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蒙药多叶棘豆中的黄酮类化学成分

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摘　要:为了进一步研究多叶棘豆的化学成分,本文采用正相硅胶柱、Sephadex LH-20 柱色谱法及制备高效液相色谱法,从蒙药多叶棘豆中分离纯化 10 个黄酮类化合物。经各种波谱分析法鉴定其结构分别为:4,4′-二甲氧基-2′-羟基查尔酮(**1**)、2′,4′-二羟基- 4-甲氧基查尔酮(**2**)、7,8-二羟基二氢黄酮(**3**)、4,2′,4′ -三羟基查尔酮(**4**)、2′,4′-二羟基二氢查尔酮(**5**)、4′-羟基二氢黄酮-7-*O*-*β*-D-葡萄糖苷(**6**)、2′,4′-二羟基查尔酮(**7**)、芹菜素(**8**)、芹菜素-7-*O*-*β*-D-葡萄糖醛酸苷(**9**)和 3′,7-二羟基-2′,4′-二甲氧基异黄烷(**10**)。其中,化合物 **1**~**9** 均为首次从该植物中分离。

关键词:多叶棘豆;黄酮类成分;结构鉴定

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Flavonoid Components of *Oxytropis myriophylla* (Pall.) DC.

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Abstract: Ten flavonoids were separated and purified from *Oxytropis myriophylla* (Pall.) DC. by silica gel column chromatography, Sephadex LH-20 and preparation HPLC, *etc*. Based on the spectral analysis, their structures were elucidated as 4,4′-dimethoxy-2′-hydroxychalcone (**1**), 2′,4′- dihydroxy-4-methoxychalcone (**2**), 7,8-dihydroxyflavonone (**3**), 4,2′,4′-trihydroxychalcone (**4**), 2′,4′-dihydroxydihydrochalcone (**5**), 4′-hydroxy flavonone-7-*O*-*β*-D-glucoside (**6**), 2′,4′-dihydroxychalcone (**7**), apigenin (**8**), apigenin-7-*O*-*β*-D-glucuronide (**9**) and 3′,7-dihydroxy-2′,4′-dimethoxyisoflavan (**10**). Compounds **1**-**9** were isolated from this plant for the first time.

Key words: *Oxytropis myriophylla* (Pall.) DC.; flavonoid component; structural identification

多叶棘豆为豆科植物狐尾藻棘豆[Oxytropis myriophylla (Pall.) DC]的干燥地上部位,蒙药名为那布其热哈·乌如吐扎,配伍入蒙药清感九味丸、七雄丸、七味水银丸、萨仁嘎日迪等 30 余种方剂[1,2],治疗风疹、流感、咽喉肿痛以及痈疮肿毒和多种出血证。虽为临床常用药物,但多叶棘豆的研究鲜有报道,化学成分的研究方面,一些学者从多叶棘豆中分离提纯了黄酮类[3-5]、木酚素类[6]、三萜皂苷[7]和生物碱类[8],但不够全面系统。为了进一步研究多叶棘豆的化学成分,充分阐明其药效物质基础,本实验经过系统的化学成分研究,分离提纯近 30 个化合物,其中已鉴定 10 个黄酮类化合物,分别为:4,4′-二甲氧基-6′-羟基查尔酮(**1**)、2′,4′-二羟基-4-甲氧基查尔酮(**2**)、7,8-二羟基二氢黄酮(**3**)、4,2′,4′ -三羟基查尔酮(**4**)、2′,4′-二羟基二氢查尔酮(**5**)、4′-羟基二氢黄酮-7-*O*-*β*-D-葡萄糖苷(**6**)、2′,4′-二羟基查尔酮(**7**)、芹菜素(**8**)、芹菜素-7-*O*-*β*-D-葡萄糖醛酸苷(**9**)和 3′,7-二羟基-2′,4′-二甲氧基异黄烷(**10**)。以上化合物中 **1**~**9** 均为首次从该植物中分离。

