

calibration curves were above 0.999. Standard addition recovery was 88.7%~110.0% and RSD 7.0%. Under the given analytical conditions, the results obtained showed that the contents of the six trace elements varied in these TCM samples, for element Cr, ranging between 0~11.82  $\mu\text{g}\cdot\text{g}^{-1}$ , Mn in 4.36~564.3  $\mu\text{g}\cdot\text{g}^{-1}$ , Cu in 0.55~13.34  $\mu\text{g}\cdot\text{g}^{-1}$ , Zn in 3.19~86.23  $\mu\text{g}\cdot\text{g}^{-1}$ , Se in 0~0.39  $\mu\text{g}\cdot\text{g}^{-1}$  and V in 0.05~12.04  $\mu\text{g}\cdot\text{g}^{-1}$ , respectively. **Conclusion:** The possible relationship between the content of the six trace elements and 13 TCM samples for dietetic therapy of diabetes mellitus was also discussed.

[Key words] traditional Chinese medicines; diabetes mellitus; medicated diet; trace elements; ICP-MS

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## 帽蕊木中的喹诺酸三萜皂苷

康文艺<sup>1,3\*</sup>, 石渊渊<sup>2</sup>, 郝小江<sup>3</sup>

(1. 河南大学 天然药物研究所, 河南 开封 475004; 2. 河南大学 医学院, 河南 开封 475004;  
3. 中国科学院 昆明植物研究所, 云南 昆明 65020)

**[摘要]** 目的:研究帽蕊木 *M. rotundifolia* 茎皮的正丁醇部位化学成分。方法:采用各种色谱法分离,运用多种波谱技术鉴定结构。结果:分离鉴定出 6 个化合物 quinovic acid-3-O- $\beta$ -D-Deoxy-glucopyranoside, 28-O- $\beta$ -D-glucopyranosyl ester(1), quinovic acid-27-O- $\alpha$ -L-Rhamnopyranosyl ester(2), quinovic acid-3-O- $\alpha$ -L-Rhamnopyranoside(3), quinovic acid-27-O- $\beta$ -D-glucopyranosyl ester(4), quinic acid-3-O- $\beta$ -D-Deoxy-glucopyranoside(5), quinovic acid-27-O- $\beta$ -6-deoxy-D-glucopyranosyl ester(6)。结论:化合物 1~6 均为首次从该植物中得到,化合物 1~4 和 6 为首次从该属中得到。

[关键词] 帽蕊木;茎皮;三萜皂苷

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帽蕊木 *M. rotundifolia* Kuntze 为茜草科 Rubiaceae 帽蕊木属植物。本属植物约有 10 种,非洲分布 4 种,亚洲 6 种。分布在印度,孟加拉,缅甸,泰国和老挝等国家<sup>[1~3]</sup>。我国仅有 1 种,产于云南南部<sup>[4,5]</sup>。帽蕊木属植物的研究主要集中在吲哚类生物碱成分上,药理研究也相当明确,主要集中在抗肿瘤、心血管疾病和抗菌活性<sup>[1~3]</sup>。作者前期的研究工作中对该植物抗菌活性的醋酸乙酯部位进行了初步研究,分离鉴定并报道了 12 个化合物<sup>[6]</sup>,在筛选抑制艾滋病病毒活性化合物研究中,进一步对该植物的正丁醇部位进行了系统的分离,又获得 6 个喹诺酸类型的三萜皂苷,并对其化学分类学做了讨论<sup>[7]</sup>,其中化合物 1~6 均为首次从该植物中得到,化合物 1~4 和 6 为首次从该属中得到。

### 1 仪器与材料

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[通讯作者] \*康文艺, E-mail: kangweny@hotmai.com

XRC-1型显微熔点测定仪(温度未校正);SEPA-300型旋光仪;Bruker Am-4000型超导核磁共振仪;Finnegan-4510型质谱仪。柱色谱材料为青岛海洋化工厂生产的 200~300 目及 4  $\mu\text{m}$  硅胶;薄层色谱材料为青岛海洋化工厂生产的 GF254 硅胶板,Sephadex LH-20(瑞典 Pharmacia 公司),C-18(德国 Merk 公司)。

帽蕊木于 2000 年 5 月采自云南省西双版纳地区,经中国科学院西双版纳植物园崔景云高级工程师鉴定为茜草科帽蕊木属植物 *M. rotundifolia*,标本见于中国科学院昆明植物所标本馆(KUN No 0334819)。

