3 结论

本实验采用的 RP-HPLC/ESI-MS可以更简便地分析葡萄籽低聚原花青素 (DP1-3)的基本组成,但由于各聚合体异构体数目繁多,其 ESI质谱行为也很相近 除单体外,还未能对各异构体的结构作出准确的鉴定

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虎杖的水溶性成分研究

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摘 要: 目的 研究虎杖的水溶性活性成分。方法 利用反相色谱的方法分离纯化,根据化合物的化学性质与光谱数据鉴定其结构,并测试其生物活性。结果 虎杖根茎的 60% 丙酮提取物中分得 6个化合物,确定其结构分别为白藜芦醇 (Ⅰ),云杉新苷 (白藜芦醇-3-*O*-β-*D*-吡喃葡萄糖苷) (Ⅱ), 2, 3-二氢-2-(4′-*O*-β-*D*-吡喃葡萄糖基-3′-甲氧基-苯基)-3-羟甲基-5-(3-羟基丙基)-7-甲氧基苯唑呋喃 (Ⅲ), 2, 6-二甲氧基-*p*-苯-1-*O*-β-*D*-吡喃葡萄糖苷 (Ⅳ), 5, 7-二羟基-异苯唑呋喃 (Ⅴ), 5, 7-二羟基-异苯唑呋喃-7-*O*-β-*D*-吡喃葡萄糖苷 (Ⅵ)。结论 化合物Ⅲ~Ⅵ为首次从该植物中分得,化合物Ⅰ~Ⅵ没有显示出 DNA裂解活性,化合物Ⅱ对 KB和 MCF-7细胞表现出较弱的细胞毒活性。

关键词: 虎杖;水溶性成分;生物活性

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Studies on water-soluble constituents in rhizome of *Polygonum cuspidatum*

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Abstract **Object** To study the chemical structures and bioactivity of the water-soluble constituents from *Polygonum cuspidatum* Sieb. et Zucc. **Methods** To isolate the constituents by reverse phase methods, and characterize their structures by the analysis of chemical property and spectral data. **Results** Six compounds were isolated from the 60% aqueous acetone extract from the rhizome of *P. cuspidatum*. Their structures were elucidated as reveratrol (Ⅰ); piceid (Ⅱ); 2, 3-dihydro-2-(4′-*O*-β-*D*-glucopyranosyl-3′-methoxy-phenyl)-3-hydroxymethyl-5-(3-hydroxypropyl)-7-methoxybenzofuran (Ⅲ); 2, 6-dimethoxy-*p*-hydroquinone-1-*O*-β-*D*-glucopyranoside (Ⅳ); 5, 7-dihydroxy-isobenzofuran (Ⅴ) and 5, 7-dihydroxy-isobenzofuran-7-*O*-β-*D*-glucopyranoside (Ⅵ), respectively. **Conclusion** Compounds Ⅲ-Ⅵ are isolated

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from the plant for the first time. Compounds Ⅰ – Ⅵ show no DNA cleavage activity. Compound Ⅱ exhibits weak cytotoxicity against two human cancer cell lines (KB and MCF-7) *in vitro*.

Key words *Polygonum cuspidatum* Sieb. et Zucc.; water-soluble constituents; bioactivity

虎杖为蓼科植物虎杖 *Polygonum cuspidatum* Sieb. et Zucc. 的根茎。多生于山谷,溪旁或岸边,为多年生灌木状草本。性味苦,平,具有祛风、利湿、破瘀、通经的功效;用于风湿,筋骨疼痛,湿热黄疸,淋浊带下,妇女经闭,产后恶露不下,疮漏下血,跌扑损伤,烫伤,恶疮癣疾等疾病。虎杖分布于江苏、浙江、江西、福建、山东、河南等中南部地区[1,2],春秋均可采挖。从虎杖中分离蒽醌类化合物、stilbene类化合物、黄酮类化合物及其他一些酚性成分已有文献报道[1,2]。虎杖有显著的抗菌、抗真菌作用与较好的抗病毒活性,对心血管系统亦有作用,其中 piceid具有广泛的心血管活性[3],虎杖还能抵抗脂质过氧化,具有抗突变与自由基清除作用,抗肿瘤作用[4],抗炎活性等。

