Stereochemical Heterogeneity in Verongid Sponge Metabolites. Absolute Stereochemistry of (+)-Fistularin-3 and (+)-11-*epi*-Fistularin-3 by Microscale LCMS-Marfey's Analysis

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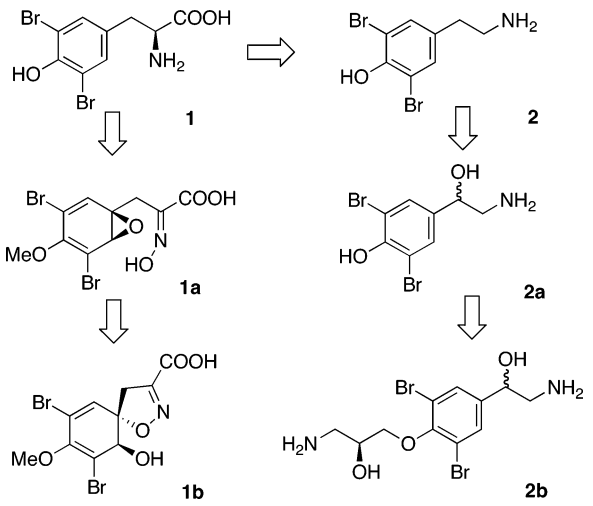
The absolute configurations of fistularin-3, 11-*epi*-fistularin-3, and a related bis-oxazolidinone were determined by microscale hydrolysis followed by derivatization with 1-fluoro-2,4-dinitrophenyl-5-L-alaninamide. Samples of fistularin-3 from Verongid marine sponges collected in the Great Barrier Reef (Australia), Baía de Todos os Santos (Brazil), and the Key Largo, Florida (USA) varied in configuration at C11, a phenomenon that may be attributed to the involvement of stereochemically promiscuous hydroxylase enzymes. Variability in C11 configuration in fistularin-3 samples may have been overlooked in previously reported encounters due to the similarity of spectroscopic properties of fistularin-3 and 11-*epi*-fistularin-3 and their coelution under chromatographic conditions. Stereochemical heterogeneity at C11 in fistularin-3 samples suggests a possibility of a native biotransformation of suitable precursor in Verongid sponges by their associated microbial flora.

Marine sponges of the family Verongidae produce a diverse array of bromine-containing metabolites with interesting biological properties. It is thought that most brominated Verongid metabolites are derived from transformations of the precursor 3,5-dibromotyrosine (**1**). This hypothesis finds support from in situ ^{14}C labeling studies that show incorporation of [U]-^{14}C-Tyr, [U]-^{14}C-Phe,[1a] and [U-^{14}C]-3,5-dibromotyrosine[1b] by the sponge *Aplysina fistularis* (*Verongia aurea*) into brominated natural products. In addition, 3,5-dibromotyrosine (**1**) has been isolated from Verongid sponges, both in its free form and as an amino acid residue in matrix protein.[2] The fate of **1** in secondary metabolism of Vergonid metabolites may follow several pathways that parallel the transformations of tyrosine in plant secondary metabolism, including decarboxylation to 3,5-dibromotyramine (**2**), or oxidative transformation and conjugation, presumably through the putative intermediates **2a** and **2b**. Higher molecular weight molecules, such as fistularin-3 (**3**), arise from oxidation of the α-amino group to a ketoxime and oxidation of the aromatic ring in **1**, through the involvement of an arene epoxide (e.g., the hypothetical **1a**), which undergoes intramolecular *endo*-trig ring opening by the ketoxime to give **1b**.[5e] Additional variations, including incorporation of 3-amino-1,2-propanediol, which is present in **3**, add interesting complexity to the structures of high molecular weight Verongid metabolites.

Fistularin-3 (**3**, $[\alpha]_D$ +104.2° (*c* 1.67, MeOH)) was first reported in 1979 by Gopichand and Schmitz from a specimen of *A. fistularis* forma *fulva* (= *Aplysina fulva*) collected in St. Thomas, Virgin Island.[3] A separate report describes the isolation of 11-*epi*-fistularin-3 (**4**) from a specimen of a non-Verongid sponge, *Agelas oroides*, collected on the Great Barrier Reef (Australia).[4] Although reisolation of **3** has been reported on numerous occasions,[5] the absolute configuration at the two secondary carbinol groups, C11 and C17, has never been defined. Compound **3** does not lend itself to X-ray analysis due to lack of suitable crystallinity, while configurational analysis by ^1H NMR using various ^1H NMR methods based on anisotropy of the derived aryl acetate esters (e.g., the modified Mosher's method) is made difficult by the presence of multiple OH groups.



