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The Native Production of the Sesquiterpene Isopterocarpolone by *Streptomyces* sp. RM-14-6

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Abstract

We report the production, isolation and structure elucidation of the sesquiterpene isopterocarpolone from an Appalachian isolate *Streptomyces* species RM-14-6. While isopterocarpolone was previously put forth as a putative plant metabolite, the current study highlights the first native bacterial production of isopterocarpolone and the first full characterization of isopterocarpolone using 1D and 2D NMR spectroscopy and HR-ESI mass spectrometry. Considering the biosynthesis of closely related metabolites (geosmin or 5-epiaristolochene), the structure of isopterocarpolone also suggests the potential participation of one or more unique enzymatic transformations. In this context, this work also sets the stage for the elucidation of potentially novel bacterial biosynthetic machinery.

Keywords

terpene; natural product; biosynthesis; cyclase; isoprenoid; bacteria

1. Introduction

Bacterial terpenoids are attractive targets given the relative simplicity of bacterial genomics, genetic manipulation and corresponding enzymology studies. Consequently, there is heightened interest in the identification and isolation of bacterial terpenes (Meguro et al., 2013; Nakano et al., 2011). Examples of bacterial sesquiterpenes include geosmin (Gerber & Lechevalier, 1965), (-)-germacradien-4-ol, (-)-*epi*-abisabolol (Nakano et al., 2011), and kandenols (Ding et al., 2012). As part of our ongoing natural product discovery initiative focused upon unique environments within the Commonwealth of Kentucky (Shaaban et al., 2013; Wang et al., 2013), herein we report the production, isolation and structure elucidation of the sesquiterpene isopterocarpolone (Scheme 1, **3**) from an Appalachian isolate

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Conflict of Interest

The authors report competing interests. JST is a co-founder of Centrose (Madison, WI).

Supplementary material

Supplementary material, including the full experimental details, bacterial strain characterization, physico-chemical properties, and full spectroscopic data of **1**, are available online

designated *Streptomyces* species RM-14-6. While **3** was previously put forth as a putative plant metabolite (*Pterocarpus santalinus*) (Kumar et al., 1974), the current study highlights the first native bacterial production of this metabolite and provides the first structural characterization/validation of **1** via 1D and 2D NMR spectroscopy and HR-ESI mass spectrometry.

2. Results and Discussion

HPLC-HRMS analysis of a crude extract from a 50 mL fermentation revealed a peak with the molecular weight of 236 Da (237.1849 [M+H]⁺). The deduced molecular formula C₁₅H₂₄O₂ from HRESIMS implied a member of the sesquiterpene class lacking a suitable match in the AntiBase (Laatsch, 2012). Scale up fermentation (8 L) of the strain followed by isolation and purification afforded 7.1 mg of **3** as a colorless oil (see supporting information). The ¹H NMR spectrum of **3** in CDCl₃ displayed a singlet peak of an olefinic proton at δ 5.83 along with four methyl group signals at δ 1.91, 1.28, 1.28 and 0.91. The methyl signal at δ 1.91 suggested the attachment to an *sp*²-carbon. Additional signals for nine aliphatic protons were observed in the range δ 2.81~1.34 ppm. The ¹³C NMR/HSQC spectra identified **3** as a sesquiterpene comprised of 15 carbon atoms including a carbonyl (δ_C 199.7), one oxygenated quaternary carbon signal at δ 74.7, and two olefinic carbons (δ_C 126.8, CH and 164.1, C_q) within an α,β -unsaturated ketone. In addition, signals for four methylenes, one quaternary carbon and four methyl groups were observed between δ_C 55.1-18.1 ppm. Thorough analyses of ¹H, ¹³C, HSQC, COSY, TOCSY and HMBC spectra cumulatively established the structure of **3** as depicted in Scheme 1 and Figure S1 (Supporting Information). The NOE interactions between H-5/H-7_{ax} and H-9_{ax}, and between H₃-14/H-6_{ax} and H-8_{ax} in the NOESY spectrum implicated a trans A/B ring juncture with H-5 and the C-10 methyl as axial and an equatorial C-7 hydroxyisopropyl (Supporting Information, Figure S1). Thus, **3** was deduced to be isopterocarpolone, a compound first reportedly isolated from *Pterocarpus santalinus* (Kumar et al., 1974) but one for which no spectroscopic or spectrometric data has been reported. The absolute structure of the isopterocarpolone was concluded to be 5*R*,7*R*,10*S*, and the determined optical rotation of **3** of $[\alpha]_D^{25} = +42^\circ$ (CHCl₃) is consistent with the previously reported value of $[\alpha]_D^{32} = +47^\circ$ (CHCl₃) (Kumar et al., 1974; Kitajima et al. 2003). Isopterocarpolone (**3**) was inactive in antibacterial, antifungal, and cytotoxicity assays (See supporting information).

To the best of our knowledge, this is the first reported isolation of isopterocarpolone from bacteria. While geosmin (**2**) and kandenol A (**5**) represent bacterial metabolites structurally related to isopterocarpolone (**3**), the plant terpenoid 5-epiaristolochene (**4**) shares an identical skeletal framework with divergence deriving through the intermediate **1** via C-1 to C-6 methyl migration and inversion of C-5 stereochemistry (Scheme 1) (Whitehead et al., 1989). Biosynthetically, the key cyclization event en route to **2** that results in the formation of decalene backbone proceeds with the elimination of an acetone molecule (Scheme 1; Jiang et al., 2006) while the genesis of **4** proceeds through germacrene A (Rising et al., 2000). For the latter, the addition of a proton at the C-5 of germacrene followed by C1-C6 cyclization and C-1→C-6 methyl and C-6→C-5 migrations has been proposed for the biosynthesis of **4**. Interestingly, neither biosynthetic strategy provides a plausible explanation for the biosynthesis of **3**. Thus, the potential participation of one or more unique enzymatic transformations in the biosynthesis of **3** is expected and, in this context, the isolation and characterization of **3** reported herein may provide an unparalleled resource for the elucidation of the underlying catalytic process.

3. Conclusions

In this study, we report the new bacterial sesquiterpene **3** from an Appalachian isolate *Streptomyces* species RM-14-6. While **3** was previously implicated as a plant metabolite, the structure of **1** is fully assigned here for the first time using 1D and 2D NMR spectroscopy and HR-ESI mass spectrometry. Considering the biosynthesis of closely related sesquiterpenes (geosmin or 5-epiaristolochene), the structure of isopterocarpolone (**3**) also suggests the potential participation of one or more unique enzymatic transformations. This work also sets the stage for the elucidation of potentially novel bacterial biosynthetic machinery.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Scheme 1.

Proposed biosynthetic divergence leading to isopterocarpolone (**3**), 5-epiaristolochene (**4**) (Whitehead et al., 1989) and geosmin (**2**) (Jiang et al., 2006) where solid arrows represent known processes and dashed arrows represent putative routes. Kandenol A (**5**) (Ding et al., 2012) represents a closely related analogue of isopterocarpolone (**3**).