DIIODOCALLOPHYCOIC ACID: A NOVEL CASE OF STRUCTURAL DIVERSITY IN MARINE NATURAL PRODUCTS

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DIIODOCALLOPHYCOIC ACID: A NOVEL CASE OF STRUCTURAL DIVERSITY IN MARINE NATURAL PRODUCTS

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[To the students of the Georgia Institute of Technology]

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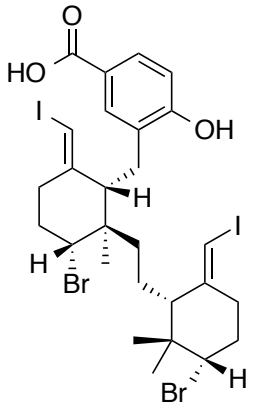
SUMMARY

Chemical investigations of the chemistry of Fijian reef systems have yielded the discovery of an iodinated diterpene-benzoate produced by the Fijian red macroalga *Callophycus* sp. Through the use of bioassay guided fractionation, NMR and mass spectrometric-based structural elucidation, diiodocallophycoic acid (**1**) was successfully isolated and structurally characterized. This novel secondary metabolite was found to contain two highly unusual exocyclic iodinated olefins and demonstrated moderate biological activity against methicillin resistant *Staphylococcus aureus* (MRSA) with an MIC of 3.9 μg/mL.



Diiodocallophycoic acid **(1)**

Figure 1 – Proposed structure of **1** based on "within ring" relative stereochemical assignments.

CHAPTER 1

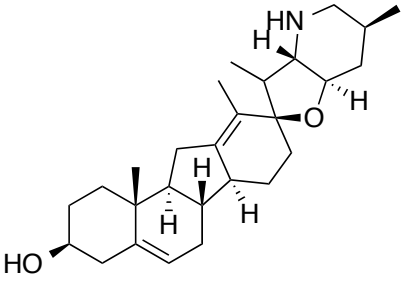
INTRODUCTION

A critical element in the success of humans against the threat of disease has been the adoption of medicinal remedies from natural sources, primarily terrestrial plant species.[1] Based on these early practices, natural product chemists have sought to isolate and characterize the molecular constituents responsible for observed biological activities of natural extracts.

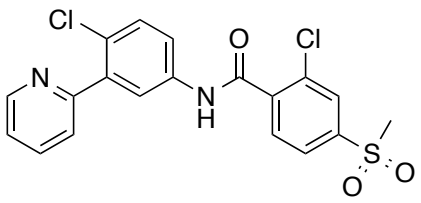
Eliciting a wide range of pharmacological effects, natural product based drugs have advanced the treatment of human disease. The clinical significance of natural therapeutics is evident given that natural product based carbon skeletons and pharmacophores have inspired the design of 74% of biologically active compounds and constitute the majority of anti-infective (69%) and anti-cancer agents (75%).[2] The wide range of medical applications and historical significance of natural products and their derivatives are exemplified by: penicillin (antibacterial agent); paclitaxel (Taxol®; ovarian and breast cancer treatment); morphine (analgesic); artemisinin and quinine (malaria treatments).[2]

Investigations of natural product biological activity and their interactions with cellular machinery have enhanced our understanding of developmental and cancer biology. The effects of the teratogen cyclopamine **(2)** (Figure 2) [3, 4], produced by *Veratrum californicum,* on fetal lamb susceptibility to the congenital disease cyclopia were found to be dependent on the gestational period in which the mother consumed *V. californicum*.[5] Further investigation revealed that instances of fetal cyclopia were due to

the inhibition of the sonic hedgehog developmental pathway[6] via binding of cyclopamine to the integrand smoothened.[7]



Cyclopamine **(2)**



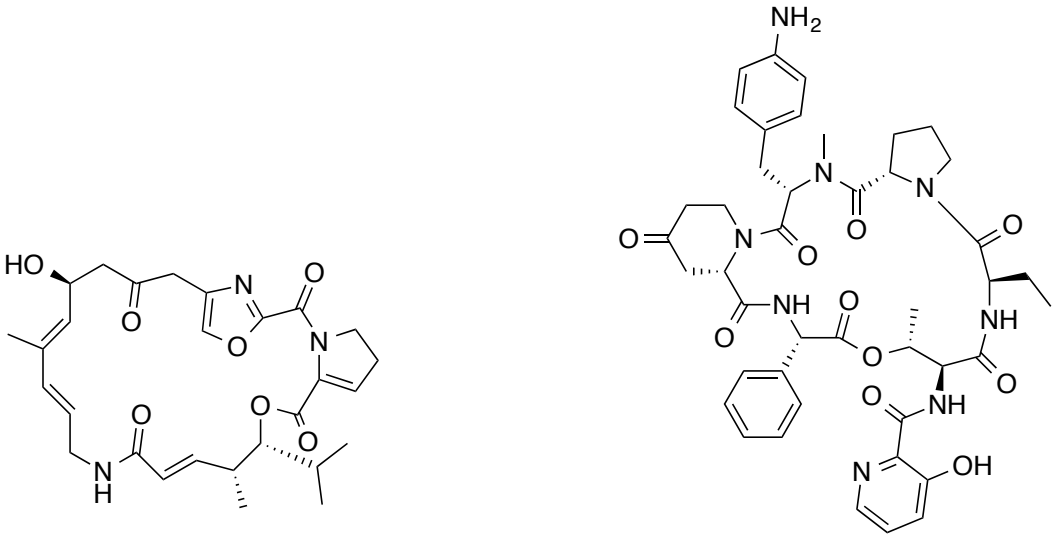
Vismodegib **(3)**

Figure 2 – Reported structures of cyclopamine and vismodegib.

