Family-level stereoselective synthesis and biological evaluation of pyrrolomorpholine spirotetral natural product antioxidants†

Alyssa L. Veranoa and Derek S. Tan*ab

The pyranose spirotetral natural products pollenopyrroside A and shensongine A (also known as xylapyrroside A, ent-capparisine B) have been synthesized by stereoselective spirocyclizations of a common C1-functionalized glycal precursor. In conjunction with our previously reported syntheses of the corresponding furanose isomers, this provides a versatile family-level synthesis of the pyrrolomorpholine spirotetral natural products and analogues. In rat mesangial cells, hyperglycemia-induced production of reactive oxygen species, which is implicated in diabetic nephropathy, was inhibited by pollenopyrroside A and shensongine A with mid-μM IC50 values, while unnatural C2-hydroxy analogues exhibited more potent, sub-μM activity.

Introduction

Pyrrolomorpholine spirotetcales are a novel family of natural products that include both pyranose and furanose isomeric forms and both epimeric configurations at the anomeric carbon (Fig. 1). In 2010, three groups contemporaneously isolated acortatarins A (3) and B (6) from the rhizome of Acorus tatarinowii,1 pollenopyrroside A (1) and B (3) from the bee-collected pollen of Brassica campestris,2 and capparisines A (3) and B (ent-2) from the mature fruit of Capparis spinosa.3,4 Acortatarin A and a pyranose isomer named acortatarin C (stereochemistry not assigned) were also isolated as bitter components of whole wheat bread crust.5

More recently, the epimeric β-spirotetcales shensongine A (2) and shensongine B (3) were isolated from capsules of the anti-arrhythmic Chinese herbal medicine Shensong Yangxin, along with shensongine C (4), a C2-hydroxy congener of acortatarin A, and pollenopyrroside B (3).6 Contemporaneously, the same β-spirotetcales xylapyrroside A (2) and B (5), were isolated from the fungus Xylaria nigripes.7

The plant-derived sources of these natural products have been used in traditional Chinese medicines for the treatment of a variety of diseases.1-3,5,6 Notably, acortatarins A and B exhibit antioxidant activity in a diabetic renal cell model, inhibiting production of reactive oxygen species (ROS) and significantly attenuating hyperglycemia-induced activation of NADPH oxidase and extracellular matrix production,5,8 hallmarks of diabetic nephropathy.9-12 The xylapyrroside also have moderate antioxidant effects and inhibit t-buty liver peroxyde-induced cytotoxicity in rat vascular smooth muscle cells.7 Importantly, oxidative stress has been shown to play a critical role in the

Fig. 1 Pyrrolomorpholine spirotetral family of natural products. Structures reflect certain stereochemical revisions made subsequent to the initial isolation reports.4 Identical structures isolated from different natural sources and given different names (parentheses) are referred to herein by the first published name.
Results and discussion

Retrosynthetic analysis of pyrrolomorpholine spiroketal natural products

The isomeric nature of the pyrrolomorpholine spiroketals presents an attractive opportunity to develop a family-level synthesis that would provide access to both furanose and pyranose congeners, as well as both anomeric stereoisomers. Our laboratory has a long-standing interest in the stereocontrolled synthesis of spiroketals from glycals, with a particular focus on natural product scaffolds for use in probe and drug discovery. Analogous to our approach to the furanose acortatarins, we envisioned that syntheses of the corresponding pyranose isomers could be achieved by stereoselective spirocyclization of a pyranoglycal intermediate 7. Retrosynthetically, this glycal intermediate 7 would originate from coupling of pyrrole-2,5-dicarboxaldehyde (8) with pyranoarabinal (pyranoribial) derivative 9, both of which can be accessed from commercially-available starting materials (10, 11).

