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## Bio-inspired synthesis of xishacorenes A, B, and C, and a new congener from fuscoldagger

Alexander R. Rovira,<sup>a</sup> Nicolas Müller,<sup>a</sup> Weiwen Deng,<sup>b</sup> Chudi Ndubaku<sup>b</sup> and Richmond Sarpong<sup>a\*</sup>

The xishacorene natural products are structurally unique apolar diterpenoids that feature a bicyclo[3.3.1] framework. These secondary metabolites likely arise from the well-studied, structurally related diterpenoid fuscold. In this manuscript, we describe the conversion of fuscold to xishacorenes A, B, and C, as well as a previously unreported congener, which we have named xishacorene D. In addition, we describe immunomodulatory activity studies of the xishacorenes, a structurally related analogue, and fuscold. These studies were aided by an accurate determination of the physical properties (e.g., molar extinction coefficient) of the highly apolar xishacorenes.

## Introduction

Polyene cyclizations are among the most powerful methods for the rapid construction of C–C bonds in carbon-based frameworks. These cyclizations, which appear in the biosyntheses of a wide array of topologically complex natural products, often proceed through concerted, stereocontrolled pathways.<sup>1</sup> Of the natural products formed through polyene cyclizations, terpene-derived secondary metabolites (terpenoids) are among the most studied. While the biosynthetic pathways that lead to many terpenoid molecules have formed the basis for their chemical syntheses, synthetic endeavors toward terpenoids that exploit polyene cyclizations can provide insights into the role of these reactions in their biosynthesis as well.<sup>2</sup>

The xishacorene diterpenoids (see 1–3, Fig. 1), were isolated in 2017 from the soft coral *Sinularia polydactyla* off the coast of the Xisha Islands in the South China Sea.<sup>3</sup> These secondary metabolites were obtained alongside a structurally-related terpenoid, fuscold (5), from which 1–3 are proposed to arise through an acid-mediated polyene cyclization (Fig. 1).<sup>3</sup> Fuscold was first isolated in 1978 by Schmitz and coworkers.<sup>4</sup> In considering the biogenesis of the xishacorenes, fuscold appears to be the likely progenitor. However, the link between these natural products has not been realized in a laboratory setting.<sup>5</sup> Furthermore, as outlined by the cyclization sequence illustrated in Fig. 1, one would have expected the isolation of compound 4, a diastereomer of 3, which we have named “xishacorene D”, alongside the other xishacorene congeners.

Evidence that the xishacorenes can arise from fuscold would provide key support for a unified biosynthesis of these natural products from geranylgeranyl pyrophosphate (GGPP, 8), as it has been shown that 5 can arise from eunicol (6) through a Cope rearrangement.<sup>6</sup>

Fuscold and its glycosylated derivatives (e.g., fuscoldside B, 8, Fig. 2A) possess significant anti-inflammatory activity.<sup>7</sup> Given their putative biosynthetic connections, analogous activity might be anticipated for the xishacorenes. A preliminary biological screening of 1–3 by Guo *et al.* revealed that these molecules possess notable activity as promoters of concavalin A (ConA)-induced T-lymphocyte proliferation.<sup>3</sup> This observed activity supports a possible link between fuscold and the xishacorenes as immune modulators.<sup>8</sup> However, the capacity of the xishacorenes to act as antagonists of immunological pathways has not previously been studied.<sup>9</sup>

In 2018, we reported the first total synthesis of xishacorene B from *R*-(–)-carvone as part of a program aimed at the synthesis of structurally complex molecules from inexpensive “chiral pool” precursors.<sup>10,11</sup> While our synthetic plan provided access to 2, it was not easily extended to the syntheses of the other xishacorenes, especially 3, which possesses an *exo* methylene group that would be difficult to install using our previously reported approach. It has been recognized that fuscold, which has been synthesized by several groups,<sup>12</sup> could serve as a precursor to all xishacorene congeners. Access to the xishacorenes from fuscold would not only provide support for their proposed biosynthesis, but also facilitate further investigations into their bioactivity.

Herein, we report a preparation of the known xishacorenes (i.e., A–C; 1–3) and the previously unreported congener xishacorene D (4), along with a fuscold-like analog (10, Fig. 2B). Furthermore, we provide revised physical data for the xishacorene natural products and biological assays of 1–5 and 10.

