Antimycobacterial Naphthopyrones from *Senna obliqua*[⊥]

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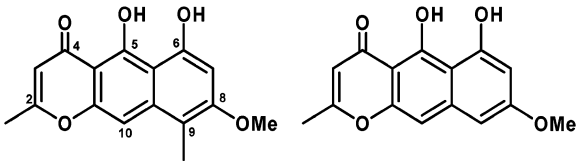
Bioactivity-directed fractionation of the methanolic extract of the stem and fruits of *Senna obliqua* led to the isolation of two known antimycobacterial natural products, quinquangulin (**1**) and rubrofusarin (**2**). Both compounds had minimum inhibitory concentrations (MICs) of 12.0 μg/mL against *Mycobacteria tuberculosis* in radiometric culture. This is the first report of antimycobacterial activity associated with naphthopyrone compounds. Their structures were determined by spectroscopic means including 1D and 2D NMR techniques and further confirmed by X-ray crystallographic analysis.

Among adults, tuberculosis (TB) is the leading cause of death worldwide due to a single infectious agent and is estimated to cause over 25% of avoidable adult deaths in developing countries.[1] During the evaluation of a number of Peruvian plants to identify potential antimycobacterial agents, the dichloromethane extract of *Senna obliqua* (G. Don) Irwin & Barneby (Fabaceae) showed strong inhibitory activity against *Mycobacterium tuberculosis*. Bioassay-directed fractionation of the methanolic extract of the stem and fruits of *S. obliqua* resulted in the isolation and identification of two known antimycobacterial naphthopyrones, quinquangulin (**1**)[2−4] and rubrofusarin (**2**).[5,6] Compounds belonging to the same class have been found in *S. longiracemosa*[6] and in several members of the closely allied genus *Cassia*.[3,7−10] However, this is the first report of antimycobacterial activity associated with naphthopyrone compounds.



1 **2**

S. obliqua is a poorly known species, primarily located at inter-Andean valleys of Ecuador and Peru.[11] Examples of this species that have been documented to date are shrubs or treelets of up to 6 m, found in gallery forests along rivers, at 200−2000 m elevation.[12,13] No phytochemical or pharmacological investigation of this species has been undertaken previously.

Table 1. NMR Data of Quinquangulin (**1**) and Rubrofusarin (**2**) in Pyriding-d_5 (500 MHz, *J* in Hz)

	1		**2**	
position	δ_H	δ_C	δ_H	δ_C
2		169.9 s		169.7 s
3	6.08 q (0.6)	106.2 d	6.08 q (0.7)	106.5 d
4		184.1 s		184.2 s
4a		102.5 s		103.3 s
5		164.0 s		163.1 s
5a		106.7 s		107.4 s
6		158.5 s		159.5 s
7	6.87 s	96.9 d	6.79 dd (2.3, 0.8)	101.1 d
8		160.0 s		163.3 s
9		108.8 s	6.83 d (2.3)	98.5 d
9a		138.8 s		141.0 s
10	7.11 s	98.1 d	7.13 s	101.4 d
10a		152.5 s		153.0 s
2-Me	2.20 d (0.5)	20.5 q	2.13 d (0.6)	20.4 q
9-Me	2.39 s	10.7 q		
MeO	3.79 s	55.9 q	3.75 s	55.4 q

The initial bioactive methanol-soluble extract of the stem and fruits of *Cassia obliqua* was fractionated using *M. tuberculosis* in radiometric culture (BACTEC 460 [Becton Dickinson Diagnostic Instrument Systems, Sparks MD]) to monitor the antimycobacterial activity of the fractions, resulting in the isolation of quinquangulin (**1**) and rubrofusarin (**2**).

Quinquangulin (**1**), bright red crystals isolated from MeOH extract of *C. obliqua*, has a molecular formula of $C_{16}H_{15}O_5$ as shown by its HRCIMS (*m/z* 287.0928 $[M + H]^+$, calcd for 287.0919). The NMR data of **1** (Table 1) were identical to those of compound **2a** reported by Li et al.,[4] who revised the structure of earlier reported quinquangulin to **1** through analysis of NMR data, especially by employing NOESY techniques. The X-ray crystallographic structure of **1** was previously reported by De Gil et al.[2] However, neither the source of the compound nor the physical and spectral data were provided in this literature. We therefore present both NMR and X-ray crystallographic data (Figure 1 and Table S1, Supporting Information) for the identity of **1**.

