Reassignment of the Configuration of Salvianolic Acid B and Establishment of Its Identity with Lithospermic Acid B

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Salvianolic acid B and lithospermic acid B are the major components of *Salvia miltiorrhiza*, which is one of the most popular herbal traditional medicines in Asian countries. Salvianolic acid B and lithospermic acid B are reported to have identical structures except for the configurational assignments of two stereocenters. Through chemical correlation between a degradation product of salvianolic acid B and synthetic material, the absolute configuration of salvianolic acid B has been corrected to establish that salvianolic acid B and lithospermic acid B are in fact the same compound.

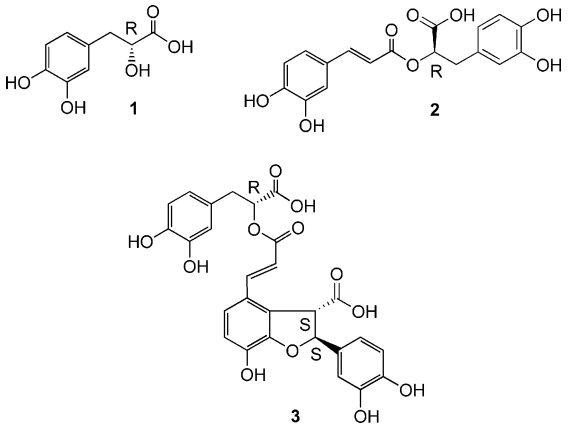
The dried root of *Salvia miltiorrhiza* (Danshen in Chinese) is one of the most popular herbal traditional medicines in Asian countries and has been used extensively for the treatment of coronary artery diseases, angina pectoris, myocardial infarction, cerebrovascular diseases, various types of hepatitis, chronic renal failure, and dysmenorrhea.[1] In the United States and European countries, Danshen products have been widely used for the treatment of cardiovascular and cerebrovascular diseases, whereas in China the specific clinical use is to treat angina pectoris. In Japan, Danshen products are used to promote circulation and improve blood flow.

Early studies of the chemical constituents of Danshen mainly focused on the lipophilic compounds. More than 30 diterpenes have been isolated and identified and their pharmacological activities studied.[2] Recent studies have focused more on the hydrophilic compounds with caffeic acid derivatives occurring as the major water-soluble components.[3] Twenty-five caffeic acid derivatives have been isolated and identified from the aqueous extracts of Danshen including caffeic acid monomers and oligomers that are called salvianolic acids or lithospermic acids.[4] Many of these derivatives have known pharmacological activities, and a wide variety of biological activities have been evaluated.[1,5] The structures of isolated caffeic acid derivatives have been elucidated and classified as caffeic acid monomers, to which 3-(3,4-dihydroxyphenyl)lactic acid (**1**) belongs, caffeic acid dimers, of which rosmarinic acid (**2**) is the simplest representative, and caffeic acid oligomers such as lithospermic acid (**3**), which is characterized by the presence of a dihydrobenzofuran nucleus (Scheme 1).

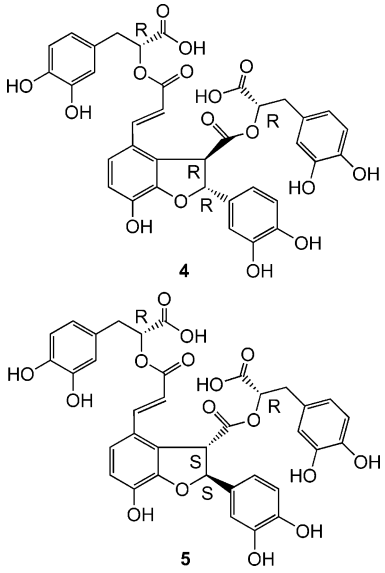
Among the polyphenolic compounds, salvianolic acid B is a major component in *S. miltiorrhiza* (Scheme 2),[6] and extensive pharmacological studies have been reported for this compound. In animal studies, salvianolic acid B has shown several beneficial cardiovascular effects.[7] The configurational assignments for salvianolic acid B were based on chemical degradation and circular dichroic correlation.[6] In particular, the α,*β*-positions of the dihydrobenzofuran core were assigned the *R*/*R*-configuration (structure **4**, Scheme 2).

Lithospermic acid B (**5**) has also been identified as a major component of *S. miltiorrhiza* (Scheme 2). The therapeutic effects of lithospermic acid B and its salt, magnesium lithospermate B, have been investigated extensively, including hepatoprotection,[8] elicitation of endothelium-dependent vasodilation,[9] lowering of blood pressure in hypertension,[10] and inhibition of HIV-1 replication.[11] The absolute configuration of lithospermic acid B was based on the chemical degradation of permethylated lithospermic acid B and circular dichroic correlation.[12] Using the exciton chirality method, the dihydrobenzofuran core was assigned the *S*/*S*-configuration (Scheme 2).

