刺五加有效成分及体外抑制二酰基甘油酰基转移酶活性研究

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摘　要:本文为研究刺五加(*Acanthopanax senticosus* Harms)的化学成分及其抑制二酰基甘油酰基转移酶(DGAT)活性。刺五加用75%乙醇提取,经硅胶、ODS、半制备HPLC进行分离纯化,结合理化性质、波谱数据鉴定化合物的结构。得到12个化合物分别鉴定为赤式-愈创木基丙三醇-β-*O*-4′-二羟基松柏醇(**1**)、(*E*)-3-(2,2-dimethyl-2*H*-chromen-6-yl)prop-2-enal(**2**)、7′*E*-4,9-dihydroxy-3,3′,5′-trimethoxy-8,4′-oxyneolign-7′-en-9′-al(**3**)、5-甲氧基去氧双松柏醇(**4**)、去氧双松柏醇(**5**)、5,5′-二甲氧基落叶松脂素(**6**)、5,5′-二甲氧基开环异落叶松树脂酚(**7**)、(7′*S*,8′*S*)-4′-*O*-甲基黄花菜木脂素(**8**)、(+)-9′-*O*-(*Z*)-阿魏酰-5,5′-二甲氧基落叶松脂素(**9**)、(+)-9′-*O*-(*E*)阿魏酰-5,5′-二甲氧基落叶松脂素(**10**)、大豆苷(**11**)和3′-甲氧基大豆苷(**12**)。其中化合物**1**~**3**和**8**~**10**为首次从该植物中分离得到。化合物**1**、**3**~**7**、**9**和**10**对DGAT1活性具有抑制作用,其IC_{50}值范围在81.5±1.2到123.2±1.1 μM之间。

关键词:刺五加;化学成分;二酰基甘油酰基转移酶;抑制剂

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Study on inhibition of diglyceride acyltransferase active components by *Acanthopanax senticosus* Harms

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Abstract:This paper studies the chemical constituents of *Acanthopanax senticosus* Harms and its inhibition of diacylglycerol acyltransferase (DGAT) activity. *A. senticosus* was extracted with 75% ethanol,and purified by silica gel,ODS and semi-preparative HPLC. The structures of the compounds were identified by physicochemical properties and spectral data. Twelve compounds were identified as *erythro*-guaiacylglycerol-β-*O*-4′-dihydroconiferyl alcohol (**1**),(*E*)-3-(2,2-dimethyl-2*H*-chromen-6-yl)prop-2-enal (**2**),7′*E*-4,9-dihydroxy-3,3′,5′-trimethoxy-8,4′-oxyneolign-7′-en-9′-al (**3**),5-methoxydehydroconiferyl alcohol (**4**),dehydroconiferyl alcohol (**5**),5,5′-dimethoxylariciresinol (**6**),5,5′-dimethoxysecoisolariciresinol (**7**),(7′*S*,8′*S*)-4′-*O*-methylcleomiscosin D (**8**),(+)-9′-*O*-(*Z*)-feruloyl-5,5′-dimethoxylariciresinol (**9**),(+)-9′-*O*-(*E*)-feruloyl-5,5′-dimethoxylariciresinol (**10**),daidzin (**11**) and 3′-methoxydaidzin (**12**),respectively. Compounds **1**-**3** and **8**-**10** were isolated from this plant for the first time. Compounds **1**,**3**-**7**,**9** and **10** showed inhibitory effect on DGAT1 activity with IC_{50} values ranging from 81.5±1.2 to 123.2±1.1 μM.

Key words:*Acanthopanax senticosus* Harms;chemical constituents;DGAT;inhibitor

甘油三酯(TG)是真核生物能量储存的主要形式,当人体摄入的能量超过其消耗的能量时,过剩的能量便以TG的形式储存在脂肪组织,同时过多的TG也会在胰岛β细胞、肝脏等组织沉积,最终导致肥胖和糖尿病等其他疾病[1]。二酰基甘油酰基转移酶(DGAT)作为催化TG合成的关键最后一步,是TG合成的限速酶[2]。DGAT有两种亚型DGAT1和DGAT2。经研究发现DGAT1/2抑制剂在预防肥胖、调节脂代谢紊乱、预防肝脂肪变性等方面有重要的药物研究价值[2]。

