

苣叶丹参的多酚类化合物*

吴志军 欧阳明安 杨崇仁⁺

(中国科学院昆明植物研究所, 昆明 650204)

K 284.1

摘要 自云南省文山州产的中药丹参代用植物苣叶丹参 (*Salvia sonchifolia* C. Y. Wu) 的根中分离得到 9 个多酚类化合物。即：原儿茶醛、原儿茶酸、咖啡酸、R-(+)-β-D-(3,4-二羟基苯基)-乳酸、3,4-二羟基苯基乙醇酮、迷迭香酸、迷迭香酸甲酯、紫草酸和紫草酸 B。它们的结构通过波谱方法得到鉴定。研究结果表明，苣叶丹参含有与正品丹参相似的酚类化合物。

关键词 鼠尾草属, 苣叶丹参, 多酚类化合物

分类号 Q 946

Polyphenolic Constituents of *Salvia sonchifolia* *

WU Zhi - Jun, OUYANG Ming - An, YANG Chong - Ren⁺

(Kunming Institute of Botany, The Chinese Academy of Sciences, Kunming 650204)

Abstract From the fresh root of *Salvia sonchifolia* C. Y. Wu, nine polyphenolic compounds, protocatechualdehyde, protocatechuic acid, caffeic acid, R-(+)-β-D-(3,4-dihydroxyphenyl)-lactic acid, 3,4-dihydroxyphenyl ethanol ketone, rosmarinic acid, methyl rosmarinate, lithospermic acid and lithospermic acid B are isolated. Their structures were identified by spectral method. The result shows that *Salvia sonchifolia* contains the phenolic constituents which are similar to that of medical *Salvia*.

Key words *Salvia*, *Salvia sonchifolia*, Polyphenols

Salvia sonchifolia C. Y. Wu is a new species endemic in the south of Yunnan Province in China (Wu, 1977). It has been used as a local substitute for "Dan-shen" (*Salvia miltiorrhiza* Bunge), a commonly used Chinese traditional medicine herb (Xu, 1990), in its native area. The chemical constituents of this plant have not been reported. Continuing our phytochemical examination of the biological active polyphenols from the species of genus *Salvia* (Tanaka *et al.*, 1996, 1997), in this paper we report the details of the isolation and identification of nine polyphenolic compounds from the polar fraction of *S. sonchifolia* collected in Wenshan county of Yunnan.

