DANSHENOLS A AND B, NEW ALDOSE REDUCTASE INHIBITORS FROM THE ROOT OF *SALVIA MILTIORHIZA* BUNGE

Rena KASIMU, Purusotam BASNET, Yasuhiro TEZUKA, Shigetoshi KADOTA,* and Tsuneo NAMBA

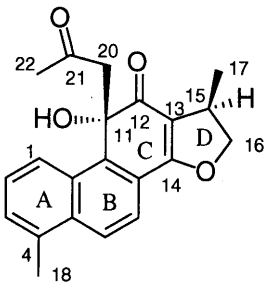
Research Institute for Wakan-Yaku (Traditional Sino-Japanese Medicines), Toyama Medical and Pharmaceutical University, Sugitani 2630, Toyama 930-01, Japan

Two new abietane-type diterpenoids, danshenols A (**1**) and B (**2**), were isolated from an MeOH extract of *Salvia miltiorhiza* BUNGE, and their structures determined by chemical and spectroscopic methods including the 2D NMR technique. Danshenol A (**1**) showed strong inhibitory activity against aldose reductase isolated from the eye lens of rats.

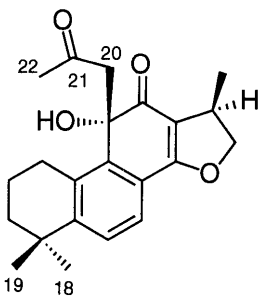
KEY WORDS danshenol; abietane-type diterpene; aldose reductase inhibitor; *Salvia miltiorhiza*; Lamiaceae

The traditional Chinese medicine "Dan-Shen (丹参)" is made from the dried root of *Salvia miltiorhiza* BUNGE (Lamiaceae) and is used to treat hematological abnormalities, heart disease, menstrual disorders, miscarriage, hepatitis, and swelling.[1] Moreover, it has been reported that the MeOH extract of "Dan-Shen" shows strong inhibitory activity against aldose reductase.[2] Thus, as one of our studies on drugs effective for the treatment of diabetes and/or diabetic complications,[3] we examined the aldose reductase-inhibitory constituents of *S. miltiorhiza* and isolated two new abietane-type diterpenes, danshenol A (**1**) and danshenol B (**2**), along with four known ones, dihydrotanshinone I[4,5] (**3**), cryptotanshinone[4,6] (**4**), tanshinone I[4] (**5**), and tanshinone IIA[4,7] (**6**). In this communication, we report the isolation and structure elucidation of the new compounds, as well as their inhibitory activity against aldose reductase.

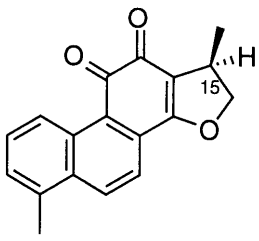
Chopped root (8 kg) of *S. miltiorhiza* was extracted successively with hot water and MeOH to give a water extract and an MeOH extract, respectively. The MeOH extract, which showed inhibitory activity against aldose reductase, was treated with EtOAc to give EtOAc-soluble and EtOAc-insoluble fractions, and then the EtOAc-soluble fraction was divided into $CHCl_3$-soluble and $CHCl_3$-insoluble fractions. The $CHCl_3$-soluble fraction was then separated by a combination of silica gel column chromatography (hexane-$CHCl_3$ solvent system) and preparative TLC (EtOAc-benzene solvent system) procedures to give two new abietane-type diterpenes, danshenol A (**1**, 7 mg) and danshenol B (**2**, 23 mg), along with four known compounds, dihydrotanshinone I (**3**, 17 mg), cryptotanshinone (**4**, 35 mg), tanshinone I (**5**, 22 mg), and tanshinone IIA (**6**, 672 mg).



1

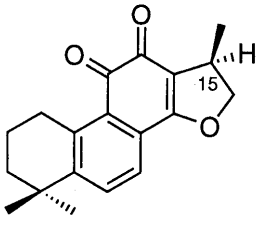


2



3

5 Δ^{15}



4

6 Δ^{15}

* To whom correspondence should be addressed.

