

牛膝种子化学成分研究

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摘要: 从牛膝(*Achyranthes bidentata* Blume.)种子中分离得到8个化合物。通过波谱分析, 分别鉴定为 *N*-反式-阿魏酰酪胺(**1**)、亚油酸甘油酯(**2**)、 β -蜕皮甾酮(**3**)、水龙骨甾酮 B(**4**)、麦角甾-7,22-二烯-3 β ,5 α ,6 β -三醇(**5**)、竹节参皂苷-1(**6**)、齐墩果酸-3-O- β -D-葡萄糖苷(**7**)和胡萝卜甾(**8**)。化合物**1**、**2**、**5**和**7**为首次从牛膝中分离。

关键词: 牛膝; 苋科; 牛膝属; 种子; 化学成分

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Chemical Constituents from Seeds of *Achyranthes bidentata* Blume.

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Abstract: Eight compounds were isolated from seeds of *Achyranthes bidentata* Blume. On the basis of the spectral data, they were identified as *N*-trans-feruloyltyramine (**1**), glycerol 1-O-9Z,12Z-octadecadienoate (**2**), β -ecdysterone (**3**), polypodine B (**4**), ergosta-7,22-diene-3 β ,5 α ,6 β -triol (**5**), oleanolic acid 28-O- β -D-glucopyranosyl ester (**6**), oleanolic acid 3-O- β -D-glucopyranosyl ester (**7**), and daucosterol (**8**). Compounds **1**, **2**, **5** and **7** were obtained from the plant at the first time.

Key words: *Achyranthes bidentata*; Amaranthaceae; *Achyranthes*; Seed; Chemical constituents

牛膝(*Achyranthes bidentata* Blume.)又名百倍、怀牛膝等, 为苋科(Amaranthaceae)牛膝属多年生草本, 在我国河南、四川、云贵等地多有栽培。牛膝以干燥根入药, 味甘、苦、酸, 性平, 入肝、肾经^[1], 有补肝肾、强筋骨、逐瘀通经和引血下行等功效^[2]。其活性成分主要有多糖、齐墩果酸类三萜皂苷、植物甾酮类等化合物^[3-5], 具有免疫调节^[6]、子宫兴奋与抗生育^[7]、抗肿瘤^[8-9]、抗炎、抗菌、镇痛等作用^[10]。有关牛膝种子化学成分的研究报道较少, 仅见其油脂组成的报道^[11]。为明确牛膝种子是否具有药用潜质, 我们对其化学成分进行了研究, 以期对牛膝的开发利用提供科学依据。

1 材料和方法

1.1 材料

牛膝(*Achyranthes bidentata* Blume.)种子购于河北安国中药材研究协会, 由中国科学院华南植物园邢福武教授鉴定, 其标本保存在中国科学院华南植物园生物有机化学研究组实验室。柱层析硅胶为青岛海洋化工厂生产, 柱层析凝胶 Sephadex LH-20 为瑞典 Amersham Bioscience 公司生产, 柱层析用聚酰胺粉和聚酰胺薄层层析板为浙江省台州市路桥四甲生化塑料厂生产, 正相薄层层析板为烟台黄务硅胶开发试验厂生产, 反相硅胶 RP-18 为日本 Nomura Chemical Co. Ltd.生产。

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1.2 仪器

核磁共振谱用 Bruker DRX-400 型超导核磁共振仪波谱测定(瑞士 Bruker 公司生产),以四甲基硅烷(TMS)为内标。ESIMS 用 MDSSCIEX API 2000 LC/MS/MS 仪,以甲醇为溶剂,直接进样测定。

