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Stereochemical assignments of rubiaquinones A–C, naphthoquinone derivatives from *Rubia yunnanensis*

Yoshihiro Suyama[a,1], Yusuke Higashino[a,1], Naonobu Tanaka[a,b], Yutaka Tatano[c], Hideki Yagi[c], Kazuyoshi Kawazoe[d], Kotaro Murakami[e], Shun-Lin Li[f], Han-Dong Sun[f], Yoshiki Kashiwada[a,*]

[a] *Graduate School of Pharmaceutical Sciences, Tokushima University, Tokushima 770-8505, Japan*
[b] *Graduate School of Bioscience and Bioindustry, Tokushima University, Tokushima 770-8513, Japan*
[c] *Faculty of Pharmaceutical Sciences, International University of Health and Welfare, Ohtawara 324-8501, Japan*
[d] *School of Pharmacy, Showa University, Shinagawa 142-8555, Japan*
[e] *Faculty of Pharmaceutical Sciences, Sojo University, Kumamoto 862-0082, Japan*
[f] *State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China*

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ABSTRACT

Three new naphthoquinone derivatives, rubiaquinones A–C (**1**–**3**), were isolated from the roots of *Rubia yunnanensis*. Rubiaquinone A (**1**) was a racemic naphthoquinone dimer consisting of a 1,4-dihydroxynaphthalene and a 4-hydroxy-1,2-naphthoquinone moieties with a 2-oxo-propyl group. Rubiaquinones B (**2**) and C (**3**) were structurally unique trimeric naphthoquinones with a racemic nature possessing one chiral axis and one chiral carbon in common. The planar structures of **1**–**3** were assigned by detailed spectroscopic analyses, and enantiomers of **1**–**3** were obtained by optical resolutions. The absolute configurations of (+)-**1** and (−)-**1** were elucidated by interpretation of the ECD spectra with the aid of TDDFT ECD calculation, while those of enantiomers obtained from **2** and **3** were assigned by analyses of the composite ECD spectra generated by summing appropriate ECD spectra of enantiomers. Rubiaquinone A (**1**) exhibited antimicrobial activity against *Bacillus subtilis*.

Plants belonging to the genus *Rubia* (Rubiaceae) are known to be a rich source of quinone derivatives[1] including dimeric naphthoquinone derivatives with diverse chemical structures and intriguing bioactivities.[2] *Rubia yunnanensis* is a perennial climbing herb distributed in mainland China.[3] The roots of *R. yunnanensis* have been used as a traditional remedy to treat cancer, vertigo, insomnia, rheumatism, tuberculosis, menstrual disorders, and contusions.[3] Rubiaquinones A–C (**1**–**3**) are racemic naphthoquinone derivatives isolated from the 70% acetone aq. extract of *R. yunnanensis* roots. Among them, **2** and **3** had a unique trimeric structure with one chiral axis and one chiral carbon. Though **1**–**3** might be acetone adducts of naphthoquinone derivatives considering their structural features, **2** and **3** were challenging targets as parts of our continuing study for the stereochemical assignments of chiral compounds from plants.[4] In this study, the absolute configurations of enantiomers obtained from **1**–**3** were investigated by analysis of ECD spectra with the aid of TDDFT ECD calculation and interpretations of composite ECD spectra. We describe herein the isolation and structure elucidation of **1**–**3** including stereochemical assignments of enantiomers obtained from **1**–**3**.

The 70% acetone aq. extract from the roots of *R. yunnanensis* (3.7 kg, dry) was partitioned with EtOAc and H_2O. Chromatographic separation of the EtOAc-soluble materials using silica gel column, Sephadex LH-20 column, and reversed phase HPLC afforded rubiaquinones A (**1**, 4.7 mg), B (**2**, 2.3 mg), and C (**3**, 6.5 mg) (Chart 1).