Danshenol A[8] (**1**) was obtained as dark yellow needles, mp 182℃ (MeOH), and showed $[\alpha]_D$ -136.4° ($CHCl_3$). Its molecular formula was determined by HR-MS measurement to be $C_{21}H_{20}O_4$ (*m/z* 336), which was C_3H_6O more than that of dihydrotanshinone I (**3**). The ^1H- and ^{13}C-NMR spectra of **1** were partially similar to those of **3**, and analyses of the ^1H-^1H and ^1H-^{13}C COSY spectra of **1** indicated the presence of the same A-, B-, and D-rings as **3** (A-ring: δ_H 9.03, d, J = 9 Hz, δ_C 125.8, C1-H; δ_H 7.52, dd, J = 9, 6.5 Hz, δ_C 126.7, C2-H; δ_H 7.44, d, J = 6.5 Hz, δ_C 128.5, C3-H; δ_H 2.73, s, δ_C 20.1, C18-H_3; B-ring: δ_H 8.10, dd, J = 9, 1 Hz, δ_C 125.5, C6-H; δ_H 7.80, d, J = 9 Hz, δ_C 120.1, C7-H; D-ring: δ_H 1.45, d, J = 6.5 Hz, δ_C 18.0, C17-H_3; δ_H 3.67, dqd, J = 9.5, 9, 6.5 Hz, δ_C 34.5, C15-H; δ_H 4.00 and 5.00, both dd, J = 9.5, 9 Hz, δ_C 81.4, C16-H_2). In addition, they showed signals due to a methylene (δ_H 3.07 and 3.30, both d, J = 12.5 Hz, δ_C 57.3, C20-H_2) and a methyl (δ_H 1.98, s, δ_C 32.1, C22-H_3) group, and the ^{13}C-NMR spectrum of **1** revealed the presence of an oxygen-bearing quaternary carbon (δ_C 79.2) and a low-field shift of two carbonyl carbons (**1**: δ_C 204.8, 196.1; **3**: δ_C 175.8, 185.3). These data suggested that danshenol A was an abietane-type diterpene with an additional C3 unit (CH_3-CO-CH_2 grouping) on the C-ring, which was supported by the fact that Eu(DPM)$_3$[9] caused a *retro*-aldol reaction to give **3**.

The location of the additional C3 unit was determined by the HMBC spectrum. The quaternary carbons C-4 (δ_C 135.2), C-5 (δ_C 134.9), and C-10 (δ_C 131.0) were assigned based on the long-range correlations with 2-H, 6-H, and 18-H_3, with 1-H, 3-H, 7-H, and 18-H_3, and with 2-H and 6-H, respectively. Similarly, the quaternary carbons C-8 (δ_C 120.4), C-9 (δ_C 141.3), C-13 (δ_C 113.6), and C-14 (δ_C 171.3) were correlated with 6-H, with 1-H and 7-H, with 15-H, 16-H_2, and 17-H_3, and with 7-H and 16-H_2, respectively. On the other hand, the carbon C-9 (δ_C 141.3) also showed long-range correlations with the methylene protons (20-H_2) on the additional C3 unit. Thus the location of the additional C3 unit was determined to be at C-11. From these and other long-range correlations observed in the HMBC spectrum, the planar structure of danshenol A was concluded to be **1**.

In the difference NOE experiments, irradiation of 17-H_3 caused the NOE increase at 22-H_3 and *vice versa*, indicating that these methyls were in *cis* relation. On the other hand, dihydrotanshinone I (**3**) prepared through the *retro*-aldol reaction by Eu(DPM)$_3$ showed $[\alpha]_D^{25}$ -146.6° ($CHCl_3$, c = 0.09) (lit.[4] $[\alpha]_D^{24}$ -328°, $CHCl_3$, c = 0.11), indicating that the configuration at C-15 is *R*. Thus the structure of danshenol A, including the absolute stereochemistry, was determined to be **1**.[10]

