三尖栝楼的抗肿瘤活性成分

周咏梅,汤 琤,张思访*

(江苏卫生健康职业学院,南京 211800)

摘 要 采用硅胶、反相高效液相色谱等柱色谱技术,结合现代波谱学方法及理化性质分析,从三尖栝楼(*Trichosanthes tricuspidata*)干燥根醇提物二氯甲烷部位中共分离鉴定了 10 个化合物,其中 1 个 hexanorcucurbitane 苷类新化合物:khekadaengoside O (**1**),以及 9 个已知化合物,分别为:khekadaengoside C-E,K(**2** ~ **5**),葫芦素 J-2-*O*-*β*-吡喃葡萄糖苷(**6**),葫芦素 K-2-*O*-*β*-吡喃葡萄糖苷(**7**),葫芦素 B,J,K(**8** ~ **10**)。利用人肝癌细胞 BEL-7402 对分离得到的化合物 **1** ~ **10** 进行体外抗肿瘤活性评价。结果表明,化合物 **8** ~ **10** 具有显著的抗肿瘤活性。

关键词 三尖栝楼;hexanorcucurbitane 苷;抗肿瘤活性;化学成分;结构鉴定

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Isolation and identification of antitumor constituents from *Trichosanthes tricuspidata*

ZHOU Yongmei, TANG Cheng, ZHANG Sifang*

Jiangsu Health Vocational College, Nanjing 211800, China

Abstract Ten compounds were isolated and purified from the dichloromethane fraction of *Trichosanthes tricuspidata* roots by silica gel and ODS column chromatographies. Their chemical structures were identified on the basis of their physical and chemical properties as well as the spectral data. These isolated compounds were elucidated as khekadaengoside O (**1**), a new hexanorcucurbitane glycoside, together with nine known compounds, including khekadaengoside C-E, K(**2**–**5**), cucurbitacin J-2-*O*-*β*-glucopyranoside(**6**), cucurbitacin K-2-*O*-*β*-glucopyranoside (**7**), cucurbitacin B, J, K (**8**–**10**). In addition, the anti-tumor activity of compounds **1**–**10** were evaluated in hepatocellular carcinoma BEL-7402 cell line. Among them, compounds **8**–**10** showed potent antitumor activity.

Key words *Trichosanthes tricuspidata*; hexanorcucurbitane glycoside; antitumor activity; chemical constituents; structural identification

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栝楼属(*Trichosanthes* L.)是葫芦科(Cucurbitaceae)中一个重要的属,全世界约80 种,广泛分布于亚洲东部及东南部。近年来,国内外对栝楼属植物的化学成分研究较多,其主要成分包括萜及其苷类、黄酮及其苷类、甾体及其苷类、苯丙素、生物碱以及氨基酸等[1-2]。药理研究证实,栝楼属中包含的三萜类成分(主要是葫芦素类)具有良好的抗肿瘤活性[3-5]。三尖栝楼(*Trichosanthes tricuspidata* L.)是栝楼属中常见的一个品种,主要分布于斯里兰卡、印度、尼泊尔等国,在我国主要分布于贵州安

收稿日期 2018-05-18 * **通信作者** Tel:13913004686 E-mail:weidazhang@126.com

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龙[6]。本课题组在从天然药物中寻找抗肿瘤成分的过程中,发现三尖栝楼根二氯甲烷提取部位具有良好的抗肿瘤活性。为进一步阐明三尖栝楼根的抗肿瘤药效物质基础,本研究采用活性导向分离手段,对其进行了系统的化学成分研究,共分离得到 10 个化合物(图 1),其中 1 个 hexanorcucurbitane 苷类新化合物:khekadaengoside O(**1**),以及 9 个已知化合物,分别为:khekadaengoside C-E,K(**2** ~ **5**),cucurbitacin J-2-*O*-*β*-glucopyranoside(**6**),cucurbitacin K-2-*O*-*β*-glucopyranoside(**7**),葫芦素 B,J,K(**8** ~ **10**)。另外,利用人肝癌细胞 BEL-7402 对分离得到的化合物 **1** ~ **10** 进行体外抗肿瘤活性评价。结果表明,化合物 **8** ~ **10** 具有显著的抗肿瘤活性。

