Extending the Record of Meroditerpenes from *Cacospongia* Marine Sponges

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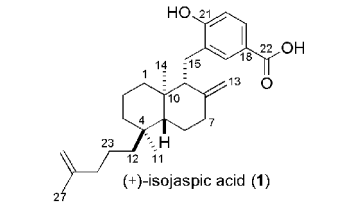
A new meroditerpene, (+)-isojaspic acid (**1**), along with two known meroditerpenes, cacospongin D (**2**) and jaspaquinol (**3**), have been isolated from a marine sponge *Cacospongia*. Comprehensive taxonomic identification distinguished this *Cacospongia* apart from morphologically similar *Psammocinia*. The absolute stereochemistry of **1** was elucidated on the basis of extensive 1D and 2D NMR techniques and analysis of the optical rotation versus (+)-zonarol (**8**), (+)-isozonarol (**9**), (−)-dactylosponol (**10**), and (+)-hyatellaquinone (**11**). Furthermore, bioactivity evaluation showed that the meroditerpenes isolated significantly inhibited *Staphylococcus epidermidis*.

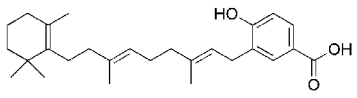
In recent years we have conducted investigations on sponge communities that are a source of cyclocinamide,[1] psymberin,[2] swinholide A,[2,3] and preswinholide A.[4] However, it has been challenging to employ natural history data, especially underwater photographs, to facilitate distinguishing between a group of drab, solitary Indo-Pacific sponges we believe to be among the *Psammocinia* taxon. As a further complication, even when the taxonomic information has been obtained, confusion about the species identification often remains. Four examples of such specimens, shown in Figure 1, include solitary sponges with similar morphologies all collected from the Milne Bay region of Papua New Guinea. We now know that these samples are among either the *Psammocinia* or *Cacospongia* genus.[5] The specimen A (coll. no. 03526) is a rich source of psymberin[2] and has been identified as *Psammocinia* aff. *bulbosa*. Similarly, specimen B (coll. no. 05411) is now identified as *P.* aff. *bulbosa*, but does not contain even trace amounts of psymberin. The specimen C (coll. no. 01236), previously identified as *Psammocinia* sp., was found in our recent work[6] to be a source of meroterpenoids also isolated from *Cacospongia*.[7] Last, specimen D (coll. no. 05401), the subject of the results described below, was initially assumed to be *Psammocinia* sp. but was identified during the course of the isolation work as an undescribed *Cacospongia*.

Results and Discussion

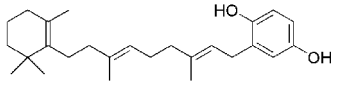
The progress on this project was facilitated once the correct taxonomic data were obtained. Our expectation was that specimen D in Figure 1, provisionally identified as *Psammocinia*, would be a source of nitrogen-containing analogues of cyclocinamide,[1] psymberin,[2] or polyketides such as swinholide A.[2,3] Two developments changed this view. First, ^{1}H NMR evaluation of the crude extract fractions coded XFH (hexane), XFD (dichloromethane), and XFM (methanol) revealed a complex array of peaks, but none appeared to be associated with the expected chemotypes (Supporting Information, Figure S8). Instead, the upfield region was packed with methyl resonances, and signals could also be observed in the aromatic range. Once the taxonomic determination of *Cacospongia* was in hand, this drew our attention to the possibility that mixtures of terpenoids would be encountered.[8] It is important to note that the overall chemistry patterns from *Cacospongia* are complex and range from terpenoids,[8] to polyketides,[9] to mixed biogenetic nitrogen-[10] and bromine-containing compounds.[11] The next step was the re-evaluation of the NMR spectra and the pooling of fractions suspected to contain terpenoids. Once completed, there were three separate fractions of interest, coded P3 (79 mg), P4 (140 mg), and P5 (340 mg). Subsequent HPLC gave the new compound (+)-isojaspic acid (**1**) (21 mg) from P3, plus known compounds cacospongin D (**2**)[7] (68 mg) from P4 and P5 and jaspaquinol (**3**)[12] (8 mg) from P5. Both **2** and **3** have co-occurred in the sponge specimen C of Figure 1, previously identified as *Psammocinia*[6] and now provisionally reassigned to *Cacospongia*. Interestingly, **3** has been reported from two unrelated sponges, *Suberea* sp.[13] and *Jaspis splendens*,[12] and a closely related structural isomer, cacospongin B (**4**),[7] has been isolated from *Cacospongia*.



