Salvianolic Acid I: A New Depside from *Salvia cavaleriei*

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Abstract

A new depside named salvianolic acid I was isolated from the aqueous extract of *Salvia cavaleriei*, along with salvianolic acids A, B, C, H, isosalvianolic acid C, lithospermic acid, and rosmarinic acid. The chemical structures were determined by spectral analysis. Two methylated caffeic acid dimers were synthesized using ferulic acid as the starting material.

Key words

Salvia cavaleriei, Lamiaceae, salvianolic acid I, depsides, methylated caffeic acid dimers.

Introduction

Salvia cavaleriei Levl. (Lamiaceae) is a herbal medicinal plant distributed in the southern part of China. It has been used in folk medicine for the treatment of dysentery, hemoptysis, boils, and fall injuries (1). The chemical constituents of this plant have not been reported. In our previous papers, we reported the isolation of several depsides from *S. miltiorrhiza* (2–5), *S. chinensis* (6), and *S. cavaleriei* var. *simplicifolia* (7). Pharmacological studies of these depsides showed that they all possess protective actions against peroxidation damage to liver microsomes, hepatocytes, and erythrocytes of rats (8). During our investigation on the biologically active components of *S. cavaleriei* we isolated eight depsides, among which salvianolic acid I is a new compound. The present paper deals with the structure elucidation of this new depside and the synthesis of two methylated caffeic acid dimers.

Materials and Methods

General experimental procedures

UV spectra were obtained on a Shimadzu UV-300 spectrometer; optical rotations were measured on a Perkin-Elmer 241; ^{1}H-NMR (90 and 500 MHz) and ^{13}C-NMR (125 MHz) spectra were recorded on Jeol FX-90Q and AM-500 spectrometers with TMS as internal standard. Mass spectra were obtained using ZAB-2F and MAT-711 spectrometers. Low pressure liquid chromatography was carried out on a UVILOG ALPC-100, using a Lichoprep RP-18 (40–60 μm) Lobar column (31 cm × 25 mm i.d.) with MeOH-H_2O-HCOOH (45 : 55 : 0.4) as eluant (flow rate 1 ml/min) and UV detector at 288 nm. SiO_2, 140–200 mesh (Qingdao Marine Chemical Factory) was used for column chromatography and SiO_2 GF_{254} for TLC.

Plant material

The whole plants of *S. cavaleriei* Levl. were collected on the Emei mountain in Sichuan province, China, and identified by Prof. Song Wan-Zhi of our Institute. A voucher specimen is deposited in the Institute of Materia Medica in Beijing.

Extraction and isolation of the depsides

The dried plant material (8 kg) was extracted with H_2O under reflux and the aqueous extract concentrated under reduced pressure. EtOH was added to the concentrate until the EtOH content was 70%. After filtering, the filtrate was concentrated to 2000 ml, acidified with 10% HCl to pH 4–5, and successively extracted with $CHCl_3$ and EtOAc. Evaporation of the EtOAc extract yielded 50 g of amorphous brown powder which was subjected to Sephadex LH-20 column chromatography (40 cm × 40 mm i.d.) and eluted with MeOH. Further isolation by preparative TLC with $CHCl_3$-MeOH-HCOOH (85 : 15 : 1) as solvent yielded salvianolic acids A, B, C, isosalvianolic acid C, lithospermic acid, rosmarinic acid, and an amorphous mixture. The latter was subjected to low pressure liquid chromatography yielding 15 mg of salvianolic acid H and 23 mg of salvianolic acid I (**1a**). Rosmarinic acid was obtained as the major component.

Salvianolic acid I (**1a**): amorphous powder, $[\alpha]_D^{14}$: +71° (EtOH, c = 0.1): UV λ_{max}^{EtOH} nm (log ε): 205 (4.76), 220 (sh, 4.50), 256 (4.09), 286 (4.39), 300 (4.35), 318 (4.38); FDMS m/z: 538 (M^+); ^{1}H-NMR, Table **1**, ^{13}C-NMR, Table **2**.