刺五加,又名五加参、刺拐棒,是五加科植物刺五加(*Acanthopanax senticosus* Harms)的根和根茎,主治脾肾阳虚,腰膝酸软,体恤乏力,失眠,多梦,食欲缺乏。目前对刺五加药效物质研究方面报道较多,

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但大多以皂苷、多糖和黄酮类较为集中。如皂苷类成分具有防治糖尿病[3]、抗肿瘤[4]、保护心脑血管[5]的作用;多糖类成分具有调节免疫功能的作用[6];酚酸类成分具有抗疲劳作用[7];黄酮类成分具有抗氧化活性和抗菌、抗炎[7]等作用。本研究主要报道从刺五加茎中筛选有效抑制 DGAT 活性化合物,并测试它们的有效抑制浓度。

1 仪器与材料

^{1}H NMR 和^{13}C NMR 用 INO-VA-500 MHz 型核磁共振仪(美国 CIL 公司,TMS 为内标);MS 用 QSTAR 质谱仪;ELISA 酶标仪(美国);DGAT 酶(美国 Biomol 公司);C_{18} 薄层色谱板 F254(Merck 公司);反相柱色谱 ODS(Nacalai Tesque 公司);高效液相色谱所用试剂为色谱纯,其余均为分析纯。

刺五加于 2014 年 10 月采于吉林省白山市长白山周边地区,经过延边大学药学院李镐教授鉴定为 *A. senticosus*。标本收藏于北华大学药学院药物化学实验室(标本号为:AO20141018)。

2 实验方法

2.1 提取与分离

将干燥粉碎的刺五加茎 5.0 kg,用 75% 乙醇室温每浸泡 3 天提取 1 次,共提取 3 次,合并乙醇提取液,减压浓缩得乙醇浸膏;将浸膏溶于水中成混悬液之后用二氯甲烷萃取,减压回收二氯甲烷溶剂,得二氯甲烷萃取物 150.0 g。将二氯甲烷萃取物经硅胶柱色谱分离,利用正己烷∶乙酸乙酯 =50∶0→0∶1(V/V)为流动相进行梯度洗脱,收集分离组分,并利用硅胶薄层色谱检测,成分相同的分离组分合并。得到 A 至 G 共 7 个分离组分。分离组分 E,经反相高效液相色谱法,使用 RP-18 柱(10 × 250 mm,10 μm),以乙腈∶水 =70∶30→100∶0(V/V)作为流动相梯度洗脱得化合物 **1**(8.4 mg)和 **4**(7.8 mg)。分离组分 G,200 目硅胶柱色谱,利用正己烷∶乙酸乙酯 =10∶1→0∶1(V/V)为流动相进行梯度洗脱,得到 G1 至 G7 个分离组分,G6 经甲醇重结晶得到化合物 **8**(10.1 mg)。组分 G5 经过 200 ~ 300 目硅胶柱色谱,利用正己烷∶乙酸乙酯 =8∶1→0∶1(V/V)为流动相进行梯度洗脱,得到 G5-1 至 G5-7 个分离组分。分离组分 G5-2,经反相高效液相色谱法,利用 80% 乙腈为流动相洗脱得到化合物 **5**(6.8 mg)和 **6**(12.1 mg);G5-3 以乙腈∶水 =80∶20→95∶5(V/V)作为流动相梯度洗脱得化合物 **2**(4.9 mg)和 **10**(5.4 mg);G5-5 以甲醇∶水 =2∶1→95∶5(V/V)作为流动相梯度洗脱得化合物 **3**(6.2 mg),**7**(3.5 mg)和 **9**(7.3 mg);G5-6 以甲醇∶水 =5∶95→20∶80(V/V)作为流动相梯度洗脱得化合物 **11**(7.1 mg)和 **12**(4.7 mg)。

