

Short Communication

Isolation of cinnamic acid derivatives from the root of *Rheum tanguticum* Maxim.ex Balf. and its significance

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Eight compounds (1-8) including two naphthalene glycosides, three tannins and three cinnamic acid derivatives were isolated from *Rheum tanguticum* Maxim.ex Balf. for the first time, except compounds 1, 6, 7 and 8. Their structures were determined mainly by NMR and MS techniques. The result is of significance for comparison and analysis between different rhubarbs by various chromatographic methods.

Key words: Rhubarb, *Rheum tanguticum*, cinnamic acid.

INTRUDUCTION

Rhubarb, one of the oldest and best-known Chinese herbal medicines, has been used for thousands of years (Science Press, 1998). Its species are widely distributed in China (Chinese Pharmacopoeia Commission, 2005), Kirgheeze desert and Europe. More than 30 species of rhubarb grow in China. It contains biologically active compounds such as anthraquinone derivatives, anthrone derivatives, stilbenes and tannins etc (Nan et al., 2009) and has the effect of purging heat, loosening the bowels, removing heat from the blood, clearing away toxins, promoting blood circulation and removing blood stasis etc. All of foregoing is focused on previous studies of Rhubarb. However, few references are available on cinnamic acid derivatives from Rhubarb no matter constituent isolation, chromatographic analysis, or pharmacological studies.

EXPERIMENTAL

Collection and preparation of plant material

The medicinal material was collected at Qinghai Province, China, in October 2007, and identified by Prof. Zhichuan Bai at the College of Horticulture and Landscape, Southwest University.

Extraction and Isolation

The root of *Rheum tanguticum* (10 kg) were dried, powered, and exhaustively extracted with methanol (95%) three times. The methanol extract (2.35 kg) which was filtered and concentrated under reduced pressure was subjected to a column chromatography on silica gel (3 kg) using petroleum ether, CHCl₃, EtOAc and methanol as the mobile phase and four fractions were obtained. The EtOAc fraction was repeatedly chromatographed over silica gel with stepped gradient of CHCl₃-MeOH and 1000 ml fractions were collected. Combined fractions 19 to 36 were purified using Sephadex LH-20 eluting with CHCl₃-CH₃OH (1:1) and subfractions 51 to 58 and subfractions 136 to 138 were afforded. The two subfractions were further purified by semi-preparative HPLC using MeOH-H₂O as the eluent to yield compounds 1 to 8 (Figure 1). These compounds including three cinnamic acid derivatives, two naphthalene glycosides, three tannins and three cinnamic acid derivatives identified as *trans*-cinnamic acid (1), P-coumaric acid glucoside (2) (Xiang et al., 2007), 4-Hydroxy-benzenepropanoic acid methyl ester (3), torachrysone -8-O-β-D-glucopyranoside (4) (Mashiko et al., 1977), 6-hydroxymusizin-8-O-β-D-glucopyranoside (5) (Mashiko et al., 1977), 4-(4'-hydroxyphenyl)-2-butanone-4'-O-β-D-(2''-O-galloyl)-glucopyranoside (6) (Gen-Ichiro and Itsuo 1983), 4-(4'-hydroxyphenyl)-2-butanone-4'-O-β-D-(2''-O-galloyl-6''-O-cinnamoyl)-glucopyranoside (7) (Yoshiki et al., 1984), 4-(4'-hydroxyphenyl)-2-butanone 4'-O-β-D-(6''-O-cinnamoyl)-glucopyranoside (8) (Nonaka et al., 1981). Compounds 2 to 5 were isolated from *R. tanguticum* for the first time. Compound 1, NMR data: ¹HNMR (600MHz, MeOD) δ: 7.51 (2H, d, J = 7.2 Hz, H-2',6'), 7.39 (1H, d, J = 15.6 Hz, H-3), 7.34 (2H, t, J = 7.2 Hz, H-4'), 7.30 (1H, t, J = 7.2 Hz, H-3', 5'), 6.51 (1H, d, H-2). ¹³CNMR (150 MHz, MeOH) δ: 127.0 (C-1'), 130.0 (C-2',6'), 128.6 (C-3',5'), 127.0 (C-4'), 137.6 (C-4), 140.8 (C-3), 175.8 (C = O). Compound 2, NMR data: ¹HNMR (600 MHz, DMSO-*d*₆)

