36.5); UV λ_{max} (MeOH) (nm) (log ε): 268(3.94), 346(3.95); ^{1}HNMR(DMSO-d_6) δ: 1.05(3H, d, J= 6.1 Hz, -CH_3), 4.4(1H, br. s, H-1 of rha.), 5.27(1H, d, J= 7 Hz, H-1 of glu.), 6.09(1H, H-6), 6.29(1H, H-8), 6.86(2H, dd, J= 8.8 Hz, H-5′, H-3′), 8.0(2H, dd, J= 8.8 Hz, H-2′, H-6′); ^{13}CNMR (DMSO-d_6) δ: 156.0(C-2), 133.2(C-3), 177.0(C-4), 161.0(C-5), 99.4(C-6), 163.8(C-7), 94.1(C-8), 156.6(C-9), 103.0(C-10), 120.8(C-1′), 130.8(C-2′, 6′), 115.0(C-3′, 5′), 160.0(C-4′), Gal: 102.3(C-1), 71.0(C-2), 73.4(C-3), 68.2(C-4), 73.0(C-5), 65.3(C-6), Rha: 100.0(C-1), 70.4(C-2), 70.6(C-3), 71.9(C-4), 68.0(C-5), 17.9(C-6). 以上数据与文献报道的山奈酚-3-O-β-D-[α-L-吡喃鼠李糖-(1→6)]-吡喃半乳糖苷(即山奈酚-3-O-β-刺槐双糖苷)数据一致[7]。

化合物Ⅸ: 黄色针状晶体(MeOH), mp 225 ℃~227 ℃; 盐酸镁粉试验阳性, Molish试验显紫红色; UV λ_{max} (MeOH) (nm) (log ε): 268(4.01), 317(4.11); ^{1}HNMR(DMSO-d_6) δ: 5.46(1H, d, J= 7 Hz, glc H-1), 6.12(1H, d, J= 16 Hz, p-OH-Cin H-2), 6.16(1H, d, J= 1.8 Hz, H-6), 6.39(1H, d, J= 1.8 Hz, H-8), 6.80(2H, d, J= 8.4 Hz, p-OH-Cin H-5, 9), 6.87(2H, br. d, J= 8.8 Hz, H-3′, H-5′), 7.35(2H, d, J= 16 Hz, p-OH-Cin H-3), 7.38(2H, d, J= 8.4 Hz, p-OH-Cin H-6, 8), 8.00(2H, br. d, J= 8.8 Hz, H-2′, 6′); ^{13}CNMR(DMSO-d_6) δ: 156.4(C-2), 133.1(C-3), 177.4(C-4), 161.2(C-5), 98.7(C-6), 164.2(C-7), 93.7(C-8), 156.4(C-9), 103.9(C-10), 120.8(C-1′), 130.8(C-2′, 6′), 115.6(C-3′, 5′), 159.9(C-4′), Glc: 101.0(C-1), 74.2(C-2), 76.2(C-3), 60.0(C-4), 74.1(C-5), 63.0(C-6), p-OH-Cin: 166.1(C-1), 113.6(C-2), 144.6(C-3), 125.0(C-4), 130.1(C-5, 9), 115.1(C-6, 8), 159.7(C-7). 以上数据与文献报道的山奈酚-3-O-β-D-(6-E-对羟基桂皮酰基)-葡萄糖苷数据一致[8]。