Scheme 1. Structures of 3-(3,4-Dihydroxyphenyl)lactic Acid (**1**), Rosmarinic Acid (**2**), and Lithospermic Acid (**3**)



Scheme 2. Reported Structures of Salvianolic Acid B (**4**) and Lithospermic Acid B (**5**)



Previous literature suggests that the structures of lithospermic acid B and salvianolic acid B differ only in the absolute configuration of the stereocenters present in the dihydrobenzofuran core. Because each is reported to be the major component from the same plant using similar isolation techniques,[13] we postulated that they are in fact the same compound. This implies that the configurational assignments for one of the structures are incorrect. For this reason

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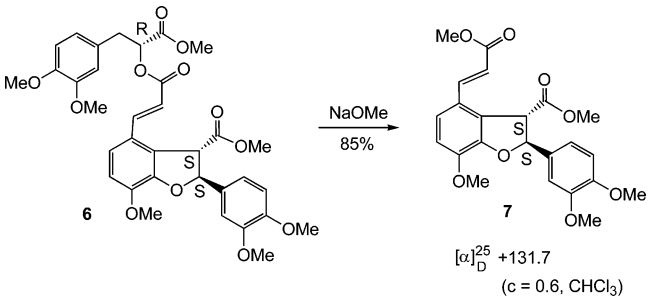
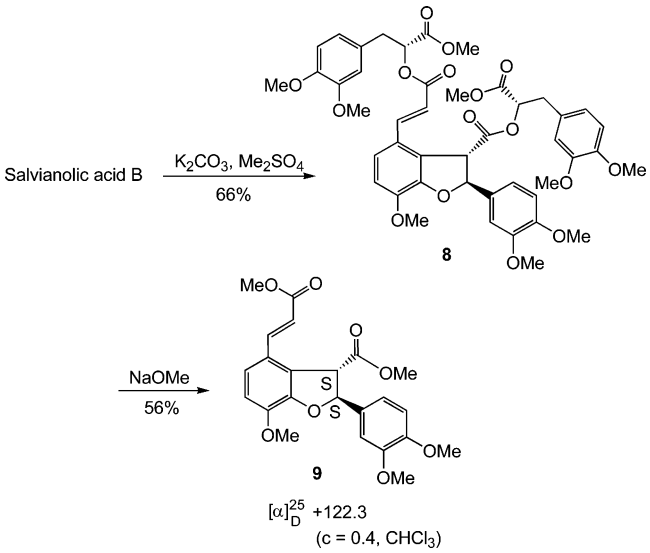
Scheme 3. Methanolysis of Lithospermic Acid Synthetic Intermediate



Scheme 4. Methylation and Degradation of Commercial Salvianolic Acid B



we have carried out further investigations on the absolute configuration of these compounds.

Recently, we reported the total synthesis of lithospermic acid (**3**, Scheme 1),[14] which has attracted interest due to its potent inhibitory activity against HIV-1 integrase.[11] The configurational assignments were established by X-ray analysis using anomalous dispersion of a bromo-substituted derivative of a late-stage synthetic intermediate.[14] Here we have used the synthetic sequence to make the configurational assignments for salvianolic acid B by chemical correlation. Specifically, the synthetic permethylated lithospermic acid derivative **6**, with *S*/*S*-configuration at the dihydrobenzofuran ring, was treated with sodium methoxide to provide **7** (Scheme 3). The specific rotation was determined to have a large positive value.

According to the literature, the configurational assignments for salvianolic acid B were established by permethylation of salvianolic acid B followed by methanolysis to provide a dihydrobenzofuran derivative, which by circular dichroic correlation was proposed to be the enantiomer of **7**.[6] Interestingly, for lithospermic acid B other researchers assigned the opposite configuration to the stereocenters present in the dihydrobenzofuran core using the same degradation method but different circular dichroism techniques.[12]

We submitted commercially available salvianolic acid B to the degradation steps previously reported (Scheme 4). After permethylation and final methanolysis of salvianolic acid B, compound **9** was found to have the same positive specific rotation and relative magnitude as that for the synthetic compound **7** (Scheme 3).[15] In addition, the CD spectra for compounds **7** and **9** are identical. These results clearly show that the absolute configurations previously assigned to the dihydrobenzofuran stereocenters of salvianolic acid B were incorrect and demonstrate that salvianolic acid B and lithospermic acid B are the same compound.

