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泽漆化学成分及其体外抗肿瘤活性研究

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摘 要:利用正相硅胶柱层析、葡聚糖凝胶 Sephadex LH-20、制备薄层层析、反相制备色谱等离手段和^{1}H、^{13}C NMR等波谱技术,从泽漆(*Euphorbia helioscopia* L.)中分离鉴定了 10个化合物,分别为 β谷甾醇(β-sitosterol, **1**),大戟甘(euphornin, **2**),大戟甘 D(euphornin D, **3**),A(euphohelioscopin A, **4**),槲皮素(quercetin, **5**),没食子酸(gallic acid, **6**),咖啡酸(caffeic acid, **7**),没食子酸乙酯(ethyl gallate, **8**),杨梅素(myrecetin, **9**),金丝桃苷(hyperoside, **10**),其中化合物 **7**、**8**、**9**是首次从该植物中分得,通过体外抗肿瘤活性研究,化合物 **6**和 **10**作为泽漆的抗肿瘤活性成分属首次报道。

关键词:泽漆;大戟科;化学成分;抗肿瘤
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Studies on the Chemical Constituents and Its Antitumor Activities from Euphorbia helioscopia L.

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Abstract: Ten compounds were isolated from the ethanol extract of *Euphorbia helioscopia* L. by repeated column chromatographies including Silica gel, Sephadex LH-20, preparative TLC, preparative HPLC, and their structures were identified by physicochemical and spectral data. The antitumor activities of the isolated compounds on LA795 cells were also conducted. The compounds are identified as β-sitosterol(**1**), euphornin(**2**), euphornin D(**3**), euphohelioscopin A(**4**), quercetin(**5**), gallic acid(**6**), caffeic acid(**7**), ethyl gallate(**8**), myrecetin(**9**) and hyperoside(**10**). Compounds **7**, **8** and **9** are isolated from *Euphorbia helioscopia* L. for the first time and **6** and **10** were reported as the antitumor constituents of *Euphorbia helioscopia* L. for the first time.

Key words: *Euphorbia helioscopia* L.; Euphorbiaceae; chemical constituents; antitumor

泽漆是大戟属(*Euphorbia* L.)植物泽漆(*Euphorbia helioscopia* Linn)的干燥全草,别名猫儿眼睛草、五凤灵芝等,分布于除新疆、西藏以外的全国各地。长期以来泽漆一直作为民间草药,味苦,性微寒,有毒,归肺、小肠、大肠经。据《本草纲目》记载,泽漆有利水消肿、消痰退热、散结杀虫等功效。临床用于治疗腹水、水肿、肺结核、颈淋巴结核、痰多喘咳、癣疮,民间还用于治疗宫颈癌、食道癌等,并具有一定疗效,因此泽漆是一味很有研究开发价值的药材。目前,泽漆的临床应用逐渐增多,而对于其化学成分研究报道很少。为了进一步开发利用泽漆资源,阐明其有效成分,为泽漆入药提供合理依据,本实验对泽漆进行了系统化学成分提取分离。

1 仪器与材料

柱色谱硅胶(100～200目,200～300目)和薄层用硅胶 GF_{254}(青岛海洋化工厂);反相制备色谱柱(YMC-Pack SL);高效液相色谱仪: Pu-1580 intelligent HPLC Pump; RI-1530 intelligent RI Detector (JASCO); Sephadex LH-20 (Pharmacia Biotech);溶剂均为分析纯。Shimadzu UV-2501PC型紫外分光光度计,NOVA 500 MHz核磁共振波谱仪(TMS内标); TECAN A-5002 Spectra型酶标仪(奥地利); RPMI1640培养基(Gibco公司);胎牛血清(天津市庆星科技有限公司);噻唑蓝(MTT, Sigma公司);二甲基亚砜(DMSO, Sigma公司);泽漆药材购自安国美威中药材有限公司,由天津大学药学院高文远教授鉴定为大戟属植物泽漆(*Euphorbia helioscopia* L.)

