

绿茎还阳参化学成分的研究

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摘要: 采用柱层析和重结晶从药用植物绿茎还阳参(*Crepis lignea*) 丙酮提取物中分离得到 7 个单体化合物, 并根据化合物的理化性质和波谱分析, 分别鉴定为: 3 β -acetoxyurs-13(18)-ene (1), lupeol acetate (2), 4-hydroxy-3-methoxycinnamaldehyde (3), (*Z*)-2-ethylidene-3-methylsuccinic acid (4),olean-12-ene-11 α -methoxy-3 β -acetate (5), α -amyrin acetate (6) 和 lupeol-9(11) en-3 β -acetate (7)。上述化合物均为首次从绿茎还阳参中分离得到。

关键词: 菊科; 还阳参属; 绿茎还阳参; 三萜

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Chemical Constituents of *Crepis lignea*

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Abstract: Seven compounds were isolated from the acetone extract of the medicinal plant *Crepis lignea* by column chromatography and recrystallization. Their structures were mainly identified by their spectral and physical data as 3 β -acetoxyurs-13(18)-ene (1), lupeol acetate (2), 4-hydroxy-3-methoxycinnamaldehyde (3), (*Z*)-2-ethylidene-3-methylsuccinic acid (4), olean-12-ene-11 α -methoxy-3 β -acetate (5), α -amyrin acetate (6) and lupeol-9(11) en-3 β -acetate (7) respectively. All compounds were obtained from *Crepis lignea* for the first time.

Key words: Compositae; *Crepis*; *Crepis lignea*; triterpenoids

绿茎还阳参(*Crepis lignea* (Vant.) Babco.) 为菊科还阳参属(*Crepis*) 多年生草本植物, 别名马尾参, 铁扫把, 细草, 万丈深等, 生于向阳山坡, 在我国主要分布四川, 贵州, 云南一带^[1]。还阳参属植物约 200 种, 我国约有 25 种^[2]。该属植物中化学成分方面研究较多的主要是茺菁还阳参(*C. napifera*)、还阳参(*C. turczaniowii*)、*C. bocconi* 等, 主要为三萜、倍半萜和酚类化合物^[3-6], 其中倍半萜类化合物具有广泛的生物活性。经文献调研, 绿茎还阳参具有一定的药用价值, 为民间常用草药, 润肺止咳, 清热解毒, 消食理气, 催乳。治支气管炎, 肺炎, 痈疽, 小儿疳积, 乳汁不足, 结膜炎; 补虚, 治体虚头晕无力^[7]。然而有关其化学成分, 国内外未见任何文献报道, 为了探寻其中的有效成分, 本研究对绿茎还阳参进行了系统的次生代谢产物研究, 分离鉴定了 7 个化合物, 这些化合物均为首次从该植物中分离得到。并对这些化合物进行抑菌活性测试, 结果均未显示明显的抑菌活性。

1 实验材料

国产 XRC-1 型显微熔点仪; VG AutoSpec 3000 及 Finnigan MAT 90 质谱仪; Bruker AM-400、DRX-500 和 AVANCE-600 核磁共振光谱仪(TMS 为内标, δ 为 ppm, J 为 Hz)。柱色谱硅胶(200~300 目)和薄层色谱硅胶 GF₂₅₄ 均为青岛海洋集团有限公司生产; MCI 填充材料为 MCI-gel CHP-20P; 凝胶为 Sephadex LH-20 (Pharmacia Fine Chemical Co. Ltd); 显色剂为 15% 的 H₂SO₄/EtOH 溶液。绿茎还阳参采集于云南省陆良县(2010 年 8 月), 由中国科学院昆明植物研究所龚洵研究员鉴定。

