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Communication

X-ray Crystallography and Unexpected Chiroptical Properties Reassign the Configuration of Haliclonadiamine

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ABSTRACT: Haliclonadiamine and papuamine are bis-indane marine natural products isolated from the marine sponge *Haliclona* sp. Their relative structures were previously reported to differ by inversion at only one of their eight shared stereocenters. Here X-ray crystallography shows the opposite to be true: papuamine has a 1*R*,3*S*,8*R*,9*S*,14*S*,15*R*,20*S*,22*R* configuration, while haliclonadiamine has a 1*S*,3*R*,8*S*,9*R*,14*R*,15*S*,20*R*,22*R* configuration. Paradoxically the ECD of each structure displays a negative Cotton effect. X-ray crystallography reveals the two structures adopt similar conformations of their 13-membered macrocyclic core that comprises a configurationally relevant diene. B97x-D/Def2-TZVPP-(MeOH)-calculated ECD supports the diene configuration with the macrocycle dominating the ECD Cotton effect for haliclonadiamine and papuamine. Additional crystallographic and chiroptical analyses of three sponge samples from geographically distant locations indicate this pair of natural products always exists as a configurationally related couple. The co-discovery of a biosynthetic precursor, halichondriamine C, present in these same Haliclona samples must be considered when discussing any biosynthetic pathway. Taken together, this work justifies a reassignment of haliclonadiamine's structure and opens the question of how this complex stereochemical relationship between haliclonadiamine and palauamine arises biosynthetically.

Papuamine[1] (**1**) and haliclonadiamine[2] (**2**) (Figure 1) are stereoisomeric marine natural product alkaloids comprising two stereoisotopic indanes. Whereas **1** is C_2-symmetric, rendering the indanes homotopic, **2** is asymmetric and the indanes are diastereotopic. Previously, through total synthesis and comparison of optical rotations with the natural products, it was concluded that **1** and **2** share the same absolute configurations at all but the C-22 center.[3−7] In this report, high-resolution crystal structures of papuamine and haliclonadiamine from multiple sponge samples reveal that **1** and haliclonadiamine (**3**) (Figure 1) are actually epimeric at all configurations except the C-22 center. This conclusion is corroborated by comparison of experimental and theoretical electronic circular dichroism (ECD) spectra. The origin of the mis-assignment stems from additional stereochemical complexity associated with atropisomerism of the macrocyclic 1,3-diene.

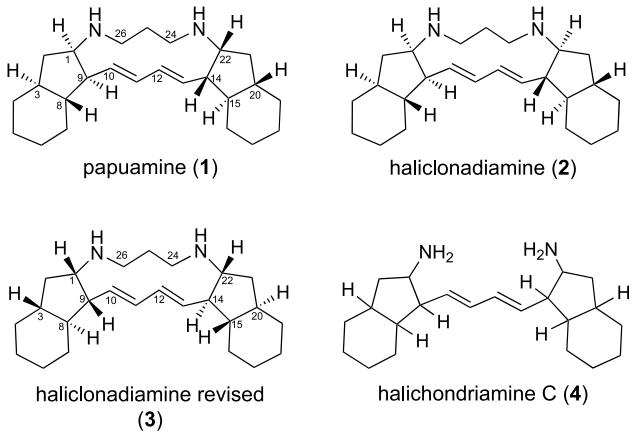

Figure 1. Structures of papuamine, haliclonadiamine, and halichondriamine C.

During an antimicrobial screen of marine natural products extracts, the organic extract C20865 from the sponge *Haliclona* sp. collected in the Caroline Islands showed modest inhibition of three standard bacterial test strains (Table S1). Activity-guided isolation led to purification of compounds **3** and **4** (Figure 1). Their structures were elucidated by HRMS and NMR characterization. The HRESIMS of **3** showed an $[M + H]^+$ peak at m/z 369.3273, corresponding to a molecular formula of $C_{25}H_{40}N_2$ and indicating seven degrees of unsaturation. Complete ^1H and ^{13}C chemical shift assignments (Table S2) and analysis of 2D NMR spectra indicated **3** to be a haliclonadiamine. Briefly, $^1H-^{13}C$ HSQC, $^1H-^1H$ COSY, and HMBC spectra identified two partial structures corresponding to contiguous spin systems from C-1 to C-22 and from C-24 to C-26 (Figure 2A). HMBC correlations from H-1 to C-26 and from H-22 to C-24, the two N atoms in the molecular formula, and the deshielded ^{13}C chemical shifts for C-1, C-22, C-24,

