文章编号:1001-6880(2014)1-0011-05

秀丽莓茎中酚类成分研究

刁玉林[1],热增才旦[1,2],王如峰[1],刘 斌[1*]

[1]北京中医药大学中药学院,北京 100102;[2] 青海大学医学院中藏药研究中心,西宁 810001

摘 要:秀丽莓(*Rubus amabilis* Focke)为蔷薇科悬钩子属植物,系常用藏药,已有 1300 多年历史。本实验从其茎中分离了 8 个化合物,通过波谱法和理化性质分别鉴定为:4-乙酰氧基-3-甲氧基苯甲酸(**1**)、2-*O*-*β*-D-吡喃葡萄糖基-6-羟基苯甲酸(**2**)、香草酸(**3**)、白藜芦醇(**4**)、2,6-二甲氧基-4-羟苯基-1-*O*-*β*-D-吡喃葡萄糖苷(**5**)、反式-4-羟基桂皮酸(**6**)、咖啡酸(**7**)及没食子酸(**8**)。其中 **1** 为新天然产物,化合物 **2** ~ **7** 均为首次从该植物中分离得到。

关键词:秀丽莓;悬钩子属;酚类;结构解析

中图分类号:R932 **文献标识码**:A

Phenolic Compounds from Stems of *Rubus amabilis* Focke

DIAO Yu-lin [1], RE-ZENG Cai-dan [1,2], WANG Ru-feng [1], LIU Bin [1*]

[1] *School of Traditional Chinese Medicine, Beijing University of Chinese Medicine, Beijing 100102, P. R. China*;

[2] *The Research Center of Chinese and Tibetan Medicine, Medicine College of Qinghai University, Xining 810001, P. R. China*

Abstract: The constituents of *Rubus amabilis* were isolated and purified by silica gel column chromatography, Sephadex LH-20, macroporous adsorption resin and TLC. The structures of the purified compounds were elucidated on the basis of spectral data and physiochemical properties. Eight compounds were isolated and their structures were identified as: 4-acetoxy-3-methoxybenzoic acid (**1**), 2-(*β*-D-glucopyranosyloxy)-6-hydroxybenzoic acid (**2**), vanillic acid (**3**), resveratrol (**4**), 2, 6-dimethoxy-4-hydroxyphenol-1-*O*-*β*-D-glucopyranoside (**5**), *trans*-4-hydroxy cinnamic acid (**6**), caffeic acid (**7**) and gallic acid (**8**). Compound **1** was a new natural product. Compounds **2-7** were isolated from *Rubus amabilis* for the first time.

Key words: *Rubus amabilis* Focke; *Rosaceae*; phenolic compounds; structural elucidation

Introduction

Rubus, belonging to *Rosaceae*, is a large genus with more than 750 known plants distributed all over the world and mainly concentrated in North America and East Asia. More than 200 different plants of this genus grow in the mainland of China, especially in southwestern China [1]. Modern pharmacological study showed its antibacterial [2], anti-inflammatory [3], anti-neoplastic [4], anti-allergic [5], anti-oxidative [6], anti-aging, free radical scavenging [7], hepatic protective [8], analgesic [9], and hypolipidemic activities [10]. The shortage of further chemical basis research restricted the development of this plant. The present article described the isolation and structural elucidation of a new natural product named 4-acetoxy-3-methoxybenzoic acid (**1**), as well as 7 known phenol derivatives, *viz*, 2-(*β*-D-glucopyranosyloxy)-6-hydroxybenzoic acid (**2**), vanillic acid (**3**), resveratrol (**4**), 2, 6-dimethoxy-4-hydroxyphenol-1-*O*-*β*-D-glucopyranoside (**5**), *trans*-4-hydroxy cinnamic acid (**6**), caffeic acid (**7**) firstly obtained from the stems of *R. amabilis* and gallic acid (**8**). (Fig. 1)

Received: April 17, 2013 Accepted: September 5, 2013

Foundation Item: This work was financially supported by the National Natural Science Foundation Project (No. 81260684) and the Research Fund for self-selected Topic of Beijing University of Chinese Medicine (No. 2013-jybzz-xs-113).

