Journal of Natural Products
Vol. 58, No. 2, pp. 201-208, February 1995

NEW AND KNOWN DIKETOPIPERAZINES FROM THE CARIBBEAN SPONGE, *CALYX* CF. *PODATYPA*[1]

MADELINE ADAMCZESKI, ANDREA R. REED, and PHILLIP CREWS*

Department of Chemistry and Biochemistry and Institute for Marine Sciences, University of California, Santa Cruz, California 95064

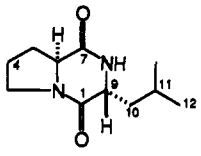
ABSTRACT.—The cyanobacteria-containing Caribbean sponge, *Calyx* cf. *podatypa*, was collected from three sites in the Bahamas. In each of the three collections, a polar solvent partition fraction contained six known compounds including five diketopiperazines [**1–4**, **6**] and phenylacetic acid, along with a new diketopiperazine, *cyclo*-(4-methyl-*R*-proline-*S*-norvaline) [**5**]. Interestingly, all six diketopiperazines are proline-derived cyclic dipeptides. This is the first example for this class of peptide derivative to be isolated from a *Calyx* sponge. Parallel studies of one of the sponge collections in which the ectosome (cyanobacteria-rich) was separated from the endosome (no cyanobacteria) revealed no significant differences in their content of diketopiperazines.

Cases where secondary metabolites have been intimated as products of the chlorophyll-containing symbionts of a sponge have attracted much attention (2). However, it is difficult to rigorously sort out which compounds are metabolites elaborated by the sponge or by the symbiont and most of the suggestions on this point are based on circumstantial rather than hard experimental evidence (3). We have been interested in probing this phenomenon but have realized that very few sponges are known which might be suitable for such research. The best candidates appear to be limited to Indo-Pacific taxa of the order Dictyoceratida, restricted to one species, *Dysidea herbacea* (4,5), and of the order Lithistida again restricted to a single species, *Theonella swinhoei* (6,7). A large number of Caribbean sponges have been mentioned in the biological literature (8) which possess either filamentous or single cell cyanobacteria (a.k.a. blue-green algae). This observation prompted us to create a library of Caribbean sponges that were rich in cyanobacteria while also having secondary metabolites to serve as a tool for further study. It was also desirable that such sponges be easy to collect and widely distributed in the Caribbean. We have located one organism, *Calyx* cf. *podatypa* (de Laubenfels) (Renieridae), which has these properties and herein report on its major secondary metabolite constituents, diketopiperazines (DKPs).

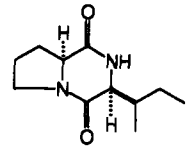
RESULTS AND DISCUSSION

Calyx cf. *podatypa* is a common Caribbean organism depicted in a popular underwater diving guide book to the Caribbean (9). It attracted our attention during an expedition to the Bahaman Islands because its morphology matched Wilkinson's profile of a sponge whose ectosome ought to contain cyanobacteria (8). Generous amounts of a cyanobacterium, tentatively identified as *Aphanocapsa*, were detected in the ectosome but not in the endosome of thin sections of this sponge using epifluorescence microscopy. A previous study of Bahaman material identified as *C. podatypa* reported a series of xestamine-type alkaloids (10), but these compounds were completely absent from our collections obtained at three widely dispersed sites. Each collection was kept separate, preserved by our standard procedure and then returned to UCSC for extraction with MeOH. Purification of the crude oil from one representative sample of the CH_2Cl_2 solvent partition fraction yielded phenylacetic acid which was accompanied by known DKPs, *cyclo*-(*S*-Pro-*R*-Leu) [**1**] (11–13); *cyclo*-(*S*-Pro-*S*-Ile) [**2**] (11); *cyclo*-(*S*-Pro-*R*-Val)

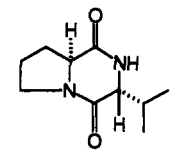
[1]Novel Sponge-Derived Amino Acids, 18. For part 17, see Crews *et al.* (1).



