

· 化学与分析 ·

过岗龙化学成分分离鉴定

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[摘要] 目的: 过岗龙为植物榼藤 *Entada phaseoloides* 的干燥藤茎, 民间常用于风湿骨痛的治疗, 为探寻过岗龙的化学物质和活性组分, 课题组对其化学成分进行系统研究。方法: 取过岗龙 9.0 kg, 粉碎后用 70% 乙醇室温浸提 3 次, 每次 24 h, 提取液减压浓缩, 加水混悬后, 依次用石油醚、乙酸乙酯和正丁醇萃取, 利用正相硅胶, LH-20 羟丙基葡聚糖凝胶 (Sephadex LH-20), 小孔树脂凝胶 (MCI) 等柱色谱和半制备高效液相色谱对正丁醇部位进行分离纯化, 应用 ¹H-NMR, ¹³C-NMR 和 MS 等现代波谱分析技术结合理化性质鉴定所得化合物的结构。结果: 从过岗龙 70% 乙醇提取物中分离得到 10 个化合物, 分别鉴定为没食子儿茶素(1), 尿嘧啶核苷(2), 表没食子儿茶素(3), 儿茶素(4), 表儿茶素(5), 原花青素 B₃(6), 原花青素 B₁(7), 圣草次苷(8), (-)-香橙素-3-O-β-D-吡喃葡萄糖苷(9) 和柚皮苷二氢查尔酮(10)。结论: 化合物 2, 6~10 为首次从该植物中分离得到。

[关键词] 榼藤属; 过岗龙; 化学成分; 类风湿关节炎

[中图分类号] R284.2; R79; R22; R2-03; R243 **[文献标识码]** A **[文章编号]** 1005-9903(2018)08-0049-05

[doi] 10.13422/j.cnki.syfjx.20180713

[网络出版地址] <http://kns.cnki.net/kcms/detail/11.3495.R.20180110.1627.007.html>

[网络出版时间] 2018-01-11 16:18

Chemical Constituents from Stems of *Entada phaseoloides*

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[Abstract] **Objective:** The stems of *Entada phaseoloides* have been used for the treatment of rheumatoid arthritis and traumatic injuries. In order to explore its bioactive constituents, 70% ethanol extract was studied systemically in chemistry. **Method:** The dried and powdered stems (9 kg) of *E. phaseoloides* were extracted for three times with 70% ethanol at room temperature (3 × 24 h). The combined ethanol extracts were extracted consecutively with petroleum ether, EtOAc and *n*-BuOH. The components of *n*-BuOH extract were isolated and purified by normal and reverse phase silica gels, Sephadex LH-20 and MCI column chromatography and semi-preparative HPLC. And their structures were confirmed based on the physicochemical properties and spectral analyses. **Result:** Ten compounds were isolated from the *n*-BuOH fraction of 70% ethanol extract from stems of *E. phaseoloides*, which were identified as epigallocatechin (1), uridine (2), galocatechin (3), catechin (4), epicatechin (5), procyanidin B₃ (6), procyanidin B₁ (7), eriocitrin (8), (-)-aromadendrin-3-O-β-D-glucopyranoside (9) and naringin dihydrochalcone (10). **Conclusion:** Compounds 2 and 6-10 were isolated from this plant for the first time.

[Key words] *Entada* genus; *E. phaseoloides*; chemical constituent; rheumatoid arthritis

[收稿日期] 20170814(022)

[基金项目] 国家自然科学基金青年项目(31600272); 湖北省自然科学基金青年项目(2016CFB189)

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过岗龙为豆科榼藤属植物榼藤的干燥藤茎,味涩、微苦,性平,有小毒,具有祛风、除湿、活络行瘀之功效,主治风湿痹痛、腰腿疼痛,系壮族地区常用药物,民间单用或配方给药用于类风湿性关节炎的治疗且疗效显著,是抗风湿、类风湿性关节炎药物过岗龙片的主药^[1],但其治疗类风湿性关节炎的药效物质基础尚未明确。现有研究发现过岗龙主要含有三萜皂苷、黄酮和酚酸类成分^[2-5],其中酚酸类成分已被证实具有较强的抗氧化活性^[4],课题组前期从过岗龙乙醇提取物发现具有抗炎活性和细胞增殖抑制作

