Chemical Constituents From the Roots of Rubia oncotricha

Li Jiang1,2*, Zhilong He1,2,3*, Yushan Nie2,4, Yang Wang2,5, Xue Ma4,5, Ting Liu2,4, Yuan Lu2,4, Yonglin Wang1,4,5 and Yongjun Li1,2,4

Abstract
Phytochemical investigation of the 70% ethanolic extract of the roots of Rubia oncotricha Hand.-Mazz. led to the isolation of 2 new anthraquinone glucosides named 1-hydroxy-2-hydroxymethyl-9,10-anthraquinone-11-O-β-D-glucopyranoside (1) and 1,3,6-trihydroxy-2-hydroxymethyl-9,10-anthraquinone-3-O-β-D-glucopyranoside (2), together with 5 known compounds (3-7). Their structures were elucidated by extensive spectroscopic data analysis (1-dimensional, 2-dimensional-nuclear magnetic resonance, and high resolution-electrospray ionization-mass spectrometry).

Keywords
Rubia, Rubia oncotricha, folk herbs, quinones, structure elucidation

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Introduction
Rubia oncotricha Hand.-Mazz., belonging to the plant family of Rubiaceae, is widely distributed in China as an endemic species. The dry roots and rhizomes of the plant have been used as Chinese folk herb for the treatment of cough, expectoration, jaundice, bronchitis, injuries, and so on by the local minorities in Guizhou province.1,2 However, there have been few reports on the chemical constituents of this plant. Previous phytochemical investigations of R oncotricha showed the isolation of anthraquinones, naphthoquinones, naphthohydroquinones, and triterpenoids,3-7 some of which exhibited biological activities, such as antitumor and antinematodal activities.6,7 During the course of searching for bioactive compounds, we performed a phytochemical study on the root and rhizome of R oncotricha. Previous studies mainly focused on the isolation and identification of low polar compounds, and there have been few reports on the highly polar chemical constituents of R oncotricha. Therefore, an n-butanol fraction of 70% EtOH extract of this species was investigated, which led to the isolation of 2 new anthraquinone glucosides (1-2) together with 5 known compounds (3-7) (Figure 1). Compounds (3-7) were isolated from genus Rubia for the first time. Herein, we described the structure elucidation of these new compounds.

Results and Discussion
Compound 1 was obtained as a yellow amorphous powder. The molecular formula of 1 was established as C21H20O9 by its high-resolution-electrospray ionization-mass spectrometry (HR-ESI-MS) at m/z 415.1025 [M-H]− (calcd for C21H19O9, 415.1023). The infrared (IR) spectrum showed absorption bands of hydroxyl (3293 cm−1), phenyl (2870 and 1591 cm−1), and carbonyl (1669 and 1633 cm−1) groups. The 1H nuclear magnetic resonance (NMR) spectrum (Table 1) showed signals for 1 unsubstituted anthraquinone aromatic ring [δH 7.6 Hz, H-3] and 7.74 (1H, d, J = 7.6 Hz, H-4) for 2 ortho-coupled protons suggested a disubstituted anthraquinone aromatic ring. One oxygen-bearing
methylene proton signals at $\delta_H$ 4.91 (1H, d, $J = 15.2$ Hz, H-11a) and 4.76 (1H, d, $J = 15.2$ Hz, H-11b) and 1 anomeric proton signal of glucose at $\delta_H$ 4.34 (1H, d, $J = 7.6$ Hz, H-1'). The $^{13}$C NMR spectrum (Table 1) showed 21 carbon signals, including 2 carbonyl carbons [$\delta_C$ 188.6 (C-9) and 181.8 (C-10)], 12 aromatic carbons [$\delta_C$ 158.5 (C-1), 135.3 (C-3), 134.7 (C-7), 134.6 (C-6), 134.1 (C-2), 133.2 (C-8a), 132.8 (C-10a), 131.8 (C-4a), 126.9 (C-5), 126.6 (C-8), 118.7 (C-4), 115.2 (C-9a)], 1 oxygen-bearing methylene carbon [$\delta_C$ 64.0 (C-11)], and 6 carbons from glucopyranosyl moiety [$\delta_C$ 102.7 (C-1'), 77.1 (C-3'), 76.7 (C-5'), 73.6 (C-2'), 70.1 (C-4'), 61.1 (C-6')]. Together, these data suggested that compound 1 revealed a structure of anthraquinone glucoside. The $\beta$-configuration of the sugar unit was assigned by high coupling constants ($J = 7.6$ Hz) of the anomeric proton. The absolute configuration of glucose was determined as D by gas chromatography (GC) analysis of its derivative of hydrolysate. In the $^1$H detected heteronuclear multiple bond correlation (HMBC) spectrum, the correlations from H-11 to C-1, C-2, and C-1' and from H-3 to C-1 and C-11 verified that the glucopyranosyl moiety was connected to C-11 (Figure 2). Therefore, the structure of 1 was assigned as 1-hydroxy-2-hydroxymethyl-9,10-anthraquinone-11-0-\(\beta\)-D-glucopyranoside.