Danshenol B[11] (**2**), yellow needles, mp 176℃ (MeOH), $[\alpha]_D$ -131.6° ($CHCl_3$), showed the molecular ion at *m/z* 354 and its molecular formula was determined as $C_{22}H_{26}O_4$ by HR-MS measurement. The ^1H-NMR spectrum of **2** was partially similar to that of cryptotanshinone (**4**) and showed the signals of three coupled methylenes (δ_H, 3.41, 1H, ddd, J = 17.5, 9, 5 Hz, 1-H; 3.12, 1H, dt, J = 17.5, 5 Hz, 1-H; 1.87 and 1.70, each 1H, m, 2-H_2; 1.69, 2H, m, 3-H_2), two coupled aromatic protons (δ_H 7.49, 1H, d, J = 8.5 Hz, 6-H; 7.41, 1H, d, J = 8.5 Hz, 7-H), a CH_3-CH-CH_2-O grouping (δ_H 1.39, 3H, d, J = 7 Hz, 17-H_3; 4.90 and 4.30, each 1H, t, J = 8.5 Hz, 16-H_2; 3.58, 1H, tq, J = 8.5, 7 Hz, 15-H), and two singlet methyls (δ_H 1.34 and 1.30, each 3H, s, 18-H_3 and 19-H_3). In addition, the signals due to the CH_3-CO-CH_2 grouping in the ^1H-NMR spectrum of **1** were also observed (δ_H 3.10 and 2.83, each 1H, d, J = 12.5 Hz, 2.06, 3H, s). Thus **2** was assumed to have the same modified C-ring as **1**, which was confirmed by the ^1H-^1H COSY, ^1H-^{13}C COSY, and HMBC spectra. On the other hand, the stereochemistry of danshenol B, including the absolute one, was determined to be **2**,[10] based on the facts that in the difference NOE experiments NOEs were observed between 17-H_3 and 22-H_3 and that Eu(DPM)$_3$ caused a *retro*-aldol reaction to give **4** ($[\alpha]_D^{25}$ -56.8°, $CHCl_3$, c = 0.09) (lit.[4] $[\alpha]_D^{24}$ -79.9°, $CHCl_3$, c = 0.18).

The inhibitory activities of **1**-**6** against aldose reductase isolated from the eye lens of rats[12] were compared with those of quercetin, a natural aldose reductase inhibitor,[13] and epalrestat, a strong aldose reductase inhibitor in clinical use,[14] with or without bovine serum albumin (BSA). The IC_{50} values of **1**-**6**, quercetin, and epalrestat without BSA were 0.10, 1.75, 1.19, 10.0, 4.80, 1.14, 5.6, and 0.038 μM, respectively, while those with 1% BSA were 29.1, 32.3, 58.1, 67.3, 39.6, 37.8,

39.8, and 0.86 μM, respectively. Further studies on these and other constituents are now in progress and will be reported elsewhere.