2.2 抑制 DGAT 活性检测

活性测定方法:96 孔板上加入样品、缓冲液[175 mM Tris-HCl(pH = 7.5),DGAT1/2,100 mM $MgCl_2$(DGAT2;5 mM $MgCl_2$)],0.2 mM *sn*-1,2-二酰基甘油,0.25 mg 无脂肪酸的牛血清白蛋白和 10 μM [1-^{14}C]甘油酰基-辅酶 A(2.75 μCi),短暂震荡,恒温 25 ℃反应 30 min,加入 1.5 mL 2-丙醇∶庚烷∶水 = 80∶20∶2(V/V/V)停止反应,用 1.0 mL 庚烷和 0.5 mL 水提取脂质涡旋后,将 1.2 mL 的有机相转移到玻璃管中,用 2.0 mL 碱性乙醇溶液[乙醇∶0.5 N NaOH∶H_2O =50∶10∶40(V/V/V)]洗涤一次,最后用液体闪烁计数器测定其放射性[8]。

3 实验结果

3.1 结构鉴定

化合物 1 黄色粉末状;ESI-MS:*m/z* 401.1 [M + Na]$^+$;^{1}H NMR(500 MHz,$(CD_3)_2CO$)δ:1.83(2H,m,H-8′),2.63(2H,m,H-7′),3.44(1H,m,H-9$_{a'}$),3.54(2H,m,H-9),3.73(1H,m,H-9$_{b'}$),3.83(3H,s,5-OCH_3),3.86(3H,s,2′-OCH_3),4.22(1H,m,H-8),4.91(1H,m,H-7),6.74(1H,d,*J* = 8.0 Hz,H-5′),6.76(1H,d,*J* = 8.0 Hz,H-3),6.87(1H,s,H-3′),6.84(1H,d,*J* = 8.0 Hz,H-2),6.95(1H,d,*J* = 8.0 Hz,H-6′),7.05(1H,s,H-6);^{13}C NMR(125 MHz,$(CD_3)_2CO$)δ:133.8(C-1),120.9(C-2),116.0(C-3),147.6(C-4),148.9(C-5),111.8(C-6),74.2(C-7),87.8(C-8),62.3(C-9),147.2(C-1′),151.8(C-2′),114.0(C-3′),138.3(C-4′),122.1(C-5′),119.7(C-6′),32.6(C-7′),35.7(C-8′),62.0(C-9′),56.8(5-OCH_3),56.5(2′-OCH_3)。以上数据与文献[8]报道基本一致,故确定为赤式-愈创木基丙三醇-β-*O*-4′-二羟基松柏醇。

化合物 2 无色油状;ESI-MS:*m/z* 237.1 [M + Na]$^+$;^{1}H NMR(500 MHz,$(CD_3)_2CO$)δ:1.47(6H,s,H-4′,H-5′),5.65(1H,d,*J* = 9.6 Hz,H-2′),6.32(1H,d,*J* = 9.6 Hz,H-1′),6.55(1H,dd,*J* = 15.6,7.6 Hz,H-8),6.83(1H,d,*J* = 8.6 Hz,H-5),7.22(1H,d,*J* = 2.0 Hz,H-2),7.33(1H,dd,*J* = 8.6,2.0 Hz,H-6),7.35(1H,d,*J* = 15.6 Hz,H-7),9.64(1H,d,*J* = 7.6 Hz,CHO);^{13}C NMR(125 MHz,

(CD_3)$_2$CO) δ:127.2(C-1),126.4(C-2),121.6(C-3),156.5(C-4),117.4(C-5),130.6(C-6),153.2(C-7),126.8(C-8),194.2(C-9),121.3(C-1′),131.5(C-2′),77.2(C-3′),28.3(C-4′),28.1(C-5′)。以上数据与文献[9]报道基本一致,故确定为(*E*)-3-(2,2-dimethyl-2*H*-chromen-6-yl)prop-2-enal。

化合物3　淡黄色油状;ESI-MS:*m/z* 403.1 [M-H]$^-$;^{1}H NMR(500 MHz,(CD_3)$_2$CO) δ:3.35(br d,*J* = 11.2 Hz,Hb-9),3.71 ~ 3.77(m,Ha-9),3.83(s,MeO-3′,5′),4.14(br s,H-8),4.85(d,*J* = 5.0 Hz,H-7),6.63(d,*J* = 7.5 Hz,H-5),6.65(dd,*J* = 15.5,7.5 Hz,H-8′),6.71(d,*J* = 7.5 Hz,H-6),6.91(br s,H-2),7.05(br s,H-2′,6′),7.51(d,*J* = 15.5 Hz,H-7′),9.52(d,*J* = 7.5 Hz,H-9′);^{13}C NMR(125 MHz,(CD_3)$_2$CO) δ:133.6(C-1),110.7(C-2),147.8(C-3),146.3(C-4),115.1(C-5),120.2(C-6),73.2(C-7),87.8(C-8),61.2(C-9),130.6(C-1′),106.7(C-2′),154.3(C-3′),139.1(C-4′),154.4(C-5′),107.1(C-6′),153.3(C7′),129.1(C-8′),193.7(C-9′),55.6(3-OMe),56.5(3′,5′-OMe)。以上数据与文献[10]报道基本一致,故确定为7′*E*-4,9-dihydroxy-3,3′,5′-trimethoxy-8,4′-oxyneolign-7′-en-9′-al。

