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spiro-Mamakone A: A Unique Relative of the Spirobisnaphthalene Class of Compounds

Sonia A. van der Sar, John W. Blunt, and Murray H. G. Munro*

Department of Chemistry, University of Canterbury, Private Bag 4800, Christchurch, New Zealand

murray.munro@canterbury.ac.nz

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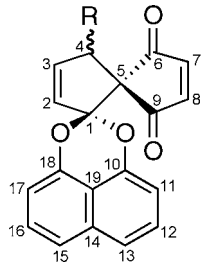
ABSTRACT



1 R = H, OH
2 R = O

A spirobisnaphthalene derivative with a new spiro-nonadiene skeleton, spiro-mamakone A (1), has been isolated from the extract of a cultured nonsporulating fungal endophyte derived from the New Zealand native tree *Knightia excelsa* (rewarewa). The carbon skeleton of spiro-mamakone A represents a new structural entity and an intriguing addition to the structurally diverse spirobisnaphthalene group of compounds. spiro-Mamakone A is potently cytotoxic and is also antimicrobial.

The spirobisnaphthalenes are a relatively new class of compounds that were first isolated 15 years ago[1] and which contain two naphthalene-derived C_{10} units bridged through a spiroketal linkage. This class of compounds is generally divided into three subclasses: those with two oxygen bridges, for example, palmarumycin CP_1,[2] those with three oxygen bridges, as in preussomerin A,[1] and those with two oxygen bridges and one C−C bridge, such as spiroxin A.[3] These compounds are normally isolated from fungi, although spirobisnaphthalenes have also been isolated on two occasions from plant sources. The most recent report is that from the fruit of *Diospyros ehretioides*.[4] However, as the authors have suggested, the source is probably an endophytic fungus, as is most likely for all spirobisnaphthalenes reported from plants.

In our continued investigation into novel bioactive compounds from New Zealand endophytic fungi, we report the isolation and structure elucidation of a new relative of the spirobisnaphthalenes, spiro-mamakone A (**1**),[5] from a nonsporulating endophytic fungus isolated from the New Zealand native tree rewarewa. The carbon skeleton of the spiro-

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(5) Compound **1**: amorphous yellow solid; $[\alpha]^{20}_D$ 0.0 (*c* 0.1, MeOH); UV λ_{max} (MeOH) (log ϵ) 230.0 (3.89), 300.0 (3.88), 314.8 (3.73), 330.0 (3.43) nm; IR (KBr disk) ν_{max} 3477, 1707, 1609, 1414, 1379, 1275, 1204, 1107, 1082 cm^{-1}; $C_{19}H_{12}O_5$ by HREIMS $[M]^+$ *m*/*z* 320.0677 (calcd 320.0685); ^1H NMR (CD_3OD, 500 MHz) δ 7.47 (1H, d, 8.8, H15/H13), 7.45 (1H, d, 8.8, H13/H15), 7.40 (1H, t, 7.8, 7.8, H12/H16), 7.36 (1H, t, 7.8, 7.8, H16/H12), 7.22 (1H, d, 5.7, H8/H7), 7.09 (1H, d, 5.7, H7/H8), 6.83 (1H, d, 7.3, H11/H17), 6.76 (1H, d, 7.3, H17), 6.40 (1H, dd, 5.8, 1.9, H3), 5.93 (1H, dd, 5.8, 2.0, H2), 5.30 (1H, br s, H4); ^{13}C NMR (CD_3OD, 75 MHz) δ 201.1 (C9/C6), 198.6 (C6/C9), 152.2 (C8/C7), 151.9 (C7/C8), 149.4 (C10/C18), 148.8 (C18/C10), 142.7 (C3), 136.1 (C14), 129.9 (C2), 129.0 (C12/C16), 128.7 (C16/C12), 122.5 (C15/C13), 122.0 (C13/C15), 114.8 (C19), 111.7 (C1), 111.0 (C11/C17), 110.6 (C17/C11), 79.1 (C4), 68.9 (C5).

