this plant for the first time. The four examined compounds exhibited certain antifungal activities against six strains of fungi *in vitro*.

Key words *Pogostemon cablin* (Blanco) Benth.; flavonoids; antifungal activity

广藿香中的黄酮类化合物

张广文[1],马祥全[1],苏镜娱[1],曾陇海[1],王发松[2],杨得坡[2]⑳
(1. 中山大学化学与化工学院,广东 广州 510275; 2. 中山大学生命科学学院,广东 广州 510275)

摘 要:目的 对广藿香全草进行研究以筛选天然抗真菌新药。方法 用色谱技术进行分离,通过 IR, UV, MS, NMR(¹H, ¹³C, DEPT)分析以及与标准品对照的方法鉴定化合物的结构。利用培养基药物浓度稀释法进行体外抗菌实验。结果 利用抗菌活性追踪,从广藿香中分离得到 8个黄酮类化合物,鉴定为: 5羟基-3′, 7, 4′-三甲氧基二氢黄酮(Ⅰ); 5羟基-7, 4′-二甲氧基二氢黄酮(Ⅱ); 3, 5-二羟基-7, 4′-二甲氧基黄酮(Ⅲ); 5羟基-7, 3, 4′-三甲氧基黄酮(Ⅳ); 5-羟基-3, 7, 3′, 4′-四甲氧基黄酮(Ⅴ); 5, 4′-二羟基-3, 7, 3′-三甲氧基黄酮(Ⅵ); 5, 4′-二羟基-7-甲氧基黄酮(Ⅶ)和 3, 5, 7, 3′, 4′-五羟基黄酮(Ⅷ)。并对化合物Ⅰ,Ⅲ,Ⅴ,Ⅵ进行了体外抗真菌活性研究。结论 除Ⅴ和Ⅵ外,均为首次从该植物中分离得到。受测的 4个黄酮类化合物具有抗真菌活性。

关键词:广藿香;黄酮类;抗真菌活性
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Pogostemon cablin (Blanco) Benth. (广藿香) is widespread in southern China. The plant has been used as Chinese herbal medicine to remove dampness, relieve summer-heat, exterior syndrome, stop vomiting and stimulate the appetite. The essential oil of *P. cablin* collected by steam distillation was found to exhibit significant antifungal and antibacterial activities *in vitro* (MIC = 0.08~1.0 mg/L)[1]. Its alcoholic extract also showed some antifungal activites (MIC < 2 mg/L). Bioassay-guided fractionation of this active extract has led to the isolation and characterization of eight flavonoids as 5-hydroxy-7, 3′, 4′-trimethoxyflavanone (Ⅰ)[2], 5-hydroxy-7, 4′-dimethoxyflavanone (Ⅱ)[3], 3, 5,-dihydroxy-7, 4′-dimethoxyflavone (Ⅲ)[4], 5-hydroxy-3, 7, 4′-trimethoxyflavone (Ⅳ)[5], 5-hydroxy-3, 7, 3′, 4′-tetramethoxyflavone (Ⅴ)[6], 5, 4′-dihydroxy-3, 7, 3′-trimethoxyflavone (Ⅵ)[6], 5, 4′-dihydroxy-7-methoxyflavone (Ⅶ)[7], and 3, 5, 7, 3′, 4′-pentahydroxyflavone (Ⅷ)[8]. We report herein on the structural elucidation of the isolated compounds and preliminary screening of the antifungal activities of compounds Ⅰ, Ⅲ, Ⅴ, and Ⅵ.

Results and discussion

Compound Ⅰ was crystalized from acetone as colorless needles. The IR spectrum showed absorptions typical of carbonyl groups, and benzene ring, and the UV spectrum was typical of a flavonoid. The ^{1}HNMR spectrum showed the presence of three methoxy groups (δ 3.81, 3.90 and 3.92), and a downfield signal (δ 12.01) due to a hydrogenbonded hydroxy group at 5 position. The aromatic protons of meta-coupled doublets (J = 2.5 Hz) at δ 6.06 and 6.08 corresponded to 6, 8 protons of ring A, three more aromatic protons at δ 6.90, 6.98 and 6.99 showed the presence of a para-substituted aromatic ring. The ^{13}CNMR spectrum showed signals of four oxygenated aromatic carbons (δ 164.2, 168.0, 149.3 and 149.6) and one carbonyl carbon (δ 195.9). These assignment were further confirmed by the DEPT spectrum which indicated the presence of one methylene, three methoxy, and six methylidyne. And with the positive-ion FABMS showed a quasimolecular peak $[M+H]^+$ at 331, the molecular formula was determined as $C_{18}H_{18}O_6$. All these data supported that the structure of flavanone Ⅰ is 5-hydroxy-7,