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拳参的 DNA裂解活性成分研究

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摘 要: 目的 研究蓼科植物拳参的水溶性成分, 并测试其 DNA裂解活性。方法 利用反相层析的方法进行分离纯化, 根据化合物的化学性质与光谱数据鉴定其结构。结果 自拳参根茎的 60% 丙酮提取物中分得 10个化合物, 确定其结构分别为没食子酸(Ⅰ), 色氨酸(Ⅱ), 2,6-二羟基苯甲酸(Ⅲ), (+)-儿茶素(Ⅳ), 绿原酸(Ⅴ), (-)-表儿茶素-5-O-β-D-吡喃葡萄糖苷(Ⅵ), (+)-儿茶素-7-O-β-D-吡喃葡萄糖苷(Ⅶ), 1-(3-O-β-D-吡喃葡萄糖基-4, 5-二羟基-苯基)-乙酮(Ⅷ), (+)-儿茶素-5-O-β-D-吡喃葡萄糖苷(Ⅸ)和(-)-表儿茶素(Ⅹ)。结论 化合物Ⅱ, Ⅲ, Ⅴ~Ⅹ为首次从该植物中分得, 化合物Ⅰ, Ⅳ, Ⅵ, Ⅶ, Ⅸ, Ⅹ具有很强的 DNA裂解活性。

关键词: 拳参; 水溶性成分; DNA裂解活性

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Studies on chemical constituents possessing DNA cleavage activity

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Abstract **Object** To study the chemical structures and DNA cleavage activity of the water-soluble constituents from *Polygonum bistorta* L. **Methods** To isolate the constituents by reverse phase chromatography, and characterize their structures by the analysis of chemical property and spectral data. **Results** Ten compounds were isolated from the 60% acetone extract of the rhizoma from *P. bistorta*. Their structures were elucidated as gallic acid (Ⅰ), tryptophan (Ⅱ), 2, 6-dihydroy-bezoic acid (Ⅲ), (+)-catechin (Ⅳ), chlorogenic acid (Ⅴ), (-)-epicatechin-5-*O*-β-*D*-glucopyranoside (Ⅵ), (+)-catechin-7-*O*-β-*D*-glucopyranoside (Ⅶ), 1-(3-*O*-β-*D*-glucopyranosyl-4, 5-dihydroxy-phenyl)-ethanone (Ⅷ), (+)-catechin-5-*O*-β-*D*-glucopyranoside (Ⅸ) and (-)-epicatechin (Ⅹ), respectively. **Conclusion** CompoundsⅡ, Ⅲ, Ⅴ-Ⅹ were isolated from the plant for the first time. CompoundsⅠ, Ⅳ, Ⅵ, Ⅶ, Ⅸ, Ⅹ showed significant DNA cleavage activity.

Key words *Polygonum bistorta* L.; water-soluble constituents; DNA cleavage activity

拳参为蓼科植物拳参 *Polygonum bistorta* L. 的根茎。中医认为,拳参性味苦、凉,具有清热利湿、凉血止血、解毒散结的功效;主治肺热咳嗽,热病惊痫,赤痢,热泻,吐血,痔疮出血,痈肿疮毒。拳参为多年生草本,分布于辽宁、内蒙古、河北、山西、陕西、宁夏、甘肃、新疆、山东等地[1,2]。其根茎含没食子酸、鞣花酸以及可水解鞣质和综合鞣质,鞣质含量在 10%左右[3]。有人用薄层层析与紫外的方法检测过其中的成分,并以脉的形式分离得到没食子酸、(+)-儿茶素、葡萄糖和β-谷甾醇的异构体[4,5]; Duwiejua等证实拳参粗提物具有抗菌活性[6],并分到 5-glutinen-3-one和 fridelanol两个具有抗炎活性的化合物及β-谷甾酸[7]。拳参还有很强的抗突变作用[4,8],一定的止血作用及护肤作用等。我们用反相层析的方法,从 60%丙酮提取物中分得 10个酚性化合物,经与文献对照,确定化合物Ⅰ~Ⅹ的结构分别为没食子酸(Ⅰ),色氨酸(Ⅱ), 2, 6-二羟基苯甲酸(Ⅲ),(+)-儿茶素(Ⅳ),绿原酸(Ⅴ),(-)-表儿茶素-5-*O*-β-*D*-吡喃葡萄糖苷(Ⅵ),(+)-儿茶素-7-*O*-β-*D*-吡喃葡萄糖苷(Ⅶ), 1-(3-*O*-β-*D*-吡喃葡萄糖基-4, 5-二羟基-苯基)-乙酮(Ⅷ),(+)-儿茶素-5-*O*-β-*D*-吡喃葡萄糖苷(Ⅸ)和(-)-表儿茶素(Ⅹ),其中化合物Ⅱ,Ⅲ,Ⅴ~Ⅹ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型红外分光光度仪测定; FAB-MS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}H 400 MHz and ^{13}C 100 MHz)测定;柱层析所用反相担体: Sephadex LH-20 (20~80 μm, Pharmacia Fine Chemical Co., Ltd.), TSK gel Toyopearl HW-40F (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);薄层层析采用 Kiesel gel 60 F_{254} (precoated plate)与 HSG F_{254} (青岛海洋化工厂)。