* Corresponding author Tel: 86-10-84738629; E-mail: liubinyn67@163.com

Materials and Methods

General experimental procedures

UV spectra were obtained on a UV 210A Shimadzu spectrometer. ^{1}H and ^{13}C NMR spectra were recorded in deuterated solvent with Bruker DRX-500 spectrometers

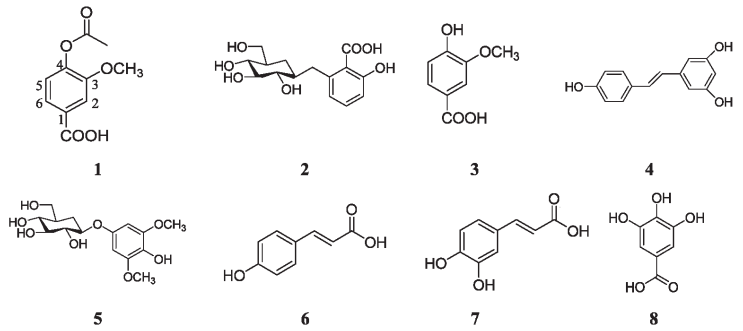

Fig. 1 Chemical structures of phenol constituents (1-8) isolated from the stems of *R. amabilis*

operating at 500 MHz for ^{1}H NMR experiments, and 125 MHz for ^{13}C NMR experiment, respectively. Coupling constants were expressed in Hertz (Hz) and chemical shifts were given on a δ (ppm) scale with tetramethylsilane as internal standard. Negative ion ESI-MS and HR-ESI-MS were recorded on a VG-20-250 and VG-ZAB-HS spectrometer (VG, Manchester, UK). Column chromatography separations were performed using Diaion HP 2MGL (Mitsubishi Chem, Beijing, China), AB-8 (The Chemical Plant of NanKai University, Tianjin, China) and Silica gel (Qingdao Haiyang Chemical Co., Qingdao, China) as stationary phase. TLC was carried on silica gel G precoated plates (Qingdao Haiyang Chemical Co., Qingdao, China). The TLC plate was monitored by spraying with 10% H_2SO_4 solution in ethanol followed by heating.

Fungal material

The dried stems of *R. amabilis* were collected from Huzhubeishan in Qinghai, China and identified by Prof. Chunsheng Liu (Beijing University of Chinese Medicines). An authentic sample was kept in School of Chinese Pharmacy, Beijing University of Chinese Medicines.

Extraction and isolation

The dried stems of *R. amabilis* (10.0 and 15.0 Kg) were powdered and extracted exhaustively with 70% EtOH and water, respectively, under reflux. The extract (extracted with 70% EtOH) was concentrated to the small volume (1 g crude herbal per mL), and applied on extraction with petroleum ether, chloroform, ethyl acetate, *n*-butanol and water. The ethyl acetate fraction was concentrated under reduced pressure, and the residue (200 g) was subjected to column chromatography (CC, 20 × 100 cm) on macroporous adsorption resin gradient eluted with $CHCl_3$-MeOH to obtain two fractions (Fraction 1: $CHCl_3$-MeOH = 45∶55-30∶70 and Fraction 2: $CHCl_3$-MeOH = 25∶75-10∶90). Fraction 1, was further fractionated on silica gel gradient eluted with $CHCl_3$-MeOH-H_2O (9∶1∶0.1, 8.5∶1.5∶0.1, 8∶2∶0.2, 7.5∶2.5∶0.3, 7∶3∶0.5, 6.5∶3.5∶0.8, 6∶4∶1 and 5∶5∶1), and ODS eluted with a step gradient of H_2O-MeOH (1∶0-0∶1) to give **2** (10 mg) and **5** (10 mg). Fraction 2 was fractionated repeatedly on silica gel gradient eluted with $CHCl_3$-CH_3OH (10∶1-0∶1), to obtain **4** (20 mg) from Fr 2. The residue (300 g) extracted with water and fractioned with ethyl acetate was subjected was subjected to CC (10 × 150cm) on silica gel ($CHCl_3$-MeOH, 1∶0-0∶1), and recrystallized to obtain **1** (20 mg), **3** (50 mg), **6** (40 mg), **7** (15mg) and **8** (37 mg).

