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Synthesis and structural revision of calafianin, a member of the spiroisoxazole family isolated from the marine sponge, *Aplysina gerardogreeni*

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Abstract—Calafianin **1** was successfully synthesized by employing a spiroisoxazoline compound, which was produced by electrochemical oxidation of the oximino-phenol derivative **7** followed by reduction. This investigation revealed a structural revision of **1**, the *trans*-relationship of two oxygen atoms between the epoxide and the isoxazoline.

Calafianin **1**,[1] isolated from the sponge, *Aplysina gerardogreeni* n. sp (Aplysinidae), is a member of such spiroisoxazoline natural products as aerothionin **2**,[2] homoaerothionin **3**, and aerophobin 1 **4** (Fig. 1).[3] In contrast to diverse biological activities of the spiroisoxazolines involving antimicrobial, cytotoxic, and antiinflammatory activities, calafianin **1** has been reported not to have significant biological activity. Previously, **1** and **2** were examined against multidrug-resistant clinical isolates of *M. tuberculosis* H37Rv,[4] whereas **2** was potently active, **1** showed no significant activity in spite of its closely related structure to **2**. No activity will make it possible to express a specific activity against new bioassay system. Accordingly, such natural product will be a promising candidate possessing high biological selectivity. Against such background, synthetic studies toward **1** were initiated for acquisition of further biological information.

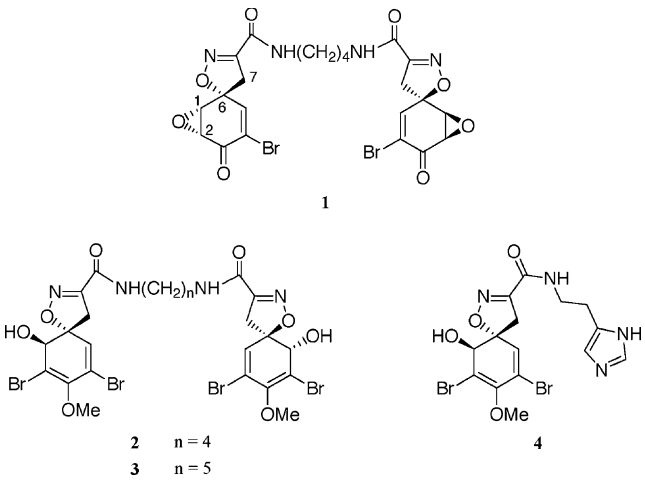

Figure 1. Proposed structure of calafianin **1** and related natural products.

Calafianin **1** consists of the spiroisoxazoline moiety and the cyclohexenone carrying the epoxide. The relative stereochemistry of the C-1, C-2, C-6 centers on the cyclohexenone was deduced to possess the *cis*-relationship of two oxygen atoms between the epoxide and the isoxazoline by the NOE correlation between H-1 and H-7 protons.[1]

In this context, we have accomplished the synthesis of these spiroisoxazoline natural products employing phenolic oxidation of oximino-phenol **7** with TTN[5] and anodic oxidation[6] as key steps. The following $Zn(BH_4)_2$ reduction of spirodienone **8** produced, provided the spiroisoxazoline derivatives involving natural *trans*-form **5** and unnatural *cis*-form **6** (Scheme 1).[7]

Keywords: *Aplysina gerardogreeni*; Calafianin; Spiroisoxazoline; Bromophenylpyruvic acid.
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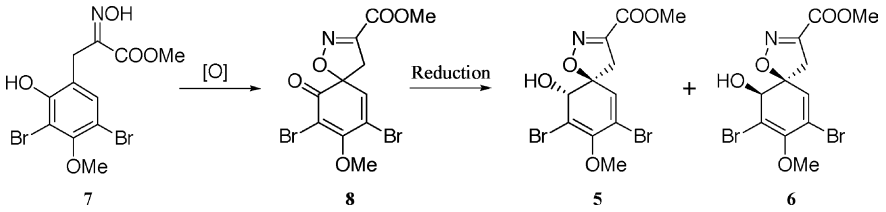