1　材料与仪器

核磁共振谱采用 Bruker ARX-500 型核磁共振谱仪;分光光度计(UV-2501PC 型,日本岛津);半制备高效液相色谱仪(LC6-AD 输液泵,SPD-10Avp 检测器,SCL-10Avp 工作站);SHINADZU PRC-ODS 色谱柱(20 mm × 250 mm, 15 μm)电子天平(AUW220D 型,日本岛津);旋转蒸发器(RE52-2 型,上海泸西分析仪器厂)。硅胶(青岛海洋化工厂,200 ~ 300 目);Sephadex LH-20(Pharmacia 公司);氘代试剂为 Cambridge Isotope Laboratories,

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InC;柱色谱试剂均为分析纯。多叶棘豆于 2013 年 12 月由内蒙古民族大学附属医院提供,并由布和巴特尔教授鉴定为豆科狐尾藻棘豆 *Oxytropis myriophylla* (PALL.) DC. 的全草。

2 提取与分离

粉碎多叶棘豆 5.0 kg,加 95% 乙醇 50 L,回流提取 3 h,滤过。药渣再用 95% 乙醇 30 L 分两次提取,滤过,合并滤液。滤液减压回收乙醇得浸膏 680.5 g。浸膏加适量水成悬浮液,依次用石油醚、二氯甲烷、乙酸乙酯和正丁醇各萃取 3 次,合并提取液,减压回收溶剂得石油醚提取物 143.0 g,二氯甲烷提取物 117.0 g,乙酸乙酯提取物 37.3 g 和正丁醇提取物 153.5 g。二氯甲烷萃取部分(100 g)加 150 g 硅胶(160 ~200 目)拌样,充分干燥,上硅胶柱色谱分离,二氯甲烷-丙酮(100∶0 ~0∶100)梯度洗脱,250 mL 收集,洗脱液经 TLC 检识合并,得 6 个流分。流分 1(120 mg)经 Sephadex LH-20 柱色谱,以二氯甲烷-甲醇(1∶1)反复纯化,并 TLC 检识得化合物 **5**(10 mg);流分 2(130 mg)以二氯甲烷-丙酮(80∶1)反复洗脱得黄色和白色两种物质,分别经反复重结晶得化合物 **7**(12 mg)和 10(5 mg);流分 3(100 mg)经二氯甲烷-丙酮(60∶1)反复洗脱,得化合物 **1**(8 mg)和 **2**(7 mg)结晶。乙酸乙酯萃取部分(20 g),经硅胶柱色谱,以二氯甲烷-甲醇(100∶1、60∶1、40∶1、20∶1)梯度洗脱,得 4 个流分。流分 2(60 mg)经 Sephadex LH-20 柱色谱,以二氯甲烷-甲醇(1∶1)反复洗脱,得化合物 **8**(5 mg);流分 3(80 mg)经 Sephadex LH-20 柱色谱,以二氯甲烷-甲醇(1∶1)反复洗脱得黄色混合物(25 mg),再经半制备高效液相色谱仪,以甲醇-水(49∶51)反复分离制备,得化合物 **3**(7 mg)和 **4**(8 mg)。正丁醇萃取部分(130 g),经硅胶柱色谱,以二氯甲烷-甲醇(100∶1、60∶1、30∶1、10∶1)梯度洗脱,得 4 个流分。流分 2(100 mg)经 Sephadex LH-20 柱色谱,以二氯甲烷-甲醇(1∶1)反复洗脱得化合物 **9**(9 mg),流分 3(90 mg)过滤,经半制备高效液相色谱仪,以甲醇-水(32∶68)反复分离制备,得化合物 **6**(6 mg)。

