文章编号:1001-6880(2014)10-1548-04

中药甘松中的白藜芦醇低聚体类成分

王忠平[1,2],陈应鹏[1,2],梁　爽[1,2],汪　娟[1,2],郑红红[1,2],吴红华[1,2]*

[1]天津中医药大学中医药研究院 天津市现代中药重点实验室-省部共建国家重点实验室培育基地,天津 300193;

[2]天津国际生物医药联合研究院中药新药研发中心,天津 300457

摘　要:采用硅胶、凝胶等柱色谱以及高效液相制备色谱等现代分离方法,从甘松(*Nardostachys chinensis* Batal.)根及根茎 70% 乙醇冷浸渗漉提取液的乙酸乙酯萃取部位中分离得到 5 个白藜芦醇低聚体类化合物,通过物理化学性质和波谱学方法鉴定其化学结构分别为:-α-viniferin(**1**)、kobophenol A(**2**)、蛇葡萄素 A(ampelopsin A,**3**)、isohopeaphenol(**4**)、grandiphenol A(**5**)。化合物 **1**~**5** 均为首次从该属植物中分离得到。

关键词:甘松;化学成分;白藜芦醇;低聚体

中图分类号:R93　　**文献标识码**:A

Resveratrol Oligomers from *Nardostachys chinensis* Batal.

WANG Zhong-ping[1,2], CHEN Ying-peng[1,2], LIANG Shuang[1,2], WANG Juan[1,2], ZHENG Hong-hong[1,2], WU Hong-hua[1,2]*

[1] *Tianjin State Key Laboratory of Modern Chinese Medicine, Institute of Traditional Chinese Medicine, Tianjin University of Traditional Chinese Medicine, Tianjin 300193, China*; [2] *Research and Development Center of Traditional Chinese Medicine, Tianjin International Joint Academy of Biotechnology and Medicine, Tianjin 300457, China*

Abstract: Five resveratrol oligomers were isolated from the 70% alcohol extracts of the dried roots and rhizomes of *Nardostachys chinensis* Batal.. On the basis of spectroscopic data and physicochemical properties, their chemical structures were identified as-α-viniferin (**1**), kobophenol A (**2**), ampelopsin A (**3**), isohopeaphenol (**4**) and grandiphenol A (**5**). This is the first report of resveratrol oligomers isolated from *Nardostachys* DC..

Key words: *Nardostachys chinensis* Batal.; chemical constituents; resveratrol; oligomers

甘松为败酱科多年生草本植物甘松的干燥根及根茎,性辛、甘、温,归脾、胃经,具有"理气止痛,开郁醒脾;外用祛湿消肿"之功效,用于脘腹胀满,食欲不振,呕吐;外用治牙痛,脚气肿毒等,现代药理研究报道其具有抗心律失常、降血压、舒张平滑肌、抗心肌缺血等作用,是稳心颗粒等中药制剂重要组成中药之一。甘松的化学成分国内外已有较多报道,主要含倍半萜、环烯醚萜、三萜等萜类成分。本研究拟通过甘松化学成分的系统分离与鉴定,为后期甘松药材的质量控制及相关中成药物质基础研究提供支持。采用硅胶、凝胶等柱色谱,以及高效液相制备色谱等现代分离方法,从甘松 70% 乙醇冷浸渗漉提取液的乙酸乙酯萃取部位中分离得到 5 个白藜芦醇低聚体类化合物,通过物理化学性质和波谱学方法鉴定其化学结构分别为:α-viniferin(**1**)、kobophenol