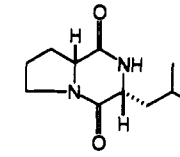
1
Cyclo-(*S*-Pro-*R*-Leu)
[α] D−91°



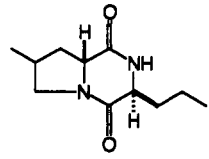
2
Cyclo-(*S*-Pro-*S*-Ile)
[α] D−197°



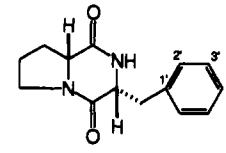
3
Cyclo-(*S*-Pro-*R*-Val)
[α] D−74°



4
Cyclo-(*R*-Pro-*R*-Leu)
[α] D+142°



5
Cyclo-(4-methyl-*R*-Pro-*S*-Nva)
[α] D+128°



6
Cyclo-(*R*-Pro-*R*-Phe)
[α] D+68°

[**3**] (13–15); *cyclo*-(*R*-Pro-*R*-Leu) [**4**] (16); *cyclo*-(*R*-Pro-*R*-Phe) [**6**] (13, 15, 17), and a new DKP, *cyclo*-(4-methyl-*R*-Pro-*S*-Nva) [**5**]. All of these compounds were observed by analytical hplc analyses of extracts from the ectosome and endosome material, respectively (Collection No. 93318, BAH #4). A parallel content of DKPs could also be inferred from resonances present in the nmr spectra of both ectosome and endosome crude extracts.

With the exception of a recent report (18) there is a paucity of ^{13}C-nmr data available in the literature for DKPs, and therefore we present such data in Table 1 to address this deficiency. The structures of the diketopiperazones **1** ([α]^{20}D −91°) and **2** ([α]^{20}D −197°), were characterized by comparison of their nmr properties shown in Tables 1 and 2 to those of synthetic DKPs. These were obtained via cyclization (19), of respectively

TABLE 1. ^{13}C-Nmr Data ($CDCl_3$, 300 MHz) of Compounds **1–6**.

Carbon	Compound					
	1	**2**	**3**	**4**	**5**	**6**
1	171.4	169.9	[a]	169.6	169.3	[a]
3	45.6	45.2	45.2	45.7	45.7	45.6
4	22.8	22.4	22.5	23.1	39.7	22.6
5	28.2	28.6	28.6	29.1	29.5	28.4
6	59.1	58.9	58.9	58.1	58.4	59.2
7	167.1	165.1	[a]	166.4	165.2	[a]
9	53.4	60.6	60.4	56.4	63.0	56.2
10	38.7	35.3	28.4	42.6	22.1	36.9
11	24.8	24.1	19.4	24.5	24.6	
12	22.8	12.2		22.3	11.4	
Me@11	21.2			21.4		
Me@10		16.0	16.1			
Me@4					15.4	
Ar@10						
1′						129.4
2′						129.2
3′						127.6
4′						129.2

[a]Sample too dilute to observe quaternary resonances.

TABLE 2. ^{1}H-Nmr Data (300 MHz, $CDCl_3$) of Compounds **1–6**.[a]

Proton	Compound					
	1	**2**	**3**	**4**	**5**	**6**
3	3.6–3.5, 2H, m	3.6–3.5, 2H, m	3.55, 1H, dt (9.1, 2.8) 3.63, 1H, m	3.52, 1H, dt (9.8, 2.7) 3.62, 1H, dt (9.0, 4.5)	3.69, 1H, dt (8.3, 3.9) 3.53, 1H, dt (10.4, 2.1)	3.7–3.6, 1H, m; 3.6–3.5, 1H, m
4	1.94–1.86, 1H, m; 2.02–1.99, 1H, m	2.0–1.9, 1H, m; 1.9–1.8, 1H, m	2.02–1.99, 1H, m; 1.93–1.88, 1H, m	1.96, 1H, m 1.88, 1H, m	1.90, 1H, m	1.9–1.8, 2H, m
5	2.13, 1H, m; 2.33, 1H, m	2.3–2.2, 1H, m; 2.1–2.0, 1H, m	2.4–2.3, 1H, m; 2.1–2.06, 1H, m	2.37, 1H, ddd (8.7, 6.4, 2.4); 2.02, 1H, m	2.40, 1H, ddd (10.8, 6.3, 4.3); 1.98, 1H, m	2.4–2.3, 1H, m; 2.1–2.0, 1H, m
6	4.12, 1H, t (8.1)	4.07, 1H, t (7.5)	4.08, 1H, dt (7.8, 1.8)	4.07, 1H, dd (6.9, 1.5)	4.08, 1H, dt (9.5, 3)	4.08, 1H, t (7.1)
N-H	5.91, 1H, br s	5.99, 1H, br s	5.72, 1H, dd (1.5, 1.2)	6.68, 1H, br s	6.31, br d (1.5)	5.60, 1H, br s
9	4.01, 1H, dd (9.4, 3.4)	3.96, 1H, br s	3.94, 1H, br s	3.92, 1H, ddd (9.9, 5.4, 4.5)	3.77, 1H, dt (5.1, 1.8)	4.27, 1H, dd (10.6, 2.6)
10	2.01, 1H, m; 1.52, 1H, ddd (14.5, 9.6, 4.9)	2.4–2.3, 1H, m	2.64, 1H, m	1.75, 1H, q (6.3) 1.63, 1H, ddd (11.1, 6.5, 1.8)	2.04, 1H, m; 1.83, 1H, m	3.6–3.5, 1H, m; 2.77, 1H, dd (14.4, 10.8)
11	1.76–1.69, 1H, m	1.5–1.4, 1H, m; 1.3–1.1, 1H, m	0.91, 3H, d (7.2)	1.66–1.60, m, under H-10	1.54, 1H, m; 1.23, 1H, m	
12	0.94, 3H, d (6.3)	0.92, 3H, t (7.4)		0.94, 3H, d (6.3)	0.92, 3H, t (7.3)	
Me@11	1.00, 3H, d (6.3)			0.97, 3H, d (6.3)		
Me@10		1.05, 3H, d (7.2)	1.06, 3H, d (7.2)			
Me@4					1.01, 3H, d (6.9)	
Ar@10						7.4–7.2, 5H