2 提取与分离

采用购自上海市药材公司的中药拳参根茎(产于山东) 10 kg,用 60%丙酮提取 3遍,浓缩蒸去丙酮,沉淀,过滤,除掉大部分脂溶性物质。滤液经浓缩至适当体积后,再加水沉淀,浓缩至小体积后,分次上 LH-20柱层析,先用水洗脱以去除大部分糖,后用梯度甲醇洗脱,其中 100%甲醇洗脱部分为鞣质类化合物。将 10%~80%梯度甲醇洗脱部分合并再上 LH-20柱,分为 4个部分,各个部分经过 Sephadex LH-20, TSK gel Toyopearl HW-40F, MCI gel CHP20P, Cosmosil ODS, TSK gel Phenyl-Toyopearl 650 M的结合应用,分离了 10个已知化合物(Ⅰ~Ⅹ)。拳参中的主要化学成分为绿原酸、没食子酸、儿茶素及大量的鞣质。

3 结构鉴定

化合物Ⅰ:白色针晶,化合物Ⅰ与没食子酸的标准品混合熔点不下降,薄层层析 Rf值、IR及 ^{1}HNMR与没食子酸相一致。

化合物Ⅱ：白色无定形粉末。^{1}HNMR数据与文献所报道的色氨酸数据相符合[9]。

化合物Ⅲ：浅红色无定形粉末，溶于水，易溶于甲醇、丙酮；^{1}HNMR(400 MHz, CD_3COCD_3) δ 6.74 (1H, t, J= 8.4 Hz, H-4), 6.54(2H, d, J= 8.4 Hz, H-3, 5)，其数据与文献中 2, 6-二羟基苯甲酸一致[10]。

化合物Ⅳ：无色结晶状物，易溶于甲醇与丙酮，不溶于水，$[\alpha]_D^{24}$+ 12°(c, 0.1 MeOH)；其光谱数据与文献所报道的 (+)-儿茶素数据一致[11,12]。

化合物Ⅴ：白色结晶状物，易溶于含水的甲醇，$[\alpha]_D^{24}$- 38°(c, 0.3 MeOH)；其光谱数据与文献报道的绿原酸数据相符合[13]。

化合物Ⅵ：白色无定形粉末，易溶于水、甲醇与丙酮，$[\alpha]_D^{24}$- 13.5°(c, 0.16 MeOH)；^{1}HNMR(400 MHz, CD_3COCD_3+ D_2O) δ 7.04(1H, d, J= 1.5 Hz, H-2′), 6.82(1H obscured, H-5′), 6.82(1H obscured, H-6′), 6.35(1H, d, J= 2.2 Hz, H-8), 6.09 (1H, d, J= 2.2 Hz, H-6), 4.92(1H, d, J= 7.8 Hz, H-1″), 4.90(1H obscured, H-2), 4.22(1H br, H-3), 3.92(1H, dd, J= 12.0, 2.1 Hz, H-6′α), 3.72 (1H, dd, J= 12.0, 5.7 Hz, H-6′β), 3.61~ 3.43 (4H, H-2′, 3′, 4′, 5′), 2.96(1H, dd, J= 4.4, 16.9 Hz, H-4α), 2.81(1H, dd, J= 3.6, 16.9 Hz, H-4β)。以上数据与文献报道的 (-)-表儿茶素-5-O-β-D-吡喃葡萄糖苷数据一致[14]。