2 提取分离

绿茎还阳参干燥粗粉(500 g) 用丙酮溶液浸提(3 \times 3 L) 浓缩后得浸膏(30 g)。将其用丙酮溶解后, 硅胶拌样, 经硅胶柱层析, 氯仿/丙酮梯度洗脱, 得到五个流份: Fr. 1(氯仿), Fr. 2(氯仿/丙酮 9:1), Fr. 3(氯仿/丙酮 8:2), Fr. 4(氯仿/丙酮 5:5) 和 Fr. 5(丙酮)。Fr. 1 反复硅胶柱层析(石油醚/乙酸乙酯 8:11), Sephadex LH-20 凝胶柱层析(丙酮) 以及重

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结晶得到化合物 **1** (10 mg) 和 **2** (7 mg) ,Fr. 2 经 MCI 柱层析 (70% 甲醇/水-甲醇) 梯度洗脱, 得到四个亚流份 (Fr. 2-1 到 Fr. 2-5) ,Fr. 2-1 依次经过 Sephadex LH-20 凝胶柱层析 (丙酮) ,硅胶柱层析 (石油醚/乙酸乙酯 5:1) 得到化合物 **3** (2 mg) 和 **4** (1.5 mg) ,Fr. 2-3 依次经过 Sephadex LH-20 凝胶柱层析 (丙酮) ,反复硅胶柱层析 (石油醚/乙酸乙酯 20:1) 得到化合物 **5** (13 mg) ,Fr. 2-4 经过反复硅胶柱层析 (石油醚/乙酸乙酯 70:1) 得到化合物 **6** (15 mg) 和 **7** (12 mg) 。

3 结构鉴定

化合物 1 白色簇状结晶 (氯仿) ,mp. 204 ~ 205 °C ,分子式为 $C_{32}H_{52}O_2$, 1H NMR (400 MHz , $CDCl_3$) δ : 4.55 (1H ,dd , J = 4.6 ,11.7 Hz ,H-3) , 2.06 (3H ,s ,3-OAc) ,1.05 (3H ,s ,H-23) ,1.00 (3H ,s ,H-25) ,0.99 (3H ,d , J = 7.0 Hz ,H-29) , 0.98 (3H ,s ,H-26) ,0.90 (3H ,d , J = 6.0 Hz ,H-30) ,0.88 (3H ,s ,H-27) ,0.87 (3H ,s ,H-28) ,0.84 (3H ,s ,H-24) 。 ^{13}C NMR (100 MHz , $CDCl_3$) δ : 170.2 (s ,3-OAc) ,134.5 (s ,C-18) ,134.0 (s ,C-13) ,80.9 (d ,C-3) ,52.3 (d ,C-5) ,50.5 (d ,C-19) ,40.9 (s ,C-17) ,38.1 (s ,C-4) ,37.7 (t ,C-22) ,37.6 (s ,C-14) ,37.4 (s ,C-8) ,35.9 (d ,C-9) ,34.7 (t ,C-12) ,33.1 (t ,C-22) ,31.9 (d ,C-20) ,31.7 (s ,C-10) ,29.6 (t ,C-1) ,29.0 (t ,C-21) ,28.2 (q ,3-OAc) ,27.9 (q ,C-23) ,27.2 (t ,C-15) ,25.2 (t ,C-16) ,25.1 (q ,C-28) ,24.2 (t ,C-2) ,22.4 (q ,C-27) ,22.2 (q ,C-30) ,21.3 (q ,C-29) ,20.5 (t ,C-11) ,19.9 (q ,C-26) ,19.0 (t ,C-6) ,16.6 (q ,C-25) ,15.6 (q ,C-24) 。EI-MS m/z (%): 468 (52) [M]⁺ ,453 (34) ,410 (47) ,393 (51) ,289 (100) ,255 (32) ,205 (62) ,189 (67) ,69 (96) 。以上波谱数据与文献^[8]比较,可鉴定化合物为 3 β -acetoxyurs-13 (18) -ene。

