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Identification of the Eight-Membered Heterocycles Hicksoanes A–C from the Gorgonian *Subergorgia hicksoni*

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Novel eight-membered heterocycles, named hicksoanes A–C, were isolated from the gorgonian *Subergorgia hicksoni*. Their structures, including the absolute configuration of all three stereocenters, were determined by means of extensive spectroscopic data. Hicksoanes have a unique structure containing a triazocane cycle, which is substituted by isoleucine and tryptophan moieties. Hicksoanes A–C showed antifeeding activity against goldfish at natural concentration (≈10 μg mL^{-1}).

Organohalides are particularly abundant in nature, especially in the marine environment; at least 4000 naturally occurring compounds of this type have been reported to date.[1] Somewhat surprisingly, the relative number of fluorinated, chlorinated, brominated, and iodinated metabolites does not correlate with the concentration levels of the halides in the ocean or their relative redox potentials. Chlorinated and brominated derivatives are predominant, whereas fluorinated and iodinated molecules are rare.[1] A series of novel cyclic peptides, keramamides, were isolated from the marine sponges *Theonella*. Many of them contain chlorinated[2] or brominated[3] tryptophan. Examples of iodine-containing natural products are, for example, nucleoside derivatives,[4] terpene derivatives, and the recently reported tasihalides that were isolated from an assemblage of a marine cyanobacterium and an unidentified red alga.[5] Iodine-containing tyrosine derivatives include, for example, the simple alkaloids dakaramine[6] and turbotoxin,[7,8] the more complex depsipeptides geodiamolides[9–12] and doliculide,[13] and the structurally intriguing calicheamicin.[14]

The diketopiperazine pharmacologically active indole alkaloids barettin and 8,9-dihydrobarettin were isolated from the cold-water sponge *Geodia barretti*.[15] Both compounds may form part of the sponge's chemical defense to deter fouling organisms, as indicated by the fact that barettin is found in water exposed to living specimens of *G. barretti* in concentrations that completely inhibit barnacles from settling.[16]

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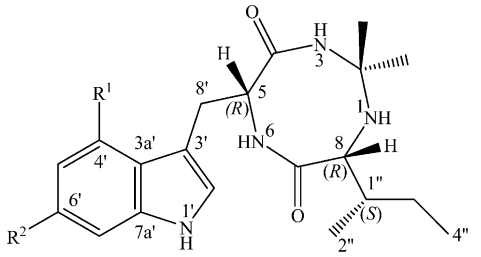
The family Subergorgiidae comprises genus *Subergorgia* with many species. Gorgonians, or sea fans, are sessile colonial cnidarians found in tropical and subtropical seawater from the northern Red Sea to the central Pacific. They are classified in the phylum Cnidaria, class Anthozoa, subclass Alcyonaria, order Gorgonacea. Individual tiny polyps form an erect, flattened, branching colony reminiscent of a fan, often brightly colored − purple, red, or yellow. Colonies of almost all species are supported by an extremely strong, flexible endoskeleton. The main structural skeleton is formed from a flexible, horny substance called gorgonian, and living polyps cover the surface. Unlike corals, sea fans do not attach themselves to a hard substrate; instead, they anchor themselves in mud or sand. They may also contain algae, which are capable of photosynthesis.

Extensive investigations of *Subergorgia* sp. by different research groups have resulted in the isolation of several sesquiterpenes of subergane,[17,18] quadrone, and α-caryophyllene types,[19] in addition to several 9,11-secosteroids.[20,21] Two oxygenated calamenene sesquiterpenes were isolated from a gorgonian *Subergorgia hicksoni*, and their structure was determined.[22] *Subergorgia tuberosa* was found to contain subergorgic acid, a novel tricyclopentanoid cardiotoxin, and suberosenone.[23,24]

During the last few years, our group has been working on a detailed analysis of the secondary metabolite composition of different organisms, and we have isolated a series of unusual compounds.[25–28] In this paper, we describe the isolation and structural elucidation of three new halogenated compounds, hicksoanes A–C (**1**–**3**) (Figure 1), from the organic extract of *Subergorgia hicksoni* and the results of an evaluation of the antifouling activity of these compounds.