<sup>a</sup>Department of Chemistry, University of California, Berkeley, California 94720, USA.  
E-mail: rsarpong@berkeley.edu

<sup>b</sup>Aduro Biotech, Inc., 740 Heinz Ave, Berkeley, California 94720, USA

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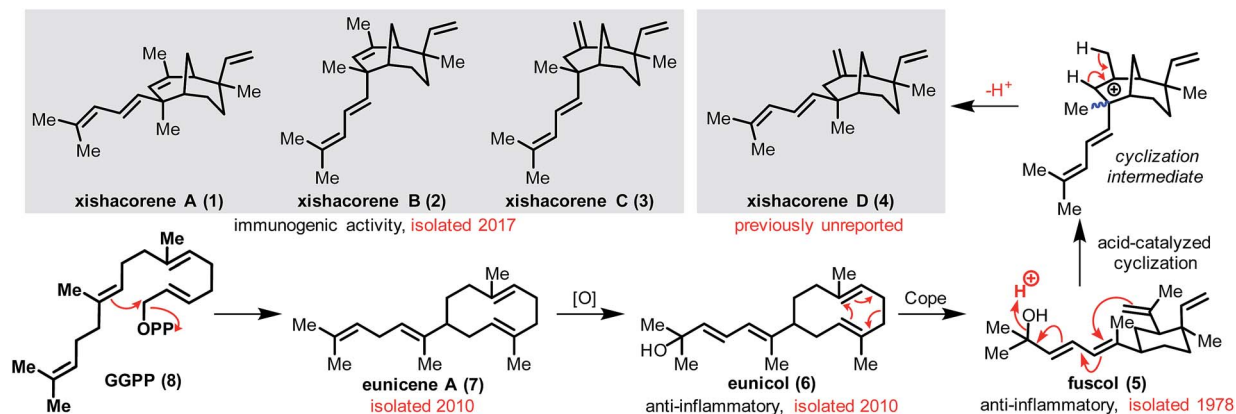


Fig. 1 Proposed biosynthesis of the xishacorene family from GGPP.

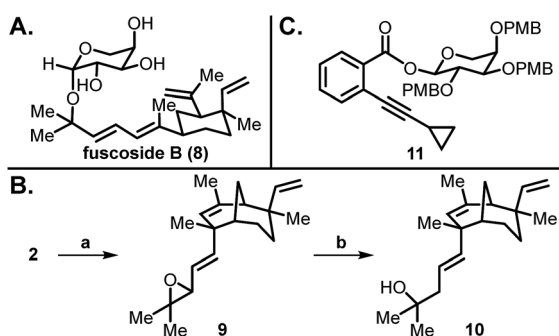


Fig. 2 (A) Structure of fuscoidin B; (B) Fuscol analogue **10** synthesis; (C) Alkyne-arabinose **11**. Conditions: (a) *m*-CPBA, DCM, 0 °C, 2 h (80% yield); (b) LiAlH<sub>4</sub>, THF, 0 °C, 4 h (87% yield).

## Results and discussion

### Synthesis of analogues of the xishacorenes

Our studies that led to the preparation of all the xishacorenes commenced with an initial goal of obtaining a library of glycosylated derivatives of fuscol and xishacorene B for biological screenings. For this purpose, we synthesized a hydroxylated xishacorene B-derived analogue of fuscol (**10**, Fig. 2B) starting from **2**, which we had previously prepared.<sup>11</sup> Epoxidation of **2** with *m*-CPBA gave epoxide **9** as a mixture of diastereomers, which upon reduction with LiAlH<sub>4</sub> yielded tertiary alcohol **10** (70% yield over two steps). With **10** in hand, we then sought to prepare fuscoidin B (**8**), which would enable us to test the glycosylation of these tertiary alcohols including **10**. While there have been several attempts to prepare the fuscoidins,<sup>13</sup> only the undesired β-anomer of **8** has been synthesized in low yield. We posited that modern glycosylation strategies, such as those developed by Yu *et al.*,<sup>14</sup> using gold catalysis with an alkyne glycoside donor (*e.g.* **11**, Fig. 2C), would enable mild, relatively neutral glycosylation conditions to access **8**.<sup>15</sup> The fuscol (**5**) required for these studies was prepared in 14 steps from *S*-(–)-verbenone by adapting the synthetic procedures reported by Kato *et al.*<sup>12a</sup>