Methylation of **1a**

Dry K_2CO_3 (50 mg) was suspended in a solution of **1a** (15 mg) in anhydrous acetone (5 ml) under an atmosphere of N_2. Me_2SO_4 (1 ml) was added dropwise and the mixture stirred for 36 h. After filtration and concentration, the residue was purified by SiO_2 VLC using a mixture of petroleum ether and EtOAc with increasing polarity. Methylated **1b** (15 mg) was obtained as a colourless gum.

Compound **1b**: HRMS m/z (%): 636.2205 (M^+, 25) ($C_{34}H_{36}O_{12}$ requires 636.2207), 414 (40), 352 (35), 222 (100), 191 (13), 181 (2), 163 (1), 151 (37).

Synthesis of 5 *and* 6

Preparation of **2**: 4 g of ferulic acid were methylated as described for the methylation of **1a** yielding 4.2 g of a methylated product, which was dissolved in 50 ml of CCl_4, and cooled in an ice bath, 3.1 ml of Br_2 were dropwise added, and the resultant white precipitate was filtered, yielding 7.2 g of **2**: ^{1}H-NMR (Me_2CO-d_6): δ 3.78, 3.80, 3.82 (each 3H, s), 4.40, 4.90 (each 1H, d, J = 8); 7.00 (3H, m).

Preparation of **3** *and* **4**: Dry K_2CO_3 (500 mg) and NaI (4 mg) were added to a solution of methyl ferulate (208 mg) and **2** (380 mg) in anhydrous acetone (20 ml), the mixture was refluxed for 5 h. After filtration and removal of the solvent, the residue was purified by TLC using petroleum ether-EtOAc (2 : 1) as solvent, yielding 350 mg of yellowish gum of **3** and **4**.

Preparation of **5** and **6**: NaOH (100 mg) was added to a solution of **3** and **4** (350 mg) in DMSO (40 ml), the mixture was stirred at 100 °C for 8 h. The mixture was then poured into H_2O and extracted with EtOAc. The EtOAc extract was dried over Na_2SO_4, after removal of the solvent, the residue was applied on TLC and developed with petroleum ether-EtOAc (2 : 1), yielding 15 mg of **5** and 25 mg of **6**.

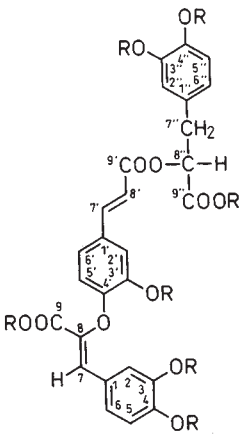
Compound **5**: yellowish gum, EI-MS *m/z* (%): 428 (M^+, 95), 397 (50), 221 (100), 165 (100); UV λ_{max}^{EtOH} nm (log ε): 205 (4.34), 215 (4.32), 230 (4.29), 258 (3.96), 295 (4.32), 305 (4.31), 317 (4.32).

Compound **6**: yellowish gum: EI-MS *m/z* (%): 428 (M^+, 100), 397 (50), 221 (80), 165 (100); UV λ_{max}^{EtOH} nm (log ε): 205 (4.40), 225 (4.31), 255 (3.99), 288 (4.30), 310 (sh, 4.19).

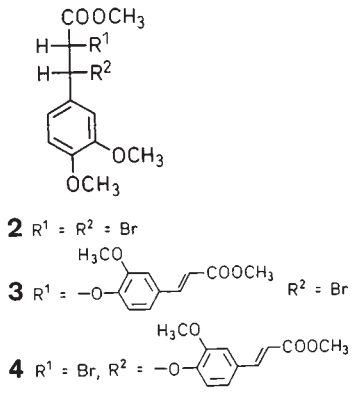
Results and Discussion

Investigation of the aqueous extract of *S. cavaleriei* yielded a new depside named salvianolic acid I (**1a**), together with seven known depsides, salvianolic acids A, B, C, H, isosalvianolic acid C, lithospermic acid, and rosmarinic acid.