In conclusion, through chemical correlation we have corrected the configurational assignments for salvianolic acid B and thereby have established that salvianolic acid B and lithospermic acid B are identical. These results have profound pharmacological implications because it means that the extensive pharmacological studies that have separately been reported for salvianolic acid B and lithospermic acid B must now both be considered when evaluating the bioactivity of this natural product. Moreover, the configurational assignments provided here are of critical importance to any future synthesis of the natural product or its analogues.

Experimental Section

General Experimental Procedures. Materials were obtained from commercial suppliers and used without further purification. Salvianolic acid B was purchased from Ivy Fine Chemicals (Cherry Hill, NJ). All organic reactions were performed under an atmosphere of N_2 in flame-dried or oven-dried glassware. Thin-layer chromatography was performed on Merck 60 F254 250 μm silica gel plates. Visualization of the developed chromatogram was performed by fluorescence quenching or $KMnO_4$ stain. Flash chromatography was carried out using Merck 60 230−240 mesh silica gel. ^{1}H and ^{13}C NMR spectra were obtained in $CDCl_3$. NMR chemical shifts are reported in ppm and referenced to residual protonated solvent. A Perkin-Elmer 241 polarimeter with a sodium lamp was used to determine optical rotations, and concentrations are reported in g/dL. CD spectra were measured with a AVIV 60DS apparatus.

Compound 4, salvianolic acid B: ^{1}H NMR ($(CD_3)_2CO$, 400 MHz) δ 7.85 (1H, d, J = 16.0 Hz, C*H*═CHCO$_2$CH$_3$), 7.63 (1H, d, J = 16.0 Hz, Ar*H*), 6.90 (1H, d, J = 8.4 Hz, Ar*H*), 6.84−6.64 (8H, m, Ar*H* × 8), 6.44 (1H, dd, J = 2.0, 8.0 Hz, Ar*H*), 6.29 (1H, d, J = 16.0 Hz, CH═C*H*CO$_2$CH$_3$), 5.88 (1H, d, J = 4.4 Hz, ArOC*H*Ar), 5.23−5.19 (2H, m, ArCH$_2$C*H*CO$_2$CH$_3$ × 2), 4.47 (1H, d, J = 4.4 Hz, ArC*H*CO$_2$R), 3.12−2.90 (4H, m, ArC*H*$_2$CHCO$_2$CH$_3$ × 2); ^{13}C NMR ($(CD_3)_2CO$, 100 MHz) δ 171.8, 171.5, 170.9, 166.9, 148.9, 146.6, 146.3, 145.9, 145.9, 145.1, 145.0, 144.8, 143.1, 133.6, 129.3, 128.9, 126.4, 124.8, 122.1, 122.0, 121.9, 118.6, 118.5, 117.6, 117.5, 117.0, 116.4, 116.4, 116.3, 113.5, 87.8, 75.1, 74.1, 57.3, 37.7, 37.3; CD (*c* 0.066, EtOH) {Φ} (nm) +10 204 (340), +8761 (320), +6592 (300), −3009 (283), −13 629 (265), +25 614 (255), +9692 (240), +3647 (223).

Compound 6, heptamethyl-(+)-lithospermic acid. Heptamethyl lithospermic acid was prepared by total synthesis as previously reported:[14] $[\alpha]^{25}_D$ +134.50 (*c* 0.49, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.71 (1H, d, J = 15.9 Hz, C*H*═CHCO$_2$CH$_3$), 7.17 (1H, d, J = 8.5 Hz, Ar*H*), 6.89−6.85 (3H, m, Ar*H* × 3), 6.81 (1H, d, J = 8.2 Hz, Ar*H*), 6.77−6.74 (3H, m, Ar*H* × 3), 6.29 (1H, d, J = 15.9 Hz, CH═C*H*CO$_2$CH$_3$), 6.01 (1H, d, J = 5.6 Hz, ArOC*H*Ar), 5.31 (1H, dd, J = 4.8, 8.2 Hz, ArCH$_2$C*H*CO$_2$CH$_3$), 4.45 (1H, d, J = 5.6 Hz, ArC*H*CO$_2$CH$_3$), 3.91 (3H, s, OC*H*$_3$), 3.84 (3H, s, OC*H*$_3$), 3.83 (3H, s, OC*H*$_3$), 3.82 (3H, s, OC*H*$_3$), 3.81 (3H, s, OC*H*$_3$), 3.71 (6H, s, CO$_2$C*H*$_3$ × 2), 3.16 (1H, dd, J = 4.7, 14.3 Hz, one of ArC*H*$_2$CHCO$_2$CH$_3$), 3.09 (1H, dd, J = 8.2, 14.3 Hz, one of ArC*H*$_2$CHCO$_2$CH$_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.4, 170.0, 165.8, 149.0, 149.0, 148.5, 148.2, 147.8, 146.1, 142.2, 132.1, 128.8, 128.1, 124.7, 124.1, 121.2, 120.6, 117.7, 116.2, 112.7, 112.2, 110.9, 110.9, 108.5, 87.2, 72.8, 55.9, 55.6, 55.6, 55.6, 55.5, 52.5, 52.0, 36.8.; FAB(+)-HRMS *m*/*z* 636.2216 (calcd for $C_{34}H_{36}O_{12}$ [M]$^+$, 636.2207).