我们曾报道用反相色谱的方法从虎杖的 60%丙酮提取物中分得 10个水溶性很强的 stilbene苷的硫酸酯盐[5]。在进一步的研究中,我们又从中分得了 6个单体成分,经过与文献对照,确定它们的结构分别为白藜芦醇(Ⅰ),白藜芦醇-3-*O*-β-*D*-吡喃葡萄糖苷(Ⅱ),2,3-二氢-2-(4′-*O*-β-*D*-吡喃葡萄糖基-3′-甲氧基-苯基)-3-羟甲基-5-(3-羟基丙基)-7-甲氧基苯唑呋喃(Ⅲ),2,6-二甲氧基-*p*-苯-1-*O*-β-*D*-吡喃葡萄糖苷(Ⅳ),5,7-二羟基-异苯唑呋喃(Ⅴ),5,7-二羟基-异苯唑呋喃-7-*O*-β-*D*-吡喃葡萄糖苷(Ⅵ)。其中化合物Ⅲ~Ⅵ为首次从该植物中分得。

1 仪器与材料

比旋光度用 Perkin-Elmer Polarimeter 341测定;红外光谱用 Hitachi 275-50型经分光光度仪测定;FABMS用 MAT-212型质谱仪测定;^{1}HNMR,^{13}CNMR,^{1}H-^{1}HCOSY,HMQC与 HMBC用 Bruker DRX-400 spectrometer (^{1}HNMR 400 MHz,^{13}CNMR 100 MHz)测定。柱色谱所用反相担体:Sephadex LH-20(20~80 μm,Pharmacia Fine Chemical CO. Ltd.),TSK gel Toyopearl HW40F(30~60 μm,Tosoh),MCI gel CHP20P(75~150 μm,Mitsubishi),Cosmosil ODS(40~80 μm,Nacalai Tesque Inc.),TSK gel Phenyl-Toyopearl 650 M(80~100 μm,Tosoh);薄层色谱采用 Kiesel gel 60 F_{254}(precoated plate)与 HSGF_{254}(青岛海洋化工厂)。

2 提取与分离

虎杖 10 kg(购自上海市药材公司),用 60%丙酮提取 3次,蒸去丙酮,沉淀,过滤,除掉大部分脂溶性物质。滤液经浓缩至适当体积,再加水沉淀部分脂溶性物质,浓缩至适当体积后,分次上 MCI gel CHP20P柱,先用水洗脱以去除相当部分的糖与大量的化合物Ⅱ,后用 50%~80%甲醇洗脱,50%~80%甲醇洗脱部分上 LH-20柱层析,用水洗脱以去除剩余的糖,然后用梯度的甲醇洗脱,分成 A, B, C, D, E, F, G共 6个组分,各组分经过 Sephadex LH-20, TSK gel Toyopearl HW40F, MCI gel CHP20P, Cosmoisl ODS, TSK Phenyl-Toyopearl 650 M的结合应用,分离得到 6个化合物(Ⅰ~Ⅵ)。

3 结构鉴定

化合物Ⅰ:白色无定形粉末,易溶于甲醇、丙酮;^{1}HNMR数据与文献所报道的白藜芦醇数据一致[6]。

化合物Ⅱ:白色针晶,易溶于甲醇、丙酮;IR,^{1}HNMR光谱数据与文献所报道的白藜芦醇-3-*O*-β-*D*-吡喃葡萄糖苷数据相一致[6]。

化合物Ⅲ:白色无定形粉末,易溶于甲醇、丙酮;^{1}HNMR(400 MHz, DMSO-d_6+ D_2O) δ 7.06(1H, d, *J*= 8.2 Hz, H-5′), 6.97(1H, br, H-2′), 6.85(1H, brd, *J*= 8.2 Hz, H-6′), 6.69(2H, br, H-4, 6), 5.46(1H, d, *J*= 6.4 Hz, H-2), 4.88(1H, d, *J*= 7.3 Hz, H-1″), 3.77(3H, s, OMe-4), 3.75(3H, s, OMe-3′), 3.71(1H, dd, *J*= 11.0, 5.6 Hz, H-3α a), 3.66(1H, brd, *J*= 12.0 Hz, H-6″α), 3.60(1H, dd, *J*= 11.0, 7.3 Hz, H-3α b), 3.42(1H, obscured, H-6″β), 3.41(2H, t, *J*= 6.4 Hz, H-5′), 3.28(1H, m, H-5″), 3.25(2H, m, H-2″, H-3″), 3.16(1H, m, H-4″), 2.50(2H, obscured, H-5α), 1.69(2H, m, H-5β)。^{13}CNMR(100 MHz, DMSO-d_6+ D_2O) δ 148.9(s, C-3′), 146.1(s, C-4′), 145.5(s, C-8), 143.4(s, C-7), 135.5(s, C-1′), 135.1(s, C-5), 128.8(s, C-9), 118.0(d, C-6′), 116.5(d, C-4), 115.3(d, C-5′), 112.4(d, C-6), 110.2(d, C-2′), 100.00(d, C-1″), 86.5(d, C-2), 77.0(d, C-5″), 76.8(d, C-3″), 73.1(d, C-2″), 69.1(d, C-4″), 63.0(t, C-3α), 60.5(t, C-6″), 60.1(t, C-5′), 55.7(q, OMe-3′), 55.6(q, OMe-7), 53.5(d, C-3), 34.7(t, C-5β), 31.6(t, C-5α)。与文献所报道的