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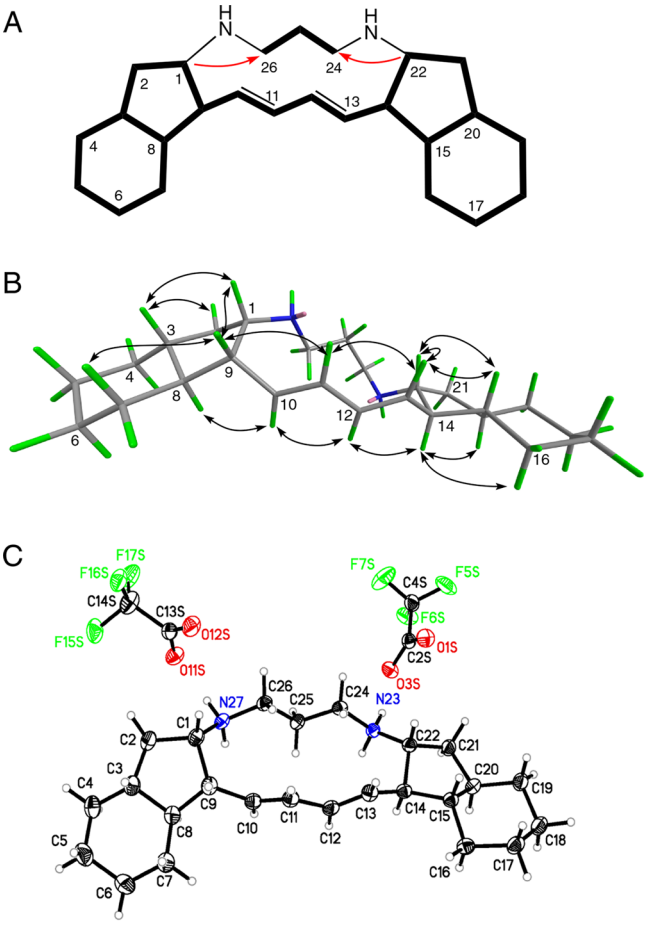

Figure 2. Revised structure of haliclonadiamine (**3**) determined by NMR and X-ray crystallography. (A) NMR assignments: COSY and HMBC correlations are shown in bold bonds and red arrows, respectively. (B) NOE correlations observed for **3**. (C) X-ray structure of the TFA salt of **3** from C20865 collected in the Caroline Islands (Supporting Information).

and C-26 linked the two fragments through the NH groups, revealing the structure of haliclonadiamine. The $[\alpha]_d$ of −20° was in agreement with previously published values (Table 1).

Using single-crystal X-ray diffraction, we confirmed the structure and determined the absolute configuration of **3** (Supporting Information and Table S3). Block crystals (ca. 1 mm^3) were formed upon evaporation from MeOH at 4 °C. Data collected on a portion of a crystal were 99.8% complete to 74.5° θ (~0.80 Å) with an average redundancy of 7.84. The crystal structure confirmed an *s-trans*-10*E*,12*E* diene and revealed a 1*S*,3*R*,8*S*,9*R*,14*R*,15*S*,20*R*,22*R* configuration for **3**. Surprisingly this configuration is opposite to that previously proposed for haliclonadiamine.[7] The absolute configuration was verified by calculation of the Flack[8] and Hooft[9] X-ray parameters that were very small (−0.05 and −0.05) for the correct enantiomer and close to the unit (1.05 and 1.05) for the inverted structure.

The unexpected finding that the configuration of **3** is opposite to that previously assigned on the strong basis of synthesis and the sign of the optical rotations (Table 1) poses several intriguing questions: (i) Are the core structures of papuamine and haliclonadiamine always enantiotopic, and is this observed across this taxon? (ii) What optical properties lead to similar $[\alpha]_d$'s for synthetic **2** and natural **3** (−5° and −20°, respectively) when they have opposite configurations? (iii) Since haliclonadiamine and papuamine co-occur in individual sponges, what biosynthetic scheme could lead to such a stereochemically complex pairing?