[a] *J* values in Hz in parentheses.

the dipeptides of Pro-Leu and of *S*-Pro-*S*-Ile. Two-dimensional nmr data (^{1}H-^{1}H and ^{1}H-^{13}C COSY) were also used to characterize two other known DKPs, **3** ([α]^{20}D −74°) and **6** ([α]^{20}D +68°). Also, the nmr data and [α]^{20}D +142° obtained for compound **4** indicated that it was a diastereomer of **1**.

The new DKP was initially recognized to be slightly different from the other proline-derived DKPs described above. Its molecular formula of $C_{11}H_{18}N_2O_2$ was proposed from the lreims M^+ *m/z* 210 which was also consistent with the APT nmr formula of $C_{11}H_{17}$. The DKP skeleton was apparent from the characteristic ^{13}C- and ^{1}H-nmr shifts of the two amides (δ 165.2 s, 169.3 s) and the N-C residues (58.4 d, 4.08 dt, and 63.0 d, 3.77 dt). The nmr shifts pinpointed the additional ring substituents as a propyl (δ 11.4 q, 0.92 t) and β-Me proline (δ 15.4 q, 1.01 d). This suggestion was consistent with ^{1}H-^{1}H COSY nmr correlations observed from H-9 to N-H, H-10, and H-10′, and from H-6 to H-5 and H-5′ and from H-4 to H-5 and a CH_3 group at position 4.

The task of assigning the absolute stereochemistry for **1–5** was begun by collecting known optical rotations for proline-containing DKPs. Summarizing a subset of data from the literature illustrates an interesting empirical trend (see Table 3). The overall sign of [α]D for DKPs in this study is either negative or positive depending only on the Pro absolute configuration being *S* or *R*, respectively (14). In addition, the actual magnitude of this [α]D varies from −134° to −185° for the *S*-Pro-*S*-Xxx substances versus −78° to −120° for *S*-Pro-*R*-Xxx compounds, where Xxx has an aliphatic side-

TABLE 3. Optical Rotations for Diketopiperazines of the General Formula *cyclo*-(*S*-Pro-*R*/*S*-Xxx).

Xxx	[α]D (°)	Ref.
S-Leu	−156 [a]	(13)
	−143 [EtOH]	(10)
	−142 [EtOH]	(11)
	−91.3 [H_2O][b]	(14)
R-Leu	−91.2 [a]	(13)
	−78 [EtOH]	(12)
S-Ile	−171 [a]	(13)
R-Ile	−109 [a][c]	(13)
S-Val	−185 [a]	(13)
	−180 [EtOH]	(12)
	−161 [EtOH]	(13)
	−134 [EtOH]	(27)
R-Val	−120 [EtOH]	(12)
	−103 [a]	(13)
S-Nva	−147 [a]	(13)
R-Nva	−100 [a]	(13)
S-Phe	−115 [EtOH]	(12)
	−91 [a]	(13)
	−83 [H_2O]	(16)
	−68 [H_2O]	(14)
R-Phe	−102 [EtOH]	(12)
	−92 [H_2O][b]	(16)
	−94 [a]	(13)

[a]=0.01 N NaOH in aqueous MeOH (1:1, v/v).

