

波棱瓜子化学成分的研究

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摘要: 目的 研究波棱瓜子 *Herpetospermum caudigerum* Wall 的化学成分。方法 波棱瓜子 95% 乙醇提取物的石油醚部位采用硅胶、Sephadex LH-20、制备薄层 TLC 和半制备 HPLC 进行分离纯化, 根据理化性质及波谱数据鉴定所得化合物的结构。结果 从中分离得到 9 个化合物, 分别鉴定为亚麻酸 (1)、亚油酸 (2)、9, 11, 15-十八碳三烯酸 (3)、甘油三亚油酸酯 (4)、9-十八碳烯酸 (5)、1-O-二十六烷酰基甘油酯 (6)、(+)- $(7'S, 7''S, 8'R, 8''R)-4, 4', 4''$ -trihydroxy-3, 5', 3''-trimethoxy-7-oxo-8-ene [8-3', 7'-O-9'', 8'-8'', 9'-O-7''] lignoid (7)、 α -菠甾醇 (8)、波棱甲素 (9)。结论 化合物 3、4、6、8 首次从该植物中分离得到。

关键词: 波棱瓜子; 化学成分; 分离鉴定

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Chemical constituents from *Herpetospermum caudigerum*

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ABSTRACT: AIM To study the chemical constituents from *Herpetospermum caudigerum* Wall. **METHODS**

The petroleum ether fraction of 95% ethanol extract from *H. caudigerum* was isolated and purified by silica, Sephadex LH-20, preparative TLC and semi-preparative HPLC, then the structures of obtained compounds were identified by physicochemical properties and spectral data. **RESULTS** Nine compounds were isolated and identified as linolenic acid (1), linoleic acid (2), 9, 11, 15-octadecatrienoic acid (3), trilinolein (4), 9-octadecenoic acid (5), 1-cerotoylglycerol (6), (+)- $(7'S, 7''S, 8'R, 8''R)-4, 4', 4''$ -trihydroxy-3, 5', 3''-trimethoxy-7-oxo-8-ene [8-3', 7'-O-9'', 8'-8'', 9'-O-7''] lignoid (7), α -spinasterol (8), herpetrione (9). **CONCLUSION** Compounds 3, 4, 6 and 8 are isolated from this plant for the first time.

KEY WORDS: *Herpetospermum caudigerum* Wall; chemical constituents; isolation and identification

波棱瓜子为葫芦科波棱瓜属一年生攀援草本植物波棱瓜 *Herpetospermum caudigerum* Wall 的干燥成熟种子, 收录于《中华人民共和国卫生部药品标准》藏药标准^[1], 主要分布在我国西藏、云南、四川等地及印度、尼泊尔等国。该药味苦、性寒, 具有泻肝火、清胆热的功效, 藏医临床常用其治疗黄疸型传染性肝炎、胆囊炎、消化不良等症。现代药理学研究表明波棱瓜子具有抗肝损伤^[2-3]、抗乙

肝病毒^[4]、抗疲劳^[5]、抗氧化^[6]等作用。课题组前期研究发现, 波棱瓜子 95% 乙醇提取物石油醚萃取部位对于 ANIT 诱发的胆汁淤积大鼠具有良好的保护作用, 其可能的药效活性成分为脂肪酸类^[7]。目前, 有关波棱瓜子石油醚萃取部位的研究, 主要是集中在其脂肪油成分的分析上^[8-9], 具体化学成分的分离研究报道很少, 课题组对其进行了化学成分研究, 从中分离得到 9 个化合

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物, 其中6个为有机酸类, 2个为木脂素类, 1个为甾醇类, 且化合物3、4、6、8首次从该植物中分离得到。

1 仪器与材料

1.1 仪器与试剂 HPLC液相色谱仪(日本岛津公司); Bruker Avance-500, 600型核磁共振光谱仪、Bruker DRX400型核磁共振仪(德国Bruker公司, TMS为内标); YMC-Pack ODS-A半制备柱, 250 mm×10 mm, 5 μm); Sephadex LH-20凝胶(美国GE公司); TLC板GF₂₅₄(德国默克尔公司); 制备TLC板和柱色谱硅胶(青岛海洋化工厂)。