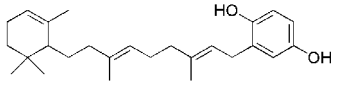
(+)-isojaspic acid (**1**)



cacospongin D (**2**)



jaspaquinol (**3**)



cacospongin B (**4**)

The structure elucidation of the new compound, (+)-isojaspic acid (**1**), $C_{27}H_{38}O_3$, began with a side-by-side evaluation of the molecular formulas derived from the NMR data shown in Table 1 and the high-resolution mass spectrometry data discussed below. The partial formula of $C_{27}H_{36}$ assessed by obtaining an NMR DEPT spectra was augmented with additional oxygen and hydrogen atoms of OH (δ_H = 3.52) and CO_2H (δ_C = 172.2) moieties clearly identified by 1D NMR. This count was used as input to evaluate the positive ion HRESITOF pseudomolecular ion cluster at *m*/*z* 411.2903 $[M + H]^+$ and confirmed that the formula shown above uniquely fits the MS data. The two functional groups noted above, plus an additional constraint of a trisubstituted benzene ring (δ_H = 6.79, J = 8.4 Hz; δ_H = 7.89, J = 2.4 Hz; δ_H = 7.83, J = 8.4, 2.4 Hz), allowed the 23 hits obtained from a dereplication search of the molecular formula, within marine natural products literature,

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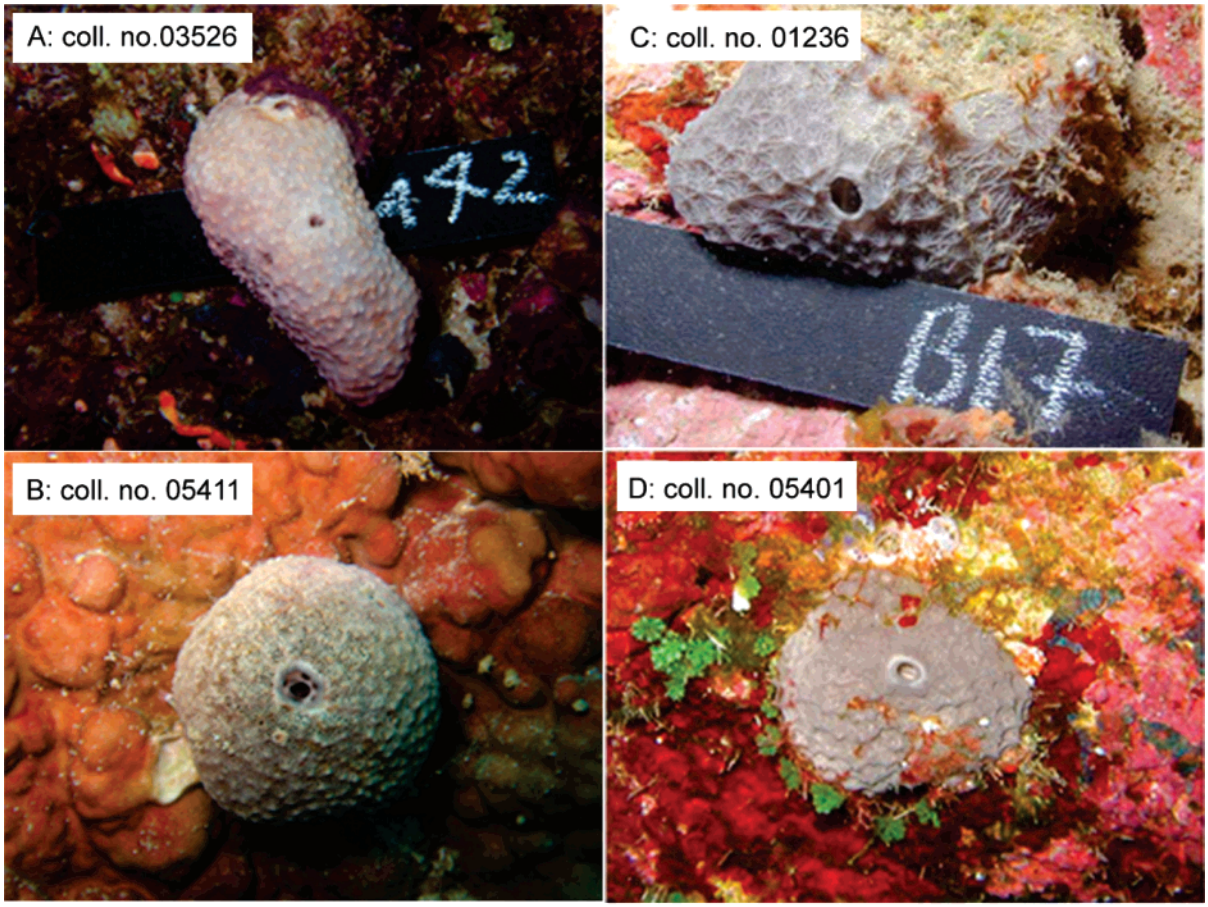