Name	R^1	R^2
Hicksoane A (**1**)	H	I
Hicksoane B (**2**)	I	H
Hicksoane C (**3**)	I	I

Figure 1. Structures of hicksoanes A–C (**1**–**3**). Hicksoane A (**1**) is {(2′′*S*,5*R*,8*R*)-8-(2′′-methybutyl)-5-[(6′-iodo-1′*H*-indol-3′-yl)methyl]-2,2-dimethyl-1,3,6-triazocane-4,7-dione}.

Results and Discussion

Our search for new compounds in marine animals included examination of *Subergorgia hicksoni* collected in the Gulf of Aqaba, Israel. The gorgonians were extracted with butanol, and the extract was separated with a Sephadex LH-20 instrument. The fractions were further purified by RP-HPLC to give three compounds, which were identified by their IR, UV, and ^{1}H and ^{13}C NMR spectroscopic data, mass spectrometry, and by chemical degradation.

Compound **1** (hicksoane A) had the molecular formula $C_{20}H_{27}IN_4O_2$, which was established by HRMS (*m*/*z* = 505.1071 [M + Na]$^+$). The UV spectrum of **1** revealed the presence of a conjugated aromatic chromophore with absorption maxima at 227 (log ε = 4.21) and 286 (log ε = 3.75). In the IR spectrum, **1** showed absorption of amino groups at 3420 and 3200 cm^{-1} and carbonyl groups at 1670 and 1655cm^{-1}, which are typical values for an amide functionality. These findings together with the absence of the amide II band near 1550 cm^{-1} in the IR spectrum suggested the presence of a lactam system in **1**.

The ^{1}H NMR spectrum obtained in [D$_6$]DMSO shows signals corresponding to protons bonded to nitrogen atoms (N–H groups), that is, 1-H, 3-H, 6-H, and 1′-H. There are signals of two methyl groups [δ = 0.75 (d, J = 7.0 Hz), 0.81 (t, J = 7.6 Hz) ppm] representative of a *sec*-butyl group, and signals from two tertiary methyl groups [δ = 1.53 (s) and δ = 1.58 (s, 2 × C-2 Me) ppm] along with signals due to several methylene and methine groups (Table 1) were observed.

The ^{1}H NMR spectrum of **1** showed four signals in the deep-field area of the spectrum that are due to the protons of the aromatic rings [δ = 7.88 (dd, 4′-H), 7.39 (dd, 5′-H), 7.70 (dd, 7′-H), 7.18 (d, 2′-H) ppm]. The values of the signals indicate that the basic structure consists of an indole skeleton; one of the substituents must clearly be at the cyclic carbon, either at C-5′ or C-6′, because with a substitution at C-4′ or C-7′ all three remaining protons would have to be directly neighboring.

Two protons [δ = 7.88 (dd), 7.39 (dd) ppm] are located on the two adjacent carbon atoms by the multiplicity and coupling constants of their ^{1}H NMR spectrum and the correlations present in the COSY spectrum (Figure 2). Protons at δ = 7.70 ppm and 7.18 ppm have no other hydrogen atoms bound to carbon atoms in their vicinity. Substitution by iodine in C-6′ was further confirmed by comparison with model compounds.[29–31]

Table 1. NMR spectroscopic data for compound **1** (measured in [D$_6$]DMSO).

No.	^{1}H NMR, δ [ppm]	COSY	HMBC	^{13}C NMR, δ [ppm]
1	2.13 (d, 1 H, J = 8.3 Hz)	8		–
2	–			77.5 (s)
C-2 Me	1.53 (s, 3 H)			27.1 (q)
C-2 Me	1.58 (s, 3 H)			27.6 (q)
3	7.95 (1 H, br. s)	8	5	–
4	–			174.1 (s)
5	4.09 (m, 1 H)	8′, 6	7	56.7 (d)
6	8.07 (d, 1 H, J = 3.1)	5	4, 8	–
7	–			170.4 (s)
8	4.17 (t, 1 H, J = 8.3 Hz)	1, 1′′	3′′	58.4 (d)
1′	11.07 (dd, 1 H, J = 2.3, 0.6 Hz)	2′, 7′	3′, 3a′, 7a′	–
2′	7.18 (ddd, 1 H, J = 2.3, 0.8, 0.5 Hz)	1′, 8′	3′, 3a′, 7a′	124.7 (d)
3′	–			109.3 (s)
3a′	–			125.0 (s)
4′	7.88 (dd, 1 H, J = 8.5, 0.5 Hz)	5′, 7′	3′, 6′, 7a′	120.3 (d)
5′	7.39 (dd, 1 H, J = 8.5, 2.0 Hz)	4′, 7′	3a′, 7′	125.8 (d)
6′	–			84.6 (s)
7′	7.70 (ddd, 1 H, J = 2.0, 0.5, 0.6 Hz)	1′, 5′, 4′	3a′, 5′	120.1 (d)
7a′	–			137.4 (s)
8′	2.97 (ddd, 1 H, J = 14.1, 8.0, 0.8 Hz), 3.25 (ddd, 1 H, J = 14.1, 2.3, 0.5 Hz)	2′, 5	2′, 3′, 3a′, 5	27.2 (t)
1′′	1.70 (m, 1 H)	8, 3′′, 2′′		37.2 (d)
2′′	0.75 (d, 3 H, J = 7.0 Hz)	1′′		15.7 (q)
3′′	1.20 (m, 1 H), 1.51 (m, 1 H)	1′′, 4′′		24.8 (t)
4′′	0.81 (t, 3 H, J = 7.6 Hz)	3′′		10.9 (q)