[b]This value is inconsistent with other reported data.

[c]Actual data of the enantiomer.

chain of three carbons or more. As a cautionary note, an opposite empirical relationship appears to hold for Pro-Ala DKPs in that the *cis*-(*S*-Pro-*S*-Ala) has an $[\alpha]_D$ range of $-85°$ to $-120°$ (14, 20, 21) while the *trans*-(*S*-Pro-*R*-Ala) has $[\alpha]_D$ values of $-140°$ to $-185°$ (14,21). Applying these trends to the DKPs isolated in this study allowed the following conclusions. Based on the overall +/− sign of $[\alpha]_D$, compounds **1–3** have an *S*-Pro, **4** and **6** have an *R*-Pro, and the β-MePro of **5** can be provisionally assigned as *R*. Examining the actual magnitudes of these rotations supported additional assignments of *R*-Leu in **1** and in **4**, *S*-Ile in **2**, and *S*-Nva can be provisionally assigned in **5**. Compound **6** has an *R*-Pro and it was concluded to have the cis geometry as shown because the shift of H-6 (δ 4.08) is diagnostic of a cis H-6/9 relationship (22). Thus the Phe was assigned as *R*.

In contrast to the stability of acyclic dipeptides, DKPs are susceptible to rapid epimerization in both acid- and base-catalyzed conditions (23). In proline-containing DKPs, epimerization occurs preferentially at H-6 and the equilibrium mixture consists of an 80–95% trans and 20–5% cis mixture of epimers (14). This situation was used to further support the stereochemical assignments proposed above. For example, when **5** was equilibrated (0.01 N NaOH in aqueous MeOH, 1:1, v/v) the optical rotation changed from $[\alpha]^{20}_D$ +91° (initial) to +81° (24 h). A decrease in the positive value of this rotation would be expected for the addition of a small amount of the *cis-S,S* isomer to the equilibrium mixture dominated by the *trans-R,S* isomer. An analogous case was reported in the literature (14) and is represented by the epimerization of respectively *trans-R*-Pro-*S*-Ile or *trans-R*-Pro-*S*-alle in which the optical rotation changed from $[\alpha]^{20}_D$ +108° to +82° for the former and from $[\alpha]^{20}_D$ +94° to +71° for the latter. Alternatively, epimerization of a *cis-R,R* **5** ought to give a mixture rich in the *trans-S,R* isomer having an overall negative rotation. An example of this situation is provided by the $[\alpha]_D$ −59° reported for the equilibrium mixture of *trans*-(*cyclo-S*-Pro-*R*-Nva) and *cis*-(*cyclo-R*-Pro-*R*-Nva) (14). A different consideration involves the possibility that the cis isomers reported above are artifacts from epimerization reactions occurring during the isolation work. We believe this to be unlikely because the relative yields of **1** (1.4 mg, *trans-S,R*) and **4** (3.7 mg, *cis-R,S*) of are opposite of the cis/trans=11/89 equilibrium ratio reported in alkaline solution for this equilibration (14).

The two peptide bonds in DKPs possess the cis geometry and the ring has a boat conformation (24) which might be a factor in the action of these compounds on metabolic functions (25). For example, it has been reported that some act as hormones, antibiotics, antitoxins, or ion carriers. According to Sammes (26), diketopiperazines are ubiquitous throughout nature and are most commonly isolated from terrestrial yeast, lichen and fungi culture filtrates and are also observed in the culture broths of marine bacteria (27) and marine actinomycetes (28). Other examples of DKPs from marine sources include the isolation of *cyclo*-(Gly-*S*-Pro) from the starfish *Luidia clatharata* (29) and of *cyclo*-(Ala-Pro) from marine bacteria associated with sponges (30). To date, DKPs have also been isolated from the following marine sponges: *Jaspis* sp. (31), *Tedania ignis* (32), *Dysidea fragilis* (31), *Dysidea herbacea* (33), *Geodia baretti* (34), and *Leucophloeus fenestrata* (35). Hence, this is the first example of DKPs to be obtained from a *Calyx* sponge. Current studies are underway to determine why the metabolites of *C. podatypa* appear to be variable.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The nmr spectra were recorded in $CDCl_3$ at 250, 300, or 500 MHz for ^1H and 62.9, 75.5, 125.7 MHz for ^{13}C. Multiplicities of ^{13}C-nmr resonances were determined from ^1H-^{13}C COSY experiments. ^1H-^1H and ^1H-^{13}C COSY nmr data were used to assign resonances of compounds **1–6**. Low- and high-resolution fabms (using a Magic Bullet matrix, 3:1 erythro/threo $C_4H_{10}O_2S_2$) were obtained at the UCSC mass spectrometry facility. Optical rotations were determined on a digital