用的三萜皂苷^[3],为进一步丰富过岗龙的化学内涵,深入探究其药效物质基础和指导临床安全用药,课题组综合应用柱色谱法和高效液相色谱法对其化学成分进行进一步分离纯化,共得到 10 个化合物,分别鉴定为没食子儿茶素(1),尿嘧啶核苷(2),表没食子儿茶素(3),儿茶素(4),表儿茶素(5),原花青素 B₃(6),原花青素 B₁(7),圣草次苷(8),(-)-香橙素-3-O-β-D-吡喃葡萄糖苷(9)和柚皮苷二氢查尔酮(10),其中化合物 2,6~10 为首次从该植物中分离得到。结构见图 1。

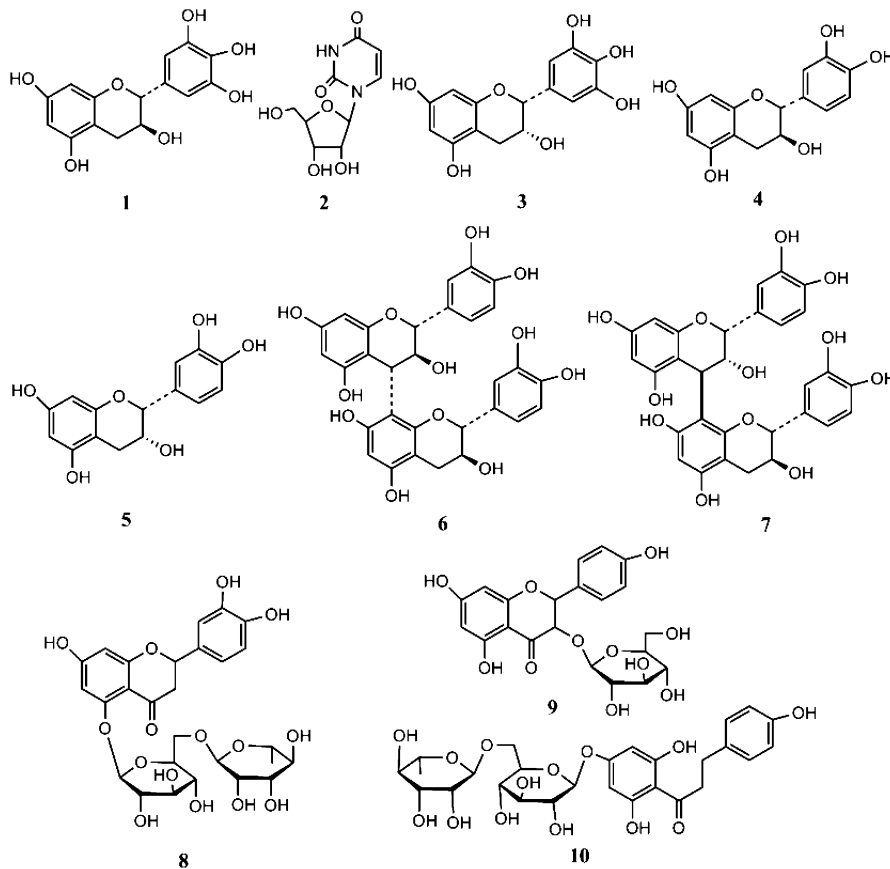


图 1 化合物 1~10 化学结构

Fig.1 Structures of compounds 1-10

1 材料

DRX-500 MHz 型超导核磁共振仪, HCT Esquire 3000 型质谱仪(德国 Bruker 公司); Ultimate 3000 型高效液相色谱仪(美国 Dionex 公司), Phenomenex 色谱柱(Luna 5u C₁₈ 100A, 10.0 mm × 250.0 mm, 5 μm); 柱色谱硅胶和高效薄层板(青岛海洋化工有限公司); ODS 反相硅胶(日本 YMC 公司, 50 μm); MCI GEL CHP20P(日本三菱化学株式会社, 75~150 μm); LH-20 羟丙基葡聚糖凝胶(Sephadex LH-

20, 美国 GE 公司); 常用分离用试剂均为国药集团化学试剂有限公司分析纯产品, 高效液相色谱试剂为 TEDIA 色谱纯产品。

过岗龙采自云南西双版纳傣族自治州, 经中南民族大学药学院刘新桥副教授鉴定为豆科植物榼藤 *Entada phaseoloides* 的藤茎, 标本现存放于中南民族大学民族药物研究院。

