

大紫丹参的多酚类化合物*

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摘要: 从云南丽江产大紫丹参 (*Salvia przewalskii* Maxim.) 的根部分离得到 11 个多酚类化合物, 其中 8 个鉴定为已知的原儿茶醛, 原儿茶酸, 咖啡酸, R-(+)- β -D-(3, 4-二羟基苯基)-乳酸, 迷迭香酸, 迷迭香酸甲酯, 紫草酸和紫草酸 B。另外 3 个为紫草酸 B 的甲酯化衍生物, 即紫草酸 B 二甲酯, 9'-紫草酸 B 单甲酯和 9''-紫草酸 B 单甲酯。它们的结构通过波谱方法得到鉴定。研究结果表明, 大紫丹参含有与正品丹参相似的酚类化合物。

关键词: 鼠尾草属; 大紫丹参; 多酚类化合物

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Polyphenolic Constituents of *Salvia przewalskii*

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Abstract: Three lithospermic acid B ester derivatives, dimethyl lithospermate B, 9'-methyl lithospermate B and 9''-methyl lithospermate B together with nine known polyphenol compounds: protocatechualdehyde, protocatechuic acid, caffeic acid, R-(+)- β -D-(3, 4-dihydroxyphenyl)-lactic acid, rosmarinic acid, methyl rosmarinate, lithospermic acid and lithospermic acid B were isolated from the dried roots of *Salvia przewalskii*.

Key words: *Salvia*; *S. przewalskii*; Polyphenols

Salvia przewalskii Maxim. distributes in Gansu, Sichuan and Yunnan Provinces in western China. In northwest Yunnan, its roots have been used as a substitute of "Dan-Shen", a commonly used crude material of traditional Chinese medicine (Jiangsu College of New Medicine, 1979). Previously studies on this species have mainly focused on the constituents of lipophilic diterpenoid quinones (Yang *et al*, 1984, 1981). As a continuation of our chemical research on the genus *Salvia* (Tanaka *et al*, 1996, 1997; Wu *et al*, 1999), we isolated eleven polyphenols from this plant collected from Lijiang County of Yunnan Province, We report here the isolation and structural determination of these compounds.

Eleven polyphenol compounds (1~11) were isolated from the MeOH extracts of dry roots of *S.*

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przewalskii by macro pore absorption resins, Sephadex LH-20 gel and reverse phase silica gel column chromatographies. Among them, compounds **1**~**8** were determined as protocatechualdehyde (**1**), protocatechuic acid (**2**), caffeic acid (**3**), R-(+)- β -D-(3,4-dihydroxyphenyl)-lactic acid (**4**), rosmarinic acid (**5**), methyl rosmarinate (**6**), lithospermic acid (**7**) and lithospermic acid B (**8**) respectively, by spectral analysis and comparison with authentic samples (Wu *et al*, 1999). Other three, compounds **9**~**11**, are lithospermic acid B ester derivatives.