During the course of our investigation of the stereochemistry of related amino alcohols with potent antifungal activity, we required a sensitive microscale method for determination of the configurations of enantiomers of 3-amino-1,2-propanediol that would be applicable to analysis of **3** and the known related bis-oxazolidinone metabolite, **5**,[6] that was obtained from a Brazilian sample of *Clavelina oblonga*.[7] We found that strong acid hydrolysis of **3** or **5** (12 M HCl, 100−110 °C, 20 h) liberated 3-amino-1,2-propanediol, which was stereochemically stable under these conditions. Treatment of the hydrolysates with 2,4-dinitrophenyl-5-fluoro-L-alaninamide (Marfey's reagent[8]) gave diastereomeric derivatives that were resolved by LCMS,

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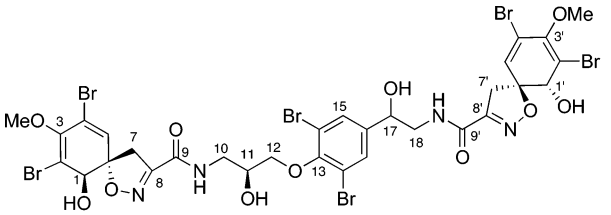

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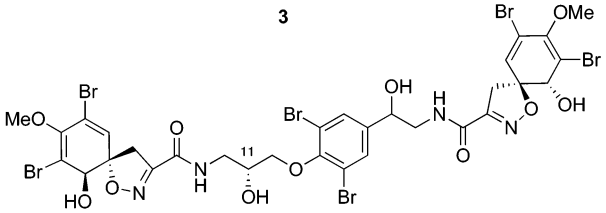

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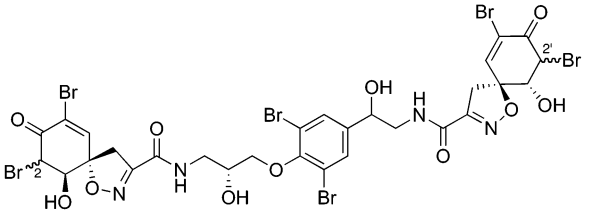
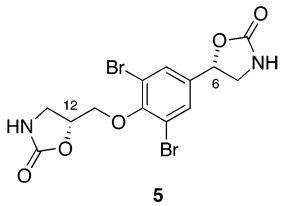


6a (2*S*,2′*S*)
6b (2*R*,2′*R*)



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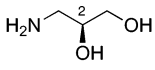
and their absolute configurations were identified by comparison with standards. When hydrolysis−Marfey's derivatization was performed on a sample of **3**, obtained from a Brazilian specimen of *Aplysina cauliformis*, we confirmed that the C11 configuration is *opposite* of that of 11-*epi*-fistularin-3 (**4**), obtained from an Australian specimen of *Agelas oroides* and proposed by König and Wright on the basis of differences in ^{13}C NMR chemical shifts between **3** and **4**.[4] The absolute configurations at C11 for **3** and **4** are 11*S* and 11*R*, respectively, and since **4** was chemically correlated by hydrolysis to the co-occurring agelorins A (**6a**) and B (**6b**), the C11 configurations of the latter compounds are both *R*.

Results and Discussion

A specimen of the sponge *A. cauliformis*, collected in Brazil, was extracted according to a standard protocol and purified by HPLC to give fistularin-3 (**3**, >95% pure, HPLC, ^1H NMR), which was identified by comparison of ^1H and ^{13}C NMR data and optical rotation ($[\alpha]_D$ +147° (*c* 0.275 MeOH), +104.2° (*c* 1.67)) with literature values.[3] The bis-oxazolidinone was isolated from an unrelated organism, *C. oblonga*, as previously described.[7] We found that vigorous hydrolysis of both compounds (12 M, 100−110 °C, 12 h) liberated 3-amino-1,2-propanediol, which provided an opportunity to investigate the absolute stereochemistry of **3**−**5**. It only remained to assign the C2 configuration of the aminodiol as either (−)-*S*-**7** or (+)-*R*-**8**.

To determine absolute stereochemistry at C11 in **3**, we prepared diastereomeric derivatives by reaction of (±)-3-amino-1,2-propanediol with chiral reagents. After some



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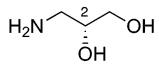

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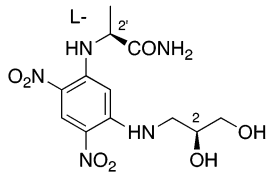

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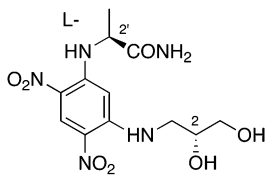

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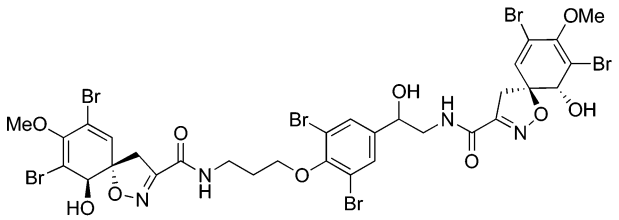

