

6. Bohlmann, F., Schmeda-Hirschmann, G., Jakupovic, J., King, R. M. and Robinson, H. (1984) *Phytochemistry* **23**, 1989.
7. Faini, F., Torres, R. and Castillo, M. (1984) *J. Nat. Prod.* **47**, 552.
8. Hoeneisen, M. and Becker, H. (1986) *J. Nat. Prod.* **49**, 360.
9. Bohlmann, F., Zdero, C., Schmeda-Hirschmann, G., Jakupovic, J., Dominguez, X. A., King, R. M. and Robinson, H. (1986) *Phytochemistry* **25**, 1175.
10. Zhang, D., Haruna, M., Mc Phail, A. T. and Lee, K. H. (1986) *Phytochemistry* **25**, 899.
11. Campbell, W. E. and Cragg, G. M. L. (1979) *Phytochemistry* **18**, 688.

*Phytochemistry*, Vol. 27, No. 1, pp. 290–292, 1988.  
Printed in Great Britain.

0031-9422/88 \$3.00 + 0.00  
Pergamon Journals Ltd.

## EPI-DANSHENSPIROKETALLACTONE FROM *SALVIA MILTIORRHIZA*

HOU WEI LUO,\* SHAOXING CHEN†, JUNNING LEE,† and JOHN K. SNYDER\*\*

Nanjing College of Pharmacy, Nanjing, China; †Department of Chemistry, Boston University, 590 Commonwealth Ave., Boston, MA 02215, U.S.A.

(Received 27 March 1987)

**Key Word Index**—*Salvia miltiorrhiza*; Labiatae; abietanoid; *epi*-Danshenspiroketallactone

**Abstract**—The structure of a new abietanoid pigment from the Chinese traditional medicine Dan-shen, *Salvia miltiorrhiza*, was isolated and its structure determined by NMR spectroscopy.

### INTRODUCTION

The dried roots of the Chinese sage, *Salvia miltiorrhiza* Bunge, are used in the traditional medicine, Dan-shen, to treat heart disease, [1] hepatitis, [2] and more recently, in the treatment of tuberculosis [3, 4] and leprosy [5]. Numerous abietanoid pigments have been isolated from this drug and identified as physiologically active natural products [6–13]. Several synthetic investigations have also been devoted to these compounds [14–21]. The activities of these Dan-shen constituents in the pure state, however, has not matched the activity of the crude drug itself [10]. For this reason, considerable effort is still being devoted to identifying the minor constituents of this traditional medicine, as well as determining the most effective mixture of components for eliciting its therapeutic value.

Recently, the structure of a new spiro lactone, named danshenspiroketallactone (**1**) was determined by X-ray analysis [12]. In our work on the constituents of Dan Shen, we have also isolated **1**, but prior to recrystallization, had been unable to separate **1** from a minor compound by any chromatographic method. We have now identified this minor component as *epi*-danshenspiroketallactone (**2**). For reasons discussed below, we demonstrate that **2** is not an artifact of the isolation process (via epimerization of **1**), but more likely that **1** results from the epimerization of **2**.

### RESULTS AND DISCUSSION

The 95% ethanolic extract of Dan-shen was fractionated as previously described [11]. The residue from evaporation of the mother liquor from the tanshinone IIA recrystallization yielded 50 mg of ether soluble material. Chromatography on silica gel with cyclohexane and cyclohexane-CH<sub>2</sub>Cl<sub>2</sub> afforded **1** and **2** as a 7:1 mixture (**1**:**2**), and tanshinlactone (**3**) [13]. An exhaustive effort to resolve **1** and **2** with normal and reverse phase HPLC using numerous solvent systems was not successful. Recrystallization from ethanol, however, gave pure **1** and a mother liquor enriched in **2**, thus accounting for the ability of the Shanghai group to obtain **1** in pure form for X-ray analysis [12]. Two additional recrystallizations ultimately afforded a 4:6 mixture of **1**:**2** (3 mg total wt). Due to the limited amount of material, no further attempts to purify **2** via additional recrystallizations were made.