化合物4　黄色粉末状;ESI-MS:*m/z* 413.1 [M + Na]$^+$;^{1}H NMR(500 MHz,(CD_3)$_2$CO) δ:1.81(2H,m,H-8′),2.62(2H,m,H-7′),3.48(1H,m,H-8),3.58(2H,m,H-9′),3.75(1H,m,H-9$_a$),3.82(6H,s,3,5-OCH_3),3.85(1H,m,H-9$_b$),3.87(3H,s,3′-OCH_3),5.46(1H,d,*J* = 5.5 Hz,H-7),6.66(2H,s,H-2,6),6.75(2H,s,H-4′,6′);^{13}C NMR(125 MHz,(CD_3)$_2$CO) δ:132.6(C-1),102.7(C-2),147.9(C-3),135.6(C-4),147.8(C-5),102.7(C-6),87.6(C-7),54.1(C-8),63.7(C-9),128.4(C-1′),146.0(C-2′),143.8(C-3′),112.7(C-4′),135.5(C-5′),116.6(C-6′),31.8(C-7′),34.3(C-8′),60.8(C-9′),55.3(3,5-OCH_3),55.2(3′-OCH_3)。以上数据与文献[11]报道基本一致,故确定为5-甲氧基去氧双松柏醇。

化合物5　黄色粉末状;ESI-MS:*m/z* 383.1 [M + Na]$^+$;^{1}H NMR(500 MHz,(CD_3)$_2$CO) δ_H:1.83(2H,m,H-8′),2.65(2H,m,H-7′),3.49(1H,m,H-8),3.57(2H,m,H-9′),3.72(1H,m,H-9$_a$),3.82(1H,m,H-9$_b$),3.83(3H,s,3-OCH_3),3.87(3H,s,3′-OCH_3),5.52(1H,d,*J* = 6.0 Hz,H-7),6.76(2H,s,H-4′,6′),6.78(1H,d,*J* = 8.0 Hz,H-5),6.81(1H,d,*J* = 8.0 Hz,H-6),6.95(1H,s,H-2);^{13}C NMR(125 MHz,(CD_3)$_2$CO) δ_C:1134.9(C-1),110.6(C-2),49.2(C-3),147.61(C-4,2′),115.9(C-5),119.8(C-6),89.1(C-7),55.6(C-8),65.0(C-9),129.7(C-1′),147.6(C-2′),145.5(C-3′),114.1(C-4′),137.3(C-5′),118.1(C-6′),33.0(C-7′),35.9(C-8′),62.5(C-9′),56.8(3-OCH_3),56.4(3′-OCH_3)。以上数据与文献[12]报道基本一致,故确定为去氧双松柏醇。

化合物6　白色粉末;EI-MS:*m/z* 420.3 [M]$^+$;^{1}H NMR(500 MHz,(CD_3)$_2$CO) δ:2.45(1H,q,8-H),2.56(1H,dd,*J* = 13.5,11.0 Hz,7′-H$_a$),2.75(1H,m,8′-H),2.95(1H,dd,*J* = 13.5,5.0 Hz,7′-H$_b$),3.83(1H,dd,*J* = 8.5,5.0 Hz,9′-H$_a$),3.73 ~ 3.81(1H,overlapped,9-H$_a$),3.84(6H,s,3′,5′-OCH_3),3.84(6H,s,3,5-OCH_3),3.91(1H,dd,*J* = 13.0,7.0 Hz,9-H$_b$),4.04(1H,dd,*J* = 8.5,7.0 Hz,9′-H$_b$),4.76(1H,d,*J* = 7.0 Hz,7-H),6.43(2H,s,2′,6′-H),6.59(2H,s,2,6-H);^{13}C NMR(125 MHz,(CD_3)$_2$CO) δ:131.7(C-1),102.8(C-2),147.3(C-3),134.4(C-4),147.3(C-5),102.7(C-6),83.2(C-7),52.8(C8),61.1(C-9),133.2(C-1′),105.3(C-2′),147.3(C-3′),134.2(C-4′),147.3(C-5′),105.4(C-6′),33.8(C-7′),42.5(C-8′),72.6(C-9′),56.4(2 × OCH_3),56.5(2 × OCH_3)。以上数据与文献[13]报道基本一致,故确定为5,5′-二甲氧基落叶松脂素。