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experimentation it was found that treatment of (±)-3-amino-1,2-propanediol with 2,4-dinitrophenyl-1-fluoro-L-alaninamide (L-FDAA) gave resolvable peaks on LCMS (single-ion monitoring) corresponding to the L-DAA diastereomers **9** and **10** (t_R 12.07, 12.29 min, respectively). The later-eluting L-DAA diastereomer (t_R 12.29 min) was assigned to (2*S*,2*S*′)-**9** by comparison of the retention times with that of the L-DAA derivative, **9** (ambient column temperature: t_R 12.29 min, single peak by co-injection), derived from optically pure (2*S*)-3-amino-1,2-propanediol (**7**). When D-FDAA was used for derivatization of **7**, a second peak with retention time of t_R 12.07 min was observed. This diastereomer is expected to have the same retention time as **10** since it corresponds to *ent*-**10**. The retention times were found to be dependent upon HPLC column temperature. Both compounds eluted at later times when the column was held at $T = 30$ °C (t_R 15.35 min; t_R 15.80 min, respectively). In both cases, the later-eluting peak was always associated with the (2*S*)-isomer. When *S*-**7** was subjected to the hydrolysis−Marfey's derivatization, a single peak was obtained corresponding to **9**, which showed the aminodiol **7** did not racemize under these conditions.

11-*epi*-Fistularin-3 (**4**) was isolated from a sample of *A. oroides* collected on the Great Barrier Reef, Australia, in 1987. Analysis of the ^1H and ^{13}C NMR spectra of **4**, together with consideration of an optical rotation ($[\alpha]_D$ +65.2° (*c* 1.04, MeOH), see Table 1) that was significantly different from that of **3** ($[\alpha]_D$ +104.2° (*c* 1.67, MeOH)), led to the assignment of **4** as the C11 epimer of **3**, but of indeterminate configuration. Upon prolonged standing at −20 °C, **4** slowly hydrolyzed to a mixture of the epimeric agelorins A (**6a**) and B (**6b**), which were also isolated as natural products from the original sponge sample. Vigorous acid hydrolysis of **4** (12 M HCl, 110 °C) followed by treatment with L-FDAA and analysis of the product by LCMS analysis, as described for **3**, gave a single peak corresponding to the L-DAA-derivative of (*R*)-3-amino-1,2-propanediol (**10**). Since it is unlikely that the C11 configuration is altered during spontaneous hydrolysis, it follows that **4**, **6a**, and **6b** share the 11*R* configuration.

Conversely, hydrolysis and analysis of a sample of **5**, obtained from the colonial tunicate *C. oblonga*, gave (*S*)-**7**. Since the relative configuration of this compound was

Table 1. Reported Stereoisomers of Fistularin-3 (**3**)

organism	location	trivial name	$[\alpha]_D$ MeOH	reference
Aplysina fistularis forma *fulva*	St. Thomas, Virgin Islands	fistularin-3 (**3**)	+104.2° (*c* 1.67)[a]	Gopichand and Schmitz[3]
Aplysina cauliformis	Brazil, Baía de Todos os Santos	fistularin-3 (**3**)	+147° (*c* 0.275) +139.3° (*c* 0.263)[b]	this work
Agelas oroides	Australia, Great Barrier Reef	11-*epi*-fistularin-3 (**4**)	+65.2° (*c* 1.04)	König and Wright.[4]
Aplysina fulva	USA, Florida, Key Largo	**3**:**4** (2:3 mixture)	+147° (*c* 0.275) +150° (*c* 1.67)	this work
Verongia aerophoba	Turkey, Gallipoli	"isofistularin-3"	+108° (*c* 2.75)	Cimino et al.[11]
Verongia aerophoba	Turkey, Gökçeada	"1-*epi*-fistularin-3"	+51.6° (*c* 0.58)	Aydogmus[12]
Verongula gigantea	Bahamas, San Salvador Island			Ciminiello et al.[5d]
Aplysina archeri	Bahamas, San Salvador Island	fistularin-3	+102° (*c* 0.1)	Gunasekera and Cross[5g]

[a] Peracetate of **3** $[\alpha]_D$ +149.4° (*c* 1.32, $CHCl_3$)[3]. [b] In acetone.

determined earlier,[7] if follows that the absolute configuration is 12*R*, 6*S* (note different numbering scheme).[9]

The diastereomeric ratio at C11 in "fistularin-3" samples appears to vary with species or location of the sponge that produces it. Extraction of a pooled collection of individual *A. fulva* specimens from Key Largo, FL, in 1995, gave a substance that eluted as a single HPLC peak and was consistent with **3** (*m/z* 1130.7 [M + Na^+], calcd 1130.7 for $C_{31}H_{30}Br_6N_4NaO_{11}$) ($[\alpha]_D$ +147° (*c* 0.275, MeOH)) based on preliminary spectroscopic characterization. Careful examination of the ^{13}C NMR spectrum of this sample revealed signals corresponding to those reported for **4**,[4] in addition to signals that appeared doubled (C2/C2′ δ 122.30 s, 122.23 s, 122.30 s; C5,5′ 132.26 s, 132.31 s, 132.37 s; C11 69.88 d, 69.90 d, and C17 71.44 s, 71.47 s), and suggested a mixture of epimers at either C17 or C11. It seemed unlikely that C17 was epimeric since only ^{13}C NMR signals for carbon atoms nearby to C11 were doubled. To resolve the issue, the sample was subjected to acid hydrolysis−Marfey's analysis as described above to afford a 2:3 mixture of L-DAA derivatives **9** and **10**, respectively (LCMS integration). Therefore, the sample of "fistularin-3" from Key Largo was epimeric at C11, not C17, and composed of a 2:3 mixture of (11*S*)-fistularin (**3**) and (11*R*)-11-*epi*-fistularin-3 (**4**).