RESULTS AND DISCUSSION

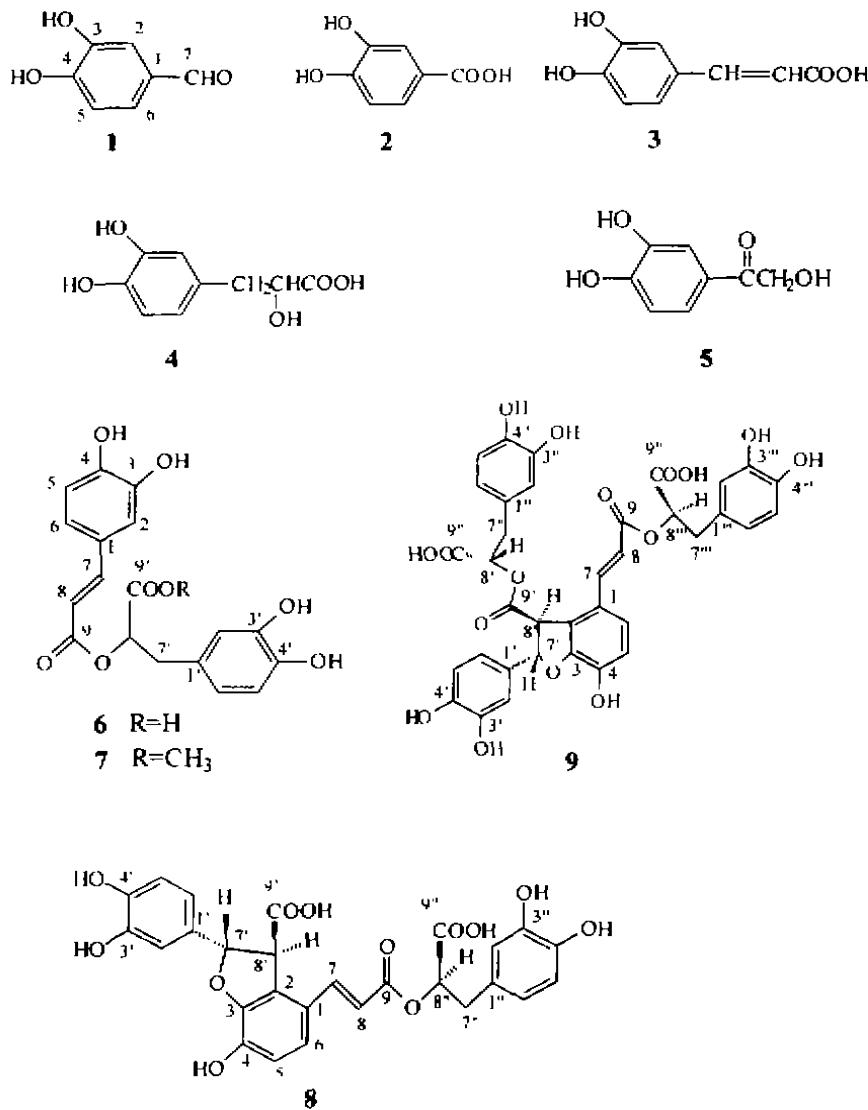
The MeOH extracts of fresh roots of *S. sonchifolia* were separation by macro pore absorption resins, Sephadex LH-20 gel and reverse phase silica gel column chromatographies to afford nine com-

* Projects supported by the Natural Science Foundation of Yunnan province

+ Author to whom correspondence should be addressed

1998-11-04 收稿, 1999-03-09 接受发表

pounds (1~9). All of these compounds are polyphenols and identified as protocatechualdehyde (1), protocatechuic acid (2), caffeic acid (3), R-(+)- β -D-(3,4-dihydroxyphenyl)-lactic acid (4), 3,4-dihydroxyphenyl ethanol ketone (5), rosmarinic acid (6), methyl rosmarinate (7), lithospermic acid (8) and lithospermic acid B (9), respectively. Among them, compound 5 is first time isolated from the genus *Salvia*, as our knowledge. Moreover, it is noticed that the phenol constituents of the roots of *S. sonchifolia* are very similar with that of *S. miltiorrhiza* Bunge. On the basis of this result, the roots of this plant could be as a valuable substitute of "Dan-Shen".



EXPERIMENTAL

General experimental procedures

Optical rotations were measured on a JASCO - 20C digital polarimeter. IR spectra were measured on a Pekin - Elmer 577 spectrometer. UV spectra were obtained on UV - 210A spectrometer. FAB -

MS spectra were measured on a VG Autospc 3000 system spectrometer. ¹H and ¹³C NMR spectra were obtained with Bruker AM - 400 spectrometer. The chemical shifts (δ) were expressed in ppm with reference to the solvent signals. Coupling constants (J) were given in Hz. Chromatographic materials were used Rp - 8 (40 ~ 60 μ m, Merck), Sephadex LH - 20 (25 ~ 100 μ m, Pharmacia Fine Chemical Co. Ltd.), MCI - gel CHP20P (75 ~ 150 μ m, Mitsubishi Chemical Industries, Ltd.) and silica gel (200 ~ 300 mesh, Qingdao Marine Chemical Factory). TLC was developed with benzene: ethyl acetate: formic acid (3:6:1, 5:4:1). The ratio of solvents was given in v/v in each case. Spot of TLC were detected by spraying 5% ethanolic ferric chloride or spraying 5% sulfuric acid following by heating.

