DITERPENOIDS FROM *SALVIA PARAMILTIORRHIZA*

Hou-Wei Luo,[a]* Xiao-Ru Sun,[a] and Masatake Niwa[b]

[a]Department of Phytochemistry, China Pharmaceutical University, Nanjing 210009, China

[b]Faculty of Pharmacy, Meijo University, Tempaku, Nagoya 468, Japan

Abstract ----- Six diterpenes including a novel compound named paramiltioic acid were isolated from *Salvia paramiltiorrhiza* Li et Huang. Their structures were characterized by physical methods.

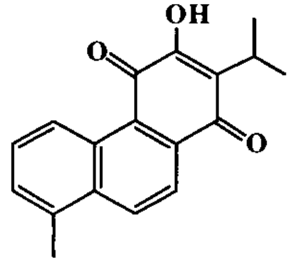
The roots of various plants of the genus *Salvia* (Labiatae) are used in China as a folk medicine to treat a wide variety of diseases.[1,2] Among them, the dried roots of *Salvia miltiorrhiza* Bunge known well as "Dan-Shen" in Chinese has been very often used and still attracts considerable attention of medicinal chemists as well as clinicians because of the exhibition of significant physiological activities such as antiinflammatory, antineoplastic and antiplatelet acttivities.[3-5] In China, chemical and pharmaceutical studies on Dan-Shen and the related plants are presently one of the most importnat national projects. A number of diterpenoids have been isolated from Dan-Shen as physiologically active constituents.[6] In the course of our studies on the constituents of *Salvia* plants,[7,8] we examined the dried roots of *Salvia paramiltiorrhiza* Li et Huang, which is used as a substitute for *Salvia miltiorrhiza* Bunge in Anhui province of China. Our preliminary examination of the root of *S. paramiltiorrhiza* showed the presence of triterpenes and diterpenes as the main chemical constituents and the triterpenes were already reported.[9] Our continuous study of the above plant led to isolate a novel rearranged abietanoid having an unusual carbon skeleton, named paramiltioic acid[10] as well as five known diterpenoids. In this paper we wish to deal with the isolation and structural characterization of these diterpenes.

Isolation and Identification As reported in the previous paper,[9] the ethanol extract of the plant was roughly separated by column chromatography. The first fraction eluted with a mixture of petroleum ether and chloroform (9 : 1) contained a known diterpene, danshexinkun B (**1**).[6] The second fraction eluted with a mixture of petroleum ether and chloroform (1 : 1) contained 4 known diterpenes; tanshinone IIA (**2**),[6] tanshinone I (**3**),[6] methylene tanshinquinone (**4**),[6] and salviolone (**5**).[11,12] The third fraction eluted with a mixture of chloroform and ethly acetate (9 ; 1) contained a novel diterpene named paramiltioic acid (**6**)[12] having a spirolactone function. Five known compounds (**1** ~ **5**) were respectively identified by the tests of mixed melting point with the authentic samples.

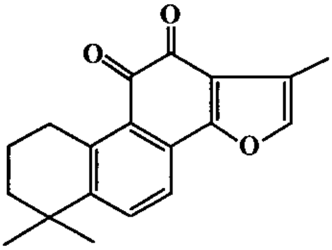
Structure of Paramiltioic acid (6) The structure of paramiltioic acid (**6**) was found to have the molecular formula $C_{19}H_{24}O_5$ determined by the high resolution mass spectrum. The infrared spectrum of **6** showed the presence of two carbonyl groups at 1761 and 1717 cm^{-1} due to an αβ-unsaturated five-membered lactone (δ_C 173.4 (s)) and an αβ-unsaturated carboxylic acid (δ_C 166.5 (s)), respectively. The H-H COSY spectrum showed a long range coupling between H-19 (δ_H 1.77 (3H, d, *J*=1.5 Hz)) and H-12 (δ_H 7.34 (1H, q, *J*=1.5 Hz) owing to an allyl coupling. The HMBC spectrum furthermore showed the correlations of the C-8 signal at δ_C 83.4 with H-7 (δ_H 3.50), H-9 (δ_H 5.85) and H-12 (δ_H 7.34). These data indicated the presence of a spirolactone structure connected to an αβ-unsaturated carboxylic acid group in **6**. The presence of an oxabicyclo[4.4.0]decane system having a gem-dimethyl group in **6** was suggested by the close relation of the ^{13}C-nmr data to those of norsalvioxide (**7**)[11] and miltipolone (**8**)[12] as shown in Table 1. In fact, the H-5 signal at δ_H 1.42 showed distinct HMBC correlations with C-4 (δ_C 34.1), C-6 (δ_C 24.3), C-11 (δ_C 38.7), C-15 (δ_C 20.8), C-16 (δ_C 32.0) and C-17 (δ_C 66.6), respectively and also the H-17a signal at δ_H 4.20

Table 1. ^{13}C-Nmr data corresponding to the oxabicyclo[4.4.0]decane system in compounds (**6**, **7** and **8**).