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nonadiene portion of the molecule is unprecedented in any naturally occurring compounds.

The endophytic fungus was isolated from surface-sterilized leaves of rewarewa, commonly known as New Zealand honeysuckle (*Knightia excelsa*), which were collected from a mixed broadleaf-podocarp forest in the Kamai-Mamaku Forest Park, Bay of Plenty, New Zealand.[6] The fungus was cultivated on 24 plates (85 mm diameter) of MYE (malt yeast extract) agar. After 24 days at 26 °C, the plates were extracted exhaustively with EtOAc. The resulting crude extract (208 mg) showed potent cytotoxicity (IC_{50} 0.12 μg/mL) against the P388 cell line. The extract was subjected to reversed-phase (C18) flash chromatography,[7] eluting with a steep, stepped gradient from MeOH/H_2O (1:3) through MeOH to DCM. Final purification was achieved by semi-preparative HPLC (Phenomenex Luna C18, 10 × 250 mm, 5 μm; 30−45% MeCN/H_2O; 5 mL/min) to yield the major compound, spiro-mamakone A (**1**) (10.7 mg), as an amorphous yellow solid.[5]

HREIMS analysis of the molecular ion (*m*/*z* 320.0677 $[M]^+$) suggested a molecular formula of $C_{19}H_{12}O_5$, which was supported by the ^{13}C NMR spectrum (19 resonances) and was indicative of 14 degrees of unsaturation. The ^1H NMR spectrum showed just one oxymethine and 10 olefinic/aromatic signals. Using ^{13}C and HSQC/DEPT experiments, the carbon resonances were assigned as one oxymethine (C-4), two carbonyls (C-6 and C-9), 14 sp^2 carbons, four of them nonprotonated (C-2, C-3, C-7, C-8, C-10-C-19), and two quaternary sp^3 carbons (C-1 and C-5).

COSY and HSQC experiments were used to define spin systems a−d (Figure 1 in blue). The remaining signals were

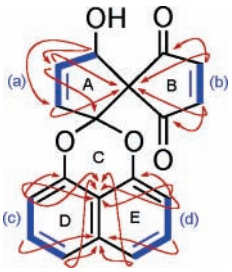

Figure 1. Important COSY and CIGAR correlations for spiro-mamakone A in CD_3OD.

assigned based on a detailed analysis of CIGAR experimental data. Protons H-11 to H-13 (spin system d) and H-15 to H-17 (spin system c) showed correlations to the two nonprotonated carbons, C-14 and/or C-19, indicating a common ring junction between the two spin systems. H-11 and H-12 were also found to correlate to C-10, whereas H-16 and H-17 correlated to C-18. The chemical shifts of C-10 and C-18, δ_C 149.4 and 148.8, respectively, suggested oxygenation. These correlations closed the ring system and defined a 1,8-dioxygenated naphthalene (rings D and E, Figure 1).

The chemical shift of the quaternary carbon at C-1 (δ_C 111.7) was ketal-like, which, when combined with the lack of CIGAR correlations linking spin systems c and d to those of a and b, suggested that C-1 formed a spiroketal bridge (ring C) between the two isolated parts of the molecule. Support for this assignment came from a substructure search in the AntiMarin database,[8] which confirmed that the spiroketal bridge-containing spirobisnaphthalenes all showed comparable ^{13}C chemical shift data for the spiroketal center. The only correlation to C-1 was from H-3 of ring A, establishing the attachment of the spiroketal bridge to ring A. Ring B was found to be a cyclopentene-dione unit by means of the correlations from H-7 and H-8 to the carbonyls at C-6 and C-9 and the quaternary carbon at C-5. Correlations to C-5 were also seen from the H-2 and H-3 olefinic protons, confirming C-5 as a spirocenter joining the two cyclopentene rings, A and B. The oxymethine proton at H-4 also showed correlations to C-6 and C-9, confirming the proximity of ring A to ring B. This analysis of the NMR spectral features accounted for all degrees of unsaturation and all but one proton. Therefore, the oxygenation at C-4 was assigned as an hydroxyl in keeping with the observed OH stretch in the IR spectrum (3477 cm^{-1}), leading to the structural assignment of spiro-mamakone A as **1**. As a result of the near-symmetry of rings B, D, and E, the NMR assignments in these regions are interchangeable.

