb used in the treatment of cardiovascular disease. We previously reported that some abietane tanshinones isolated from the chloroform extract of 'Dan-Shen' showed significant cytotoxicity against four human tumor cell lines, nasopharyngeal carcinoma (KB), cervical carcinoma (Hela), colon adenocarcinoma (Colo-205), and laryngeal epidermoid carcinoma (Hep-2). Based on those results, we suggested that the *ortho*-quinone in ring C and the intact ring D of tanshinones are required for cytotoxicity (Lin, Chang & Chen, 1991, 1993, 1995; Wu, Chang & Chen, 1991, Lee, Wu, Chang, Lin & King, 1987, Chang, Wu, Chen & Lin, 1990; Lin & Chang, 1991, 1993; Lin, Chang & Chen, 1993). However, there are few examples of tanshinones with *para*-quinone moieties to clarify any relationship between structure and cytotoxicity. This fact prompted us to search further for analogs of tanshinone leading to the isolation and characterization of **1** and **2**.

2. Results and discussion

The $CHCl_3$-soluble part of the EtOH extract of the root of *Salvia miltiorrhiza* was subjected to repeated silica gel chromatography and preparative TLC to give a new abietane diterpenoid, neocryptotanshione II (**1**), and the known abietane diterpenoid, 6,12-dihydroxyabieta-5,8,11,13-tetraen-7-one (**2**).

Compound **1** was obtained as yellowish needles from $CHCl_3$, mp 129–130° and molecular formula of $C_{17}H_{18}O_3$ (HR-EIMS). The UV absorbances (250, 255, 280, 355 nm) and the IR spectrum (3340, 1665, 1645 cm^{-1}), similar to those of neocryptotanshinone (**3**), indicated the presence of a 2-hydroxyl-1,4-naphthoquinone moiety (Lee et al., 1987). The basic skeleton was established by comparing its ^{1}H-NMR spectrum with that of **3** (Lee et al., 1987). The ^{1}H-NMR spectrum ($CDCl_3$) revealed an AX pattern for two *ortho*-aromatic proton signals at δ 7.63 (*d*, J = 8.1 Hz) and δ 7.92 (*d*, J = 8.1 Hz), a geminal dimethyl group at δ 1.31 (*s*, 6H), and three methylene groups at δ 1.63 (*m*), δ 1.73 (*m*), and δ 3.24 (*t*, J = 6.0 Hz) similar to those of **3**. However, a vinylmethyl signal at δ 2.03 (*s*, 3H) of **1** replaced an isopropyl group of **3**. These data when taken together suggest **1** to be neocryptotanshinone II. Analyses of HMBC, HMQC, and COSY-45 data furnished the complete ^{1}H- and ^{13}C-NMR spectral assignments (Table 1) for **1**.

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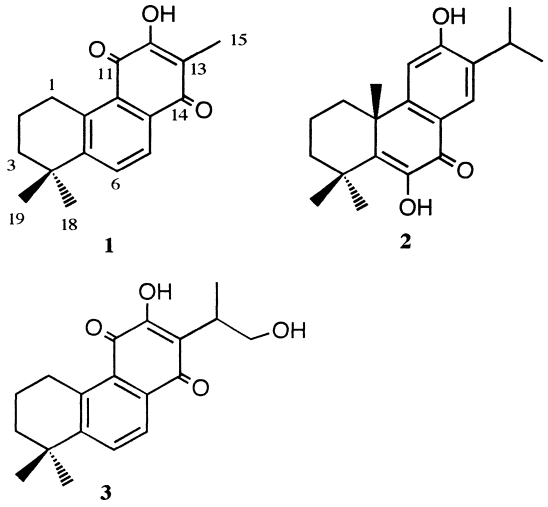
The known abietane-type diterpenoid, 6,12-dihydroxyabieta-5,8,11,13-tetraen-7-one (**2**) was identified by comparison of its physical and spectral data ($[\alpha]_D^{25}$, mass, ^{1}H- and ^{13}C-NMR, UV, IR) with literature values (Su, Fang & Cheng, 1994). This is the first report of compound **2** from a *Salvia* species.



3. Experimental

3.1. General

Mps: uncorr; ^{1}H-NMR (300 MHz), ^{13}C-NMR (80 MHz) and 2D NMR (500 MHz): $CDCl_3$ using the solvent peak as int. standard; MS: direct inlet system; UV: MeOH; IR: KBr disc.

3.2. Plant material

See Ref. Wu et al., 1991.