化合物7　白色粉末;EI-MS:*m/z* 422.4 [M]$^+$;^{1}H NMR(500 MHz,(CD_3)$_2$CO) δ:1.83(2H,m,8,8′-H),2.62(2H,dd,*J* = 13.5,6.0 Hz,7,7′-H$_a$),2.73(2H,dd,*J* = 13.5,7.5 Hz,7,7′-H$_b$),3.55(2H,dd,*J* = 12.0,5.0 Hz,9,9′-H$_a$),3.81(3H,s,-OCH_3),3.83 ~ 3.86(2H,9,9′-H$_b$),6.31(4H,s,Ar-H);^{13}C NMR(125 MHz,(CD_3)$_2$CO) δ:131.3(C-1),105.4(C-2),146.6(C-3),132.7(C-4),146.8(C-5),105.4(C-6),43.6(C-7),36.3(C-8),61.2(C-9),131.4(C-1′),105.4(C-2′),146.7(C-3′),132.6(C-4′),146.6(C-5′),105.4(C-6′),43.5(C-7′),36.7(C-8′),61.2(C-9′),56.1(4 × OCH_3)。以上数据与文献[13]报道基本一致,故确定为5,5′-二甲氧基开环异落叶松树脂酚。

化合物8 白色粉末;ESI-MS:*m/z* 453.1 [M + Na]$^+$;^{1}H NMR(500 MHz,$(CD_3)_2CO$)δ:3.53(1H,m,H-9′),3.94(1H,m,H-9′),3.95(9H,s,3′,4′,5′-OCH_3),3.97(3H,s,6-OCH_3),4.03(1H,dt,H-8′),5.07(1H,d,*J* = 8.0 Hz,H-7′),6.24(1H,d,*J* = 9.0 Hz,H-3),6.56(1H,s,H-5),6.69(2H,s,H-2′,H-6′),7.95(1H,d,*J* = 9.0 Hz,H-4);^{13}C NMR(125 MHz,$(CD_3)_2CO$)δ:161.1(C-2),112.2(C-3),138.1(C-4),92.7(C-5),152.2(C-6),149.6(C-7),132.2(C-8),140.1(C-9),103.4(C-10),126.1(C-1′),103.1(C-2′),152.7(C-3′),137.3(C-4′),152.8(C-5′),103.1(C-6′),77.2(C-7′),78.2(C-8′),61.3(C-9′),56.4(6-OCH_3),56.4(3′-OCH_3),60.7(4′-OCH_3),56.4(5′-OCH_3)。以上数据与文献[9]报道基本一致,故确定为(7′*S*,8′*S*)-4′-*O*-甲基黄花菜木脂素。