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的全草。

2 提取分离

泽漆干燥全草 10 kg,粉碎,体积分数为 95%工业乙醇热回流提取 3次,提取液合并减压浓缩,浓缩后得浸膏,加适量水,分别用石油醚、乙酸乙酯、正丁醇萃取,依次得 180、100、60 g浸膏。石油醚部分经硅胶 (2. 8 kg, 100～200目)柱层析分离,以石油醚-乙酸乙酯系统 (50∶1, 20∶1, 10∶1, 5∶1, 3∶1, 2∶1, 1∶1, 1∶3)梯度洗脱,通过 TLC合并类似组分,经过硅胶柱色谱、凝胶渗透色谱以及制备高效液相色谱、制备薄层色谱纯化,分别得到化合物 **1** (615 mg), **2** (626 mg), **3** (30 mg), **4** (22 mg)。乙酸乙酯部分 100 g,以硅胶 (1. 5 kg, 100～200目)柱层析分离,以石油醚-乙酸乙酯 (4∶1, 3∶1, 2∶1, 1∶1, 1∶2),乙酸乙酯-甲醇 (20∶1, 10∶1, 5∶1)梯度洗脱,通过 TLC合并类似组分,经过硅胶柱色谱、凝胶渗透色谱以及制备高效液相色谱、制备薄层色谱纯化,分别得到化合物 **5** (10. 7 mg), **6** (35. 5 mg), **7** (18 mg), **8** (24. 6 mg), **9** (12. 2 mg), **10** (130 mg)。

3 结构鉴定

化合物 1 白色针状晶体, mp. 136～138 ℃(石油醚-乙酸乙酯)。Liebermann-Burchard 反应阳性。^{1}H NMR (500 MHz, $CDCl_3$) δ: 0. 68 (3H, s), 0. 81 (3H, s), 0. 83 (3H, d), 0. 84 (3H, d), 0. 92 (3H, d), 1. 01 (3H, s), 3. 52 (1H, m H-3), 5. 35 (1H, brs H-6)。上述数据与化合物 β-谷甾醇[1]数据一致。

化合物 2 无色针晶 (甲醇), UV λ_{max}^{MeOH} nm: 239, ESI-MS $[M + Na]^+$: 602. 2。^{1}H NMR (500 MHz, $CDCl_3$) δ: 0. 89 (3H, s), 0. 96 (6H, m), 0. 96 (3H, s), 1. 19 (3H, s), 1. 75 (3H, s), 1. 96 (3H, s), 2. 22 (3H, s)为 8个 CH_3 信号, 5. 09 (1H, dd), 5. 65 (1H, dd), 5. 74 (1H, dd)为双键上的质子信号, 7. 44 (2H, m), 7. 46 (1H, m), 8. 08 (2H, m)显示有苯环存在。^{13}C NMR (125 MHz, $CDCl_3$)波谱显示,该化合物存在 5个连氧的 C (δ 72. 9, 73. 5, 80. 7, 81. 0, 83. 8),两个双键 (δ 120. 1, 128. 7, 133. 8, 138. 3)。^{13}C NMR (125 MHz, $CDCl_3$) δ: 171. 2, 169. 6, 169. 0, 165. 6 (each -C=O), 138. 3 (C-5), 133. 8 (C-6), 132. 8 (C-4′), 130. 2 (C-1′), 129. 8 (×2, C-3′, 5′), 128. 7 (C-11), 128. 5 (×2, C-2′, 6′), 120. 1 (C-12), 83. 8 (C-15), 81. 0 (C-3), 80. 7 (C-7), 73. 5 (C-9), 72. 9 (C-14), 47. 9 (C-13), 46. 2 (C-8), 39. 7 (C-10), 39. 5 (C-4), 36. 7 (C-2), 32. 4 (C-1), 21. 0, 21. 1, 22. 6 (each for -CH_3), 20. 2 (C-20), 19. 9 (C-19), 19. 4 (C-18), 16. 2 (C-17), 13. 5 (C-16)。上述数据与化合物 Euphorin[2]数据一致。

化合物 3 无色油状物质 (甲醇)。^{1}H NMR (500 MHz, $CDCl_3$) δ: 0. 87 (3H, s), 0. 88 (3H, s), 0. 93 (3H, d), 1. 08 (3H, d), 1. 54 (3H, s), 1. 66 (3H, s), 1. 93 (3H, s), 2. 10 (3H, s), 2. 28 (3H, s)为 9个 CH_3 信号,与化合物 2比较,增加了一个 CH_3 信号,^{13}C NMR (125 MHz, $CDCl_3$)数据比较,增加了 22. 7和 169. 9两个 C信号,即多了一个乙酰基。^{13}C NMR (125 MHz, $CDCl_3$) δ: 170. 4, 169. 9, 169. 5, 169. 3, 166. 1 (each -C=O), 136. 0 (C-5), 132. 9 (C-6), 132. 6 (C-4′), 129. 4 (×2, C-3′, 5′), 129. 3 (C-1′), 129. 2 (C-11), 128. 3 (×2, C-2′, 6′), 121. 2 (C-12), 92. 1 (C-15), 83. 8 (C-3), 73. 9 (C-7), 73. 9 (C-9), 73. 1 (C-14), 43. 5 (C-13), 41. 8 (C-8), 39. 8 (C-10), 39. 6 (C-4), 36. 5 (C-2), 32. 3 (C-1), 21. 2, 21. 3, 22. 5, 22. 7 (each for -CH_3), 21. 1 (C-20), 20. 5 (C-19), 20. 2 (C-18), 17. 0 (C-17), 15. 7 (C-16)。上述数据与化合物 Euphorin D[2]数据一致。

