Antibacterial Lignans and Triterpenoids from Rostellularia procumbens

Yongli Zhang1,2, Fukai Bao1, Juanjuan Hu¹, Shengwang Liang³, Yu Zhang1, Guanhua Du4, Caijun Zhang3, Yongxian Cheng1

Abstract

One new lignan, rostellulin A (1), four known lignans, justin B (2), justicinid C (3), cinapthalide A (4), and justicinid A (5), and four known triterpenoids, ursolic acid (6), eusapchic acid (7), 2α-hydroxysapurscic acid (8), and tormentic acid (9), have been isolated from the whole plants of Rostellularia procumbens. Their structures were established on the basis of spectral data, including extensive NMR experiments. To our knowledge, compounds 6–9 are known compounds but not previously isolated from R. procumbens. 4 was previously reported from other Rostellularia species. Antibacterial activities of 1–9 were evaluated against eight bacterial strains with the agar dilution method, and they were found to possess antimicrobial activity with MIC values in the range of 1.56–100 μg/mL. None of the lignans exhibited cytotoxic activity against HCT-8 and Bel-7402 cells at concentrations up to 5 μg/mL.

The widespread use of antibiotics in human diseases and agriculture leads to a serious problem of bacterial resistance [1]. Currently, there is a growing interest for search for antimicrobial substances from natural sources [2]. Traditional Chinese medicine, having been used for the treatment of infectious disorders for centuries, may serve as a rich source for new classes of antibiotics. Rostellularia procumbens (Justicia procumbens), belonging to the family of Acanthaceae, is a commonly used folk medicine for the treatment of lumbago, fever, cough, and especially for the treatment of infections of the urinary systems in China [3]. The abundance of lignans in the genus Rostellularia (Justicia) and their broad biological effects has made it a research topic of considerable interest in recent years. To date, a broad spectrum of bioactivities including cytotoxicity, antipallete activity, antiviral property, TNF-α inhibitory effect, and the apoptotic induce effect of justicidin A have been well studied [4, 5, 6, 7, 8, 9, 10, 11, 12, 13]. However, so far there have been no attempts to study the antibacterial activities of chemical constituents in R. procumbens, although this herb is most impressive for its effectiveness in the treatment of infectious diseases [3]. In this paper, we describe the isolation, structural characterization of compound 1, and antimicrobial activities of compounds 1–9 (Fig. 1) from R. procumbens.

Rostellular A (1) was obtained as a white amorphous powder, its molecular formula was assigned as C23H28O9 by HR-ESI-MS (m/z = 495.1629 [M+Na]+, calc. for C23H28O9Na: 495.1531) and NMR spectra. The 13C-NMR and DEPT spectra showed 22 signals for 25 carbons, which were attributable to two methyl, two methoxy groups, four sp3 methylenes, including a dioxymethylene (δ = 100.5) and two oxygen-bearing substituents (δ = 66.5, 63.7), three sp3 methines, four sp2 methines, eight quaternary olefinic carbons, and two carbonyl groups. These data suggested that 1 was an arylphene-type lignan with one symmetrical unit in the structure. The symmetrical moiety was readily identified as a 4-hydroxy-3,5-dimethoxylbenzene ring by interpretation of the 1H-NMR spectrum. 13C-NMR data comparison between 1 and 2 revealed that a methylene (δ = 35.5) and a methine signal (δ = 122.3) in 2 were replaced by an sp3 methine signal (δ = 48.2) and a quaternary carbon (δ = 128.6) in 1. This difference implied that a ring was formed via a C-6 to C-7 bond in 1, which was evident from HMBC correlations of H-5 to C-7 and H-7 to C-6. The relative stereochemistry at C-7, C-8, and C-9 in 1 was determined by means of ROESY and selective 1D NOE irradiation experiments. ROESY interactions of H-7' and H-8 that indicated these two protons are spatially vicinal. Upon irradiation of H-8', no Overhauser effects were detected between the signals of H-7' and H-8', and H-8 and H-8. In combination with ROESY interactions, this established the relative configurations for H-7', H-8', and H-8 to be R*, S*, and R*, respectively. Hence, the structure of rostellulin A was deduced as shown (Fig. 1).