Figure 1. Similar morphologies of *Psammocinia* aff. *bulbosa*. (A and B) and *Cacospongia* sp. (C and D).

to be pruned to just four reasonable candidate structures. These consisted of (−)-jaspic acid (**5**),[12] (−)-subersic acid (**6a**),[13] (+)-subersic acid (**6b**),[14] and (+)-makassaric acid (**7**).[14] Closer inspection of the 2D NMR data of **1** revealed resonances for the 3-alkyl-4-hydroxybenzoic acid portion of substructure **A** that were identical to those observed for **5** and **7**. A series of 2D NMR correlations outlined in Figure 2 were observed and interpreted to visualize the full substructure **A** and substructure **B**. At this point it was clear that **1** possessed a new framework because the key elements of structures **5**−**7** were missing. The one or more distinctive features of **1** included the two geminal disubstituted double bonds (δ_C = 107.8, δ_H = 4.70 and 4.83 d, ^2J = 1.2 Hz; δ_C = 110.1, δ_H = 4.70 and 4.72 d, ^2J = 1.2 Hz), the requirement for only two additional carbocyclic rings, and the geminal structural relationship of a methylene and methyl group. Finally, it was apparent that carbon frameworks of partial structures **A** and **B** were each isomeric to similar arrays present in **5**.

The stereochemical assignments deduced for **1** were based on several lines of reasoning. This began with a comparison of ^{13}C chemical shifts to those of **5** and to related drimane[15] type merosesquiterpenes. Next was an analysis of ^{1}H *J* values, and a final corroboration utilized results obtained from NOE data. The C-11 methyl was confidently assigned as axial on the basis of the δ_C = 21.4, which was similar to that of (+)-zonorol (**8**) (δ_C = 21.7),[16] (+)-isozonorol (**9**) (δ_C = 21.9),[16] (−)-dactylosponol (**10**) (δ_C = 21.9),[17] and (+)-hyatellaquinone (**11**) (δ_C = 21.7)[18] and significantly upfield relative to the equatorial C-11 methyl (δ_C = 28.9) in **5** (Supporting Information, Figure S9). A parallel pattern of shift differences was observed between CH_2-12 equatorial (δ_C = 38.3) versus the CH_2-12 axial (δ_C = 28.9) groups of **1** and **5**, respectively. Somewhat similar arguments were used to assign the relative axial/equatorial stereochemistry at C-5, C-9, and C-10. Most importantly, the large ^3J = 12.6 Hz defined H-5 as axial, the axial C-14 methyl was based on diagnostic chemical shifts (**1** δ_C = 15.3; **5**, **8**−**10** δ_C = 14.0−15.0), and the equatorial CH_2-15 was similarly assigned (**1** δ_C = 23.7, **8** δ_C = 23.9, **11** δ_C = 24.4). Additional substantiation for these assignments was provided by the NOESY data outlined in Figure 3, with the axial C-14 methyl as one anchor point and the axial H-1 as the other.