化合物Ⅶ：浅黄色无定形粉末，易溶于水、甲醇与丙酮，$[\alpha]_D^{24}$- 29.7°(c, 0.12 MeOH)；^{1}HNMR(400 MHz, CD_3COCD_3+ D_2O) δ 6.92(1H, d, J= 1.5 Hz, H-2′), 6.82(1H, d, J= 8.1 Hz, H-5′), 6.75(1H, dd, J= 8.1, 1.5 Hz, H-6′), 6.32(1H, d, J= 1.8 Hz, H-8), 6.10(1H, d, J= 1.8 Hz, H-6), 4.87(1H, d, J= 7.8 Hz, H-1″), 4.62(1H, d, J= 7.8 Hz, H-2), 4.05(1H, m, H-3), 3.84(1H, dd, J= 12.0, 2.1 Hz, H-6′α), 3.72(1H, dd, J= 12.0, 5.7 Hz, H-6′β), 3.60~ 3.40(4H, H-2′, 3′, 4′, 5′), 2.91(1H, dd, J= 5.4, 16.3 Hz, H-4α), 2.56(1H, dd, J= 8.3, 16.3 Hz, H-4β)；^{13}CNMR(100 MHz, CD_3COCD_3+ D_2O) δ 158.4(s, C-7), 157.3(s, C-5), 156.7(s, C-8a), 145.9(s, C-3′), 145.8(s, C-4′), 131.9(s, C-1′), 120.1(d, C-6′), 116.0(d, C-5′), 115.5(d, C-2v), 103.6(d, C-4a), 102.1(d, C-1″), 97.5(d, C-6), 96.8 (d, C-8), 82.7(d, C-2), 77.7(d, C-5″), 77.6(d, C-3″), 74.5(d, C-2″), 71.2(d, C-4″), 68.1(d, C-3), 62.6(t, C-6″), 28.7(t, C-4)。与文献报道的 (+)-儿茶素-7-O-β-D-吡喃葡萄糖苷数据一致[15]。

化合物Ⅷ：白色无定形粉末，易溶于甲醇、丙酮。UV λ_{max} (MeOH): 321, 290, 225 nm; IR ν_{max}^{KBr} cm^{-1}: 3 406, 1 630, 1 601, 1 456, 1 367, 1 279, 1 076, 1 026; FAB-MS m/z: 369[M+ K]$^+$ 和 353[M+ Na]$^+$；^{1}HNMR(400 MHz, D_2O) δ 6.15(1H br, H-6), 5.99(1H, br, H-2), 5.19(1H, d, J= 7.4 Hz, H-1′), 3.97(1H, br d, J= 12.5 Hz, H-6′α), 3.78(1H, dd, J= 12.5, 5.6 Hz, H-6′β), 3.75~ 3.48(4H, m, H-2′, 3′, 4′, 5′), 2.65(3H, s, Me)；^{13}CNMR(100 MHz, D_2O) δ 207.7(s, C= O), 167.4(s, C-3), 166.6(s, C-5), 163.0(s, C-4), 108.3(s, C-1), 102.1 (2C, d, C-2, 6), 92.2(d, C-1′), 78.9(d, C-5′), 78.8 (d, C-3′), 75.4(d, C-2′), 71.9(d, C-4′), 63.3(t, C-6′), 35.2(q, Me)。其光谱数据与文献中 1-(3-O-β-D-吡喃葡萄糖基-4, 5-二羟基-苯基)-乙酮一致[16]。