1.2 材料 藏药波棱瓜药材于2013年6月自西藏林芝地区采集, 经西藏农牧学院动物科技系植物生物技术教研室兰小中教授鉴定为正品, 凭证标本(2013-CM-01)保存于西南大学药学院药物分析教研室。

2 提取与分离

干燥的成熟波棱瓜子27 kg, 粉碎, 室温下用95%乙醇提取3次, 合并提取液, 减压浓缩, 得2.1 kg总浸膏。将浸膏分散在3 L水中制成混悬液, 依次经过石油醚、乙酸乙酯、正丁醇萃取。减压浓缩得到石油醚萃取物1 500 g、乙酸乙酯萃取物175 g、正丁醇萃取物125 g。从石油醚萃取物中取出200 g样品, 经过正相硅胶色谱(2 000 g)分离, 以石油醚-乙酸乙酯(1:0~0:1)梯度洗脱, 得到25个组分(Fr.1~25)。Fr.2经过制备薄层TLC(石油醚:乙酸乙酯=95:5)得到化合物1(30 mg); Fr.6经过凝胶柱色谱得7个亚组份(Fr.6-1~7), Fr.6-3经过半制备HPLC, 流动相为甲醇-水(85:15)分离, 得化合物2(4 mg), Fr.6-3经过制备薄层TLC(石油醚:乙酸乙酯=9:1)得化合物3(7 mg); Fr.10经过硅胶柱色谱, 石油醚-乙酸乙酯体系(9:1~5:5)分离得到6个亚组份(Fr.10-1~6), Fr.10-2经过制备薄层TLC(石油醚:乙酸乙酯=85:15)分离得到化合物4(15 mg)、5(13 mg); Fr.12经过凝胶柱色谱分离得到5个亚组份(Fr.12-1~5), Fr.12-5经半制备HPLC, 流动相为甲醇-水(35:65)分离, 得到化合物7(5 mg); Fr.13洗脱流份中有白色结晶析出, 重结晶得到化合物6(30 mg); Fr.23经过硅胶柱色谱, 得到7个亚组份(Fr.23-1~7), Fr.23-5经半制备HPLC, 流动相为甲醇-水(36:64)分离, 得到化合物8(4 mg)、9(3 mg)。

3 结构鉴定

化合物1: 无色油状物, 分子式C₁₈H₃₀O₂。¹H-NMR(CDCl₃, 400 MHz) δ: 5.21~5.36(6H, m, H-9, 10, 12, 13, 15, 16), 2.74(4H, t, J=6.14 Hz, H-11, 14), 2.28(2H, t, J=7.50 Hz, H-2), 2.01(4H, m, H-8, 17), 1.57(2H, m, H-3), 1.19-1.25(8H, m, H-4, 5, 6, 7), 0.91(3H, t, J=7.52 Hz, H-18); ¹³C-NMR(CDCl₃, 100 MHz) δ: 178.23(C-1), 130.96(C-16), 129.24(C-9), 127.29(C-12), 127.25(C-13), 126.76(C-15), 126.12(C-10), 32.90(C-2), 19.54(C-4), 28.55(C-5), 28.12(C-6), 28.06(C-7), 28.02(C-8), 24.62(C-11), 26.19(C-14), 24.53(C-3), 23.66(C-17), 13.24(C-18)。以上数据与文献[10]基本一致, 故鉴定为亚麻酸。

化合物2: 黄色油状物, 分子式C₁₈H₃₂O₂。¹H-NMR(CDCl₃, 400 MHz) δ: 5.33~5.38(4H, m, H-9, 10, 12, 13), 2.77(2H, m, H-11), 2.23(2H, m, H-2), 2.05(4H, m, H-8, 14), 1.64(2H, m, H-3), 1.26~1.33(14H, m, H-4~7, H-15~17), 0.89(3H, t, J=6.90 Hz, H-18); ¹³C-NMR(CDCl₃, 100 MHz) δ: 175.45(C-1), 130.24(C-13), 130.04(C-9), 128.08(C-10), 127.92(C-12), 35.93(C-2), 31.54(C-16), 29.61(C-7), 29.36(C-15), 29.24(C-4), 29.21(C-5), 29.13(C-6), 27.22(C-8), 27.20(C-14), 25.65(C-11), 25.52(C-3), 22.58(C-17), 14.08(C-18)。以上数据与文献[10]基本一致, 故鉴定为亚油酸。

