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# NEW RED PIGMENTS FROM LICHEN *LETHARIELLA SINENSIS*

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**Abstract** – New red pigments were isolated from a lichen thallus, *Lethariella sinensis* by Wei & Jiang. The structures of the red pigments, rubrosinensiquinones A (**1**), B (**2**), and C (**3**), were determined by spectroscopic analyses.

The lichens *Lethariella* spp. are used for a health-promoting tea named Lu xing cha or Hong xue cha in Yunnan, China and Tibet.[1] During our studies on secondary metabolites from *Lethariella* spp., we discovered three new red pigments, rubrocashmeriquinone (**4**), 7-chlororubrocashmeriquinone (**5**), and 7-chlorocanarione (**6**), along with one known yellow pigment, canarione (**7**) (Figure 1), and studied their antioxidant activity.[2] In this paper, we report additional new red pigments, named rubrosinensiquinones A (**1**), B (**2**), and C (**3**) from *Lethariella sinensis*.

The MeOH and $H_2O$ extracts of *L. sinensis* were analyzed by TLC to reveal the presence of several red spots, which successfully guided the subsequent chromatographic separation of each extract. The two new red pigments (**1** and **2**) were isolated from the MeOH extract. Whereas, **3** was isolated from the $H_2O$ extract. The structures of **1**, **2**, and **3** (Figure 2) were determined to be 1,2-quinone derivatives based on the following spectroscopic data. Compound **1**, dark red amorphous, was suggested to have the molecular formula $C_{20}H_{17}NO_8$ by its positive HR-ESI (electronspray ionization) MS spectral data *m*/*z* 400.1014 $[M+H]^+$, (calcd for $C_{20}H_{18}NO_8$, $[M+H]^+$ 400.1032). The IR spectrum exhibited absorptions for hydroxy

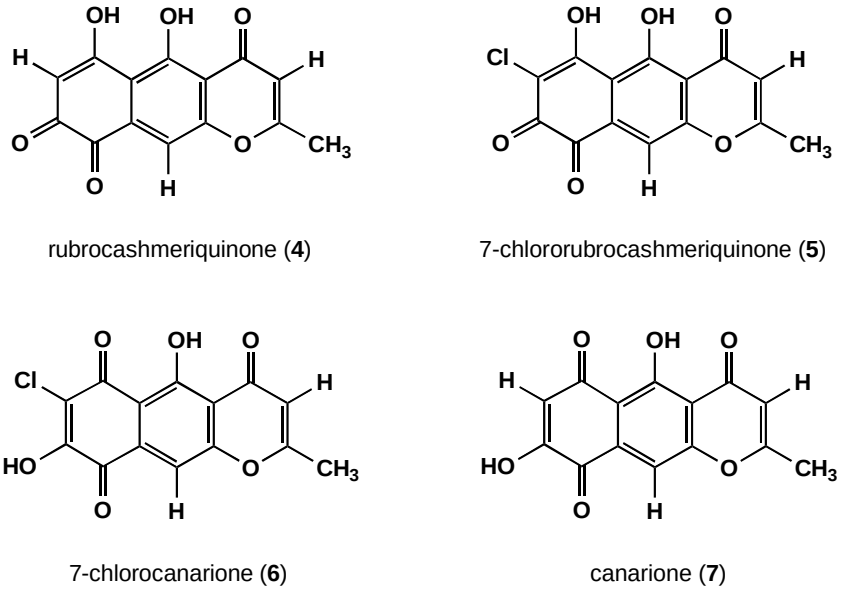


Figure 1. Structures of isolated compounds from *Lethariella* spp. (**4**-**7**)