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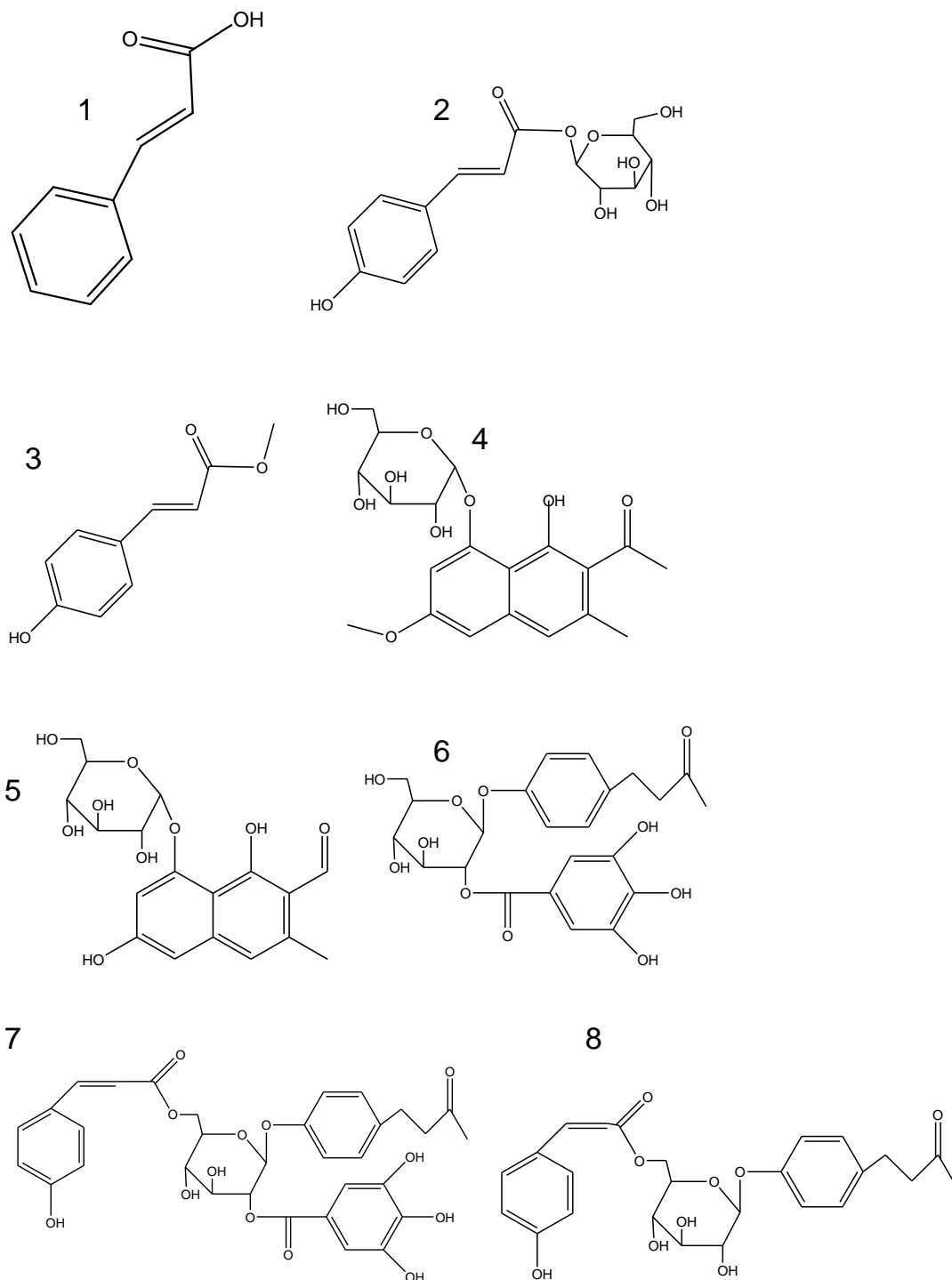


Figure 1. Structures of compounds 1–8.

