Phytochemistry 145 (2018) 153–160

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Rubipodanones A-D, naphthohydroquinone dimers from the roots and rhizomes of *Rubia podantha*

Zhe Wang [a, b, 1], Si-Meng Zhao [b, 1], Yan-Yun Hu [a, 1], Li Feng [a], Li-Mei Zhao [b], Ying-Tong Di [b], Ning-Hua Tan [a, b, *]

[a] School of Traditional Chinese Pharmacy and State Key Laboratory of Natural Medicines, China Pharmaceutical University, Nanjing 211198, People's Republic of China

[b] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, People's Republic of China

ARTICLE INFO

Article history:
Received 30 August 2017
Received in revised form
30 October 2017
Accepted 1 November 2017

Keywords:
Rubia podantha
Rubiaceae
Naphthohydroquinone dimers
Rubipodanones A-D
Cytotoxicity
NF-κB signaling pathway

ABSTRACT

Four previously undescribed naphthohydroquinone dimers named rubipodanones A-D, together with 19 known quinones containing three known napthohydroquinone dimers named rubioncolin C, methyl 5-hydroxy-dinaphtho[1,2-2′,3′]furan-7,12-dione-6-carboxylate and rubialatin B, were isolated from the roots and rhizomes of *Rubia podantha*. Their structures and absolute configurations were determined mainly by NMR, X-ray diffraction, and computational methods. Rubipodanones C and D, the glycosides of rubipodanone A and a pair of C-3 epimer, are the first identified dimeric napthohydroquinone glycosides from the *Rubia* plants. All naphthohydroquinone dimers were evaluated for their cytotoxicities against ten tumor cell lines and effects on the tumor-associated NF-κB signaling pathway, and rubioncolin C showed the best cytotoxicity with IC_{50} value of 1.53 μM and NF-κB inhibitory activity with IC_{50} value of 2.97 μM. These results also demonstrated that the key roles of C-3 configuration and sugar group for biological activities of rubipodanone C.

1. Introduction

The genus *Rubia* of the Rubiaceae family comprises about 70 species that are widely distributed around the world, 36 of which occur in China. Previous chemical studies on *Rubia* species have led to the isolation and characterization of more than 250 specialised metabolites with different chemical profiles, including Rubiaceae-type cyclopeptides (Tan and Zhou, 2006; Zhao et al., 2011; Xu et al., 2013), quinones (Xu et al., 2014), and triterpenes (Xu et al., 2013), which exhibit wide bioactivities containing anti-tumor, anti-inflammatory, anti-oxidant, anti-bacteria and anti-platelet aggregation (Tan and Zhou, 2006; Zhao et al., 2011; Xu et al., 2013, 2014). Among them, rubidate, a naphthoquinone synthetic derivative, has been used in the clinic as a market remedy for raising the amount of leukocyte in China. It is worth noting that napthohydroquinone dimers have drawn more attentions from the chemists for their distinctive structures. Despite this, only eleven napthohydroquinone dimers have been isolated from the *Rubia* plants (Qiao et al., 1990a; Itokawa et al., 1993; Hassanean et al., 2000; Ibraheim and Gouda, 2010; Zhao et al., 2014; Zhao et al., 2017), and some of them have been synthesized (Lumb and Trauner, 2005; Lumb et al., 2008; Yang et al., 2015). *Rubia podantha* Diels, mainly distributed in the southwest of China, is a perennial climbing herb. Its roots and rhizomes have been used as a substitute for the traditional Chinese medicine *R. cordifolia* recorded in Chinese Pharmacopoeia (2015 Version), which have been used for treating menoxenia, metrorrhagia, hematemesis and contusion for a long time. Recently, we performed phytochemical investigations for *R. podantha*, and obtained seven Rubiaceae-type cyclopeptides and described cytotoxic and NF-κB signaling pathway activities of RA-V and rubipodanin A (Wang et al., 2015). In the present study, four previously undescribed napthohydroquinone dimers, named as rubipodanones A-D (**1**–**4**), together with 19 known quinones (**5**–**23**) containing three napthohydroquinone dimers (**5**–**7**), were isolated from the roots and rhizomes of *R. podantha*. Herein, details of the isolation, structural

* Corresponding author. School of Traditional Chinese Pharmacy and State Key Laboratory of Natural Medicines, China Pharmaceutical University, Nanjing 211198, People's Republic of China.
E-mail address: nhtan@cpu.edu.cn (N.-H. Tan).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.phytochem.2017.11.002
0031-9422/

elucidation and bioactivities of napthohydroquinone dimers (**1**–**7**) (Fig. 1) are described.