Intrigued by this result, we extracted discrete pieces of frozen *A. fulva* from the same collection to examine the variability between individual animals. The results of the hydrolysis−LCMS analysis are shown in Figure 1. Of the five pieces examined, two contained almost pure 11*R*-**4** (**4**/**3**, 35:4, 80% diastereomeric excess), while the other three contained mixtures of variable composition (**4**/**3**, 31:4−4:3), which demonstrates that stereochemical variability occurs between individuals of the same species.

The C17 configuration in **3** was not explicitly determined. Proksch and co-workers reported that *Aplysina* spp. release **5** by a spontaneous chemical fragmentation of **3** that is induced upon tissue damage and catalyzed by an undefined enzyme.[10] It is unlikely that changes in configuration occur during this transformation, and it is reasonable to suggest that the configuration of C17 in **3** is the same as that of **5**, from which it purportedly derives; however we have not been able to effect a conversion of **3** to **5**.

Anomalies with respect to the configuration of fistularin-3 and composition of its stereoisomers permeate the literature. Cimino first reported "isofistularin-3"[11] as a stereoisomer that differed from **3** by ^1H NMR chemical shifts, even though the $[\alpha]_D$'s of the two were nearly the same (Table 1). One of us (G.M.K.) characterized the structure of 11-*epi*-fistularin-3 (**4**) as an isomeric compound obtained from the Great Barrier Reef (Australia).[4] Compound **4** showed the same Cotton effects in the CD spectra as did both of our samples of **3** (Brazil, Key Largo; MeOH, λ_{max} 251 ($\Delta\epsilon$ +19.0), 285 (+17.8)), which supports the same configurations about the spiro-isoxazoline rings. A third report, which claimed yet another isomer of **3** from *A. fistularis* from Turkey ("1-*epi*-fistularin-3", $[\alpha]_D$ +51.6° (*c* 0.58, MeOH)),[12] offered a somewhat equivocal argument for an isomeric C1 epimer; however, this seems unlikely

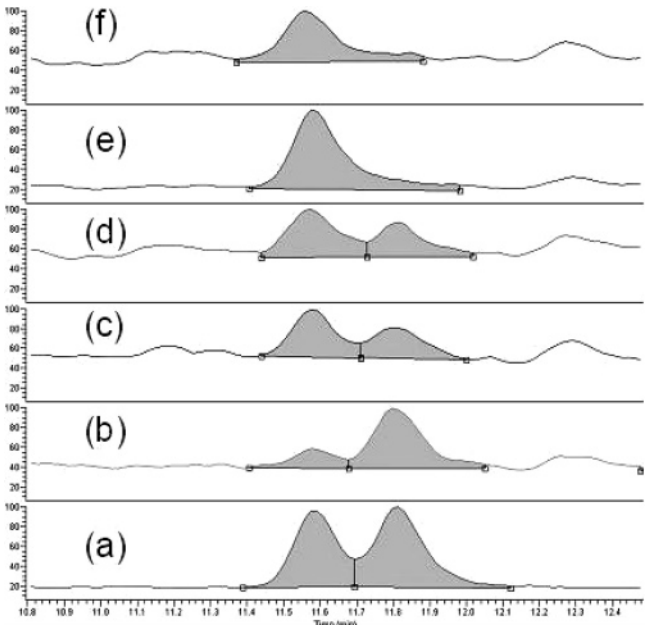
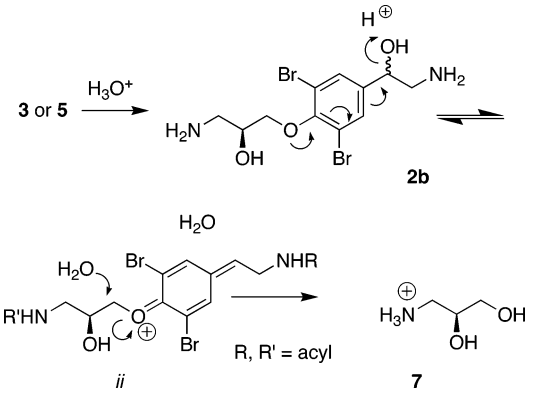