Synthesis of shensongine A via acid-catalyzed spirocyclization of pyranoarabinal intermediate 16

Thus, we synthesized pyrrole-2,5-dicarboxaldehyde (8) as previously reported. To access the pyranoglycal coupling partner 14, commercially available 3,4-di-O-acetyl-D-arabinal (11) was deacetylated then silylated to provide triisopropylsilyl-protected glycal 12 (Fig. 3). This protecting group change was necessary for efficient coupling by instead using the more stable mesylate 14b, which provided the desired coupling product 15 in 71% yield. Aqueous base was required for this N-alkylation due to the tendency of pyrrole monomers to dimerize in pyranose C1-iodomethyl glycal 14a. However, attempted coupling of the iodide 14a with pyrrole 8 under basic conditions led to poor yields of pyrrolomethylglycal 15, due to the susceptibility of the C1-iodomethyl arabinal 14a to decomposition. This is in contrast to the observed stability of the corresponding furanose C1-iodomethyl glycal in our acortatarin syntheses. However, we were able to achieve efficient coupling by instead using the more stable mesylate 14b, which provided the desired coupling product 15 in 71% yield. Aqueous base was required for this N-alkylation due to the tendency of pyrrole monomers to dimerize in
Nonaqueous conditions.\textsuperscript{20} Monoreduction of the dialdehyde\textsuperscript{21} with NaBH\textsubscript{4} gave the pivotal spirocyclization precursor 16, from which pollenopyrroside A and shensongine A would be accessed. Careful monitoring of the reaction allowed for minimal over-reduction and minimal remaining starting material.

Treatment of arabinal 16 with catalytic Brønsted acids led to exclusive formation of the thermodynamic $\beta$-spiroketal 17 (Fig. 4). Desilylation furnished shensongine A (2, xylapyrroside A, ent-capparisine B), whose optical rotation, NMR, and high-resolution mass spectral data matched those reported for the natural products.

Attempted synthesis of pollenopyrroside A via mercury-mediated spirocyclization of arabinal intermediates 16 and 18

In contrast, stereoselective access to the $\alpha$-anomer, pollenopyrroside A, proved to be more challenging due to the inherent thermodynamic preferences of this [6,6]-spiroketal system. Formation of the contrathermodynamic $\alpha$-anomer was overwhelmingly disfavored under numerous spirocyclization conditions evaluated.\textsuperscript{22} By analogy to our previous work in the corresponding furanoribal-derived [6,5]-spiroketal system,\textsuperscript{14} we first attempted Hg-mediated spirocyclization of pyranoarabinal 16, expecting preferential anti-mercuration to form $\beta$-mercurnium intermediate 19, followed by stereoinvertive cyclization to form an $\alpha$-spiroketal intermediate (not shown), which could be reduced to the desired $\alpha$-spiroketal 23 (Fig. 5). However, under these conditions, we instead observed exclusive formation of the $\beta$-spiroketal 17. We attributed this undesired selectivity to steric conflicts between the bulky 3- and/or 4-O-TIPS groups and the morpholine ring en route to the $\alpha$-anomer 23. In addition, the 3-O-TIPS protecting group would preclude an intramolecular hydrogen bond with the morpholine oxygen suggested by the crystal structure of pollenopyrroside A,\textsuperscript{2} which may stabilize the $\alpha$-configuration. To avoid these issues, we attempted Hg-mediated spirocyclization with the fully deprotected glycal substrate 18. However, the undesired $\beta$-anomer 2 was again formed exclusively. We attributed this undesired stereoselectivity to syn-mercuration directed by the free C3-hydroxyl group to form the corresponding $\alpha$-mercurinium intermediate 21, followed by stereoinvertive cyclization to $\beta$-spiroketal intermediate 22a. Isolation and NMR analysis of the intermediate 2-mercurial spiroketals as the corresponding chlorides 20\textsubscript{b} and 22\textsubscript{b} provided stereochemical assignments consistent with these mechanistic interpretations.\textsuperscript{23} Use of other mercury salts and less-hindered protecting groups, including a conformationally restricted cyclic carbonate, did not improve stereoselectivity (Table S1\textsuperscript{†}).\textsuperscript{22} Synthesis of pollenopyrroside A via metal-catalyzed spirocyclization of arabinal intermediate 18