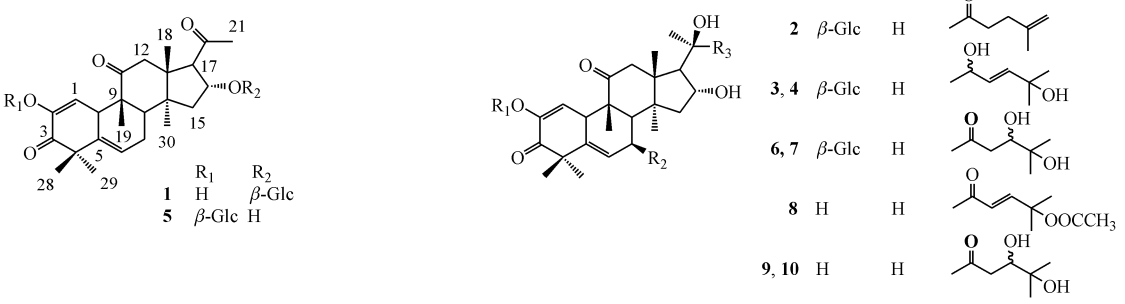

Figure 1　Chemical structures of compounds **1**–**10**

1　材　料

1.1　仪器和试剂

Ultrashield Plus 600 核磁共振仪(德国布鲁克公司);P-1020 型旋光仪、FT/IR-480 Plus 红外光谱仪(日本 Jasco 公司);DU-7 型紫外分光光度仪(美国贝克曼公司);硅胶 G(200 ~ 300 目,青岛海洋化工有限公司);6210 型 ESI/TOF 质谱仪、1200 高效液相色谱仪(DAD 检测器,美国安捷伦公司);ODS 色谱柱(250 mm × 19.0 mm,5 μm,Waters Atlantis T3 C_{18});MPLC 液相色谱仪[利穗科技(苏州)有限公司];GF_{254} 制备薄层色谱板(烟台江友硅胶开发有限公司)。其他试剂均为市售分析纯。

1.2　药　材

三尖栝楼干燥根购自南京海源中药饮片有限公司,经江苏卫生健康职业学院张思访实验师鉴定为葫芦科栝楼属植物三尖栝楼(*T. tricuspidata* L.)的根,标本(No. 20170506)存于江苏卫生健康职业学院药学院标本室。

2　方　法

2.1　提取与分离

取三尖栝楼干燥根 5 kg,用甲醇回流提取 3 次(3 × 30 L),每次 2 h,过滤,合并滤液,浓缩至无醇味,浓缩液(0.5 L)加适量的水(2 L)使混悬,依次用二氯甲烷和正丁醇萃取(3 × 3 L,每次 30 min)。细胞毒实验显示活性部位为二氯甲烷萃取部位,因此将二氯甲烷部位(30 g)经硅胶柱色谱(8 cm × 50 cm,200 ~ 300 目,1 kg),以石油醚-乙酸乙酯梯度洗脱(10∶1 → 0∶1)得到 4 个流份(Frs. A ~ D)。Fr. C(8 g)再经硅胶柱色谱(4 cm × 50 cm,200 ~ 300 目,0.4 kg),以石油醚-乙酸乙酯(3∶1)洗脱,得到 6 个流份(Frs. C1 ~ C6)。Frs. C2(1.1 g)再用制备 TLC(氯仿-乙酸乙酯,5∶1)得到化合物 **8**(17 mg)。Frs. C3(980 mg)再用制备 TLC(氯仿-乙酸乙酯,4∶1)得到化合物 **9**(38 mg)和 **10**(53 mg)。Fr. D(12 g)再经硅胶柱色谱(4 cm × 60 cm,200 ~ 300 目,0.6 kg),以二氯甲烷-丙酮(20∶1 → 1∶1)梯度洗脱,得到 4 个流份(Frs. D1 ~ D4)。Fr. D2(3 g)进一步用 MPLC(甲醇-水,10∶90 → 90∶10)得到 6 个流份(Frs. D2-1 ~ D2-6)。Fr. D2-2(560 mg)经制备 HPLC(乙腈-水,42∶58)分离得到化合物 **1**(24 mg)和化合物 **2**(22 mg)。Fr. D2-3(430 mg)经制备 HPLC(乙腈-水,35∶65)分离得到化合物 **5**(68 mg)。Fr. D3(3.5 g)用制备 HPLC(乙腈-水,30∶70→60∶40 分离分离得到化合物 **3**(39 mg)和化合物 **4**(28 mg);Fr. D4(2 g)用制备 HPLC(乙腈-水,45∶55)分离得到化合物 **6**(21 mg)和 **7**(37 mg)。

2.2 化合物 **1** 酶水解[7]

将化合物 **1**(5 mg)溶于甲醇 0.5 mL 中,加入质量浓度为 5 mg/mL 的橙皮苷酶水溶液 20 mL,37 ℃水浴搅拌 2 d。接着反应液用乙酸乙酯萃取并减压浓缩至干,得到化合物 **1a**(3.8 mg)。通过与文献[8]的 NMR 及其他物理和光谱数据比较,确定化合物 **1a** 为 hexanorcucurbitacin I。

2.3 MTT 实验

人肝癌细胞 BEL-7402 与不同浓度化合物 **1** ~ **10** 在 37 ℃条件下共培养 72 h。根据文献[9]方法通过 MTT 实验对细胞增殖进行测定,每组均平行测定 3 次,顺铂作为阳性对照。

