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Merocyclophanes A and B, antiproliferative cyclophanes from the cultured terrestrial Cyanobacterium *Nostoc* sp.

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ABSTRACT

The cell extract of a cultured terrestrial *Nostoc* sp. (UIC 10062), obtained from a sample collected at Grand Mere State Park in Michigan, displayed antiproliferative activity against the HT-29 human colon cancer cell line. Bioactivity-guided fractionation of the cell extract, combined with LC–MS analysis, led to the isolation of two cyclophanes, named merocyclophanes A and B (**1** and **2**). Their structures were determined by various spectroscopic techniques including HRESIMS, and 1D and 2D NMR analyses. The stereoconfiguration was assigned on the basis of X-ray crystallographic and CD analyses. The structures of merocyclophanes A and B (**1** and **2**) established a hitherto unknown [7.7]paracyclophane skeleton in nature, as characterized by α-branched methyls at C-1/14. Merocyclophanes A and B (**1** and **2**) displayed antiproliferative activity against the HT-29 human colon cancer cell line with IC_{50} values of 3.3 and 1.7 μM, respectively.

1. Introduction

Cyanobacteria (blue-green algae) have been shown to be prolific producers of bioactive secondary metabolites (Tan, 2007; Wagoner et al., 2007; Harada, 2004). Several terrestrial cyanobacterial species belonging to the order *Nostocales* have been reported to produce naturally occurring paracyclophanes. Of these, nostocyclyne A, an acetylenic [14]paracyclophane, was isolated from the natural bloom of *Nostoc* sp. and displayed antimicrobial activity against Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* (Ploutno and Carmeli, 2000). Additionally, cylindrocyclophanes and nostocyclophanes, the two classes of cyanobacterial [7.7]paracyclophanes isolated from the cultured *Nostoc* sp. and *Cylindrospermum* sp., have exhibited a broad spectrum of biological activities, including antibacterial, antifungal and cytotoxic activities (Moore et al., 1990, 1992; Chen et al., 1991; Bui et al., 2007; Chlipala et al., 2010). As regards their formation, the polyketide biosynthetic pathway to these natural cyclophanes was determined by an isotope precursor administration experiment, and involves dimerization of two acetate-derived nonaketides and subsequent modification by chlorination, oxidation and/or methylation, resulting in diverse chemical structures (Bobzin and Moore, 1993).

Recently, several cyclindrocyclophanes were reported from a terrestrial *Nostoc* sp. (UIC 10022A) obtained from the material collected in the city of Chicago. These compounds displayed inhibitory activity against the 20S proteasome and were found to be cytotoxic against cancer cell lines, including HT-29, NCI-H460, SF268 and MCF7 cells (Chlipala et al., 2010). Herein, structure elucidation and biological activity of two additional cyclophanes, named merocyclophanes A and B (**1** and **2**), that were isolated from a second *Nostoc* sp. (UIC 10062) are reported. This strain was obtained from a sample collected at Grand Mere State Park in Michigan, and the merocyclophanes were named in recognition of the collection site. Their structures were determined using various spectroscopic methods including HRESIMS, and 1D and 2D NMR analyses. The stereoconfiguration was assigned by a combination of X-ray crystallographic and CD analyses, and the structures of merocyclophanes A and B (**1** and **2**) established a new [7.7]paracyclophane carbon skeleton, as characterized by the presence of α-branched methyls at C-1/14.

2. Results and discussion

Nostoc sp. (UIC 10062) was isolated from a sample collected at Grand Mere State Park in Michigan, and cultured in Z media (Falch et al., 1995). The freeze-dried cells were extracted with a mixture

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of CH_2Cl_2 and MeOH (1:1, v/v) and dried *in vacuo*, with the resulting extract (156.6 mg) subjected to bioassay-guided fractionation. This cell extract showed antiproliferative activity against the HT-29 human colon cancer cell line (IC_{50} 13.1 μg/mL), and was fractionated using Diaion HP-20 resin and an increasing amount of iPrOH in H_2O. Fractions eluting at 70 (5.0 mg) and 80 (6.5 mg)% iPrOH were found to be active, with IC_{50} values of 0.9 and 1.2 μg/ml, respectively. LC–MS and UV analyses of the active fractions also indicated the presence of two cyclophanes with molecular weights of 552 and 566. Reversed-phase HPLC of these fractions yielded merocyclophanes A (**1**, 2.4 mg, 0.11%) and B (**2**, 0.9 mg, 0.04%) as minor compounds (Fig. 1).

Merocyclophane A (**1**) was obtained as white amorphous powder, and the molecular formula was determined as $C_{36}H_{56}O_4$ by HRESIMS analysis (m/z 551.4170 $[M-H]^-$). The total numbers of proton and carbon signals, determined by analysis of ^1H and 2D NMR spectra, were only half of those required by the molecular formula, thus indicating the presence of C_2 axis of symmetry in **1**. The structure of **1** was elucidated by analysis of 2D NMR spectra including COSY, HSQC and HMBC (Fig. 2). The appearance of only two aromatic singlets (H-10/23, δ_H 6.04 and H-12/25, δ_H 6.00) indicated the presence of two tetrasubstituted aromatic rings. The carbon chemical shifts of C-9/22 (δ_C 158.5) and C-13/26 (δ_C 156.9), together with HMBC correlations from H-7/20 (δ_H 3.10) to C-9/22 (δ_C 158.5) and C-13/26 (δ_C 156.9), identified these partial structures as two 2,5-dialkylresorcinol moieties. Sequential COSY correlations from H1/14 to H7/20 combined with HMBC correlations from H-10/23 (δ_H 6.04) and H-12/25 (δ_H 6.00) to C-1/14 (δ_C 41.8) and from H-7/20 (δ_H 3.10) to C-9/22 (δ_C 158.5) and C-13/26 (δ_C 156.9) completed the [7.7]paracyclophane core. Additional sequential COSY correlations from the H-7/20 to the triplet methyls H_3-30/34 via three methylenes (H_2-27/31, H_2-28/32 and H_2-29/33) further expanded the carbon chains. Structure determination was completed by an HMBC correlation from the doublet methyl (H_3-35/36, δ_H 1.15) to the aromatic carbon (C-11/24, δ_C 146.6) combined with a COSY correlation between H-1/14 (δ_H 2.30) and H-35/36 (δ_H 1.15), placing methyl groups at C-1/14.