1.3 提取和分离

牛膝种子干粉(12 kg)用 90% 乙醇浸泡提取 3 次,每次 3 d。乙醇提取液减压浓缩后加水成悬浮液,用三氯甲烷萃取得到水相和三氯甲烷部分。三氯甲烷部分提取物(125 g)经硅胶柱层析,以氯仿-甲醇 98:2、95:5、88:12、50:50 和纯甲醇进行梯度洗脱,相应得到 F1 ~ F5 共 5 个部分。F2 部分(34 g)经反复正相硅胶柱层析,并结合反相硅胶 ODS 柱层析分离,得到化合物 **1** (93 mg)。F3 部分(12 g)经正相硅胶柱层析,以氯仿-甲醇 50:1 洗脱得到化合物 **2** (6.5 mg),然后以氯仿-甲醇 95:5 ~ 90:10 梯度洗脱得到 5 个组分,继而分别用 Sephadex LH-20 柱层析纯化得到化合物 **3** (36.5 mg)、**4** (51.3 mg)、**5** (12.3 mg)、**6** (6.5 mg)和 **7** (20.4 mg)。F4 部分(13 g)经反复正相硅胶柱层析,并结合甲醇重结晶得到化合物 **8** (200 mg)。

1.4 结构鉴定

化合物 1 白色晶体,熔点:143 ~ 144℃,分子式为 $C_{18}H_{19}NO_4$, 分子量为 313;正离子 ESIMS m/z : 314 $[M+H]^+$, 336 $[M+Na]^+$, 352 $[M+K]^+$; 1H NMR (400 MHz, CD_3OD): tyramine moiety: δ_H 7.01 (2H, d, $J=8.4$ Hz, H-2', H-6'), 6.71 (2H, d, $J=8.4$ Hz, H-3', H-5'), 3.45 (2H, t, $J=7.4$ Hz, H-1), 2.72 (2H, t, $J=7.4$ Hz, H-2); caffeoyl moiety: δ_H 7.44 (1H, d, $J=15.6$ Hz, H-3), 7.36 (1H, d, $J=2.0$ Hz, H-2'), 6.97 (1H, dd, $J=8.4$ Hz, 2.0 Hz, H-6'), 6.78 (1H, d, $J=8.4$ Hz, H-5'), 6.39 (1H, d, $J=15.6$ Hz, H-2), 3.79 (3H, s, OCH_3); ^{13}C NMR (100 MHz, CD_3OD): tyramine moiety: δ_C 156.7 (C-4'), 131.3 (C-1'), 130.7 (C-2', 6'), 116.4 (C-3', 5'), 42.4 (C-1), 35.7 (C-2); caffeoyl moiety: δ_C 169.1 (C-1), 149.6 (C-4'), 149.1 (C-3'), 142.0 (C-3), 128.1 (C-1'), 123.1 (C-6'), 118.6 (C-2), 116.2 (C-2'), 114.1 (C-5'), 56.2 (OCH_3)。对照文献[12]的数据,确定该化合物为 *N*-反式-阿魏酰酪胺。

化合物 2 油态,分子式为 $C_{21}H_{38}O_4$, 分子量为 354;负离子 ESIMS m/z : 353 $[M-H]^-$, 389 $[M+$

$Cl]^-$; 1H NMR (400 MHz, $CDCl_3$): δ_H 5.38 (1H, m, H-9), 5.36 (1H, m, H-10), 5.35 (1H, m, H-12), 5.32 (1H, m, H-13), 4.17 (2H, m, H-1'), 3.94 (1H, m, H-2'), 3.70 (2H, d, $J=8.0$ Hz, H-3'), 2.78 (2H, dd, $J=8.0$ Hz, 2.0 Hz, H-11), 2.37 (2H, t, $J=8.0$ Hz, H-2), 2.07 (2H, m, H-17), 1.63 (2H, m, H-8), 1.31 (2H, m, H-6, 7), 1.28 (2H, m, H-4, 5), 1.25 (2H, m, H-3), 0.89 (3H, t, $J=8.0$ Hz, H-18); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 174.3 (C-1), 130.2 (C-13), 129.9 (C-12), 128.0 (C-10), 127.8 (C-9), 70.2 (C-1'), 65.0 (C-2'), 63.3 (C-3'), 34.1 (C-2), 31.5 (C-17), 29.6 (C-16), 29.4 (C-15), 29.3 (C-14), 29.1 (C-11), 29.0 (C-8), 27.1 (C-7), 25.6 (C-5, 6), 24.8 (C-4), 22.5 (C-3), 14.1 (C-18)。 1H NMR 和 ^{13}C NMR 数据与文献[13]对照,确定该化合物是亚油酸甘油酯。