To confirm the spiro-nonadiene portion of spiro-mamakone A (**1**), the allylic alcohol was oxidized (MnO_2, $CHCl_3$, rt). This cleanly afforded the tri-keto analog, 4-oxo-spiro-mamakone A (**2**).[9] The nonequivalence of the ring B chemical shifts observed in the ^1H and ^{13}C NMR spectra of **1** are a consequence of the stereocenter at C-4; hence, oxidation at this stereocenter should render ring B symmetric. The NMR data for **2** confirmed that this indeed was the case. The H-7 and H-8 olefinic protons appeared as one singlet integrating for two protons at δ_H 7.00. Likewise, both the olefinic and carbonyl carbons showed identical chemical shifts of δ_C 150.8 and 194.2, respectively. Furthermore, oxidation also results in symmetrization of the intact naphthalene subunit. This is a consequence of the newly introduced mirror plane, which passes through both spiro centers, making rings D and E symmetrically equivalent. This change from C_1 to C_s symmetry is also reflected in the simplified NMR spectra of **2** and provides further support for the proposed structure of **1**.

(6) A voucher specimen (UOC-E484) has been deposited in the collection at the School of Biological Sciences, University of Canterbury, New Zealand.

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(8) A new database compiled from the MarinLit and AntiBase databases (J. W. Blunt and M. H. G. Munro, University of Canterbury, and H. Laatsch, University of Goettingen).

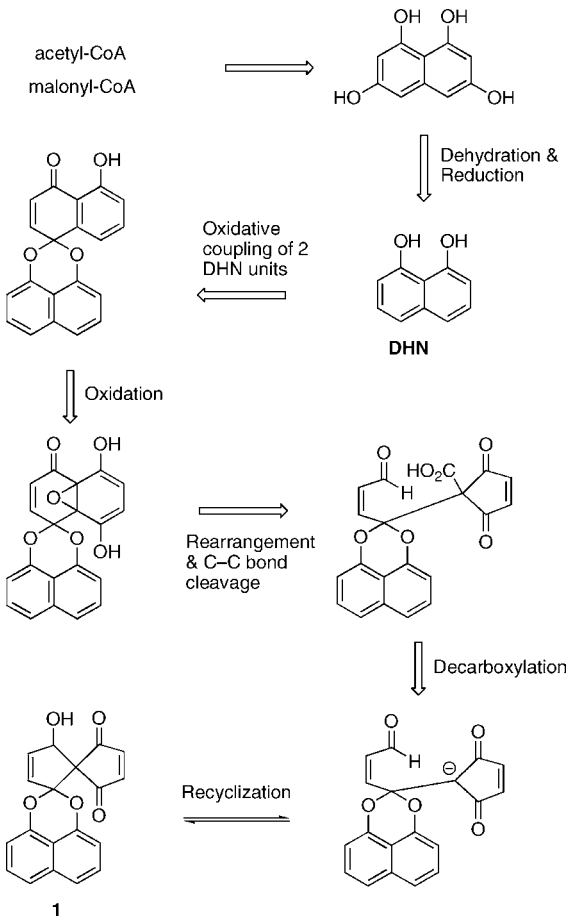
(9) Compound **2**: amorphous yellow solid; UV λ_{max} ($CHCl_3$) (log ϵ) 232.0 (3.89), 295.0 (4.04), 313.9 (3.85), 330.5 (3.66) nm; IR (KBr disk) ν_{max} 1763, 1701, 1612, 1414, 1377, 1270, 1256, 1126, 1047 cm^{-1}; $C_{19}H_{10}O_5$ by HREIMS $[M]^+$ *m*/*z* 318.0533 (calcd 318.0528); ^1H NMR ($CDCl_3$, 500 MHz) δ 7.77 (1H, d, 5.8, H3), 7.49 (2H, d, 8.3, H15/H13), 7.41 (2H, t, 8.3, 8.3, H16/H12), 7.00 (2H, s, H7/H8), 6.90 (2H, d, 8.3, H17/H11), 6.69 (1H, d, 5.8, H2); ^{13}C shifts determined from HSQC-DEPT and CIGAR experiments, δ 196.0 (C4), 194.2 (C9/C6), 157.0 (C3), 150.8 (C8/C7), 146.2 (C10/C18), 137.7 (C2), 134.0 (C14), 127.6 (C16/C12), 121.6 (C15/C13), 113.4 (C19), 110.7 (C17/C11), 105.8 (C1), 70.5 (C5).