化合物 4 无色油状物质 (甲醇)。^{1}H NMR (500 MHz, $CDCl_3$)波谱显示, 0. 91 (3H, s), 1. 07 (3H, s), 1. 09 (3H, s), 1. 16 (3H, s), 1. 56 (3H, s), 1. 65 (3H, s)为 6个 CH_3 信号, 1. 93 (3H, s)为 -OAc上的质子信号, ^{13}C NMR (125 MHz, $CDCl_3$)有三个羰基信号 δ: 194. 8, 169. 8, 167. 0,四个双键信号 δ: 145. 4, 143. 5, 142. 1, 140. 3, 133. 3, 126. 7, 121. 5, 121. 1。^{13}C NMR (125 MHz, $CDCl_3$) δ: 194. 8, 167. 0, 169. 8 (each C=O), 145. 4 (C-2′), 143. 5 (C-3′), 142. 1 (C-4′), 140. 3 (C-5′), 133. 4 (C-5), 126. 7 (C-12), 121. 5 (C-13), 121. 1 (C-6), 96. 5 (C-7), 81. 8 (C-3), 77. 0 (C-15), 41. 0 (C-11), 40. 8 (C-9), 33. 4 (C-4), 30. 5 (C-2), 30. 4 (C-8), 29. 8 (C-1), 29. 2 (C-6′), 24. 5 (C-7′), 22. 8 (-CH_3), 21. 8 (C-20), 19. 1 (C-10), 19. 0 (C-19), 18. 5 (C-18) 16. 4 (C-17), 12. 4 (C-8′)。上述数据与化合物 Euphohelioscopin A[2]数据一致。

化合物 5 黄色针晶 (甲醇), UV λ_{max}^{MeOH} nm: 376, 256。^{1}H NMR (500 MHz, C_5D_5N) δ: 8. 61 (1H, d, J = 2. 5 Hz, H-2′), 8. 11 (1H, dd, J = 8. 5, 2. 5 Hz, H-6′), 7. 38 (1H, d, J = 8. 5 Hz, H-5′), 6. 75 (1H, d, J = 2. 5 Hz, H-8), 6. 71 (1H, d, J = 2. 5 Hz, H-6); ^{13}C NMR

(125 MHz, C_5D_5N) δ: 150.5 (C-2), 138.2 (C-3), 177.6 (C-4), 157.8 (C-5), 99.5 (C-6), 165.8 (C-7), 94.6 (C-8), 162.7 (C-9), 104.8 (C-10), 123.9 (C-1′), 117.0 (C-2′), 148.0 (C-3′), 147.4 (C-4′), 117.0 (C-5′), 121.3 (C-6′)。**上述数据与化合物槲皮素**[3]**数据一致。**

化合物 6　白色针状结晶, ^{1}H NMR (500 MHz, CD_3OD) δ: 7.07 (2H, s, H-2, 6); ^{13}C NMR (125 MHz, CD_3OD) δ: 122.3 (C-1), 110.7 (C-2), 146.5 (C-3), 139.7 (C-4), 146.5 (C-5), 110.7 (C-6), 170.6 (C=O)。**上述数据与化合物没食子酸**[4]**数据一致。**

化合物 7　白色结晶 (甲醇), ^{1}H NMR (500 MHz, CD_3OD) δ: 7.51 (1H, d, *J* = 16 Hz, H-7), 7.03 (1H, dd, *J* = 3.0, 8.0 Hz, H-2), 6.94 (1H, dd, *J* = 3.0, 9.0 Hz, H-6), 6.76 (1H, d, *J* = 8.0 Hz, H-5), 6.19 (1H, d, *J* = 16 Hz, H-8); ^{13}C NMR (125 MHz, CD_3OD) δ: 128.1 (C-1), 115.3 (C-2), 146.9 (C-3), 149.5 (C-4), 116.7 (C-5), 122.9 (C-6), 147.1 (C-7), 115.8 (C-8), 171.2 (C-9)。**上述数据与化合物咖啡酸**[5]**数据一致。**