3 结构鉴定

化合物 1 鲜黄色结晶(甲醇),紫外光下显黄色荧光,易溶于甲醇和乙醇。^{1}H NMR(500 MHz,DMSO-d_6) δ:13.6 (1H,s,2′-OH),3.98 (6H,s,4,4′-OCH_3),8.14 (1H,d,J = 9.0 Hz,H-6′),6.37 (1H,dd,J = 9.0,2.0 Hz,H-3′),6.24 (1H,d,J = 2.0 Hz,H-5′),7.78 (1H,d,J = 15.5 Hz,α-H),7.76 (1H,d,J = 15.5 Hz,β-H),7.36 (2H,d,J = 9.0 Hz,H-2,6),6.93(1H,d,J = 9.0 Hz,H-3,5)。^{13}C NMR(126 MHz,DMSO-d_6) δ:191.7 (-CO-),113.1 (C-1′),163.5 (C-2′),109.0 (C-3′),166.4(C-4′),103.1 (C-5′),133.2 (C-6′),117.9 (α-C),144.5 (β-C),126.2 (C-1),131.6 (C-2,6),116.3 (C-3,5),160.8 (C-4),55.7(OCH_3)。据以上数据与文献[9]对照,鉴定该化合物为 4,4′-二甲氧基-2′-羟基查尔酮。

化合物 2 鲜黄色结晶(甲醇),紫外光下显黄色荧光,易溶于甲醇和乙醇。^{1}H NMR(500 MHz,DMSO-d_6) δ:13.5 (1H,s,2′-OH),10.1 (1H,s,4′-OH),3.91 (3H,s,4-OCH_3),8.15 (1H,d,J = 9.0 Hz,H-6′),6.35 (1H,dd,J = 9.0,2.0 Hz,H-3′),6.21 (1H,d,J = 2.0 Hz,H-5′),7.75 (1H,d, J = 15.5 Hz,α-H),7.73 (1H,d, J = 15.5 Hz,β-H),7.34 (2H,d, J = 9.0 Hz,H-2,6),6.91(1H,d,J = 9.0 Hz,H-3,5)。^{13}C NMR(126 MHz,DMSO-d_6) δ:192.0 (-CO-),113.6 (C-1′),163.1 (C-2′),108.1 (C-3′),166.3 (C-4′),103.4 (C-5′),133.1 (C-6′),118.3 (α-C),144.1 (β-C),126.0 (C-1),131.3 (C-2,6),116.5 (C-3,5),161.4 (C-4),56.9 (OCH_3)。据以上数据与文献[10]对照,鉴定该化合物为 2′,4′-二羟基-4-甲氧基查尔酮。

化合物 3 淡黄色结晶(甲醇),易溶于甲醇和乙醇。^{1}H NMR (500 MHz,MeOD) δ:7.40 (2H,d,J = 8.5 Hz,H-2′,6′),7.32 (2H,d,J = 8.5 Hz,H-3′,5′),7.27 (1H,d,J = 8.5 Hz,H-4′),5.80 (1H,d,J = 8.5 Hz,H-5),5.79 (1H,dd,J = 8.5,2.0 Hz,H-6),5.36 (1H,dd,J = 13.0,3.0 Hz,H-2),2.99 (1H,dd,J = 16.5,13.0 Hz,H-3α),2.65 (1H,dd,J = 16.5,2.5 Hz,H-3β);^{13}C NMR (126 MHz,MeOD) δ:197.1 (C-4),168.2 (C-7),165.5 (C-8),164.7 (C-9),140.5 (C-1′),129.7 (C-3′,5′),129.6 (C-4′),127.4(C-2′,6′),103.2 (C-10),97.4(C-6),96.4(C-5),80.4(C-2),44.2(C-3)。据以上数据并与文献[11]对照,鉴定该化合物为 7,8-二羟基二氢黄酮。

化合物 4 鲜黄色结晶(甲醇),紫外光下显黄色荧光,易溶于甲醇和乙醇。^{1}H NMR(500 MHz,DM-

SO-d_6) δ: 13.1 (1H, s, 2′-OH), 10.1 (1H, s, 4′-OH), 9.78 (1H, s, 4-OH), , 8.11 (1H, d, J = 9.0 Hz, H-6′), 7.70 (1H, d, J = 15.5 Hz, α-H), 7.73 (1H, d, J = 15.5 Hz, β-H), 7.30 (2H, d, J = 9.0 Hz, H-2,6), 6.91 (1H, d, J = 9.0 Hz, H-3,5), 6.33 (1H, dd, J = 9.0, 2.0 Hz, H-3′), 6.22 (1H, d, J = 2.0 Hz, H-5′); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 192.0 (-CO-), 114.2 (C-1′), 161.8 (C-2′), 108.7 (C-3′), 163.1 (C-4′), 103.7 (C-5′), 133.0 (C-6′), 117.8 (α-C), 144.7 (β-C), 126.2 (C-1), 131.8 (C-2,6), 116.1 (C-3,5), 159.8 (C-4)。据以上数据并与文献[12]对照,鉴定该化合物为4,2′,4′-三羟基查尔酮。