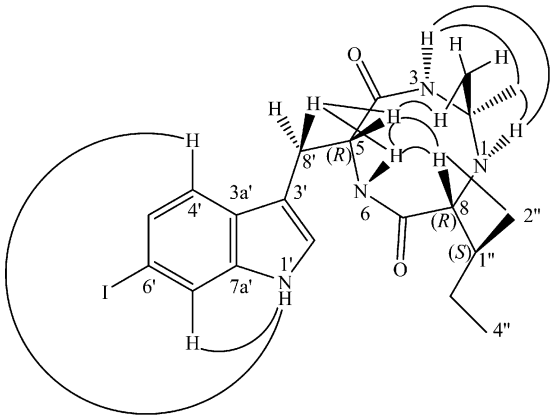


Figure 2. The NOESY correlations of hicksoane A (**1**).

The ^{13}C NMR spectrum of **1** analyzed by(the DEPT method indicated the presence of two amide carbonyl groups [δ = 174.1 (s, C-4), 170.4 (s, C-7)], an iodo-bearing sp^2 carbon atom [δ = 84.6 (s, C-6′)], and four methyl groups [δ = 27.1 (q, C-2 Me), 27.6 (q, C-2 Me), 15.7 (q, C-2′′), 10.9 (q, C-4′′)] together with four sp^2 methine carbon atoms, three quaternary sp^2 carbon atoms, one quaternary sp^3 carbon atoms, and three sp^3 methine and two methylene groups (Table 1). Detailed analysis of the ^1H and ^{13}C NMR spectra with the aid of COSY and HMBC (see also Table 1) coupled with the structural information from the UV and IR spectra determined its total planar structure.

The iodo-tryptophan moiety in **1** was converted into aspartic acid (Asp) by ozonolysis followed by oxidation with hydrogen peroxide, whereas the Ile segment in compound **1** was transformed into 2-methylbutyric acid by treatment with HClO (chloramine T) and Jones reagent. Asp was determined by using chiral HPLC analysis with the use of an OA-5000 column, which offers chiral discrimination by ligand-exchange interaction in the reverse-phase mode. 2-Methylbutyric acid was determined on a capillary column with a stationary chiral phase, this time by gas chromatography as described previously.[32] These results suggest the structure of **1**, which was partially determined, and show that the absolute configuration of two out of the three chiral centers is (5*R*,1′′*S*).

The remaining third chiral center was determined from the fact that **1** showed large values for the corresponding coupling constants ($^3J_{1''\text{-H},8\text{-H}}$ = 8.3 Hz) in the *allo*-Ile units, which suggested restricted rotation and an *anti* relationship of these protons (Figure 3). This contention was reinforced by the absence of NOEs between these protons (1′′-H and 8-H). In NOE experiments, the N-H (1-H) showed a significant NOE with 2′′-H_3 and no NOE with 3′′-H_2 and/or 4′′-H_3, which suggested that this unit is the *allo*-Ile (Figure 3). Thus, the structure of hicksoane A was assigned as **1**, that is, {(2′′*S*,5*R*,8*R*)-8-(2′′-methybutyl)-5-[(6′-iodo-1′*H*-indol-3′-yl)methyl]-2,2-dimethyl-1,3,6-triazocane-4,7-dione} as shown in Figure 1.