### 2 提取分离

帽蕊木茎皮 6.4 kg 粉碎后,用 95% 乙醇室温下冷浸 3 次,每次 7 d。回收乙醇,将浸膏悬浮于水中,依次用醋酸乙酯和正丁醇萃取。正丁醇部分 44 g 经过 200~300 目硅胶柱色谱,氯仿-甲醇梯度洗脱(95:5~6:4)。氯仿-甲醇(8:2)部分经硅胶 H 柱色谱,氯仿-甲醇-水(8:2:0.2)洗脱,以 TLC 检测合并,

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经 Sephadex LH - 20柱色谱,甲醇洗脱得到化合物**1**(57 mg),**2**(34 mg),**3**(54 mg)。氯仿-甲醇(1:1)部分经 Sephadex LH - 20柱色谱,氯仿-甲醇(1:1)洗脱,TLC检测合并后,经C-18柱色谱,甲醇水(7:3)洗脱得到**4**(56 mg),**5**(107 mg)和**6**(28 mg)。

### 3 结构鉴定

**化合物1** 白色粉末(甲醇),HRFAB-MS  $m/z$  793.4403,Calcd. 793.4374。 $\text{mp}$  275~277<sup>o</sup>,[ $\eta$ ]<sub>D</sub><sup>24</sup> +50(*c* 0.1, MeOH)。<sup>1</sup>H-NMR(400 MHz, C<sub>5</sub>D<sub>5</sub>N) : 6.35(1H, d, *J* = 8.0 Hz, H-1), 5.98(1H, br s, H-12), 4.40(1H, d, *J* = 8.0 Hz, H-4), 4.24(1H, t, *J* = 8.1 Hz, H-3), 4.15(1H, t, *J* = 8.0 Hz, H-2) 4.09(1H, m, H-5), 4.42, 4.44(2H, dd, *J* = 12.51, 4.58 Hz, H-6), 4.67(1H, d, *J* = 7.66 Hz, H-1), 3.96(1H, t, *J* = 8.08 Hz, H-3), 3.78(1H, m, H-2), 3.78(1H, m, H-2), 3.69(1H, t, *J* = 8.08 Hz, H-5), 1.65(3H, d, *J* = 5.90 Hz, H-6), 3.26(1H, dd, *J* = 11.04, 4.80 Hz, H-3), 2.67(1H, d, *J* = 12.02 Hz, H-18), 1.12(3H, s, H-23), 0.73(3H, s, H-24), 0.72(3H, s, H-25), 1.15(3H, d, *J* = 5.90 Hz, H-29), 0.80(3H, d, *J* = 5.80, H-30), <sup>13</sup>C-NMR(100 MHz, C<sub>5</sub>D<sub>5</sub>N) : 39.5(C-1), 26.9(C-2), 88.2(C-3), 39.5(C-4), 55.8(C-5), 18.6(C-6), 37.5(C-7), 40.2(C-8), 47.3(C-9), 36.4(C-10), 23.4(C-11), 129.6(C-12), 133.3(C-13), 56.8(C-14), 26.2(C-15), 25.5(C-16), 49.0(C-17), 54.7(C-18), 39.0(C-19), 37.5(C-20), 30.3(C-21), 37.2(C-22), 28.0(C-23), 17.1(C-24), 16.7(C-25), 18.8(C-26), 178.1(C-27), 176.5(C-28), 19.3(C-29), 21.2(C-30), 106.7(C-1), 75.9(C-2), 78.4(C-3), 76.9(C-4), 72.7(C-5), 18.1(C-6), 95.7(C-1), 74.2(C-2), 78.9(C-3), 71.2(C-4), 79.3(C-5), 62.3(C-6)。参考文献[9],确定为 quinovic acid-3-O-6-deoxy-glucopyraosyl, 28-D-glucopyranosyl ester。

**化合物2** 白色粉末(甲醇),Negative FAB-MS  $m/z$  632[M]<sup>+</sup>。<sup>1</sup>H-NMR(400 MHz, C<sub>5</sub>D<sub>5</sub>N) : 6.00(1H, br s, H-12), 6.30(1H, br s, H-1), 4.56(1H, d, *J* = 11.6 Hz, H-6), 4.41(1H, dd, *J* = 11.6, 5.1 Hz, H-6), 4.20(2H, m, H-3, 4), 3.90(2H, m, H-2, 5), 3.19(1H, dd, *J* = 11.3, 4.2 Hz, H-3), 2.62(1H, d, *J* = 11.4 Hz, H-18), 1.22(3H, d, *J* = 5.6 Hz, H-