polarimeter. Hplc was performed using a 10-μm normal phase column. The solvent system used in this study was hexanes-EtOAc (3:7) with a flow rate of 1.0 ml/min.

ANIMAL MATERIAL.—The sponges were collected with scuba at a depth of 40 to 100 feet from open reef environments at three coral reef habitats in the southeastern Bahamas: off Cape Verde, near Crooked Island Passage from BAH#4 site, 22°30′, 73°00′ (Collection Nos. 93318 endosome and 93318 ectosome); off Fish Cay reef near Bight of Acklins from BAH#9 site 22°08′, 74°20′ (Collection No. 93318 whole); and near Little San Salvador Island from BAH#10 site 24°36′, 75°58′ (Collection No. 93347). They were identified as *Calyx* cf. *podatypa* (De Laubenfels, 1934), order Haplosclerida (36), family Oceanapiidae (37), by M.C. Diaz. The species has been previously classified in the order Petrosida (38,39), family Oceanapiidae. It is important to mention that this species has also been referred to as *Haliclona podatypa* and *Pachypellina podatypa* (38,39) but presently is considered to belong to the genus *Calyx* (40). The specimens were massive-amorphous to lobate in shape, sometimes consisting of repent branches. The sizes of the specimens were variable, but were usually more than 5-cm thick. When observed *in situ*, the color of the sponge was burgundy red on the ectosome and tan on the endosome. Under atmospheric conditions, the color changed to brownish-tan. The external color is attributed to the presence of cyanobacteria in the outermost tissue layers which were verified to be present by epifluorescence microscopy of freshly collected material. The surface of the specimens had a smooth texture with a consistency varying from very soft and fragile to compressible. The surface of some of the sponges contained a dense population of brown zooanthids. The oscules were round and slightly elevated from the body surface. The skeleton formed from oxeas or strongyloxeas (220–350×6–8 μm), consisted of multispicular tracts, loosely oriented, and spicules were arranged in an isotropic fashion. A thick tangential spicule conglomeration was observed at the surface.

EXTRACTION AND ISOLATION.—Sponges were preserved by being immersed in a EtOH-H_2O (1:1) solution. After approximately 24 h, this solution was decanted and discarded. The damp organisms of each collection were separately placed in Nalgene bottles and shipped back to the home laboratory at ambient temperature. Next, 100% MeOH was added and the organisms were soaked for at least 24 h after which time the extraction solvent was decanted and evaporated at room temperature *in vacuo*. This procedure was repeated two more times. The MeOH extract was then successively partitioned between equal volumes of aqueous MeOH (percent adjusted to produce a biphasic solution) and hexanes (FH fraction) followed by CH_2Cl_2 (FD fraction). The remaining H_2O solubles were extracted with 2-BuOH. The actual isolation work was initially done on the CH_2Cl_2 fraction from Collection No. 93318 from BAH#4 collection site. The semipure oil was chromatographed over Sephadex LH-20 using MeOH-CH_2Cl_2 (1:1) as eluent to yield six main fractions, the last of which gave phenylacetic acid as a pure compound. Fractions containing low-field peaks in the ^{13}C-nmr spectra were combined and subjected to further purification via Si gel flash cc using a solvent gradient of hexanes-EtOAc (1:1) to 100% EtOAc followed by MeOH-CH_2Cl_2 (15:85) and adding 5% Et_3N. The fractions containing compounds of similar polarity were monitored by tlc and nmr. The fifth fraction displayed low-field signals in the ^{13}C-nmr spectrum (i.e., contained the 6 DKPs) and was further purified by hplc. In order of increasing polarity the following DKPs were isolated as amorphous solids: *cyclo*-(*S*-Pro-*R*-Leu) [**1**] 1.4 mg; *cyclo*-(*S*-Pro-*S*-Ile) [**2**] 1.3 mg; *cyclo*-(*S*-Pro-*R*-Val) [**3**] 3.3 mg; *cyclo*-(*R*-Pro-*R*-Leu) [**4**] 3.7 mg; *cyclo*-(4-methyl-*R*-Pro-*S*-Nva) [**5**], 1.7 mg; and *cyclo*-(*R*-Pro-*R*-Phe) [**6**], 4.5 mg. Similar work up and hplc purification of Collection No. 93318 "endosome" BAH#4 gave identical results. Comparison of the ^{1}H- and ^{13}C-nmr spectra as well as the hplc traces of all four collections showed that they were similar in content of compounds **1–6**.