Now implemented as a therapeutic target in the treatment of various hedgehog associated cancers, such as basal cell carcinoma[8], synthetic studies of smoothened inhibitors led to the discovery of the marketed chemotherapeutic vismodegib **(3)** (Figure 2).[9, 10]

The use of natural products as therapeutics can be attributed in part to their evolutionary history and the ecological interactions resulting from their development.[11] While it should be noted that human exploitation of natural products has no bearing on their native functions or evolutionary history, microbial defensive compounds have had dramatic impacts on human health. In the case of soil dwelling microbes such as members of the *Streptomyces* genus, the up-regulation of defensive compounds (i.e. antibiotics) is hypothesized to contribute to periods of intensified competition when nutrients are limited.[12] Evidence for such claims can be drawn from members of the *Streptomyces* genus through the production of streptogramin antimicrobial compounds.[13] Classified as type A or B, members of this family of molecules are coproduced and act synergistically to inhibit bacterial peptide translation via binding to ribosomal active sites.[14, 15, 16, 17] In the case of *S. pristinaespralis,* the independent genes responsible for the

production of the structurally unrelated pristinamycins A **(4)** and B **(5)** (Figure 3) were found to be clustered on the same chromosome over a 200 kb region. Based on these results, it has been hypothesized that the utilization of two independent metabolic pathways evolved based on the constant presence of competitors with resistance to one of the two components.[13, 18]



Pristinamycin A **(4)** Pristinamycin B **(5)**

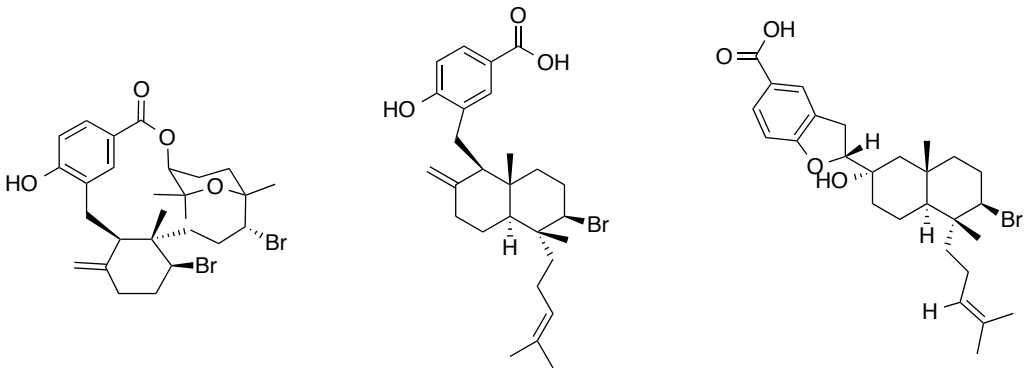
Figure 3 – Reported examples of streptogramins isolated from *S. pristinaespralis.*

The rise of drug resistant bacterial populations represents a constant threat to the global human population. Drug resistant strains, such as methicillin resistant *Staphylococcus aureus* (MRSA), were first documented in the early 1950's[19] and have since been observed in a wide spectrum of infections ranging from skin lesions to septic arthritis.[20] Driven in part by the improper usage of antibiotics (e.g. patient failure to finish a regimen) bacterial populations have demonstrated remarkable adaptability.[21, 22] The 1950's implementation of erythromycin as a treatment for *S. aureus* saw drug resistant strains surface in less than a year, accounting for 70% of sampled *S. aureus* cases in

Boston City Hospital.[23] The rise of bacterial resistance has classically been attributed to mutations within bacterial resistance genes (r genes) resulting in the degradation or inhibition of prescribed treatments.[21, 22] β-lactam based drugs, such as penicillins, which generally act to disrupt cell wall biosynthesis are especially susceptible to hydrolytic degradation via β-lactamases.[24] The accumulated discovery of over 1,000 β-actamases (native and mutant forms) and their propensities for horizontal gene transfer via conjugation has highlighted the dangers of bacterial r gene plasticity and communicability.[11, 25] Studies of vancomycin have revealed the periodic evolution of resistant strains[26] based on the increase of minimal inhibitory concentration (MIC) values from ≤ 1 to ≥1.5 μg/mL.[27] The threat of emerging, untreatable bacterial strains has necessitated the pursuit of alternative antibiotic sources.

In light of evolutionary arguments and the high degree of biodiversity in marine ecosystems[28, 29], one could predict that the frequency and complexity of ecological interactions within such environments would result in a wide range of natural products stemming from diverse metabolic pathways.[30] Studies of marine sponges have resulted in the discovery of numerous structurally diverse and biologically active compounds,[31] such as the polyether macrolide halichondrin B[32], now marketed as the derivatized chemotherapeutic Halaven®.[33] The metabolic complexity of marine sponge taxa has classically been attributed to their interactions with symbiotic microbes.[34, 31] This hypothesis has been further validated through the identification of two chemically distinct microbial symbiotes, ubiquitous in the marine sponge *Theonella swinhoei*. Genetic analysis confirmed that these microbial communities were responsible for the production of numerous polyketide and ribosomal peptide based natural products.[35]