化合物 2 白色针状结晶 (丙酮) ,mp. 201 ~ 203 °C ,分子式为 $C_{32}H_{52}O_2$, 1H NMR (400 MHz , $CDCl_3$) δ : 4.68 (1H ,s ,H-29b) ,4.57 (1H ,s ,H-29a) ,4.47 (1H ,dd , J = 5.7 ,10.6 Hz ,H-3) ,2.37 (1H ,dt , J = 5.8 ,11.0 Hz ,H-19) ,2.04 (3H ,s ,OAc) ,1.91 (1H ,dt , J = 10.1 ,13.5 Hz ,H-2a) ,1.68 (3H ,s ,H-30) ,1.02 (3H ,s ,H-25) ,0.93 (3H ,s ,H-28) ,0.85 (3H ,s ,H-23) ,0.84 (3H ,s ,H-24) ,0.83

(3H ,s ,H-26) ,0.78 (3H ,s ,H-27) 。 ^{13}C NMR (100 MHz , $CDCl_3$) δ : 171.0 (s ,3-OAc) ,150.9 (s ,C-20) ,109.3 (t ,C-29) ,80.9 (d ,C-3) ,55.3 (d ,C-5) ,50.3 (d ,C-9) ,48.2 (d ,C-18) ,48.0 (d ,C-19) ,41.2 (s ,C-17) ,41.1 (s ,C-14) ,40.3 (s ,C-4) ,40.0 (t ,C-16) ,38.3 (t ,C-7) ,38.0 (d ,C-13) ,37.8 (s ,C-10) ,35.8 (s ,C-8) ,35.5 (t ,C-21) ,34.2 (t ,C-15) ,29.8 (t ,C-12) ,27.9 (q ,3-OAc) ,27.4 (t ,C-2) ,25.0 (t ,C-11) ,23.7 (t ,C-2) ,21.3 (q ,C-23) ,20.9 (t ,C-6) ,19.5 (q ,C-30) ,18.2 (t ,C-1) ,18.0 (q ,C-28) ,16.5 (q ,C-24) ,16.1 (q ,C-25) ,15.9 (q ,C-26) ,14.5 (q ,C-27) 。以上波谱数据与文献^[9]比较,鉴定化合物为 lupeol acetate。

化合物 3 白色粉末,分子式为 $C_{10}H_{10}O_3$, 1H NMR (600 MHz , $CDCl_3$) δ : 9.65 (1H ,d , J = 7.8 Hz ,H-9) ,7.40 (1H ,d , J = 15.8 Hz ,H-7) ,7.13 (1H ,dd , J = 1.8 ,8.2 Hz ,H-6) ,7.07 (1H ,d , J = 1.8 Hz ,H-2) ,6.96 (1H ,d , J = 8.2 Hz ,H-5) ,6.60 (1H ,dd , J = 7.8 ,15.8 Hz ,H-8) ,5.99 (1H ,s ,4-OH) ,3.95 (3H ,s ,3-OAc) 。 ^{13}C NMR (150 MHz , $CDCl_3$) δ : 193.8 (d ,C-9) ,153.2 (d ,C-7) ,148.9 (s ,C-4) ,146.9 (s ,C-3) ,126.4 (s ,C-1) ,126.4 (d ,C-8) ,124.1 (d ,C-6) ,114.9 (d ,C-5) ,109.3 (d ,C-2) ,56.0 (q ,3-OAc) 。以上波谱数据与文献^[10]比较,鉴定化合物为 4-hydroxy 3-methoxycinnamaldehyde。