2. Results and discussion

Rubipodanone A (**1**) was obtained as light yellowish crystals. Its molecular formula was determined by HRESIMS ([M+Na]$^+$, 465.1305, calcd 465.1309) as $C_{27}H_{22}O_6$, which was in accordance with the ^{1}H and ^{13}C NMR spectroscopic data (Table 1). The IR spectrum showed the absorptions at 3443, 1663, 1641, 1282 cm^{-1}, indicating the existence of hydroxyl, carbonyl, and phenyl groups. The ^{1}H NMR spectrum showed two pairs of AA′BB′ type aromatic protons at δ_H 8.19 (1H, dd, J = 7.6, 1.2 Hz), 8.04 (1H, dd, J = 7.6, 1.2 Hz), 7.75 (1H, td, J = 7.6, 1.2 Hz), 7.62 (1H, td, J = 7.6, 1.2 Hz), and 8.05 (1H, dd, J = 7.5, 1.3 Hz), 7.85 (1H, d, J = 7.5), 7.69 (1H, td, J = 7.5, 1.3 Hz), 7.63 (1H, td, J = 7.5, 1.3 Hz); one aromatic proton at δ_H 7.16 (1H, s); one olefinic proton at δ_H 4.74 (1H, m); one methylene group at δ_H 3.09 (1H, dd, J = 12.8, 7.6 Hz), 2.78 (1H, dd, J = 12.8, 7.6 Hz); one hydroxyl group at δ_H 13.46 (1H, s); one methoxyl group at δ_H 3.67 (3H, s); and two methyl groups at δ_H 1.42 (3H, s), 1.30 (3H, s). The ^{13}C NMR spectrum displayed three unsaturated ketonic carbonyl groups at δ_C 198.0 (s), 185.6 (s), and 184.2 (s); one carbonyl group at δ_C 172.1 (s); 12 aromatic carbons at δ_C 134.3 (d), 133.3 (s), 132.3 (s), 132.0 (d), 126.3 (d), 125.7 (d), and 134.1 (d), 134.1 (d), 132.2 (s), 131.7 (s), 127.2 (d), 126.3 (d); six olefinic carbons at δ_C 165.3 (s), 101.6 (s), 154.0 (s), 137.3 (d), 137.0 (s), and 116.4 (d); one methylene group at δ_C 36.3 (t); one methoxyl group at δ_C 52.0 (q); two methyl groups at δ_C 25.8 (q), 17.9 (q); as well as one quaternary carbon at δ_C 54.2 (s). **1** was presumed to be a naphthohydroquinone dimer on the basis of these data.

Detailed interpretation of HMBC and ^{1}H-^{1}H COSY correlations (Fig. 2) allowed the elucidation of the molecular structure. The HMBC correlations from δ_H 13.46 (1-OH) to δ_C 165.3 (C-1), δ_C 101.6 (C-2), and δ_C 133.3 (C-9); from δ_H 8.19 (H-8) to δ_C 132.3 (C-10) and δ_C 165.3 (C-1); from δ_H 8.04 (H-5) to δ_C 198.0 (C-4); together with the ^{1}H-^{1}H COSY correlations of H-5/H-6/H-7/H-8 gave a naphthohydroquinone moiety. Similarly, another naphthoquinone moiety was established based on the HMBC correlations from δ_H 7.85 (H-8′) to δ_C 184.2 (C-1′) and 131.7 (C-10′); from δ_H 8.05 (H-5′) to δ_C 185.6 (C-4′) and 132.2 (C-9′); from δ_H 7.16 (H-3′) to δ_C 184.2 (C-1′), δ_C 154.0 (C-2′) and 131.7 (C-10′); together with the ^{1}H-^{1}H COSY correlations of H-5′/H-6′/H-7′/H-8′. The HMBC correlation from δ_H 7.16 (H-3′) to δ_C 54.2 (C-3) suggested the C-3/C-2′ linkage of the two naphthoquinone moieties. The HMBC correlations from δ_H 1.42 (H-14) and δ_H 1.30 (H-15) to δ_C 137.0 (C-13); from δ_H 4.74 (H-12) to δ_C 17.9 (C-14) and δ_C 25.8 (C-15); from δ_H 3.09 (H-11a) and δ_H 2.78 (H-11b) to δ_C 137.0 (C-13), δ_C 101.6 (C-2), and δ_C 54.2 (C-3); from δ_H 4.74 (H-12) to δ_C 54.2 (C-3); along with the ^{1}H-^{1}H COSY correlation of H-11a/H-12 and H-11b/H-12, indicated an isopentene group at C-3 position. The methoxycarbonyl group was substituted at C-2 position, which was proved by the HMBC correlation from δ_H 3.67 (H-17) to δ_C 172.1 (C-16) and chemical shift of C-2 with an extraordinary high field shift. Thus, the planar structure of **1** was established. The compound contained only one chiral center at C-3 position, and the absolute configuration was further determined as 3*R* by X-ray diffraction using Hooft parameter (Hooft et al., 2008) (Fig. 3) and the calculated electronic circular dichroism (ECD) using the time-dependent density functional theory (TD-DFT) of the Gaussian 09 program package (Frisch et al., 2010) (Fig. 4a) (for details of the calculate ECD, see the Supplementary Information).