Studies of Fijian marine organisms have served to demonstrate that complex ecological interactions could serve as a hallmark of metabolic diversity, leading to novel natural products. In the case of red macroalgal genera, such as *Peyssonnelia* and *Callophycus,* susceptibility to fungal invasion may have selected for the development of surface confined terpenoids, serving as antimicrobial agents.[36, 37] Additional investigations into *Callophycus* spp. have revealed a host of novel halogenated macrolide and non-macrolide diterpene shikimates. [38, 39, 40, 41] Bromophycolide P **(6)** [42] and callophycoic acid G **(7)** [40, 41] (Figure 4) demonstrated anti-MRSA activity with MICs of 1.4 and 1.6 μg/mL respectively. Further work with *Callophycus* sp. collection G-0807, resulted in the discovery of five new halogenated diterpene-benzoic acid metabolites, bromophycoic acids A-E[41]. Bromophycoic acid A **(8)** was notably effective against MRSA, having an MIC of 1.6 μg/mL, comparable to currently marketed antibiotics. [43]



Bromophycolide P **(6)** Callophycoic acid G **(7)** Bromophyoic acid A **(8)**

Figure 4 – Reported biologically active natural products from *Callophycus* spp.

While past assessments of *Callophycus* sp. collection G-0807 have successfully resulted in the identification of five bromophycoic acid analogs, unpublished spectroscopic and spectrometric data indicated the presence of additional structurally

related metabolites. Due to the comparability of known bromophycoic acid A to current treatment options and past instances of metabolite analog diversity exhibited by the *Callophycus* genus [38, 39, 40, 41], this study sought to discover novel secondary metabolites demonstrating anti-MRSA activity. In order to isolate novel analogs from previously generated algal extracts, a combination of biological assays and chromatographic techniques—thin layer chromatography (TLC), high performance liquid chromatography (HPLC), liquid chromatography coupled to mass spectrometry (LC-MS) and solid phase extraction (SPE)—were utilized in a process known as bioassay guided fraction. Structural elucidation of the isolated active species required the use of various spectrometric and spectroscopic techniques, including ^{1}H & ^{13}C nuclear magnetic resonance (NMR) and high-resolution mass spectrometry (HR-MS).

CHAPTER 2

EXPERIMENTAL

Biological material

Collection G-0807 was procured on April 6th, 2010 between 10 and 20 m on channel walls and the reef slope near the Mango Bay Resort, Viti Levu, Fiji (18° 14' 12'' S, 177° 46' 48'' E). This collection was identified as *Callophycus* sp. through a series of 18S rDNA analyses and morphological comparisons as previously described. [44] Voucher specimens were preserved in aqueous formaldehyde and stored at the University of the South Pacific.

General Procedures

HPLC based fractionations and purifications were conducted using a Waters 1525 pump coupled to a Waters 2487 dual-wavelength absorbance detector set for 254 & 277 nm and operated via Waters Empower Version 3.20 software. All LC-MS based analyses utilized a Waters 2695 Separation Module coupled to a Waters 2996 photodiode-array UV detector and a Micromass ZQ 2000 mass spectrometer with electrospray ionization in negative mode. Optical rotations were collected on a Jasco DIP 360 Digital Polarimeter. LC-MS associated chromatographic separations were performed using a Grace Altima C_{18} -silica column (3 μm, 2.1 x 100 mm) and gradient mobile phase of 70% to 95% aqueous acetonitrile containing 0.1% aqueous acetic acid over 30 min at a flow rate of 1 mL/min . High-resolution mass spectrometry was conducted on a LTQ OrbiTrap in negative ion mode.

NMR spectra (^{1}H, DEPT-135, HMBC, HSQC, COSY and ROESY) were obtained on a Bruker DRX-500 instrument, equipped with either a 5 mm inverse or broadband probe. All HPLC purifications were directed through ^{1}H NMR spectroscopy in place of traditional bioassay guided fractionation in order to monitor structurally relevant compounds based on published spectral data.[41] All solvents were either HPLC or Optima grade (Fisher Scientific Co.) and NMR solvents were purchased from Cambridge Isotope Laboratories.

Isolation

A 250 mL equivalent of frozen algal mass corresponding to *Callophycus* sp. collection G-0807 was thawed and exhaustively extracted with methanol followed by 1:1 methanol/dichloromethane. Filtered extracts were pooled and reduced *in vacuo*. The resulting crude extract was first partitioned against hexanes and methanol/water (9:1), which was followed by an adjustment of the methanol/water fraction with water to 3:2 methanol/water and partitioned against chloroform. The three resulting fractions were reduced *in vacuo* to dryness. The chloroform fraction was then fractionated by C_{18}-silica flash chromatography (Supelco Envi-18, 10g) using a stepwise gradient from 70% aqueous methanol to 100% methanol to generate 11 fractions. The generated 11 fractions were combined based on TLC yielding 4 fractions. Based on LC-MS data, the relevant fraction was further purified via HPLC using a Grace Alltima C_{18}-silica column (5μ, 10 x 250 mm) with a gradient of 10% aqueous methanol with 0.1% formic acid to 100% methanol with 0.1% formic acid over 20 min at a flow rate of 3 mL/min. The relevant sub-fraction was further purified by HPLC on a Grace Altima C_{18}-silica column (5 μm, 4.6 x 250 mm) utilizing a gradient from 80% aqueous acetonitrile to 100% acetonitrile

over 26 min at 3 mL/min. The relevant fraction was further purified via HPLC using a Phenomenex Phenyl Hexyl column (5 μm, 9.4 x 250 mm) with a gradient from 70% aqueous acetonitrile to 100% acetonitrile over 28 min at 1 mL/min to yield **1** as a white residue.