2 提取和分离

过岗龙(9.0 kg)粉碎后用 70% 乙醇室温浸提 3

次,每次24 h,提取液减压浓缩,加水混悬,依次用石油醚、乙酸乙酯和水饱和正丁醇萃取,得正丁醇萃取物173.9 g。将正丁醇萃取物经正相硅胶柱(200~300目)色谱分离,用CH₂Cl₂-CH₃OH(10:0,9:1,6:1,4:1,6:4,0:10)梯度洗脱,洗脱液经TLC检测合并,共得到13个组分(Fr. 1~Fr. 13)。其中Fr. 4(4.60 g)经MCI柱色谱分离,以H₂O-CH₃OH梯度洗脱,经HPLC(H₂O-CH₃CN,75:25)再次分离纯化得化合物1(7.5 mg);Fr. 5(13.2 g)经MCI柱色谱分离,以H₂O-CH₃OH(90:10~0:100)梯度洗脱得到5个组分(Fr. 5.1~Fr. 5.5),Fr. 5.1经Sephadex LH-20柱色谱分离(CH₃OH洗脱),经HPLC[H₂O-CH₃CN(0~5 min,97:3;5~10 min,97:3~95:5;10~15 min,95:5~90:10;15~20 min,90:10~80:20)]进一步纯化得化合物2(4.0 mg);Fr. 5.2经Sephadex LH-20柱色谱分离(CH₃OH洗脱),HPLC(H₂O-CH₃CN 78:22)纯化得化合物3(6.7 mg),4(27.1 mg),5(28.6 mg),6(5.8 mg),7(6.8 mg);Fr. 5.3经Sephadex LH-20柱色谱分离(CH₃OH洗脱),HPLC[H₂O-CH₃CN(0~5 min,80:20;5~25 min,80:20~70:30)]纯化得化合物8(12.9 mg),9(7.0 mg)和10(5.0 mg)。

3 结构鉴定

化合物1 白色粉末(CH₃OH),ESI-MS m/z 305 [M-H]⁻,分子式为C₁₅H₁₄O₇。¹H-NMR(500 MHz,CD₃OD)δ:6.59(2H,br s,H-2',6'),5.94(1H,d,J=2.0 Hz,H-8),5.83(1H,d,J=2.0 Hz,H-6),4.58(1H,d,J=7.5 Hz,H-2),4.08(1H,m,H-3),2.80(1H,dd,J=16.0,5.5 Hz,H-4a),2.60(1H,dd,J=16.0,8.0 Hz,H-4b);¹³C-NMR(125 MHz,CD₃OD)δ:157.8(C-7,9),157.5(C-5),146.3(C-3',5'),133.9(C-4'),132.3(C-1'),107.2(C-2',6'),100.9(C-10),96.5(C-6),95.7(C-8),82.9(C-2),68.9(C-3),28.5(C-4)。以上数据与文献[6]报道一致,可推断化合物1为没食子儿茶素。

化合物2 白色粉末(CH₃OH),ESI-MS m/z 243 [M-H]⁻,分子式为C₉H₁₂N₂O₆。¹H-NMR(500 MHz,CD₃OD)δ:8.02(1H,d,J=8.0 Hz,H-6),5.90(1H,d,J=4.5 Hz,H-1'),5.68(1H,d,J=8.0 Hz,H-5),4.18(1H,m,H-4'),4.03(2H,m,H-2',H-3'),3.84(1H,dd,J=12.5,2.5 Hz,H-5'a),3.76(1H,dd,J=12.0,3.0 Hz,H-5'b)。¹³C-NMR(125 MHz,CD₃OD)δ:165.0(C-4),152.5(C-2),142.7(C-6),102.7(C-5),90.8(C-1'),86.4(C-4'),75.7