Cyclo-(S-*Pro*-S-*Leu*) [**1**].—Colorless amorphous solid; $[\alpha]^{20}D$ −90.6° (*c*=0.14, EtOH); hplc R_t=120 min; nmr data in accordance with the structure are shown in Tables 1 and 2.

Cyclo-(S-*Pro*-S-*Ile*) [**2**].—Colorless amorphous solid, $[\alpha]^{20}D$ −197° (*c*=0.13, EtOH); hplc R_t=145 min; nmr data in accordance with the structure are shown in Tables 1 and 2.

Cyclo-(S-*Pro*-R-*Val*) [**3**].—Colorless amorphous solid, $[\alpha]^{20}D$ −74.15° (*c*=0.26, EtOH); hplc R_t=165 min; nmr data in accordance with the structure are shown in Tables 1 and 2.

Cyclo-(R-*Pro*-R-*Leu*) [**4**].—Colorless amorphous solid, $[\alpha]^{20}D$ −142.14° (*c*=0.28, EtOH); hplc R_t=180 min; nmr data in accordance with the structure are shown in Tables 1 and 2.

Cyclo-(*4-methyl*-R-*Pro*-S-*Nva*) [**5**].—Colorless amorphous solid, $[\alpha]^{20}D$ +128° (*c*=0.1, EtOH); $[\alpha]^{20}D$ +91° (*c*=0.001, 0.01 N NaOH in aqueous MeOH (1:1, v/v), lrfabms *m/z* M^+ 210° ($C_{11}H_{18}N_2O_2$); hplc R_t=215 min; nmr data in accordance with the structure are shown in Tables 1 and 2.

Cyclo-(R-*Pro*-R-*Phe*) [**6**].—Colorless amorphous solid, $[\alpha]^{20}D$ +68° (*c*=0.45, EtOH); hplc R_t=225 min; nmr data in accordance with the structure are shown in Tables 1 and 2.

CONVERSION OF PROLYL-LEUCINE DIPEPTIDE INTO *CYCLO*-(PRO-LEU) [**1**].—To a stirred solution of 10 g

of phenol under N_2 at 140–150°, was added 22.4 mg of the dipeptide, Pro-Leu, also dissolved in the molten phenol. Heating was continued for 3 h. Phenol was removed by washing the reaction mixture twice with a saturated solution of K_2CO_3. Next, H_2O was added to the phenoxide mixture and the alkaline solution was extracted three times with CH_2Cl_2. The CH_2Cl_2 was evaporated at room temperature *in vacuo* to afford the cyclized product in 82% yield after purification via hplc with a R_t of 110 min; nmr, see Tables 1 and 2.

CONVERSION OF *S*-PROLYL-*S*-ISOLEUCINE-DIPEPTIDE INTO *CYCLO*-(*S*-PRO-*S*-ILE) [**2**].—The procedure was identical to that described above except that 33.7 mg of the dipeptide and 483.3 mg of the phenol were used. Following hplc purification, the diketopiperazine, *cyclo*-(*S*-Pro-*S*-Ile), was afforded in 87% yield, the hplc R_t was 165 min; $[\alpha]_D$ −197° (c=0.13, EtOH); nmr, see Tables 1 and 2.

ACKNOWLEDGMENTS

We are grateful to Prof. F.J. Schmitz for supplying samples of the dipeptides Pro-Leu and *S*-Pro-*S*-Ile. Financial support was from NIH grants CA52955, CA47135, and an MBRS program grant, GM08132. We appreciate assistance in the collection of sponges by Ms. M. Cristina Diaz (UCSC) and Mr. Mustafa Varoglu (UCSC). Taxonomic analysis was conducted by Ms. Diaz. The sponges were collected during an expedition on the R/V Columbus-Islin supported by an NSF grant to Prof. W. Fenical (UCSD), CHE-86-20217.