### An unexpected cyclization of fuscol

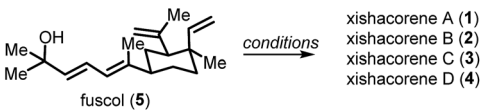
Upon treatment of **5** with glycoside **11** at 23 °C using the standard Yu glycosylation conditions (Table 1, entry 1), we observed a complex mixture of products, which co-eluted as a single, apolar band by normal-phase chromatography. Using reversed-phase chromatography, we unexpectedly isolated small quantities of the xishacorenes along with several unidentifiable decomposition products. Upon lowering the reaction temperature to –20 °C, xishacorenes A–C (**1**, **2**, and **3**), as well as the previously unreported diastereomer **4**, were isolated (Table 1, entry 2) in appreciable yields, with **3** as the major product. Following the screening of various acid conditions (Table 1, entries 5–10), we found the use of BF<sub>3</sub>·OEt<sub>2</sub> at –78 °C to be optimal, giving the xishacorenes in a combined 49% isolated yield after 15 minutes (Table 1, entry 8). Intriguingly, cyclization was not observed using the triflimide gold complex in the absence of glycoside **11**,<sup>16</sup> nor did the desired cyclization occur with bis(trifluoromethane)sulfonimide (Tf<sub>2</sub>NH) alone.<sup>17</sup>

### Analysis of the physical properties of the xishacorenes

Following our preparation of **1–4**, we sought to verify their reported physical properties. The originally reported molar extinction coefficient ( $\epsilon$ ) values for each of the xishacorenes varied significantly (by factors of 10–20), which was surprising given their close structural similarity. Likewise, the reported  $\epsilon$  value for xishacorene B is unexpectedly high (147 910 M<sup>–1</sup> cm<sup>–1</sup>), and is more characteristic of a highly conjugated  $\pi$  system.<sup>18</sup> As  $\epsilon$  values are particularly important for the accurate determination of solution concentrations for biological studies, we remeasured these values in cyclohexane before pursuing bioactivity assays. We found the corresponding  $\epsilon$  values to equal 9448, 11 145, 18 594, and 9721 M<sup>–1</sup> cm<sup>–1</sup> for **1**, **2**, **3**, and **4** respectively. We also measured  $\lambda_{\text{max}}$  values for these compounds and obtained values consistent with what would be anticipated for these molecules (239–240 nm for **1–4** in cyclohexane).<sup>19</sup> We hypothesize that the inconsistencies between the previously reported values and those we measured may arise from the insolubility of these secondary metabolites in methanol, which had been used as the solvent in the original report.



Table 1 Fuscol cyclization

			
Entry	Conditions <sup>a</sup>	Yield	Ratio (1 : 2 : 3 : 4)
1	<b>11</b> , Ph <sub>3</sub> PAuNTf <sub>2</sub> (20 mol%), 4 Å MS, rt	Trace	—
2	<b>11</b> , Ph <sub>3</sub> PAuNTf <sub>2</sub> (20 mol%), 4 Å MS, −20 °C	40%	1 : 1 : 2.5 : 1.25
3	<b>11</b> , Ph <sub>3</sub> PAuNTf <sub>2</sub> (20 mol%), 4 Å MS, −78 °C	n.r.	—
4	<b>11</b> , (4-CF <sub>3</sub> C <sub>6</sub> H <sub>4</sub> ) <sub>3</sub> PAuCl (20 mol%), 4 Å MS, rt	n.r.	—
5	Ph <sub>3</sub> PAuNTf <sub>2</sub> (20 mol%), 4 Å MS, rt	n.r.	—
6	Tf <sub>2</sub> NH (20 mol%), rt	n.r.	—
7	Tf <sub>2</sub> NH (1 equiv.), −78 °C	Decomp.	—
8	BF <sub>3</sub> ·OEt <sub>2</sub> (1 equiv.), −78 °C	49%	1.5 : 1 : 3 : 1.5
9	BF <sub>3</sub> ·OEt <sub>2</sub> (1 equiv.), Et <sub>2</sub> O, −78 °C	Decomp.	—
10	SnCl <sub>4</sub> (1 equiv.), −78 °C	Decomp.	—
11	Silica, rt <sup>b</sup>	n.r.	—
12	TFA, 0 °C <sup>b</sup>	Decomp.	—
13	CSA, 0 °C <sup>b</sup>	Decomp.	—

<sup>a</sup> All reactions run in DCM unless otherwise noted. <sup>b</sup> Previously reported.

