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Nematicidal quinone derivatives from three *Rubia* plants

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ABSTRACT

Five new quinone derivatives, rubiasins D-F (**1**–**3**), rubialatones A and B (**4** and **5**), have been isolated from the roots and rhizomes of three *Rubia* species, *Rubia alata*, *R. wallichiana* and *R. schumanniana*, together with 26 known quinones (**6**–**31**). Their structures have been elucidated on the basis of NMR, MS spectra and computational methods. The compounds have been evaluated for their toxicity to the saprophytic nematode *Caenorhabditis elegans* and the root-knot nematode *Meloidogyne incognita*. Compounds **1**, **6** and **7** showed toxicity to *C. elegans* with the LC_{50} values at 8.50, 9.44 and 44.82 μg/mL, respectively; and **6** showed toxicity to *M. incognita* with the LC_{50} value at 35.22 μg/mL. Meanwhile, compounds **1** and **6** showed inhibitory effect on egg hatch of *C. elegans* with the LC_{50} values at 5.60 and 48.95 μg/mL.

1. Introduction

The genus *Rubia*, which belongs to the family Rubiaceae, distributes widely around the world covering about 70 species. The roots and rhizomes of *Rubia* plants have been used as herbal drugs for the treatment of hepatitis, menoxenia, and tuberculosis in China, India, Japan, and Korea. *Rubia*, meaning red in Latin, indicated the existence of red pigments in the roots and rhizomes of the genus. Chemical studies on the *Rubia* plants have revealed that quinone derivatives were the main constituents of the pigments.[1–7] Besides the quinones, cyclopeptides[1,8–19] and terpenoids[1,3,8,20] have also been isolated from *Rubia* species.

The roots and rhizomes of *Rubia* plants were recorded as a nontoxic drug in Chinese traditional herbal books. However, it has been described that the extracts were toxic to invertebrates, such as earthworm, cysticercus, and snail, which indicated the possibility to discover the chemicals for pesticide development from *Rubia* plants. Our recent investigation has shown that 1,4-naphthoquinone, could trigger nematode lethality by inducing oxidative stress and activating insulin/IGF signaling.[21] During our chemical investigations on the roots and rhizomes of *Rubia* plants, five new quinone derivatives, rubiasins D-F (**1**–**3**), rubialatones A and B (**4** and **5**) (Fig. 1), have been isolated from the roots and rhizomes of three *Rubia* species, *Rubia alata, R. wallichiana* and *R. schumanniana*, together with 26 known quinones (**6**–**31**). Compounds **1**–**3** were dearomatized quinone derivatives sharing similar skeletons with rubiasins A-C, which were reported by Chang et al. in 2000.[22] These compounds were further evaluated for their toxicity to the saprophytic nematode *Caenorhabditis elegans* and the root-knot nematode *Meloidogyne incognita*. Herein, we reported the isolation, structure elucidation, and nematicidal activity of these compounds.

2. Results and discussion

Five new quinone derivatives, rubiasins D-F (**1**–**3**), rubialatones A and B (**4** and **5**), have been isolated from the roots and rhizomes of three *Rubia* species, *R. alata*, *R. wallichiana* and *R. schumanniana* after repeated column chromatography over silica gel, Sephadex LH-20, RP-18 and HPLC preparation. Moreover, by comparing the NMR and MS data with literature, 26 known quinone derivatives were identified as 1,4-naphthoquinone (**6**),[23] 2-methoxy-1,4-

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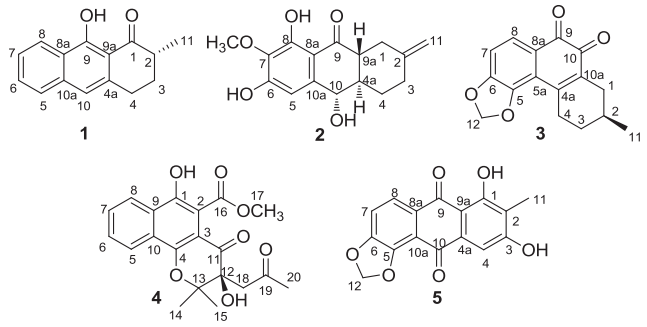

Fig. 1. New compounds isolated from three *Rubia* species.