化合物5 白色粉末(甲醇),紫外光下显黄色荧光,易溶于甲醇和乙醇。^{1}H NMR (500 MHz, $CDCl_3$) δ: 12.74 (1H, s, 2′-OH), 7.63 (1H, d, J = 8.4 Hz, H-6′), 7.30 (2H, m, H-3,5), 7.23 (3H, m, H-2,4,6), 6.35 (1H, d, J = 2.4 Hz, H-3′), 6.37 (1H, dd, J = 5.0, 2.4 Hz, H-5′), 3.24 (2H, t, J = 10.0, 5.0 Hz, α-H), 3.05 (2H, t, J = 10.0, 5.0 Hz, β-H); ^{13}C NMR (126 MHz, $CDCl_3$) δ: 203.6 (CO), 165.2 (C-4′), 162.5 (C-2′), 140.8 (C-1), 132.2 (C-6′), 128.6 (C-3,5), 128.4 (C-2,6), 126.3 (C-4), 113.9 (C-1′), 107.7 (C-5′), 103.6 (C-3′), 39.70 (α-C), 30.3 (β-C)。据以上数据并与文献[13]对照,鉴定该化合物为2′,4′-二羟基二氢查尔酮。

化合物6 淡黄色粉末(甲醇),易溶于甲醇和乙醇,盐酸-镁粉反应阳性,Molish反应为阳性,提示该化合物为黄酮苷。^{1}H NMR (500 MHz, DMSO-d_6) δ: 7.51 (1H, d, J = 8.5 Hz, H-5), 6.39 (1H, dd, J = 8.5, 2.0 Hz, H-6), 6.16 (1H, d, J = 2.0 Hz, H-8), 7.33 (2H, d, J = 8.5 Hz, H-2′,6′), 6.71 (2H, d, J = 8.5 Hz, H-3′,5′), 5.36 (1H, dd, J = 13.0, 3.0 Hz, H-2), 3.01 (1H, dd, J = 16.5, 13.0 Hz, H-3α), 2.49 (1H, dd, J = 16.5, 3.0 Hz, H-3β); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 79.3 (C-2), 43.9 (C-3), 190.4 (C-4), 128.5 (C-5), 112.0 (C-6), 165.1 (C-7), 103.3 (C-8), 162.8 (C-9), 111.0 (C-10), 130.8 (C-1′), 127.1 (C-2′,6′), 113.4 (C-3′,5′), 159.1 (C-4′)。糖的碳信号δ: 100.1 (C-1″), 75.1 (C-2″), 76.8 (C-3″), 70.2 (C-4″), 77.4 (C-5″)及61.8 (C-6″),提示该糖是葡萄糖,并且在^{1}H NMR谱中给出了端基质子信号δ 5.01 (1H, d, J = 6.5 Hz),显示为β糖苷键。在HMBC谱中端基质子信号δ 5.01 (1H, d, J = 6.5 Hz)与165.1 (C-7)相关,说明葡萄糖连接在7位。其数据与文献[14]基本一致,故鉴定该化合物为4′-羟基二氢黄酮-7-*O*-β-D-葡萄糖苷。

化合物7 黄色粉末(甲醇),紫外光下显黄色荧光,易溶于甲醇和乙醇。^{1}H NMR (500MHz, $CDCl_3$) δ: 13.36 (1H, s, 2′-OH), 7.57 (1H, d, J = 15.5 Hz, α-H), 7.89 (1H, d, J = 15.5 Hz, β-H), 7.68 (2H, m, H-3,5), 7.45 (3H, m, H-2,4,6), 7.84 (1H, d, J = 8.4 Hz, H-6′), 6.45 (1H, dd, J = 8.4, 2.4Hz, H-5′), 6.43 (1H, d, J = 2.4 Hz, H-3′); ^{13}C NMR (126 MHz, $CDCl_3$) δ: 192.0 (CO), 166.5 (C-2′), 162.7 (C-4′), 144.7 (β-C), 134.7 (C-1), 132.1 (C-6′), 130.8 (C-4), 129.0 (C-2,6), 128.6 (C-3,5), 120.3 (α-C), 114.6 (C-1′), 107.8 (C-5′), 103.8 (C-3′)。其数据与文献[13]基本一致,故鉴定该化合物为2′,4′-二羟基查尔酮。