Table 1. Optical Rotations of Natural and Synthetic Papuamine and Haliclonadiamine

reference or source	compound or source	$[\alpha]_d$
	Papuamine	
Baker et al., 1988[1]	natural papuamine·HCl	−140
″	natural papuamine	−150
Barrett et al., 1994[4]	*syn*-(+)-papuamine·HCl	+138.6
McDermott et al., 1996[7]	*syn*-(+)-papuamine·HCl	+179.7
Borzilleri and Weinreb, 1994[5]	*syn*-(−)-papuamine·HCl	−108
Borzilleri et al., 1995[6]	*syn*-(−)-papuamine·HCl	−140
″	*syn*-(−)-papuamine	−140
C29959, Palau	natural **1**	−150
C18963, Papua New Guinea	natural **1**	−103.5
	Haliclonadiamine	
Fahy et al., 1988[2]	natural haliclonadiamine	−18.2
McDermott et al., 1996[7]	*syn*-(−)-haliclonadiamine	−5.0
Taber and Wang, 1997[10]	*syn*-(−)-haliclonadiamine	nr
C20865, Caroline Islands	natural **3**, TFA salt	−20
C29959, Palau	natural **3**	−31.7
C18963, Papua New Guinea	natural **3**	−22.7

To address the first question, we used LC-MS to analyze additional samples of *Haliclona* sp. collected in geographically distinct locations for the presence of both papuamine and haliclonadiamine, reported to co-occur in some sponge samples.[2,11] We identified two such collections, C29959 from Palau and C18963 from Papua New Guinea (Figure S21). We isolated and crystallized both compounds from the extract of C29959. Single-crystal X-ray diffraction of papuamine confirmed the previously proposed configuration 1*R*,3*S*,8*R*,9*S*,-14*S*,15*R*,20*S*,22*R*;[4,5] we again observed the 1*S*,3*R*,8*S*,9*R*,-14*R*,15*S*,20*R*,22*R* configuration in this second crystal structure of haliclonadiamine (**3**) (Figure 3 and Tables S4 and S5). Optical rotations of **1** and **3** isolated from each of the three *Haliclona* extracts had similar negative values and were in agreement with the original reports for each natural product (Table 1).[1,2]

We next sought to independently probe the chiroptical properties of both natural products by performing UV−Vis and ECD computations of **1** and **3** in MeOH for comparison with experimental ECD curves. We performed full geometry optimizations on both the X-ray and optimized structures at the B97D/Def2-TZVPP level of theory, followed by wB97xD/Def2-TZVPP/−/B97D/Def2-TZVPP determinations of the UV−Vis and ECD spectra (Figures S25−S28 and Supporting Information). As can be seen in Figure 4A, for both **1** and **3** we observe excellent agreement between the calculated and experimental data. Despite **1** and **3** having opposite configurations at seven of eight chiral centers, both compounds display negative experimental and theoretical CD curves and closely aligned minima. Because the chromophore in this pair of compounds is the diene, this suggested the 13-membered cores have the same chiroptical chromophoric configurations.

To test this hypothesis, we carried out ECD calculations on the core macrocycles of **1** and **3** that are stripped of all tetrahedral stereocenters but retain the same conformation as the optimized structures used in the first set of calculations. As

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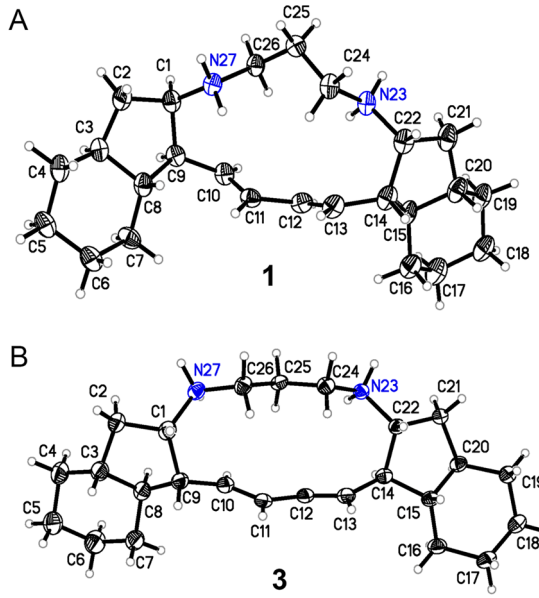

Figure 3. ORTEP renderings of the crystal structures of (A) papuamine (**1**) and (B) haliclonadiamine (**3**) isolated from a second extract, C29959, collected in Palau. **1** and **3** from this single specimen have respective 1*R*,3*S*,8*R*,9*S*,14*S*,15*R*,20*S*,22*R* and 1*S*,3*R*,8*S*,9*R*,-14*R*,15*S*,20*R*,22*R* configurations.