CHAPTER 3

RESULTS

Extracts from *Callophycus* sp. collection G-0807 were found to contain the novel diterpene benzoate diiodocallophycoic acid (**1**) exhibiting notable structural diversity and moderated biological activity against MRSA (MIC 3.9 μg/mL) and VREF (MIC 7.8 μg/mL) (Table 1). Diiodocallophycoic acid (**1**) was found to exhibit $[\alpha]_D^{25}$ $-1.9x10^{-1}$ ($4.0x10^{-4}$ g/mL) and an HRESI $[M-H]^-$ of *m/z* of 816.8888 with an isotopic peak intensity ratio of 1:3:1, indicative of two bromine atoms. The molecular formula corresponding to this mass was determined to be $C_{27}H_{34}Br_2I_2O_3$, (Δ= 0.27 ppm) consistent with a diterpene-benzoate carbon skeleton, as demonstrated by previous molecules from the genus of macroalgae. [41]

Table 1 – Biological Assay Data for **1**

	Antibacterial MIC [a] (μg/mL)	
	MRSA	VREF
Diiodocallophycoic Acid (**1**)	3.9	7.8

[a]MRSA= methicillin resistant *Staphylococcus aureus;* VREF= vanomycin resistant *Enterococcus facium*

The observed aromatic protons associated with C-17 (δ 128.7), C-18 (δ 114.4), and C-4 (δ 125.6) (Table 1) were found to be indicative of a tri-substituted aromatic ring based on the singlet at δ 7.69, doublet at δ 6.86 (*J*= 8.4 Hz) and doublet of doublets at δ 7.60 (*J*= 8.4, 1.9 Hz) (Figure 5). Further inspection of previously reported callophycoic acids [40], bromophycolides [39, 45, 46] and bromophycoic acids [41] all strongly pointed to the presence of a benzoate moiety. The previously described *J* coupling(s) observed between protons at δ 7.60 and δ 6.85 allowed for the assignments of C-17 and C-18. The assignment of C-1 (δ 168.1) was first inferred based upon the highly deshielded carbon resonance indicative of a carbonyl moiety. The orientation of the carbonyl was established with respect to C-17 (*ortho*) and C-4 (*meta*) due to HMBC correlations from H-17 (δ 7.60) and H-3 (δ 7.69) to the carbonyl center C-1.

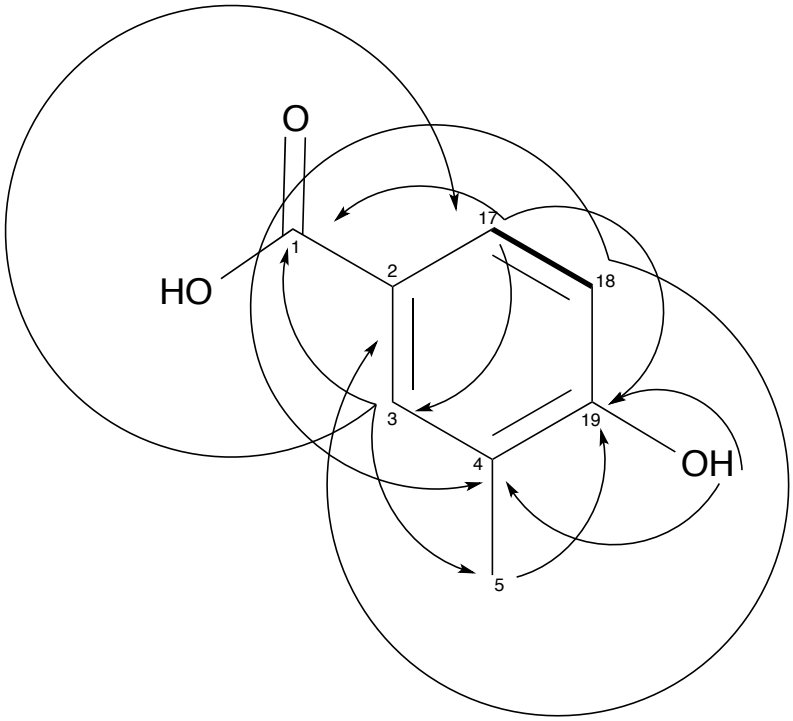


Figure 5 -- Key HMBC (solid single headed arrows) and COSY (bolded lines) correlations corresponding to the benzoate moiety.

These assignments were further supported by reciprocal HMBC correlations from H-3 to C-17 and from H-17 to C-3. Due to the favorability of three bond HMBC correlations in aromatic systems, C-2 (δ 121.2) and C-4 (δ 125.6) were assigned based on correlations from H-18 (δ 6.86). Due to the resonance of a proton at δ 10.41 exhibiting HBMC correlations to C-4 and C-19, the presence of a phenol was inferred, accounting for the last oxygen atom. The assignment of C-19 (δ 159.1) as the phenolic carbon was determined based on HMBC correlations from both H-17 and the phenolic proton at δ 10.41. The assignment of C-5 (δ 26.1) was based on HMBC correlations from H-3 to this carbon and H-5a (δ 2.60) to C-19, bridging the two aromatic and aliphatic.