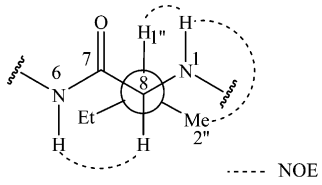


Figure 3. The correlations around the chiral center (C-8) of hicksoane A (**1**).

HRMS data allowed us to assign the molecular formula $C_{20}H_{27}IN_4O_2$ to hicksoane B (**2**), which is the same as that of hicksoane A; this thus suggests that the two compounds must be positional isomers. Except for the indole moiety in both the ^1H and ^{13}C NMR spectra of **2** ([D_6]DMSO, Table 2), the signals of the carbon atoms are almost identical to the signals exhibited by hicksoane A (**1**). In contrast, in this case the three aromatic protons of the benzene ring in the ^1H NMR spectrum appear as two doublets and one triplet, which indicates that all three atoms are mutually adjacent. By following the same reasoning as above, the proton at δ = 7.09 ppm was assigned to 2′-H; this excludes the possibility of iodine substitution on the five-membered ring. Therefore, the presence of *ortho* coupling between two pairs of the remaining aromatic protons points to a 4′- or a 7′-iodo substitution pattern. The key COSY correlation between the signals at δ = 7.54 (5′-H), 7.03 (6′-H), and 7.25 ppm (7′-H) rules out the second possibility, which was also confirmed by comparing the ppm values with those of

Table 2. NMR spectroscopic data for compounds **2** and **3** (measured in [D_6]DMSO).[a]

No.	^1H NMR of **2**, δ [ppm]	^{13}C NMR of **2**, δ [ppm]	^1H NMR of **3**, δ [ppm]	^{13}C NMR **3**, δ [ppm]
1′	10.84 (d, 1 H, *J* = 2.3 Hz)	–	10.95 (d, 1 H, *J* = 2.3 Hz)	–
2′	7.09 (ddd, 1 H, *J* = 2.3, 0.8, 0.5 Hz)	122.5 (d)	7.13 (ddd, 1 H, *J* = 2.3, 0.8, 0.5 Hz)	122.9 (d)
3′	–	111.2 (s)	–	112.0 (s)
3a′	–	137.6 (s)	–	135.4 (s)
4′	–	91.2 (s)	–	92.1 (s)
5′	7.54 (dd, 1 H, *J* = 8.5, 1.8 Hz)	130.3 (d)	7.92 (d, 1 H, *J* = 1.7 Hz)	138.4 (d)
6′	7.03 (dd, 1 H, *J* = 8.5, 7.9 Hz)	123.8 (d)	–	91.0 (s)
7′	7.25 (dd, 1 H, *J* = 1.8, 7.9 Hz)	111.9 (d)	7.63 (d, 1 H, *J* = 1.7 Hz)	119.6 (d)
7a′	–	138.4 (s)	–	139.7 (s)
8′	2.96 (ddd, 1 H, *J* = 14.1, 8.0, 0.8 Hz), 3.24 (ddd, 1 H, *J* = 14.1, 2.3, 0.5 Hz)	27.1 (t)	3.01 (ddd, 1 H, *J* = 14.1, 8.0, 0.8 Hz), 3.28 (ddd, 1 H, *J* = 14.1, 2.3, 0.5 Hz)	27.1 (t)

[a] All chemical shift values (both ^1H and ^{13}C) identical to those in compound **1** have been omitted and only values relating to the indole moiety are retained.

the model compound, that is, D,L-*N*-acetyl-4-bromotryptophan methyl ester and/or with 4-chloroindole acetic acid and its esters.[33,34]

Compound **3** (hicksoane C) possesses the molecular formula $C_{20}H_{26}I_2N_4O_2$ as was determined by HRMS. Unlike compound **1** and/or **2**, this compound is characterized by the replacement of an additional hydrogen atom with an iodine atom. The ^{1}H NMR spectrum of **3** shows a doublet (δ = 7.13 ppm) and two mutually coupled doublets (δ = 7.92 and 7.63 ppm) in the aromatic region in a *meta* relationship (Table 2), as clearly indicated by the J = 1.7 Hz coupling constant. Further, the resonances in the aliphatic region are nearly superimposable with the same signals of hicksoane A and/or B and the signal at δ = 7.13 ppm was confidently attributed to 2′-H on the basis of its HMBC correlation peak with C-8′. Also, the NOESY correlation peak of the doublet at δ = 7.63 ppm with the proton at N-1′ rules out the possibility of substitution at C-7′, which thus allows unambiguous assignment of **3** as the 4′,6′-diiodo derivative, that is, hicksoane C.