spiro-Mamakone A (**1**) showed no optical activity and was therefore proposed to be racemic. To confirm this, (*R*)- and (*S*)-Mosher esters of the alcohol at C-4 were prepared.[10] HPLC analysis of the Mosher esters established the racemic nature of **1**. This was concluded from the identical nature of the two peaks, for each ester, which were the two diastereomeric esters formed from each of the starting (*R*)- and (*S*)-acid chlorides. ESIMS confirmed that the two diastereomeric peaks observed for each experiment were those of the Mosher's ester (*m*/*z* 559 $[M + Na]^+$).

spiro-Mamakone A (**1**) was found to show potent *in vitro* cytotoxicity (0.33 μM) toward the P388 murine luekemia cell line and was also particularly effective against the following microorganisms: *Bacillus subtilis* (12 mm), *Trichophyton mentagrophytes* (10 mm), and *Cladosporium resinae* (6 mm) in the agar diffusion assay.[11,12] 4-Oxo-spiromamakone A (**2**) was also cytotoxic (1.13 μM) in the same range as that of **1**. Inhibition of growth of the same range of microorganisms however, was significantly lower (inhibition zones of 3 mm) than that of **1**.

It is most probable that the biosynthesis of spiro-mamakone A proceeds as for the regular spirobisnaphthalenes with both halves of the molecule being derived from dihydroxynaphthalene (DHN).[13] Our working biogenetic hypothesis (Scheme 1) suggests an epoxy derivative (or equivalent) as the key intermediate. Such epoxides have been found previously among the spirobisnaphthalene metabolites.[14−18] Several steps involving rearrangement, C−C bond cleavage, decarboxylation, and recyclization could then lead to the formation of the spiro-nonadiene system. The suggested final step in the scheme is a Knoevenagel-type addition. Although in principle the *re* and *si* faces of the formyl group are distinguishable, with this combination of functionalities the isolation of spiro-mamakone A (**1**) as a racemate is not unreasonable and lends some credence to this biogenetic hypothesis.

Scheme 1. Potential biogenesis of the spiro-mamakone A (**1**) skeleton



Future work will focus on the synthesis of spiro-mamakone A and analogs for structure/activity studies within this new group. Biosynthetic studies will also be included.

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Supporting Information Available: ^{1}H, ^{13}C, HSQC-DEPT, and CIGAR NMR spectra for **1**; ^{1}H, HSQC-DEPT, and CIGAR NMR data for **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) A solution of (*S*)-MTPA chloride (5.8 μL) in pyridine (50 μL) and a small crystal of DMAP were added to spiro-mamakone A (2.5 mg). After 2 h, the solvent was evaporated, the residue dissolved in MeOH (0.5 mL), and the solution analyzed by HPLC (Phenomenex Luna C18, 250 × 4.6, 5 μm; solvents: A water + 0.05% TFA, B MeCN; linear gradient: 0 min 10% B, 2 min 10% B, 14 min 75% B, 24 min 75% B; 40 °C; 1 mL min^{-1}). To generate the (*S*)-MPTA ester, the same experimental procedure for the production of the (*R*)-MPTA ester was followed, except that (*R*)-MTPA chloride was used.

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(12) In each case, 40 μg of material was applied to each disk for antimicrobial assays.

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