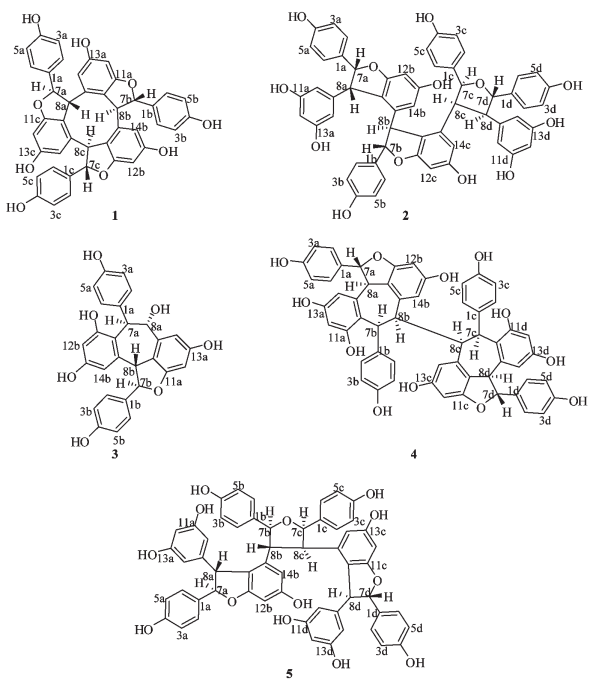

图 1　化合物 1~5 的化学结构

Fig. 1　Chemical structures of compounds **1-5**

收稿日期:2014-04-23　　接受日期:2014-08-05

基金项目:科技部国家国际合作专项项目(2013DFA31620)

* 通讯作者 Tel:86-22-59596163;E-mail:wuhonghua2003@163.com

A(**2**)、蛇葡萄素 A(ampelopsin A,**3**)、isohopeaphenol(**4**)、grandiphenol A(**5**)。化合物 **1**~**5** 均为首次从该属植物中分离得到(图 1)。

1 仪器与材料

BRUKER AV Ⅲ核磁共振波谱仪(瑞士 Bruker 公司),Agilent 1260 制备型液相色谱仪(美国 Agilent 公司),METTLER TOLEDO 万分之一天平(梅特勒-托利多仪器有限公司),SHZ-D(Ⅲ)循环水式多用真空泵、DLSB-5/25 型低温冷却液循环泵、RE-52A 旋转蒸发仪(巩义市英峪予华仪器厂),TH-II 型薄层加热器(上海科哲生化科技有限公司),ZF-20D 暗箱式紫外分析仪(上海顾村电光仪器厂),薄层层析硅胶 GF_{254}、柱层析硅胶(青岛海洋化工厂),反相 C_{18} 柱层析硅胶 ODS-A-HG(日本 YMC 公司),Sephadex LH-20 葡聚糖凝胶(瑞士 GE Healthcare 公司),D101 大孔吸附树脂(天津市海光化工有限公司);所用试剂均为分析纯。

本研究所用药材购自河北安国药材市场,经天津中医药大学中药学院李天祥副教授鉴定为败酱科甘松属植物甘松 *Nardostachys chinensis* Batal. 的根及根茎,标本存放于天津中医药大学中药新药研发中心中药化学与分析部(No. B20604126)。

2 提取与分离

甘松 20 kg,8 倍量 70% 乙醇浸泡 3 次,第 1 次 7 d,后 2 次各 3 d,合并提取液,减压回流至无醇味,得浸膏约 3 kg。药渣用 70% 乙醇煮 2 h,合并提取液,减压回流至无醇味,得浸膏约 400 g。合并两次浸膏得总浸膏 3.4 kg,加适量蒸馏水混悬,依次用石油醚(沸程 60~90 ℃)、乙酸乙酯、正丁醇萃取,减压回收溶剂,得石油醚萃取物 320 g、乙酸乙酯萃取物 1130 g、正丁醇萃取物 600 g。萃取后水层经 70% 乙醇醇沉,所得上清液浓缩回收成浸膏并与正丁醇萃取物合并,再通过 D101 大孔吸附树脂柱乙醇-水梯度洗脱,得水洗脱部位 950 g、30% 乙醇洗脱部位 190 g、50% 乙醇洗脱部位 176 g、70% 乙醇洗脱部位 140 g、95% 乙醇洗脱部位 70 g。95% 乙醇洗脱部位合并入乙酸乙酯萃取物得乙酸乙酯部位。

乙酸乙酯部位 1.2 kg 经硅胶柱色谱,二氯甲烷-甲醇梯度(100∶0~0∶100)洗脱,得 15 个组分(Fr. A、B、C、……)。组分 Fr. J(40 g)再经硅胶柱色谱(乙酸乙酯等度洗脱),得 4 个组分(Fr. JA、JB、JC、JD),其中组分 Fr. JB(3.1 g)经凝胶柱(甲醇洗脱)后,利用硅胶柱及制备高效液相色谱分离得到化合物 **1**(60 mg)、**2**(84 mg)、**3**(10 mg)、**4**(5 mg)、**5**(50 mg)。