From both the <sup>1</sup>H and <sup>13</sup>C-NMR spectra it was apparent that **1** and **2** were epimers (Table I) with only the <sup>1</sup>H- and <sup>13</sup>C-nuclei of the isoprene moieties differing in their chemical shift values. The mass spectrum also indicated only a single molecular ion (EIMS: M<sup>+</sup> at *m/z* 268). The <sup>1</sup>H-homonuclear coupling connectivities of the isoprene moieties of **1** and **2** could be independently mapped in the <sup>1</sup>H-homonuclear COSY spectrum (Fig. 1) of the initially obtained mixture (prior to recrystallization). The <sup>1</sup>H-aromatic signals of **1** and **2** coincided in benzene-*d*<sub>6</sub>.

Additional NMR evidence revealed **2** to be the C-15

\* Authors to whom correspondence should be addressed.

Table 1.  $^{13}\text{C}$  NMR chemical shifts of compounds **1** and **2** (100 MHz, 93.93 kG,  $\text{CDCl}_3$ , TMS as int. standard)\*

C	1	2
1	118.1 <i>d</i>	118.0 <i>d</i>
2	131.8 <i>d</i>	131.9 <i>d</i>
3	128.8 <i>d</i>	128.8 <i>d</i>
4	135.0 <i>s</i>	135.0 <i>s</i>
5	122.0 <i>s</i>	122.0 <i>s</i>
6	128.4 <i>d</i>	128.4 <i>d</i>
7	122.0 <i>d</i>	122.0 <i>d</i>
8	146.9 <i>s</i>	146.9 <i>s</i>
9	129.2 <i>s</i>	129.2 <i>s</i>
10	133.3 <i>s</i>	133.3 <i>s</i>
11	168.2 <i>s</i>	168.2 <i>s</i>
13	113.1 <i>s</i>	113.1 <i>s</i>
14	77.5 <i>t</i>	77.5 <i>t</i>
15	32.9 <i>d</i>	33.7 <i>d</i>
16	45.6 <i>t</i>	44.5 <i>t</i>
17	17.7 <i>q</i>	18.4 <i>q</i>
18	20.2 <i>q</i>	20.2 <i>q</i>

\* Multiplicities were assigned with a DEPT experiment which also revealed the signals for C-14 which were overlapped by the chloroform triplet, and distinguished the C-5 and C-7 resonances.

epimer of **1**. A 2D-NOE experiment (benzene- $d_6$ , on the originally isolated mixture of **1**:**2**, 7:1) revealed NOE's between the aromatic H-7 doublet and the H-15 multiplet of the major component (spirolactone **1**) and between the H-7 doublet and the Me-17 doublet of the minor component (spirolactone **2**). Both of these NOE's were confirmed by NOE difference experiments and could be reciprocated, enhancements observed on the H-7 signal by irradiation of the H-15 multiplet of **1** and the Me-17 doublet of **2**. Furthermore, other enhancements observed upon irradiation of the H-15 multiplet of **2** and the Me-17 doublet of **1** were in accord with their proposed structures, and enabled the assignments of all  $^1\text{H}$ -signals (Fig. 1).

In an effort to determine whether **2** is an artifact of the isolation procedure, a solution of the mixture enriched in **2** (**1**:**2**, 4:6) in  $\text{CHCl}_3$  was stirred in the presence of silica gel for 24 h. A solution of pure **1** was also exposed to silica gel under identical conditions. Under these conditions, **2** epimerized to **1**, producing a 2:1 mixture (**1**:**2**). In contrast, **1** was stable under the same conditions, and showed no evidence of epimerization even when a catalytic amount of HCl (0.1 N) was added.

These results do not unambiguously establish that **2** is the natural product and that **1** is an artifact of the isolation scheme. It is clear, however, that some of **1**, though perhaps not the entire amount of **1** isolated, was indeed produced from **2** during the silica gel chromatographies in the separation scheme.

#### EXPERIMENTAL

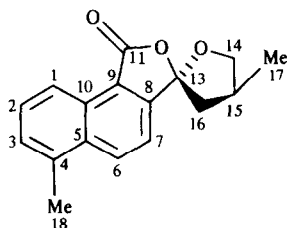
$^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra were recorded at 93.93 kG (400 MHz for  $^1\text{H}$ , 100 MHz for  $^{13}\text{C}$ ) in  $\text{CDCl}_3$  unless otherwise stated, TMS as int. standard. DEPT experiments confirmed the multiplicities of the  $^{13}\text{C}$  signals of **2** and revealed the doublet at 77.46 (C-14), Table 1.