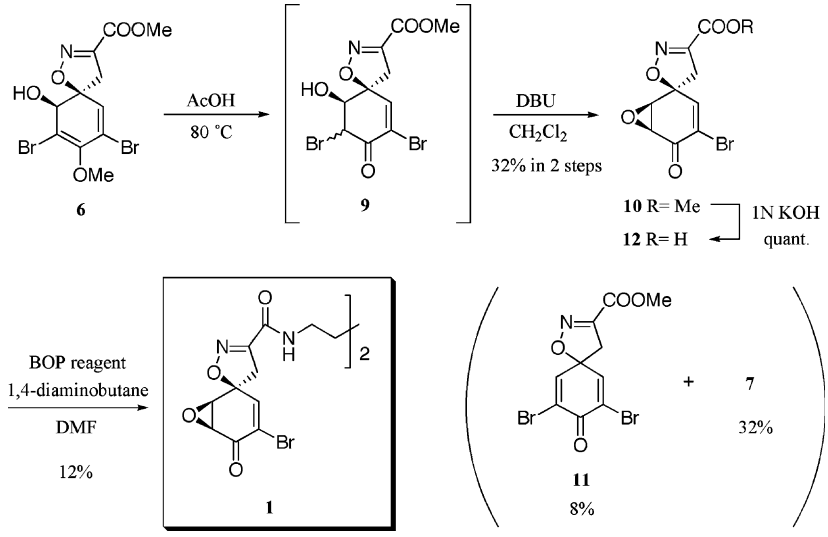
Scheme 1. Synthesis of the spiroisoxazoline structure.

We describe herein the findings obtained in the synthesis of calafianin **1** by utilizing the *trans*- and *cis*-spiroisoxazolines **5** and **6**.

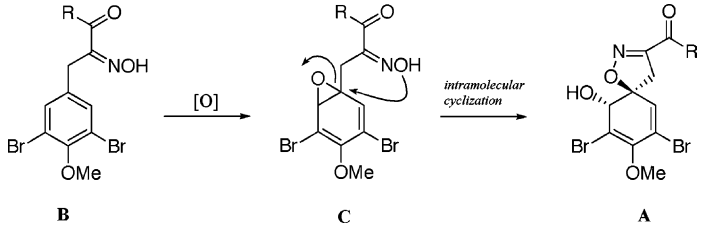
At the outset, synthesis of methyl ester **10** carrying a partial-structure of calafianin **1**, was performed. The *cis*-spiroisoxazoline **6** was submitted to cleavage of methyl enol ether under acidic conditions to give **9**, which was basified with DBU to afford the epoxy derivative **10** in 32% yield (Scheme 2). A main reason of the low yield might be the labile property of the *cis*-spiroisoxazoline **6** under the acidic or basic conditions, which gave the spirodienone **11**[8] by dehydration, along with **7** by a ring-opening of the isoxazoline moiety as byproducts. However, this synthetic approach starting from *cis*-alcohol **6**, might be an effective suitable method for stereoselective construction of the epoxide moiety. The proposed structure of calafianin **1**[9] was successfully obtained by condensation of carbonic acid **12** with 1,4-diaminobutane in 12% yield.[13]

Unfortunately, comparison of the ^{1}H NMR data of the synthetic sample with reported data[1] indicated a clear difference in the region of H-2, H-5, and H-7. Christophersen et al. mentioned determination of the relative stereochemistry, based on the spectroscopic evidence: in particular, the NOE correlation between H-1 and H-7 indicated that the H-1 proton exists at the same side of C-7. In contrast, a series of spiroisoxazoline natural products, such as aerothionin **2**, homoaerothionin **3** and aerophobin-1 **4**, have a *trans* vicinal relationship between a hydroxy group and an oxygen atom in the spiroisoxazoline unit (Fig. 1). A hypothesis of the biosynthesis indicated that spiroisoxazoline **A** is prepared from *p*-methoxy phenyl derivative **B** by epoxidation, followed by intramolecular nucleophilic attack to the epoxide of the arene oxide **C** (Scheme 3).[10]

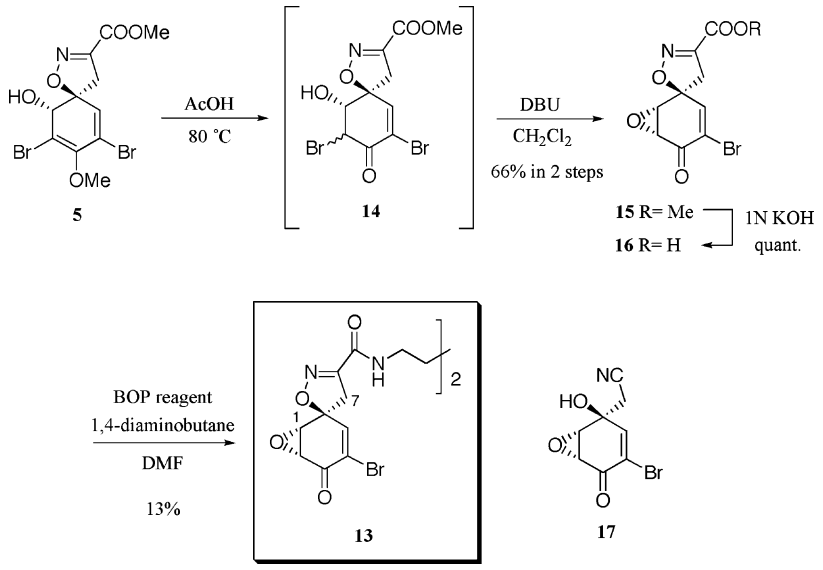
On the basis of these considerations, we expected **1** might possess the relative stereochemistry of **13**, which has *trans*-relationship between the epoxy group and