化合物8 黄色结晶(甲醇),盐酸-镁粉反应阳性,推测为黄酮类化合物。在^{1}H NMR (500 MHz, DMSO-d_6): δ 6.77 (1H, s, H-3), 6.18 (1H, d, J = 2.0 Hz, H-6), 6.46 (1H, d, J = 2.0 Hz, H-8), 7.90 (2H, d, J = 8.5 Hz, H-2′,6′), 6.93 (2H, d, J = 8.5 Hz, H-3′,5′), 12.90 (1H, s, 5-OH)。其数据与文献[15]中谱学数据基本一致,故鉴定为芹菜素。

化合物9 淡黄色粉末(甲醇),盐酸-镁粉反应阳性,Molish反应为阳性,提示该化合物为一黄酮苷。^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.96 (1H, s, 5-OH), 6.85 (1H, s, H-3), 6.46 (1H, d, J = 2.0 Hz, H-6), 6.87 (1H, d, J = 2.0 Hz, H-8), 7.95 (2H, d, J = 9.0 Hz, H-2′,6′), 6.95 (2H, d, J = 9.0 Hz, H-3′,5′), 5.17 (1H, d, J = 9.0 Hz, H-1″), 3.29 (3H, m, J = 9.0 Hz, H-2″,3″,5″), 3.81 (1H, d, J = 9.0 Hz, H-4″); ^{13}C NMR (126 MHz, MSO-d_6) δ: 164.2 (C-2), 102.9 (C-3), 181.9 (C-4), 161.3 (C-5), 99.3 (C-6), 162.5 (C-7), 94.6 (C-8), 156.8 (C-9), 105.3 (C-10), 120.7 (C-1′), 128.5 (C-2′,6′), 161.0 (C-4′), 115.9 (C-3′,5′), 99.3 (C-1″), 71.5 (C-2″), 72.8 (C-3″), 74.7 (C-4″), 76.0 (C-5″), 170.5 (6″-COOH)。其数据与文献[16]中谱学数据基本一致,故鉴定为芹菜素-7-*O*-β-D-葡萄糖醛酸苷。

化合物10 白色粉末(甲醇),易溶于甲醇和乙

醇。^{1}H NMR (500 MHz, $CDCl_3$) δ 6.96 (1H, d, J = 8.2 Hz, H-5), 6.80 (1H, d, J = 8.7 Hz, H-6′), 6.47 (1H, d, J = 8.7 Hz, H-5′), 6.40 (1H, dd, J = 11.8, 2.4 Hz, H-6), 6.38 (1H, d, J = 2.4 Hz, H-8), 4.35 (1H, d, J = 1.5 Hz, H-2), 4.07 (1H, t, J = 10.0 Hz, H-2), 3.95 (3H, s, OCH_3), 3.87 (3H, s, OCH_3), 3.55 (1H, m, H-3), 3.03 (1H, dd, J = 16.1, 10.3 Hz, H-4), 3.01 (1H, ddd, J = 16.1, 12.1, 1.7 Hz, H-4); ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.2 (C-7), 154.9 (C-9), 151.1 (C-4′), 147.5 (C-3′), 135.4 (C-2′), 130.4 (C-5), 121.8 (C-6′), 120.3 (C-1′), 114.8 (C-10), 107.9 (C-6), 103.7 (C-5′), 103.2 (C-8), 69.7 (C-2), 61.0 (OCH_3), 55.8 (OCH_3), 32.1 (C-3), 30.2 (C-4)。其数据与文献[17]基本一致,故鉴定为3′,7-二羟基-2′,4′-二甲氧基异黄烷。

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