epi-Danshenspiroketallactone (**2**). The mother liquor from the recrystallization of **1** in EtOH (anhydrous) [13] was evapd to yield a mixture (6 mg) of **1** and **2** (3:1). Two additional recrystallizations of the residue from EtOH (dry) afforded 3 mg of a 4:6 mixture of **1**:**2**.  $^1\text{H}$  NMR for **2**:  $\delta$  1.33 (3H, *d*,  $J$  = 7.0 Hz, H-17), 2.24 (1H, *dd*,  $J$  = 13.3, 4.6 Hz, H-16), 2.72 (1H, *dd*,  $J$  = 13.3, 9.7 Hz, H-16), 2.76 (3H, *s*, H-18), 2.7 (1H, *m*, H-15, overlapped by H-18 methyl), 3.95 (1H, *dd*,  $J$  = 7.5, 8.6 Hz, H-14), 4.44 (1H, *dd*,  $J$  = 7.5, 7.5 Hz, H-14), 7.47 (1H, *d*,  $J$  = 6.7 Hz, H-3), 7.54 (1H, *d*,  $J$  = 8.7 Hz, H-7), 7.60 (1H, *dd*,  $J$  = 6.7, 8.3 Hz, H-2), 8.35 (1H, *d*,  $J$  = 8.7 Hz, H-6), 8.87 (1H, *d*,  $J$  = 8.3 Hz, H-1).  $^1\text{H}$  NMR, benzene- $d_6$ :  $\delta$  0.92 (3H, *d*, H-17), 1.76 (1H, *dd*, H-16), 2.1–2.2 (2H, *m*, H-16, H-15), 2.27 (3H, *s*, H-18), 3.66 (1H, *dd*, H-14), 3.98 (1H, *dd*, H-14), 7.06 (1H, *d*, H-3), 7.20 (1H, *d*, H-7), 7.26 (1H, *dd*, H-2), 7.82 (1H, *d*, H-6), 9.30 (1H, *d*, H-1).

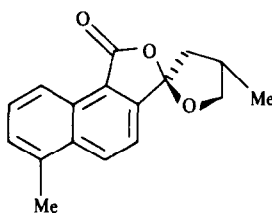
**Acknowledgements**—We gratefully thank the Research Corporation, the Donors of The Petroleum Research Fund administered by the American Chemical Society, and The Camille and Henry Dreyfus Foundation, for support.

#### REFERENCES

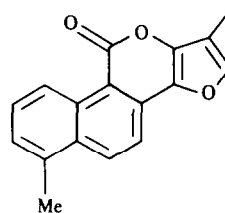
- (1976) in *Zhong Cao Yao Xue* (Nanjing Yao Xue Yuan), Part 3, p. 947 'Zhong Cao Yao Xue' Bian Xie Zu, Jiang Su Ren Min Chu Ban She, Nanjing.
- Zhou, Q. (1985) in *Advances in Chinese Materials Research* (H. M. Chang, H. W. Yeung, W. W. Tso, and A. Koo, eds), p. 215. World Scientific, Singapore.
- Chien, M. K. (1978) *Acta Chim. Sin.* **36**, 199.
- Luo, H. (1985) *Acta Pharm. Sin.* **20**, 542.
- Tong, J. (1986) *J. Clin. Dermatology* **4**, 210.
- Okumura, Y., Kakisawa, H., Kato, M. and Hirata, Y. (1961) *Bull. Chem. Soc. Jpn* **34**, 895.
- Takiura, K. and Koizumi, K. (1962) *Chem. Pharm. Bull.* **10**, 112.
- Kakisawa, H., Hayashi, T., Okazaki, I. and Ohashi, M. (1968) *Tetrahedron Letters* 3231.
- Kakisawa, H., Hayashi, T. and Yamazaki, T. (1969) *Tetrahedron Letters*, 301.