3 结果和讨论

3.1 结构鉴定

化合物 **1** 为无定形粉末,根据 HR-ESI-MS 确定其分子式为 $C_{30}H_{42}O_{10}$(*m/z* 585.259 68[M + Na]$^+$, Calcd. 585.259 7)。$[\alpha]_D^{25}$ -15.6°(*c* 1.5, MeOH);UV(MeOH):λ_{max} 242(lg ε 4.02) nm;IR(KBr, ν_{max}):3 408,1 688,1 668,1 585,1 379,1 072,1 034 cm^{-1}。^{13}C NMR 谱(CD_3OD,150 MHz)(表 1)共显示了 30 个碳原子信号,其中包括 1 个 β-葡萄糖单元信号和一组 24 个碳信号的苷元部分,表明化合物 **1** 可能含有一个 hexanorcucurbitacin 骨架,与已知化合物 khekadaengoside K(**5**)[8]核磁数据很相似,除了前者 β-葡萄糖单元是连接在 C-16 上而不是 C-2。用橙皮苷酶对化合物 **1** 进行酶解得到化合物 **1a**,通过与文献[8]光谱数据对比,确定为 hexanorcucurbitacin I($C_{24}H_{32}O_5$),进一步证明其骨架结构。HMBC(图 2)谱显示在 H-1′(4.68)到 C-16(δ 71.3)之间以及 H-16(4.88)到 C-1′(δ 99.8)之间存在远程相关进一步证明了葡萄糖的连接位置在 C-16 上。因此,化合物 **1** 最终鉴定为 2,16-二羟基-(22-27)-hexanorcucurbit-5-烯-11,20-二酮 16-*O*-β-吡喃葡萄糖苷(khekadaengoside O)(图 2)。

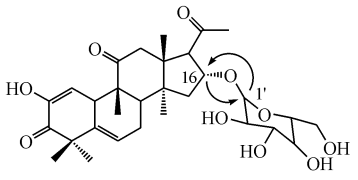

Figure 2 Significant HMBC correlations of compound **1**

Table 1 ^{1}H NMR(600 MHz) and ^{13}C NMR(150 MHz) spectral data of compound **1** in CD_3OD

Position	δ_H	δ_C
1	6.13 d(2.4)	122.1
2	–	145.9
3	–	198.5
4	–	48.5
5	–	136.2
6	5.87 br. s	120.9
7	2.45 m 2.13 d(12.9)	23.4
8	2.10 m	42.0
9	–	49.4
10	3.75 br. s	35.1
11	–	213.6
12	3.50 d(14.5) 2.50 d(14.4)	47.2
13	–	48.9
14	–	49.9
15	2.01 m 1.57 d(14.6)	45.0
16	4.88 t(7.4)	71.3
17	3.24 d(6.8)	66.6
18-CH_3	0.72 s	18.9
19-CH_3	1.06 s	19.3
20	–	209.4
21-CH_3	2.22 s	30.5
28-CH_3	1.31 s	27.0
29-CH_3	1.34 s	19.5
30-CH_3	1.46 s	17.4
1′	4.68 d(7.6)	99.8
2′	3.36-3.55 m	73.0
3′	3.36-3.55 m	76.3
4′	3.36-3.55 m	69.3
5′	3.36-3.55 m	76.8
6′	4.06dd(12.2,2.4) 3.88dd(12.0,3.4)	60.6

化合物 **2** 无定形粉末(甲醇),$[\alpha]_D^{25}$ -53.6°(*c* 2.5, MeOH),ESI-MS *m/z*:659.3[M - H]$^-$。^{1}H NMR(C_5D_5N,600 MHz) δ:6.43(1H,d,*J* = 2.4 Hz,H-1),5.63(1H,br. s,H-6),2.16(1H,m,H-7a),1.87(1H,m,H-7b),1.92(1H,m,H-8),3.72(1H,br. s,H-10),3.38(1H,d,*J* = 14.2 Hz,H-12a),2.95(1H,d,*J* = 14.4 Hz,H-12b),1.89(1H,m,H-15a),1.68(1H,d,*J* = 12.2 Hz,H-15b),4.93(1H,t,*J* = 7.6 Hz,H-16),2.91(1H,d,*J* = 7.1 Hz,H-17),1.40(3H,s,18-CH_3),1.53(3H,s,19-CH_3),1.62(3H,s,21-CH_3),3.33(1H,m,H-23a),3.08(1H,m,H-23b),2.58(2H,t,*J* = 8.1 Hz,H-24),4.82(1H,s,H-26a),4.76(1H,s,H-26b),