By comparison of their spectral data with those reported in the literature, compounds 2–9 (Fig. 1) were identified to be justin B (2) [10], justicicinid C (3) [14], cinaphthalide A (4) [15], justicidin A (5) [15], ursolic acid (6) [16], eusapchic acid (7) [17], 2α-hydroxyursolic acid (8) [17], and tormentic acid (9) [16]. To the best of our knowledge, compound 4 has been previously reported from other Rostellularia species, and compounds 6–9 were isolated from R. procumbens for the first time.

The antimicrobial properties of the isolated compounds were tested against eight disease-associated microorganisms. The results (Table 1) showed that compound 8 was found to be most effective against Staphylococcus epidermidis with an MIC value of 1.56 μg/mL, and 7 inhibited the growth of Salmonella typhimurium with an MIC of 3.125 μg/mL; these data are comparable with those of cefadine. All tested bacteria were not sensitive to 2, except for Staphylococcus aureus with an MIC of 12.50 μg/mL. In addition, the same MIC values of 12.50 μg/mL were observed for 3 against Salmonella paratyphi B, 4 against Escherichia coli, 8 against Shigella flexneri and Salmonella typhimurium. In addition, to observe the selectivity of the isolated compounds, the cytotoxic assay was performed with 5-FU (5-fluorouracil) as positive control (IC50 = 0.43 μg/mL and 0.37 μg/mL
Fig. 1 The structures of compounds 1–9.

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*Left column is for colfadin and right is for gentamicin.

Table 1 Antimicrobial activities of compounds 1–9 (MIC values, μg/mL)

Materials and Methods

General: Melting point was determined on an XRC-1 micro-melting apparatus. Optical rotation was determined on a JASCO-20C digital polarimeter. UV spectrum was recorded on a Shimadzu UV-240PC spectrophotometer. IR spectrum was obtained with a Bruker Tensor 27 FT-IR spectrophotometer with KBr pellets. 1H-NMR (400 MHz) and 13C-NMR (100 MHz) spectra were recorded at 300 K on a Bruker AM-400 spectrometer with TMS as an internal reference. 1H-1H COSY, HMOC, HMBC, and ROESY spectra were measured with a DRX-500 spectrometer. EI-MS (70 eV) were recorded on a VG Auto Spec-3000 spectrometer. HR-ESI-MS was carried our with an API QSTAR Pulsar 1 spectrometer. Silica gel (200–300 mesh and 10–40 μm) for column chromatography and GF254 for TLC were obtained from Qingdao Marine Chemical Factory (Qingdao, P. R. China). Sephadex LH-20 was obtained from Amersham Pharmacia Biotech (Upsala, Sweden). Fractions were monitored by TLC and spots were visualized after spraying with 10% H2SO4 in ethanol followed by heating.

Plant material: The whole plants of R. procumbens were collected in Malong, Yunnan Province, in July 2005. The plant material was identified by Prof. Heng Li, and a voucher specimen (No.
CHYX0354) is deposited at the State Key Laboratory of Phytochemistry, Kunming Institute of Botany, The Chinese Academy of Sciences.