Extraction and isolation

S. sonchifolia C. Y. Wu was collected in Wenshan, Yunnan province in October 1995. A voucher specimen is kept in the Herbarium of Kunming Institute of Botany. The fresh roots (6 kg) were extracted with MeOH (3 × 10 L) under reflux and then concentrated in vacuum to give crude extract. The extract was suspended in water and then extracted with chloroform (3 × 1L). The soluble of water layer portion was first subjected to a macro pore resin D101, after washing with H₂O, eluting with MeOH to give a MeOH fraction (10g). This MeOH fraction was subjected to MCI - gel CHP20P column chromatography with H₂O containing increasing proportions of MeOH to give four fractions (Fr. A ~ D). The fraction A (1g) was separated by Sephadex LH - 20 (40% ~ 80% MeOH) and MCI - gel CHP20P (30% ~ 50% MeOH) column chromatography to yield protocatechualdehyde (1, 50mg), protocatechuic acid (2, 40mg) and caffeic acid (3, 150mg). The fraction B (4g) was chromatographed over MCI - gel CHP20P (40% ~ 70% MeOH) and Rp - 8 gel column (30% ~ 50% acetone) to afford R - (+) - β -D - (3,4 - dihydroxyphenyl) - lactic acid (4, 200mg) and 3,4 - dihydroxyphenyl ethanol ketone (5, 50mg). The fraction C (3.5g) was separated by Rp - 8 gel column (30% ~ 70% acetone) to give rosmarinic acid (6, 50mg) and methyl rosmarinate (7, 30mg). The fraction D (1g) was subjected to MCI - gel CHP20P (30% ~ 50% acetone) and Rp - 8 gel column (30% ~ 60% acetone) to afford lithospermic acid (8, 30mg) and lithospermic acid B (9, 50 mg).

Identification of compounds

Protocatechualdehyde (1): A yellow powder; FAB - MS m/z 137 [M(C₇H₆O₃) - H]⁻; ¹³C NMR (CD₃OD): δ 130.71 (C - 1), 115.42 (C - 2), 147.00 (C - 3), 153.62 (C - 4), 116.22 (C - 5), 126.41 (C - 6), 193.14 (C - 7); ¹H NMR (CD₃OD): δ 7.26 (d, J = 1.8Hz, H - 2), 6.89 (d, J = 7.6Hz, H - 5), 7.27 (dd, J = 1.8, 8.2Hz, H - 6), 9.64 (s, - CHO) (Chen *et al.*, 1981).

Protocatechuic acid (2): A yellow powder; FAB - MS m/z 153 [M(C₇H₆O₄) - H]⁻; $[\alpha]_D^{25}$ + 75.88°(c 0.48, CH₃OH); IR ν_{max}^{KBr} cm⁻¹: 3166, 2734, 1717, 1609, 1521, 1449, 1399, 1288, 1263, 1179, 1117, 1067, 979, 868, 812, 589, 457; UV (CH₃OH) λ_{max} nm: 205, 252, 288, 325; ¹³C NMR (CD₃OD): δ 123.17 (C - 1), 115.80 (C - 2), 146.02 (C - 3), 151.49 (C - 4), 117.79 (C - 5), 123.92 (C - 6), 170.21 (C - 7); ¹H NMR (CD₃OD): δ 7.41 (d, J = 2.1Hz, H

-2), 6.80 (d, J=8.2Hz, H-5), 7.43 (s, br., H-6).