30), 1.12, 1.07, 0.93, 0.83(S, CH<sub>3</sub> ×4), 0.78(3H, d, *J* = 6.0, H-29)。<sup>13</sup>C-NMR(100 MHz, C<sub>5</sub>D<sub>5</sub>N) : 39.2(C-1), 29.1(C-2), 79.3(C-3), 40.2(C-4), 55.8(C-5), 18.9(C-6), 37.6(C-7), 40.8(C-8), 47.4(C-9), 37.4(C-10), 23.5(C-11), 129.6(C-12), 133.3(C-13), 56.0(C-14), 26.2(C-15), 25.2(C-16), 49.0(C-17), 54.7(C-18), 39.1(C-19), 37.5(C-20), 30.4(C-21), 36.5(C-22), 28.6(C-23), 19.3(C-24), 16.7(C-25), 18.2(C-26), 173.6(C-27), 180.2(C-28), 16.6(C-29), 21.2(C-30), 95.7(C-1), 72.7(C-2), 71.9(C-3), 73.9(C-4), 68.2(C-5), 18.3(C-6)。与文献[9]对照,确定为 quinovic acid-27-O-β-Rhamnopyranosyl ester。

**化合物3** 白色粉末(甲醇)。Negative FAB-MS  $m/z$  631[M - 1]<sup>+</sup>。<sup>1</sup>H-NMR(400 MHz, C<sub>5</sub>D<sub>5</sub>N) : 6.00(1H, br s, H-12), 5.17(1H, br s, H-1), 3.34(1H, dd, *J* = 12.0, 4.8 Hz, H-3), 2.64(1H, d, *J* = 11.3 Hz, H-18), 1.20, 1.06, 0.88, 0.84(each 3H, s) 1.16(3H, d, *J* = 6.0 Hz, H-29), 0.73(3H, d, *J* = 5.8 Hz, H-30), 4.38(1H, t, *J* = 7.6 Hz, H-2), 4.15(1H, overlap, H-3), 4.35(1H, m, H-4), 4.15(1H, overlap, H-5), 1.68(3H, d, *J* = 6.0 Hz, H-6)。<sup>13</sup>C-NMR(100 MHz, C<sub>5</sub>D<sub>5</sub>N) : 39.4(C-1), 26.5(C-2), 88.2(C-3), 40.0(C-4), 55.5(C-5), 18.7(C-6), 37.8(C-7), 39.0(C-8), 47.2(C-9), 38.8(C-10), 23.4(C-11), 129.0(C-12), 134.2(C-13), 56.9(C-14), 26.4(C-15), 25.6(C-16), 48.8(C-17), 55.0(C-18), 39.4(C-19), 37.5(C-20), 30.6(C-21), 37.2(C-22), 28.1(C-23), 18.9(C-24), 16.8(C-25), 18.9(C-26), 178.1(C-27), 180.5(C-28), 18.4(C-29), 21.4(C-30), 103.8(C-1), 72.2(C-2), 71.4(C-3), 73.6(C-4), 68.8(C-5), 18.2(C-6)。与文献[9]对照,确定为 quinovic acid-3-O-β-Rhamnopyranoside。

**化合物4** 白色粉末(甲醇)。Negative FAB-MS  $m/z$  647[M - 1]<sup>+</sup>。<sup>1</sup>H-NMR(400 MHz, C<sub>5</sub>D<sub>5</sub>N) : 6.35(1H, d, *J* = 8.1 Hz, H-1), 5.97(1H, br s, H-12), 3.18(1H, dd, *J* = 11.5, 4.8 Hz, H-3), 2.66(1H, d, *J* = 11.2 Hz, H-18), 1.20, 1.10, 0.92, 0.87(each 3H, s), 1.13(3H, d, *J* = 6.0 Hz, H-29), 0.72(3H, d, *J* = 6.2 Hz, H-30)。<sup>13</sup>C-NMR(100 MHz, C<sub>5</sub>D<sub>5</sub>N) : 39.2(C-1), 29.1(C-2), 79.3(C-3), 40.2(C-

4), 55.8(C-5), 18.9(C-6), 37.6(C-7), 40.8(C-8), 47.4(C-9), 37.4(C-10), 23.5(C-11), 129.6(C-12), 133.3(C-13), 56.0(C-14), 26.2(C-15), 25.2(C-16), 49.0(C-17), 54.7(C-18), 39.1(C-19), 37.5(C-20), 30.4(C-21), 36.5(C-22), 28.6(C-23), 19.3(C-24), 16.7(C-25), 18.2(C-26), 176.6(C-27), 180.2(C-28), 16.6(C-29), 21.2(C-30), 96.7(C-1), 75.9(C-2), 78.5(C-3), 76.9(C-4), 72.1(C-5), 62.7(C-6)。与文献[10]对照,确定为quonovic acid-27-O-*D*-glucopyranosyl ester。