Compound 2 was isolated as a yellow amorphous powder. The molecular formula of 2 was established as C$_{21}$H$_{20}$O$_{11}$ by its HR-ESI-MS at $m/z$ 447.0920 [M-H]$^-$ (calcld for C$_{21}$H$_{19}$O$_{11}$, 447.0921). The IR spectrum showed absorption bands of hydroxyl (3455 cm$^{-1}$), phenol (3196 and 1598 cm$^{-1}$), and carbonyl (1662 cm$^{-1}$) groups. The $^1$H NMR spectrum (Table 1) showed 1 ABX coupling system anthraquinone aromatic benzene [$\delta_H$ 8.09 (1H, d, $J = 8.8$ Hz, H-8), 7.48 (1H, d, $J = 2.4$ Hz, H-5), and 7.24 (1H, dd, $J = 2.8$, 8.8 Hz, H-7)], 1 isolated aromatic proton [$\delta_H$ 7.42 (1H, s, H-4)]. One hydroxymethyl proton signal [$\delta_H$ 4.62 (1H, d, $J = 11.2$ Hz, H-11a)], 4.54 (1H, d, $J = 11.2$ Hz, H-11b)] and an anomeric proton signal of sugar unit [$\delta_H$ 5.06 (1H, d, $J = 7.6$ Hz, H-1')]. The $^{13}$C NMR spectrum (Table 1) showed 21 carbon signals, including 2 carbonyl carbons [$\delta_C$ 186.3 (C-9) and 181.7 (C-10)], 12 aromatic carbons [$\delta_C$ 164.3 (C-6), 161.8 (C-3), 161.6 (C-1), 135.3 (C-10a), 133.8 (C-4a), 129.7 (C-8), 124.1 (C-8a), 123.8 (C-2), 121.7 (C-7), 112.9 (C-5), 111.1 (C-9a), 106.1 (C-4'), 1 hydroxymethyl carbon [$\delta_C$ 50.9 (C-11)], and 6 carbons from glucopyranosyl moiety [$\delta_C$ 100.9 (C-1'), 77.4 (C-3'), 76.0 (C-5'), 73.4 (C-2'), 69.4 (C-4'), 60.4 (C-6')]. The above data obtained indicated that 2 was also an anthraquinone glucoside derivative and the 1-dimensional (1D)-NMR data of 2 was similar to 1,3,6-trihydroxy-2-hydroxymethyl-9,10-anthraquinone-3-O-(6'-O-acetyl)-\(\beta\)-D-glucopyranoside by comparison of their NMR data.8 The C-6' signal of 2 (at $\delta_C$ 60.4) was shifted downfield to $\delta_C$ 63.4 in 1,3,6-trihydroxy-2-hydroxymethyl-9,10-anthraquinone-3-O-(6'-O-acetyl)-\(\beta\)-D-glucopyranoside due to the C-6' acetyl group. The configuration of the anomeric proton of glucose was proposed as $\beta$ on the basis of its coupling constant ($J = 7.6$ Hz). The absolute configuration of glucose was determined as D by GC analysis of its derivative of hydrolysate. In the HMBC spectrum, the glucose moiety was determined to be bound to C-3 of the anthraquinone based on the correlations between H-1'/C-3 and H-4'/ C-2, C-3.
Additional HMBC correlation peaks were also observed between H-11/C-1, H-11/C-2, H-5/C-6, H-5/C-7, H-7/C-5, H-8/C-6, and H-8/C-9. These results confirmed that 2 hydroxyl groups were present at C-1 and C-6, and 1 hydroxymethyl was present at C-2. Thus, compound 2 was elucidated as 1,3,6-trihydroxy-2-hydroxymethyl-9,10-anthraquinone-3-O-β-D-glucopyranoside.