The biological activity of iodo-containing compounds has been repeatedly tested; thus, plakohypaphorines A–F display antihistamine activity, and they show a specific antagonism of the noncompetitive type.[35] Extracts from *Subergorgia suberosa* were found to possess inhibitory effects on fungi, especially against *Tritirachium* sp. at a minimum inhibitory concentration of 12 mg mL^{-1}.[36] Antifungal activity was also detected in some marine organisms from India against food spoilage *Aspergillus* strains, where the extracts from *Subergorgia suberosa* were active against *A. fresenii*, *A. japonicus*, and *A. niger*.[37] Antifouling activity was described in two compounds, barettin and 8,9-dihydrobarettin from the marine sponge *Geodia barrette*. The compounds inhibit the settlement of cyprid larvae of the barnacle *Balanus improvisus*. The activities of these brominated cyclic dipeptides were in the range of antifouling agents in use today, as shown by their EC_{50} values of 0.9 and 7.9 μM, respectively.[38]

Four synthetic analogues, namely, 4-chloro-L-tryptophan and 5- or 6- or 7-bromo-D,L-tryptophans, and hicksoanes A–C were tested for possible structure–activity relationships. As shown in Table 3, compounds **1**–**3** but not the standards deter feeding by goldfish in aquarium bioassays at concentrations of 5–100 μg mL^{-1}, which is equal to their natural concentrations. We hypothesize that isolated compounds **1**–**3** are part of the chemical defense system of gorgonians, which deters fouling organisms. This theory is supported by the fact that hicksoanes A–C were found in gorgonians at a concentration that is about 10-fold higher than that causing a 50% reduction in pellet eating (Table 3).

Compound **3** having two atoms of iodine had a higher activity than its counterparts **1** and **2**. As shown in the literature[39] and confirmed by our results, halogenation increases the antifeeding activity.

Hicksoanes A–C are exceptional in two respects. The first is the presence of iodine atoms in the molecule, specifically in the Trp moiety, in the 4- or 6-positions or in the 4- and 6-positions. This is highly unusual as other halogens, viz., Cl or Br atoms, have solely been found in these positions. Some of the chlorine-containing compounds are, for example, the widely distributed 4-chloroindolyl acetic acid,[40] which is a known auxin derivative, and microsclerodermins C–E, which are antifungal cyclic peptides from the lithistid marine sponges *Theonella* sp. and *Microsclerodermа* sp.[41] Brominated derivatives have been identified as clionamide, which is a 6-bromotryptophan derivative from the sponge *Cliona celata*,[42] celenamides A and B, which are linear peptide alkaloids from the sponge *C. celata*,[43] and convolutindole A, which is a nematocidal brominated alkaloid from the marine bryozoan *Amathia convolute*.[44]

The biosynthesis of hicksoanes A–C proceeds presumably under the participation of haloperoxidases and nonribosomal peptide biosynthetic machinery. Both haloperoxidases producing hypohalogenic acid as the actual halogenating agent and NADH-dependent halogenases transforming the substrate so that the halide ion may be used directly as a nucleophile have been proposed to catalyze this reaction.[45] The structure of hicksoane as a natural product is also unprecedented, as an eight-membered cycle − 1,3,6-triazocane − is not found in nature and has only been prepared synthetically.[46]

Thus, hicksoanes A–C represent a new group of compounds with an eight-membered cycle that, at the same time, contains iodine atom(s).

Table 3. Bioactivities of hicksoanes A–C (**1**–**3**) and commercial compounds.