Rubiaquinone A (**1**)[5] was obtained as a yellow amorphous solid. The HRESIMS gave the pseudomolecular ion peak at m/z 395.0899 ($[M+Na]^+$, Δ+0.4 mmu), suggesting the molecular formula of $C_{23}H_{16}O_5$. The IR spectrum implied the presence of hydroxy ($3416\ cm^{-1}$) and carbonyl (1707 and $1670\ cm^{-1}$) functionalities. Analysis of the ^1H and ^{13}C NMR spectra revealed the presence of one pentasubstituted benzene, two 1,2-disubstituted benzenes, one α,β-unsaturated ketone, and one 2-oxo-propyl group (Table 1). Interpretation of the ^1H–^1H COSY and HMBC spectra of rubiaquinone A (**1**) disclosed the presence of a 1,4-dihydroxynaphthalene moiety (C-1′–C-8′a) (Fig. 1). The existence of a 4-hydroxy-1,2-naphthoquinone moiety (C-1–C-8a) with a 2-oxo-propyl group (C-9–C-11) at C-1 was also suggested by analysis of the 2D NMR spectra. Given the unsaturation degree of **1** and the

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* Corresponding author.
E-mail address: kasiwada@tokushima-u.ac.jp (Y. Kashiwada).
[1] These authors contributed equally to this work.

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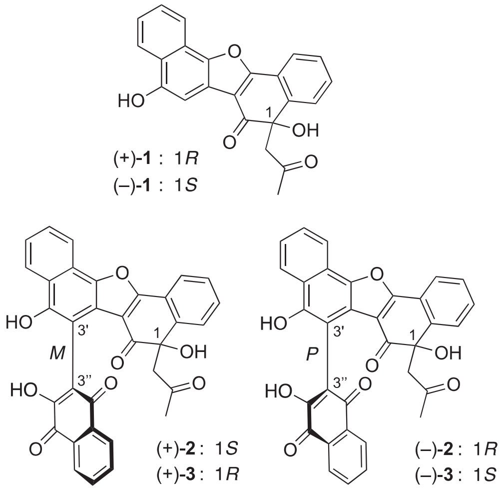

Chart 1. The structures of rubiaquinones A–C (**1**–**3**).

chemical shifts of C-1′ (δ_C 144.3), C-2′ (δ_C 120.3), C-3 (δ_C 113.4), and C-4 (δ_C 161.4), the connectivity between a 1,4-dihydroxynaphthalene and a 4-hydroxy-1,2-naphthoquinone moieties forming a furan ring was deduced. Thus, the planar structure of **1** was assigned as shown in Fig. 1.

Rubiaquinone A (**1**) did not show an optical rotation, which prompted us the optical resolution of **1** on chiral HPLC to give a pair of enantiomer {(+)-**1** and (−)-**1**}.[6,7] The experimental ECD spectra of (+)-**1** and (−)-**1** were correlated with those TDDFT calculated spectra of 1*R*-**1** and 1*S*-**1** (Fig. 2),[8] suggesting the absolute configurations of C-1 for (+)-**1** and (−)-**1** to be *R* and *S*, respectively.

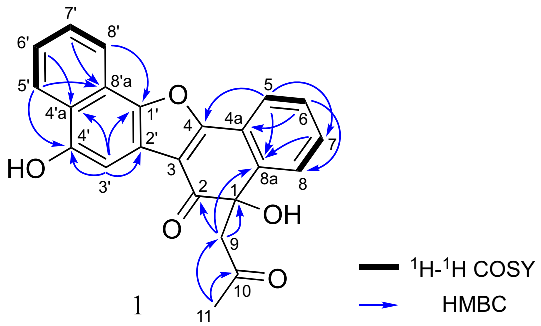

Fig. 1. Selected 2D NMR correlations for rubiaquinone A (**1**).