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作者简介:张广文(1975-),男,中山大学化学与化工学院在读直博生,现从事天然药物研究。

3′, 4′-trimethoxyflavanone (Ⅰ)[2]. Compounds Ⅲ~Ⅵ were determined as flavone from its similar spectral data (IR, ^{1}H, ^{13}C and DEPT) to those of compound Ⅰ except without the signals at δ 2.81 and 3.11 in ^{1}HNMR, but with an additional peak of methoxy or hydroxy at position 3. All the formulae were determined by the aid of FABMS. Compounds Ⅶ, Ⅷ were confirmed by comparing with their ^{13}CNMR spectra reported in literature.

Chemical structural formulae of compound Ⅰ~Ⅷ were shown in Fig 1.

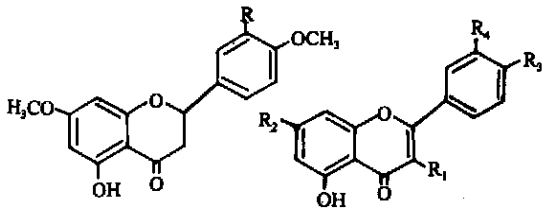

Fig. 1 The chemical structure of compounds Ⅰ~Ⅷ

Experimental section

General experimental procedures. Melting points were determined on a 5X micromelting point apparatus and were uncorrected. UV spectra were obtained on a Rayleigh UV-1200 spectrophotometer in MeOH. IR spectra were recorded on a Bruker EQUINOX-55 infrared spectrophotometer with KBr pellets. FABMS were measured on a VG ZAB-HS mass spectrometer. NMR (^{1}H, ^{13}C, DEPT) spectra were obtained on Varian Unity IN-OVA-500 spectrometer with $CDCl_3$ or DMSO-d_6 as solvent and TMS as internal standard. The chemical shifts are expressed on the δ scale. TLC analyses was performed on precoated Silica gel 60 F_{254} plates (Qingdao Haiyang Chemical Group CO. of China). Silica gel 60 H (particle size, 38 μm, Qingdao) was used for vacuum liquid chromatography (VLC) and preparative TLC. Spots on the plate were observed under UV_{254} light and visualized by spraying with vanillin-H_2SO_4 followed by heating. All solvents were AR reagents purchased from Guangzhou Chemical Reagent Factory.

Plant material. The plant *P. cablin* were collected in Wuzhou, Guangxi autonomous region, PRC in 1999.

Extraction and isolation. The whole plant (2 kg) was extracted three times with 95% EtOH at room temperature. Evaporation of the EtOH in vacuo gave a gum (71.8 g) which was partitioned between petroleum ether (60℃~90℃) and 90% MeOH-H_2O. The MeOH-soluble portion was further partitioned between $CHCl_3$ and 65% MeOH-H_2O. Each fraction was submitted to a bioassay against fungi: *Crytococus neoformans*, *Candida albicans*, *Mucor globosus*, *Chaetomium globosum*, *Rhizopus nigricans* and *Scopulariopsis brevicaulis*. From this bioassay, the $CHCl_3$ extract (15.2 g) was found to have antifungal activities (MIC < 2.0 mg/L). Bioassay-guided fractionation of the $CHCl_3$ extract (15.2 g) was subjected to VLC using a step gradient of petroleum ether (60℃~90℃) and EtOAc to yield Ⅰ (39 mg), Ⅱ (6 mg), Ⅲ (21 mg), Ⅳ (12 mg), Ⅴ (70 mg),), and Ⅵ (25 mg). Fraction 17 (56 mg) eluted with 30% Pet-EtOAc was further purified by preparative TLC (eluent 20% MeOH-$CHCl_3$) to yield Ⅶ (8 mg), and Ⅷ (5 mg).