化合物3: 无色油状物, 分子式C₁₈H₃₀O₂。¹H-NMR(CDCl₃, 400 MHz) δ: 5.99~6.11(2H, m, H-10, 11), 5.60~5.69(4H, m, H-9, 12, 15, 16), 2.34(2H, t, J=7.52 Hz, H-2), 2.07(6H, m, H-8, 13, 14, 17), 1.62(2H, tt, J=7.26, 7.20 Hz, H-3), 1.30~1.38(8H, m, H-4, 5, 6, 7), 0.88(3H, t, J=7.13 Hz, Me-18); ¹³C-NMR(CDCl₃, 100 MHz) δ: 178.07(C-1), 133.49(C-16), 133.27(C-9), 129.89(C-12), 129.75(C-15), 129.53(C-11), 129.42(C-10), 32.85(C-2), 31.72(C-13), 27.92(C-14), 23.65(C-3), 28.06(C-4), 28.26(C-5), 30.51(C-6), 31.46(C-7), 27.98(C-8), 21.21(C-17), 12.90(C-18)。以上数据与文献[11]基本一致, 故鉴定为9, 11, 15-十八碳三烯酸。

化合物4: 无色油状液体, 分子式 $C_{57}H_{98}O_6$ 。 1H -NMR ($CDCl_3$, 400 MHz) δ : 5.24~5.40 (12H, overlap), 4.29 (2H, dd, $J=4.08, 11.88$ Hz), 4.14 (2H, dd, $J=5.94, 11.89$ Hz), 2.79 (2H, dt, $J=6.23, 11.31$ Hz), 2.31 (2H, dt, $J=2.83, 7.65$ Hz), 2.03 (2H, dt, $J=6.84, 12.81$ Hz), 1.27 (66H, overlap), 0.89 (9H, overlap); ^{13}C -NMR ($CDCl_3$, 100 MHz) δ : 173.46 (C-1), 173.46 (C-1''), 172.70 (C-1'), 34.03 (C-2), 34.03 (C-2''), 34.19 (C-2'), 24.8 (C-3), 24.8 (C-3''), 24.8 (C-3'), 29.1 (C-4), 29.1 (C-4''), 29.0 (C-4'), 29.19 (C-5), 29.19 (C-5''), 29.21 (C-5'), 29.13 (C-6), 29.13 (C-6''), 29.15 (C-6'), 29.63 (C-7), 29.63 (C-7''), 29.64 (C-7'), 27.22 (C-8), 27.22 (C-8''), 27.21 (C-8'), 130.22 (C-9), 130.22 (C-9''), 130.22 (C-12), 130.22 (C-12''), 130.01 (C-9'), 130.01 (C-12'), 129.46 (C-10), 129.46 (C-10''), 129.46 (C-10''), 129.46 (C-13), 129.46 (C-13''), 129.46 (C-13''), 27.21 (C-11), 27.21 (C-11''), 27.20 (C-11'), 29.37 (C-12), 29.37 (C-12''), 29.36 (C-13), 29.36 (C-13''), 29.36 (C-13''), 24.31 (C-14), 24.31 (C-14''), 24.31 (C-14''), 22.89 (C-15), 22.89 (C-15''), 22.89 (C-15''), 32.0 (C-16), 32.0 (C-16''), 32.0 (C-16''), 22.70 (C-17), 22.70 (C-17''), 22.70 (C-17''), 14.04 (C-18), 13.99 (C-18''), 13.85 (C-18''), 68.91 (CHO), 62.11 (CH_2O)。以上数据与文献 [12] 基本一致, 故鉴定为甘油三亚油酸酯。