**化合物5** 白色粉末(甲醇), HRFAB-MS *m/z*: 631.3877, Cacl. d Mass 631.3846. mp 237~238°。[ $\alpha$ ]<sub>D</sub><sup>25</sup> +25 (c 0.002, MeOH)。<sup>1</sup>H-NMR (400 MHz, C<sub>5</sub>D<sub>5</sub>N) : 5.77 (1H, br s, H-12), 4.63 (1H, d, *J*=7.88 Hz, H-1), 3.91 (1H, t, *J*=8.76 Hz, H-2), 4.05 (1H, t, *J*=8.76 Hz, H-3), 3.64 (1H, t, *J*=8.76 Hz, H-4), 3.73 (1H, m, H-5), 2.74 (1H, d, *J*=11.4 Hz, H-18), 2.69 (1H, dd, *J*=4.84, 11.40 Hz, H-9), 1.61 (3H, d, *J*=5.80 Hz, H-6), 1.09 (3H, s, H-24), 0.90 (3H, s, H-23), 0.84 (3H, s, H-25), 1.05 (3H, s, H-26), 1.18 (3H, d, *J*=6.0 Hz, H-29), 0.76 (3H, d, *J*=6.10 Hz, H-30), <sup>13</sup>C-NMR (100 MHz, C<sub>5</sub>D<sub>5</sub>N) : 39.2(C-1), 29.1(C-2), 79.3(C-3), 40.2(C-4), 55.8(C-5), 18.9(C-6), 37.6(C-7), 40.8(C-8), 47.4(C-9), 37.4(C-10), 23.5(C-11), 129.6(C-12), 133.3(C-13), 56.0(C-14), 26.2(C-15), 25.2(C-16), 49.0(C-17), 54.7(C-18), 39.1(C-19), 37.5(C-20), 30.4(C-21), 36.5(C-22), 28.6(C-23), 19.3(C-24), 16.7(C-25), 18.2(C-26), 176.2(C-27), 180.1(C-28), 16.6(C-29), 21.2(C-30), 95.6(C-1), 75.9(C-2), 78.5(C-3), 77.0(C-4), 72.1(C-5), 18.1(C-6)。与文献[10]对照,确定为quonovic acid-27-O-*6*-deoxy-*D*-glucopyranosyl ester。

**化合物6** 白色粉末,[ $\alpha$ ]<sub>D</sub><sup>15.9</sup>+65.7(c 0.035, MeOH); Negative FAB-MS *m/z*: 631 [M - 1]。<sup>1</sup>H-NMR (400 MHz, C<sub>5</sub>D<sub>5</sub>N) : 3.29 (1H, dd, *J*=11.4, 4.6 Hz, H-3), 6.00 (1H, br s, H-12), 2.68 (1H, d,

*J*=10.6 Hz, H-18), 0.82 (3H, s, H-23), 1.20 (3H, s, H-24), 0.96 (3H, s, H-25), 0.87 (3H, s, H-26), 1.15 (3H, d, *J*=6.0 Hz, H-29), 0.72 (3H, d, *J*=6.3 Hz, H-30), 6.36 (1H, d, *J*=8.1 Hz, H-1), 4.39 (1H, t, *J*=7.8 Hz, H-2), 4.23 (1H, t, *J*=8.4 Hz, H-3), 4.31 (1H, t, *J*=8.8 Hz, H-4), 4.05 (1H, m, H-5), 1.66 (3H, d, *J*=5.9 Hz, H-6)。<sup>13</sup>C-NMR (100 MHz, C<sub>5</sub>D<sub>5</sub>N) : 39.2(C-1), 29.1(C-2), 79.3(C-3), 40.2(C-4), 55.8(C-5), 18.9(C-6), 37.6(C-7), 40.8(C-8), 47.4(C-9), 37.4(C-10), 23.5(C-11), 129.6(C-12), 133.3(C-13), 56.0(C-14), 26.2(C-15), 25.2(C-16), 49.0(C-17), 54.7(C-18), 39.1(C-19), 37.5(C-20), 30.4(C-21), 36.5(C-22), 28.6(C-23), 19.3(C-24), 16.7(C-25), 18.2(C-26), 176.2(C-27), 180.1(C-28), 16.6(C-29), 21.2(C-30), 95.6(C-1), 75.9(C-2), 78.5(C-3), 77.0(C-4), 72.1(C-5), 18.1(C-6)。与文献[10]对照,确定为quonovic acid-27-O-*6*-deoxy-*D*-glucopyranosyl ester。