5-Hydroxy-7, 3′, 4′-trimethoxyflavanone (Ⅰ)[1]: colorless needles, mp 153℃~154℃; UV (MeOH) λ_{max} (log ε) nm: 228 (sh, 4.41), 286 (4.30), 333 (3.52); IR (KBr) ν_{max} cm^{-1}: 1 638, 1 517, 1 265, 1 158, 1 026; FABMS m/z 331 (100) [M+H]$^+$; ^{1}HNMR ($CDCl_3$) δ 2.81 (1H, dd, J= 3.5, 17 Hz, Heq-3), 3.11 (1H, dd, J= 12.5, 16.5 Hz, Hax-3), 3.81, 3.90, 3.92 (all 3H, s, OCH_3), 5.36 (1H, dd, J= 3.5, 13 Hz, H-2), 6.06 (1H, d, J= 2.0 Hz, H-6), 6.08 (1H, d, J= 2.5 Hz, H-8), 6.90 (1H, dd, J= 1.5, 7.0 Hz, H-5′), 6.98 (1H, dd, J= 2.75 Hz, H-6′), 6.99 (1H, d, J= 2 Hz, H-2′), 12.01 (1H, s, 5-OH); ^{13}CNMR ($CDCl_3$) δ 43.4 (C-3), 55.7 (7-OCH_3), 56.0 (3′, 4′-OCH_3), 79.2 (C-2), 94.3 (C-8), 95.1 (C-6), 103.2 (C-10), 109.4 (C-5′), 111.2 (C-2), 118.8 (C-6′), 130.8 (C-1′), 149.3 (C-3′), 149.6 (C-4′), 162.8 (C-9), 164.2 (C-5), 168.0 (C-7), 195.9 (C=O).

5-Hydroxy-7, 4′-dimethoxyflavanone (Ⅱ): colorless needles; mp 115 ℃ ~ 116 ℃; it was identified by comparing various spectral data with those reported in the literature.

3, 5,-Dihydroxy-7, 4′-dimethoxyflavone (Ⅲ): yellow needles, mp 184 ℃ ~ 186 ℃; IR (KBr) ν_{max} cm^{-1}: 3 437, 1 661, 1 596, 1 497, 1 348, 1 210; FABMS m/z 315 (50) [M+ H]$^+$; ^{1}HNMR ($CDCl_3$) δ 3.89, 3.90 (all 3H, s, OCH_3), 6.37 (1H, d, J= 2.5 Hz, H-6), 6.49 (1H, d, J= 2.5 Hz, H-8), 6.58 (1H, s, 3-OH), 7.03 (2H, d, J= 9 Hz, H-3′, 5′), 8.17 (2H, d, J= 9 Hz, H-2′, 6′), 11.76 (1H, s, 5-OH); ^{13}CNMR ($CDCl_3$) δ 55.4, 55.8 (4, 7-OCH_3), 92.2 (C-8), 97.9 (C-6), 104.0 (C-10), 114.1 (C-3′, 5′), 123.2 (C-1), 129.4 (C-2′, 6′), 135.7 (C-3), 145.7 (C-2), 156.9 (C-5), 160.9 (C-4′), 161.2 (C-9), 165.8 (C-7), 175.2 (C-O).

5-Hydroxy-3, 7, 4′-trimethoxyflavone (Ⅳ): yellow needles, mp 146 ℃ ~ 148 ℃; ^{1}HNMR ($CDCl_3$) δ 3.86, 3.88, 3.90 (all 3H, s, OCH_3), 6.36 (1H, d, J= 2.5 Hz, H-6), 6.45 (1H, d, J= 2 Hz, H-8), 7.02 (2H, d, J= 8 Hz, H-3′, 5′), 8.08 (2H, d, J= 8.5 Hz, H-2′, 6′), 12.72 (1H, s, 5-OH).