2,3-二氢-2-(4′-*O*-β-*D*-吡喃葡萄糖基-3′-甲氧基-苯基)-3-羟甲基-5-(3-羟基丙基)-7-甲氧基苯唑呋喃[7,8]。

化合物Ⅳ:白色无定形粉末,易溶于甲醇、丙酮、DMSO; UV λ_{max} (MeOH): 277, 256(sh), 208 nm; ^{1}HNMR(400 MHz, DMSO-d_6) δ: 9.20(1H, s, 4-OH), 6.06(2H, d, H-3, 5), 4.64(1H, d, *J*= 6.9 Hz, H-1′), 3.68(6H, s, OMe-2, 6), 3.59(1H, brd, *J*= 11.4 Hz, H-6′α), 3.41(1H, dd, *J*= 11.4, 5.6 Hz, H-6′β), 3.2~2.9(4H, m, H-2′, 3′, 4′, 5′)。与文献所报道的 2,6-二甲氧基-*p*-苯-1-*O*-β-*D*-吡喃葡萄糖苷数据一致[9,10]。

化合物Ⅴ:白色结晶状物,易溶于丙酮、氯仿; IR ν_{max}^{KBr} cm^{-1}: 3 350, 1 716, 1 616, 1 487, 1 350, 1 215, 1 164, 1 053; ^{1}HNMR(400 MHz, D_2O) δ: 6.48(1H, br, H-4), 6.36(1H, br, H-6), 5.17(2H, s, H-3); ^{13}CNMR(400 MHz, D_2O) δ: 171.5(s, C=O), 166.5(s, C-3), 159.1(s, C-5), 152.1(s, C-7), 104.4(s, C-8), 103.4(d, C-4), 102.0(d, C-6), 70.3(t, C-3); EI-MS *m/z*: 166[M]$^+$ (80%)。与文献所报道的 5,7-二羟基-异苯唑呋喃数据一致[11]。

化合物Ⅵ:白色结晶状物,易溶于丙酮、氯仿; UV λ_{max} (MeOH): 295, 260, 238, 212 nm; IR ν_{max}^{KBr} cm^{-1}: 3 419, 1 716, 1 621, 1 608, 1 359, 1 209, 1 064, 1 043; ^{1}HNMR(400 MHz, CD_3COCD_3 + D_2O) δ: 6.73(1H, br, H-4), 6.58(1H, br, H-6), 5.18(2H, s, H-3), 5.06(1H, d, *J*= 7.2 Hz, H-1′), 3.88(1H, dd, *J*= 11.9, 1.8 Hz, H-6′α), 3.65(1H, dd, *J*= 11.9, 6.0 Hz, H-6′β), 3.60~3.40(4H, m, H-2′, 3′, 4′, 5′)。^{13}CNMR(400 MHz, CD_3COCD_3 + D_2O) δ: 167.8(s, C=O), 164.7(s, C-3), 157.1(s, C-5), 151.7(s, C-7), 104.5(s, C-8), 102.1(d, C-4), 102.0(d, C-6), 99.6(d, C-1′), 77.1(d, C-5′), 76.6(d, C-3′), 73.0(d, C-2′), 69.4(d, C-4′), 68.2(t, C-3), 60.5(t, C-6′); FABMS *m/z*: 367[M+K]$^+$, 351[M+Na]$^+$, 329[M+1]$^+$。与文献所报道的 5,7-二羟基-异苯唑呋喃-7-*O*-β-*D*-吡喃葡萄糖苷数据一致[11]。

4 化合物的 DNA裂解活性测定

根据改进的 Hetch方法进行[12]。化合物Ⅰ~Ⅵ没有显示出 DNA裂解活性。细胞毒活性根据文献报道的方法用 MTT法测定[13],化合物Ⅱ对 KB细胞的 IC_{50}值为 70 μg/mL,对 MCF-7细胞的 IC_{50}值为 66 μg/mL。

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通　知

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