Rubrofusarin (**2**) was isolated as bright orange needles from the mother liquor of **1** by recrystallization in MeOH.

[⊥] Dedicated to the late Dr. Monroe E. Wall and to Dr. Mansukh C. Wani of Research Triangle Institute for their pioneering work on bioactive natural products.

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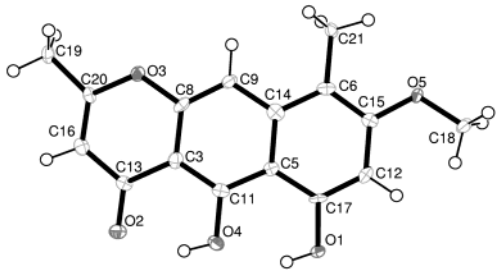

Figure 1. X-ray structure of compound **1** drawn by ORTEP. (The numbering scheme does not follow that of Chemical Abstracts.)

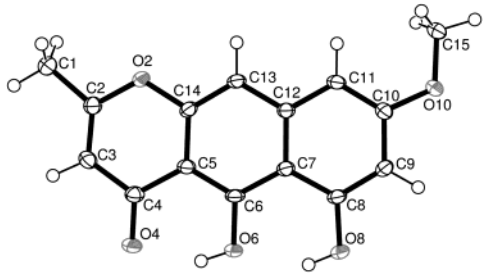

Figure 2. X-ray structure of compound **2** drawn by ORTEP. (The numbering scheme does not follow that of Chemical Abstracts.)

It was shown to have a molecular formula of $C_{15}H_{13}O_5$ by HRCIMS ($[M + H]^+$ *m/z* 273.0787). ^1H and ^{13}C NMR spectra showed that **2** is an analogue of **1** (Table 1), containing one less methyl group at C-9. The NMR data of **2** were found to be identical with those reported in the literature.[6] Rubrofusarin (**2**) was primarily isolated as a mold metabolite by Ashley et al.[14] Although Strout et al.[5] previously reported an X-ray crystallographic structure for **2**, they did not supply X-ray crystallographic data. For the purpose of confirming the structure, the NMR and X-ray crystallographic data (Figure 2 and Table S1, Supporting Information) of **2** are also included in current paper.

Naphthopyrones are often pigments and have been commonly isolated from fungi of the genus *Aspergillus*.[15] Naphthopyrones have also been found in the marine echinoderms *Comantheria briareu*, *Comantheria rotalaria*, and *Comanthus parvicirrus timorensis*,[16,17] as well as from the lichen *Flavoparmelia euplecta*.[18] There are scattered reports of naphthopyrones in higher plants, in the Malpighiaceae from *Mascagnia rigida*,[19] in the Eriocaulaceae from *Paepalanthus* bromelioides,[20] in the Rhamnaceae from *Berchemia racemosa*,[21] and in the Lamiaceae from *Coleus forskohleii*.[22]

A majority of naphthopyrones that have been reported to date in higher plants are found in the genera *Senna* and *Cassia* of the subtribe Cassinae, tribe Cassieae, of the subfamily Caesalpinioideae of the Fabaceae; from *Senna longiracemosa*,[6] and several species in the closely allied genus *Cassia*, including *C. obtusifolia*,[8,23] *C. pudibunda*,[10] and *C. tora*;[7,9,24−26] and in *C. quinquagulata*.[3] The isolation of quinquangulin (**1**) and rubrofusarin (**2**) from *Senna obliqua* in the present study provides yet another example of naphthopyrone compounds in the subtribe Cassinae and may serve to underscore the chemotaxonomic significance of this class of compound in the taxa.

A number of these naphthopyrone compounds have exhibited in vitro activity, including antimicrobial,[26] hepatoprotective,[8,9] antimutagenic,[25] cytotoxic,[3] and antiallergenic[8] activities; reversal of drug resistance in KB cells;[27] strong hypotensive activity in cats; and an LD_{50} value of 150 mg/ kg in mice.[22] Both of our isolated quinquangulin (**1**) and rubrofusarin (**2**) demonstrated minimum inhibitory concentrations (MICs) at 12.0 μg/mL against *M. tuberculosis* in radiometric culture (Table 2). This is the first report of antimycobacterial activity associated with this compound class.