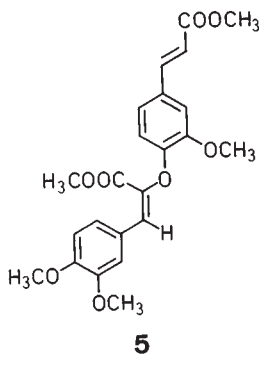
Salvianolic acid I (**1a**), an amorphous powder, showed UV maxima at 205, 220, 286, and 318 nm indicating the presence of a cinnamoyl group. The FD-MS displayed M^+ at *m/z* = 538, while the HR-MS of its methylat-



1a R = H
1b R = CH_3



2 R^1 = R^2 = Br
3 R^1 = –O– ; R^2 = Br
4 R^1 = Br, R^2 = –O– ;



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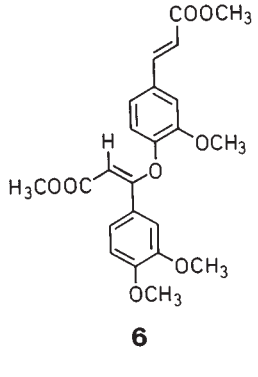

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Table 1 ^{1}H-NMR spectral data of **1a**, **1b**, **5**, and **6** (500 MHz).

H	1a	1b	5	6
2-H	7.30 (d, *J* = 2)	7.37 (d, *J* = 2)	7.38 (d, *J* = 2)	7.53 (d, *J* = 2)
5-H	6.80 (d, *J* = 8)	6.82 (d, *J* = 8)	6.84 (d, *J* = 8)	7.20 (d, *J* = 8)
6-H	7.12 (dd, *J* = 2/8)	7.19 (dd, *J* = 2/8)	7.19 (dd, *J* = 2/8)	7.31 (dd, *J* = 2/8)
7-H	7.37 (s)	7.38 (s)	6.03 (s)	5.04 (s)
3-OCH_3		3.84 (s)	3.76 (s)	3.82 (s)
4-OCH_3		3.86 (s)	3.79 (s)	3.83 (s)
9-OCH_3		3.77 (s)	3.60 (s)	3.49 (s)
2′-H	7.18 (d, *J* = 2)	7.12 (d, *J* = 2)	7.20 (d, *J* = 2)	7.33 (d, *J* = 2)
5′-H	6.79 (d, *J* = 8)	6.75 (d, *J* = 8)	6.78 (d, *J* = 8)	6.91 (d, *J* = 8)
6′-H	7.01 (dd, *J* = 2/8)	6.97 (dd, *J* = 2/8)	7.00 (dd, *J* = 2/8)	7.31 (dd, *J* = 2/8)
7′-H	7.60 (d, *J* = 16)	7.61 (d, *J* = 16)	7.50 (d, *J* = 16)	7.66 (d, *J* = 16)
8′-H	6.39 (d, *J* = 16)	6.33 (d, *J* = 16)	6.35 (d, *J* = 16)	6.57 (d, *J* = 16)
3′-OCH_3		3.98 (s)	3.98 (s)	3.96 (s)
9′-OCH_3			3.65 (s)	3.74 (s)
2″-H	6.79 (d, *J* = 2)	6.79 (m)		
5″-H	6.76 (d, *J* = 8)	6.79 (m)		
6″-H	6.68 (dd, *J* = 2/8)	6.79 (m)		
7″-H	3.04 (dd, *J* = 4/14)	3.13 (dd, *J* = 4/14)		
	3.14 (dd, *J* = 8/14)	3.18 (dd, *J* = 8/14)		
8″-H	5.22 (dd, *J* = 4/8)	5.36 (dd, *J* = 4/14)		
3″-OCH_3		3.74 (s)		
4″-OCH_3		3.86 (s)		
9″-OCH_3		3.74 (s)		

Compounds **1a**, **5**, and **6** were measured in Me_2CO-d_6, **1b** in $CDCl_3$ (*J* values in Hz).