An interesting correlation of absolute stereochemistry with patterns of optical rotation signs has been previously discussed for sponge drimane type core meroditerpenoids.[19] We extend this dialogue with the following observations. Unrelated sponges have been a source of enantiomeric subersic acids 5*R*,10*R*-(−)-**6a**[13] and 5*S*, 10*S*-(+)-**6b**,[14] which differ in stereochemistry across the *trans*-decalin ring. We previously noted that the 5*S*,10*S* *trans*-decalin stereochemistry seemed reasonable for (−)-jaspic acid (**5**) ([α] = −22.9)[12] because of its optical properties similar to those of 5*S*,9*S*,-10*S*-(−)-dactylosponol (**10**, [α] = −14)[17] and rotation sign opposite that of 5*R*,9*R*,10*R*-(+)-isozonorol (**9**, [α] = +28), whose absolute stereochemistry has been set through synthesis.[20] Likewise, the 5*R*, 9*R*, 10*R* stereostructure established by enantiomerically controlled syntheses of both (+)-zonorol (**8**, [α] = +17)[20] and (+)-hyatellaquinone (**11**, [α] = +16)[21] appears to be a relevant model for the absolute stereochemistry shown here of (+)-**1** ([α] = +9.3).

The brief bioactivity evaluation of the compounds isolated focused on their antimicrobial properties. A sample of (−)-**5**, obtained from our pure compound library, was tested alongside (+)-**1**, **2**, and **3** against *Bacillus subtillus*, *Escherichia coli*, and *Staphylococcus epidermidis*. All four compounds (+)-**1**, **2**, **3**, and (−)-**5** showed significant antimicrobial activity against *S. epidermidis* (MIC = 2.5, 20, 5.0, and 2.5 μg/mL, respectively) though weaker in comparison with vancomycin[22] (MIC = 0.625 μg/mL). Alternatively, (+)-**1**, **2**, **3**, and (−)-**5** showed no activity toward *E. coli* and *B. subtillus* (MIC > 400 μg/mL).

Table 1. ^{1}H and ^{13}C NMR Data for (+)-Isojaspic Acid (**1**) in $CDCl_3$[a]

position	δ_C (mult.)	δ_H (mult., *J* in Hz)	gCOSY	gHMBC
1ax	39.1 (CH_2)	1.16 (m)	2ax	5, 10,[b] 14[b]
1eq		1.89 (dt, 12.6, 3.0)		5
2ax	19.4 (CH_2)	1.56 (m)	1ax	10[b]
2eq		1.69 (m)	3eq	10[b]
3ax	44.3 (CH_2)	1.13 (m)		5
3eq		1.25 (td, 13.2, 4.8)	2eq	
4	36.1 (qC)			
5	52.8 (CH)	1.32 (dd, 12.6, 1.8)	6	3,[b] 4, 6, 9, 10[b]
6	24.3 (CH_2)	1.34 (m)	5	4, 5
7	37.7 (CH_2)	1.34 (m)		5, 6,[b] 8, 9, 10
8	149.0 (qC)			
9	56.3 (CH)	2.26 (dd, 8.4, 7.8)	15	8, 10, 13, 14, 15
10	40.5 (qC)			
11	21.4 (CH_3)	0.86 (s)		3, 4, 5, 12
12a	38.3 (CH_2)	2.05 (td, 12.6, 4.8)	12b, 23	
12b		2.39 (ddd, 12.6, 3.6, 2.4)	12a	3, 5
13a	107.8 (CH_2)	4.70 (d, 1.2)		8
13b		4.83 (d, 1.2)		
14	15.3 (CH_3)	0.84 (s)		1, 9, 10
15	23.7 (CH_2)	2.79 (m)	9	8, 10,[b] 16,[b] 17,[b] 21
16	128.8 (qC)			
17	132.7 (CH)	7.89 (d, 2.4)		15, 16,[b] 21, 22
18	121.7 (qC)			
19	129.9 (CH)	7.83 (dd, 8.4, 2.4)	20	17, 21, 22
20	115.4 (CH)	6.79 (d, 8.4)	19	15,[b] 16, 18, 21, 22[b]
21	158.9 (qC)			
22	172.2 (qC)			
23	21.4 (CH_2)	1.34 (m)	12a, 24	24, 25[b]
24	38.9 (CH_2)	1.98 (t, 7.2)	23	23, 25, 26, 24
25	146.4 (qC)			
26a	110.1 (CH_2)	4.70 (d, 1.2)		
26b		4.72 (d, 1.2)		
27	22.7 (CH_3)	1.73 (s)		24, 25, 26
OH		3.52 (s)		