Compound[a]	5 μg mL^{-1}[b]	10 μg mL^{-1}[b]	50 μg mL^{-1}[b]	100 μg mL^{-1}[b]	Nat. conc.[c] μg mL^{-1}
1	58.4 ±5.1	11.3 ±1.9	5.4 ± 1.1	0.7 ± 0.3	41.9
2	69.1 ± 7.3	12.4 ± 2.0	9.1 ± 2.2	1.0 ± 0.4	12.4
3	26.5 ± 3.4	5.3 ± 1.7	3.1 ± 0.8	0.0	8.3
4-Chloro-L-tryptophan[d]	100 ± 1.7	100 ± 1.8	99.6 ± 1.7	99.1 ± 1.6	–
5-Bromo-D,L-tryptophan[e]	100 ± 1.8	100 ± 1.2	99.7 ± 1.4	99.3 ± 2.1	–
6-Bromo-D,L-tryptophan[e]	100 ±2.2	100 ± 1.4	99.2 ± 1.5	99.4 ± 1.9	–
7-Bromo-D,L-tryptophan[e]	100 ± 1.8	100 ± 1.7	99.1 ± 2.2	98.5 ± 2.3	–

[a] Aquarium assay results from feeding goldfish on pellets treated with **1**–**3** and standards. All control pellets (100 pieces) were eaten in all assays by 100 fish. Three replicate assays were performed at each concentration (mean ± SD is indicated in columns 2 to 5). [b] Concentration of pure compound **1**–**3** and standards in the pellet. [c] Natural concentration of **1**–**3** in *Subergorgia hicksoni*. Gorgonian volume was determined by displacement of water with frozen material according to Pawlik.[48] [d] Fulcrum Scientific Ltd., 35–36 Lion Chambers, John William St., Huddersfield HD1 1ER, UK. [e] Biosynth AG, Rietlistrase 4, CH-9422 Staad, Switzerland.

Experimental Section

General Experimental Procedures: UV spectra were measured in MeOH within the range 200–350 nm with a Cary 118 (Varian) apparatus. A Perkin–Elmer Model 1310 (Perkin–Elmer, Norwalk, CT, USA) IR spectrophotometer was used for scanning IR spectroscopy of heterocycles in KBr tablets. NMR spectra were recorded with a Bruker AMX 500 spectrometer (Bruker Analytik, Karlsruhe, Germany) at 500.1 MHz (^{1}H) and 125.7 MHz (^{13}C) in a deuterated DMSO and/or CD_3OD. High-resolution mass spectra were recorded with a VG 7070E-HF spectrometer (70 eV). HRMS (FAB, positive ion mode) was performed with a PEG-400 matrix. HPLC was carried out by using a Shimadzu gradient LC system (Shimadzu, Kyoto, Japan). Gas chromatography analysis was performed with a Hewlett–Packard HP 5980 gas chromatograph (Hewlett–Packard, Czech Republic). An FS capillary column HYDRODEX β-3P (ID 0.25 mm, length 25 m) with the stationary phase [heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin] from Macherey–Nagel GmbH & Co. KG, Düren, Germany was used. Oven temperature: 50 to 150 °C at 2 °C min^{-1}, then to 240 °C at 5 °C min^{-1}, carrier gas helium, 20 cm s^{-1}, detector FID, 300 °C, injection of 1 μL mixture in dichloromethane (for standards: containing 0.5 mg mL^{-1} of each sample), split (100:1), 300 °C. The following compounds: (*S*)-(+)-2-methylbutyric acid, (±)-2-methylbutyric acid, L- and D-Asp were purchased from Sigma–Aldrich (Prague, Czech Republic); 4-chloro-L-tryptophan from Fulcrum Scientific Ltd. (UK), and 5- and 6- and 7-bromo-D,L-tryptophans from Biosynth AG (Switzerland).

Animal Material: The gorgonians *Subergorgia hicksoni* (phylum Cnidaria, class Anthozoa, subclass Alcyonaria, order Gorgonacea, family Subergorgiidae) were collected by hand (scuba diving) from rocks 11–17 m deep in the Red Sea, Gulf of Aqaba (Eilat, Israel), on October 30, 2001. The voucher specimens are deposited in the Inter-University collection (Eilat). Fresh gorgonians were placed in ethanol and stored at –18 °C under an atmosphere of nitrogen.

Extraction and Isolation of Hicksoanes: The gorgonians (ca. 750 g wet weight) were extracted with BuOH for 24 h. After evaporation, the crude extract was chromatographed on a Sephadex LH-20 column (100 × 5 cm) with elution with MeOH. The organic fractions (8 mL) were checked by two-dimensional TLC (silica-gel plates, *n*BuOH/AcOH/H_2O, 12:3:5 and $CHCl_3$/MeOH/H_2O, 40:9:1). Fractions 23–27 were further separated by RP-HPLC on a C18-Bondapak column (30 cm × 7.8 mm, flow rate 2.0 mL min^{-1}) with MeOH/H_2O (4:1) to yield three compounds: **1** (31.4 mg, 0.0042%), **2** (9.3 mg, 0.0012%), and **3** (6.2 mg, 0.0008%).

