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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甲单酯。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, Sephdex LH－20 gel and reverse phase silica gel column chromatographies. Among them, compounds **1**～**8** were determined as protocatechualdehye (**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**) respectevely, by spectral analysis and comparison with authentic samples (Wu *et al*，1999). Other three, compounds **9**～**11**, are lithospermic acid B ester derivatives.

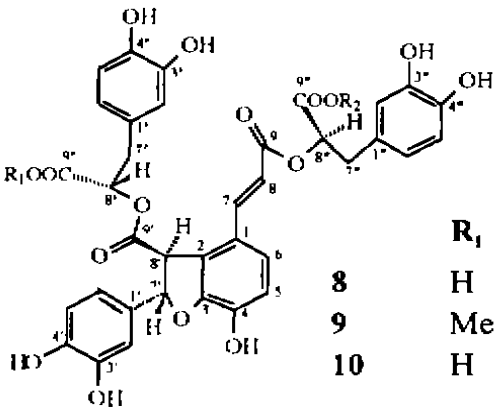
Compound **9** was obtained as a yellow amorphous powder, which is in a dark green color with $FeCl_3$ reagent on TLC. It showed a quasimolecular ion peak at m/z 745 $[M(C_{38}H_{34}O_{16})-H]^-$ in negative FAB mass spectrum. The ^{13}C NMR spectrum showed the signals of a double bond at δ 143.96 and 116.48; four carbonyl groups at δ 172.18 (2×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＝15.9Hz); three oxo－methine protons at δ 4.39, 4.96 and 5.20; two methoxy groups at δ 3.65 and 3.64; two benzoylic 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 $[M(C_{37}H_{32}O_{16})-H]^-$ 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 indicats 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).



	R_1	R_2
8	H	H
9	Me	Me
10	H	Me
11	Me	H

The dried roots of *Salvia przewalskii* Maxim. (10kg) which collected in Lijiang County, Yunnan Province, were extracted with 60% acetone at room temperature (4 × 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 rosmarinate (**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-dihy roxyphenyl)-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℃) = +93.43°(c = 0.59, MeOH); IRν_{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) λ_{max} nm: 206, 255, 289, 304, 332; ^{1}H 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℃) = +98.23° (c = 0.68, MeOH); IRν_{max}^{KBr} cm^{-1}: 3387, 2958, 1731, 1611, 1519, 1446, 1363, 1178, 1114, 1072, 1043, 976, 935, 867, 811, 779, 724, 589; UV (MeOH) λ_{max} nm: 206, 254, 289, 306, 329; ^{1}H 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℃) = +98.20° (c = 0.65, MeOH); IRν_{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; ^{1}H 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 ^{1}H NMR spectral data of compounds **8**, **9**, **10** and **11** (400MHz, CD_3OD)

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, 6)	7.57(1H,d,16.0)
8	6.24(1H,d,15.9)	6.26(1H,d,15.9)	6.23(1H,d, 6)	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 beinterchanged in each column

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