(3440 $cm^{-1}$) and carbonyl (1650, 1630 $cm^{-1}$) groups. The $^{1}H$ and $^{13}C$ NMR data of **1** are shown in Table 1. Both spectroscopic signal patterns of **1** are similar to those of **4–7**. Therefore, we suggest that these are similar naphthoquinone derivatives. In addition, the UV spectrum (MeOH) of **1** showed a longer wavelength absorption (515 nm) than that of 1,4-naphthoquinone derivative **7**. Generally, the UV absorptions of 1,2-quinones tend to be at longer wavelengths than 1,4-quinones. For example 1,4-benzoquinone absorbs at 242, 281, and 434 nm, whereas 1,2-benzoquinone absorbs at 390 and 610 nm.[3] Thus, the structure of **1** was determined to be a 1,2-quinone derivative. HMBC (Hetero-nuclear Multiple-Bond Connectivity) and $^{1}H$-$^{1}H$ COSY data are presented in Figure 2. The methylene proton at δ 3.97 (H-7'), with an HMQC (Hetero-nuclear Multiple Quantum Coherence) correlation with the δ 47.5 (C-7') methylene carbon, showed HMBC correlations with the aromatic quaternary carbons at δ 106.3 (C-7), 172.9 (C-6), 183.7 (C-8), the methylene carbon at δ 52.6 (C-5'), and the methine carbon at δ 67.7 (C-2'). HMQC and $^{1}H$-$^{1}H$ COSY data of **1** indicated the presence of the fragment, δ 67.7 (C-2') - δ 28.3 (C-3') - δ 23.3 (C-4') - δ 52.6 (C-5'). Additionally, the structure of **1** contains nitrogen and carboxylic acid moieties according to the molecular formula of $C_{20}H_{17}NO_8$. The methine carbon at δ 67.7 (C-2') and methylene carbon at δ 52.6 (C-5') shifted downfield because these carbons were near the nitrogen. As a result, the structure of the proline (amino acid) with a methylene group on the nitrogen atom was considered. Furthermore this proline moiety was connected via a methylene group to C-7 of the quinone moiety. Comparing to the optical rotation data of proline derivatives,[4,5] it was indicated that compound 1 with negative rotation was a derivative of (-)-*S*-proline. The IR data of **1** (1620 $cm^{-1}$) suggested the presence of a zwitter ion ($COO^{-}$ and $NH^{+}$) on the proline moiety. Compound **1** was a new pigment, which we named rubrosinensiquinone A.

Compound **2**, dark red amorphous, was suggested to have the molecular formula $C_{18}H_{15}NO_8$ by its positive HR-ESIMS spectral data *m/z* 374.0856 $[M+H]^+$, (calcd for $C_{18}H_{16}NO_8$, $[M+H]^+$ 374.0876). The IR spectrum exhibited presences of hydroxy (3450 $cm^{-1}$) and carbonyl (1650, 1640, 1630, and 1610 $cm^{-1}$) groups. The $^1H$ and $^{13}C$ NMR data of **2** are shown in Table 1. The UV spectrum (MeOH) of **2** was similar to that of **1**. HMBC data is presented in Figure 2. The methylene proton at δ 4.05 (H-4'), with an HMQC correlation with the δ 49.6 (C-4') methylene carbon, showed HMBC correlations with the aromatic quaternary carbons at δ 104.0 (C-7), 173.0 (C-6), 183.7 (C-8), the methylene carbon at δ 57.7 (C-2'), and the methyl carbon at δ 40.6 (1'-$\underline{C}H_3$). The methyl proton at δ 2.68 (1'-C$\underline{H_3}$) showed an HMBC correlation with the methylene carbons at δ 49.6 (C-4') and δ 57.7 (C-2'). The methylene proton at δ 3.45 (H-2') showed an HMBC correlation with the carbonyl carbon at δ 164.7 (C-3'). From the above, the 1-methylglycine with a methylene group on the nitrogen atom was considered as residual moiety. Furthermore 1-methylglycine moiety was connected via a methylene group to C-7 of the quinone moiety. Compound **2** was a new pigment, and named rubrosinensiquinone B.