化合物 3 白色晶体,熔点:235 ~ 237℃, $[\alpha]_D^{25} + 62.0^\circ$ (c 0.8, $CHCl_3$), 分子式为 $C_{27}H_{44}O_7$, 分子量为 480;正离子 ESIMS m/z : 481 $[M+H]^+$, 503 $[M+Na]^+$; 1H NMR (400 MHz, CD_3OD): δ_H 5.80 (1H, d, $J=2.0$ Hz, H-7), 3.93 (1H, q, $J=3.0$ Hz, H-3), 3.83 (1H, dt, $J=12.0, 4.2, 3.0$ Hz, H-2), 3.34 (1H, dd, $J=10.6$ Hz, 1.8 Hz, H-22), 3.30 (1H, ddd, $J=11.3$ Hz, 7.1 Hz, 2.6 Hz, H-9), 2.38 (1H, t, $J=7.5$ Hz, H-17), 2.36 (1H, dd, $J=13.2$ Hz, 4.8 Hz, H-5), 1.19 (3H, s, H-27), 1.18 (3H, s, H-21), 1.17 (3H, s, H-26), 0.92 (3H, s, H-19), 0.88 (3H, s, H-18); ^{13}C NMR (100 MHz, CD_3OD): δ_C 206.5 (C-6, C=O), 167.9 (C-8), 122.1 (C-7), 85.2 (C-14), 78.4 ~ 77.8 (C-20), 77.8 (C-22), 68.7 (C-2), 68.6 (C-3), 68.5 (C-25), 51.8 (C-5), 50.5 (C-17), 48.4 (C-13), 42.4 (C-24), 39.3 (C-10), 37.4 (C-1), 37.0 (C-15), 35.1 (C-9), 31.8 (C-12), 30.2 (C-27), 30.0 (C-26), 29.7 (C-23), 24.0 (C-19), 21.5 (C-16), 21.5 (C-11), 21.0 (C-21), 18.0 (C-18)。光谱数据与文献[14]报道的 β -蜕皮甾酮一致,确定该化合物为 β -蜕皮甾酮。

化合物 4 白色粉末,熔点:252 ~ 253℃, $[\alpha]_D^{25} + 93.0^\circ$ (c 0.5, MeOH), 分子式为 $C_{27}H_{44}O_8$, 分子量为 496;正离子 ESIMS m/z : 497 $[M+H]^+$, 519 $[M+Na]^+$; 1H NMR (400 MHz, CD_3OD): δ_H 5.97 (1H, br.s, H-7), 4.06 (1H, s, H-3), 4.05 (1H, s, H-2), 3.43 (1H, m, H-22), 3.32 (1H, m, H-9), 2.48 (1H, m, H-17), 1.31 (3H, s, H-21), 1.30 (3H, s, H-27), 1.30 (3H, s, H-26), 1.02 (3H, s, H-19), 1.00 (3H, s, H-

18); ^{13}C NMR (100 MHz, CD_3OD): δ_{C} 202.4 (C-6, C=O), 167.6 (C-8), 120.5 (C-7), 85.0 (C-14), 80.2 (C-5), 78.4 (C-22), 77.9 (C-20), 71.3 (C-21), 70.1 (C-25), 70.1 (C-3), 68.3 (C-2), 50.4 (C-17), 49.0 (C-13), 45.4 (C-10), 42.3 (C-24), 38.9 (C-9), 36.1 (C-4), 34.2 (C-1), 32.5 (C-12), 31.7 (C-15), 30.0 (C-27), 29.0 (C-26), 27.3 (C-23), 22.4 (C-11), 21.4 (C-16), 18.1 (C-18), 16.9 (C-19)。与文献[15]数据基本一致, 确定该化合物为水龙骨甾酮 B。