The second ring system was characterized via the observed methyl singlet at δ 0.85 corresponding to Me-24 (δ 17.5), which exhibited HMBC correlations to C-6 (δ 48.7), C-7 (δ 44.3) and C-23 (δ 62.7) (Figure 6). The inclusion of bromine to C-23 was rationalized based on the down field resonance of this carbon (δ 62.7) and its associated proton H-23 (δ 4.80). C-22 (δ 33.4) was then assigned via COSY correlations between H-23 and both H-22a (δ 1.94) and H-22b (δ 2.20), consistent with the observed multiplicity of H-23 (doublet of doublets).

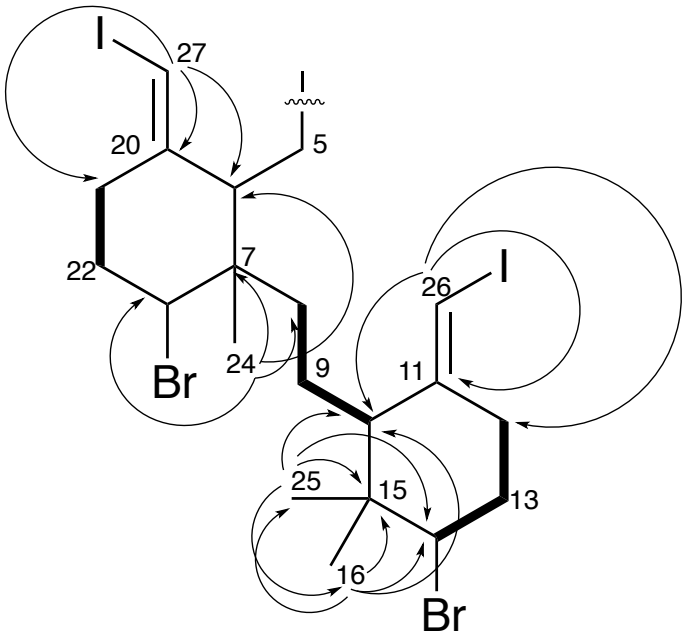


Figure 6 -- Key HMBC (solid single headed arrows; from proton to carbon) and COSY (bolded lines; *J coupling*) correlations.

An additional COSY correlation between H-22a and H-21b (δ 2.60) allowed for the assignment of C-21 (δ 35.9). The assignment of the exocyclic olefin corresponding to C-27 (δ 75.6) and C-20 (δ 146.6) was facilitated by HMBC correlations from the proton H-27 (δ 5.95; singlet) to C-20, C-21 and C-6. It was determined that the relatively up-field chemical shift of C-27 was confirmed based on published results. [47] The two-carbon bridge between the two isoprenoid rings consisting of C-8 (δ 37.5) and C-9 (δ 18.4) was elucidated based on an HMBC correlation from Me-24 to C-8 (δ 37.5) and a COSY correlation between H-8a (δ 1.35) and H-9 (δ 1.73). An additional COSY correlation between H-9 and H-10 (δ 2.05) linked the two aliphatic ring systems. An inspection of the ^{1}H NMR data indicated the presence of two methyl singlets at δ 1.16 and δ 0.85 corresponding to Me-16 (δ 27.5) and Me-25 (δ 17.5), respectively. HMBC correlations between these methyl groups and the quaternary carbon C-15 (δ 41.7) established a germinal relationship. Carbons C-14 (δ 65.9) and C-10 (δ 54.8) were assigned based on

HMBC correlations from both Me-16 and Me-25. Based on pervious carbon and proton resonance arguments the final bromine atom was assigned to C-14. COSY correlations between H-14 (δ 4.41) and H-13b (δ 2.20), as well as H-13a (δ 1.84) and H-12b (δ 2.60) facilitated the assignments of C- 13 (δ 33.8) and C-12 (δ 35.9) respectively. Utilizing the previous rationale, an additional exocyclic olefin moiety, consisting of C-11 (δ 147.7) and C-26 (δ 74.5) was identified and further supported by HMBC correlations from H-26 (δ 6.02) to both C-11 and C-10.

Table 2 –^{13}C and ^{1}H NMR Spectroscopic Data for **1** (500 MHz; DMSO-d_6)[a]

Carbon no.	δ ^{13}C	δ ^{1}H ($J_{H,H}$)
1	167.2	
2	121.2	
3	131.0	7.69 d (1.9)
4	125.6	
5	25.3	2.60 m
		2.93 dd (12.1,10.8)
6	48.7	2.93 m
7	44.3	
8	37.5	1.35 dt (16.2,7.1)
		1.56 m
9	18.4	1.73 m
10	54.8	2.05 dd (6.7,5.8)
11	147.7	
12	35.9	2.13 td (12.3, 4.2)
		2.60 m
13	33.8	1.84 m
		2.2 m
14	65.9	4.41 dd (10.2, 4.2)
15	41.7	
16	27.5	1.16 s
17	128.7	7.60 dd (8.4, 1.9)
18	114.4	6.86 d (8.4)
19	159.1	

20	146.6	
21	35.9	2.13 td (12.3, 4.2)
		2.60 m
		1.94 ddd (23.3,12.3,
22	33.4	4.1)
		2.20 m
23	62.7	4.80 dd (10.8, 4.2)
24	17.5	0.85 s
25	17.5	0.85 s
26	74.5	6.02 s
27	75.6	5.95 s
OH		10.42 s

[a] ^{13}C and ^{1}H NMR chemical shifts for **1** were inferred by DEPT-135, HSQC, and HMBC experiments rather than by a ^{13}C NMR spectrum.