**1**



**2**



**3**

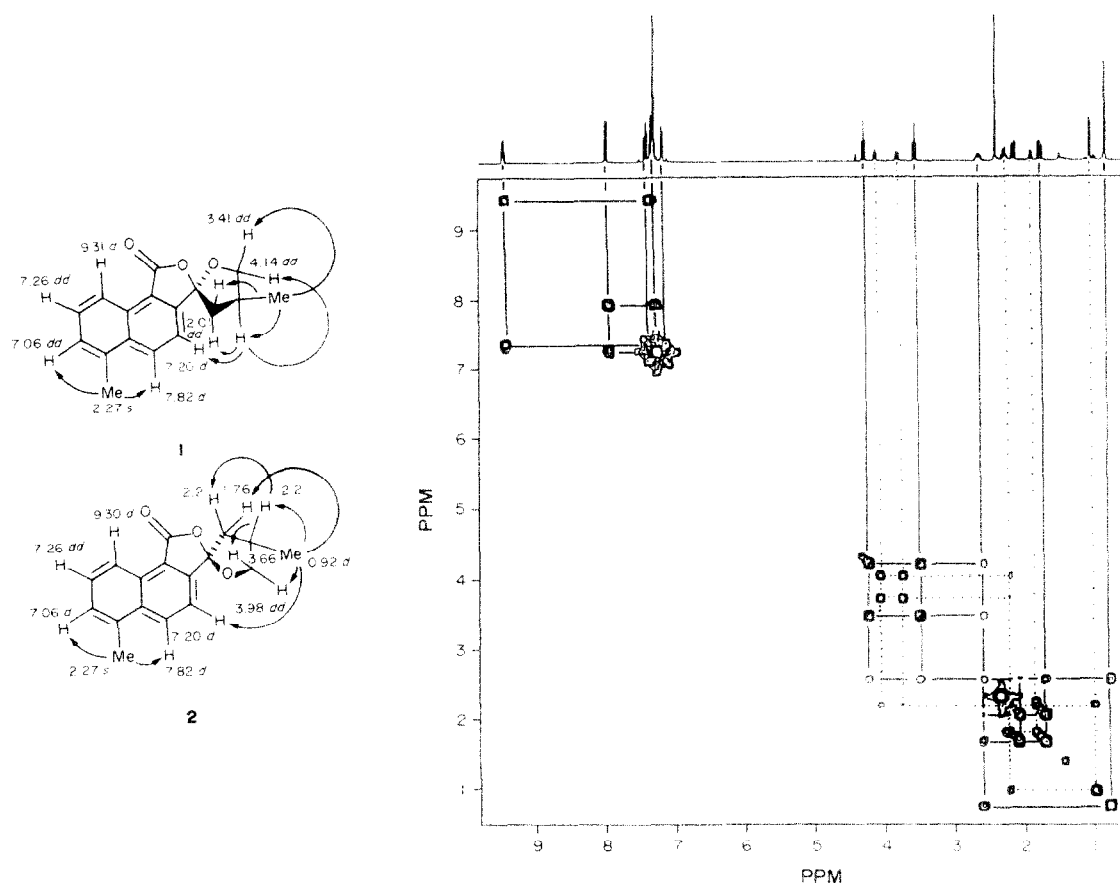


Fig. 1. COSY spectrum of the mixture of **1** and **2** obtained from the mother liquor after two recrystallizations of the danshenspiroketallactone fraction: **1**, solid line; **2**, dotted line. Aromatic signals of **1** and **2** coincide. Observed NOE's used to assign  $^1\text{H}$  signals of **1** and **2** are indicated by arrows.

10. Onitsuka, M., Fujiu, M., Shinma, N. and Maruyama, H. B. (1983) *Chem. Pharm. Bull.*, **31**, 1670.
11. Luo, H., Wu, B., Wu, N., Yong, Z., Niwa, M. and Hirata, Y. (1985) *Phytochemistry*, **24**, 815.
12. Kong, D., Liu, X., Teng, M. and Rao, Z. (1985) *Acta Pharm. Sin.*, **20**, 747.
13. Luo, H., Ji, J., Wu, M., Yong, Z., Niwa, M. and Hirata, Y. (1986) *Chem. Pharm. Bull.*, **34**, 3167.
14. Kakisawa, H. and Inouye, Y. (1968) *J. Chem. Soc., Chem. Commun.* 1327.
15. Baille, A. C. and Thomson, R. H. (1968) *J. Chem. Soc. (C)*, 48.
16. Kakisawa, H., Tateishi, M. and Kusumi, T. (1968) *Tetrahedron Letters* 3783.
17. Inouye, Y. and Kakisawa, H. (1969) *Bull. Chem. Soc. Jpn* **42**, 3318.
18. Tateishi, M., Kusumi, T. and Kakisawa, H. (1971) *Tetrahedron* **27**, 237.
19. Imai, S. (1971) *J. Sci. Hiroshima Univ., Ser. A* **35**, 171.
20. Huot, R. and Brassard, P. (1974) *Can. J. Chem.* **52**, 88.
21. Knapp, S. and Sharma, S. (1985) *J. Org. Chem.* **50**, 4996.