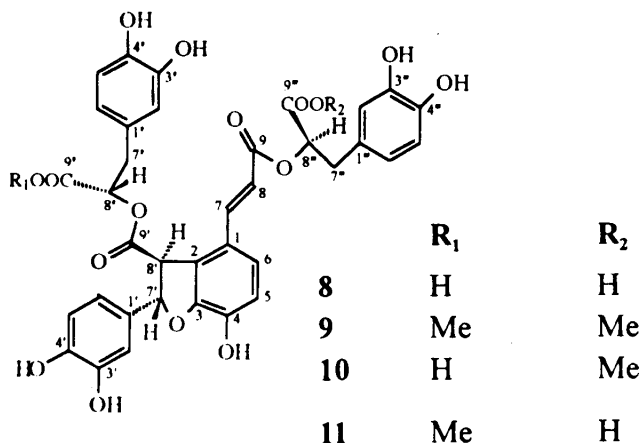
Compound **9** was obtained as a yellow amorphous powder, which is in a dark green color with FeCl_3 reagent on TLC. It showed a quasimolecular ion peak at m/z 745 $[\text{M}(\text{C}_{38}\text{H}_{34}\text{O}_{16}) - \text{H}]^-$ in negative FAB mass spectrum. The ^{13}C NMR spectrum showed the signals of a double bond at δ 143.96 and 116.48; four carbonyl groups at δ 172.18 ($2 \times \text{C}$), 171.19 and 167.96; three oxo-methines at δ 88.16, 74.75 and 75.61; a methine at δ 57.46; two methylenes at δ 37.39 and 37.78; two methoxy groups at δ 52.65 and 52.79; and 24 aromatic carbons at δ 113.37~149.00. Its ^1H NMR spectrum showed the signals of a pair of doublet peaks due to trans-olefinic protons at δ 6.26, 7.63 (each 1H, d, $J = 15.9\text{Hz}$); three oxo-methine protons at δ 4.39, 4.96 and 5.20; two methoxy groups at δ 3.65 and 3.64; two benzylic methylenes in δ 2.83~3.02; and the signals at δ 6.20~7.20 belonging to four aromatic ring protons. A comparison of the ^1H and ^{13}C NMR spectral data of **9** with that of compound **8**, showed that **9** was quite similar to **8**. The difference between them is that there are only more two methoxy group signals appeared in **9**. Moreover, the carbonyl groups of C-9'' and C-9''' at δ 173.52 and δ 172.17 in **8** were upfield shifted to δ 172.18 (-1.34) and δ 171.19 (-0.98) in **9**, respectively. It indicated that two additional methoxy groups were linked at the position carbonyl C-9'' and C-9''' in **9**. Therefore, the structure of **9** was identified as dimethyl lithospermate B.

Compound **10** and **11** were obtained as yellow powders, both had the same quasimolecular ion peak at m/z 731 $[\text{M}(\text{C}_{37}\text{H}_{32}\text{O}_{16}) - \text{H}]^-$ in their negative FAB mass spectra, which were 14 mass units larger than that of compound **8**. By comparison the spectral data of UV, IR, ^1H and ^{13}C NMR, compounds **10** and **11** were closely similar to those of **8** and **9**. This suggests that both **10** and **11** are mono-methyl ester of **8**. By observation of the ^{13}C NMR spectral data of **10** together with that of **8**, the C-9'' carbonyl group at δ 173.52 was upfield shifted to δ 172.20 (-1.32) in **10**. It suggests that an additional methoxy group links C-9'' carbonyl group and the structure of compound **10** could be 9''-methyl lithospermate B. In the same way, by comparison the ^{13}C NMR spectrum of **11** with that of **8**, only the C-9''' carbonyl group at δ 172.17 was upfield shifted to δ 171.20 (-0.97), while the other carbon signals were almost unaffected. It indicates that this additional methoxy group links C-9''' carbonyl group. Therefore, the structure of **11** was established as 9'''-methyl lithospermate B.

It is the first isolation of mono- and di-methoxy esters of lithospermic acid B in natural forms.

Experimental

The instruments and chromatographic materials used throughout this work are the same as described in the reference (Wu *et al*, 1999).



The dried roots of *Salvia przewalskii* Maxim. (10kg) which collected in Lijiang County, Yunnan Province, were extracted with 60% acetone at room temperature ($4 \times 20L$), then concentrated in vacuum to evaporate the acetone. Keeping the water solution for one day, after filtration, the filtrate was acidified with 10% HCl and then extracted with EtOAc. The 100 g of EtOAc extract (230g) was chromatographed on a silica gel column (200 ~ 300 mesh) with benzene: ethyl acetate: formic acid (5:4:1) as developed solvent system and giving three fractions (Fr. A-C). Protocatechualdehyde (**1**, 100mg), protocatechuic acid (**2**, 200mg), caffeic acid (**3**, 200mg) and methyl rosmarinat (**6**, 50mg) were obtained from fraction A (5g), after subjected to Sephadex LH-20 (30% ~ 60% acetone) and MCI-gel CHP20P (30% ~ 50% acetone) column chromatographies. R - (+) - β - D - (3, 4 - dihydroxyphenyl) - lactic acid (**4**, 2g), rosmarinic acid (**5**, 7g), dimethyl lithospermate B (**9**, 150mg), 9'' - methyl lithospermate B (**10**, 100mg) and 9''' - methyl lithospermate B (**11**, 200mg) were obtained from fraction B (30g), after separation by Sephadex LH - 20 (30% ~ 50% acetone) and MCI - gel CHP20P (30% ~ 60% acetone) column chromatographies. The fraction C (20g) was subsequently chromatographed over MCI - gel CHP20P (30% ~ 50% acetone) and Rp - 8 gel (30% ~ 40% acetone) column to afford lithospermic acid (**7**, 1.5g) and lithospermic acid B (**8**, 4.5g).