Table 2 ^{13}C-NMR spectral data of **1a** (in Me_2CO-d_6, 125 MHz).

C		C		C	
1	125.84	1′	130.82	1″	129.45
2	118.19	2′	116.66	2″	117.65
3	146.35	3′	146.35	3″	146.13
4	148.78	4′	148.33	4″	145.23
5	115.68	5′	116.50	5″	116.38
6	124.95	6′	122.15	6″	121.66
7	129.22	7′	146.67	7″	37.87
8	139.31	8′	116.44	8″	74.91
9	167.02	9′	168.15	9″	173.68

ed product (**1b**) showed M^+ at m/z = 636.2205 (calcd. for $C_{34}H_{36}O_{12}$ 636.2207) and diagnostic fragments of a β-(3,4-dimethoxyphenyl)lactic ester (m/z = 222, 191, 181, 163, 151). The ^{1}H-NMR spectrum of **1a** (Table 1) resembled that of salvianolic acid H (7), having three sets of ABX aromatic protons. Thus, **1a** might be an isomer of salvianolic acid H. NOE experiments on the methylated compound **1b** showed an enhancement of the aromatic proton doublet at δ = 7.12 (d, J = 2 Hz) upon irradiation of the methoxy signal at δ = 3.98; on the other hand, a nuclear Overhauser effect was observed between the olefinic proton singlet at δ = 7.38 and the carboxymethyl signal at δ = 3.77. Structure **1b** was in agreement with these results. In order to verify this structure, a methylated caffeic acid dimer was synthesized using ferulic acid as starting material. Methylation and bromine addition to ferulic acid yielded a pair of enantiomers (**2**) whose favoured conformation should be in the *"anti"* form. A neighbouring group mechanism during substitution of either bromine with methyl ferulate yielded two pairs of C-8 (**3**) and C-7 (**4**) substituted enantiomers with retention of configuration. HBr elimination yielded the dimers **5** and **6** with the *trans*-configuration. The C-7 olefinic proton singlet in the ^{1}H-NMR spectrum of **6** (δ = 6.03) was shifted to higher field in comparison to that of **1b**. This can be explained in terms of a shielding effect of the *cis*-aromatic nucleus. Attempts to isomerize the C-7 (8) double bond of **5** to the *cis*-configuration were unsuccessful. Based on these results, structure **1a** was elucidated for salvianolic acid I. The fact that the known depsides from this plant possess an *R*-(+)-β-(3,4-dihydroxyphenyl)lactic moiety suggested that the C-8″ atom of **1a** may also have an *R* configuration. This was supported by a dextrorotatory optical rotation value of this compound. A relatively high-field shift of the olefinic proton singlet at δ = 5.04 in the ^{1}H-NMR spectrum of **6** was in agreement with the calculated value for this type of trisubstituted olefinic moiety (9).

Acknowledgements

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References

[1] Compilation of Chinese Herb Medicine (1972) Vol. 1, p. 365, People's Publishing House, Beijing.
[2] Li, L.-N., Tan, R., Chen, W.-M. (1984) Planta Med. 50, 227–228.
[3] Ai, C.-B., Li, L.-N. (1988) Lloydia 51, 145–149.
[4] Ai, C.-B., Li, L.-N. (1991) Chinese Chem. Lett. 2, 17–18.
[5] Ai, C.-B., Li, L.-N. (1992) Planta Med. 58, 197–199.
[6] Qian, T.-X., Li, L.-N. (1992) Phytochemistry 31, 1068–1070.
[7] Zhang, H.-J., Li, L.-N. (1993) Chinese Chem. Lett. 4, 501–504.
[8] Liu, G.-T., Zhang, T.-M., Wang, B.-E., Wang, Y.-W. (1992) Biochem. Pharmacol. 43, 147–152.
[9] Gordon, A. J., Ford, R. A. (1972) The Chemist's Companion, p. 269, Wiley-Interscience, New York, London, Sydney, Toronto.