The relative stereochemistry of **1** was assessed based ROESY NMR data and associated NOE signals. The elucidation of stereocenters C-6 (δ 48.7), C-7 (δ 44.3) and C-23 (δ 62.7) was initially facilitated through the establishment of two distinct molecular faces (Figure 7). The lower face was deduced based on NOE signals between Me-24 (δ 0.85) and H-22a (δ1.94), which oriented both constituents on a common ring face axial to their geminal partners.

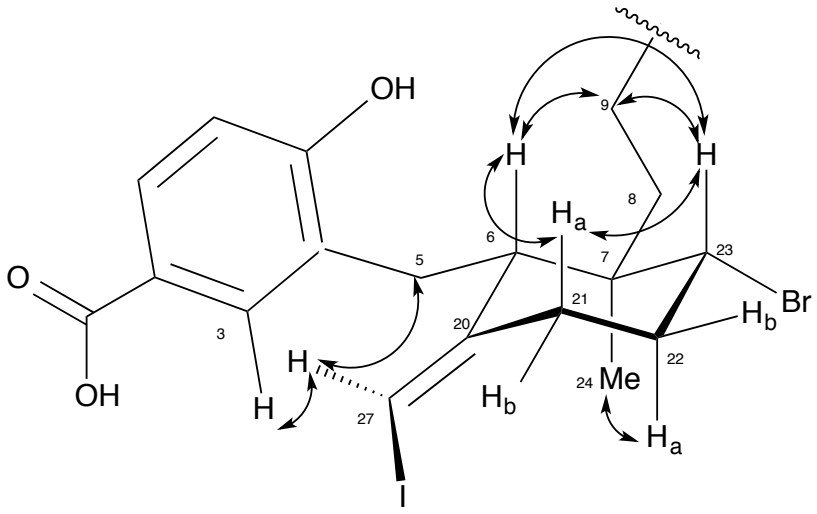


Figure 7 – Relevant NOEs for the first the assignment of stereogenic centers C-6, C-7 and C-23

Pairwise NOE signals between H-21a (δ 2.13), H-23 (δ 4.80) and H-6 (δ 2.93), indicative of 1,3 diaxial interactions, allowed for the orientation of these protons on the upper ring face. This determination was suggested by a lack of NOEs between Me-24 and these protons and was solidified based on NOE signals between H-23 and both H-8b (δ 1.56) and H-9 (δ 1.73). Thus, C-6, C-7 and C-23 were found to be in *R* configurations. The *E* assignment of the excocyclic olefin was confirmed via two NOE(s) between H-27 (δ 5.95), the aromatic proton H-3 (δ 7.69) and the H-5b (δ 2.93). While H-6 (δ 2.93) and H-5a (δ 2.93) are notably equivalent protons, a lack of NOEs between H-27 and H-21a or H-21b further supported the assignment.

In light of carbon resonances, Me-16 (δ 27.5) was found to be equatorial relative to its germinal partner Me-25 (δ 17.5) (Figure 8). NOE signals between H-14 (δ 4.41) and both H-10 (δ 2.05) and H-12a (δ 2.13), indicative of a common molecular face, along with correlations between Me-16 (δ 1.16; equatorial) and H-10, lead to the *S* assignment of the C-14 (δ 65.9) stereocenter. The C-10 (δ 54.8) stereocenter was

assigned to the *S* configuration based on NOE correlations between H-13a (δ 1.84) and both H-9 (δ 1.73) and Me-25 (δ 0.85; axial) in addition to a correlation between Me-16 and H-10 (δ 2.05). The *E* isomer assignment of the exocyclic olefin was notably ambiguous based upon within-ring NOE correlations and was thus determined through conformational arguments and (see Figure 9).

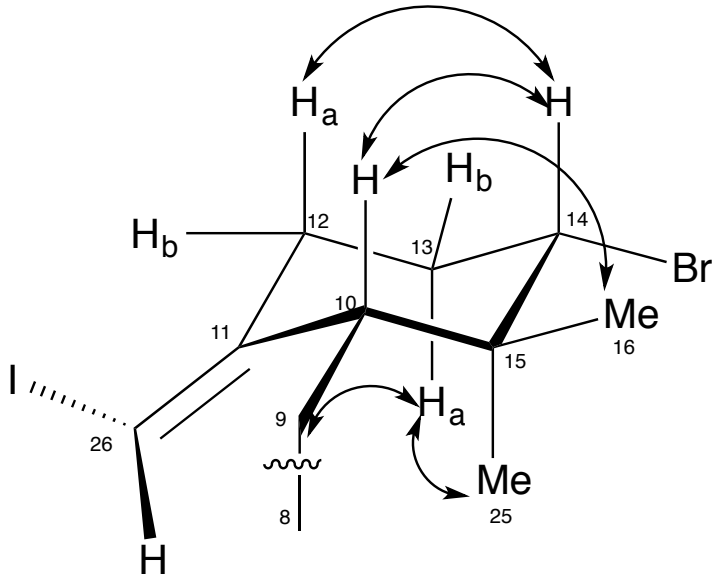
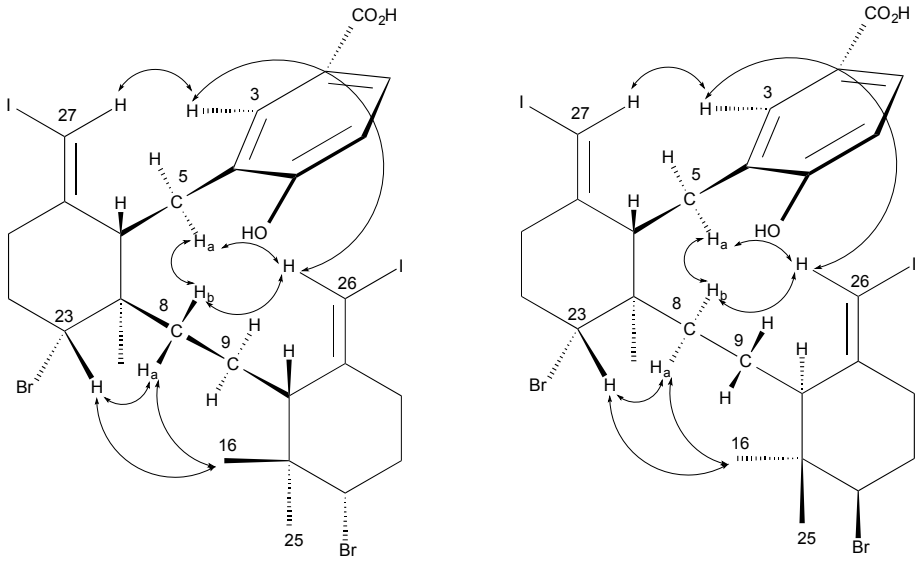