Structural identification

4-acetoxy-3-methoxybenzoic acid (1) was obtained as a pale white crystal (MeOH). Bromocresol green reaction showed yellow points in TLC indicated carboxyl contained in the structure. UV (MeOH) λmax (log ε): 246sh (2.83) nm. Its mass spectrum showed a molecular ion peak at *m/z* 211 ($[M+H]^+$) consistent with the molecular formula $C_{10}H_{10}O_5$ elucidated from ^{1}H NMR, ^{13}C NMR, HSQC and HMBC results. The ^{1}H NMR spectrum of **1** displayed characteristic signals for a substituted aromatic ring in the form of ABX system at δ 7.44 (1H, d, J = 1.5 Hz, H-2), 7.34 (1H, dd, J = 8.0, 1.5 Hz, H-6) and 6.72 (1H, d, J = 7.8 Hz, H-5). Two three-proton signals as a singlet at δ 1.76 (3H, s) and 3.50 (3H, s) were attribu-

ted to the protons of methyl and methoxyl, respectively. The ^{13}C NMR spectrum of 1 exhibited signals for a aromatic ring at δ_C 113.3 (C-2), 114.2 (C-5), 122.5 (C-6), 129.5 (C-1), 146.4 (C-3) and 148.3 (C-4). The carbonyl signals appeared at δ_C 170.0 and 173.8, respectively, and one of them might be assigned to an ester carbonyl group. In addition, the signals at δ_C 23.4 and 55.4 were assigned to -CH_3 and -OCH_3 carbons, respectively. Based on above evidence, two possible similar structures could be deduced as shown in Fig. 2.

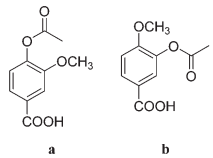

Fig. 2 The possible structures of compound 1: (a) 4-acetoxy-3-methoxybenzoic acid; (b) 3-acetoxy-4-methoxybenzoic acid.

The HMBC spectrum of **1** showed correlations of C-4 with δ_H 7.44, 7.34 and 6.72, and -OCH_3 with δ_C 146.4 and 148.3. This demonstrated that the structure of this compound should be structure **a** because there would be no correlation of δ_H 7.34 (H-6) with δ_C 148.3 observed in structure **b**. This structure was further confirmed by the stronger correlation signal of δ_H 3.75 (-OCH_3) with δ_C 146.4 (Fig. 3). Additionally, the isolation of compound **3** could further prove the given structure. Finally, the spectra data were given in Table 1.

Table 1 ^{1}H NMR (500 MHz, DMSO-d_6), ^{13}C NMR (125 MHz, DMSO-d_6)* and HMBC data of compound 1

Position	δ_H (J, Hz)	δ_C	HMBC
1	–	129.5	–
2	7.44 (1H, d, 1.5)	113.3	C-4, 6
3	–	146.4	–
4	–	148.3	–
5	6.72 (1H, d, 7.8)	114.2	C-1, 3, 4
6	7.34 (1H, dd, 8.0, 1.5)	122.5	C-2, 4
C=O of -COOH	–	173.8	–
C=O of -$COCH_3$	–	170.0	–
-OCH_3	3.50 (3H, s)	55.4	C-3, 4
-CH_3	1.76 (3H, s)	23.4	–

* Assignments were confirmed by HSQC and HMBC experiments

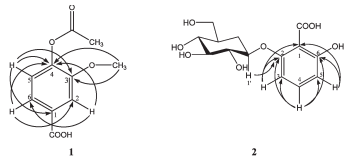

Fig. 3 Selective HMBC correlations for 1 and 2 (H→C)

2-(β-D-glucopyranosyloxy)-6-hydroxybenzoic acid (2) was obtained as yellowish powder (MeOH) with positive $FeCl_3$ and Molish reaction results, which indicated this compound may be a phenolic glycoside. The ESI-MS (m/z 315 [M-H]$^-$, 653 [2M + Na]$^-$) in combination with HR-ESI-MS (m/z): 315.0714 [M-H]$^-$, calcd 315.0716 revealed a molecular formula $C_{14}H_{18}O_8$, suggesting the presence of six degrees of unsaturation. In the ^{1}H NMR spectrum of 2, three downfield peaks at δ_H 6.64 (1H, d, J = 7.8 Hz), 7.07 (1H, t, J = 7.8 Hz) and 6.43 (1H, d, J = 7.8 Hz) were attributed to H-3, H-4 and H-5 in an ABC spin coupling system, respectively. One single-proton signal at δ 4.43 with a coupling constant of 7.2 Hz showed the existence of a β oriented glucosyl group. Five multiplets at δ_H 3.25-3.74 were assignable to the glucosyl group.