Compound **3**, dark red amorphous, was suggested to have the molecular formula $C_{14}H_8O_6$ by its HR-EI (electron ionization) MS spectral data *m/z* 272.0319, (calcd for $C_{14}H_8O_6$, $[M]^+$ 272.0321). The IR spectrum exhibited presences of hydroxy (3440 $cm^{-1}$) and carbonyl (1660 $cm^{-1}$) groups. The $^1H$ and $^{13}C$ NMR data of **3** are shown in Table 1. Both spectroscopic signal patterns were similar to those of **4.** The UV spectrum (MeOH) of **3** showed almost the same absorption at 503 nm compared to **1** and **2**. HMBC and LSPD (long-range selective proton decoupling) data are presented in Figure 2. The aromatic methine proton of δ 6.06 (H-3) showed LSPD (long-range selective proton decoupling) correlations with the methyl carbon at δ 19.3 (2-$\underline{C}H_3$), the aromatic methine carbon at δ 111.8 (C-3), and the aromatic quaternary carbon at δ 164.4 (C-4). The hydroxyl proton at δ 17.34 (4-OH) showed HMBC correlations with aromatic quaternary carbons at δ 116.6 (C-4a), 164.4 (C-4), and the aromatic methine carbon at δ 111.8 (C-3). From the above, we deduced that the 5-OH of **4** switched positions with the carbonyl group at position 4 of **4** in **3** (Figure 2). Compound **3** was a new pigment, and named rubrosinensiquinone C.

Antioxidant activities of the isolated compounds were determined by the CUPRAC-BCS (copper (II) reduction assay with bathocuproine disulfonic acid disodium salt) method.[5] The principle of this assay is based on Cu(II) reducing activity. As a result, **2** was found to have antioxidant activity (0.19), **3** showed equivalent antioxidant activity (0.10) to canarione (0.06), and **1** showed no effect (activity is expressed as mM trolox equivalents /mM).