δ : 10.05 (1H, s, 4-OH), 7.64 (1H, d, $J = 16.2$ Hz, H-7), 7.58 (2H, d, $J = 8.4$ Hz, H-2, 6), 6.80 (2H, d, $J = 8.4$ Hz, H-3, 5), 6.39 (1H, d, $J = 16.2$ Hz, H-8), 5.46 (1H, d, $J = 8.4$ Hz, anomeric H), 4.60~3.10 (6H, m, sugar H). ^{13}C NMR (150MHz, DMSO- d_6) δ : 124.9 (C-1), 130.5 (C-2), 113.6 (C-3), 160.0 (C-4), 115.8 (C-5), 130.6 (C-6), 145.9 (C-7), 115.8 (C-8), 165.3 (C-9), 94.2 (C-1'), 77.8 (C-3'), 76.4 (C-5'), 72.5 (C-2'), 69.5 (C-4'), 60.6 (C-6'). Compound 3, NMR data:

^1H NMR (600MHz, DMSO- d_6) δ : 7.61 (1H, d, $J = 16.2$ Hz, H-7), 7.44 (2H, d, $J = 8.4$ Hz, H-2, 6), 6.79 (2H, d, $J = 8.4$ Hz, H-3, 5), 6.31 (1H, d, $J = 16.2$ Hz, H-8), 3.76 (3H, s, OCH₃), 4.60~3.10 (6H, m, sugar-H). ^{13}C NMR (150MHz, DMSO- d_6) δ : 127.0 (C-1), 131.3 (C-2,6), 114.8 (C-3,5), 162.1 (C-4), 145.9 (C-7), 162.1 (C-8), 170.0 (C=O). Compound 4, NMR data: ^1H NMR (600 MHz, MeOD) δ : 2.28 (3H, s, Ar-CH₃), 2.58 (3H, s, COCH₃), 5.09 (1H, d, $J = 8.4$ Hz,