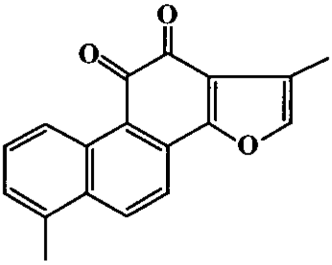
C	6	7	8	C	6	7	8
1	28.6	28.6	29.9 ppm	7	73.4	70.2	74.4 ppm
2	18.3	18.9	19.0	11	38.7	37.7	40.6
3	40.1	41.3	41.0	17	66.6	67.8	67.4
4	34.1	33.9	34.5				
5	42.9	43.1	41.5	15	20.8	21.0	21.3
6	24.3	30.3	29.2	16	32.0	32.9	32.7



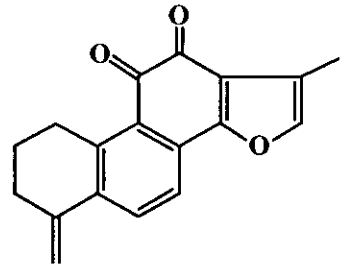
1



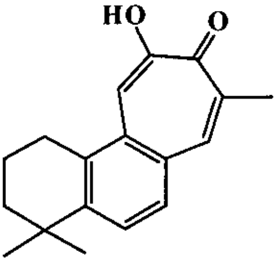
2



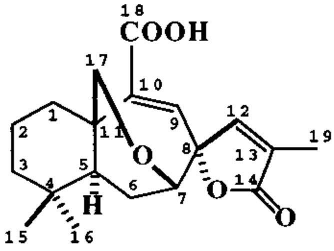
3



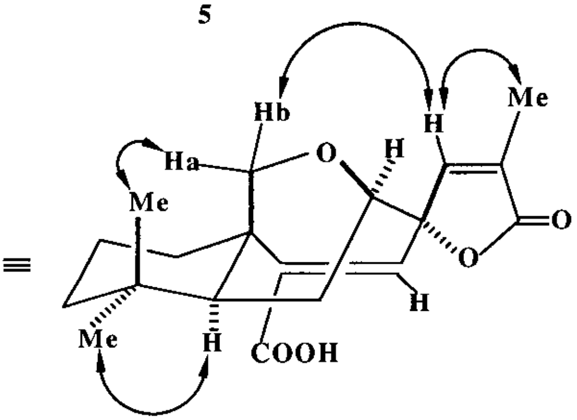
4



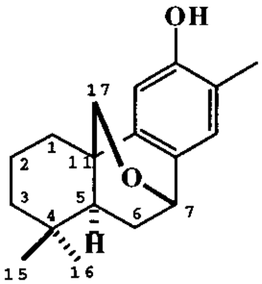
5



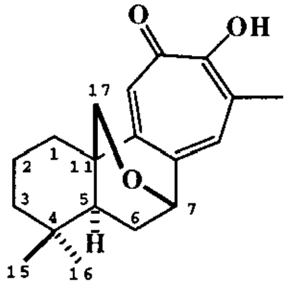
6



Relative stereochemistry for **6** on the basis of the NOESY indicated by arrows.



7



8

correlated to C-7 (δ_C 73.4) and C-11 (δ_C 38.7), respectively. The relative stereochemistry of paramiltioic acid (**6**) was assigned as follows. The NOESY spectrum of **6** showed the cross peaks between H-5 and H-16, H-15 and Ha-17, H-12 and Hb-17, and H-12 and H-19, respectively.

Additionally, the *trans*-configuration of A/B ring junction was also supported by the appearance of a cross peak owing to W-type coupling between H-7 (δ_H 3.50 (1H, br d, J=2.4 Hz)) and H-9 (δ_H 5.85 (1H, br s)).[13]

On the basis of the data mentioned above, the structure of paramiltioic acid should be represented by **6**.