naphthoquinone (**7**),[24] 3-amino-2-methoxycarbonyl-1,4-naphthoquinone (**8**),[25] 2-carboxy-3-hydroxy-1,4-naphthoquinone (**9**),[26] mollugin (**10**),[27] 1′-methoxy-2′-hydroxydihydromollugin (**11**),[28] rubialatin A (**12**),[4] rubialatin B (**13**),[4] methyl 5-hydroxy-dinaphtho [1,2-2′3′]furan-7,12-dione-6-carboxylate (**14**),[29] 1-hydroxy-2-methyl-9,10-anthraquinone (**15**),[30] 1,2-dimethoxy-9,10-anthraquinone (**16**),[31] 1-hydroxy-2-hydromethyl-3-methoxy-9,10-anthraquinone (**17**),[32] 1-hydroxy-5,6-dimethoxy-2-methyl-9,10-anthraquinone (**18**),[33] digiferruginol (**19**),[34] 2,3-dimethoxy-6-methyl-9,10-anthraquinone (**20**),[35] 1-hydroxy-3-methoxy-2-methyl-9,10-anthraquinone (**21**),[32] 2-carbomethoxy-3-prenyl-1,4-naphthohydroquinone-1,4-di-*O*-*β*-D-glucopyranoside (**22**),[27] 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone-3-*O*-*α*-L-rhamnopyranosyl-(1 → 2)- (4′,6′-*O*-diacetyl)-*β*-D-glucopyranoside (**23**),[27] 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone-3-*O*-*α*-L-rhamnopyranosyl-(1 → 2)- (3′-*O*-acetyl)-*β*-D-glucopyranoside (**24**),[27] 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone-3-*O*-*α*-L-rhamnopyranosyl-(1 → 2)- (6′-*O*-acetyl)-*β*-D-glucopyranoside (**25**),[30] 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone-3-*O*-*α*-L-rhamnopyranosyl-(1 → 2)-(4′-*O*-acetyl)-β-D-glucopyranoside (**26**),[36] rubiayannone A (**27**),[37] lucidin primeveroside (**28**),[30] 1-hydroxy-2-hydroxymethylene-9,10-anthraquinone-11-*O*-*β*-D-glucopyranosyl-(1 → 6)-*β*-D-glucopyranoside (**29**),[34] 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone-3-*O*-*β*-D-xylopyranosyl-(1 → 2)-(6′-*O*-acetyl)-*β*-D-glucopyranoside (**30**),[38] 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone-3-*O*-(6′-*O*-acetyl)-*β*-D-glucopyranoside (**31**),[39] respectively.

Rubiasin D (**1**) was obtained as yellow crystals. Its molecular formula was determined by HRESIMS ($[M+H]^+$, 227.1070, calcd 227.1072) as $C_{15}H_{14}O_2$, indicating nine degrees of unsaturation. The IR spectrum showed the absorptions at 3440, 3433, 1633, 1619 cm^{-1}, indicating the existence of hydroxyl, carbonyl, and phenyl groups. The ^1H NMR spectrum (Table 1) showed a pair of AA′BB′ type aromatic protons at δ_H 8.36 (1H, d, J = 8.2 Hz), 7.61 (1H, d, J = 8.2 Hz), 7.55 (1H, td, J = 8.2, 1.2 Hz), and 7.41 (1H, td, J = 8.2, 1.2 Hz); one single aromatic proton at δ_H 6.99 (1H, s); one hydroxyl group at δ_H 14.29 (1H, s); one methyl group at δ_H 1.32 (3H, d, J = 7.0 Hz); and several other multiplet proton signals at δ_H 3.02 (2H, m), 2.69 (1H, m), 2.17 (1H, m), and 1.85 (1H, m). The ^{13}C NMR spectrum (Table 2) displayed one ketonic carbonyl group at δ_C 207.9 (s); 10 aromatic carbons at δ_C 163.4 (s), 138.6 (s), 137.4 (s), 130.4 (d), 126.9 (d), 125.1 (d), 124.5 (d), 124.0 (s), 116.3 (d), and 111.2 (s), which indicated a naphthoquinone skeleton; one methine at δ_C 42.8 (d); two methylenes at δ_C 31.2 (t), 29.3 (t); as well as one methyl at δ_C 15.8 (q). On the basis of NMR data and unsaturation degrees, **1** was presumed to be a dearomatized quinone derivative. Detailed analysis of 2D NMR data (Fig. 2) allowed the construction of the planar structure. The HMBC correlations from δ_H 1.32 (H-11) to δ_C 207.9 (C-1), δ_C 42.8 (C-2), and δ_C 31.2 (C-3); from δ_H 3.02 (H-4) to δ_C 111.2 (C-9a); from δ_H 2.17 (H-3) and 1.85 (H-3) to δ_C 138.6 (C-4a); together with the COSY correlations of H-11/H-2/H-3/H-4 built an unsaturated cyclohexanone fragment with an α-methyl substitution. The HMBC correlations from δ_H 14.29 (9-OH) to δ_C 163.4 (C-9), δ_C 124.0 (C-8a), and δ_C 111.2 (C-9a); from δ_H 3.02 (H-4) to δ_C 116.3 (C-10); from δ_H 6.99 (H-10) to δ_C 124.0 (C-8a), and δ_C 111.2 (C-9a) gave a hydroxyl substituted benzene ring. Further, the HMBC correlations from δ_H 7.61 (H-5) to δ_C 116.3 (C-10); from δ_H 8.36 (H-8) to δ_C 163.4 (C-9) and δ_C 137.4 (C-10a); together with the COSY correlations of H-5/H-6/H-7/H-8 suggested the naphthoquinone fragment. Thus, the planar structure of **1** was elucidated. Further comparison of the $[\alpha]_D$ of **1** ($[\alpha]^{22.5}_D$ +31.1 (c 0.05, MeOH)) with those of (+)-(*R*)-6-methylcyclhex-2-en-1-one ($[\alpha]^{23.0}_D$ +86.0 (c 1.46, $CHCl_3$))[40] and (−)-(S)-6-methylcyclhex-2-en-1-one ($[\alpha]^{23.0}_D$ −65.6 (c 1.80, $CHCl_3$))[41] indicated the 2*R* configuration in **1**. Compound **1** was then elucidated as (+)-(2*R*)-rubiasin D.