Extraction and isolation: The air-dried and powdered whole plants of *R. procumbens* (17 kg) were extracted with 80% ethanol under reflux (2 × 25 l). After removal of the solvent by evaporation, the residues (1700 g) were suspended in H2O (3 l), and then partitioned with EtOAc (3 × 3 l) and n-BuOH (3 × 3 l), successively. The EtOAc extract (1650 g) was subjected to silica gel column chromatography (CC) (8.5 × 120 cm, 200–300 mesh, 3.0 kg) eluted with petroleum ether (60–90 °C)–Me2CO (4:1, 20 l) to give four fractions (Fr. A–D). Fr. A (14 g) was further fractionated by silica gel CC (5 × 70 cm, 200–300 mesh, 600 g) with increasing polarity of petroleum ether (60–90 °C)–EtOAc (4:1, 3:1, 2:1, 1:1, 0:1, each 1.5 l) to yield three fractions (Fr. A1–A3). Fr. A3 (5 g) was subjected to vacuum liquid chromatography (VLC) (3.5 × 10 cm, 10–40 μm) eluted with CHCl3–Me2CO (20:1, 2 l) to give four fractions A3.1–A3.4. Final purification of fr. A3.1 by VLC (2 × 7 cm, 10–40 μm) with petroleum ether (60–90 °C)–EtOAc (5:1, 400 mL) as solvents, and Sephadex LH-20 (1.5 × 70 cm) with CHCl3–MeOH (6:4, 200 mL) afforded compounds 2 (9 mg) and 3 (27 mg). Fr. A.3 was repeatedly chromatographed by VLC (2 × 5 cm, 10–40 μm) with petroleum ether (60–90 °C)–Me2CO (5:1, 200 mL) and Sephadex LH-20 (1.5 × 70 cm) with MeOH to yield compound 6 (10 mg). Fr. B (75 g) was subjected to silica gel CC (7.5 × 70 cm, 200–300 mesh, 15 kg) eluted with CHCl3–MeOH–H2O (80:18:2, 15 l) to yield six fractions (Fr. B1–B6). Silica gel CC (5 × 70 cm, 200–300 mesh, 900 g) of fr. B1 (31 g) with gradient CHCl3–Me2CO as eluent (15:1:0–0:1, each 800 mL) to afford eight fractions B1.1–B1.8. Compounds 1 (8 mg) and 4 (7 mg) were obtained from fr. B1.3 (300 mg): by VLC (2 × 7 cm, 10–40 μm) with CHCl3–Me2CO (15:1, 300 mL) and Sephadex LH-20 gel filtration with CHCl3–MeOH, Rechromatized by repeated chromatography of fr. B1.2 (700 mg) by VLC (2 × 7 cm, 10–40 μm) with CHCl3–Me2CO (10:1, 400 mL) and Sephadex LH-20 with CHCl3–MeOH (6:4, 200 mL) afforded 5 (12 mg) and 7 (120 mg). From fr. B1.4 (3 g), compounds 8 (60 mg) and 9 (70 mg) were purified by VLC (3.5 × 10 cm, 10–40 μm) using increasing polarity of CHCl3–Me2CO (10:1–5:1, each 100 mL) followed by gel filtration on Sephadex LH-20 with MeOH as eluent.

**Antimicrobial assay:** Antibacterial properties were tested with the agar dilution method [18]. The bacterial strains employed were *Staphylococcus aureus* CMCC26001 (CMCC; National Center for Medical Culture Collections; Beijing, China), *Escherichia coli* CMCC44103, *Salmonella typhimurium* CMCC80087, and *Shigella flexneri* CMCC51335, and the following clinically isolated strains: *Staphylococcus epidermidis*, *Bacillus subtilis*, *Salmonella paratyphi*-A, *Salmonella paratyphi*-B. Cefradine and gentamycin (both from Sigma; Shanghai, China) were used as reference standards; plates containing only MHA medium and 1% DMO in MHA medium served as negative and solvent controls. The tests were performed in triplicate and repeated once.

**Cytotoxic assay:** The cytotoxic effects against human HCT-8 (gel center, Institute of Basic Medical Science, Chinese Academy of Medical Science, Beijing Union Medical College, Beijing, China) and Bel-7402 cell lines (Cell center, Wuhan University, Wuhan, China) were assayed *in vitro* by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide] method [19], with 5-FU (fluorouracil, Shanghai Huatian Pharm. Inc., Shanghai, China) as a positive control. The final concentration of each tested compound was 5 μg/mL. All experiments were run in triplicate.

**Acknowledgements**

This work was supported in part by the "Xi-Bu-Zhi-Guang" Project funded by the Chinese Academy of Sciences [2005–2006] and the Talent Scholarship of Yunnan Youth (No. 2007TPY01-01). We thank Mr. Ming Shi at the Department of Microbiology and Immunology, Kunming Medical College, for his assistance in antimicrobial assays. Prof. Matthias Hamburger at the Department of Pharmaceutical Biology, University of Basel, Switzerland, for contributing to language editing and for a critical reading of this paper is also gratefully acknowledged.

**References**