EXPERIMENTAL

Melting points were determined on a micro melting point apparatus Buchi-520 and are uncorrected. Optical rotations were measured with a JASCO DIP-181 automatic polarimeter. ^1H Nmr spectra were taken on JEOL FX-90Q (90 MHz) and JEOL JNM GX-400 (400 MHz) spectrometeres. ^{13}C Nmr spectra were recorded on a JEOL JNM GX-400 spectrometer (100 MHz). Chemical shifts are presented in terms of δ_H and δ_C (ppm) with $CHCl_3$ (7.26 and 77.0 ppm, respectively) or DMSO-d_6 (2.5 and 39.5 ppm, respectively) in the deuterated solvent as an internal standard. The following abbreviations are used: s = singlet, d = doublet, dd = double doublet, ddd = double double doublet, t = triplet, q = quartet, h = heptet, m = multiplet. Coupling constans (J values) are given in herz (Hz). Mass spectra (ms) were recorded on Hitachi M-80 and JEOL HX-110. Ir spectra were recorded on a JASCO FT/IR-5000.

Plant material

The roots of *Salvia paramiltiorrhiza* were obtained in Anhui Province of China in 1987. The plant specimen was identified by Mr. Yang-Hui Jian of Department of Pharmacognosy, China Pharmaceutical University. A voucher specimen has been deposited in the Herbarium of China Pharmaceutical University.

Extraction and Isolation

The roots (10 kg) were extracted 3 times with EtOH (50 l) for 5 days at room temperature to give an extract. The extract (400 g) was subjected to column chromatography on silica gel (1700 g) using a mixture of petroleum ether and chloroform (9 : 1), petroleum ether and chloroform (1 : 1), and chloroform and ethyl acetate (9 : 1), each 2 l, respectively, to give 3 fractions. Each fraction was repeatedly chromatographed on silica gel with the following solvent system : Fr.1, petroleum ether - chloroform (9 : 1); Fr. 2, petroleum ether - chloroform (2 : 1); Fr. 3, chloroform - ethyl acetate (5 : 1). Fr. 1 gave compound (**1**) (15 mg, 0.00015%). Fr. 2 gave compounds (**2**) (750 mg, 0.0075%), (**3**) (890 mg, 0.0089%), (**4**) (20 mg, 0.0002%), and (**5**) (100 mg, 0.001%). Fr. 3 gave compound (**6**) (60 mg, 0.0006%).

Compound (1)

Red needles, mp 187 - 188 °C (petroleum ether - CH_2Cl_2). ^1H Nmr, δ_H ($CDCl_3$) 1.34 (6H, d, J=7.3 Hz), 2.73 (3H, s), 3.41 (1H, h, J=7.3 Hz), 7.45 (1H, d, J=7.0 Hz), 7.61 (1H, dd, J=7.0, 8.8 Hz), 8.26 (1H, d, J=8.8 Hz), 8.40 (1H, d, J=8.8 Hz), 9.42 (1H, d, J=8.8 Hz). No depression of the melting point was shown in mixing into an authentic sample of danshexinkun B.

Compound (2)

Red needles, mp 205 - 206 °C (petroleum ether - CH_2Cl_2). ^1H Nmr, δ_H ($CDCl_3$) 1.31 (6H, s), 2.26 (3H, s), 3.19 (2H, t, J=6.6 Hz), 7.24 (1H, s), 7.55 (1H, d, J=8.4 Hz), 7.63 (1H, d, J=8.4 Hz). No depression of the melting point was shown in mixing into an authentic sample of tanshinone IIA.

Compound (3)

Red needles, mp 220 °C (petroleum ether - CH_2Cl_2). ^1H Nmr, δ_H ($CDCl_3$) 2.29 (3H, s), 2.70 (3H, s), 7.30 (1H, s), 7.35 (1H, d, J=7.3 Hz), 7.55 (1H, dd, J=8.8, 7.3 Hz), 7.82 (1H, d, J=8.8 Hz), 8.31 (1H, d, J=8.8 Hz), 9.26 (1H, d, J=8.8 Hz). No depression of the melting point was shown in mixing into an authentic sample of tanshinone I.

Compound (4)

Purplish red needles, mp 174 - 175 °C (petroleum ether - CH_2Cl_2). ^1H Nmr, δ_H ($CDCl_3$) 2.26 (3H, d, J=1.5 Hz), 5.06 (1H, s), 5.48 (1H, s), 7.23 (1H, q, J=1.5 Hz), 7.53 (1H, d, J=8.0 Hz), 7.86 (1H, d, J=8.0 Hz). No depression of the melting point was shown in mixing into an authentic sample of methylene tanshiquinone.