5-Hydroxy-3, 7, 3′, 4′-tetramethoxyflavone (Ⅴ): yellow needles, mp 162 ℃ ~ 164 ℃; UV (MeOH) λ_{max} (log ε) nm: 254 (4.53), 268 (4.46′), 351 (4.50); IR (KBr) ν_{max} cm^{-1}: 3 426, 1 656, 1 594, 1 512, 1 433, 1 380, 1 324, , 1 272, 1 253, 1 235, 1 211, 1 192, 1 159, 823; FABMS m/z 359 (75) [M+ H]$^+$; ^{1}HNMR ($CDCl_3$) δ 3.87, 3.88, 3.97, 3.98 (all 3H, s, OCH_3), 6.35 (1H, d, J= 2.5 Hz, H-6), 6.44 (1H, d, J= 2 Hz, H-8), 6.99 (1H, d, J= 8 Hz, H-5′), 7.69 (1H, d, J= 2.5 Hz, H-2′), 7.73 (1H, dd, J= 2, 8.5 Hz, H-6′).

5, 4′-Dihydroxy-3, 7, 3′-trimethoxyflavone (Ⅵ): yellow needles, mp 171 ℃ ~ 173 ℃; IR (KBr) ν_{max} cm^{-1}: 3 312, 1 650, 1 595, 1 506, 1 350, 1 159, 833; FABMS m/z 345 (100) [M+ H]$^+$; ^{1}HNMR ($CDCl_3$) δ 3.86, 3.88, 3.90 (all 3H, s, OCH_3), 6.00 (1H, s, 4′-OH), 6.36 (1H, d, J= 2 Hz, H-6), 6.44 (1H, d, J= 2.5 Hz, H-8), 7.04 (1H, d, J= 8 Hz, H-5′), 7.67 (1H, dd, J= 2, 8 Hz, H-6′), 7.70 (1H, d, J= 2 Hz, H-2′), 12.61 (1H, s, 5-OH); ^{13}CNMR ($CDCl_3$) δ 55.8, 56.1, 60.2 (3, 7, 3′-OCH_3), 92.2 (C-8), 97.8 (C-6), 106.0 (C-10), 110.9 (C-2′), 114.6 (C-5′), 122.5 (C-1′), 122.7 (C-6′), 138.9 (C-3), 146.3 (C-3′), 148.3 (C-4′), 155.9 (C-2), 156.7 (C-5), 162.1 (C-9), 165.4 (C-7), 178.7 (C= O).

5, 4′-Dihydroxy-7-methoxyflavone (Ⅶ): yellow amorphous powder, mp 286 ℃ ~ 288 ℃; ^{1}HNMR (DMSO-d_6) δ 3.87 (3H, s, OCH_3), 6.34 (1H, d, J= 2.5 Hz, H-6), 6.68 (1H, d, J= 2.5 Hz, H-8), 6.74 (1H, s, H-3), 6.89 (2H, d, J= 9 Hz, H-3′, 5′), 7.91 (2H, d, J= 9 Hz, H-2′, 6′), 12.93 (1H, s, 5-OH); ^{13}CNMR (DMSO-d_6) δ 55.8 (7-OCH_3), 91.8 (C-8), 97.3 (C-6), 102.7 (C-3), 103.9 (C-10), 115.8 (C-3′, 5′), 121.1 (C-1′), 128.3 (C-2′, 6′), 157.2 (C-5), 161.0 (C-9), 161.3 (C-4′), 164.0 (C-2), 164.8 (C-7), 181.6 (C= O).

3, 5, 7, 3′, 4′-Pentahydroxyflavone (Ⅷ): yellow amorphous powder, mp> 300 ℃; ^{13}CNMR (DMSO-d_6) δ 93.8 (C-8), 98.7 (C-6), 103.6 (C-10), 115.2 (C-2′), 115.4 (C-5′), 119.9 (C-6′), 121.8 (C-1′), 135.9 (C-3), 145.0 (C-3′), 147.2 (C-2), 147.7 (C-4′), 156.0 (C-5), 156.0 (C-9), 163.9 (C-7), 175.8 (C= O).

Antifungal assays. The test organisms were *Crytococus neoformans*, *Candida albicans*, *Mucor globosus* (AS 3.349), *Chaetomium globosum* (AS 3.963), *Rhizopus nigricans* (AS 3.31) and *Sporotariopsis brevicaulis*.

Antifungal assays were carried out by the doubling dilutions method using a modified procedure. Fungi suspensions were obtained from 5 ~ 10 days cultured in Sabouraud agar to ca. 10^5 ~ 10^7 cells/mL in fresh sterile water. The four pure compounds were dissolved in EtOH or DMSO to 10 mg/mL as stock solutions. The required amount of stock solutions was pipetted into 50 ℃ sterile Sabluraud agar in order to obtain 1.0 mg/mL solutions. After solidified and inoculated, the culture plates were kept in a thermotank, and cultured at their respective temperatures for 48 h (see table 1, below).