Based on the intramolecular hydrogen bond between the 3-hydroxyl group and morpholine oxygen postulated from the crystal structure of pollenopyrroside A,\textsuperscript{2} we next pursued a chelation-based approach to favor formation of the desired $\alpha$-spiroketal (Table 1). Such metal-chelation approaches have been used previously to access contrathermodynamic spiroketales.\textsuperscript{24} Treatment of $\beta$-spiroketal 2 with various metal salts at reflux in CH\textsubscript{3}CN or dioxane over 24 h had no effect upon the $\alpha$ : $\beta$ ratio (Table S2\textsuperscript{†}).\textsuperscript{22} Treatment of the glycal

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure5.png}
\caption{Attempted synthesis of pollenopyrroside A (1) via mercury-mediated spirocyclization. HMDS = hexamethyldisilazide.}
\end{figure}
Table 1 Metal chelation-based spirocyclization approaches to pollenopyrroside A

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Equiv.</th>
<th>dr (α : β)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MgCl₂</td>
<td>3.0</td>
<td>n.r.b</td>
</tr>
<tr>
<td>2</td>
<td>ZnCl₂</td>
<td>3.0</td>
<td>n.r.</td>
</tr>
<tr>
<td>3</td>
<td>Ti(O-iPr)₄</td>
<td>2.0</td>
<td>Decomp.c</td>
</tr>
<tr>
<td>4</td>
<td>Sc(OTf)₃</td>
<td>3.0</td>
<td>60 : 40d</td>
</tr>
<tr>
<td>5</td>
<td>Sc(OTf)₃ + DTBMPf</td>
<td>3.0</td>
<td>n.r.</td>
</tr>
<tr>
<td>6</td>
<td>TIOH</td>
<td>0.2</td>
<td>0 : 100</td>
</tr>
<tr>
<td>7</td>
<td>ScCl₃</td>
<td>3.0</td>
<td>n.r.</td>
</tr>
<tr>
<td>8</td>
<td>ScCl₃ + TIOHf</td>
<td>3.0</td>
<td>60 : 40</td>
</tr>
<tr>
<td>9</td>
<td>MgCl₂ + TIOHf</td>
<td>3.0</td>
<td>50 : 50</td>
</tr>
<tr>
<td>10</td>
<td>ZnCl₂ + TIOHf</td>
<td>3.0</td>
<td>50 : 50</td>
</tr>
</tbody>
</table>

a Determined by 1H-NMR. b n.r. = no reaction. c Decomp. = decomposition of starting material. d 25% isolated yield of α-spiroketal 1. e 1.0 equiv. DTBMP. f 0.5 equiv. TIOH; DTBMP = 2,6-di-tert-butyl-4-methylpyridine.

The spirocyclization precursor 18 with MgCl₂, ZnCl₂, or Ti(O-iPr)₄ led to no reaction or decomposition (Table 1, entries 1–3). However, treatment of glycal 18 with Sc(OTf)₃ led to a 60 : 40 ratio of diastereomeric spiroketal products, favoring the desired α-anomer 1 (entry 4). We have recently used Sc(OTf)₃ in stereoselective spirocyclizations of exo-glycal epoxides to form benzannulated spiroketaloids. In those reactions, solvent played a dramatic role, with the catalyst acting as a Lewis acid in THF but as a mild source of triflic acid in CH₂Cl₂. To assess the mechanistic basis for the observed selectivity in this substrate system, we carried out a control experiment with DTBMP (2,6-di-tert-butyl-4-methylpyridine) as an acid scavenger, but no reaction occurred (entry 5), suggesting that the catalyst might, indeed, be serving as a triflic acid source. However, when the reaction was carried out with TIOH alone, we observed complete diastereoselectivity for the undesired β-spiroketal (2) (entry 6). Moreover, no reaction was observed upon treatment with ScCl₃ alone (entry 7). In contrast, treatment of glycal 18 with both ScCl₃ and TIOH together (entry 8) recapitulated the 60 : 40 dr observed with Sc(OTf)₃ (entry 4). Notably, combination of MgCl₂ or ZnCl₂ with TIOH also afforded a 50 : 50 mixture of the two diastereomeric spiroketals 1 and 2. Taken together, these results suggest that the α-spiroketal 1 is formed under kinetic control, and that both a Lewis acid and Brønsted acid are required to overcome the inherent complete selectivity for the β-spiroketal 2. This is in contrast to our previous study with exoglycal epoxides, in which Sc(OTf)₃ served as either a Lewis acid or a Brønsted acid source exclusively, depending upon solvent selection. Unfortunately, the diastereomeric mixture was difficult to separate, resulting in only a 25% isolated yield of pollenopyrroside A (1).