1.69(3H,s,27-CH_3),1.20(3H,s,28-CH_3),1.30(3H,s,29-CH_3),1.04(3H,s,30-CH_3),5.46(1H,d,J=7.6 Hz,H-1′),4.21(1H,dd,J=8.8,7.8 Hz,H-2′),4.28(1H,dd,J=8.8,8.5 Hz,H-3′),4.47(1H,dd,J=9.4,8.9 Hz,H-4′),4.06(1H,m,H-5′),4.70(1H,dd,J=12.4,1.3 Hz,H-6′a),4.56(1H,dd,J=11.4,3.3 Hz,H-6′b)。[13] C NMR(C_5D_5N,150 MHz) δ:120.9(C-1),146.9(C-2),197.1(C-3),49.5(C-4),136.9(C-5),120.9(C-6),23.9(C-7),41.8(C-8),51.0(C-9),35.6(C-10),213.8(C-11),49.7(C-12),49.1(C-13),48.5(C-14),46.5(C-15),70.4(C-16),59.0(C-17),20.2(C-18),18.3(C-19),80.1(C-20),25.5(C-21),215.0(C-22),32.1(C-23),35.6(C-24),145.3(C-25),110.4(C-26),22.7(C-27),20.3(C-28),27.6(C-29),20.8(C-30),100.6(C-1′),74.3(C-2′),78.5(C-3′),70.8(C-4′),78.8(C-5′),62.0(C-6′)。以上数据与文献报道基本一致[8],故鉴定化合物**2** 为 khekadaengoside C。

化合物**3**　无定形粉末(甲醇),$[\alpha]_D^{25}$ −73.6°(*c* 1.5,MeOH),ESI-MS *m/z*:677.4[M − H]$^-$。^{1}H NMR(C_5D_5N,600 MHz) δ:6.45°(1H,d,J=2.4 Hz,H-1),5.64(1H,brs,H-6),2.15(1H,m,H-7a),1.97(1H,m,H-7b),1.97(1H,m,H-8),3.67(1H,br.s,H-10),3.18(1H,d,J=14.2 Hz,H-12a),2.85(1H,d,J=14.2 Hz,H-12b),1.98(1H,m,H-15a),1.82(1H,d,J=12.4 Hz,H-15b),5.23(1H,t,J=7.6 Hz,H-16),2.81(1H,d,J=6.5 Hz,H-17),1.40(3H,s,18-CH_3),1.54(3H,s,19-CH_3),1.58(3H,s,21-CH_3),4.60(1H,d,J=5.3 Hz,H-22),6.52(1H,dd,J=15.6,5.4 Hz,H-23),6.58(1H,d,J=15.5 Hz,H-24),1.50(3H,s,26-CH_3),1.54(3H,s,27-CH_3),1.24(3H,s,28-CH_3),1.28(3H,s,29-CH_3),0.99(3H,s,30-CH_3),5.50(1H,d,J=7.8 Hz,H-1′),4.20(1H,dd,J=9.0,7.8 Hz,H-2′),4.29(1H,dd,J=9.0,8.6 Hz,H-3′),4.48(1H,dd,J=9.3,9.0 Hz,H-4′),4.05(1H,m,H-5′),4.70(1H,dd,J=12.2,2.1 Hz,H-6′a),4.56(1H,dd,J=12.2,3.5 Hz,H-6′b)。[13] C NMR(C_5D_5N,150 MHz) δ:120.8(C-1),146.7(C-2),197.1(C-3),49.5(C-4),136.9(C-5),120.9(C-6),23.9(C-7),41.8(C-8),51.0(C-9),35.6(C-10),214.2(C-11),49.9(C-12),49.1(C-13),48.5(C-14),45.6(C-15),71.4(C-16),56.6(C-17),20.2(C-18),18.2(C-19),76.9(C-20),24.8(C-21),81.6(C-22),126.1(C-23),141.6(C-24),69.8(C-25),30.7(C-26),30.9(C-27),20.3(C-28),27.6(C-29),20.9(C-30),100.6(C-1′),74.3(C-2′),78.5(C-3′),70.8(C-4′),78.8(C-5′),62.1(C-6′)。以上数据与文献报道基本一致[8],故鉴定化合物**3** 为 khekadaengoside D。