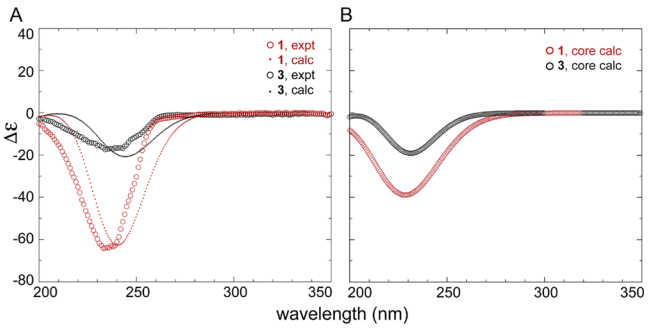

Figure 4. Experimental and calculated CD spectra for **1** (red) and **3** (black). (A) Experimental and calculated CD spectra shown in open circles and dots, respectively. (B) Calculated CD spectra of the central macrocyclic core of **1** and **3**.

seen in Figure 4B, their calculated curves are similar to experimental curves, indicating the indane rings do not appreciably affect the CD. Turning to the optical rotations the magnitude and sign of the contribution from the diene may also explain the observed α_D values of **2** and **3**. Natural haliclonadiamine, **3**, has a mean $[\alpha]_D$ of −23.2° while that of epi-haliclonadiamine (**2**) is −5°. If the negative contribution of the diene is greater than the positive contribution from the saturated indane, a small net, negative optical rotation could be observed. A similar phenomenon was reported by Mislow and Djerassi wherein different stereoisomers of two sesquiterpenes and a biphenyl displayed near superimposable rotatory dispersion curves attributed to the shared positions of the respective double bond and carbonyl π-electrons.[12]

The third question as to how papuamine and haliclonadiamine may be biosynthesized remains. Baldwin and colleagues proposed that **1** may be derived from a tandem ene reaction involving the C_{22} hydrocarbon docosa-2,8,14,20-tetraenedial and 1,3-diaminopropane (Scheme 1A).[13] In addition to **1** and **3**, we isolated sub-milligrams of a new C_{22} bis-indane, halichondriamine C (**4**) from *Haliclona* sp. (see Supporting Information for structure determination).[14] The halichondriamines could be biosynthetic intermediates poised to react with malonyl dialdehyde, or derailed biosynthetic products formed on the way to papuamine and haliclonadiamine (Scheme 1B). Nonetheless, to obtain **1** and **3** enantiomeric precursors must exist. Though these could be formed non-enzymatically through a tandem ene reaction, **1** and **3** contain eight stereocenters such that a non-enzymatic route is less likely to lead to stereoselective ring formation always apparent in the natural products. For this reason, we hypothesize that the producer contains two enzymes, each dedicated to the biosynthesis of one of the bis-indane enantiomers (Scheme 1B,C). A less likely alternative would be the occurrence of a single promiscuous cyclase that could accommodate either conformer of the C-22 precursors shown in Scheme 1. There is precedent for the former in the plant *Solidago canadensis* where separate enzymes lead to production of (+)- and (−)-germacrene D in a single plant specimen.[15] For the latter, cyclization of the proposed C_{22} tetraene or hexaene conformers **i** or **iv** is reminiscent of cyclases that contain large binding sites to accommodate complex substrates such as squalene-hopene cyclase that converts squalene to the pentacyclic triterpene hopene. X-ray crystal structures of this enzyme in complex with squalene or aliphatic inhibitors revealed a large binding site that accommodates the "folded" conformation of squalene.[16−18]

To form the final natural products, **ii** (or **v**) could be oxidized to the C-1, C-22 keto intermediates and reaction with 1,3-diaminopropane or ammonia would produce **3** or **4**. Reaction of **4** with the common metabolite malonyl dialdehyde could also lead to **1** or **3** (Scheme 1B,C). It remains to be seen whether the steps leading to macrocyclization use separate enzymes dedicated to each enantiomer (where formation of haliclonadiamine would require epimerization at C-22) or a single enzyme capable of recognizing a shared conformation of the last intermediate in the biosynthesis. Substrate-controlled stereoselective cyclization has been observed in lanthionine biosynthesis, leading to epimers at the site of ring closure.[19]