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## Quinovic acid triterpenoid saponins from bark of *M itragyna rotundifolia*

KANG Wen-yi<sup>1,3</sup>, SHI Yuan-yuan<sup>2</sup>, HAO Xiao-jiang<sup>3</sup>

(1 Institute of Natural Products, Henan University, Kaifeng 475004, China;

(2 Medical College of Henan University, Kaifeng 475004, China;

(3 Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, China)

**[Abstract]** Objective: To study the chemical constituents from the bark of *M itragyna rotundifolia*. Method: Column chromatographic techniques were applied to isolate constituents. A combination of IR, MS and NMR spectroscopy was used to identify structures of constituents. Result: Six compounds were isolated from the *n*-BuOH fraction and their structures were elucidated as quinovic acid-3-O-*D*-6-deoxy-glucopyranoside, 28-O-*D*-glucopyranosyl ester (1), quinovic acid-27-O-*L*-rhamnopyranosyl ester (2), quinovic acid-3-O-*L*-rhamnopyranoside (3), quinovic acid-27-O-*D*-glucopyranosyl ester (4), quinovic acid-3-O-*D*-6-deoxy-glucopyranoside (5), quinovic acid-27-O-*D*-6-deoxy-*D*-glucopyranosyl ester (6). Conclusion: Compounds 1~6 were isolated for the first time from the plant. Compounds 1~4 and 6 were isolated for the first time from the genus.

**[Key words]** *M itragyna rotundifolia*; bark; triterpenoid saponins

[责任编辑 牛泽宇 ]

## 冬虫夏草及其代用品中腺苷和虫草素的LC-MS/MS定量分析研究

杨钊\*, 迟少云, 张春辉, 吴爱英

(山东省青岛市药品检验所, 山东 青岛 266071)

**[摘要]** 目的:建立定量测定冬虫夏草及其代用品中腺苷和虫草素的 LC-MS/MS 方法。方法: HPLC-ESI-MS/MS 法,用 90% 甲醇为提取溶剂,采用多反应监测 (MRM) 模式测定。结果: 腺苷的回归方程  $Y = 89.04X + 506.85$ ,  $r = 0.9997$ , 虫草素的回归方程  $Y = 99.66X + 1251.34$ ,  $r = 0.9988$ , 线性范围均为  $5.0 \sim 1000.0 \mu\text{g} \cdot \text{L}^{-1}$ ; 腺苷和虫草素的检出限 (LOD) 分别为  $0.44, 0.31 \mu\text{g} \cdot \text{L}^{-1}$ , 加样回收率分别为 98.12%, 97.94%。结论: 该法灵敏、选择性好、快速, 可用于冬虫夏草及其代用品中腺苷和虫草素的定量测定和质量控制。

**[关键词]** LC-MS/MS; 冬虫夏草; 腺苷; 虫草素

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冬虫夏草为麦角菌科真菌冬虫夏草 *Cordyceps sinensis* (Berk.) Sacc. 寄生在蝙蝠蛾科昆虫幼虫上的子座及幼虫尸体的复合体, 具有补肺益肾、止血化痰等功效。由于其稀缺, 人们利用从冬虫夏草中分离的不同菌种经深层发酵培养虫草人工菌丝体作为其替代物, 以满足市场需求。研究表明冬虫夏草中

含有腺苷、虫草素、甘露醇及微量元素、矿质元素等成分, 尤以腺苷具有明显的药理作用, 腺苷已被用作冬虫夏草的质控指标<sup>[1]</sup>。腺苷、虫草素等活性成分的含量以往采用紫外分光光度法<sup>[2]</sup>、高效液相色谱法<sup>[3]</sup>、毛细管区带电泳法<sup>[4]</sup>测定。以上方法存在选择性差, 灵敏度低等缺点。本研究用 LC-MS/MS 法, 采用多反应检测 (MRM) 模式, 同时测定了冬虫夏草及其代用品中腺苷和虫草素的含量。

### 1 仪器与试药

Waters 2695 型高效液相色谱仪, 美国 Waters 公

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[通讯作者] \*杨钊, Tel: (0532) 85735184, E-mail: yangzhao@yahoo.com.cn

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