化合物Ⅸ：白色结晶状物，易溶于水、甲醇与丙酮，$[\alpha]_D^{24}$- 30.8°(c, 0.04 MeOH)；^{1}HNMR(400 MHz, CD_3COCD_3+ D_2O) δ 6.92(1H, d, J= 2.0 Hz, H-2′), 6.84(1H, d, J= 8.1 Hz, H-5′), 6.76(1H, dd, J= 8.1, 2.0 Hz, H-6′), 6.36(1H, d, J= 2.2 Hz, H-8), 6.05(1H, d, J= 2.2 Hz, H-6), 4.91(1H, d, J= 7.4 Hz, H-1″), 4.60(1H, d, J= 7.9 Hz, H-2), 4.04(1H overlapped, H-3), 3.95(1H, dd, J= 12.0, 2.1 Hz, H-6″α), 3.75(1H, dd, J= 12.0, 5.7 Hz, H-6″β), 3.60~ 3.47(4H, m, H-2″, 3″, 4″, 5″), 3.07(1H, dd, J= 5.5, 16.4 Hz, H-4α), 2.60(1H, dd, J= 8.6, 16.4 Hz, H-4β)。与文献报道的 (+)-儿茶素-5-O-β-D-吡喃葡萄糖苷数据一致[17]。

化合物Ⅹ：无色结晶状物，易溶于甲醇与丙酮，不溶于水；^{1}HNMR(400 MHz, CD_3COCD_3) δ 7.02 (1H, d, J= 2.0 Hz, H-2′), 6.76(1H, dd, J= 8.1, 2.0 Hz, H-6′), 6.63(1H, d, J= 8.1 Hz, H-5′), 5.99 (1H, br, H-8), 5.90(1H, br, H-6), 4.84(1H, br, H-2), 4.19(1H, m, H-3), 2.68~ 2.86(2H, m, H-4)。与文献报道的 (-)-表儿茶素数据一致[11,18]。

4 化合物的 DNA裂解活性测定

4.1 实验方法(改进的 HechtIFC)[19]：1)样品溶于在 50% MeOH-H_2O或 MeOH-DMSO 溶液中，然后将之加入反应混合物进行反应。反应混合物包括 10 mmol/L Tris HCl (pH 8.0) 21.5 μL, 1 000 μmol/L Cu^{++} 2 μL, 250 μg/mL pSP 64 DNA 2 μL，样品 1 μL。反应混合物中样品浓度为 100 μg/mL。2) 37 ℃温育 60 min。3)加入 5 μL 0.124 5% 的溴酚蓝终

止反应。4)电泳: 0.1%的琼指糖凝胶, 1× Tris缓冲液中电泳 3 h。5) EB荧光显色后拍照,计算与对照组的比率。

4.2 结果: 化合物Ⅰ,Ⅳ,Ⅵ,Ⅶ,Ⅸ,Ⅹ有很强的 DNA裂解活性,Ⅴ有一定的 DNA裂解活性。

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灵芝一共生真菌的脑苷类成分研究

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摘 要: 目的 为了从灵芝 *Ganoderma lucidum* 中寻找新的天然抗肿瘤活性产物,对其次生代谢产物进行了较系统的研究。方法 利用柱层析和制备 RP-HPLC从灵芝一共生真菌 (*Calcarisporium arbuscula*)的发酵菌丝体中分得 4个脑苷类化合物。结果 通过光谱分析鉴定,分别为: (4*E*, 8*E*, 3′*E*, 2*S*, 3*R*, 2′*R*)-2′-羟基-3′-十六烯酰基-1-*O*-*D*-吡喃葡萄糖基-9-甲基-4, 8-二氢鞘氨二烯醇 (Ⅰ), (4*E*, 8*E*, 2*S*, 3*R*, 2′*R*)-2′-羟基十六烷酰基-1-*O*-β-*D*-吡喃葡萄糖基-9-甲基-4, 8-二氢鞘氨二烯醇 (Ⅱ), (4*E*, 8*E*, 3′*E*, 2*S*, 3*R*, 2′*R*)-2′-羟基-3′-十八烯酰基-1-*O*-*D*-吡喃葡萄糖基-9-甲基-4, 8-二氢鞘氨二烯醇 (Ⅲ), 2-(2-羟基二十四烷酰氨基)-1, 3, 4-十八烷三醇 (Ⅳ)。结论 Ⅰ~Ⅳ均首次从齿梗孢属真菌中分离得到。

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