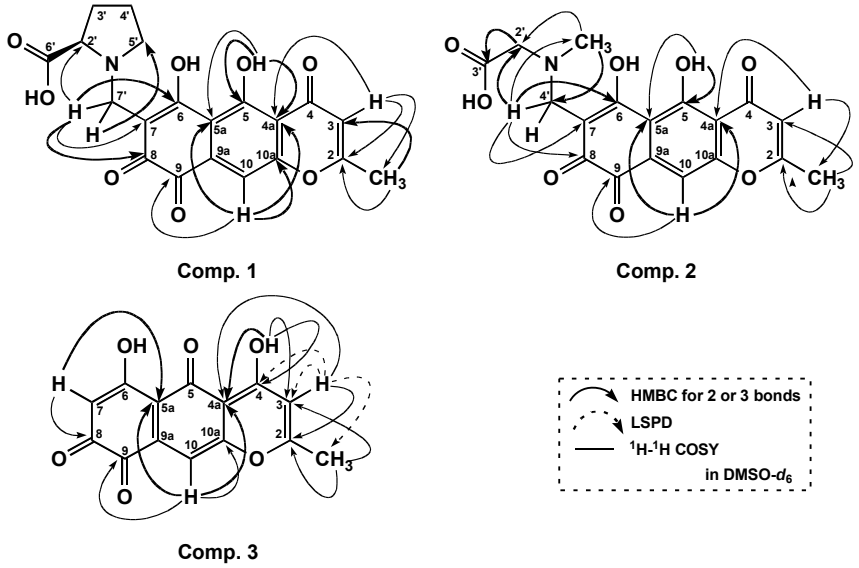


Figure 2. HMBC correlations of **1-3**

Table 1. $^1$H and $^{13}$C NMR spectral data of **1-3** in DMSO-$d_6$

| Position | 1 | | 2 | | 3 | |
|---|---|---|---|---|---|---|
| | $^\delta$C | $^\delta$H (mult., $J$ in Hz) | $^\delta$C | $^\delta$H (mult., $J$ in Hz) | $^\delta$C | $^\delta$H (mult., $J$ in Hz) |
| 2 | 164.8 | | 163.2 | | 163.1 | |
| 3 | 112.1 | 6.04 (1H, s) | 112.1 | 6.10 (1H, s) | 111.8 | 6.06 (1H, s) |
| 4 | 175.9 | | 175.8 | | 164.4 | |
| 4a | 116.6 | | 116.5 | | 116.6 | |
| 5 | 162.7 | | 162.6 | | 176.0* | |
| 5a | 111.3 | | 111.3 | | 111.8 | |
| 6 | 172.9 | | 173.0 | | 173.4* | |
| 7 | 106.3 | | 104.0 | | 104.5 | 5.26 (1H, s) |
| 8 | 183.7 | | 183.7 | | 184.2 | |
| 9 | 182.5 | | 182.2 | | 184.0 | |
| 9a | 134.5 | | 134.5 | | 134.4 | |
| 10 | 105.7 | 7.13 (1H, s) | 105.7 | 7.17 (1H, s) | 104.7 | 7.09 (1H, s) |
| 10a | 159.1 | | 159.0 | | 158.8 | |
| 2' | 67.7 | 3.70 (1H, dd, 3.7, 9.2) | 57.7 | 3.45 (2H, s) | | |
| 3' | 28.3 | 1.95 (1H, m), 2.06 (1H, m) | 164.7 | | | |
| 4' | 23.3 | 1.56 (1H, m), 1.80 (1H, m) | 49.6 | 4.05 (2H, s) | | |
| 5' | 52.6 | 2.94 (1H, m), 3.39 (1H, m) | | | | |
| 6' | 169.6 | | | | | |
| 7' | 47.5 | 3.97 (2H, s) | | | | |
| 2-$CH_3$ | 19.3 | 2.25 (3H, s) | 19.4 | 2.31 (3H, s) | 19.3 | 2.30 (3H, s) |
| 1'-$CH_3$ | | | 40.6 | 2.68 (3H, s) | | |
| 4-OH | | | | | | 17.34 (1H, s) |
| 5-OH | | 16.33 (1H, s) | | 16.34 (1H, s) | | |

*: may be interchanged

## EXPERIMENTAL

### General

The IR spectra were measured with a JASCO IR Report-100 infrared spectrophotometer. The $^{1}H$ and $^{13}C$ NMR spectra and HMQC, HMBC, COSY, and LSPD were recorded using a JEOL JNM-AL-400 ($^{1}H$ 400 and $^{13}C$ 100 MHz) and a JEOL JNM-LA 500 ($^{1}H$ 500 and $^{13}C$ 125 MHz) spectrometer in DMSO-$d_6$ using TMS as internal standard. The MS spectra were obtained using a JEOL JMS-700 and Thermo LTQ Orbitrap XL. Column chromatography was carried out on silica gel 60N (spherical, neutral, 63-210 μM, Kanto Chemical Co., Inc.) and PEGASIL PREP ODS-7515-12-A (Senshu Chemical Co., Inc.) and GE Healthcare Sephadex LH-20. HPLC was carried out using JASCO PU-2080 PLUS pump (flow late, 1.5 mL/min), equipped with a JASCO UV-2075 PLUS UV/VIS Detector.

### Plant material

The lichen thalli (Useaceae), *L. sinensis* Wei and Jiang, was collected and identified by Dr. Wang Li-Song in Yunnan, China, in 2005. A voucher specimen is deposited at the Department of Pharmacognosy, Meiji Pharmaceutical University.

### Extraction and isolation

The lichen thallus (1.0 kg) was extracted with $Et_2O$, acetone, MeOH, and $H_2O$ three times each. The MeOH extract (75.4 g) was subjected to column chromatography (C. C.) on silica gel using a $CHCl_3$-MeOH stepwise gradient system ($CHCl_3$-MeOH 1:0 → 10:1 → 5:1 → 2:1 → 1:1 → 0:1 → $CHCl_3$-MeOH-$H_2O$ 6:4:1) to yield Fraction 1 ($CHCl_3$-MeOH 1:0), Fraction 2 ($CHCl_3$-MeOH 10:1), Fraction 3 ($CHCl_3$-MeOH 10:1), Fraction 4 ($CHCl_3$-MeOH 5:1), Fraction 5 ($CHCl_3$-MeOH 5:1 and 2:1), Fraction 6 ($CHCl_3$-MeOH 2:1 and 1:1), Fraction 7 ($CHCl_3$-MeOH 0:1), Fraction 8 ($CHCl_3$-MeOH 0:1 and $CHCl_3$-MeOH-$H_2O$ 6:4:1), and Fractions 9 ($CHCl_3$-MeOH-$H_2O$ 6:4:1). Fr. 7 was repeatedly chromatographed on ODS using MeOH-$H_2O$ to obtain compound **1** (10.4 mg). Compound **2** (2.6 mg) was isolated from Fr. 8 by the combination of ODS column chromatography (eluant: MeOH-$H_2O$ 1:9 → 3:7 → 1:1 → 7:3 → 1:0) and ODS HPLC (eluant: 25% MeOH).