(C-3'),71.3(C-2'),62.3(C-5')。以上波谱数据与文献[7]报道一致,可推断化合物2为尿嘧啶核苷。

化合物3 白色粉末(CH₃OH),ESI-MS m/z 305 [M-H]⁻,分子式为C₁₅H₁₄O₇。¹H-NMR(500 MHz,CD₃OD)δ:6.50(2H,br s,H-2',6'),5.95(1H,d,J=2.5 Hz,H-8),5.83(1H,d,J=2.5 Hz,H-6),4.58(1H,d,J=7.5 Hz,H-2),4.06(1H,br s,H-3),2.94(1H,dd,J=16.0,5.5 Hz,H-4a),2.68(1H,dd,J=16.0,8.0 Hz,H-4b);¹³C-NMR(125 MHz,CD₃OD)δ:158.0(C-9),157.7(C-7),157.4(C-5),145.8(C-3',5'),133.9(C-4'),132.3(C-1'),108.3(C-2',6'),100.4(C-10),96.3(C-6),95.9(C-8),79.4(C-2),67.5(C-3),29.8(C-4)。以上数据与文献报道[8]一致,可推断化合物3为表没食子儿茶素。

化合物4 白色粉末(CH₃OH),ESI-MS m/z 289 [M-H]⁻,分子式为C₁₅H₁₄O₆。¹H-NMR(500 MHz,CD₃OD)δ:6.83(1H,s,H-2'),6.50(2H,m,H-5',6'),5.92(1H,d,J=2.0 Hz,H-8),5.85(1H,d,J=2.0 Hz,H-6),4.56(1H,d,J=7.5 Hz,H-2),3.99(1H,dd,J=13.0,7.5 Hz,H-3),2.85(1H,dd,J=16.0,5.5 Hz,H-4a),2.51(1H,dd,J=16.0,7.5 Hz,H-4b);¹³C-NMR(125 MHz,CD₃OD)δ:157.8(C-9),157.6(C-7),156.9(C-5),146.9(C-4'),146.2(C-3'),132.2(C-1'),120.1(C-6'),116.1(C-5'),115.2(C-2'),100.8(C-10),96.3(C-6),95.5(C-8),82.8(C-2),68.8(C-3),28.5(C-4)。以上数据与文献[8]报道一致,可推断化合物4为儿茶素。

化合物5 白色粉末(CH₃OH),ESI-MS m/z 289 [M-H]⁻,分子式为C₁₅H₁₄O₆。¹H-NMR(500 MHz,CD₃OD)δ:6.97(1H,s,H-2'),6.76(2H,m,H-5',6'),5.94(1H,d,J=2.0 Hz,H-8),5.91(1H,d,J=2.0 Hz,H-6),4.80(1H,d,J=7.5 Hz,H-2),4.16(1H,m,H-3),3.34(2H,d-like,J=16.5 Hz,H-4);¹³C-NMR(125 MHz,CD₃OD)δ:158.0(C-9),157.7(C-7),157.4(C-5),145.9(C-4'),145.8(C-3'),132.3(C-1'),119.4(C-6'),115.9(C-5'),115.3(C-2'),100.0(C-10),96.3(C-6),95.9(C-8),79.9(C-2),67.5(C-3),29.8(C-4)。以上数据与文献[8]报道一致,可推断化合物5为表儿茶素。

化合物6 黄色粉末(CH₃OH),ESI-MS m/z 577 [M-H]⁻,分子式为C₃₀H₂₆O₁₂。¹H-NMR(500 MHz,CD₃OD)δ:6.95(2H,br s,H-10,10'),6.75(2H,dd,J=8.0,3.0 Hz,H-14),6.73(2H,m,H-13,

13'), 6.66 (2H, dd, $J = 8.0, 2.0$ Hz, H-14'), 6.06 (1H, s, H-6'), 5.88 (1H, d, $J = 2.0$ Hz, H-6), 5.78 (1H, d, $J = 2.0$ Hz, H-8), 4.54 (1H, d, $J = 7.5$ Hz, H-2), 4.50 (1H, m, H-2'), 4.40 (1H, d, $J = 8.5$ Hz, H-4), 4.34 (1H, m, H-3'), 4.24 (1H, d, $J = 8.5$ Hz, H-3), 2.76 (1H, dd, $J = 16.0, 5.5$ Hz, H-4'a), 2.49 (1H, dd, $J = 16.0, 8.0$ Hz, H-4'b); $^{13}\text{C-NMR}$ (125 MHz, CD_3OD) δ : 158.7 (C-9), 157.1 (C-7), 155.9 (C-5, 5', 7'), 154.9 (C-9'), 146.1 (C-12), 145.8 (C-11, 12'), 145.8 (C-11'), 132.7 (C-9), 132.0 (C-9'), 120.7 (C-14), 119.9 (C-14'), 116.0 (C-13, 10', 13'), 115.6 (C-10), 108.2 (C-8'), 102.3 (C-10, 10'), 97.4 (C-6'), 97.0 (C-6), 96.2 (C-8), 84.0 (C-2'), 82.5 (C-2), 73.7 (C-3), 68.9 (C-3'), 38.6 (C-4), 28.8 (C-4')。以上数据与文献[8]报道一致,可推断化合物 6 为原花青素 B₃。