Rubipodanone B (**2**) was obtained as a yellowish oil. Its molecular formula was determined by HRESIMS ([M+Na]$^+$, 465.1310, calcd 465.1309) as $C_{27}H_{22}O_6$, which was in accordance with the ^{1}H and ^{13}C NMR spectroscopic data (Table 1). The IR spectrum showed the absorptions at 3442, 1693, 1640, 1268 cm^{-1}, indicating the existence of hydroxyl, carbonyl, and phenyl groups. 1D NMR spectra showed typical naphthohydroquinone dimer characteristics. Further analysis of 2D NMR data constructed the molecular architecture (Fig. 2). Similar to **1**, a naphthoquinone moiety could be determined based on the HMBC correlations from δ_H 7.97 (H-8) to δ_C 133.1 (C-10) and δ_C 191.4 (C-1); from δ_H 8.21 (H-5) to δ_C 133.2 (C-9) and δ_C 192.3 (C-4); together with the ^{1}H-^{1}H COSY correlations of H-5/H-6/H-7/H-8. The HMBC correlations from δ_H 10.08 (4′-OH) to δ_C 149.3 (C-4′), δ_C 101.4 (C-3′), and δ_C 125.3 (C-10′); from δ_H 7.90 (H-

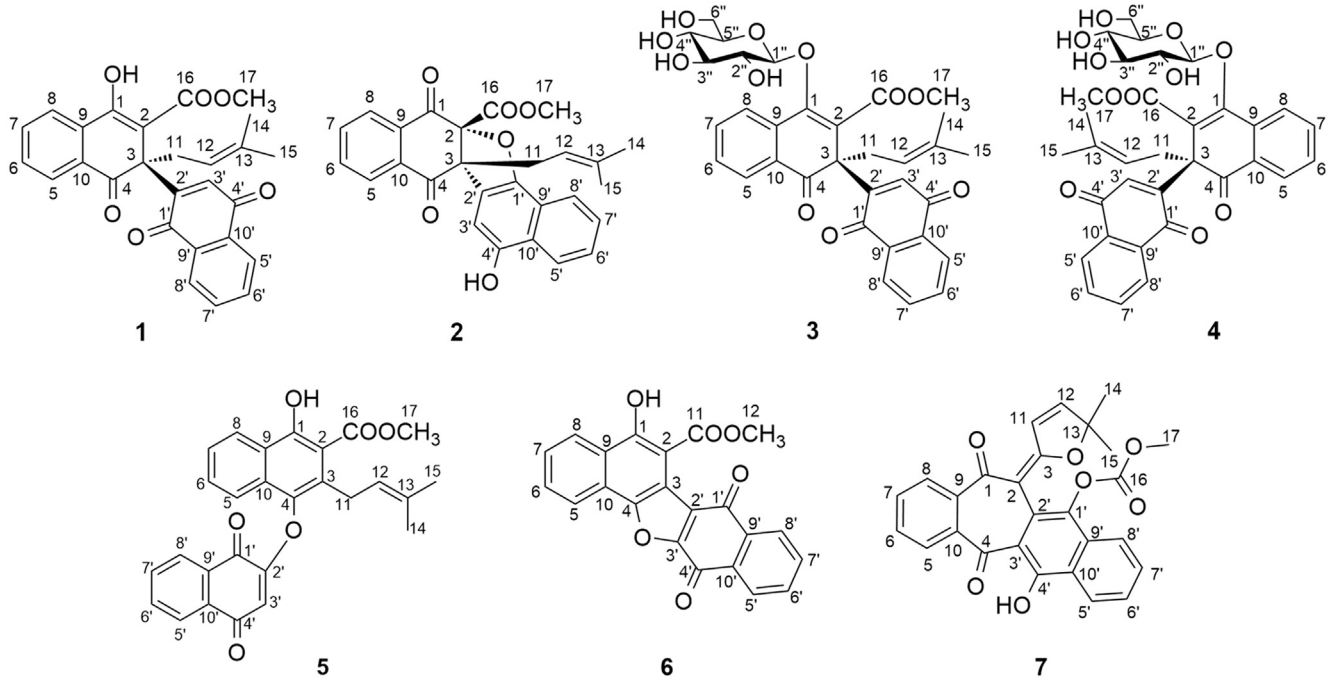

Fig. 1. Chemical structures of **1**–**7**.