化合物**4**　无定形粉末(甲醇),$[\alpha]_D^{25}$ −35.4°(*c* 1.5,MeOH),ESI-MS *m/z*:677.4[M − H]$^-$。^{1}H NMR(C_5D_5N,600 MHz) δ:6.44(1H,d,J=2.4 Hz,H-1),5.64(1H,br.s,H-6),2.14(1H,m,H-7a),1.98(1H,m,H-7b),1.98(1H,m,H-8),3.68(1H,brs,H-10),3.24(1H,d,J=14.2 Hz,H-12a),2.88(1H,d,J=14.2 Hz,H-12b),1.98(1H,m,H-15a),1.82(1H,d,J=12.4 Hz,H-15b),5.32(1H,t,J=7.6 Hz,H-16),2.84(1H,d,J=6.5 Hz,H-17),1.39(3H,s,18-CH_3),1.56(3H,s,19-CH_3),1.60(3H,s,21-CH_3),5.12(1H,d,J=5.4 Hz,H-22),6.51(1H,dd,J=15.6,5.4 Hz,H-23),6.58(1H,d,J=15.5 Hz,H-24),1.50(3H,s,26-CH_3),1.53(3H,s,27-CH_3),1.24(3H,s,28-CH_3),1.25(3H,s,29-CH_3),0.99(3H,s,30-CH_3),5.50(1H,d,J=7.8 Hz,H-1′),4.20(1H,dd,J=9.0,7.8 Hz,H-2′),4.29(1H,dd,J=9.0,8.6 Hz,H-3′),4.46(1H,dd,J=9.3,9.0 Hz,H-4′),4.06(1H,m,H-5′),4.68(1H,dd,J=12.2,2.1 Hz,H-6′a),4.56(1H,dd,J=12.2,3.5 Hz,H-6′b)。[13] C NMR(C_5D_5N,150 MHz) δ:120.9(C-1),146.7(C-2),197.1(C-3),49.5(C-4),137.0(C-5),121.2(C-6),23.9(C-7),41.9(C-8),51.1(C-9),35.6(C-10),214.4(C-11),49.9(C-12),49.2(C-13),48.5(C-14),46.6(C-15),71.4(C-16),57.2(C-17),20.2(C-18),18.1(C-19),76.9(C-20),21.6(C-21),76.6(C-22),126.8(C-23),142.3(C-24),69.8(C-25),30.7(C-26),30.6(C-27),20.3(C-28),27.5(C-29),20.9(C-30),100.6(C-1′),74.3(C-2′),78.5(C-3′),70.8(C-4′),78.6(C-5′),62.0(C-6′)。以上数据与文献报道基本一致[8],故鉴定化合物**4** 为 khekadaengoside E。

化合物**5**　白色无定形粉末(甲醇),$[\alpha]_D^{25}$

-15.4°(*c* 1.5, MeOH), ESI-MS *m/z*: 561.4 [M-H]$^-$。^{1}H NMR(C_5D_5N, 600 MHz) δ: 6.38 (1H, d, *J* = 2.4 Hz, H-1), 5.64 (1H, br. s, H-6), 5.32 (1H, t, *J* = 7.4 Hz, H-16), 5.42 (1H, d, *J* = 7.6 Hz, H-1′), 4.65 (1H, dd, *J* = 12.2, 2.4 Hz, H-6′a), 4.57 (1H, dd, *J* = 12.0, 3.4 Hz, H-6′b), 3.74 (1H, br. s, H-10), 4.20 (1H, dd, *J* = 9.0, 7.6 Hz, H-2′), 4.28 (1H, dd, *J* = 9.0, 8.8 Hz, H-3′), 4.46 (1H, dd, *J* = 9.3, 9.1 Hz, H-4′), 4.03 (1H, m, H-5′), 3.43 (1H, d, *J* = 6.8 Hz, H-17), 3.45 (1H, d, *J* = 14.5 Hz, H-12a), 2.70 (1H, d, *J* = 14.4 Hz, H-12b), 2.14 (1H, m, H-7a), 1.80 (1H, d, *J* = 12.9 Hz, H-7b), 2.18 (3H, s, 21-CH_3), 1.92 (1H, m, H-8), 1.92 (1H, m, H-15a), 1.36 (1H, dd, *J* = 14.6, 3.5 Hz, H-15b), 1.45 (3H, s, 30-CH_3), 1.40 (3H, s, 29-CH_3), 1.24 (3H, s, 28-CH_3), 1.56 (3H, s, 19-CH_3), 0.98 (3H, s, 18-CH_3); ^{13}C NMR (C_5D_5N, 150 MHz) δ: 212.6 (C-11), 208.3 (C-20), 196.9 (C-3), 146.8 (C-2), 137.1 (C-5), 120.4 (C-1), 120.8 (C-6), 100.7 (C-1′), 78.7 (C-5′), 78.2 (C-3′), 74.3 (C-2′), 71.2 (C-16), 70.2 (C-4′), 67.5 (C-17), 61.9 (C-6′), 49.1 (C-14), 50.3 (C-9), 49.8 (C-13), 49.4 (C-4), 47.8 (C-12), 46.1 (C-15), 41.9 (C-8), 35.5 (C-10), 32.4 (C-21), 20.9 (C-28), 23.5 (C-7), 27.4 (C-29), 18.2 (C-19), 20.1 (C-18), 19.8 (C-30)。以上数据与文献[8]报道一致,故化合物**5**鉴定为khekadaengoside K。