化合物 5 白色晶体, 熔点: 254 ~ 255℃, $[\alpha]_{\text{D}}^{25} + 30.0^\circ$ (c 0.5, Pydine), 分子式为 $\text{C}_{28}\text{H}_{46}\text{O}_3$, 分子量为 430; 正离子 ESIMS m/z : 431 $[\text{M} + \text{H}]^+$, 453 $[\text{M} + \text{Na}]^+$; ^1H NMR (400 MHz, CD_3OD): δ_{H} 5.24 (1H, m, H-7), 5.20 (1H, m, H-22, 23), 4.32 (1H, m, H-3), 3.04 (1H, bd, $J = 4.0$ Hz, H-6), 1.06 (3H, s, H-19), 1.05 (3H, d, $J = 7.0$ Hz, H-21), 0.95 (3H, d, $J = 7.0$ Hz, H-28), 0.86 (3H, d, $J = 6.5$ Hz, H-27), 0.85 (3H, d, $J = 7.0$ Hz, H-26), 0.66 (3H, s, H-18); ^{13}C NMR (100 MHz, CD_3OD): δ_{C} 141.8 (C-8), 136.3 (C-22), 132.3 (C-23), 120.4 (C-7), 76.3 (C-5), 74.3 (C-6), 67.8 (C-3), 56.4 (C-17), 55.3 (C-14), 43.9 (C-13), 43.9 (C-9), 43.3 (C-24), 41.8 (C-20), 40.9 (C-12), 40.1 (C-4), 38.2 (C-10), 33.9 (C-25), 33.5 (C-2), 32.6 (C-1), 28.6 (C-16), 23.6 (C-15), 22.6 (C-11), 21.5 (C-21), 20.3 (C-27), 19.9 (C-26), 18.9 (C-19), 17.9 (C-28), 12.6 (C-18)。与文献[16]对照, 确定该化合物为麦角甾-7,22-二烯-3 β ,5 α ,6 β -三醇。

化合物 6 白色粉末, 熔点: 228 ~ 230℃, $[\alpha]_{\text{D}}^{25} + 36.8^\circ$ (c 0.4, MeOH), 分子式为 $\text{C}_{36}\text{H}_{58}\text{O}_8$, 分子量为 619; 正离子 ESIMS m/z : 620 $[\text{M} + \text{H}]^+$, 655 $[\text{M} + 2\text{NH}_4]^+$; ^1H NMR (400 MHz, CD_3OD): δ_{H} 5.36 (1H, d, $J = 8.4$ Hz, H-1'), 1.14 (3H, s, H-27), 0.96 (3H, s, H-23), 0.94 (3H, s, H-30), 0.92 (3H, s, H-24), 0.90 (3H, s, H-29), 0.79 (3H, s, H-26), 0.76 (3H, s, H-25); ^{13}C NMR (100 MHz, CD_3OD): δ_{C} 178.1 (C-28), 144.9 (C-13), 123.8 (C-12), 95.7 (C-1'), 79.7 (C-5'), 78.7 (C-3'), 78.3 (C-3), 73.9 (C-2'), 71.1 (C-4'), 62.4 (C-6'), 56.8 (C-5), 48.6 (C-9), 47.2 (C-17), 42.6 (C-14), 40.7 (C-8), 39.9 (C-1), 39.8 (C-4), 38.2 (C-10), 34.9 (C-21), 33.5 (C-29), 33.1 (C-22), 33.1 (C-7), 31.5 (C-20), 28.9 (C-15), 28.7 (C-23), 27.9 (C-2), 26.3 (C-27), 24.5 (C-11), 24.0 (C-30), 23.9 (C-16), 19.5 (C-6), 17.7 (C-26), 16.3 (C-24), 16.0 (C-25)。与文