Compound (5)

Pale yellow plates, mp 178 - 179 °C (petroleum ether - CH_2Cl_2). Ms, m/z 268 (M^+). ^1H Nmr, δ_H ($CDCl_3$) 1.36 (6H, s), 1.71 (2H, m), 1.94 (2H, m), 2.48 (3H, s), 3.10 (2H, t, J=7.0 Hz), 7.53 (1H, d, J=8.5 Hz), 7.56 (1H, d, J=8.5 Hz), 7.96 (1H, s), 8.05 (1H, s), 8.45 (1H, s, disappeared after addition of D_2O). No depression of the melting point was shown in mixing into an authentic sample of salviolone.

Compound (6)

Colorless needles, mp 200 - 201 °C (MeOH). $[\alpha]_D^{27}$ -86.9° (*c* 0.06, MeOH). HRms, *m/z* 332.1532 [M$^+$] ($C_{19}H_{24}O_5$ requires: 332.1617). Uv (MeOH) λ_{max} 225 nm (log ε 3.65). Ir (KBr) ν 3300 ~ 2400, 1761, 1717, 1684, 1658, 942 cm^{-1}. ^{1}H Nmr, δ_H (CDCl$_3$) 0.83 (3H, s, H-16), 1.03 (3H, s, H-15), 1.13 (1H, m, H-3), 1.25 (1H, br d, *J*=8.5 Hz, H-1), 1.42 (1H, ddd, *J*=12, 5.9, 1.5 Hz), 1.44 (1H, m, H-3), 1.52 (1H, m, H-1), 1.54 (2H, m, H-2), 1.77 (3H, d, *J*=1.5 Hz, H-19), 1.81 (1H, superimposed on the peak of 19-CH$_3$, H-6), 1.96 (1H, ddd, J=14, 12, 1.5 Hz, H-6), 3.45 (1H, dd, *J*=10, 1.5 Hz, Hb-17), 3.50 (1H, br d, *J*=2.4 Hz, H-7), 4.20 (1H, d, *J*=10 Hz, Ha-17), 5.85 (1H, br s, H-9), 7.34 (1H, q, J=1.5 Hz, H-12). ^{13}C Nmr, δ_C (CDCl$_3$) 10.0 (C-19), 18.3 (C-2), 20.8 (C-15), 24.3 (C-6), 28.6 (C-1), 32.0 (C-16), 34.1 (C-4), 38.7 (C-11), 40.1 (C-3), 42.9 (C-5), 66.6 (C-17), 73.4 (C-7), 83.4 (C-8), 117.9 (C-9), 128.9 (C-13), 149.1 (C-12), 153.1 (C-10), 166.5 (C-18), 173.4 (C-14).

ACKNOWLEDGEMENT

We are grateful to Mr. Yang-Hui Jian (C. P. U.) for identification of the plant material.

REFERENCES AND NOTES

1. T. Yokozawa, H. Y. Chung, H. Oura, G. Nonaka, and I. Nishioka, *J. Med. Pharm. Soc. WAKAN-YAKU*, 1988, **5**, 163.
2. W. Cheng, 'Dan-Shen: Biology and its Application,' ed. by R. Xu, Science Press Inc., Beijing, 1990, pp. 180 ~ 185.
3. Y. Gao, L. Wang, and K. Tang, *Chin. Integrated Trad. Western Med.*, 1983, **3**, 300.
4. W. Wu, W. Chang, A. Lee, H. Lin, and M. King, *J. Med. Sci.*, 1985, **6**, 159.
5. H. Luo, X. Hu, and N. Wang, *J. Acta Pharm. Sinica*, 1988, **23**, 830.
6. B. Yang, 'Dan-Shen: Biology and its Application,' ed. by R. Xu, Science Press Inc., Beijing, 1990, pp. 81 ~ 157.
7. N. Wang, M. Niwa, and H. Luo, *Phytochemistry*, 1988, **27**, 299.
8. X. Lu, H. Luo, and M. Niwa, *Planta Medica*, 1990, **56**, 87.
9. X. Sun, H. Luo, T. Sakai, and M. Niwa, *Shoyakugaku Zasshi*, 1992, **46**, 202.

10. X. Sun, H. Luo, T. Sakai, and M. Niwa, *Tetrahedron Lett.*, 1991, **32**, 5797.

11. G Haro, T. Kusumi, M. Ishituka, H. Kakisawa, W. Zhao, J. Chen, and Y. Guo, *Tetrahedron Lett.*, 1988, **29**, 4603.

12. G. Haro, M. Mori, O. Ishituka, T. Kusumi, Y. Inouye, and H. Kakisawa, *Bull. Soc. Chem. Jpn.*, 1991, **64**, 3422.

13. A dreiding molecular model of the *cis*-isomer showed no such favorable W-arrangement of the bonds between H-7 and H-9.

Received, 25th July, 1994