化合物 4 白色粉末,分子式为 $C_7H_{10}O_4$, 1H NMR (600 MHz , $CDCl_3$) δ : 6.88 (1H ,dd , J = 2.1 ,7.3 Hz ,H-5) ,3.37 (1H ,d , J = 7.4 Hz ,H-3) ,1.92 (3H ,dd , J = 1.1 ,7.4 Hz ,H-6) ,1.45 (3H ,d , J = 7.5 Hz ,H-7) 。 ^{13}C NMR (150 MHz , $CDCl_3$) δ : 177.9 (s ,C-4) ,169.0 (s ,C-1) ,135.4 (d ,C-5) ,132.6 (s ,C-2) ,38.5 (d ,C-3) ,15.6 (q ,C-7) ,14.8 (q ,C-6) 。结合 2D NMR 数据,并与文献^[11]比较得出化合物为 (*Z*)-2-ethylidene-3-methylsuccinic acid。

化合物 5 白色固体,分子式为 $C_{33}H_{54}O_3$, 1H NMR (500 MHz , $CDCl_3$) δ : 5.46 (1H ,d , J = 3.6 Hz ,H-12) ,4.45 (1H ,dd , J = 4.8 ,11.7 Hz ,H-3) ,3.19 (3H ,s ,11-OMe) ,1.99 (3H ,s ,3-OAc) ,1.24 (3H ,s ,H-27) ,1.09 (3H ,s ,H-25) ,1.03 (3H ,s ,H-26) ,0.91 (3H ,s ,H-29) ,0.89 (3H ,s ,H-30) ,0.89 (3H ,s ,H-24) ,0.88 (3H ,s ,H-23) ,0.86 (3H ,s ,H-28) 。 ^{13}C NMR (125 MHz , $CDCl_3$) δ : 171.1 (s ,3-OAc) ,150.4 (s ,C-13) ,123.3 (d ,C-12) ,81.4 (d ,C-3) ,

76.6 (d, C-11) 56.4 (d, C-5) 54.2 (q, H-1-OMe), 53.0 (d, C-9) 48.1 (d, C-18) 47.8 (t, C-19) 42.5 (s, C-8) 41.6 (s, C-14) 38.2 (C-4) 40.7 (t, C-1) 38.1 (t, C-22) 38.0 (s, C-10) 35.7 (t, C-21), 34.7 (t, C-7) 32.1 (s, C-17) 31.0 (s, C-20) 33.9 (q, C-29) 21.5 (q, 3-OAc) 24.4 (q, C-30) 28.8 (q, C-28) 27.3 (q, C-27) 17.8 (q, C-26) 17.5 (q, C-25) 17.4 (q, C-24) 28.8 (q, C-23) 27.9 (t, C-16) 27.4 (t, C-15) 19.4 (t, C-6) 23.9 (t, C-2)。EI-MS m/z (%): 498 (95) $[M]^+$ 482 (32), 466 (51), 451 (21), 407 (25), 293 (29), 273 (49), 248 (100), 232 (47)。以上波谱数据与文献^[12]比较, 故鉴定化合物为 olean-12-ene-11 α -methoxy-3 β -acetate。

化合物 6 白色簇状结晶(丙酮), mp. 231 ~ 232 °C, 分子式 $C_{32}H_{52}O_2$, 1H NMR (400 MHz, $CDCl_3$) δ : 5.70 (1H, t, $J = 4.8$ Hz, H-12) 4.48 (1H, dd, $J = 4.1, 11.4$ Hz, H-3) 2.04 (3H, s, 3-OAc) 1.05 (3H, s, H-23) 1.03 (3H, d, $J = 6.5$ Hz, H-29) 1.02 (3H, s, H-25) 0.97 (3H, s, H-26) 0.95 (3H, s, H-27) 0.93 (3H, d, $J = 7.0$ Hz, H-30) 0.90 (3H, s, H-28) 0.87 (3H, s, H-24)。 ^{13}C NMR (100 MHz, $CDCl_3$) δ : 170.8 (s, 3-OAc) 142.9 (s, C-13) 124.8 (d, C-12) 80.4 (d, C-3) 53.1 (d, C-18) 52.0 (d, C-5) 39.9 (d, C-9) 34.9 (d, C-19) 31.9 (d, C-20) 39.1 (s, C-14) 38.8 (s, C-8) 37.6 (s, C-4) 37.5 (t, C-22) 36.9 (t, C-21), 35.8 (s, C-10) 35.7 (t, C-7) 35.0 (s, C-17) 31.2 (t, C-15) 29.1 (t, C-16) 27.9 (q, 3-OAc) 27.8 (q, C-23) 25.1 (q, C-28) 24.1 (t, C-1) 24.1 (t, C-11) 23.7 (q, C-27) 22.5 (t, C-6) 22.4 (q, C-30) 22.0 (t, C-2) 21.3 (q, C-24) 17.9 (q, C-29) 17.8 (q, C-26) 17.2 (q, C-25)。EI-MS m/z (%): 468 (8) $[M]^+$ 453 (9), 424 (12), 423 (49) 422 (100) 407 (51) 394 (25) 393 (28), 379 (30)。以上波谱数据与文献^[13, 14]比较, 故鉴定化合物为 α -amyrin acetate。