Rubiasin E (**2**) was obtained as yellow powder. The HRESIMS ($[M-H]^-$, 289.1071, calcd 289.1075) gave the molecular formula as $C_{16}H_{18}O_5$, with eight degrees of unsaturation. The ^1H and ^{13}C NMR spectra (Tables 1 and 2) showed typical dearomatized anthracene skeleton. And 1D and 2D NMR spectra and the unsaturation degrees suggested that **2** might have two dearomatized benzene rings, together with a terminal double bond, *i.e.* δ_H 4.84 (1H, s) and 4.81 (1H, s) (H-11), δ_C 109.4 (t, C-11) and δ_C 148.0 (s, C-2). Further detailed 2D NMR analysis (Fig. 2) established the structure. The HMBC correlations from δ_H 4.82 (H-11) to δ_C 148.0 (C-2), δ_C 34.9 (C-1), and δ_C 34.3 (C-3) confirmed that the terminal double bond was substituted at C-2 position. The HMBC correlations from δ_H 2.79 (H-4α) to δ_C 51.0 (C-9a) and δ_C 148.0 (C-2); from δ_H 4.71 (H-10) to δ_C 32.0 (C-4); from δ_H 2.15 (H-1), 2.15 (H-4a) and δ_H 2.43 (H-9a) to δ_C 203.8 (C-9); together with the COSY correlations of H-1/H-9a/H-4a/H-10 and H-4a/H-4/H-3 showed a hydrogenated naphthoquinone fragment which had a ketonic group at C-9 position. In ^1H NMR spectrum, the hydroxyl group δ_H 13.72 was strongly shifted to downfield, which meant the hydroxyl group must be substituted at C-8 position to form the H-bond. Then, subsequent HMBC analysis constructed the complete structure. The correlations from δ_H 13.72 (8-OH) to δ_C 158.7 (C-8), δ_C 110.4 (C-8a), and δ_C 134.8 (C-7); from δ_H 7.59 (H-5) to δ_C 72.5 (C-10), δ_C 110.4 (C-8a), δ_C 134.8 (C-7), and δ_C 159.7 (C-6) established a five-substituted benzene ring. The methoxyl group was substituted at C-7 position, which was confirmed by the HMBC correlation from δ_H 3.96 (-OCH_3) to δ_C 134.8 (C-7). Thus, the planar structure was established. The NOE correlation of H-9a/H-10 revealed that the H-9a and H-10 was cofacial and arbitrarily assigned as β-oriented, which was further confirmed by the correlations of H-9a/H-4β, H-4β/H-10. Thus, **2** could be one of four possible stereochemical structures, *i.e.* (10*S*, 4a*R*, 9a*R*)-**2** (**2a**), (10*R*, 4a*S*, 9a*S*)-**2** (**2b**), (10*S*, 4a*R*, 9a*S*)-**2** (**2c**) and (10*R*, 4a*S*, 9a*R*)-**2** (**2d**) (Fig. 3a). The absolute configuration of **2** was determined by the ECD spectrum calculation using the time-dependent density functional theory (TD-DFT) method of the Gaussian 09 program package.[42,43] All the ECD spectra for four possible configurations were calculated at the B3LYP/6-311G (d,p) level in methanol (Fig. 3b), and the calculated ECD spectrum of **2a** resembled the experimental ECD spectrum. Accordingly, compound **2** was determined to be (+)-(10*S*, 4a*R*, 9a*R*)-rubiasin E.

Rubiasin F (**3**) was obtained as yellow powder. The HREIMS ($[M]^+$, 270.0888, calcd 270.0892) established the molecular formula as $C_{16}H_{14}O_4$, with ten degrees of unsaturation. The ^1H and ^{13}C NMR (Tables 1 and 2) showed a typical dearomatized benzene ring, *i.e.* δ_H 2.78 (1H, overlap) (H-1), δ_H 1.98 (1H, m) (H-1), and δ_C 31.7 (t, C-1); δ_H 1.69 (1H, br s) (H-2), and δ_C 27.7 (d, C-2); δ_H 1.85 (1H, m) (H-3), δ_H 1.21 (1H, m) (H-3), and δ_C 29.4 (t, C-3); δ_H 2.78 (1H, overlap) (H-4), δ_H 2.39 (1H, m) (H-4), and δ_C 23.4 (t, C-4). 2D NMR (Fig. 2) showed **3** had a phenanthrene rather than an anthracene skeleton,