A lack of complete dissolution of the xishacorenes could explain the significant variations of the reported values from those we measured. Similarly, as noted in our previous report,<sup>11</sup> our measured optical rotation of synthetic xishacorene B differed from that reported for the naturally isolated material ( $[\alpha]_D^{20}$  of −11.3 reported, whereas we had found it to be −19.5 measured). We observed similar deviations for **1** and **3** as well and have provided updated  $[\alpha]_D^{20}$  values obtained using CHCl<sub>3</sub> as the solvent (see the ESI†).<sup>20</sup>

### Preliminary biological assays

With the revised analytical properties of the xishacorenes determined, we undertook a series of biological assays to ascertain their bioactivity. Initial cell viability studies using THP1-Dual™ reporter cells with each of the assayed compounds confirmed that the majority of cells survived at 100 μM, whereas we observed dramatic cell death at 1 mM, as determined by a CellTiter-Glo® assay (Fig. 3A). In order to determine the immunogenic activity of the xishacorenes, THP-

1-Dual™ cells featuring a secreted luciferase reporter to monitor activity of the IRF pathway were used. Each of the assayed compounds was dissolved in DMSO to create stock solutions. These solutions were then added to the media prior to incubation with or without a known STING (stimulator of interferon genes) agonist (ML RR-S2 cGAMP).<sup>21</sup> After 24 hours of incubation, none of the assayed compounds emerged as antagonists of the IRF pathway, as monitored by luciferase expression. However, fuscol and hydroxylated xishacorene B derivative **10** were found to inhibit IRF activity induced by ML RR-S2 cGAMP (Fig. 3B). These observations suggest that the hydrophilic nature of fuscol and the fuscol-like analogue, unlike the highly apolar xishacorenes, is key to potency. Our future studies will seek to prepare other oxygenated variants of the xishacorenes that may have enhanced hydrophilicity.

## Conclusions

In conclusion, we have demonstrated that fuscol undergoes an acid-mediated cyclization to produce the xishacorenes, supporting the proposed biosynthesis of this unique family of diterpenoids. Our isolation of xishacorene D also raises the possibility that this compound might co-occur alongside the other xishacorene congeners and may have been missed in the original isolation, since we obtained xishacorene D in a very small amount from the cyclization of fuscol. Bioactivity studies of fuscol and the xishacorenes, facilitated by a redetermination of the physical properties of the latter compounds, have provided unique insight into the importance of hydrophilic substituents to their bioactivity.

## Conflicts of interest

WD and CN are employees and shareholders of Aduro Biotech, Inc.

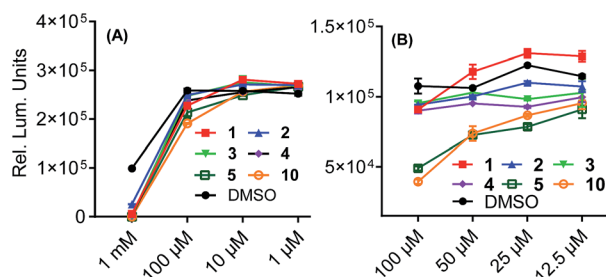
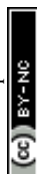


Fig. 3 THP-1 cell assay of compounds **1**–**5** and **10** after 24 h of incubation. (A) CellTiter-Glo® assay measure of cell viability at each concentration. (B) Activity assay of compounds following stimulation with ML RR-S2 cGAMP (STING Promoter).



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- 17 Note that entries 11–13 represent previous attempts from the original isolation report.
- 18 For example ( $\log \epsilon$ ) values of organic compounds, see: E. Pretsch, P. Bühlmann and M. Badertscher, *Structure determination of organic compounds*, Springer, Berlin, 4th rev. and enl. edn, 2009, vol. xv, p. 433.
- 19 The original report provides a  $\lambda_{\max}$  difference of 16 nm between xishacorenes A and B, a surprisingly large shift when considering the two contain identical chromophoric functional groups. We believe the observed  $\lambda_{\max}$  values align more closely to those predicted by Woodward–Feiser Rules as well.
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