化合物**6** 无定形粉末(甲醇), $[\alpha]_D^{25}$ -45.4° (*c* 1.5, MeOH), ESI-MS *m/z*: 693.3 [M-H]$^-$。^{1}H NMR(C_5D_5N, 600 MHz) δ: 6.46 (1H, d, *J* = 2.4 Hz, H-1), 5.63 (1H, brs, H-6), 2.13 (1H, m, H-7a), 1.90 (1H, m, H-7b), 1.93 (1H, m, H-8), 3.68 (1H, br. s, H-10), 3.28 (1H, d, *J* = 14.2 Hz, H-12a), 2.88 (1H, d, *J* = 14.2 Hz, H-12b), 1.93 (1H, m, H-15a), 1.68 (1H, d, *J* = 12.4 Hz, H-15b), 5.02 (1H, t, *J* = 7.6 Hz, H-16), 3.04 (1H, d, *J* = 6.5 Hz, H-17), 1.40 (3H, s, 18-CH_3), 1.52 (3H, s, 19-CH_3), 1.62 (3H, s, 21-CH_3), 3.65 (1H, dd, *J* = 15.9, 9.4 Hz, H-23a), 3.36 (1H, dd, *J* = 15.9, 1.4 Hz, H-23b), 4.55 (1H, dd, *J* = 9.4, 1.4 Hz, H-24), 1.49 (3H, s, 26-CH_3), 1.51 (3H, s, 27-CH_3), 1.20 (3H, s, 28-CH_3), 1.27 (3H, s, 29-CH_3), 0.99 (3H, s, 30-CH_3), 5.49 (1H, d, *J* = 7.8 Hz, H-1′), 4.20 (1H, dd, *J* = 9.0, 7.8 Hz, H-2′), 4.28 (1H, dd, *J* = 9.0, 8.6 Hz, H-3′), 4.46 (1H, dd, *J* = 9.3, 9.0 Hz, H-4′), 4.05 (1H, m, H-5′), 4.68 (1H, dd, *J* = 12.2, 2.1 Hz, H-6′a), 4.56 (1H, dd, *J* = 12.2, 3.5 Hz, H-6′b)。^{13}C NMR (C_5D_5N, 150 MHz) δ: 120.9 (C-1), 146.8 (C-2), 197.1 (C-3), 49.5 (C-4), 137.0 (C-5), 120.8 (C-6), 23.9 (C-7), 41.9 (C-8), 51.0 (C-9), 35.6 (C-10), 213.9 (C-11), 49.5 (C-12), 49.2 (C-13), 48.6 (C-14), 46.2 (C-15), 70.4 (C-16), 57.7 (C-17), 20.2 (C-18), 18.2 (C-19), 80.4 (C-20), 24.6 (C-21), 216.0 (C-22), 41.1 (C-23), 75.6 (C-24), 72.2 (C-25), 24.7 (C-26), 27.6 (C-27), 20.3 (C-28), 27.5 (C-29), 20.9 (C-30), 100.4 (C-1′), 74.3 (C-2′), 78.5 (C-3′), 70.7 (C-4′), 78.6 (C-5′), 62.0 (C-6′)。以上数据与文献报道基本一致[10],故鉴定化合物**6**为葫芦素 J-2-*O*-β-吡喃葡萄糖苷。

化合物**7** 无定形粉末(甲醇), $[\alpha]_D^{25}$ -66.3° (*c* 1.5, MeOH), ESI-MS *m/z*: 693.3 [M-H]$^-$。^{1}H NMR(C_5D_5N, 600 MHz) δ: 6.45 (1H, d, *J* = 2.4 Hz, H-1), 5.63 (1H, brs, H-6), 2.13 (1H, m, H-7a), 1.90 (1H, m, H-7b), 1.93 (1H, m, H-8), 3.68 (1H, br. s, H-10), 3.28 (1H, d, *J* = 14.2 Hz, H-12a), 2.89 (1H, d, *J* = 14.2 Hz, H-12b), 1.93 (1H, m, H-15a), 1.68 (1H, d, *J* = 12.4 Hz, H-15b), 4.94 (1H, t, *J* = 7.6 Hz, H-16), 2.94 (1H, d, *J* = 6.5 Hz, H-17), 1.40 (3H, s, 18-CH_3), 1.51 (3H, s, 19-CH_3), 1.71 (3H, s, 21-CH_3), 3.65 (1H, dd, *J* = 14.6, 2.4 Hz, H-23a), 3.62 (1H, dd, *J* = 14.6, 9.0 Hz, H-23b), 4.64 (1H, dd, *J* = 9.0, 2.4 Hz, H-24), 1.49 (3H, s, 26-CH_3), 1.50 (3H, s, 27-CH_3), 1.20 (3H, s, 28-CH_3), 1.29 (3H, s, 29-CH_3), 0.99 (3H, s, 30-CH_3), 5.45 (1H, d, *J* = 7.8 Hz, H-1′), 4.20 (1H, dd, *J* = 8.8, 7.8 Hz, H-2′), 4.28 (1H, dd, *J* = 8.8, 8.8 Hz, H-3′), 4.46 (1H, dd, *J* = 9.3, 8.8 Hz, H-4′), 4.03 (1H, m, H-5′), 4.68 (1H, dd, *J* = 12.0, 2.4 Hz, H-6′a), 4.56 (1H, dd, *J* = 12.0, 3.7 Hz, H-6′b)。^{13}C NMR (C_5D_5N, 150 MHz) δ: 120.8 (C-1), 146.8 (C-2), 197.1 (C-3), 49.4 (C-4), 137.0 (C-5), 120.8 (C-6), 23.8 (C-7), 41.7 (C-8), 51.0 (C-9), 35.5 (C-10), 213.9 (C-11), 49.2 (C-12), 49.2