Table 1. $^1$H and $^{13}$C NMR Spectroscopic Data of Compounds 1 and 2 in DMSO-$d_6$ (δ in ppm, J in Hz).

| No. | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
|-----|------------|------------|------------|------------|
| 1   | 158.5      | 161.6      |            |            |
| 2   | 134.1      | 123.8      |            |            |
| 3   | 8.04 (d, 7.6) | 134.6  | 161.8      |            |
| 4   | 7.74 (d, 7.6) | 118.6  | 7.42 (s)   | 106.1      |
| 4a  | 8.19 (m)   | 131.8      |            |            |
| 5   | 7.95 (overlap) | 135.2  | 7.48 (d, 2.4) | 112.9      |
| 6   | 7.95 (overlap) | 134.7  | 7.24 (dd, 2.4, 8.8) | 121.7      |
| 7   | 8.24 (m)   | 126.6      | 8.09 (d, 8.8) | 129.7      |
| 8   | 133.2      |            |            |            |
| 9   | 188.6      |            |            |            |
| 9a  | 115.2      |            |            |            |
| 10  | 181.8      |            |            |            |
| 10a | 132.8      |            |            |            |
| 11  | 4.91 (d, 15.2) | 64.0   | 4.62 (d, 11.2) | 50.9       |
| 12  | 4.76 (d, 15.2) | 6.45  | 4.54 (d, 11.2) | 60.9       |
| 1’  | 3.43 (d, 7.6) | 102.7  | 5.06 (d, 7.6) | 100.9      |
| 2’  | 3.10 (overlap) | 73.6   | 3.29 (overlap) | 73.4       |
| 3’  | 3.14 (overlap) | 77.1   | 3.41 (overlap) | 77.4       |
| 4’  | 3.08 (overlap) | 70.0   | 3.19 (overlap) | 69.4       |
| 5’  | 3.31 (overlap) | 76.7   | 3.32 (overlap) | 76.0       |
| 6’  | 3.46 (dd, 6.0, 11.6) | 61.0 | 3.55 (dd, 5.2, 11.8) | 60.4       |
| 7   | 3.70 (dd, 5.6, 11.6) | 3.71 (dd, 5.0, 11.8) |

Abbreviations: NMR, nuclear magnetic resonance; DMSO-$d_6$, dimethylsulfoxide-$d_6$; $^1$H at 400 MHz and $^{13}$C at 100 MHz in DMSO-$d_6$.

Conclusions

In the present work, 2 new anthraquinone glucosides (1-2), along with 5 known compounds (3-7) were isolated and identified from the roots of R oncotricha and characterized using 1D and two-dimensional (2D)-NMR and HR-ESI-MS spectroscopic analysis. Compounds 3-7 were isolated from the plants of genus Rubia for the first time. The current study provided more information on the chemical composition of R oncotricha, which lays a foundation for the development of this plant used as a Chinese folk herb.