Synthesis of pollenopyrroside A via methanol-catalyzed kinetic spirocyclization of arabinial intermediate 16

Thus, to provide more stereoselective access to pollenopyrroside A (1), we turned to our previously reported methanol-catalyzed kinetic spirocyclization of glycal epoxides. While we recognized that this approach would introduce the need for deoxygenation of the resulting 2-hydroxyl group, this was balanced by the much higher stereoselectivity expected with this spirocyclization. In addition, this epoxidation–spirocyclization strategy would provide access to the non-natural 2-hydroxypryanose analogues for biological evaluation, complementing the known 2-hydroxy natural products in the furanose series (4, 6). Accordingly, anti-epoxidation of glycal 16 with DMDO was followed by spirocyclization with inversion of configuration in methanol to give the desired α-spiroketal 24 in >98 : 2 dr (Fig. 6a). Desilylation of 24 provided a 2-hydroxy analogue of pollenopyrroside A, 25, for biological evaluation below. Alternatively, Barton–McCombie deoxygenation of 24 at the 2-position and desilylation provided pollenopyrroside A (1) in 46% yield over four steps from glycal precursor 16.

For completeness, we also carried out a stereocomplementary spirocyclization of the glycal epoxide derived from 16 using our previously established Ti(O-iPr)₄-catalyzed kinetic spirocyclization with retention of configuration to provide the corresponding β-spiroketal 27 with complete β-diastereoselectivity (Fig. 6b). Desilylation then provided the 2-hydroxy analogue of shensongine A, 28, for biological evaluation below.

Acid equilibration studies of natural products 1 and 2 and 2-hydroxy analogues 25 and 28

Acid equilibration experiments under aqueous conditions showed that pollenopyrroside A (1) was kinetically stable at pH ≥ 5, but underwent complete conversion to its C1-epimer shensongine A (2) at pH ≤ 4. Conversely, shensongine A was stable down to pH 1, indicating that this β-spiroketal is thermodynamically favored (Fig. 6c). In the 2-hydroxypryanose series, both 25 and its C1-epimer 28 were kinetically stable down to pH 1. Accordingly, epimerization would not be expected for any of these compounds in biological assays under physiologic conditions at pH 7.4.

Evaluation of antioxidant activity of pyrrolomorpholine spiroketals

Previous reports have demonstrated the antioxidant activity of acortatarin A against high glucose-induced oxidative stress in rat mesangial cells suggesting that this scaffold may have therapeutic potential for the treatment of diabetic nephropathy. Thus, we evaluated the antioxidant activity of the complete D-enantiomeric family of furanose and pyranose pyrrolomorpholine spiroketal natural products and analogues. Intracellular ROS levels were measured using the cell-permeable 2',7'-dichlorofluorescein diacetate (DCFH-DA), which is converted to the highly fluorescent 2',7'-dichlorofluorescein (DCF)
Rat mesangial cells are highly sensitive to hyperglycemic conditions, and a significant increase in ROS was observed after 3 h exposure to 30 mM d-glucose. Inhibition of high glucose-induced oxidative stress was evaluated by concomitant treatment with the pyrrolomorpholine spiroketalts, or with 1 mM acetylcysteine, a precursor of the antioxidant glutathione, as a positive control. Consistent with the previous report, acortatarins A (3) and B (6) reduced high glucose-induced ROS generation in a dose-dependent manner, with IC_{50} values of 4.6 and 11 μM, respectively, returning ROS levels to that of normal glucose conditions (Table 2, entries 2, 3). Consistent with the previous report, acortatarins A (3) and B (6) reduced high glucose-induced RO...
Strikingly, the 2-hydroxy analogues natural products pollenopyrroside A (mesangial cells indicated similar activities for the anomeric action of these compounds and additional analogues are ongoing and will be reported in due course.