化合物 8　白色针状结晶 (MeOH), ^{1}H NMR (500 MHz, CD_3OD) δ: 7.04 (2H, s, Ar-H), 4.30 (2H, q, *J* = 7.1 Hz, $-OCH_2$), 1.35 (3H, t, *J* = 7.1 Hz, $-CH_3$); ^{13}C NMR (125 MHz, CD_3OD) δ: 122.1 (C-1), 110.3 (C-2), 146.6 (C-3), 139.8 (C-4), 146.6 (C-5), 110.3 (C-6), 168.7 (C=O), 61.8 ($-OCH_2$), 14.7 ($-CH_3$)。**上述数据与化合物没食子酸乙酯**[5]**数据一致。**

化合物 9　黄绿色粉末 ($CHCl_3$/MeOH 1∶1), ^{1}H NMR (500 MHz, DMSO-d_6) δ: 7.23 (2H, s, H-2′, 6′), 6.40 (1H, d, *J* = 2.1 Hz, H-6), 6.17 (1H, d, *J* = 2.1 Hz, H-8); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 146.8 (C-2), 135.8 (C-3), 175.7 (C-4), 160.7 (C-5), 98.1 (C-6), 163.9 (C-7), 93.2 (C-8), 156.1 (C-9), 102.9 (C-10), 120.8 (C-1′), 107.2 (C-2′), 145.7 (C-3′), 135.8 (C-4′), 145.7 (C-5′), 107.2 (C-6′)。**上述数据与化合物杨梅素**[6]**数据一致。**

化合物 10　淡黄色粉末,分子式为 $C_{21}H_{20}O_{12}$, UV λ_{max}^{MeOH} nm: 359, 257。^{1}H NMR (500 MHz, DMSO-d_6) δ: 7.66 (1H, d, *J* = 4.0 Hz, H-2′), 7.65 (1H, d, *J* = 8.0 Hz, H-6′), 6.81 (1H, d, *J* = 8.0 Hz, H-5′), 6.39 (1H, d, *J* = 4.0 Hz, H-8), 6.19 (1H, d, *J* = 4.0 Hz, H-6), 5.37 (1H, d, *J* = 8.0 Hz, H-1″); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 156.2 (C-2), 133.5 (C-3), 177.5 (C-4), 161.2 (C-5), 98.6 (C-6), 164.1 (C-7), 93.5 (C-8), 165.3 (C-9), 103.9 (C-10), 122.0 (C-1′), 116.0 (C-2′), 148.5 (C-3′), 144.8 (C-4′), 115.2 (C-5′), 121.1 (C-6′), 101.8 (C-1″), 71.2 (C-2″), 73.2 (C-3″), 67.9 (C-4″), 75.8 (C-5″), 60.1 (C-6″)。**上述数据与化合物金丝桃苷**[7]**数据一致。**

4　单体化合物的生物活性

参照文献[8]**方法,采用** MTT**法对部分单体化合物进行了体外抑制小鼠肺腺癌** LA795**细胞生长活性筛选,结果表明化合物 6、10在浓度为** 100 μg/mL **时抑制率较高,观察到细胞坏死性细胞毒活性 (见表** 1)。**化合物 1、2、8显示活性较低,其余化合物在本实验条件下未检测到活性。**

表 1　**泽漆中化合物对** LA795**细胞生长的抑制作用**

Table 1　Inhibitory effects of compounds from *E. helioscopia* L. on LA795 cell proliferation

化合物 Compound	细胞生长抑制率 Inhibitory rate on cell proliferation (%)	
	100 μg/mL	10 μg/mL
1	13.24	-
2	33.21	9.48
6	93.29	-
8	37.77	38.52
10	90.34	-

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有一个结合位点。

从硫酸粘菌素的紫外吸收光谱和 ALP溶液的荧光光谱比较,二者有重叠的部分。根据 Forster非辐射能量转移理论[10],可以认为 ALP内部的 Trp残基与硫酸粘菌素之间发生了能量转移作用,从而 ALP荧光发生淬灭。

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