[a] Measured at 600 MHz (^{1}H) and 150 MHz (^{13}C). [b]Optimized for 4 Hz coupling.

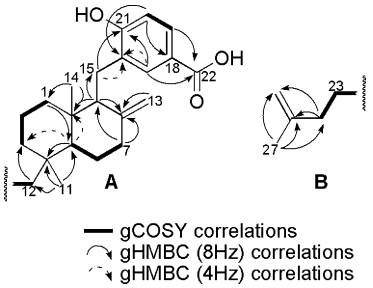


Figure 2. Key 2D NMR correlations of substructures **A** and **B** of (+)-**1**.

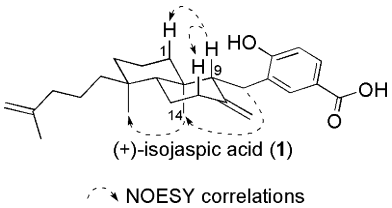
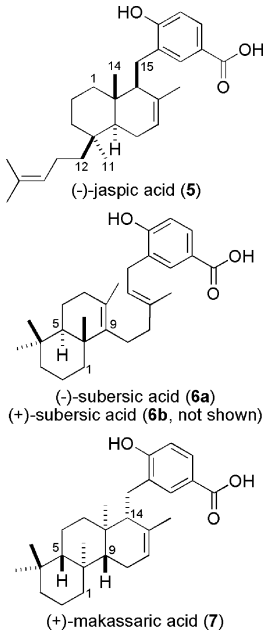


Figure 3. Key 1D NOESY correlations of (+)-**1**.

This is the first report of the isolation of diverse meroditerpenes from a *Cacospongia*. The circumstance of observing meroditerpenoids cacospongin D (**2**), jaspaquinol (**3**), and (−)-jaspic acid (**5**) from three vastly unrelated sponges, *Cacospongia*, *Suberea*, and *Jaspis*, is also noteworthy. These results should provide the stimulus for future side-by-side evaluation of the biosynthetic machinery present in these unrelated sponges. An additional challenge evident from this work is the need for total synthetic efforts to affirm the absolute stereochemistry relationships proposed to date for the meroterpenoid isomers 4*S*,5*R*,9*R*,10*S*-(+)-isojaspic acid (**1**), 4*R*,5*S*,9*S*,10*S*-(+)-**5**,[12] 5*R*,10*R*-(−)-subersic acid (**6a**),[13] and 5*S*,8*S*,9*S*,10*S*,-14*R*-(+)-makassaric acid (**7**).[14]



Experimental Section

General Experimental Procedures. Standard pulse sequences were used for the ^{1}H, ^{13}C, gHMQC, gHMBC (for 8 Hz), and gCOSY NMR experiments, which were run on a Varian UNITY INOVA-600 spectrometer (600 and 150 MHz for ^{1}H and ^{13}C, respectively). The delay time was modified to 4 ms for long-range gHMBC (optimized for 4 Hz) experiments. DEPT (used to determine ^{13}C NMR multiplicities) and 1D-NOESY experiments were run on a Varian UNITY INOVA-500 spectrometer (500 and 125.7 MHz for ^{1}H and ^{13}C, respectively). Chemical shifts from residual solvent protons and carbon were used as reference (7.26 and 77.0 ppm for ^{1}H and ^{13}C, respectively). High-resolution mass measurements were obtained on a benchtop ESITOF mass spectrometer. HPLC was performed with a reversed-phase preparative column (using a tandem 254 nm UV−evaporative light scattering detector (ELSD) monitoring system) and a reversed-phase semipreperative C18 5 μm column (254 or 310 nm UV detection).