This strategy that required subsequent C2-deoxygenation to a natural product, this route also provided convenient access to the corresponding 2-hydroxy analogues, which have not yet been described in the pyranose series but are known as natural products in the isomeric furanose series (i.e.: 4, 6). Evaluation of antioxidant activity against hyperglycemia-induced ROS in rat mesangial cells indicated similar activities for the analogues are potent, sub-

isomers, and led to the discovery of novel 2-hydroxy analogues which may be useful for mechanistic studies, including target identification efforts. Further investigation into the mechanisms of action of these compounds and additional analogues are ongoing and will be reported in due course.

Acknowledgements

We thank Prof. Anthony Sauve (Weill Cornell Medicine) for helpful discussions and access to cell culture facilities, Dr Kristin Kirschbaum (University of Toledo) for helpful discussions, and Dr George Sukenick and Dr Rong Wang, (MSK) for helpful discussions and access to cell culture facilities, Dr

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Several of the original stereochemical assignments have been revised based on total synthesis and/or X-ray crystallographic analysis using CuKα radiation, which enables assignment of absolute configuration. The revised structures based on current understanding are shown in Fig. 1. See ESI† Section F for a complete discussion and relevant references.

Notes and references

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12 A. tatarinowii root (50 kg) yielded 7.3 mg acortatarin A (0.000015 wt%) and 3.4 mg acortatarin B (0.000007 wt%) (ref. 1); B. campestris pollen (15 kg) yielded 6 mg pollonpyrroside A (0.000040 wt%) and 5 mg pollonpyrroside B (acortatarin A) (0.000033 wt%) (ref. 2); C. spinosa powdered fruits (10 kg) yielded 4 mg capparisine A (acortatarin A) (0.000040 wt%) and 5 mg capparisine B (0.000050 wt%) (ref. 3); powdered Shensong Yangxin (2 kg) yielded 8.1 mg shensongine A (0.000045 wt%), 2.8 mg shensongine B (0.000140 wt%), 15.5 mg shensongine C (0.000775 wt%), as well as 14.8 mg pollonpyrroside B (0.000740 wt%) (ref. 6); X. nigripes mycelium (20 kg) yielded 4.0 mg xylapyrroside A (0.000020 wt%) and 21.3 mg xylapyrroside B (0.000107 wt%), as well as 1.2 mg pollonpyrroside A (0.000006 wt%) and 20.1 mg acortatarin A (0.000100 wt%) (ref. 7).


22 See ESIF for complete details.

23 NMR analysis of the 2-mercurial intermediates, isolated after brine workup prior to NaBH₄ reduction, indicated that substrate 16 undergoes anti-mercuration, while 18 undergoes syn-mercuration, presumably due to directing effects of the free hydroxyl groups.

![Diagram](image-url)


31 Ref. 7 provided shensongine A in 13.7% yield over 11 steps, with the key spirocyclization proceeding in >98:2 diastereoselectivity to the thermodynamic β-spiroketal. Ref. 13f provided pollenenpyrroside A in 2.7% yield and shensongine A in 8.8% yield over 14 steps with the key spirocyclization proceeding in 1:3 α/β dr. Ref. 13g provided pollenenpyrroside A in 5.6% yield and shensongine A in 0.79% yield over 11 steps, with the key spirocyclization proceeding in 1:2 α:β dr as an inseparable mixture of unassigned diastereomers.