化合物9 白色粉末状;EI0MS:*m/z* 596.2 [M]$^+$;^{1}H NMR(500 MHz,$(CD_3)_2CO$)δ:2.47(1H,dd,*J* = 13.0,12.0 Hz,H-7_a),2.61(1H,m,H-8′),2.70(1H,m,H-8),2.83(1H,dd,*J* = 13.0,5.0 Hz,H-7_b),3.72(1H,dd,*J* = 8.5,6.0 Hz,H-9_a),3.84(12H,s,3,5,3′,5′-OCH_3),3.93(3H,s,3″-OCH_3),4.04(1H,dd,*J* = 8.5,7.0 Hz,H-9_b),4.33(2H,dd,*J* = 12.5,7.0 Hz,H-9′),4.75(1H,d,*J* = 6.5 Hz,H-7′),5.73(1H,d,*J* = 13.5 Hz,H-8″),6.34(2H,s,H-2,H-6),6.52(2H,s,H-2′,H-6′),6.81(1H,d,*J* = 13.5 Hz,H-7″),6.86(1H,d,*J* = 8.5 Hz,H-5″),7.12(1H,dd,*J* = 8.5,2.0 Hz,H-6″),7.77(1H,d,*J* = 13.5 Hz,H-2″);^{13}C NMR(125 MHz,$(CD_3)_2CO$)δ:131.3(C-1),105.4(C-2),147.3(C-3),133.4(C-4),147.7(C-5),105.3(C-6),33.9(C-7),42.8(C-8),72.9(C-9),133.8(C-1′),102.7(C-2′),147.3(C-3′),134.4(C-4′),147.3(C-5′),102.7(C-6′),83.6(C-7′),49.4(C-8′),62.7(C-9′),127.5(C-1″),112.9(C-2″),145.6(C-3″),147.6(C-4″),114.2(C-5″),126.2(C-6″),144.7(C-7″),115.7(C-8″),166.4(C-9″),56.4(3-OCH_3),56.4(5-OCH_3),56.4(3′-OCH_3),56.4(5′-OCH_3),55.8(3″-OCH_3)。以上数据与文献[9]报道基本一致,故确定为(+)-9′-*O*-(*Z*)-阿魏酰-5,5′-二甲氧基落叶松脂素。

化合物10 白色粉末;ESI-MS:*m/z* 619.2 [M + Na]$^+$;^{1}H NMR(500 MHz,$(CD_3)_2CO$)δ:2.52(1H,dd,*J* = 13.5,11.0 Hz,H-7_a),2.63(1H,m,H-8′),2.71(1H,m,H-8),2.85(1H,dd,*J* = 13.5,5.0 Hz,H-7_b),3.75(1H,dd,*J* = 8.0,6.0 Hz,H-9_a),3.86(12H,s,3,5,3′,5′-OCH_3),3.93(3H,s,3″-OCH_3),4.07(1H,dd,*J* = 8.0,6.5 Hz,H-9_b),4.31(1H,dd,*J* = 12.0,7.5 Hz,H-$9'_a$),4.51(1H,dd,*J* = 12.0,7.0 Hz,H-$9'_b$),4.83(1H,d,*J* = 7.0 Hz,H-7′),6.25(1H,d,*J* = 16.5 Hz,H-8″),6.47(2H,s,H-2,H-6),6.55(2H,s,H-2′,H-6′),6.93(1H,d,*J* = 8.0 Hz,H-5″),6.96(1H,d,*J* = 2.0 Hz,H-2″),7.04(1H,dd,*J* = 8.0,2.0 Hz,H-6″),7.48(1H,d,*J* = 16.5 Hz,H-7″);^{13}C NMR(125 MHz,$(CD_3)_2CO$)δ:131.2(C-1),105.3(C-2),147.2(C-3),133.4(C-4),147.2(C-5),105.3(C-6),33.8(C-7),42.9(C-8),72.7(C-9),133.6(C-1′),102.7(C-2′),147.1(C-3′),134.3(C-4′),147.2(C-5′),102.8(C-6′),83.9(C-7′),49.3(C-8′),62.9(C-9′),126.9(C-1″),109.6(C-2″),146.9(C-3″),148.4(C-4″),114.9(C-5″),123.3(C-6″),145.5(C-7″),114.7(C-8″),167.2(C-9″),56.4(3-OCH_3),56.5(5-OCH_3),56.4(3′-OCH_3),56.5(5′-OCH_3),56.1(3″-OCH_3)。以上数据与文献[9]报道基本一致,故确定为(+)-9′-*O*-(*E*)阿魏酰-5,5′-二甲氧基落叶松脂素。