Figure 1. LCMS analysis (C_{18} HPLC, column temperature T = 30 °C; selected ion-monitoring, *m/z* 344) of L-DAA-3-amino-1,2-propanediol samples: (a) standard L-DAA-(±)-3-amino-1,2-propandiol (shaded peaks in order of elution: *R*-**10**, t_R = 11.57 min, *S*-**9**, t_R = 11.81 min; (b−f) **9** and **10** derived by hydrolysis (12 M HCl, aq, ~100 °C, 12 h) and derivatization of **3** from five randomly selected discrete pieces of *Aplysina fulva* (Key Largo, Florida). Column conditions were slightly different from those given in the Experimental Section. Ion-current normalized to 100 in each chromatogram.

since the ^{13}C NMR shift differences about the two identical spiro-isoxazoline rings were relatively minor and could be accommodated by a C11 epimer. Comparison of the NMR data of the latter compound with those of **4**, and their similar optical rotations, suggests the two compounds are identical.



Vigorous hydrolysis of **3** or **5** (12 M HCl, aq) gave 3-amino-1,2-propanediol, by initial cleavage of the amide or oxazolidinone groups, followed by acid-catalyzed S_N2 attack of H_2O at the primary CH_2OAr group. Facile S_N2 hydrolysis of the aryl ether bond in **3** and **5** may be explained by a stepwise mechanism, which invokes elimination of the benzylic C17 oxygen and the resultant delocalized cation *ii*. Intermediate *ii* is a good leaving group that is readily displaced by H_2O as a neutral quinone methide. Although other mechanisms are possible, we may exclude those that epimerize C2 of **7** by neighboring group participation since we found that exposure of optically pure standard (*S*)-**7** to hydrolysis conditions (12 M HCl, 110 °C) returns starting material without racemization.

Stereochemical heterogeneity in marine chiral natural products has been observed in a few instances, but is relatively rare. For example, Crews reported variable enantiomeric composition of the sesquiterpenes furodysin and furodysidin from *Dysidea herbacea* collected from different geographic locations in the Pacific.[13] The linear oxidized furanoditerpene, 12-acetoxyambliofuran, was shown to be a 1:3 mixture of enantiomers on the basis of analysis of the corresponding Mosher's esters,[14] and the chiral 2-azacyclopropene, dysidazirine, is found in either enantiomeric modification in *Dysidea fragilis* depending on the location in the Pacific.[15] In the present work, the results of analysis of three sponge samples—one from Brazil, another from Australia, and the third from the United States, together with the report of "1-*epi*-fistularin-3"[12] from the Aegean Sea—are best reconciled with the conclusion that "fisturalin-3"-like metabolites from Verongid sponges exhibit considerable stereochemical heterogeneity at C11, not only between species and geographic location but between individuals of the same species.

It is important to stress that *optical activity is not a reliable determinant for C11 configuration* in **3** or **4** as shown by the range of reported $[\alpha]_D$'s (Table 1). In our hands, careful measurements of freshly purified samples (HPLC) of optically pure **3** and the 2:3 mixture of **3** and **4** from Key Largo gave the same optical rotation (each $[\alpha]_D$ +147° (*c* 0.275, MeOH)) when measured at identical concentrations.[16] We must conclude that the lower $[\alpha]_D$'s reported for **3** and **4** are the result of other factors including purity of the samples used for measurements.

Variable C11 epimer composition is clearly revealed by Marfey's analysis of the acid hydrolysates of **3** and **4**, as demonstrated here, and presents an instructive object lesson. The co-occurrence of C11 epimers, in the same sample, can easily be overlooked by interpretation of NMR data, only, due to remoteness of the C11 center from other stereocenters and its diminished influence upon critical chemical shifts. Stereoisomerism at C11 in **3** and **4** is discernible by ^{13}C NMR in mixtures approaching a composition of 1:1 by slight signal doubling ($\Delta\delta \leq 0.1$ ppm) of a few (not all) signals; however, this can easily escape detection under conditions where the inferior set of peaks merge as "shoulders" into the dominant peaks.

We propose that the biochemical precursor to **3** and **4** is deoxyfistularin-3 (**11**)—itself a natural product characterized from *A. fistularis*[5c]—and that the biochemical origin of variable C11 configuration composition in **3** and **4** may involve cytochrome P_{450}-dependent enzymes with promiscuous stereoselectivity for H-abstraction and hydroxylation of the 3-amino-1-propanol unit in the precursor molecule **11**. Imperfect stereofidelity leading to formation of **3** and **4**, although counter to the conventional dogma that enzymes are always stereoselective, should not be so surprising since selection for abstraction of the C2 prochiral hydrogens in the 3-amino-1-propanol moiety of pseudosymmetric **11** is subject to less steric discrimination. Other examples of nonstereoselective cytochrome P_{450}-dependent enzymic hydroxylations can be found in the biotransformations of natural products added to cultured eubacteria and fungi.[17] Under these conditions, oxidation of compounds by cultures of metabolically active bacteria is relatively facile and conversions are surprisingly high. Alternatively, it is conceivable that oxidation of **11** to **3** and **4** is carried out by two different enzymes with high stereo*fidelity*, but different stereo*selectivity*. If the latter is true, the variation of C11 configuration with geographic location of the producing organism—or between different specimens of the same species demonstrated here for *A. fulva* from Key Largo—may be the result of differential levels of expression (or specific activities) of the respective enzymes.[18]

Since many sponges harbor large populations of symbiotic bacteria, the possibility of *native* biotransformation of metabolites in Verongid sponges by their associated microbial flora cannot be discounted and, in fact, constitutes a hypothesis suggested by our results.