化合物 7 黄色粉末 (CH_3OH), ESI-MS m/z 577 $[\text{M} - 1]^-$, 分子式为 $\text{C}_{30}\text{H}_{26}\text{O}_{12}$ 。 $^1\text{H-NMR}$ (500 MHz, CD_3OD) δ : 6.88 (1H, d, $J = 2.0$ Hz, H-10), 6.84 (1H, d, $J = 2.0$ Hz, H-10'), 6.73 (3H, m, H-13, 13', 14'), 6.69 (1H, d, $J = 2.0$ Hz, H-14), 6.05 (2H, s, H-6, 8), 6.00 (1H, s, H-6'), 4.86 (overlapping, H-2), 4.58 (1H, d, $J = 13.0$ Hz, H-2'), 4.56 (1H, d, $J = 6.0$ Hz, H-4), 4.00 (1H, br s, H-3), 3.96 (1H, dd, $J = 13.0, 8.0$ Hz, H-3'), 2.77 (1H, br d, $J = 14.0$ Hz, H-4'a), 2.46 (1H, dd, $J = 14.0, 7.5$ Hz, H-4'b); $^{13}\text{C-NMR}$ (125 MHz, CD_3OD) δ : 159.5 (C-9), 159.4 (C-7), 158.1 (C-5'), 156.0 (C-5, 5'), 155.7 (C-7'), 155.0 (C-9'), 146.2 (C-12, 12'), 146.0 (C-11), 145.8 (C-11'), 132.4 (C-9, 9'), 120.1 (C-14), 119.3 (C-14'), 116.2 (C-13, 10', 13'), 115.3 (C-10), 108.3 (C-8'), 101.5 (C-10, 10'), 96.9 (C-6'), 96.3 (C-6, C-8), 82.6 (C-2'), 77.3 (C-2), 72.7 (C-3), 68.0 (C-3'), 37.7 (C-4), 28.6 (C-4')。以上数据与文献[8]报道一致,可推断化合物 7 为原花青素 B₁。

化合物 8 黄色粉末 (CH_3OH), ESI-MS m/z 595 $[\text{M} - 1]^-$, 分子式为 $\text{C}_{27}\text{H}_{32}\text{O}_{15}$ 。 $^1\text{H-NMR}$ (500 MHz, CD_3OD) δ : 6.95 (1H, d, $J = 1.5$ Hz, H-2'), 6.79 (2H, m, H-5', 6'), 6.18 (2H, m, H-6, 8), 5.36 (1H, m, H-2), 2.78 (2H, m, H-3); Glc, 4.97 (1H, d, $J = 7.0$ Hz, H-1), 4.00 (1H, dd, $J = 11.0, 4.0$ Hz, H-6a), 3.60 (1H, m, H-6b), 3.33 ~ 3.68 为糖上其他质子信号; Rha, 4.72 (1H, d, $J = 4.0$ Hz, H-1), 3.63 (1H, m, H-5), 3.33 ~ 3.68 为糖上其他质子信号,

1.19 (3H, d, $J = 6.0$ Hz, CH_3); $^{13}\text{C-NMR}$ (CD_3OD , 125 MHz) δ : 198.5 (C-4), 166.9 (C-7), 164.9 (C-9), 164.5 (C-5), 146.9 (C-4'), 146.5 (C-3'), 131.7 (C-1'), 119.3 (C-6'), 114.9 (C-2'), 114.7 (C-5'), 102.0 (C-10), 98.0 (C-8), 97.0 (C-6), 80.7 (C-2), 44.3 (C-3); Glc: 101.2 (C-1), 77.8 (C-3), 77.1 (C-5), 74.7 (C-2), 71.5 (C-4), 67.6 (C-6); Rha: 101.2 (C-1), 74.7 (C-4), 72.5 (C-2), 72.1 (C-3), 69.5 (C-5), 17.9 (C-6), 以上数据与文献[9]报道一致,可推断化合物 8 为圣草次苷。