Compound 7. Methanolysis of Synthetic Heptamethyllithospermic Acid. To a solution of 25 mg (0.038 mmol) of heptamethyllithospermic acid in 3 mL of $CHCl_3$ was added dropwise 1.5 equiv of NaOMe (0.094 mmol, 157 μL of a 0.6 N solution in MeOH) at 0 °C. The reaction mixture was stirred for 1 h at 0 °C and one further hour at room temperature. After addition of 50 μL of HOAc and 50 μL of H_2O the solvent was evaporated under reduced pressure and the crude product was purified by column chromatography [silica gel, elution with CH_2Cl_2−Et_2O (95:5)], yielding **7** as an off-white solid (14 mg, 0.033 mmol, 85%): $[\alpha]^{25}_D$ +131.7 (*c* 0.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.67 (1H, d, J = 15.6 Hz, C*H*═CHCO$_2$CH$_3$), 7.18 (1H, s, J = 8.4 Hz, Ar*H*), 6.90−6.86 (3H, m, Ar*H* × 3), 6.80 (1H, d, J = 8.0 Hz, Ar*H*), 6.26 (1H, d, J = 16.0 Hz, CH═C*H*CO$_2$CH$_3$), 6.00 (1H, d, J = 5.6 Hz, ArOC*H*Ar), 4.45 (1H, d, J = 6.0 Hz, ArC*H*CO$_2$CH$_3$), 3.91 (3H, s, OC*H*$_3$), 3.84 (3H, s, OC*H*$_3$), 3.83 (3H, s, OC*H*$_3$), 3.75 (3H, s, CO$_2$C*H*$_3$), 3.75 (3H, s, CO$_2$C*H*$_3$); ^{13}C NMR ($CDCl_3$, 100 MHz)

δ 171.8, 167.3, 149.3, 149.2, 148.4, 146.1, 141.1, 132.3, 125.0, 124.5, 120.5, 118.0, 117.3, 112.9, 111.1, 108.7, 87.5, 56.1, 55.9, 52.8, 51.6; FAB(+)-HRMS *m*/*z* 428.1479 (calcd for $C_{23}H_{24}O_8$ $[M]^+$, 428.1471); CD (*c* 0.066, $CHCl_3$) {Φ} (nm) +10 547 (340), +13 237 (320), +10 230 (300), +40 481 (265), +90 233 (256), +15 654 (240), +5344 (227).

Compound 8. Methylation of Salvianolic Acid B. To a suspension of 40 mg (0.0557 mmol) of salvianolic acid B and 20 equiv of K_2CO_3 (1.11 mmol, 154 mg) in 5 mL of dry acetone was added 30 equiv of Me_2SO_4 (1.67 mmol, 158 μL). The reaction mixture was heated to reflux for 5 h. After filtration of the inorganic salts the solvent was evaporated under reduced pressure and the crude product was purified by column chromatography [silica gel, benzene−acetone (9:1)], yielding **8** as an off-white solid (31 mg, 0.037 mmol, 66%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.52 = (1H, d, *J* = 16.0 Hz, C*H*═CHCO$_2$CH$_3$), 7.12 (1H, d, *J* = 8.4 Hz, Ar*H*), 6.87−6.73 (7H, m, Ar*H* × 7), 6.65 (1H, d, *J* = 8.0 Hz, Ar*H*), 6.52 (2H, m, Ar*H*), 6.16 (1H, d, *J* = 16.0 Hz, CH═C*H*CO$_2$CH$_3$), 5.99 (1H, d, *J* = 5.6 Hz, ArOC*H*Ar), 5.28−5.20 (2H, m, ArCH$_2$C*H*CO$_2$CH$_3$ × 2), 4.40 (1H, d, *J* = 6.0 Hz, ArC*H*CO$_2$R), 3.92 (3H, s, OC*H*$_3$), 3.83 (3H, s, OC*H*$_3$), 3.82 (3H, s, OC*H*$_3$), 3.82 (3H, s, OC*H*$_3$), 3.81 (3H, s, OC*H*$_3$), 3.79 (3H, s, OC*H*$_3$), 3.77 (3H, s, OC*H*$_3$), 3.67 (3H, s, CO$_2$C*H*$_3$), 3.66 (3H, s, CO$_2$C*H*$_3$), 3.11−2.94 (4H, m, ArC*H*$_2$CHCO$_2$CH$_3$ × 2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.2, 170.2, 169.3, 165.9, 149.2, 149.2, 148.7, 148.6, 148.5, 148.0, 147.8, 146.2, 142.0, 132.2, 128.4, 127.9, 124.5, 124.3, 121.5, 120.8, 120.8, 118.0, 116.2, 112.8, 112.3, 111.1, 108.6, 87.2, 74.1, 73.1, 56.3, 56.1, 55.9, 55.8, 55.8, 55.7, 55.6, 52.4, 52.2, 37.1, 36.6; FAB(+)-HRMS *m*/*z* 844.2950 (calcd for $C_{45}H_{48}O_{16}$ $[M]^+$, 844.2942).