The ^{13}C NMR spectrum of **2** exhibited the presence of aromatic carbons at δ_C 112.0 (C-1), 160.0 (C-2), 108.9 (C-3), 131.3 (C-4), 112.1 (C-5) and 164.0 (C-6), carbonyl carbon at δ_C 170.9 (C=O), anomeric carbon of the glucosyl group at δ_C 106.5 (C-1′) and the remaining five carbons of glucosyl group at between δ_C 61.5 and 78.0. The HMBC spectrum of **2** showed correlations of H-1′ with δ_C 160.0, 78.0 and 76.3, which indicated that the glucosyl group linked the carbon at δ_C 160.0 of the aromatic ring to form glycoside. The HMBC correlations showed the linkages as illustrated in Fig. 3.

The ^{1}H-^{1}H COSY spectrum of **2** showed two sets of optional spin coupling proton correlation signals (δ_H 4-H/3-H and 4-H/5-H) in the aromatic ring, which confirmed the structure given above. Complete ^{1}H and ^{13}C NMR assignments were accomplished by a combination of 2D NMR techniques, including HMQC, HMBC, and ^{1}H-^{1}H COSY.

The spectra data were given as follows: ^{1}H NMR (in DMSO-d_6, 500 MHz) δ: 6.64 (1H, d, J = 7.8 Hz, H-

3),6.43 (1H,d,J=7.8 Hz,H-5),7.07 (1H,t,J=7.8 Hz,H-4),4.43 (1H,d,J=7.2 Hz,H-1′),3.25 (1H,m,H-2′),3.51 (1H,m,H-3′),3.39 (1H,m,H-4′),3.49 (1H,m,H-5′),3.74,3.65 (2H,m,H-6′). ^{13}C NMR (in DMSO-d_6,125 MHz) δ:112.0 (C-1),160.0 (C-2),108.9 (C-3),131.3 (C-4),112.1 (C-5),164.0 (C-6),170.9 (C=O),106.5 (C-1′),73.9 (C-2′),76.4 (C-3′),70.4 (C-4′),78.0 (C-5′),61.5 (C-6′). ESI-MS (m/z) (negative mode): 315 [M-H]$^-$, 653 [2M+Na]$^-$. HR-ESI-MS: m/z 315.0714 [M-H]$^-$ (calculated for $C_{14}H_{18}O_8$, 315.0716).

Vanillic acid (**3**) was obtained as colourless needle crystal (MeOH). ^{1}H NMR (CD_3OD,500 MHz) δ: 7.56 (1H,brs,H-2),7.54 (1H,d,J=8.8 Hz,H-6),6.82 (1H,d,J=8.8 Hz,H-5),3.88 (3H,s,-OCH_3); ^{13}C NMR (CD_3OD,125 MHz) δ:123.1 (C-1),113.9 (C-2),152.7 (C-3),148.7 (C-4),115.8 (C-5),125.3 (C-6),170.0 (C=O),56.4 (-OCH_3).

Resveratrol (**4**) was obtained as white crystal (MeOH). ^{1}H NMR (500 MHz,CD_3OD) δ:7.34 (2H,d,J=8.4 Hz,H-2′,H-6′),6.96 (1H,d,J=16.2 Hz,H-α),6.80 (1H,d,J=16.2 Hz,H-β),6.76 (2H,d,J=8.4 Hz,H-3′,H-5′),6.43 (2H,d,J=1.8 Hz,H-2,H-6),6.15 (1H,t,J=2.1 Hz,H-4); ^{13}C NMR (125 MHz,CD_3OD) δ:138.6 (C-1),103.1 (C-2,6),157.0 (C-3,5),100.0 (C-4),127.7 (C-1′),126.1 (C-2′,6′),113.9 (C-3′,5′),155.8 (C-4′),124.3 (C-α),126.7 (C-β). ESI-MS m/z 227 [M-H]$^-$.