Figure 8 –Relevant NOEs for the assignment of stereogenic centers C-10 and C-14

Conformational information derived from the ROESY spectrum allowed for the prediction of two possible epimers of **1**. NOE correlations between the aromatic proton H-3 (δ 7.69) and both vinylic protons H-27 (δ 5.95) and H-26 (δ 6.02) (Figure 9) confined molecular conformations such that both olefins pointed in the same direction.



(6R, 7R, 23R, 10S, 14S) (6R, 7R, 23R, 10R, 14R)

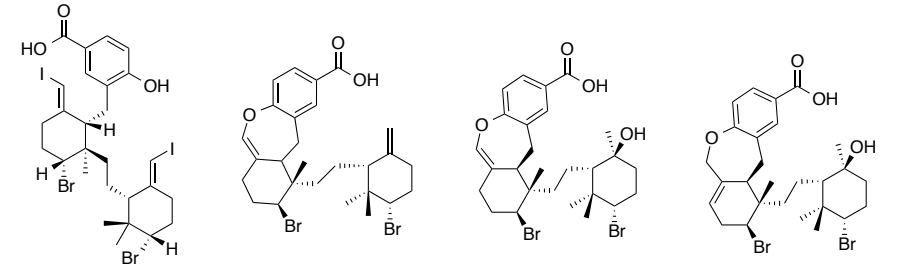
Figure 9 -- Conformational analysis of **1** based on observed NOEs and the two possible epimers reflected by this data.

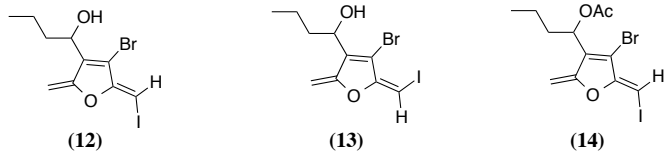
Mutual NOE correlations between H-26, H-8b, and H-5a (δ 6.02, 1.56, and 2.60, respectively) established a mutual orientation for these protons, which was further supported by the lack of NOEs among H-5b, H-26 and H-8a (δ 1.35). These NOE correlations also fixed the C-26 olefin geometry as the *E*-isomer. Similarly, mutual NOE correlations between Me-16 (δ 1.16; equatorial), H-8a (δ 1.35), and H-23 (δ 4.80) indicated that these protons were also oriented towards each other. While an NOE signal between H-23 and H-8b was observed, any ambiguity about the orientation of the C-8 protons was dismissed due to a lack of correlations between Me-16 and the diasteriotopic protons with opposing orientation (H-8a, H-5a, and H-26). The Relative stereochemistry for the whole molecule could not be assigned due to the existence of two epimers, which were structurally consistent with the observed NOES. Due to the prevalence of diasterotopic protons on **1,** overlap in the ROESY spectrum gave rise to notable ambiguity.

CHAPTER 3

DISCUSSION

The discovery of **1** represents a significant contribution to the study of natural products in light of the unprecedented structural diversity demonstrated by this compound. While instances of analogous vinyl iodine moieties have been reported in polyacetylenes **(15) (16)** and polyhalogenated furanones **(12-14)** isolated from *Placospongia* sp. (marine sponge), and *Delisea pulchra* (red alga) respectively [47, 48], **1** is distinguished by two exocylic iodinated olefins. Curiously, the aliphatic ring systems of **1** maintained notable structural similarities as demonstrated by: the *E* configuration and identical molecular orientation of both olefins towards the benzoate (Figure 9) and analogous sites of bromination on C-23 and C-14.





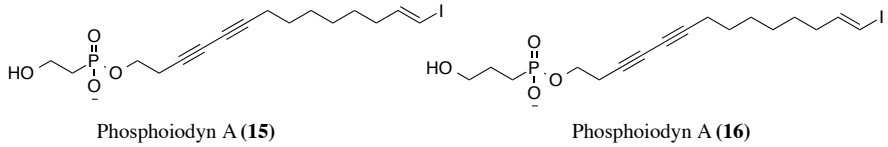


Figure 10 – Structurally related natural products from *Calliphycus* sp (1-11); reported examples of vinyl iodogroups in marine natural products analogous to those seen in 1 (12-16)

These similar structural units differed primarily in the proposed relative stereochemistry and retention of the isoprene head (Me-25 & Me-26) in the "C" ring. In comparison to the closely related nonmacrolide diterpene shikimates from *Callophycus sp.*, **1** was further differentiated by the retention of the phenolic moiety, which was generally functionalized into 3, 5 and 7 membered heterocycles. [41] [40] Given the irregular nature of this structure, **1** was found to be the first example of a natural product with these detailed functionalizations.