Compound 9. Degradation of Nonamethylsalvianolic Acid B. To a solution of 14 mg (0.0166 mmol) of nonamethyl salvianolic acid B in 3 mL of $CHCl_3$ was added dropwise at 0 °C 3 equiv of NaOMe (0.0498 mmol, 83 μL of a 0.6 N solution in MeOH). The reaction mixture was stirred for 1 h at 0 °C and one further hour at room temperature. After addition of 50 μL of HOAc and 50 μL of H_2O the solvent was evaporated under reduced pressure and the crude product was purified by column chromatography [silica gel, CH_2Cl_2−Et_2O (95:5)], yielding **9** as an off-white solid (4 mg, 0.009 mmol, 56%): $[\alpha]_D^{25}$ +122.3 (*c* 4.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.68 (1H, d, *J* = 16.0 Hz, C*H*═CHCO$_2$CH$_3$), 7.18 (1H, d, *J* = 8.8 Hz, Ar*H*), 6.90−6.85 (3H, m, Ar*H* × 3), 6.80 (1H, d, *J* = 8.4 Hz, Ar*H*), 6.26 (1H, d, *J* = 16.0 Hz, CH═C*H*CO$_2$CH$_3$), 6.00 (1H, d, *J* = 5.6 Hz, ArOC*H*Ar), 4.46 (1H, d, *J* = 5.6 Hz, ArC*H*CO$_2$CH$_3$), 3.92 (3H, s, OC*H*$_3$), 3.84 (3H, s, OC*H*$_3$), 3.84 (3H, s, OC*H*$_3$), 3.75 (3H, s, CO$_2$C*H*$_3$), 3.71 (3H, s, CO$_2$C*H*$_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.8, 167.3, 149.3, 149.2, 148.4, 146.1, 141.1, 132.3, 125.0, 124.5, 120.5, 118.0, 117.3, 112.9, 111.1, 108.7, 87.5, 56.1, 55.9, 52.8, 51.6; FAB(+)-HRMS 428.1479 *m*/*z* (calcd for $C_{23}H_{24}O_8$ $[M]^+$, 428.1471); CD (*c* 0.066, $CHCl_3$) {Φ} (nm) +7841 (340), +10 051 (320), +8561 (300), +27 046 (265), +66 119 (256), +14 445 (240), +1246 (227).

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Supporting Information Available: Includes ^{1}H and ^{13}C NMR spectra of compounds **4**, **7**, **8**, and **9** and CD spectra of compounds **4**, **7**, and **8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) Salvianolic acid was extracted from *S. miltiorrhiza* Radix with water and isolated by MCI-gel and Sephadex LH-20 column chromatography (ref 6). Lithospermic acid B was extracted from *S. miltiorrhiza* Radix with EtOH and H_2O and isolated by SiO_2 and Sephadex LH-20 column chromatography (ref 12).

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(15) The NMR data for **9** are identical to the NMR data reported by Ai and Li for the same degradation product in their configurational assignment of salvianolic acid B.[6] The specific rotation of **9** in EtOH [+88 (*c* 0.1, EtOH)] also has the same sign as the specific rotation reported by Ai and Li [+41 (*c* 0.092, EtOH)]. The CD spectrum of compound **9** [Supporting Information] also corresponds to the data reported by Ai and Li.[6] This comparison of analytical data confirms that the same degradation product, i.e., identical structure and configuration, was obtained in both of our studies.

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