Experimental

General

HR-ESI-MS was measured on a Bruker Daltonics micro TOF-Q II mass spectrometer (Bruker Daltoniks GmbH). 1D and 2D NMR were recorded on a JEOL ECS 400 NMR spectrometer (Jeol). Ultraviolet (UV) spectra were acquired on a UV-2700 spectrometer (Shimadzu Corporation). Optical rotation values were measured on an AUTOPOL spectrometer (Rudolph Co., Ltd). IR spectra were recorded on an IR Tracer-100 spectrometer (Shimadzu Corporation). Column chromatography was performed with macroporous resin.
(D101, Tianjin Haiguang Chemical Co., Ltd), silica gel (200-300 mesh and 300-400 mesh, Qingdao Haiyang Chemical Co., Ltd), Toyopearl HW-40C (Tocho Corporation), Toyopearl HW-40F (Tocho Corporation) and Sephadex LH-20 (Pharmacia Biotech). Semipreparative high-performance liquid chromatography (HPLC) was performed on Shimadzu LC-20AP with ACE C18-PFP columns (10 × 250 mm, 5 μm) (Phenomenex). Thin layer chromatography was performed on silica gel GF 254 plates (Qingdao Haiyang Chemical Co. Ltd). GC analysis was carried out on a Shimadzu-2010 plus gas chromatograph (Shimadzu Corporation) using a ZB-5 capillary column (30 m × 0.25 mm i.d. × 0.25 μm).

Plant Material

The roots and rhizomes of _R. oncotricha_ were purchased in September 2018 in Guiyang, Guizhou Province, China, and were identified by Associate Professor Qingde Long from the Department of Medicinal Botany and Pharmacognosy (School of Pharmacy, Guizhou Medical University). The specimen (No. 20180227) was stored at the Guizhou Provincial Key Laboratory of Pharmaceutical Preparations at Guizhou Medical University.

Extraction and Isolation

The dried root and rhizome of _R. oncotricha_ (5.0 kg) were extracted with 70% (v/v) EtOH 3 times. After evaporation of the solvent under reduced pressure, the crude extract (592.0 g) was dissolved in water and then partitioned in turn with petroleum ether, ethyl acetate, and n-butanol. The n-butanol was evaporated under reduced pressure to yield 215.0 g. The n-butanol extract (215.0 g) was chromatographed on silica gel column chromatography eluted gradient with a gradient of CHCl<sub>3</sub>-MeOH (30:1, 20:1, 15:1, 10:1, 7:1, 4:1, 1:1, v/v) to give 11 fractions (Fr.1-11).

Fraction Fr.2 (44.1 g) was subjected to silica gel column chromatography eluting with EtOAc-MeOH (15:1 to 1:1, v/v) afforded 9 subfractions (Fr.2.1-2.9). Fr.2.2 (2.7 g) was fractionated by silica gel column chromatography eluted with EtOAc-MeOH (15:1-1:1, v/v) and Toyopearl HW-40F column chromatography to give 11 fractions (Fr.1-11).

Compound 1 (10.0 mg) was subjected to silica gel column chromatography eluted with EtOAc-MeOH (15:1-1:1, v/v) to obtain 5 subfractions (Fr.1.1-1.5). Fr.1.2 was further subjected to repeated Sephadex LH-20 column chromatography (CHCl<sub>3</sub>/MeOH 1:1) and Sephadex LH-20 column chromatography (MeOH) to get compound 1 (10.0 mg). Fr.1.3 (6.9 mg) was subjected to silica gel column chromatography eluted with EtOAc-MeOH (15:1-1:1, v/v) and Toyopearl HW-40F column chromatography (MeOH) to obtain compound 2 (9.0 mg).

Acid Hydrolysis and Determination of Absolute Sugar Configuration

Each compound (0.5 mg) was hydrolyzed with 2 M HCl (2.0 ml) at 95 °C for 3 h. After cooling, the reaction mixture was partitioned between water and ethyl acetate 3 times. The aqueous layer was repeatedly evaporated to dryness with methanol, until neutral. The residue was dissolved in pyridine (0.4 mL), then L-cysteine methyl ester hydrochloride (1.0 mg) was added. The mixture was reacted at 60 °C for 1 h, and then trimethylsilyl imidazole (0.15 mL) was added to the reaction mixture at 60 °C for another 1 h, after the reaction was
completed, the reaction solution was blown dry with nitrogen. The reaction residue was dissolved in water (1.0 mL), and then n-hexane (0.5 mL) was added for extraction 3 times, and the n-hexane layer was analyzed by GC. The absolute configurations of the monosaccharides were confirmed to be D-glucose by comparison of the retention times of the authentic sample \( t_R (\text{D-glucose}) = 29.196 \text{ min}, t_R (\text{L-glucose}) = 29.558 \text{ min} \) (Supplemental material).