化合物 7 白色针状结晶(丙酮), mp. 105 ~ 107 °C, 分子式 $C_{32}H_{52}O_2$, 1H NMR (400 MHz, $CDCl_3$) δ : 5.54 (1H, m, H-11) 4.51 (1H, dd, $J = 5.8, 10.4$ Hz, H-3) 2.05 (3H, s, 3-OAc) 1.08 (3H, d, $J = 5.9$ Hz, H-30) 1.01 (3H, s, H-25) 0.92 (3H, s, H-28) 0.90 (3H, d, $J = 6.4$ Hz, H-29) 0.85

(3H, s, H-23) 0.84 (3H, s, H-24) 0.83 (3H, s, H-26) 0.78 (3H, s, H-27)。 ^{13}C NMR (100 MHz, $CDCl_3$) δ : 170.8 (s, 3-OAc) 157.0 (s, C-9) 118.9 (d, C-11) 80.6 (d, C-3) 58.7 (d, C-5) 58.2 (d, C-13) 54.5 (d, C-18) 53.7 (d, C-19) 41.1 (t, C-12) 41.0 (s, C-14) 39.8 (s, C-4) 39.6 (t, C-16), 38.3 (t, C-7) 37.8 (s, C-10) 35.8 (s, C-8) 35.5 (t, C-21) 35.1 (d, C-20) 34.9 (t, C-1) 34.1 (t, C-15) 29.8 (t, C-12) 27.9 (q, 3-OAc) 27.4 (t, C-2) 25.0 (t, C-11) 23.2 (t, C-2) 22.2 (q, C-23), 22.1 (q, C-30) 21.2 (q, C-28) 19.5 (q, C-24), 19.4 (t, C-6) 18.9 (q, C-25) 16.8 (q, C-26) 16.6 (q, C-27)。以上波谱数据与文献^[15]比较, 故鉴定化合物为 lupeol-9(11)en-3 β -acetate。

4 结论

从绿茎还阳参中分离得到了7个化合物, 包括5个三萜、1个酚类和1个小分子化合物。目前从绿茎还阳参中分离得到的主要次生代谢产物为三萜类化合物, 而同属其它植物(如: 羌藜还阳参、还阳参、*C. bocconi*)中主要次生代谢产物为倍半萜类化合物。因此, 从化学角度上该属植物的同源性还有待进一步研究。

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肪酸等活性物质发挥的作用,使血脂代谢达到平衡。

目前,心脑血管疾病是世界最大的难题,其发病率、死亡率、用药额均占首位^[1]。心脑血管病的发生主要原因是血管壁硬化,而血管壁硬化的原因是脂物质的沉积。实验证明,林蛙卵酶解液可以明显改善去卵巢大鼠的高血脂症状,有效的抑制肝细胞受损,并且可以降低大鼠的脂肪系数和肝脏系数,这说明林蛙卵可以很好的预防心脑血管疾病。

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