献[17]对照, 确定该化合物是竹节参皂苷-1。

化合物 7 白色粉末, 熔点: 226 ~ 228℃, $[\alpha]_{\text{D}}^{25} + 46.8^\circ$ (c 0.9, MeOH), 分子式为 $\text{C}_{36}\text{H}_{58}\text{O}_8$, 分子量为 619; 正离子 ESIMS m/z : 620 $[\text{M} + \text{H}]^+$, 642 $[\text{M} + \text{Na}]^+$; ^1H NMR (400 MHz, CD_3OD): δ_{H} 5.19 (1H, br.s, H-12), 4.19 (1H, d, $J = 8.0$ Hz, H-1'), 1.09 (3H, s, H-27), 1.07 (3H, s, H-23), 1.05 (3H, s, H-30), 0.97 (3H, s, H-24), 0.87 (3H, s, H-29), 0.75 (3H, s, H-26), 0.71 (3H, s, H-25); ^{13}C NMR (100 MHz, CD_3OD): δ_{C} 180.7 (C-28), 143.8 (C-13), 121.6 (C-12), 105.5 (C-1'), 88.0 (C-3), 76.4 (C-3'), 75.0 (C-5'), 73.8 (C-2'), 71.9 (C-4'), 63.0 (C-6'), 55.0 (C-5), 47.1 (C-9), 45.5 (C-19), 45.5 (C-17), 41.5 (C-18), 41.5 (C-4), 39.9 (C-14), 39.9 (C-8), 38.9 (C-1), 38.7 (C-10), 36.3 (C-22), 32.8 (C-29), 32.8 (C-21), 32.8 (C-7), 30.4 (C-20), 27.6 (C-23), 27.6 (C-15), 27.6 (C-2), 25.6 (C-27), 23.4 (C-30), 23.4 (C-16), 23.4 (C-11), 16.9 (C-6), 16.9 (C-25), 16.5 (C-26), 15.2 (C-24)。与文献[18]数据比对, 确定化合物为齐墩果酸-3-O- β -D-葡萄糖苷。

化合物 8 白色粉末, 分子式为 $\text{C}_{35}\text{H}_{60}\text{O}_6$ 。10% 硫酸-乙醇溶液显紫红色。与胡萝卜甙对照品共薄层, Rf 值一致(展开剂: CHCl_3 : MeOH = 5: 1), 斑点重叠。故鉴定该化合物为胡萝卜甙。

2 结果和讨论

从牛膝种子的氯仿萃取部分分离得到 8 个化合物, 分别鉴定为 *N*-反式-阿魏酰酪胺(**1**)、亚油酸甘油酯(**2**)、 β -蜕皮甾酮(**3**)、水龙骨甾酮 B(**4**)、麦角甾-7,22-二烯-3 β ,5 α ,6 β -三醇(**5**)、竹节参皂苷-1(**6**)、齐墩果酸-3-O- β -D-葡萄糖苷(**7**)和胡萝卜甙(**8**)。化合物 **1**、**2**、**5**、**7** 为首次从牛膝中分离得到。

依据文献报道, *N*-反式阿魏酰酪胺(**1**)在一定程度上能有效抑制凝血酶、胶原质、PAF 及 ADP 等因子引起的血小板凝聚, 并在抑制 P-388 和 HL-60 等肿瘤细胞生长、抗乙型肝炎病毒以及抑制黑色素生物合成等方面具有活性^[12,19-21]。 β -蜕皮甾酮(**3**)在影响糖代谢、调节基因表达及抗脂质过氧化和内皮细胞损伤方面有一定功效, 在影响和调节生殖系统功能方面也有一定作用^[5]。麦角甾-7,22-二烯-3 β ,5 α ,6 β -三醇(**5**)具有一定抗炎活性^[22]。竹节参皂苷能促进神经突生长和增强胆碱系统功能, 对改善学习记忆应具有一定调节作用^[23]。齐墩果酸-3-O- β -D-

葡萄糖苷(7)为一具潜在抗肿瘤活性的化合物^[24]。本研究报道牛膝种子中存在丰富的药用活性成分,有潜在的药用开发价值。

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