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Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Statement of Human and Animal Rights

This article does not contain any studies with human or animal subjects.

Statement of Informed Consent

There are no human subjects in this article and informed consent is not applicable.

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Ethical Approval

Ethical Approval is not applicable for this article.

ORCID iDs

Li Jiang https://orcid.org/0000-0002-1553-2136
Zhilong He https://orcid.org/0000-0002-8706-1660

Supplemental Material

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References

1. Editorial Committee of Chinese Flora of Chinese Academy of Sciences. Flora of China. Vol. 71. China Science Publishing & Media Ltd; 1999.
2. Guizhou Medical Products Administration. Quality standards of traditional Chinese medicine and ethnic medicine in Guizhou Province. 2003. Guizhou Science and Technology Publishing House Co., Ltd; 2003.
3. Qiao YF, Takeya K, Itoh K, Iitaka Y. Three novel naphthohydroquinone dimers from Rubia oncotricha. Chem Pharm Bull. 1990;38(10):2896-2898. doi:10.1248/cpb.38.2896
4. Itokawa H, Qiao YF, Takeya K. Anthraquinones, naphthquinones and naphthohydroquinones from Rubia oncotricha. Phytochemistry. 1991;30(2):637-640. doi:10.1016/0031-9422(91)87342-4
5. Itokawa H, Qiao YF, Takeya K. New arborane type triterpenoids from Rubia cordifolia var. Pratensis and R. oncotricha. Chem Pharm Bull. 1990;38(5):1435-1437. doi:10.1248/cpb.38.1435
6. Zhao SM, Wang Z, Chen XQ, Huang MB, Tan NH. (±)-Rubionicolin D, a pair of enantiomeric naphthohydroquinone dimers from Rubia oncotricha. Tetrahedron Lett. 2017;58(31):3041-3043. doi: 10.1016/j.tetlet.2017.06.063
7. Wang Z, Zhao SM, Zeng GZ, Tan NH. Chemical constituents from roots and rhizomes of Rubia oncotricha and their cytotoxic activities. Chin J Chin Mater Med. 2018;43(22):4462-4468. doi: 10.19540/j.cnki.cjcmm.2018.0119
8. Fan JT, Kuang B, Zeng GZ, et al. Biologically active arborane-type triterpenoids and anthraquinones from Rubia yunnanensis. J Nat Prod. 2011;74(10):2069-2080. doi: 10.1021/np2002918.
9. Wang L, Li F, Yang CY, Khan AA, Liu X, Wang MK. Neolignans, lignans and glycoside from the fruits of Melia toosendan. Fitoterapia. 2014;99:92-98. doi: 10.1016/j.fitote.2014.09.008
10. Dong LP, Ni W, Dong JY, Li JZ, Chen CX, Liu HY. A new neolignan glycoside from the leaves of Acer truncatum var. polosu. J Nat Prod. 2007;70(5):784-788. doi: 10.1021/np070565+
11. Su DM, Tang WZ, Hu YC, et al. Lignan glycosides from Neolasmollis integrifoliola. J Nat Prod. 2008;71(5):784-788. doi: 10.1021/np070565+
12. Lv LS, Shao X, Wang LY, Huang DR, Ho CT, Sang SM. Stilbene glucoside from Polygonum multiflorum Thunb.: a novel natural inhibitor of advanced glycation end product formation by trapping of methylglyoxal. J Agric Food Chem. 2010;58(4):2239-2245. doi: 10.1021/jf904122q
13. Yan FL, Dong L, Liu ZL, Chao SJ, Hao YB. Study on chemical constituents of Astragalesis var. polosu. J Xinxia Med Coll. 2007;24(6):548-550. doi:10.3969/j.issn.1004-7239.2007.06.003
14. Inoshiri S, Saiki M, Kohda H, Otsuka H, Yamasaki K. Monoterpenic glucosides from Berchemya racemosa. Phytochemistry. 1988;27(9):2869-2871. doi: 10.1016/0031-9422(88)80678-2