Conclusion

In conclusion, we have developed a reliable microscale method for assignment of configuration and quantitative measurement of C11 epimer ratios in Verongid metabolites bearing 3-amino-1,2-diol units that is independent of $[\alpha]_D$ and NMR measurements. The method was used to assign the C11 absolute configuration and reveal stereoheterogeneity in fistularin-3 isomers and should find application in stereochemical surveys of brominated tyrosine natural products and related molecules.

Experimental Section

General Experimental Procedures. All solvents were AR grade and used as supplied or distilled from glass. Flash chromatography was carried out with EM Science silica (43–63 μm). LCMS measurements were made using an Agilent series 1100 HPLC with a Phenomenex Luna reversed-phase column (C_{18} 100 mm × 2.00 mm, 3 μm) fitted with a guard column and connected to a ThermoFinnigan MSQ single quadrupolar mass spectrometer. The column temperature was kept at either ambient temperature or 30 °C. Solutions were analyzed in ESIMS mode with selected ion monitoring, span 2.0 amu, dwell 1.00 s, cone 90 V, probe temperature 350 °C.

1-Fluoro-2,4-dinitrophenyl-5-L-alaninamide (L-FDAA) was purchased from Pierce (Rockford, IL), and D-FDAA was synthesized from D-alaninamide using a modification of the procedure published by Marfey.[8]

Purification of Fistularin-3 (3) from *Aplysina cauliformis* (Brazil). The sponge *A. cauliformis* was collected at the Baía de Todos os Santos (Salvador, Bahia, Brazil, at the Quebra Mar Norte station, S 12°57.7′, W 38°31.1′), on August 1, 1999, and immediately frozen. After lyophilization, the animal (164 g, dry weight) was blended with CH_3CN (3 L) and re-extracted in the same solvent (2 × 2 L). The remaining marc was extracted with MeOH (3 L). The MeOH extract (A) and CH_3CN extract (B) were separately evaporated, to give brownish gums (A = 6.14 g; B = 0.930 g).

Extract B was subjected to flash chromatography on silica gel (gradient of MeOH in CH_2Cl_2) to give seven fractions. The seventh fraction was purified by C_{18} reversed-phase HPLC (Inertsil ODS-2, 125 Å, 5 μm particle, 250 × 9.4 mm, 4:6 H_2O/MeOH; detection at λ = 254 nm, followed by Dynamax C_{18}, 5 μm, 10 × 250 mm, 1:1 H_2O/CH_3CN, 4.0 mL/min) to yield fistularin-3 (**3**, 33 mg, 0.002% wet weight): UV (MeOH) 224 nm (ϵ 32 000 ± 100), 282 (13 100 ± 100); CD (MeOH) λ 252 nm ($\Delta\epsilon$ +19.7), 286 (+19.7); ^{1}H and ^{13}C NMR data, identical with the published values;[3] ESIMS *m/z* 1130.7 [M + Na^+] calc 1130.7.

Purification of "Fistularin-3" from *Aplysina fulva* (Key Largo, FL). (a) Extraction: Bulk Collection. Samples of *A. fulva* (total ~1.4 kg) were collected by hand with assistance from scuba in 1995 at Dry Rocks near Key Largo, FL, then pooled and frozen (−20 °C) until needed. A portion (253.93 g wet) was blended with mechanical stirring in 1:2 CH_2Cl_2/MeOH and the mixture filtered through a Büchner funnel. The residue was washed with the same solvent and the filtrate concentrated to remove CH_2Cl_2. The remaining aqueous MeOH was extracted with an equal volume of hexanes to obtain a hexane-soluble fraction after concentration (2.65 g). Water (40% v/v) was added to the aqueous MeOH fraction and the mixture partitioned against $CHCl_3$. The $CHCl_3$/MeOH-soluble fraction was concentrated (3.14 g), dissolved in 3:97 MeOH/CH_2Cl_2, and applied to a silica flash chromatography, which was eluted using a solvent gradient (1.5−100% MeOH/$CHCl_3$). The crude fraction obtained with 10% MeOH was submitted to final purification by reversed-phase HPLC (Dynamax C_{18}, 5 μm, 10 × 250 mm, 1:1 H_2O/CH_3CN, 4.0 mL/min) to provide two major components: 11-oxoaerothionin[19] (18.5 mg, 0.072% wet wt) and a later-eluting single peak comprising an inseparable 2:3 mixture of fistularin-3 (**3**) and 11-*epi*-fistularin-3 (**4**, 27.6 mg, 0.011% wet wt), respectively: $[\alpha]_D$ +150° (*c* 1.67, MeOH); UV (MeOH) 224 nm (ϵ 32 000 ± 100), 282 (13 100 ± 100); CD (MeOH) λ_{max} 251 ($\Delta\epsilon$ +19.0), 285 (+17.8); ^{1}H NMR and ^{13}C NMR spectra were identical with the literature values for **4**[4] except for some signals that were doubled (see text and Table 2); ESIMS *m/z* 1130.7 [M + Na^+] calc 1130.7.