Collection and Identification. The sponge (coll. no. 05401) was gathered using scuba in Milne Bay, Papua New Guinea (GPS = 11°26.686′ S: 154°24.155′ E) at depths of 30−60 ft. The globular (1−3 cm diameter) sponge had a gray ectosome with a purple-gray endosome and a soft reticulated surface containing an apical oscule and was identified as a *Cacospongia* sp.[23] Voucher samples have been deposited at the Zoological Museum of Amsterdam for sponges including ZMAPOR 19842 = UCSC coll. no. 03526, ZMAPOR 19843 = UCSC coll. no. 05411, and ZMAPOR 19847 = UCSC coll. no. 05401. Voucher specimens and photographs are also available from the Crews laboratory.

Biological Assays. The antimicrobial assays were performed as previously described[24] with a slight modification in that (+)-**1**, **2**, **3**, and (−)-**5** were further tested at a maximum concentration of 40.0 μg/mL, which was serially diluted to 0.625 μg/mL, in order to accommodate their potencies against *Staphylococcus epidermidis*.

Extraction and Isolation. The freshly collected sponge (70 g wet) was preserved in an ethanol−seawater (1:1) solution for approximately 24 h, after which the solution was decanted and discarded in the field. The preserved sponge was then packed in bottles and shipped to the UC Santa Cruz lab at ambient temperature. After an accelerated solvent extraction (ASE) apparatus (as shown in Supporting Information Scheme S1) was employed to afford a hexanes fraction (XFH, 5.6 g), a dichloromethane fraction (XFD, 2.2 g), and a methanol fraction (XFM, 1.8 g), approximately 45% of each ASE crude was fractionated by reversed-phase (RP) preparative HPLC (PrepHPLC) with CH_3CN−H_2O (65:35 to 1:0), to afford eight fractions each (P1−P8). The three ASE fractions afforded the same P1−P8 fractions, as monitored by

retention times, mass spectrometry, and NMR, differing only by peak intensities in the PrepHPLC chromatorgram. Accordingly, fractions of interest were pooled from the XFH, XFD, and XFM to afford P3, P4, and P5. Fraction P3 (79 mg) was fractionated by RP semipreperatory HPLC (HPLC) with CH_3CN-H_2O (7:3 to 1:0 with 0.1% formic acid buffer), affording eight fractions (P3H1−P3H8), P3H2 contained the new (+)-isojaspic acid (**1**) (21 mg). P4 (140 mg) was fractionated using the same methodology as with P3 with two additional HPLC sequences using an isocratic CH_3CN (with 0.1% formic acid buffer) system to yield cacospongin D (**2**) (3.7 mg) in fraction P4H2.2.2. P5 (340 mg total) was fractionated by HPLC using the same methodology as with P4, though using only one isocratic sequence to afford P5H6.1 and P5H6.2, identified as the known compounds **2** (64 mg) and jaspaquinol (**3**) (8.5 mg), respectively.

(+)-Isojaspic acid (1): yellow oil; $[\alpha]^{27}_D$ +9.3 (*c* 0.4, $CHCl_3$); NMR data, see Table 1); HRESIMS *m*/*z* 411.2903 $[M + H]^+$ (calcd for $C_{27}H_{39}O_3$, 411.2894).

Cacospongin D (2): spectral data in accordance with published data.[7]

Jaspaquinol (3): spectral data in accordance with published data.[12]

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Supporting Information Available: NMR spectra, MS data, optical rotation data, and isolation scheme of (+)-isojaspic acid (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

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