Caffeic acid (3): A white powder; FAB-MS m/z 179 [M(C₉H₈O₄) - H]⁻; IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3432, 3237, 1646, 1619, 1522, 1450, 1354, 1280, 1218, 1174, 1119, 1011, 973, 935, 900, 850, 816, 801, 779, 698, 574; UV (CH₃OH) λ_{max} nm: 216, 229, 289, 317; ¹³C NMR (CD₃OD): δ 127.88 (C-1), 115.22 (C-2), 146.69 (C-3), 149.32 (C-4), 116.50 (C-5), 122.80 (C-6), 147.00 (C-7), 115.78 (C-8), 170.98 (C-9); ¹H NMR (CD₃OD): δ 6.95 (d, J=2.2Hz, H-2), 6.87 (d, J=8.6Hz, H-5), 6.98 (dd, J=2.1, 8.4Hz, H-6), 7.62 (d, J=15.8Hz, H-7), 6.30 (d, J=15.8Hz, H-8) (Li et al., 1994).

R-(+)-β-D-(3,4-dihydroxyphenyl)-lactic acid (4): A white needle crystal from H₂O; FAB-MS m/z 197 [M(C₉H₁₀O₅) - H]⁻; [α]_D^{25.5} + 11.74° (c 0.30, CH₃OH); IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3476, 3255, 3026, 2956, 2588, 1739, 1600, 1528, 1511, 1449, 1370, 1288, 1239, 1187, 1109, 1083, 1015, 960, 924, 902, 831, 780, 729, 626, 575; UV (CH₃OH) λ_{max} nm: 205, 225, 279; ¹³C NMR (D₂O): δ 129.30 (C-1), 117.20 (C-2), 143.70 (C-3), 142.81 (C-4), 116.22 (C-5), 121.80 (C-6), 38.91 (C-7), 71.33 (C-8), 177.12 (C-9); ¹H NMR (D₂O): δ 6.80 (s, br., H-2), 6.88 (d, J=7.9Hz, H-5), 6.75 (dd, J=2.1, 7.9Hz, H-6), 2.82~3.05 (2H, m, H-7), 5.40 (m, H-8) (Chen et al., 1981).

3,4-dihydroxyphenyl ethanol ketone (5): A yellow powder; FAB-MS m/z 167 [M(C₈H₈O₄) - H]⁻; IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3504, 3373, 3145, 2595, 1668, 1608, 1523, 1422, 1340, 1297, 1197, 1135, 1090, 1014, 982, 936, 894, 810, 775, 713, 635, 622; UV (CH₃OH) λ_{max} nm: 207, 231, 277, 308; ¹³C NMR (CD₃OD): δ 127.80 (C-1), 115.54 (C-2), 146.82 (C-3), 152.59 (C-4), 116.11 (C-5), 122.39 (C-6), 198.77 (C-7), 65.82 (C-8); ¹H NMR (CD₃OD): δ 7.44 (d, J=2.1Hz, H-2), 6.85 (d, J=8.2Hz, H-5), 7.34 (dd, J=1.6, 8.4Hz, H-6), 2.86 (2H, s, H-8).

Rosmarinic acid (6): A pale yellow powder; FAB-MS m/z 359 [M(C₁₈H₁₆O₈) - H]⁻; IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3201, 1691, 1599, 1524, 1447, 1426, 1264, 1118, 1070, 979, 855, 814, 703, 595; UV (CH₃OH) λ_{max} nm: 204, 217, 228, 286, 329; ¹H NMR (CD₃OD): δ 7.04 (d, J=2.0Hz, H-2), 6.92 (d, J=7.9Hz, H-5), 6.78 (dd, J=1.6, 7.8Hz, H-6), 7.54 (d, J=15.8Hz, H-7), 6.25 (d, J=15.8Hz, H-8), 6.84 (d, J=2.0Hz, H-2'), 6.63 (d, J=7.8Hz, H-5'), 6.61 (dd, J=2.0, 8.0Hz, H-6'), 3.07 (2H, m, H-7'), 5.20 (m, H-8'); ¹³C NMR data: Table 1. (Charles et al., 1976).