化合物 9 黄色粉末 (CH_3OH), ESI-MS m/z 449 $[\text{M} - 1]^-$, 分子式为 $\text{C}_{21}\text{H}_{22}\text{O}_{11}$ 。 $^1\text{H-NMR}$ (500 MHz, CD_3OD) δ : 7.36 (2H, d, $J = 8.0$ Hz, H-2', 6'), 6.80 (2H, d, $J = 8.0$ Hz, H-3', 5'), 5.91 (1H, d, $J = 1.0$ Hz, H-6), 5.89 (1H, d, $J = 1.0$ Hz, H-8), 5.27 (1H, d, $J = 10.0$ Hz, H-2), 5.00 (1H, d, $J = 10.0$ Hz, H-3); Glc, 4.90 (overlapping, H-1), 3.70 (1H, m, H-6a), 3.58 (1H, dd, $J = 11.0, 6.0$ Hz, H-6b), 3.06 ~ 3.30 为糖上其他质子信号; $^{13}\text{C-NMR}$ (125 MHz, CD_3OD) δ : 196.1 (C-4), 169.1 (C-7), 165.5 (C-9), 164.2 (C-5), 159.4 (C-4'), 130.5 (C-2', 6'), 128.6 (C-1'), 116.3 (C-3', 5'), 102.3 (C-10), 97.4 (C-6), 96.3 (C-6), 83.6 (C-2), 77.6 (C-3); Glc, 102.4 (C-1), 78.3 (C-5), 77.8 (C-3), 74.7 (C-2), 71.3 (C-4), 62.7 (C-6)。以上数据与文献[10]报道一致,可推断化合物 9 为 (-)-香橙素-3-O- β -D-吡喃葡萄糖苷。

化合物 10 黄色粉末 (CH_3OH), ESI-MS m/z 581 $[\text{M} - 1]^-$, 分子式为 $\text{C}_{27}\text{H}_{34}\text{O}_{14}$ 。 $^1\text{H-NMR}$ (500 MHz, CD_3OD) δ : 7.07 (2H, d, $J = 8.0$ Hz, H-2, 6), 6.38 (2H, $J = 8.0$ Hz, H-3, 5), 6.15 (1H, d, $J = 2.0$ Hz, H-3'), 5.96 (1H, d, $J = 2.0$ Hz, H-5'), 3.42 (2H, m, H- α), 2.86 (2H, $J = 7.5$ Hz, H- β); Glc, 5.00 (1H, d, $J = 7.0$ Hz, H-1), 4.00 (1H, d-like, $J = 11.0$, H-6a), 3.62 (1H, d-like, $J = 11.0$, H-6b), 3.33 ~ 3.68 为糖上其他质子信号; Rha, 4.69 (1H, d, $J = 2.0$ Hz, H-1), 3.63 (1H, m, H-5), 3.33 ~ 3.68 为糖上其他质子信号, 1.19 (3H, d, $J = 6.0$ Hz, $-\text{CH}_3$); $^{13}\text{C-NMR}$ (125 MHz, CD_3OD) δ : 205.3 (CO), 166.1 (C-4'), 164.4 (C-2'), 160.8 (C-6'), 155.0 (C-4), 132.5 (C-1), 129.0 (C-2, C-6), 114.7 (C-3, C-5), 100.0 (C-1'), 97.1 (C-5'), 94.3 (C-3'), 45.0 (C- α), 29.5 (C- β); Glc, 101.0 (C-1), 77.1 (C-3), 75.9 (C-5), 72.7 (C-2), 69.9 (C-4), 66.3 (C-6); Rha, 101.0 (C-1),

73.4(C-4), 71.0(C-2), 70.7(C-3), 68.5(C-5), 16.5(C-6)。以上数据与文献[11]报道一致,可推断化合物 10 为柚皮苷二氢查尔酮。

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[责任编辑 顾雪竹]