Rubiaquinone B (**2**)[9] was isolated as a red amorphous solid, and the molecular formula of **2**, $C_{33}H_{20}O_8$, was elucidated by the HRESIMS (*m*/*z* 566.1070 $[M+Na]^+$, Δ+1.4 mmu). The ^{1}H NMR spectrum of **2** showed the resonances due to three exchangeable protons and 12 aromatic protons as well as the signals of a 2-oxo-propyl group (Table 1). In addition to the ^{13}C NMR signals (C-1–C-11 and C-1′–C-8′a) similar to those of **1**, rubiaquinone B (**2**) displayed ten carbon signals (C-1″–C-8″a). Interpretation of the ^{1}H–^{1}H COSY and HMBC spectra indicated the presence of a 2-hydroxy-1,4-naphthoquinone moiety (C-1″–C-8″a) (Fig. 3). The connectivity between C-3″ of the

Table 1
^{1}H and ^{13}C NMR data for rubiaquinones A–C (**1**–**3**) in DMSO-d_6.

	1		**2**		**3**	
Position	^{13}C	^{1}H (*J* in Hz)	^{13}C	^{1}H (*J* in Hz)	^{13}C	^{1}H (*J* in Hz)
1	74.4	–	77.2	–	77.2	–
2	194.5	–	194.2	–	194.1	–
3	113.4	–	114.5	–	114.5	–
4	161.4	–	161.1	–	161.0	–
4a	123.5	–	123.2	–	123.0	–
5	122.1	8.06 (m)	122.2	8.09 (brd, 7.5)	122.2	8.09 (d, 7.5)
6	128.4	7.53 (m)	128.4	7.52 (td, 7.5, 1.2)	128.4	7.51 (t, 7.5)
7	130.5	7.52 (m)	130.1	7.47 (td, 7.5, 1.2)	130.2	7.46 (t, 7.5)
8	127.9	7.74 (brd, 8.8)	127.5	7.58 (brd, 7.5)	127.4	7.57 (d, 7.5)
8a	144.1	–	143.2	–	143.2	–
9	54.7	3.56 (d, 17.3) 3.52 (d, 17.3)	54.7	2.99 (d, 14.8) 2.93 (d, 14.8)	55.1	3.05 (d, 14.8) 3.00 (d, 14.8)
10	206.1	–	205.0	–	205.3	–
11	30.5	1.96 (3H, s)	31.4	1.89 (3H, s)	31.5	1.91 (3H, s)
1-OH		6.23 (brs)		6.04 (s)		6.10 (s)
1′	144.3	–	144.8	–	144.9	–
2′	120.3	–	121.8	–	121.5	–
3′	99.5	7.42 (s)	107.2	–	107.0	–
4′	151.7	–	148.4	–	148.7	–
4′a	123.7	–	124.4	–	124.4	–
5′	123.7	8.26 (brd, 8.2)	124.0	8.35 (d, 8.0)	124.0	8.33 (d, 8.0)
6′	125.3	7.58 (td, 8.2, 1.2)	125.4	7.61 (t, 8.0)	125.4	7.60 (t, 8.0)
7′	128.0	7.73 (m)	127.6	7.76 (m)	127.6	7.75 (m)
8′	119.6	8.34 (brd, 8.2)	119.7	8.41 (d, 8.0)	119.7	8.41 (d, 8.0)
8′a	121.0	–	120.8	–	120.8	–
4′-OH		10.44 (brs)		8.95 (s)		8.97 (s)
1″			182.6	–	182.5	–
2″			147.0	–	147.5	–
3″			110.5	–	110.4	–
4″			181.0	–	181.0	–
4″a			134.4	–	134.6	–
5″			125.8	7.92 (d, 7.4)	125.9	7.97 (d, 7.3)
6″			134.4	7.82 (td, 7.4, 1.2)	134.4	7.83 (t, 7.3)
7″			131.9	7.76 (m)	131.9	7.77 (m)
8″			125.5	8.08 (d, 7.4)	125.6	8.07 (d, 7.3)
8″a			131.1	–	131.2	–
2″-OH				6.36 (s)		6.32 (s)