Methyl rosmarinate (7): A yellow amorphous powder; FAB-MS m/z 373 [M(C₁₉H₁₈O₈) - H]⁻; [α]_D²⁴ + 29.41° (c 0.32, CH₃OH); IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3386, 1732, 1700, 1629, 1604, 1522, 1445, 1368, 1286, 1159, 1113, 1072, 978, 855, 813; UV (CH₃OH) λ_{max} nm: 205, 217, 287, 301, 332; ¹H NMR (CD₃OD): δ 7.05 (d, J=1.8Hz, H-2), 6.96 (d, J=8.0Hz, H-5), 6.78 (dd, J=1.8, 8.0Hz, H-6), 7.55 (d, J=16.0Hz, H-7), 6.25 (d, J=16.0Hz, H-8), 6.82 (d, J=2.0Hz, H-2'), 6.62 (d, J=8.0Hz, H-5'), 6.60 (dd, J=2.0, 8.0Hz, H-6'), 3.05 (2H, m, H-7'), 5.19 (dd, J=5.4, 11.2Hz, H-8'), 3.69 (3H, s, OCH₃); ¹³C NMR data: Table 1. (Kohda et al., 1989).

Table I ^{13}C NMR spectral data of 6-9 (100 MHz CD_3OD)

C	6	7	8	9
1	127.57	127.62	124.66	124.58
2	114.35	114.20	126.82	126.14
3	145.76	146.15	148.70	148.90
4	149.29	149.73	146.58	144.95
5	116.30	116.50	118.40	118.35
6	123.09	123.16	122.00	122.09
7	147.59	147.91	143.90	143.46
8	116.32	115.71	116.39	116.45
9	168.34	168.30	168.10	167.96
1'	129.27	128.76	133.30	133.50
2'	117.56	117.53	113.40	113.36
3'	146.37	146.76	146.00	146.49
4'	144.87	145.32	145.10	145.68
5'	116.48	116.32	116.38	116.45
6'	121.90	121.81	118.40	118.35
7'	37.66	37.66	88.40	88.12
8'	74.47	74.62	57.20	57.69
9' - OCH ₃		52.63		
1''			129.20	129.18
2''			117.48	117.54
3''			146.38	144.95
4''			145.10	146.32
5''			116.41	116.45
6''			122.00	122.21
7''			37.71	37.67
8''			74.62	74.51
9''			173.60	173.52
1'''				128.84
2'''				117.27
3'''				144.95
4'''				145.68
5'''				116.45
6'''				121.73
7'''				37.28
8'''				75.40
9'''				172.17

Lithospermic acid (8): A yellow amorphous powder; FAB-MS m/z 537 [$M(\text{C}_{27}\text{H}_{22}\text{O}_{12}) - \text{H}]^-$; $[\alpha]_{D}^{24.5} + 156.18^\circ$ (c 0.42, CH_3OH); IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3400, 1720, 1700, 1604, 1508, 1450, 1360, 1290; UV (CH_3OH) λ_{max} nm: 203, 220, 255, 288, 310; ^1H NMR (CD_3OD): δ 6.72 (d, $J = 8.0\text{Hz}$, H-5), 7.03 (d, $J = 8.4\text{Hz}$, H-6), 7.85 (d, $J = 16.0\text{Hz}$, H-7), 6.29 (d, J

δ = 16.0Hz, H - 8), 6.85 (s, br., H - 2'), 6.77 (s, br., H - 5'), 6.70 (m, H - 6'), 5.87 (d, J = 5.2Hz, H - 7'), 4.28 (d, J = 5.2Hz, H - 8'), 6.77 (s, br., H - 2''), 6.69 (d, J = 7.8Hz, H - 5''), 6.63 (m, H - 6''), 2.98 (2H, m, H - 7''), 5.05 (m, H - 8''); ^{13}C NMR data; Table 1. (Johnson et al., 1963; Charles et al., 1976).