Relative to the thousands of natural product structures reported to date, the number of enantiomeric compounds discovered is small. Divergent stereochemistry leads to different structural outcomes ranging from a related pair of natural products possessing enantiomeric fragments or core moieties,[20] to enantiomeric final products being synthesized.[15,21,22] Among marine sponge-derived natural products enantiodivergent synthesis was recently uncovered for the sceptrin family of alkaloids including massadine and ageliferin through the combination of total synthesis and analysis of natural material for each. Those studies showed that within that family, the configuration is determined by the type of cycloaddition reaction that occurs, ultimately leading to divergent core configurations.[23] In the work reported here, an alternate mechanism may be at play where enantiomeric precursors are formed prior to condensation with the C_3 unit that results in macrocyclic ring formation. Because marine sponges exist as complex host−microbiome assemblages,[24−26] additional interdisciplinary studies involving natural products chemistry, synthesis and/or biosynthesis and metagenomics may be required to determine the mechanisms leading to enantiomeric synthesis and production of **1** and **3**. Finally, the chiroptical properties of papuamine and haliclonadiamine represent an unusual case in which the conformation of an

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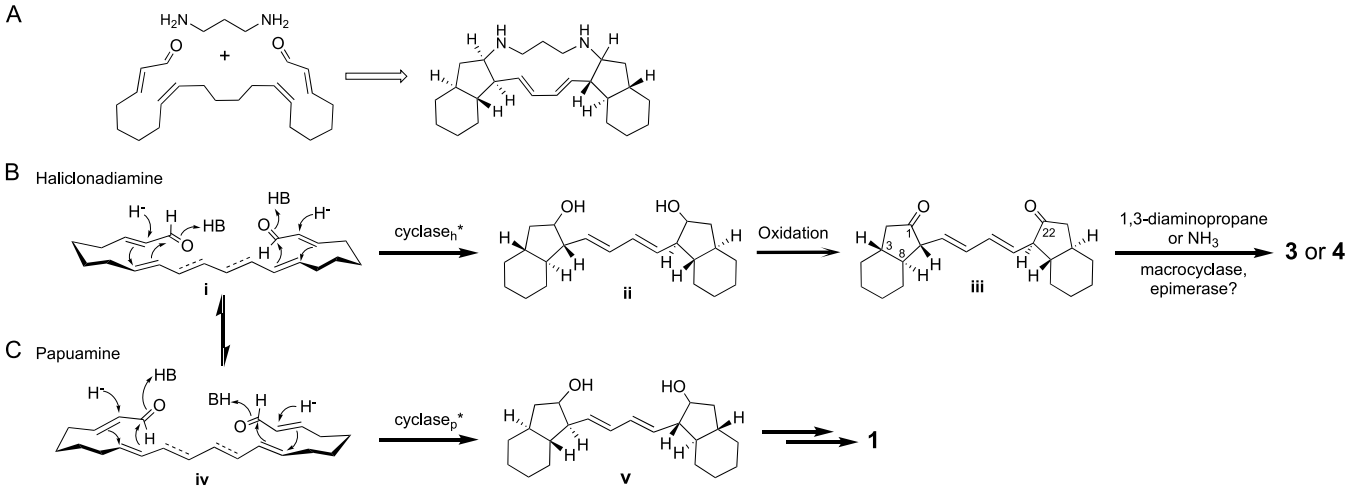
Scheme 1. Biosynthetic Proposals for 1 and 3[a]



[a]Part A shows the biosynthetic scheme proposed by Baldwin[13] starting with docosa-2,8,14,20-tetraenedial and diamino propane. In panels B and C, docosa-2,8,14,20-tetraenedial (or hexaenedial) can adopt inverted conformation **i** or **iv**. Enzyme-mediated cyclizations produce enantiotopic intermediates **ii** and **v**. Oxidation to ketone **iii** and reaction with 1,3-diaminopropane or NH_3 yields **3** or **4**. An epimerase may be needed to invert the stereochemistry at C-22. The analogous pathway leading to papuamine is shown in panel C.

achiral diene-containing macrocycle drives the sign of the CD rather than the stereocenters.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacs.9b12926.

Discussion of general, biological, and computational methods; Figures S1−S30 and Tables S1−S5, including NMR spectra and description of structure determination for **3** and **4**, X-ray data and statistics, calculated ECD curves and energies, and biological activities (PDF)

X-ray crystallographic data for **1** from extract C20865 (CIF)

X-ray crystallographic data for **3** from extract C29959 (CIF)

X-ray crystallographic data for **3** from extract C29959 (CIF)

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Notes

The authors declare no competing financial interest.

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