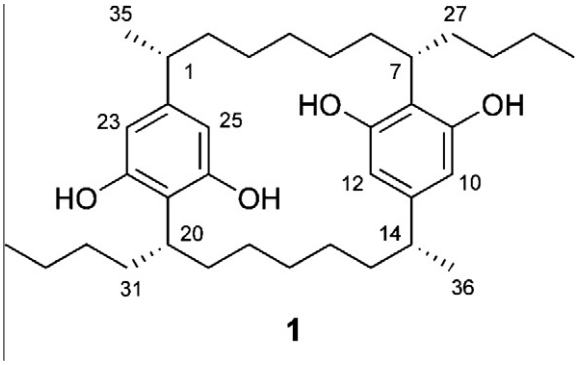

Fig. 1. Structures of merocyclophanes A (**1**) and B (**2**).

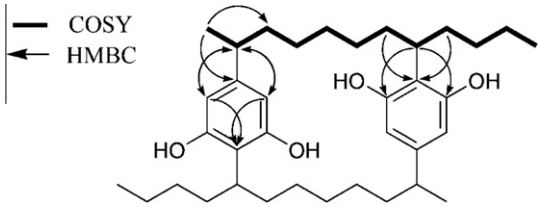

Fig. 2. Key 2D correlations used for structure determination of **1**.

Structure **1** established the presence of four stereogenic centers, whose stereoconfiguration was determined by a combination of X-ray crystallographic and CD spectral analyses. Merocyclophane A (**1**) was crystallized from CH_3CN. Single-crystal X-ray analysis established the relative configuration of **1** as shown in Fig. 3. The absolute configurations at C-1/14 and C-7/20 were established by comparison of the CD spectrum of **1** with those reported for the nostocyclophanes (Chen et al., 1991). Cotton effects observed between 220 and 230 nm and between 270 and 280 nm arise from $\pi-\pi^*$ transitions of a benzene chromophore. According to the benzene sector rule, the chlorine-bearing stereogenic carbons in the nostocyclophanes, three carbons away from the benzene chromophore, should not affect Cotton effects in these regions (Smith, 1998). The CD spectrum of **1** exhibited negative Cotton effects at 227 ($\Delta\varepsilon$, −4.35) and 277 ($\Delta\varepsilon$, −2.73), similar to those observed for the nostocyclophanes, suggesting the same absolute configuration. Therefore, the absolute configurations of the four stereogenic carbons C-1/14 and C-7/20 in **1** were assigned as "*R*" and "*S*", respectively.

Merocyclophane B (**2**) was obtained as purple amorphous powder, and the HRESIMS data (m/z 565.3954 $[M-H]^-$) established the molecular formula as $C_{36}H_{54}O_5$. The ^1H NMR spectrum of **2** closely resembled **1**, except for one of the two aromatic rings. This resulted in an unsymmetric structure. The presence of a hydroxyquinone in **2** was suggested by analysis of the UV spectrum. A chromophore corresponding to the quinone absorption was observed at 521 nm, which shifted to 413 nm in the presence of acid (0.04 v/v% TFA in MeOH). This was consistent with the observed color change from deep purple to yellow upon addition of TFA. The appearance of the down-field shifted carbon chemical shift of C-23 (δ_C 132.6 compared to δ_C 104.5 in **1**) further supported the presence of the hydroxyquinone moiety in **2**. However, significant line broadening of NMR signals was observed in the hydroxyquinone moiety, causing a number of expected correlations to be absent in the HMBC spectrum, even at the elevated temperature (343 K). Thus, the carbon chemical shifts of C-21, C-22, C-25 and C-26 could not be detected. The presence of the unsymmetrical hydroxyquinone moiety in **2** raised the possibility of two conformations arising from hindered rotation around the C1–C24 and C20–C21 bonds, resulting in the formation of two diastereotopic atropisom-

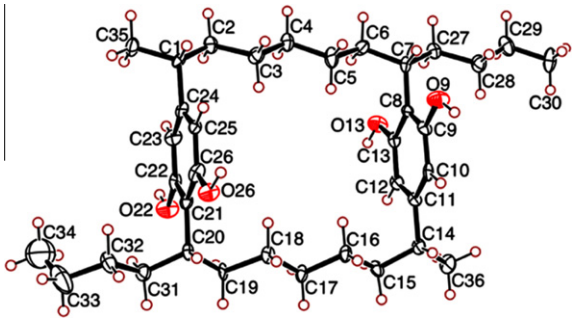

Fig. 3. ORTEP drawing of merocyclophane A (**1**).