Lithospermic acid B (9): $C_{36}H_{30}O_{16}$, an amorphous yellowish powder; FAB - MS m/z 717 [M - H]⁻; $[\alpha]_D^{24.2} + 83.76^\circ$ (c 0.39, CH₃OH); IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3371, 1727, 1611, 1521, 1447, 1361, 1287, 1180, 1114, 1072, 975, 866, 812, 589; UV (CH₃OH) λ_{max} nm: 205, 249, 288, 329; ^1H NMR (CD₃OD): 6.85 (d, J = 8.4Hz, H - 5), 7.15 (d, J = 8.5Hz, H - 6), 7.53 (d, J = 15.9Hz, H - 7), 6.24 (d, J = 15.9Hz, H - 8), 6.79 (d, J = 2.1Hz, H - 2'), 6.77 (d, J = 8.2Hz, H - 5'), 6.67 (dd, J = 1.8, 8.0Hz, H - 6'), 5.87 (d, J = 4.6Hz, H - 7'), 4.38 (d, J = 4.7Hz, H - 8'), 6.77 (d, J = 2.0Hz, H - 2''), 6.72 (d, J = 8.1Hz, H - 5''), 6.64 (dd, J = 2.0, 8.1 Hz, H - 6''), 3.08 (2H, dd, J = 4.0, 8.0 Hz, H - 7''), 5.21 (dd, J = 3.7, 7.0 Hz, H - 8''), 6.54 (d, J = 2.1 Hz, H - 2''), 6.60 (d, J = 8.0 Hz, H - 5''), 6.33 (dd, J = 2.0, 8.0 Hz, H - 6''), 3.04 (2H, dd, J = 4.0, 8.0 Hz, H - 7''), 5.12 (m, H - 8''); ^{13}C NMR data; Table 1. (Yokozawa et al 1988; Tanaka et al., 1989).

References

- Charles J K, Richard C H, Carmack M, et al., 1976. The polyphenolic acids of *Lithospermum ruderale*. II. Carbon - 13 nuclear magnetic resonance of lithospermic and rosmarinic acid. *J Org Chem.*, 41(13): 449 ~ 451
- Chen Z W, Gu W H, Wang W Z, 1981. Study on the polyphenolic compounds of *Salvia miltiorrhiza*. *Yaoxue Tongbao*, 9(16): 24
- Johnson G, Sunderwirth S G, Gibian H et al., 1963. *Lithospermum ruderale*: partial characterization of the principal polyphenol isolated from the roots. *Phytochemistry*, 2: 145 ~ 150
- Kohda H, Takeda O, Tanaka S, et al., 1989. Isolation of inhibitors of adenylate cyclase from Dan - shen, the root of *Salvia miltiorrhiza*. *Chem Pharm Bull*, 37(5): 1287 ~ 1290
- Li J, Li L L, Song W Z, 1994. Chemical constituents of *Salvia bowleyana*. *Zhong Cao Yao*, 25(7): 347 ~ 349
- Tanaka T, Morimoto S, Nonaka Gen - ichiro et al., 1989. Magnesium and ammonium - potassium lithospermates B, the active principle having a uremia - preventive effect from *Salvia miltiorrhiza*. *Chem Pharm Bull*, 37(2): 340 ~ 344
- Tanaka T, Nishimura A, Kouno I, et al., 1996. Isolation and characterization of yunnaneic acids A - D, four novel caffeic acid metabolites from *Salvia yunnanensis*. *J Nat Prod.*, 59: 843 ~ 849
- Tanaka T, Nishimura A, Kouno I, et al., 1997. Four new caffeic acid metabolites, yunnaneic acids E - H, from *Salvia yunnanensis*. *Chem Pharm Bull*, 45(10): 1596 ~ 1600
- Wu C Y, 1977. Flora Yunnanica, 1. Beijing: Sciences Press, 679 ~ 681
- Xu R S, 1990. Danshen - biology and its application. Beijing: Chinese Scientific Publication House, 23
- Yokozawa T, Hae Y C, Oura H, et al., 1988. Isolation of the active component having the uremia - preventive effect from *Salvia miltiorrhiza* Radix extract. *Chem Pharm Bull*, 36(1): 316 ~ 320