For the spectral data of compound **1** ~ **8**, see reference (Wu *et al*, 1999).

Dimethyl lithospermate B (9): A yellow amorphous powder; FAB-MS m/z : 745 [$M(C_{38}H_{34}O_{16}) - H$] $^-$; $[\alpha]_D(23.8^\circ C) = +93.43^\circ (c = 0.59, MeOH)$; $IR_{\nu_{max}}^{KBr} cm^{-1}$: 3404, 2957, 1734, 1611, 1521, 1446, 1365, 1287, 1178, 1114, 1070, 1043, 978, 934, 867, 811, 779, 732, 687, 589; UV (MeOH) $\lambda_{max} nm$: 206, 255, 289, 304, 332; 1H and ^{13}C NMR data in Table 1 and 2.

9'' - methyl lithospermate B (10): A yellow amorphous powder, FAB - MS m/z : 731 [$M(C_{37}H_{32}O_{16}) - H$] $^-$; $[\alpha]_D(24.4^\circ C) = +98.23^\circ (c = 0.68, MeOH)$; $IR_{\nu_{max}}^{KBr} cm^{-1}$: 3387, 2958, 1731, 1611, 1519, 1446, 1363, 1178, 1114, 1072, 1043, 976, 935, 867, 811, 779, 724, 589; UV (MeOH) $\lambda_{max} nm$: 206, 254, 289, 306, 329; 1H and ^{13}C NMR data in Table 1 and 2.

9''' - methyl lithospermate B (11): A yellow amorphous powder; FAB - MS m/z : 731 [$M(C_{37}H_{32}O_{16}) - H$] $^-$; $[\alpha]_D(24.50^\circ C) = +98.20^\circ (c = 0.65, MeOH)$; $IR_{\nu_{max}}^{KBr} cm^{-1}$: 3386,

2958, 1732, 1611, 1520, 1446, 1364, 1178, 1114, 1072, 1043, 977, 935, 867, 811, 779, 724, 589; UV (MeOH) λ_{\max} nm: 206, 254, 289, 305, 328; ^1H and ^{13}C NMR data in Table 1 and 2.

Table 1 ^{13}C NMR spectral data of compounds **8**, **9**, **10** and **11** (100MHz, CD_3OD)

C	8	9	10	11
1	124.58	124.60	124.60	124.60
2	126.14	126.22	126.17	126.17
3	148.90	149.00	148.97	148.97
4	144.95	145.15	145.09	145.00
5	118.35	118.67	118.37	118.37
6	122.09	121.96	122.05	121.98
7	143.46	143.96	143.65	143.78
8	116.45	116.48	116.46	116.46
9	167.96	167.96	168.04	167.91
1'	133.50	133.49	133.57	133.48
2'	113.36	113.37	113.37	113.37
3'	146.49	146.70	146.46	146.64
4'	145.68	145.25	145.99	145.84
5'	116.45	116.48	116.46	116.46
6'	118.35	118.35	118.37	118.37
7'	88.12	88.16	88.17	88.17
8'	57.69	57.46	57.78	57.45
9'	172.44	172.18	172.47	172.47
1''	129.18	128.54	128.93	129.27
2''	117.54	117.49	117.29	117.51
3''	144.95	145.15	145.00	145.09
4''	146.32	146.51	146.64	146.46
5''	116.45	116.48	116.46	116.46
6''	122.21	122.16	122.05	122.23
7''	37.67	37.78	37.76	37.76
8''	74.51	74.75	74.68	74.68
9''	173.52	172.18	172.20	173.49
1'''	128.84	128.54	128.76	128.53
2'''	117.27	117.31	117.29	117.51
3'''	144.95	145.15	145.00	145.09
4'''	145.68	145.99	145.84	145.99
5'''	116.45	116.48	116.46	116.46
6'''	121.73	121.83	121.98	121.77
7'''	37.28	37.39	37.41	37.41
8'''	75.40	75.61	75.53	75.53
9'''	172.17	171.19	172.20	171.20
9'' - OCH_3		52.79	52.82	
9''' - OCH_3		52.65		52.66