3 结构鉴定

化合物 1 黄色粉末(甲醇);$C_{42}H_{30}O_9$,易溶于甲醇,UV 254 nm 显暗斑,UV 365 nm 无荧光;10% 硫酸乙醇溶液显棕黄色。(+)-ESI-MS *m/z* 679.4 $[M+H]^+$,(-)-ESI-MS *m/z* 677.6 $[M-H]^-$。CD(c = 0.0008, CH_3OH) $\Delta\varepsilon$:224 (14.1),259 (-5.0),278 (1.5),302 (-4.6)。^{1}H NMR (DMSO-d_6, 600 MHz) δ:9.59 (2H, br s, 4c/13c-OH),9.56 (1H, br s, 4b-OH),9.47 (1H, br s, 4a-OH),9.45 (1H, br s, 13b-OH),9.42 (1H, br s, 13a-OH),7.20 (2H, d, J = 8.6 Hz, H-2c/6c),7.00 (2H, d, J = 8.6 Hz, H-2b/6b),6.92 (2H, d, J = 8.7 Hz, H-2a/6a),6.73 (4H, d, J = 8.6 Hz, H-3b/5b/3c/5c),6.64 (2H, d, J = 8.6 Hz, H-3a/5a),6.61 (1H, d, J = 1.2 Hz, H-14b),6.46 (1H, d, J = 1.8 Hz, H-14c),6.20 (1H, d, J = 1.7 Hz, H-12b),6.17 (1H, d, J = 1.8 Hz, H-12c),6.14 (1H, d, J = 1.9 Hz, H-12a),5.98 (1H, s, H-7a),5.96 (1H, d, J = 1.9 Hz, H-14a),5.88 (1H, d, J = 10.2 Hz, H-7c),4.91 (1H, d, J = 5.6 Hz, H-7b),4.62 (1H, d, J = 10.2 Hz, H-8c),4.61 (1H, d, J = 5.6 Hz, H-8b),3.78 (1H, s, H-8a);^{13}C NMR (DMSO-d_6, 150 MHz) δ:160.6 (C-13c),160.2 (C-11b/11c),159.5 (C-11a),158.9 (C-13b),158.7 (C-13a),158.0 (C-4b),157.9 (C-4c),157.4 (C-4a),140.6 (C-9b),139.0 (C-9a),137.5 (C-9c),131.1 (C-1c),130.6 (C-1a),130.2 (C-1b),128.2 (C-2b/6b/2c/6c),127.7 (C-2a/6a),119.7 (C-10b),118.6 (C-10c),118.0 (C-10a),115.7 (C-3b/5b/3c/5c),115.3 (C-3a/5a),108.1 (C-14a),105.6 (C-14b),105.2 (C-14c),97.4 (C-12a),96.5 (C-12c),96.1 (C-12b),94.7 (C-7b),88.9 (C-7c),85.6 (C-7a),53.9 (C-8b),51.2 (C-8c),45.0 (C-8a)。2D-NMR 相关谱如图 2 所示。综上所述,并参考文献[1]报道的数据,鉴定化合物 **1** 为 α-viniferin。

化合物 2 褐色粉末(甲醇);$C_{56}H_{44}O_{13}$,易溶于甲醇,UV 254 nm 显暗斑,UV 365 nm 显蓝色荧光;10% 硫酸乙醇溶液显黄褐色。(+)-ESI-MS *m/z* 925.6 $[M+H]^+$,(-)-ESI-MS *m/z* 923.5 $[M-H]^-$。