(C-13),48.6(C-14),46.4(C-15),70.4(C-16),58.7(C-17),20.2(C-18),18.2(C-19),80.3(C-20),25.6(C-21),215.5(C-22),40.8(C-23),74.6(C-24),72.2(C-25),27.0(C-26),25.0(C-27),20.3(C-28),27.5(C-29),20.8(C-30),100.6(C-1′),74.3(C-2′),78.4(C-3′),70.7(C-4′),78.6(C-5′),61.9(C-6′)。以上数据与文献报道基本一致[10],故鉴定化合物 **7** 为葫芦素 K-2-*O*-*β*-吡喃葡萄糖苷。

化合物 **8**　白色结晶(氯仿),mp:180 ~ 182 ℃,ESI-MS *m/z*:557.3[M − H]$^-$。^{1}H NMR($CDCl_3$,600 MHz) *δ*:1.24(1H,m,H-1a),2.34(1H,m,H-1b),4.44(1H,m,H-2),5.82(1H,m,H-6),1.98(1H,m,H-7a),2.44(1H,m,H-7b),1.99(1H,d,*J* = 8.7 Hz,H-8),2.78(1H,d,*J* = 12.8 Hz,H-10),2.68(1H,d,*J* = 14.8 Hz,H-12a),3.25(1H,d,*J* = 14.8 Hz,H-12b),1.42(1H,m,H-15a),1.89(1H,m,H-15b),4.39(1H,m,H-16),2.52(1H,d,*J* = 7.2 Hz,H-17),1.00(3H,s,18-CH_3),1.09(3H,s,19-CH_3),1.46(3H,s,21-CH_3),6.52(1H,d,*J* = 16.0 Hz,H-23),7.08(1H,d,*J* = 16.0 Hz,H-24),1.57(3H,s,26-CH_3),1.58(3H,s,27-CH_3),1.34(3H,s,28-CH_3),1.31(3H,s,29-CH_3),1.39(3H,s,30-CH_3),2.00(3H,s,32-CH_3)。^{13}C NMR($CDCl_3$,150 MHz) *δ*:35.9(C-1),71.7(C-2),212.8(C-3),49.9(C-4),140.2(C-5),120.2(C-6),23.9(C-7),42.3(C-8),48.1(C-9),33.6(C-10),211.8(C-11),48.6(C-12),50.6(C-13),48.0(C-14),45.1(C-15),71.4(C-16),58.2(C-17),19.6(C-18),19.8(C-19),78.3(C-20),23.6(C-21),202.3(C-22),120.4(C-23),151.7(C-24),79.1(C-25),26.0(C-26),26.2(C-27),21.3(C-28),29.2(C-29),18.9(C-30),169.8(C-31),21.6(C-32)。以上数据与文献报道基本一致[11],故鉴定化合物 **8** 为葫芦素 B。

化合物 **9**　无定形粉末(氯仿),$[\alpha]_D^{25}$ −35.8°(*c* 1.5,$CHCl_3$),ESI-MS *m/z*:531.3[M − H]$^-$。^{1}H NMR($CDCl_3$,600 MHz) *δ*:5.96(1H,d,*J* = 2.7 Hz,H-1),5.74(1H,br.s,H-6),2.03(1H,m,H-7a),2.03(1H,m,H-7b),2.37(1H,m,H-8),3.50(1H,br.s,H-10),3.22(1H,d,*J* = 14.4 Hz,H-12a),2.70(1H,d,*J* = 14.2 Hz,H-12b),1.88(1H,m,H-15a),1.88(1H,m,H-15b),4.47(1H,t,*J* = 7.6 Hz,H-16),2.64(1H,d,*J* = 7.1 Hz,H-17),1.02(3H,s,18-CH_3),1.42(3H,s,19-CH_3),1.40(3H,s,21-CH_3),2.99(1H,dd,*J* = 16.6,9.5 Hz,H-23a),2.56(1H,dd,*J* = 16.6,1.2 Hz,H-23b),3.95(1H,dd,*J* = 9.5,1.2 Hz,H-24),1.20(3H,s,26-CH_3),1.23(3H,s,27-CH_3),1.36(3H,s,28-CH_3),1.25(3H,s,29-CH_3),0.99(3H,s,30-CH_3)。^{13}C NMR($CDCl_3$,150 MHz) *δ*:114.9(C-1),144.8(C-2),198.7(C-3),47.5(C-4),137.0(C-5),120.6(C-6),23.6(C-7),41.7(C-8),50.9(C-9),34.6(C-10),212.8(C-11),48.8(C-12),48.9(C-13),48.4(C-14),45.6(C-15),71.6(C-16),55.8(C-17),20.0(C-18),18.2(C-19),79.4(C-20),23.6(C-21),214.1(C-22),39.3(C-23),74.3(C-24),72.2(C-25),25.7(C-26),24.6(C-27),20.3(C-28),27.9(C-29),20.2(C-30)。以上数据与文献报道基本一致[10],故鉴定化合物 **9** 为葫芦素 J。