Table 2 ¹H NMR spectral data of compounds 8, 9, 10 and 11 (400MHz, CD₃OD)

H	8	9	10	11
5	6.85(1H, d, 8.4)	6.85(1H, d, 8.5)	6.84(1H, d, 8.4)	6.84(1H, d, 8.4)
6	7.15(1H, d, 8.5)	7.18(1H, d, 8.5)	7.15(1H, d, 8.4)	7.17(1H, d, 8.3)
7	7.53(1H, d, 15.9)	7.63(1H, d, 15.9)	7.53(1H, d, 16)	7.57(1H, d, 16.0)
8	6.24(1H, d, 15.9)	6.26(1H, d, 15.9)	6.23(1H, d, 16)	6.30(1H, d, 16.0)
2'	6.79(1H, d, 2.1)	6.78(1H, d, 2.0) ^{a, b)}	6.77(1H, d, 2.0) ^{a, b)}	6.77(1H, d, 2.0) ^{a)}
5'	6.77(1H, d, 8.2) ^{a)}	6.71(1H, d, 8.5) ^{a, c)}	6.76(1H, d, 8.1) ^{a)}	6.77(1H, d, 8.2) ^{a)}
6'	6.67(1H, dd, 1.8, 8.0) ^{a, b)}	6.65(1H, dd, 2.0, 8.0) ^{a, d)}	6.67(1H, dd, 2.0, 8.1)	6.67(1H, dd, 1.8, 8.0)
7'	5.87(1H, d, 4.6)	5.83(1H, d, 4.4)	5.80(1H, d, 4.5)	5.87(1H, d, 4.8)
8'	4.38(1H, d, 4.7)	4.39(1H, d, 4.5)	4.38(1H, d, 4.6)	4.38(1H, d, 4.6)
2''	6.77(1H, d, 2.0) ^{a)}	6.76(1H, d, 2.0) ^{a, b)}	6.76(1H, d, 2.0) ^{a, b)}	6.77(1H, d, 2.0) ^{a)}
5''	6.72(1H, d, 8.2)	6.70(1H, d, 8.5) ^{a, c)}	6.71(1H, d, 8.3)	6.70(1H, d, 8.0)
6''	6.64(1H, dd, 2.0, 8.1) ^{a, b)}	6.64(1H, dd, 2.1, 8.2) ^{a, d)}	6.63(1H, dd, 2.0, 8.0) ^{a)}	6.64(1H, dd, 2.0, 8.0)
7''	3.08(2H, dd, 4.0, 8.0) ^{a, c)}	2.98(2H, m) ^{a)}	2.94(2H, m) ^{a)}	2.98(2H, m) ^{a)}
8''	5.21(1H, dd, 3.7, 7.0)	5.19(1H, m)	5.18(1H, m)	5.19(1H, m)
2'''	6.54(1H, d, 2.1)	6.57(1H, d, 1.5)	6.54(1H, d, 2.1)	6.54(1H, d, 2.1)
5'''	6.60(1H, d, 8.0)	6.60(1H, d, 8.1)	6.61(1H, d, 8.4) ^{a)}	6.60(1H, d, 8.0)
6'''	6.33(1H, dd, 2.0, 8.0)	6.38(1H, m)	6.30(1H, m)	6.33(1H, dd, 2.0, 8.0)
7'''	3.04(2H, dd, 4.0, 8.0) ^{a, c)}	2.98(2H, m) ^{a)}	2.94(2H, m) ^{a)}	2.98(1H, m) ^{a)}
8'''	5.12(1H, m)	4.96(1H, s, br.)	4.95(1H, s, br.)	4.96(1H, s, br.)
9'' - OCH ₃		3.64(3H, s)	3.64(3H, s)	
9''' - OCH ₃		3.65(3H, s)		3.65(3H, s)

a) Overlapping with other signals; b - d) Assignments may be interchanged in each column

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