Table 1 Antifungal activities of compounds Ⅰ, Ⅲ, Ⅴ **and** Ⅵ

Fungal strain (culture temperature and hour)	Ⅰ	Ⅲ	Ⅴ	Ⅵ
Crytococus neoforma (37℃, 48 h)	-	-	-	+
Candida albicans (37℃, 48 h)	-	+	+	+
Mucor globosu (26℃, 48 h)	+	-	+	+
Chaetomium globosum (26℃, 48 h)	+	+	+	+
Rhizopus nigricans (26℃, 24 h)	-	-	+	-
Scopulariopsis brevicattlis (26℃, 48 h)	-	-	+	+

Inhibitory concentration was defined as follows: - = less than 1.0 mg/mL; + = more than 1.0 mg/mL.

Inoculation methods were performed as described in the literature[1]

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木莓根部化学成分的研究

赵 卫权, 王 生,王明奎[20]

(中国科学院成都生物研究所,四川 成都 610041)

摘 要: 目的 研究木莓根部的化学成分。方法 采用正、反相硅胶柱层析分离纯化,通过理化性质和光谱分析鉴定其化学结构。结果 从木莓根部甲醇提取物中分离并鉴定了 10个化合物,它们分别为: 胡萝卜苷 (Ⅰ)、乌苏酸 (Ⅱ)、3β, 19α-二羟基-2-氧-乌苏-12-烯-28-酸 (Ⅲ)、2α, 3β, 23α-三羟基乌苏-12, 18-二烯-28-酸 (Ⅳ)、2α, 3β, 23α-三羟基乌苏-12, 19-二烯-28-酸 (Ⅴ)、2α, 3β, 19α, 23α-四羟基齐墩果-12-烯-28-酸 (Ⅵ)、2α, 3β-二羟基乌苏-12, 18-二烯-28-酸 (Ⅶ)、2α, 3β, 19α-三羟基乌苏-12-烯-28-酸 (Ⅷ)、2α, 3β, 19α-三羟基齐墩果-12-烯-28-酸 (Ⅸ)和蔷薇酸 (Ⅹ)。结论 以上成分均为首次从该植物中分得。

关键词: 木莓;悬钩子属;三萜

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Studies on chemical constituents in radicular part of *Rubus swinhoei*

ZHAO Wei-quan, DING Li-sheng, WANG Ming-kui

(Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu Sichuan 610041, China)

Key words *Rubus swinhoei* Hance; *Rubus* L.; triterpenoids

木莓 *Rubus swinhoei* Hance系蔷薇科悬钩子属植物,主要分布在陕西、江苏、安徽、浙江、福建、湖北、湖南、台湾、广东、广西、四川、云南等。其根有凉血止血、活血调经、收敛解毒之功效,用于牙痛、疮漏、疔肿疮肿、月经不调等症状[1],但其药用成分未见报道。为探索该植物的有效成分,揭示其生理活性物质,使之在中医临床上应用更为广泛,并为悬钩子属药用植物活性成分的研究以及深层次开发提供基础资料,本文对木莓根部甲醇提取物的化学成分进行了初步研究,结果显示其中主要成分为三萜类化合物和鞣质。我们已经报道了其中一个结构新颖的三萜成分木莓酸的结构[2]。本文继续报道其余的 10个成分。它们分别是: 胡萝卜苷 (daucosterol, Ⅰ)、乌苏酸 (ursolic acid, Ⅱ)、3β, 19α-二羟基-2-氧-乌苏-12-烯-28-酸 (2-oxo-pomolic acid, Ⅲ)、2α, 3β, 23α-三羟基乌苏-12, 18-二烯-28-酸 (pinfaenoic acid,

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作者简介: 赵卫权 (1975-),男,江苏六合人,助理工程师,硕士。1997年 6月毕业于南京大学生物系获学士学位, 2000年于中科院成都生物研究所获硕士学位,研究方向为植物化学。现在成都军区防化技术大队工作。Tel (028) 6684044 E-mail: weiquanzhao@163.com