Table 2. Antimycobacterial Activity of Extracts and Compounds Isolated from *Senna oblique* [tested against *M. tuberculosis* (ATCC 27294) in radiometric culture]

extract	% inhibition at 50 μg/mL	MIC (μg/mL)
dichloromethane	90	24.0
methanolic	89	
chloroform partition	93	
fraction F2	96	
fraction F2B	98	
fraction F2B2	99	
compound	**% inhibition at 50 μg/mL**	**MIC (μg/mL)**
quinquangulin (**1**)	99	12.0
rubrofusarin (**2**)	99	12.0

Experimental Section

General Experimental Procedures. UV spectra were measured with a Beckman DU-7 spectrometer. IR spectra were obtained with an ATI Mattson Genesis series FT-IR spectrometer. 1D and 2D NMR spectra were recorded on a Bruker DRX-500 instrument. Chemical shifts (δ) are expressed in ppm with reference to the solvent signals. All NMR experiments were obtained using standard Bruker pulse sequences supplied by the manufacturer. HREIMS were obtained on a Finnigan MAT 95 instrument. Column chromatography was carried out using Si gel G (Merck, 70−230 mesh). Thin-layer chromatography (TLC) was performed on precoated 1.0 mm thick Merck Si gel 60 F_{254} glass plates. TLC plates were dipped into a 5% (w/v) aqueous solution of H_2SO_4 and heated at 120 °C on a hot plate to visualize the spots. All solvents were double-distilled prior to use. X-ray crystallographic data collection for compound **1** was carried out on a Bruker APEX CCD area detector equipped with a standard focus sealed X-ray tube and Mo Ka radiation.[28] The data for compound **2** were collected on a MAR-165 area detector at the Southeast Regional Collaborative Access Team (SER-CAT) beamline 22-ID. For both structures, the direct methods program SIR-92 was used to locate the non-hydrogen atoms.[29] Repeated cycling with least-squares refinement on F^2 using SHELX-97 and difference Fourier maps yielded the final structure and was used to identify hydrogen atom positions.[30] All non-hydrogen atoms were refined with anisotropic Gaussian displacement parameters using the WinGX package.[31] The ORTEP diagrams are drawn with 50% probability ellipsoids.[32]

Plant Material. *Senna obliqua* (G. Don) Irwin & Barneby was collected in the district of Yarinacocha, Province of Coronel Portillo, Department of Ucayali, Republic of Peru, in August of 1998. The late Dr. Rupert Barneby of the New York Botanical Garden kindly provided the species determination. A voucher specimen has been deposited at the herbarium of the Field Museum of Natural History, Chicago, IL, under accession number 2200195.

Extraction and Isolation. The air-dried stem and fruits of *S. obliqua* (1.6 kg) were combined, milled, and extracted with MeOH (3 × 6.5 L). The successive extracts were concentrated in vacuo to give a residue (430 g), which was submitted to solvent partitioning between $CHCl_3$ and H_2O to afford a $CHCl_3$-soluble fraction (33.3 g) and a H_2O-soluble fraction (397.5 g). The $CHCl_3$ extract showed significant antimycobacterial activity (93% inhibition at 50 μg/mL) (Table 2) and was subjected to fractionation using a Si gel column (1.2 kg, 70−230 mesh) eluted with $CHCl_3$ and increasing concentrations of acetone, which resulted in seven fractions (F1−F7). Only fraction F2 showed strong activity with 96% inhibition of *M. tuberculosis* at 50 μg/mL. F2 (6.25 g) was then subjected to further fractionation using a Si gel column (500 g) eluting with

a gradient $CHCl_3$−acetone mixture in increasing polarity, resulting in seven subfractions (F2A−F2G). Fraction F2B showed 98% inhibition of *M. tuberculosis* at 50 μg/mL. F2B (5.03 g) was subjected to a third fractionation on a Si gel column eluted with a gradient $CHCl_3$−acetone mixture in increasing polarity, resulting in five fractions (F2B1−F2B5). Fraction F2B2 (1.35 g), which resulted from the elution of $CHCl_3$−acetone (95:5), showed 99% inhibition of *M. tuberculosis* at 50 μg/mL. Workup of this fraction yielded compound **1** (881 mg) by crystallization from a saturated EtOAc solution. The mother liquor of compound **1** was then evaporated to dryness (327 mg). Compound **2** (122 mg) was obtained by crystallization from a saturated MeOH solution of this residue. Both compounds showed MIC values of 12.0 μg/mL against *M. tuberculosis*.