Compound **1** (14.5 mg, 0.03 mmol) was dissolved by sonication in neutral 1% aqueous solution of chloramine-T (*N*-chloro-*p*-toluenesulfonamide sodium salt; 10.26 mg, 0.045 mmol), and the reaction was allowed to run for 10 min.[47] Excess alkali was neutralized with acetic acid. The reaction products were oxidized by Jones reagent under synchronous stirring for 10 min at 50 °C. The reaction mixture was neutralized with 0.1 M NaOH, evaporated to dryness, suspended in methanol, and extracted with diethyl ether. The mixture of compounds in the methanolic solution was treated with 14% borontrifluoride–diethyl ether (2 min under reflux), diluted with water, extracted with hexane, and dried. The hexane solution was directly injected into the gas chromatograph.

Determination of the Stereochemistry of ITrp: Hicksoane A (**1**, 5 mg) in AcOH (1 mL) was treated with ozone at –78 °C for 1 min. After removal of the excess amount of ozone by a stream of nitrogen, the mixture was treated with 30% H_2O_2 (0.5 mL) at room temperature for 3 h. The reaction mixture was hydrolyzed with 6 N HCl (0.25 mL) at 110 °C for 5 h. The hydrolysate was subjected to chiral HPLC analyses [SUMICHIRAL OA-5000, 4.6 mm I.D. × 15 cm; room temperature, flow rate, 1.0 mL min^{-1}; eluent: 2 mM copper(II) sulfate in water-2-propanol (95:5), detection at UV 254 nm]. The retention times of authentic L- and D-Asp were found to be 9.7 and 12.5 min, respectively. The retention time of Asp in the degradation product of **1** was found to be 12.5 min (i.e. D-Asp).

Hicksoane A (1): Amorphous pale-yellow powder (31.4 mg). $[\alpha]_D^{20}$ = +31 (*c* = 0.01, MeOH). UV (MeOH): λ (log ε) = 227 (4.21), 286 (3.75) nm. IR (KBr): $\tilde{\nu}$ = 3420, 3200, 1670, 1655, 1455, 1330, 1200, 800, 760 cm^{-1}. HRMS (FAB): calcd. for $C_{20}H_{27}IN_4NaO_2$ [M + Na]$^+$ 505.1076; found 505.1071. See Table 1 for NMR spectroscopic data.

Hicksoane B (2): Amorphous pale-yellow powder (9.3 mg). $[\alpha]_D^{20}$ = +37 (*c* = 0.009, MeOH). UV (MeOH): λ (log ε) = 228 (4.17), 285 (3.65) nm. IR (KBr): $\tilde{\nu}$ = 3410, 3200, 1673, 1650, 1450, 1330, 805, 765 cm^{-1}. HRMS (FAB): calcd. for $C_{20}H_{27}IN_4NaO_2$ [M + Na]$^+$ 505.1076; found 505.1069. See Table 2 for NMR spectroscopic data.

Hicksoane C (3): Amorphous yellow powder (6.2 mg). $[\alpha]_D^{20}$ = +41 (*c* = 0.007, MeOH). UV (MeOH): λ (log ε) = 225 (4.37), 284 (3.86) nm. IR (KBr): $\tilde{\nu}$ = 3415, 3205, 1675, 1660, 1460, 1333 cm^{-1}. HRMS (FAB): calcd. for $C_{20}H_{26}I_2N_4NaO_2$ [M + Na]$^+$ 631.0043; found 631.0050. See Table 2 for NMR spectroscopic data.

Bioassay for Antifeeding Activity Assay: Purified compounds **1**–**3** and standards (see Table 3) were dissolved in a minimal volume of methanol and mixed with alginate-based food matrix[48] (100 μL) until all organic- and water-soluble components were distributed uniformly throughout the paste. The alginate food matrix was then dispensed by the use of a 0.1-mL syringe into a $CaCl_2$ solution (0.25 M), which formed a strand that was allowed to harden for 2 min. The hardened strand was rinsed with filtered water and cut into 3 mm pellets with a scalpel. Control pellets were prepared identically but without the addition of natural or synthetic compounds. Feeding assays were performed with goldfish (*Carassius auratus*).

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