2,6-dimethoxy-4-hydroxyphenol-1-*O*-β-D-glucopyranoside (**5**) was obtained as white powder (MeOH) with positive $FeCl_3$ and Molish reaction. ^{1}H NMR (500 MHz,CD_3OD) δ:3.69 (6H,s,2×-OCH_3),4.43 (1H,d,J=9.0 Hz,H-1′),6.13 (2H,s,H-3,5); ^{13}C NMR(125 MHz,CD_3OD) δ:129.3 (C-1),154.7 (C-2,6),94.6 (C-3,5),156.3 (C-4),106.2 (C-1′),75.7 (C-2′),77.8 (C-3′),71.4 (C-4′),78.2 (C-5′),62.7 (C-6′),56.8 (2×-OCH_3). ESI-MS m/z 331 [M-H]$^-$.

***Trans*-4-hydroxy cinnamic acid** (**6**) was obtained as white crystal (acetone) with positive $FeCl_3$ and bromophenol blue reaction results and mp 210 ~ 212 ℃. ^{1}H NMR(acetone-d_6,500 MHz) δ:7.55 (2H,d,J=8.5 Hz,H-2,6),6.90 (2H,d,J=8.5 Hz,H-3,5),7.61 (1H,d,J=16.0 Hz,H-β),6.33 (1H,d,J=16.0 Hz,H-α); ^{13}C NMR (acetone-d_6,125 MHz) δ:126.8 (C-1),130.6 (C-2,6),116.4 (C-3,5),160.2 (C-4),115.5 (C-α),145.3 (C-β),167.9 (C=O of COOH).

Caffeic acid (**7**) was obtained as yellow cubic crystal (MeOH) with mp 223-225 ℃. ^{1}H NMR (500 MHz,DMSO-d_6) δ:7.40 (1H,d,J=16 Hz,H-7),7.01 (1H,d,J=1.5 Hz,H-2),6.95 (1H,dd,J=8.0,1.5 Hz,H-6),6.75 (1H,d,J=8.0 Hz,H-5),6.16 (1H,d,J=16 Hz,H-8); ^{13}C NMR (125 MHz,DMSO-d_6) δ:125.7 (C-1),114.6 (C-2),144.6 (C-3),148.1 (C-4),115.1 (C-5),121.1 (C-6),145.6 (C-7),115.8 (C-8),167.9 (C-9).

Gallic acid (**8**) was obtained as colourless needle crystal ($CHCl_3$-MeOH) with mp 235-238 ℃. Positive $FeCl_3$-$K_3Fe(CN)_6$ and bromocresol green reaction results showed the existence of phenolic hydroxyl group and carboxyl group. The same R_f values were obtained with gallic acid reference substance in different solvent systems. ^{1}H NMR (500 MHz,CD_3OD) δ:7.05 (2H,s,H-2,6); ^{13}C NMR (125 MHz,CD_3OD) δ:121.9 (C-1),110.3 (C-2,6),146.3 (C-3,5),139.5 (C-4),170.4 (C=O of COOH).

References

1 Chinese Herbal Editorial Committee. Flora of China. Beijing: Beijing Science and Technology Press, 1985. Vol 37: 210-218.

2 Puupponen PR, Nohynek L, Alakomi HL, *et al*. The action of berry phenolics against human intestinal pathogens. *BioFactors*, 2005, 23: 243-251.

3 Choi J, Lee KT, Ha J, *et al*. Antinociceptive and antiinflammatory effects of Niga-ichigoside F_1 and 23-hydroxytormentic acid obtained from *Rubus coreanus*. *Biol Pharm Bull*, 2003, 26: 1436-1441.

4 Lee JH, Ham YA, Choi SH, *et al*. Activity of crude extract of *Rubus crataegifolius* roots as a potent apoptosis inducer and DNA topoisomerase I inhibitor. *Arch Pharmacol Res*, 2000, 23: 338-343.

(下转第 49 页)