3.3. Extraction and isolation

The dried and powdered roots (45 kg) of *Salvia miltiorrhiza* were extracted with 95% EtOH (150 l × 3). The combined EtOH extracts were concentrated under reduced pressure 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 silica gel 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 silica gel using *n*-hexane, and then *n*-hexane–$CHCl_3$ (9:1), *n*-hexane–$CHCl_3$ (4:1) and $CHCl_3$ as eluents. The sub-fraction between known danshexkun B and tanshinone I eluted with *n*-hexane–$CHCl_3$ (4:1) was further purified by preparative TLC using *n*-hexane–$CHCl_3$ (4:1) as mobile phase to give **1** (16 mg) and **2** (14 mg).

3.4. Neocryptotanshinone II (*1*)

Yellowish needles: mp 129–130°; $[\alpha]_D^{25}$ + 3.8 ($CHCl_3$, *c* 1.0); IR (KBr) ν_{max} cm^{-1}: 3340, 2965, 1665, 1645, 1560, 1390, 1320, 1280, 1270, 1140, 1090, 1080, 860, 760; UVλ_{max} nm (log ε): 250 (*sh*, 4.45), 255 (4.50), 280 (4.38), 290 (*sh*, 4.34), 355 (3.74); ^{1}H- and^{13}C-NMR spectral data ($CDCl_3$): see Table 1; HR-EIMS $[M]^+$ *m*/*z*: 270.1275 ($C_{17}H_{18}O_3$ requires 270.1256); EIMS *m*/*z* (rel. int.): 270 $[M]^+$ (90), 255 (100), 241 (40), 227

Table 1
^{1}H- and ^{13}C-NMR Spectral Data (δ/ppm) and 2D NMR spectral data for compound **1** ($CDCl_3$)

Position	δ_H mult (*J* Hz)	COSY-45 correlations (H#)	δ_C (mult)[a]	HMBC (*J* = 8 Hz) correlations (C#)
1	3.24 *t* (6.0)	2	30.0 *t*	2, 3, 5, 9, 10
2	1.73 *m*	1, 3	19.4 *t*	1, 3, 4, 10
3	1.63 *m*	2	37.9 *t*	1, 5, 18, 19
4	–	–	34.4 *s*	–
5	–	–	155.6 *s*	–
6	7.63 *d* (8.1)	7	131.2 *d*	4, 8, 10
7	7.92 *d* (8.1)	6	124.2 *d*	5, 6, 14
8	–	–	130.3 *s*	–
9	–	–	128.6 *s*	–
10	–	–	140.1 *s*	–
11	–	–	181.5 *s*	–
12	–	–	151.4 *s*	–
13	–	–	121.7 *s*	–
14	–	–	187.9 *s*	–
15	2.03 *s*	–	9.0 *q*	12, 13, 14
18	1.31 *s*	–	31.9 *q*	3, 5
19	1.31 *s*	–	31.9 *q*	3, 5

[a] Multiplicities were obtained from DEPT experiment.

(70), 213 (10), 199 (10), 171 (30), 165 (15), 128 (15), 115 (15), 83 (35).

6,12-*Dihydroxyabieta*-5,8,11,13-*tetraen*-7-*one* (**2**). Oil; $[\alpha]_D^{25}$-8.5 ($CHCl_3$, *c* 1.0); spectral data consistent with literature values (Su et al., 1994).

References

Lin, H. C., Chang, W. L., & Chen, G. L. (1991). *Chin. Pharm. J*, *43*, 501–504.

Lin, H. C., & Chang, W. L. (1993). *Chin. Pharm. J*, *45*, 85–87.

Lin, H. C., Chang, W. L., & Chen, C. F. (1995). *Chin. Pharm. J*, *47*, 77–80.

Wu, W. L., Chang, W. L., & Chen, C. F. (1991). *Amer. J. Chin. Med*, *19*, 207–216.

Lee, A. R., Wu, W. L., Chang, W. L., Lin, H. C., & King, M. L. (1987). *J. Nat. Prod*, *50*, 157–160.

Chang, W. L., Wu, W. L., Chen, Y. C., & Lin, H. C. (1990). *Chin. Pharm. J*, *42*, 183–185.

Lin, H. C., & Chang, W. L. (1991). *Chin. Pharm. J*, *43*, 11–17.

Lin, H. C., & Chang, W. L. (1993). *Chin. Pharm. J*, *45*, 21–27.

Lin, H. C., Chang, W. L., & Chen, C. F. (1993). *Chin. Pharm. J*, *45*, 615–618.

Su, W. C., Fang, J. M., & Cheng, Y. S. (1994). *Phytochemistry*, *35*, 1279–1284.