anomeric H), 4.00~3.30 (6H, m, sugar H), 3.86 (3H, s, OCH₃), 6.84,7.00 (each 1H, d, J = 2.4 Hz, aromatic H), 7.02 (1H, s, aromatic H). ¹³CNMR (150 MHz, MeOD) δ: 20.4 (Ar-CH₃), 32.7 (COCH₃), 56.2 (OCH₃), 62.6 (C-6'), 71.5 (C-4'), 75.1 (C-3'), 78.3 (C-5'), 79.0 (C-6'), 102.7 (C-1'), 104.5 (C-5), 104.6 (C-7), 110.5 (C-9), 120.5 (C-4), 124.2 (C-2), 135.6 (C-10), 139.4 (C-3), 153.9 (C-1), 157.4 (C-8), 160.6 (C-6), 208.3 (CO). Compound 5, NMR data: ¹HNMR (600 MHz, MeOD) δ: 2.27 (3H, s, Ar-CH₃), 2.58 (3H, s, COCH₃), 5.09 (1H, d, J = 8.4Hz, anomeric H), 4.00~3.30 (6H, m, sugar H), 6.69, 6.97 (each 1H, d, J = 1.8Hz, aromatic H), 6.91 (1H, s, aromatic H). ¹³CNMR (150 MHz, MeOD) δ: 20.5(Ar-CH₃), 32.7 (COCH₃), 62.6 (C-6'), 71.4 (C-4'), 75.1 (C-2'), 78.3 (C-5'), 79.0 (C-6'), 104.5 (C-1'), 104.7 (C-5), 105.6 (C-7), 109.8 (C-9), 120.0 (C-4), 123.4 (C-2), 135.5 (C-10), 139.7 (C-3), 154.3 (C-1), 157.6 (C-8), 158.4 (C-6), 208.4 (CO). Compound 6, NMR data: ¹HNMR (600MHz, MeOD) δ: 7.12 (2H, s, galloyl H), 7.03 (2H, d, J=8 Hz, H-2', 6'), 6.87 (2H, d, J = 8 Hz, H-3',5'), 5.13 (2H, m, anomeric H), 4.55 (1H, dd, J = 2.5, 12.0 Hz, H-6"), 4.39 (1H, dd, J = 8.0, 12.0 Hz, H-6"), 3.5 to 4.0 (5H, m, sugar-H), 2.70 (4H, m, H-3, 4), 2.07 (3H, s, H-1). ¹³CNMR (150 MHz, MeOD) δ:211.4 (C-2), 167.8 (C-7"), 157.5 (C-4'), 146.6 (C-3", 5"), 140.1 (C-4"), 137.0 (C-1'), 130.5 (C-2", 6"), 121.6 (C-1"), 118.2 (C-3', 5'), 110.5 (C-2", 6"), 101.5 (C-1"), 78.5 (C-3"), 76.4 (C-5"), 75.5 (C-2"), 71.7 (C-4"), 62.6 (C-6"), 46.0 (C-4), 30.1 (C-1, 3). Compound 7, NMR data: ¹HNMR (600 MHz, MeOD) δ: 2.03 (3H, s, COCH₃), 2.64(4H, m, H-3,4), 3.5 to 4.0 (3H, m, sugar H), 4.43 (1H, dd, J = 6,12Hz, H-6"), 4.59 (1H, dd, J = 2,12Hz, H-6"), 5.09 to 5.20 (2H, m, H-1", 2"), 6.38,7.65 (each 1H, d, J = 16.2Hz, olefinic H), 6.84,6.95 (each 2H, d, J = 8.4 Hz, aromatic H), 6.84,7.49 (each 2H, d, J = 8.4 Hz, aromatic H), 7.09 (2H, s, galloyl-H). ¹³CNMR (150 MHz, MeOH) δ: 30.5 (C-1), 30.9 (C-3), 46.3 (C-2), 64.7 (C-6"), 72.4 (C-4"), 75.4 (C-5"), 75.8 (C-2"), 76.4 (C-3"), 101.4 (C-1"), 110.5 (galloyl C-2,6), 115.3 (C-α), 117.1 (C-3", C-5"), 118.4 (C-3', C-5'), 121.6 (galloyl C-1), 127.3 (C-1"), 130.4 (C-2', C-6"), 131.5 (C-2", C-6"), 136.9 (C-1'), 139.1 (galloyl C-4), 146.6 (galloyl C-3,5), 147.0 (C-β), 157.3 (C-4'), 161.6 (C-4"), 167.8 (COOH), 169.0 (COOH), 211.2 (C-2). Compound 8, NMR data: ¹HNMR (600 MHz, MeOD) δ: 2.05 (3H, s, COCH₃), 2.61 (4H, m, 3,4-H), 3.3 to 3.8 (4H, m, sugar-H), 6.37,7.63 (each 1H, d, J = 16.2Hz, olefinic-H), 6.84,7.49 (each 2H, d, J = 8.4Hz, aromatic-H), 6.97,7.01 (each 2H, d, J = 8.4 Hz, aromatic-H). ¹³CNMR (150 MHz, MeOH) δ: 30.4 (C-1), 30.4 (C-3), 46.3 (C-2), 65.2 (C-6"), 72.6 (C-4"), 75.4 (C-5"), 75.9 (C-2"), 78.5 (C-3"), 102.9 (C-1"), 115.6 (C-α), 117.5 (C-3", C-5"), 118.5 (C-3', C-5'), 127.6 (C-1"), 130.7 (C-2', C-6"), 131.8 (C-2", C-6"), 136.8 (C-1'), 147.3 (C-β), 157.7 (C-4'), 161.9 (C-4"), 169.4 (COOH), 211.4 (C-2).

RESULTS AND DISCUSSION

To date, about 200 compounds mainly in 6 different types of skeletons, including anthraquinones, anthrones, stilbenes, flavan, acylglucosides, and pyrones, have been isolated from the genus *Rhume*. A number of researches have been done systematically, no matter their chromatographic analysis, or biological activities. But, their reports mainly focused on the most common compounds such as anthraquinone, anthrone, stilbenes and tannins etc from rhubarb.

This paper describes cinnamic acid derivatives from the plant of rhubarb for the first time. So far, quality control and evaluation system of rhubarb on Pharmacopoeia of the People's Republic of China have never referred to this class of compounds. The presence of them can be considered as a chromatographic marker for

R. tanguticum compared with the other species of rhubarb, but more research is still needed to confirm it. The occurrence of cinnamic acid derivatives from *R. tanguticum* can be used as a very important phytochemical analysis of the components of the plant, and further chromatographic studies on rhubarb, too. Therefore, their quality control and evaluation system are preferably consummated.

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