The $H_2O$ extract (64.3 g) was further extracted with MeOH. This MeOH extract (1.9 g) was partitioned into a MeOH-soluble part (1.4 g) and an insoluble part (0.5 g). The MeOH-insoluble part (0.5 g) was subjected to column chromatography on ODS using a MeOH-$H_2O$ stepwise gradient system (10% MeOH → 30% MeOH→ 50% MeOH →70% MeOH → 100% MeOH) to yield Fraction A (10% and 30% MeOH), Fraction B (30% MeOH), Fraction C (30% MeOH), Fraction D (30% and 50% MeOH), Fraction E (50% MeOH), and Fraction F (50 and 100% MeOH). Fr. E was subjected to Sephadex LH-20 column

chromatography using MeOH to give compound **3** (0.5 mg).

Compound **1**: rubrosinensiquinone A

Dark red amorphous. $[\alpha]_D$ -941.2° (c 0.0034, DMSO); UV (DMSO) $\lambda_{max}$ (log ε) nm: 281 (3.86), 299 (sh, 3.78), 380 (3.32), 436 (sh, 2.97), 515 (3.03); IR $\upsilon_{max}$ (KBr) $cm^{-1}$: 3440, 1650, 1630, 1580, 1560, 1540, 1460, 1260, 1200, 1120, 1090, 960. Positive HR-ESIMS *m/z* 400.1014 $[M+H]^+$ (calcd for $C_{20}H_{18}NO_8$, $[M+H]^+$ 400.1032). Positive ESIMS *m/z* 400 $[M+H]^+$ 285, 257. The $^1H$ and $^{13}C$ NMR data are presented in Table 1. HMBC and $^1H$-$^1H$ COSY data are presented in Figure 2.

Compound **2**: rubrosinensiquinone B

Dark red amorphous. UV (DMSO) $\lambda_{max}$ (log ε) nm: 277 (3.66), 378 (3.12), 524 (2.74); IR $\upsilon_{max}$ (KBr) $cm^{-1}$: 3450, 2350, 1650, 1640, 1630, 1610, 1580, 1560, 1540, 1500, 1450, 1090. Positive HR-ESIMS *m/z* 374.0856 $[M+H]^+$ (calcd for $C_{18}H_{16}NO_8$ $[M+H]^+$ 374.0876). Positive ESIMS *m/z* 374 $[M+H]^+$ 285, 257. The $^1H$ and $^{13}C$ NMR data are presented in Table 1. HMBC data is presented in Figure 2.

Compound **3**: rubrosinensiquinone C

Dark red amorphous. UV (MeOH) $\lambda_{max}$ (log ε) nm: 227 (4.22), 279 (4.04), 378 (3.49), 503 (3.23); IR $\upsilon_{max}$ (KBr) $cm^{-1}$: 3440, 1660, 1580, 1480, 1270, 1185, 1110, 1090, 960. HR-EIMS *m/z* 272.0319 (calcd for $C_{14}H_8O_6$ $[M]^+$ 272.0321). EIMS *m/z* (rel. int. %): 272 ($[M^+]$ 100), 244 (86), 216 (21). The $^1H$ and $^{13}C$ NMR data are presented in Table 1. HMBC and LSPD data are presented in Figure 2.

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