(b) Extraction: Individual Animals. Individual pieces of frozen animals (*N* = 5, labeled (b)−(f), each 0.47−2.0 g) were selected at random from the bulk pooled collection of *A. fulva* and extracted twice with MeOH (2 × 10−11 mL, twice), and the filtered MeOH extracts were processed individually as follows. Water (10% v/v) was added followed by partition against hexane. The aqueous MeOH layer was adjusted to 40% v/v H_2O and partitioned against an equal volume (1*V*) of $CHCl_3$ followed by a second extraction ($CHCl_3$, 0.1*V*). The combined MeOH/$CHCl_3$ layer was separated, after centrifugation, and concentrated to dryness (12.5−50 mg). An aliquot of each sample (6.0 mg) was loaded onto a preparative TLC plate (1 mm × 20 cm × 20 cm), which was developed twice (7% MeOH/$CHCl_3$). The UV-active bands centered on the plate at the R_f for authentic **3** were excised and extracted with MeOH. The recovered fractions (0.6−3.1 mg) were verified to contain **3** or **4** and 11-oxoaerothionin by ESIMS and ^{1}H NMR before submission to hydrolysis−Marfey's analysis and LCMS separation of the derivatives **9** and **10** (selective ion monitoring) similar to those described below. The ratios of **9**:**10** (corresponding to **3**:**4**) were measured by integration of the MS ion current of each LC peak and are as follows: (b) 3:11, (c) 4:3, (d) 3:2, (e) 35:4, (f) 31:4. Racemic standard sample [(a) (±)-3-amino-1,2-propanediol] gave a ratio of 1:1 **9**:**10**.

Table 2. ^{13}C NMR Data for 11-*epi*-Fistularin-3 (75.5 MHz, d_6-acetone)[4] and a Mixture of **3** and **4** (100 MHz, d_6-acetone)

carbon	11-*epi*-fistularin-3 (**4**)[4]	**3/4**
1, 1′	75.0 CH, 75.1 CH	75.2 CH, 75.3 CH
2, 2′	122.0 qC, 122.1 qC	122.20 qC, 122.23 qC, 122.30 qC[a]
3, 3′	148.6 qC, 148.6 qC	148.8 qC, 148.8 qC
4, 4′	113.7 qC, 113.7 qC	113.9 qC, 113.9 qC
5, 5′	132.0 CH, 132.1 CH	132.26 qC, 132.31 qC, 132.37 qC[a]
6, 6′	91.8 qC, 91.8 qC	91.9 qC, 91.9 qC
7, 7′	39.8 CH_2, 39.9 CH_2	40.09 CH_2, 40.13 CH_2
8, 8′	154.9 qC, 155.0 qC	155.2 qC, 155.3 qC
9, 9′	160.4 qC, 160.5 qC	160.5 qC, 160.6 qC
10	43.4 CH_2	43.6 CH_2
11	69.7 CH	69.88 CH, 69.90 CH
12	75.7 CH_2	75.9 CH_2
13	152.5 qC	152.7 qC
14, 14′	118.3 qC, 118.3 qC	118.4 qC, 118.3 qC
15, 15′	131.3 CH, 131.3 CH	131.5 CH, 131.5 CH
16	142.9 qC	143.3 qC
17	71.3 CH	71.44 CH, 71.47 CH
18	47.5 CH_2	47.7 CH_2
OCH_3	60.2 CH_3, 60.2 CH_3	60.3 CH_3, 60.2 CH_3

[a] Expected fourth peak is overlapped.

Purification of Bis-oxazolidinone 5. Compound **5** was purified from the colonial tunicate *C. oblonga,* as previously described,[7,20] to give pure bis-oxazolidinone **5** ($[\alpha]_D^{24}$ −9.2° (*c* 0.25, MeOH)).

Determination of Absolute Configuration of Oxazolidinone 5.[7,20] Oxazolidinone **5** (44.5 μg) was suspended in 12 M HCl (1 mL) and heated in a sealed glass vial at 100−110 °C for 24 h. The solution was evaporated to dryness under a N_2 stream and the residue treated as follows.