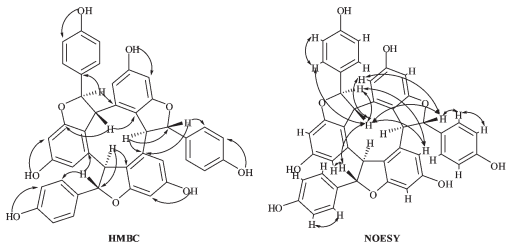

图2 化合物1的HMBC和NOESY相关谱

Fig. 2 Key HMBC and NOESY correlations of compound **1**

^{1}H NMR (DMSO-d_6,400 MHz) δ:9.61,9.47,9.29,9.02,8.96,8.91 (each 1H,br s,-OH),8.82 (2H,br s,-OH),8.60 (2H,br s,-OH),7.27 (2H,d,J=8.4 Hz,H-2a/6a),6.96 (2H,d,J=8.4 Hz,H-2d/6d),6.79 (2H,d,J=8.4 Hz,H-3a/5a),6.69 (2H,d,J=8.4 Hz,H-3d/5d),6.50 (2H,d,J=8.4 Hz,H-3c/5c),6.41 (1H,d,J=1.4 Hz,H-12b),6.36 (2H,d,J=8.4 Hz,H-2c/6c),6.28 (2H,d,J=8.3 Hz,H-3b/5b),6.22 (1H,d,J=1.2 Hz,H-14c),6.17 (2H,d,J=8.4 Hz,H-2b/6b),5.92 (1H,br s,H-12d),5.87 (1H,d,J=1.3 Hz,H-12a),5.84 (2H,br s,H-10a/14a),5.78 (2H,d,J=1.3 Hz,H-12c/14b),5.51 (2H,d,J=1.3 Hz,H-10d/14d),5.41 (1H,br s,H-7a),4.99 (1H,d,J=5.8 Hz,H-7c),4.97 (1H,d,J=12.0 Hz,H-7d),4.85 (1H,d,J=3.5 Hz,H-7b),4.18 (1H,br s,H-8a),3.42 (1H,d,J=3.5 Hz,H-8b),3.10 (1H,dd,J=4.7,5.0 Hz,H-8c),2.88(1H,dd,J=10.5,5.6 Hz,H-8d);^{13}C NMR (DMSO-d_6,100 MHz) δ:160.6 (C-11b),160.4 (C-11c),159.2 (C-13b),158.5 (C-11a/13a),157.84 (C-11d/13d),157.82(C-13c),157.4 (C-4a),157.2 (C-4d),156.8 (C-4b),155.4 (C-4c),146.1 (C-9a),143.8 (C-9b),138.0 (C-9d),135.3 (C-9c),133.5 (C-1a),132.6 (C-1d),132.1 (C-1b),130.4 (C-1c),128.4 (C-2d/6d),127.0 (C-2c/6c),126.7 (C-2b/6b),126.0 (C-2a/6a),123.2 (C-10c),118.0 (C-10b),115.7 (C-3a/5a),115.5 (C-3d/5d),115.1 (C-3b/5b),114.9 (C-3c/5c),109.5 (C-14c),107.5 (C-14b),107.4 (C-10d/14d),105.5 (C-10a/14a),102.1 (C-12d),101.1 (C-12a),95.5 (C-12b),95.2 (C-12c),92.5 (C-7b),91.7 (C-7a),84.1 (C-7d),83.8 (C-7c),60.4 (C-8d),56.1 (C-8a),51.0 (C-8c),50.4 (C-8b)。以上数据与文献[2]报道基本一致,鉴定化合物**2**为kobophenol A。

化合物3 黄色粉末(甲醇);$C_{28}H_{22}O_7$,易溶于甲醇,UV 254 nm显暗斑,UV 365 nm无荧光;10%硫酸乙醇溶液显紫红色。(+)-ESI-MS m/z 471.3 [M+H]$^+$,(-)-ESI-MS m/z 469.4 [M-H]$^-$。^{1}H NMR (DMSO-d_6,400 MHz) δ:9.55,9.26,9.19,9.09,9.02 (each 1H,br s,-OH),6.99 (2H,d,J=8.5 Hz,H-2b/6b),6.74 (2H,d,J=8.3 Hz,H-2a/6a),6.69 (2H,d,J=8.5 Hz,H-3b/5b),6.55 (2H,d,J=8.6 Hz,H-3a/5a),6.42 (1H,d,J=1.9 Hz,H-14a),6.27 (1H,d,J=1.7 Hz,H-12b),6.04 (1H,d,J=1.9 Hz,H-14b),5.98 (1H,br s,H-12a),5.67 (1H,d,J=11.3 Hz,H-7b),5.23 (1H,br t,J=4.6,4.9 Hz,H-8a),5.15 (1H,d,J=4.6 Hz,H-7a),4.85 (1H,d,J=4.9 Hz,8a-OH),3.95 (1H,d,J=11.3 Hz,H-8b);^{13}C NMR (DMSO-d_6,100 MHz) δ:158.9 (C-11a),158.14 (C-13b),158.11 (C-13a),158.05 (C-4a),156.2 (C-4b),155.4 (C-11b),141.5 (C-9b),140.0 (C-9a),131.7 (C-1b),129.6 (C-1a),129.3 (C-2b/6b),128.0 (C-2a/6a),118.1 (C-10b),117.7 (C-10a),115.6 (C-3b/5b),115.0 (C-3a/5a),110.0 (C-14a),104.1 (C-14b),100.9 (C-12b),96.3 (C-12a),87.4 (C-7b),69.6 (C-8a),48.5 (C-8b),42.9 (C-7a)。以上数据与文献[3]报道基本一致,鉴定化合物**3**为蛇葡萄素A(ampelopsin A)。