Serving as evidence of a common biosynethic origin, **1** was found to conserve the C_{27} skeleton indicative of other diterpene shikimates associated with *Callophycus* sp.[40, 41] The structural similarity of **1** and callophycoic acid E (**9**) further corroborated this hypothesis.[40] Based on the *R, R, R, S, S* epimer of **1,** biosynthesis of diiodcallophycoic acid (**1**) commenced with the alkylation of benzoic acid via electrophilic aromatic substitution with geranylgeranyl diphospate (GGP) as proposed in (Figure 11).[49, 40, 39, 45] Bromination and subsequent cyclizations of the diterpene-benzoate substrate were facilitated by the presence of bromonium ions, requiring oxidative catalytic machinery.[50,51, 52]

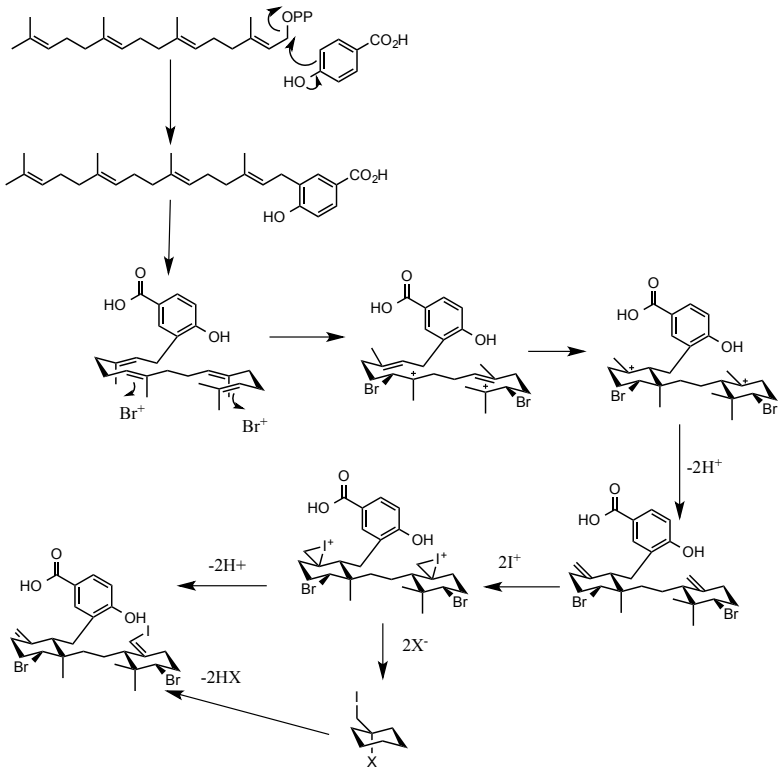


Figure 11 – Proposed biosynthetic scheme for the *R, R, R, S, S* confirmation of 1

The addition of iodine(s) to the exocyclic olefin utilized a similar oxidative mechanism, followed by elimination reactions or direct deprotonation of the iodonium three membered transition state(s). The formation of electrophilic halogens (e.g. Br^+ and Cl^+) in the biosynthesis of terpene based natural products has been attributed largely to vanadium haloperoxidases, hypothesized to additionally facilitate the oxidation of iodine.[50, 52, 53]

Based on the proposed *R,R,R,S,S* epimer, **1** could represent a notable exception to the otherwise ubiquitous trend of enantioselectivity as seen in **9, 10** and **11.** [36] Based on the proposed biosynthetic mechanism for these molecules [36], the preferential formation of *S*. stereocenters arose from the formation of carbocation intermediates, which promoted

intramolectular nucleophilic substitution by terpenoid olefins and subsequent inversion of configuration. [54] While the all *R* molecule is possible, such a finding would be equally unprecedented in related diterpene shikimates. [41] [40] In light of the observed structural novelty of **1,** further investigations into biosynthetic origins of this molecule could result in a host of equally diverse natural products.

Diiodocallophycoic acid **(1)** demonstrated confounding biological activity, potentially alluding to the interaction of additional medicinally potent components. Unreported preliminary MRSA screening of crude algal extracts from G-0807 containing **1** indicated an MIC of 3.9 μg/mL, equivalent to that of the pure compound (Table 2). This finding was troubling given that the biological activity of a mixture *should* increase, as the active component is concentrated through purification. Based on these findings, the possibility of a synergist constituent lost during fractionation should be considered and further explored.[55] Alternatively, extracts from G-0807 are known to contain bromophycoic acid (A), which was reported to elicit notable potency against MRSA (MIC of 1.6 μg/mL), potentially skewing the preliminary screening.[41]

In light of the moderate and confound biological activity of **1** compared to currently marketed therapeutics for both MRSA and VREF, new sources of antibiotics are still necessary.[56, 57] Further investigations into the chemical diversity of marine environments remain a pivotal resource in combating human illness due to drug resistant bacterial infections. The Fijian ecosystem has been distinguished as source of structurally novel and biologically active families of natural products as demonstrated by members of the *Callophycus* genus.[38, 39, 40, 41, 58, 46]

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