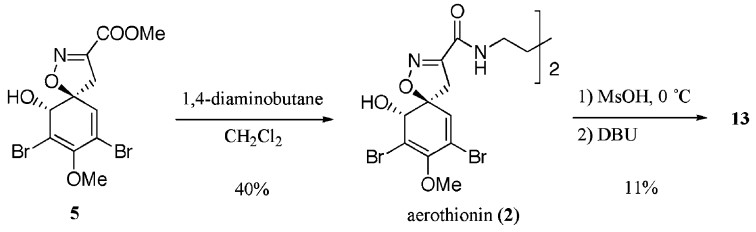
Scheme 2. Synthesis of proposed structure of calafianin **1**.



Scheme 3. Biosynthetic hypothesis of natural spiroisoxazolines.



Scheme 4. Synthesis of **13**, revised structure of calafianin **1**.



Scheme 5. Synthesis of **13**, prepared through aerothionin **2**.

the oxygen atom in the spiroisoxazoline ring, if this natural product was prepared through the same biosynthetic cascade as those of other spiroisoxazolines. This hypothesis prompted us to synthesize **13** from **5**.

The spiroisoxazoline **5** was treated under acidic conditions to give the bromohydroxy derivative **14** as a diastereomeric mixture (ca. 2:1). The epoxy derivative **15**,[11] which has *trans*-relationship between two oxygen atoms, was synthesized from **14** by intramolecular cyclization under DBU conditions in high yield, without production of spirodienone **11** as a by-product. The carbonic acid **16**, prepared from **15** with hydrolysis, was submitted to the same procedure as in the case of **1** to afford **13**,[12,13] the spectral data of which was superimposable to those reported.[1,14] Especially, NOE correlation between H-1 and H-7 was observed. Accordingly, the relative stereochemistry of calafianin **1** should be revised to **13**, as shown in Scheme 4. Alternatively, **13** was obtained from aerothionin **2**,[2] which was prepared from **5**[5a,b] in two steps (Scheme 5). In this reaction, preparation of **2** under acidic conditions succeeded upon using strong acid, methane sulfonic acid, while acetic acid effected no reaction.

In conclusion, the proposed structure of calafianin **1** and its stereo isomer **13** were successfully synthesized by using the spiroisoxazolines **5** and **6** as an efficient demonstration of conversion of spiroisoxazoline structure into other framework of natural product. This synthesis enabled the structural revision of **1** to **13**, which has *trans*-relationship between the epoxy group and the oxygen atom in the spiroisoxazoline ring.

Acknowledgements

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9. Synthetic sample of **1**: IR (film) 3375, 1703, 1666 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 1.52 (4H, br s), 3.19 (4H, br s), 3.41 (2H, d, J = 18.4 Hz), 3.56 (2H, d, J = 18.4 Hz), 3.81 (2H, d, J = 4.0 Hz), 4.12 (2H, d, J = 2.8, 4.0 Hz), 7.29 (2H, d, J = 2.8 Hz), 8.61 (2H, t, J = 6.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 26.3, 38.5, 43.3, 51.8, 55.0, 85.4, 119.7, 144.2, 154.6, 159.7, 185.8.
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12. Compound **13**: IR (film) 3410, 1701, 1670 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 1.48 (4H, br s), 3.20 (4H, br s), 3.60 (2H, d, J = 18.0 Hz), 3.67 (2H, d, J = 18.0 Hz), 3.92 (2H, d, J = 3.6 Hz), 4.12 (2H, dd, J = 2.4, 4.0 Hz), 7.48 (2H, d, J = 2.4 Hz), 8.64 (2H, t, J = 6.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 26.3, 38.5, 43.3, 52.9, 56.8, 83.9, 122.7, 143.7, 154.8, 158.2, 185.9.
13. The reason of the low yield of this condensation reaction may be the instability of carbonic acid **12** or **16**. In the case of **16**, the nitrile **17** was easily produced by decarboxylation and the following heterolysis of the N–O bond, as a by-product. Improvement of the condition is in progress.
14. Synthetic product **13** might be a diastereomer mixture. Unfortunately, up to now we have not been able to accomplish their separation. This problem was also encountered in the synthesis of aerothionin **2**.[5]