化合物4 黄色粉末(甲醇);$C_{56}H_{42}O_{12}$,易溶于甲醇,UV 254 nm显暗斑,UV 365 nm无荧光;10%硫酸乙醇溶液显红褐色。(-)-ESI-MS m/z 451.8 [M/2-H]$^-$,905.6 [M-H]$^-$,推测化合物为一对称结构。^{1}H NMR (DMSO-d_6,400 MHz) δ:9.56 (2H,br s,-OH),9.35 (2H,s,-OH),9.14 (2H,br s,-OH),8.99 (2H,br s,-OH),8.40 (2H,s,-OH),7.02 (4H,d,J=8.5 Hz,H-2a/6a/2d/6d),6.74 (4H,d,J=8.0 Hz,H-2b/6b/2c/6c),6.72 (4H,d,J=8.5 Hz,H-3a/5a/3d/5d),6.51 (4H,d,J=8.5 Hz,H-3b/5b/3c/5c),6.36 (2H,d,J=1.2 Hz,H-12a/12d),6.03 (2H,br s,H-14a/14d),5.64 (2H,d,J=12.0 Hz,H-7a/7d),5.60 (2H,d,J=1.8 Hz,H-12b/12c),5.53 (2H,br s,H-7b/7c),4.86 (2H,d,J=1.8 Hz,H-14b/14c),4.01 (2H,d,J=12.0 Hz,H-8a/8d),3.67 (2H,br s,H-8b/8c);^{13}C NMR (DMSO-d_6,150 MHz) δ:158.3 (C-11b/11c),158.1 (C-

11a/11d),157.8 (C-4a/4d),156.4 (C-13a/13d),156.2 (C-13b/13c),155.0 (C-4b/4c),141.0 (C-9a/9d),139.8 (C-9b/9c),133.9 (C-1b/1c),129.6 (C-2a/6a/2d/6d),129.4 (C-1a/1d),128.4 (C-2b/6b/2c/6c),119.4 (C-10a/10d),117.1 (C-10b/10c),115.6 (C-3a/5a/3d/5d),114.9 (C-3b/5b/3c/5c),110.6 (C-14b/14c),105.2 (C-14a/14d),100.4 (C-12a/12d),94.6 (C-12b/12c),87.3 (C-7a/7d),48.7 (C-8a/8d),47.5 (C-8b/8c),40.5 (C-7b/7c)。2D-NMR 相关谱如图 3 所示。综上所述,并参考文献[4]报道的数据,鉴定化合物 **4** 为 isohopeaphenol。