(a) Marfey's Derivatiation with L-FDAA. The hydrolysate was redissolved in water (100 μL) and the solution treated with either 1-fluoro-2,4-dinitrophenyl-5-L-alaninamide (L-FDAA) or D-FDAA (1% w/v acetone solution, 100 μL) followed by 1.0 M $NaHCO_3$ (20 μL). The mixture was heated (82 °C, 10 min), cooled, and quenched with 1.0 M HCl (20 μL). Standard samples of (±)- and (*S*)-(−)-3-amino-1,2-propanediol (Aldrich) were also converted to their L-DAA derivatives by the same procedure.

(b) LC Analysis. The solutions obtained by derivatization, as described above, were analyzed by LC-MS using an Agilent series 1100 HPLC with a Phenomenex Luna C_{18} column (100 mm × 2 mm, 3 μm) interfaced with a Thermo Finnigan MSQ quadrupole mass spectrometer using the parameters described under General Experimental Procedures. For HPLC analysis at ambient column temperature (*T* ~24 °C) the retention times for **10** and **9** were t_R = 12.07 and 12.29 min, respectively.

The L-DAA derivative of the hydrolysate obtained from bis-oxazolidinone **5** had a retention time of t_R = 12.05 min, consistent with **9** and revealing the 12*R* configuration for **5**.

Conversion of 11-*epi*-Fistularin-3 (4) to Agelorins A (6a) and B (6b). 11-*epi*-Fistularin (**4**) was obtained from a sample of *A. oroides* Schmidt (Agelasidae, Axinellidae) collected from the Great Barrier Reef (Australia) and purified as described elsewhere.[4] After prolonged standing at −20 °C (~11 years) compound **4** was found to have hydrolyzed to give agelorins A (**6a**, t_R = 13.35 min) and B (**6b**, t_R = 13.55 min), which were purified by HPLC (Knauer Eurospher C_{18}, 250 × 8 mm, 3:2 CH_3CN/H_2O, 1.5 mL/min) and exhibited ^{1}H NMR spectral data consistent with literature values.[4]

Determination of the Absolute Configuration of Fistularin-3 (3) from *Aplysina cauliformis* (Brazil). Fistularin-3[3] (**3**, 0.8 mg) was suspended in 12 M HCl (1 mL) and the mixture heated in a sealed glass vial at 100−110 °C for 17 h. The solution was evaporated to dryness under a N_2 stream to obtain the hydrolysate, which was dissolved in water (100 μL) and treated with L-FDAA as described for **5**, above.

The absolute configuration of the aminopropanediol portion was found to be *S* (>90% ee).

Determination of Configuration for 11-*epi*-Fistularin-3 (4). 11-*epi*-Fistularin-3 (**4**, 1.0 mg) was suspended in 12 M HCl (1 mL) and heated in a sealed glass vial at 100 °C for 20 h. The solution was evaporated to dryness under N_2 to obtain the crude hydrolysate.

(a) Marfey's Derivatization.[8] The hydrolysate was redissolved in water (100 μL) and combined with either 1-fluoro-2,4-dinitrophenyl-5-L-alaninamide (L-FDAA) or D-FDAA (1% w/v in acetone, 100 μL) and aqueous $NaHCO_3$ (1.0 M, 20 μL). This solution was heated at 80 °C for 10 min, then cooled and treated with HCl (aq 1.0 M, 20 μL). This procedure was repeated with the standards (±)- and (*S*)-(−)-3-amino-1,2-propanediol.

(b) LC Analysis. The solutions of DAA derivatives prepared as described above were analyzed by injection (6 μL) onto an MSQ LC-MS fitted with a reversed-phase column (Phenomenex Luna, C_{18} 100 × 2 mm, 3 μm) in line with a quadrupole mass spectrometer as described under General Experimental Procedures. LC parameters were as follows: column temp 30 °C, flow rate 0.40 mL/min, initial 90% solvent A (H_2O + 0.1% formic acid) 10% solvent B (CH_3CN), at 15 min 70% A, at 20 min 100% B hold for 5 min, at 28 min 90%, hold for 2 min. MSQ parameters were as follows: selected ion monitoring at *m/z* 344 $[M + H]^+$. Retention times for the two peaks due to (*S*)- and (*R*)-DAA derivatives of (±)-3-amino-1,2-propanediol were t_R = 15.35 min and t_R = 15.80 min, respectively (standard L-DAA-(*S*)-(−)-3-amino-1,2-propanediol eluted at t_R =15.81 min). Analysis of the 11-*epi*-fistularin-derived L-DAA derivative gave a retention time of t_R = 15.27 min, corresponding to an 11*R* configuration for *epi*-fistularin-3 (**4**).

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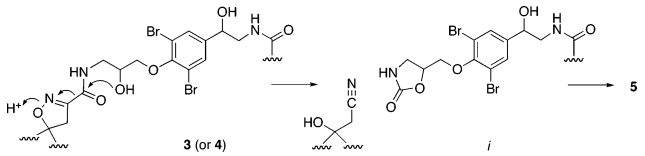

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