Quinquangulin (1): red needles (from EtOAc); mp 194−195 °C; UV (MeOH) λ_{max} (log ϵ) 230 (3.5), 267 (3.9), 336 (0.7), 349 (0.8), and 428 (1.1) nm; IR (film) ν_{max} 1620.9, 1584.2, 1424.2, 1401.0, 1320.0, 1276.7, 1181.2, 1157.1, 1134.9, 1081.9, 903.5, 828.3 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRCIMS *m/z* 287.0928 $[M + H]^+$ (calcd for $C_{16}H_{15}O_5$: 287.0919).

Rubrofusarin (2): orange needles (from MeOH); mp 212−214 °C; UV (MeOH) λ_{max} (log ϵ) 233 (3.7), 271 (4.0), 326 (0.8), and 407 (1.3) nm; IR (film) ν_{max} 1658.5, 1624.7, 1482.0, 1422.2, 1370.2, 1242.9, 1157.1, 1087.7, 1032.7, 954.6, 912.2, 841.8, 822.5, 748.3 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HREIMS *m/z* 273.0787 $[M + H]^+$ (calcd for $C_{15}H_{13}O_5$: 273.0763).

X-ray Crystal Structure of Quinquangulin (1). A crystal, 0.1 mm on edge, obtained from MeOH. Cell parameters: $a = 10.924(2)$ Å, $b = 8.1390(16)$ Å, $c = 14.324(3)$ Å, $\beta = 99.44(3)°$, $V = 1256.3(4)$ $Å^3$, space group $P2_1/c$, $Z = 4$, $D_{calc} = 1.514$ mg/cm^3, $\lambda = 0.71073$ Å, μ(Mo Kα) = 0.133 mm^{-1}, $F(000) = 600$, $T = 100(1)$ K. Data collection yielded 8742 reflections, resulting in 2204 unique, averaged reflections. Full-matrix least-squares refinement on F^2 led to a final $R(4\sigma_F)$, R(all), and GOF of 0.108, 0.192, and 1.37. Crystallographic data, excluding structure factors, have been deposited with the Cambridge Crystallographic Data Centre with deposition number CCDC 000000. Copies of the information can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44-0-1223-336033, e-mail: deposit@ccdc.cam.ac.uk).

X-ray Crystal Structure of Rubrofusarin (2), A crystal, 0.005 × 0.005 × 0.060 mm, obtained from MeOH. Cell parameters: $a = 7.442(7)$ Å, $b = 22.88(2)$ Å, $c = 7.023(7)$ Å, $V = 1185(1)$ $Å^3$, space group $P2_1/c$, $Z = 4$, $D_{calc} = 1.526$ g/cm^3, $\lambda = 0.71000$ Å, μ(Mo Kα) = 0.116 mm^{-1}, $F(000) = 568$, $T = 100(1)$ K. Data collection yielded 4985 reflections, resulting in 2492 unique, averaged reflections. Full-matrix least-squares refinement on F^2 led to a final $R(4\sigma_F)$, R(all), and GOF of 0.061, 0.091, and 0.979. Crystallographic data, excluding structure factors, have been deposited with the Cambridge Crystallographic Data Centre with deposition number CCDC 000000. Copies of the information can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44-0-1223-336033, e-mail: deposit@ccdc.cam.ac.uk).

Bioassay Evaluation Procedures. Both extracts and compounds obtained in this investigation were evaluated for antimycobacterial activity against *M. tuberculosis* (ATCC 27294) in radiometric culture according to established protocols,[33] the results of which are reported in Table 2.

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Supporting Information Available: ORTEP drawings of **1** and **2** and Table S1, comparison of bond lengths and angles in the X-ray structures of **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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