REFERENCES AND NOTES

1) Pharmacopoeia Committee of the Health Ministry of the People's Republic of China (ed.), "Pharmacopoeia of People's Republic of China," Vol. 1, Guangdong Scientific Technologic Publisher, Guangdong, 1995, pp.62-63; Chiang Su New Medicinal College (ed.), "Dictionary of Chinese Crude Drugs," Shanghai Scientific Technologic Publisher, Shanghai, 1977, pp. 478-482; Namba T., "The Encyclopedia of Wakan-Yaku (Traditional Sino-Japanese Medicines) with Color Pictures," Vol. I, Hoikusha Publishing Co., Ltd., Osaka, 1993, pp. 24-25.
2) Zhang J., Zhou Y., *China Journal of Chinese Materia Medica*, **14**, 557-559 (1989).
3) Our recent publications on this line are the following: Basnet P., Kadota S., Pandey R. R., Takahashi T., Kojima Y., Shimizu M., Takata Y., Kobayashi M., Namba T., *J. Trad. Med.*, **12**, 109-117 (1995); Basnet P., Kadota S., Hase K., Namba T., *Chem. Pharm. Bull.*, **43**, 1558-1564 (1995).
4) Onitsuka M., Fujiu M., Shinma N., Maruyama H. B., *Chem. Pharm. Bull.*, **31**, 1670-1675 (1983).
5) Ikeshiro Y., Hashimoto I., Iwamoto Y., Mase I., Tomita Y., *Phytochemistry*, **30**, 2791-2792 (1991).
6) Ikeshiro Y., Mase I., Tomita Y., *Phytochemistry*, **28**, 3139-3141 (1989); Tomita Y., Ikeshiro Y., *J. Chem. Soc., Chem. Commun.*, **1987**, 1311-1313.
7) Luo H.-W., Wu B.-J., Wu M.-Y., Yong Z.-G., Niwa M., Hirata Y., *Phytochemistry*, **24**, 815-817 (1985).
8) Danshenol A (**1**): dark yellow needles, mp 182℃ (MeOH), $[\alpha]_D^{25}$ -136.4° ($CHCl_3$, c=0.07). UV λ_{max} (MeOH) nm (log ε): 231 (4.32), 265 (sh), 275 (4.19), 287 (4.04), 299 (3.90), 343 (sh), 356 (3.42), 372 (sh), 403 (3.12). IR ν_{max} (KBr) cm^{-1}: 3350, 1600, 1380, 1160, 1170, 910. EI-MS *m/z*: 336 (M^+), 296, 279, 268, 253. HR-MS: 336.1395 ($C_{21}H_{20}O_4$ requires 336.1361). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 204.8 (s, C-21), 196.1 (s, C-12), 171.3 (s, C-14), 141.3 (s, C-9), 135.2 (s, C-4), 134.9 (s, C-5), 131.0 (s, C-10), 128.5 (d, C-3), 126.7 (d, C-2), 125.8 (d, C-1), 125.5 (d, C-6), 120.4 (s, C-8), 120.1 (d, C-7), 113.6 (s, C-13), 81.4 (t, C-16), 79.3 (s, C-11), 57.3 (t, C-20), 34.5 (d, C-15), 32.1 (q, C-22), 20.1 (q, C-18), 18.0 (q, C-17).
9) We tried to determine the relative stereochemistry between C11-OH and 15-H or 17-H_3 through an Eu(DPM)$_3$-induced shift study, but the reagent caused a *retro*-aldol reaction.
10) The possibility that danshenols A (**1**) and B (**2**) were artifacts has been excluded, because we did not use acetone in the isolation procedure; the TLC analysis (AcOEt-benzene=13:87) of the MeOH extract indicated the presence of **1** (Rf, 0.24) and **2** (Rf, 0.33); and the treatment of dihydrotanshinone I (**3**) with silica gel in refluxing acetone resulted in recovery of **3**.
11) Danshenol B (**2**): yellow needles, mp 176℃ (MeOH), $[\alpha]_D^{25}$ -131.6° ($CHCl_3$, c = 0.10). UV λ_{max} (MeOH) nm (log ε): 214 (4.18), 230 (4.10), 275 (4.06), 287 (3.84), 299 (3.90), 343 (sh), 356 (3.42), 372 (sh), 403 (3.12). IR ν_{max} (KBr) cm^{-1}: 3350, 1620, 1460, 1260, 1175, 1085, 1020, 790. EI-MS *m/z*: 354 (M^+), 311, 296, 268, 253. HR-MS: 354.1866 ($C_{22}H_{26}O_4$ requires 354.1831). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 205.1 (s, C-21), 196.0 (s, C-12), 171.6 (s, C-14), 140.9 (s, C-9), 137.3 (s, C-5), 132.6 (s, C-10), 127.3 (d, C-6), 122.2 (d, C-7), 120.3 (s, C-8), 112.5 (s, C-13), 81.5 (t, C-16), 79.6 (s, C-11), 54.8 (t, C-20), 38.7 (t, C-3), 35.3 (s, C-4), 34.7 (q, C-18), 34.4 (d, C-15), 32.2 (q, C-22), 31.7 (q, C-19), 29.2 (t, C-1), 19.9 (t, C-2), 17.9 (q, C-17).
12) Terashima S., Shimizu M., Nakayama H., Ishikura M., Ueda Y., Imai K., Suzui A., Morita N., *Chem. Pharm. Bull.*, **38**, 2733-2736 (1990); Kador P. F., Sharpless N. E., *Biophys. Chem.*, **8**, 81-85 (1978).
13) Varma S. D., Mikuni I., Kinoshita J. H., *Science*, **188**, 1215-1216 (1975).
14) Terashima H., Hama K., Yamamoto R., Tsuboshima M., Kikkawa R., Hatanaka I., Shigeta Y., *J. Pharmacol. Exp. Ther.*, **229**, 226-230 (1984).

(Received January 9, 1997; accepted February 13, 1997)