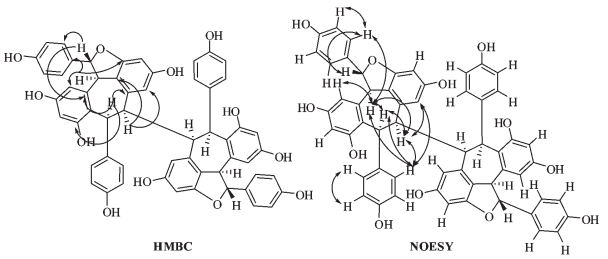

图 3 化合物 4 的 HMBC 和 NOESY 相关谱

Fig. 3 Key HMBC and NOESY correlations of compound **4**

化合物 5 黄褐色粉末(甲醇);$C_{56}H_{44}O_{13}$,易溶于甲醇,UV 254 nm 显暗斑,UV 365 nm 显蓝色荧光;10%硫酸乙醇溶液显褐色。(+)-ESI-MS *m/z* 925.4 $[M+H]^+$,(-)-ESI-MS *m/z* 923.6 $[M-H]^-$。^{1}H NMR (DMSO-d_6,400 MHz) δ: 8.92-9.58 (9H,br d,-OH),8.67 (1H,br s,-OH),6.74 (2H,d,J = 8.5 Hz),6.49-6.58 (12H,m,overlapped),6.41 (1H,br s,H-14a),6.40 (2H,d,J = 8.2 Hz),6.20 (1H,br s),6.14 (1H,br s),6.06 (1H,d,J = 1.4 Hz,H-12c),6.03 (2H,d,J = 1.8 Hz),5.86 (1H,d,J = 1.5 Hz,H-12b),5.52 (1H,d,J = 1.5 Hz,H-14b),5.17 (1H,d,J = 4.2 Hz,H-7a),5.03 (1H,d,J = 4.5 Hz,H-7d),4.88 (1H,d,J = 10.1 Hz,H-7c),4.46 (1H,d,J = 9.3 Hz,H-7b),3.96 (1H,d,J = 4.2 Hz,H-8a),3.91 (1H,d,J = 4.6 Hz,H-8d),3.77 (1H,dd,J = 9.6,11.6 Hz,H-8b),3.56 (1H,dd,J = 11.2,10.8 Hz,H-8c);^{13}C NMR (DMSO-d_6,100 MHz) δ: 160.2,159.3,159.0,158.6,157.8,157.7,157.5,157.4,156.4,147.6 (C-9a),147.4 (C-9d),137.6 (C-9c),135.3 (C-9b),132.2,131.8,131.7,129.4,128.4,128.3,128.2,122.1,121.7,115.6,115.5,115.4,114.8,109.7 (C-14b),106.0 (C-10a/14a/10d/14d),104.5 (C-14c),101.5,101.4,96.1 (C-12b),95.5 (C-12c),93.9 (C-7d),92.4 (C-7a),84.2 (C-7c),81.2 (C-7b),58.2 (C-8b),54.3 (C-8a),53.4 (C-8d),50.4 (C-8c)。2D-NMR 相关谱如图 4 所示。综上所述,并参考文献[5]报道的数据,鉴定化合物 **5** 为 grandiphenol A。

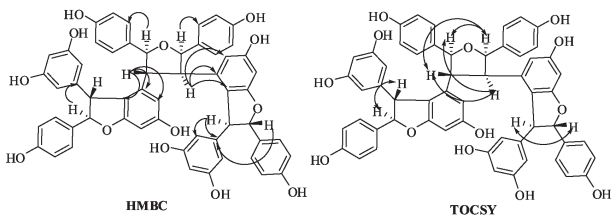

图 4 化合物 5 的 HMBC 和 TOCSY 相关谱

Fig. 4 Key HMBC and TOCSY correlations of compound **5**

参考文献

1 Pryce R J, Langcake P. α-Viniferin: an antifungal resveratrol trimer from grapevines. *Phytochemistry*, 1977, 16: 1452-1454.

2 Kurihara H, Kawabata J, Ichikawa S, *et al*. Oligostilbenes from *Carex kobomugi*. *Phytochemistry*, 1991, 30: 649-653.

3 Oshima Y, Ueno Y, Hikino H, *et al*. Ampelopsins A, B and C, new oligostilbenes of *Ampelopsis brevipedunculata* var. Hancei. *Tetrahedron*, 1990, 46: 5121-5126.

4 Ito J, Niwa M, Oshima Y. A new hydroxystilbene tetramer named isohopeaphenol from *Vitis vinifera* 'Kyohou'. *Heterocycles*, 1997, 45: 1809-1813.

5 Ito T, Tanaka T, Linuma M, *et al*. Two new resveratrol (5-[(1E)-2-(4-Hydroxyphenyl) ethenyl]-benzene-1, 3-diol) tetramers with a tetrahydrofuran ring from *Dipterocarpus grandiflorus*. *Helv Chim Acta*, 2004, 87: 479-495.