化合物5: 白色粉末, 分子式 $C_{37}H_{70}O_5$ 。 1H -NMR ($CDCl_3$, 500 MHz) δ : 5.34 (2H, d, $J=4.47$ Hz), 4.17 (2H, td, $J=5.55, 11.50$ Hz), 3.69 (1H, dd, $J=4.21, 11.83$ Hz), 3.59 (1H, dd, $J=6.15, 11.89$ Hz), 2.34 (2H, t, $J=7.98$ Hz), 1.26 (48H, overlap), 0.87 (6H, t, $J=7.21$ Hz); ^{13}C -NMR ($CDCl_3$, 125 MHz) δ : 174.32 (C-1), 174.28 (C-1''), 130.01 (C-9), 129.67 (C-10), 70.28 (C-19), 65.12 (C-20), 63.38 (C-21), 34.16 (C-2), 34.14 (C-2''), 31.89 (C-16), 31.87 (C-14''), 29.06~29.74 (C-4, 5, 6, 7, 12, 13, 14, 15, 4', 5', 6', 7', 8', 9', 10', 11', 12', 13''), 27.20 (C-8), 27.14 (C-11), 24.90 (C-3), 24.88 (C-3''), 22.65 (C-17), 22.64 (C-15''), 14.05 (C-18), 14.05 (C-16'')。以上数据与文献 [13] 基本一致,

故鉴定为9-十八碳烯酸。

化合物6: 白色粉末, 分子式 $C_{29}H_{58}O_4$ 。 1H -NMR (Acetone- d_6 , 400 MHz) δ : 4.05 (1H, dd, $J=11.22, 6.28$ Hz), 3.82 (1H, d, $J=5.50$ Hz), 3.54 (2H, dd, $J=11.22, 6.28$ Hz, H-2), 2.98 (44H, brs, H-4'~25'), 0.88 (3H, m, 26'-Me); ^{13}C -NMR (Acetone- d_6 , 100 MHz) δ : 173.02 (C-1''), 69.84 (C-2), 65.28 (C-1), 63.00 (C-3), 33.66 (C-2''), 31.72 (C-24''), 29.52, 29.48, 28.37, 28.4 (C-4'~23'), 24.75 (C-3''), 22.41 (C-25''), 13.44 (C-26'')¹。以上数据与文献 [14] 基本一致, 故鉴定为1-O-二十六烷酰基甘油酯。

化合物7: 白色针状结晶 (甲醇), mp 122.0~25.0 °C, 分子式 $C_{30}H_{30}O_9$ 。 1H -NMR ($CDCl_3$, 400 MHz) δ : 7.57 (1H, d, $J=1.55$ Hz, H-2), 7.55 (1H, dd, $J=1.73, 8.24$ Hz, H-6), 6.90 (2H, m, H-2'', 6''), 6.89 (1H, d, $J=3.84$ Hz, H-2''), 6.88 (1H, d, $J=1.93$ Hz, H-6''), 6.82 (2H, dd, $J=8.21, 1.90$ Hz, H-5, 5''), 6.39 (1H, s, H-4), 6.07 (1H, s, H-4''), 6.02 (1H, d, $J=1.18$ Hz, H-9), 5.73 (1H, d, $J=1.11$ Hz, H-9''), 5.59 (1H, s, H-4''), 4.74 (2H, t, $J=4.92$ Hz, H-7', 7''), 4.25 (2H, ddd, $J=8.71, 6.85, 1.60$ Hz, H-9', 9''), 3.94 (3H, s, 3-OMe), 3.91 (3H, s, 5'-OMe), 3.88 (4H, m, 3''-OMe, H-9''), 3.11 (2H, m, H-8', 8''); ^{13}C -NMR ($CDCl_3$, 100 MHz) δ : 196.23 (C-7), 150.44 (C-4), 147.20 (C-5''), 146.73 (C-3), 146.44 (C-3''), 145.57 (C-4''), 145.29 (C-3''), 142.66 (C-4''), 132.93 (C-1''), 132.71 (C-1''), 129.48 (C-1), 126.12 (C-8), 124.86 (C-6), 123.98 (C-9), 119.27 (C-6''), 118.99 (C-6''), 114.28 (C-5), 113.58 (C-5''), 111.48 (C-2), 108.81 (C-2''), 108.61 (C-2''), 85.90 (C-7''), 85.82 (C-7'), 71.78 (C-9''), 71.72 (C-9''), 56.25 (C-2-OMe), 56.10 (C-5'-OMe), 56.00 (C-3''-OMe), 54.24 (C-8''), 54.19 (C-8'')¹。以上数据与文献 [15] 基本一致, 故鉴定为 (+)- $(7'S, 7''S, 8'R, 8''R)$ -4, 4', 4''-trihydroxy-3, 5', 3''-trimethoxy-7-oxo-8-ene [8-3', 7'-O-9'', 8'-8'', 9'-O-7''] lignoid。