化合物 **10**　无定形粉末(氯仿),$[\alpha]_D^{25}$ −65.8°(*c* 1.5,$CHCl_3$),ESI-MS *m/z*:531.3[M − H]$^-$。^{1}H NMR($CDCl_3$,600 MHz) *δ*:5.95(1H,d,*J* = 2.7 Hz,H-1),5.75(1H,br.s,H-6),2.02(1H,m,H-7a),2.02(1H,m,H-7b),2.37(1H,m,H-8),3.50(1H,br.s,H-10),3.21(1H,d,*J* = 14.4 Hz,H-12a),2.70(1H,d,*J* = 14.4 Hz,H-12b),1.87(1H,m,H-15a),1.87(1H,m,H-15b),4.38(1H,t,*J* = 7.6 Hz,H-16),2.54(1H,d,*J* = 7.1 Hz,H-17),1.02(3H,s,18-CH_3),1.42(3H,s,19-CH_3),1.39(3H,s,21-CH_3),2.99(1H,dd,*J* = 16.1,1.2 Hz,H-23a),2.66(1H,dd,*J* = 16.1,9.8 Hz,H-23b),3.92(1H,dd,*J* = 9.5,1.7 Hz,H-24),1.19(3H,s,26-CH_3),1.23(3H,s,27-CH_3),1.36(3H,s,28-CH_3),1.25(3H,s,29-CH_3),1.00(3H,s,30-CH_3)。^{13}C NMR($CDCl_3$,150 MHz) *δ*:114.9(C-1),144.7(C-2),198.7(C-3),47.6(C-4),137.0(C-5),120.7(C-6),23.5(C-7),41.6(C-8),50.8(C-9),34.7(C-10),212.8(C-11),48.9(C-12),48.9(C-13),48.4(C-14),45.7(C-15),71.1(C-16),57.5(C-17),19.9(C-18),18.2(C-19),79.4(C-20),24.3(C-21),215.5(C-22),38.2(C-23),74.3(C-24),72.2(C-25),25.7(C-26),24.6(C-27),20.2(C-28),27.9(C-29),20.2(C-30)。以上数据与文

献报道基本一致[10],故鉴定化合物 **10** 为葫芦素 K。

3.2 MTT 实验

化合物 **1** ~ **10** 对人肝癌细胞 BEL-7402 的细胞毒活性结果见表 2。

Table 2 Cytotoxic activities of compounds **1-10** isolated from *T. tricuspidata* against BEL-4702 cell lines

Compound	IC_{50}/(μmol/L)	Compound	IC_{50}/(μmol/L)
1	31.3 ± 1.9	**7**	> 100
2	73.7 ± 1.7	**8**	3.3 ± 0.3
3	85.9 ± 3.0	**9**	7.7 ± 0.6
4	80.4 ± 2.2	**10**	8.6 ± 0.3
5	49.4 ± 2.4	Cisplatin	1.8 ± 0.4
6	> 100		

由表 2 表明,化合物 **8** ~ **10** 显示出很强的抑制人肝癌细胞 BEL-7402 增殖活性(IC_{50} 为 3.3 ~ 8.6 μmol/L),与阳性对照顺铂活性相近(IC_{50} 为 1.8 μmol/L)。同时,化合物 **1** ~ **5** 显示轻度抗肿瘤活性(IC_{50} 为 31.3 ~ 85.9 μmol/L),化合物 **6** 和 **7** 的抗肿瘤活性很弱,IC_{50} 大于实验设定的最高浓度 100 μmol/L。对上述初步活性结果分析发现,活性最强的 3 个化合物 **8** ~ **10** 均是葫芦素类三萜苷元,与文献报道一致[12],而其他 7 个活性一般的化合物均是葫芦素三萜苷类,表明该类三萜化合物的苷元比其苷类抗肿瘤作用更强,深入的构效关系有待进一步研究。

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