化合物11 白色粉末;ESI-MS:*m/z* 417.1 [M + H]$^+$;^{1}H NMR(500 MHz,$(CD_3)_2CO$)δ:3.12 ~ 3.70(6H,m,Glc-H-2″-6″),5.12(1H,d,*J* = 7.5 Hz,Glc-H-1″),6.81(2H,d,*J* = 8.0 Hz,H-3′,5′),7.11(1H,dd,*J* = 8.0,2.0 Hz,H-6),7.18(1H,d,*J* = 2.0 Hz,H-8),7.41(2H,d,*J* = 8.0 Hz,H-2′,6′),8.06(1H,d,*J* = 8.0 Hz,H-5),8.34(1H,s,H-2);^{13}C NMR(125 MHz,$(CD_3)_2CO$)δ:154.1(C-2),124.5(C-3),174.9(C-4),127.1(C-5),115.9(C-6),162.1(C-7),102.9(C-8),158.1(C-9),119.3(C-10),123.4(C-1′),131.1(C-2′,6′),114.9(C-3′,5′),158.2(C-4′),101.0(C-1″),73.6(C-2″),76.9(C-3″),69.8(C-4″),75.9(C-5″),58.9(C-6″)。以上数据与文献[14]报道一致,故确定为大豆苷。

化合物12 白色粉末;ESI-MS:*m/z* 447.2 [M + H]$^+$;^{1}H NMR(500 MHz,$(CD_3)_2CO$)δ:3.74(3H,s,3′-OCH_3),5.13(1H,d,*J* = 7.5 Hz,Glc-H-1″),6.83(1H,d,*J* = 8.0 Hz,H-5′),7.06(1H,dd,*J* = 8.0,2.0 Hz,H-6),7.15(1H,dd,*J* = 8.0,2.0

Hz,H-6′),7.18(1H,d,J = 2.0 Hz,H-8),7.28(1H,d,J = 2.0 Hz,H-2′),8.11(1H,d,J = 8.0 Hz,H-5),8.44(1H,s,H-2);^{13}C NMR(125 MHz,$(CD_3)_2CO$)δ:153.8(C-2),123.6(C-3),175.5(C-4),127.1(C-5),115.8(C-6),162.2(C-7),104.1(C-8),156.9(C-9),119.2(C-10),125.1(C-1′),115.2(C-2′),146.8(C-3′),147.1(C-4′),113.3(C-5′),122.3(C-6′),56.3($3'$-OCH_3),101.2(C-1″),74.2(C-2″),77.3(C-3″),70.6(C-4″),76.9(C-5″),60.8(C-6″)。以上数据与文献[14]报道一致,故确定为3′-甲氧基大豆苷。

3.2　活性实验结果

以次苦参黄素作为为阳性对照品,对所得化合物**1**~**12**进行了体外抑制DGAT1/2活性实验(见表1)。其中,化合物**1**、**6**~**7**和**9**~**10**对DGAT1有较好的抑制作用,而化合物**1**~**12**均对DGAT2无抑制作用。

表1　化合物1~12对DGAT1/2的抑制作用(n = 3)

Table 1　Inhibitory effects of compounds **1-12** on DGAT1/2 (n = 3)

化合物 Compound	IC_{50}(μM)[a]	
	二酰基甘油酰基转移酶Ⅰ DGAT1	二酰基甘油酰基转移酶Ⅱ DGAT2
1	97.1±1.4	>200
2	>200	>200
3	103.9±1.2	>200
4	123.2±1.1	>200
5	121.7±1.3	>200
6	83.2±1.1	>200
7	81.5±1.2	>200
8	>200	>200
9	91.1±1.4	>200
10	93.4±1.5	>200
11	>200	>200
12	>200	>200
次苦参黄素[b] Kuraridine[b]	10.7±1.3	18.4 ± 1.1

注:[a] 通过回归分析确定IC_{50}值,并表示为三次重复的平均值±SD;[b] 阳性对照。

Note:[a]IC_{50} values were determined by regression analyses and expressed as mean ± SD of three replicates;[b]Positive control.

4　结论

虽然现阶段已有大量设计和合成DGAT抑制剂的相关文献,但从植物中筛选天然DGAT抑制剂的研究报道较少。通过本次实验从刺五加茎中提取分离到十二个化合物,包括九个木脂素类(**1**和**3**~**10**),一个香豆素类(**2**)和两个黄酮苷类(**11**和**12**)化合物。其中,木脂素类化合物**6**、**7**、**9**、**10**对DGAT1有较好的抑制作用。本研究虽然没有对刺五加木脂素类化合物与抑制DGAT构效关系进行深入研究,但为今后刺五加资源的合理开发,并进一步探索该类型化合物在细胞及动物体内的作用机制,以及寻找潜在的新型天然DGAT抑制剂提供了基础理论依据。

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