化合物8: 白色结晶 (氯仿), mp 152.0~156.0 °C, 分子式 $C_{29}H_{48}O$ 。 1H -NMR ($CDCl_3$, 600 MHz) δ : 0.55 (3H, s, 18-CH₃), 0.80 (3H, s, 19-CH₃), 0.82 (3H, d, $J=7.40$ Hz, 26-CH₃), 0.83

(3H, t, $J=6.30$ Hz, 27-CH₃), 0.87 (3H, d, $J=6.28$ Hz, 29-CH₃), 1.03 (3H, d, $J=6.63$ Hz, 21-CH₃), 3.60 (1H, m, 3-H), 5.04 (1H, dd, $J=8.9, 8.6$ Hz, H-22 or 23), 5.16 (2H, dd, $J=8.6, 8.7$ Hz, 22 or 23-H); ¹³C-NMR (CDCl₃, 150 MHz) δ : 139.56 (C-8), 138.07 (C-22), 129.53 (C-23), 117.46 (C-7), 71.06 (C-3), 55.94 (C-17), 55.14 (C-14), 51.23 (C-24), 49.50 (C-9), 43.30 (C-13), 40.71 (C-5), 40.30 (C-12), 39.49 (C-12), 38.03 (C-2), 37.17 (C-1), 34.24 (C-10), 31.81 (C-25), 31.51 (C-4), 29.66 (C-6), 28.36 (C-16), 25.36 (C-28), 23.00 (C-15), 21.56 (C-11), 21.32 (C-21), 20.89 (C-26), 18.94 (C-27), 13.01 (C-19), 12.39 (C-29), 12.05 (C-18)。以上数据与文献[16]基本一致, 故鉴定为 α -波甾醇。

化合物9: 淡黄色粉末, 分子式C₃₀H₃₂O₁₀。¹H-NMR (CDCl₃, 400 MHz) δ : 7.28~7.61 (2H, m, H-2, 6), 6.76~6.89 (6H, m, H-2', 2'', 5, 5'', 6', 6''), 5.20 (1H, dd, $J=4.64, 8.12$ Hz, H-8), 4.68 (1H, d, $J=5.18$ Hz, H-7''), 4.60 (1H, d, $J=5.12$ Hz, H-7'), 4.14~4.26 (4H, m, H-9', 9''), 3.89 (6H, s, 3'', 3'-OCH₃), 3.88 (3H, s, 3-OCH₃), 2.93~3.07 (2H, m, H-8', 8''); ¹³C-NMR (CDCl₃, 100 MHz) δ : 199.06 (C-7), 150.60 (C-4), 146.97 (C-3''), 146.74 (C-3), 146.40 (C-3'), 145.27 (C-4''), 142.20 (C-4'), 132.93 (C-1''), 132.89 (C-1'), 128.97 (C-1), 124.22 (C-6), 122.35 (C-5''), 118.95 (C-6'), 118.14 (C-6''), 114.28 (C-5''), 114.06 (C-5), 110.66 (C-2''), 108.62 (C-2), 107.55 (C-2''), 85.83 (C-7''), 85.70 (C-7''), 71.69 (C-9''), 71.60 (C-9''), 63.91 (-CH₂OH), 56.11 (3-OCH₃), 55.98 (3'-OCH₃), 55.96 (3''-OCH₃), 54.22 (C-8''), 54.10 (C-8''), 47.94 (C-8)。以上数据与文献[17]基本一致, 故鉴定为波棱甲素。

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