LITERATURE CITED

1. P. Crews, J.J. Farias, R. Emrich, and P.A. Keifer, *J. Org. Chem.*, **59**, 2932 (1994).
2. J. Kobayashi and M. Ishibashi, *Chem. Rev.*, **93**, 1753 (1993).
3. D.J. Faulkner, H.-Y. Hee, M.D. Unson, C. Brewley, and M.J. Garson, *Gazz. Chim. Ital.*, **123**, 301 (1993).
4. C.R. Wilkinson, in: "Algae and Symbioses: Plants, Animals, Fungi, Viruses, Interactions Explored." Ed. by W. Reisser, Biopress Ltd., Bristol, UK, 1992, p. 111.
5. M.J. Garson, in: "Sponges in Time and Space." Ed. by R.W.M. van Soest, T.M.G. van Kempen, and J.C. Braekman, A.A. Balkema, Rotterdam, 1994, p. 435.
6. C.A. Bewley and D.J. Faulkner, *J. Org. Chem.*, **59**, 4849 (1994).
7. N. Fusetani, S. Matsunaga, *Chem. Rev.*, **93**, 1793 (1993).
8. K. Reutzler, in: "New Perspectives in Sponge Biology." Smithsonian Institution Press, Washington, D.C., 1990, p. 455, and references therein.
9. P. Humann, in: "Reef Creature Identification: Florida, Caribbean, Bahamas," Ed. by N. Deloach, New World Publications, Vaughan Press, Orlando, Florida, 1992.
10. D.B. Stierle and D.J. Faulkner, *J. Nat. Prod.*, **54**, 1134 (1991).
11. J.L. Johnson, W.G. Jackson, and T.E. Eble, *J. Am. Chem. Soc.*, **73**, 2947 (1951).
12. E. Fischer and G. Reif, *Lieb. Ann. Chem.*, **363**, 118 (1908).
13. I.Z. Siemion, *Org. Magn. Res.*, **3**, 545 (1971).
14. A. Loffet, in: "Peptides 1976. Proceedings of the Fourteenth European Peptide Symposium," Wepion, Belgium, April 11–17, 1976. Ed. de l'Université de Bruxelles, pp. 641–646.
15. Y. Kodaira, *Agric. Biol. Chem.*, **25**, 261 (1961).
16. W. Pickenhagen, P. Dietrich, B. Keil, J. Polonsky, F. Nouaille, and E. Lederer, *Helv. Chim. Acta*, **58**, 1078 (1975).
17. H. Ott, A.J. Frey, and A. Hofmann, *Tetrahedron*, **19**, 1675 (1963).
18. J. Su, Y. Zhong, L. Zeng, S. Wei, Q. Wang, T.C.W. Mak, and Z. Zhou, *J. Nat. Prod.*, **56**, 637 (1993).
19. K.D. Kopple and H.G. Ghazarian, *J. Org. Chem.*, **33**, 862 (1968).
20. I.Z. Siemon, *Org. Magn. Reson.*, **8**, 432 (1976).
21. B.W. Bycroft and G.R. Lee, *J. Chem. Soc., Chem. Commun.*, 988 (1975).
22. P.E. Young, V. Madison, and E.R. Blout, *J. Am. Chem. Soc.*, **98**, 5365 (1976).
23. S. Steinberg and J.L. Bada, *Science*, **213**, 544 (1981).
24. I.Z. Siemion and B. Picur, *Org. Magn. Reson.*, **22**, 171 (1984).
25. A. Ohta, F. Yamamoto, Y. Arimura, and T. Watanabe, *J. Heterocyclic Chem.*, **19**, 781 (1982).
26. P.G. Sammes, *Fortschr. Chem. Org. Naturstoffe*, **32**, 51 (1975).
27. A.A. Stierle, "Investigation of Biologically Active Metabolites from Symbiotic Microorganisms." Ph.D. Dissertation, Montana State University, Bozeman, MT, 1988.
28. J. Trischman, "Chemical Investigations of Microbial Isolates from Estuarine and Extreme Marine Environments." Ph.D. Dissertation, University of California, San Diego, La Jolla, CA, 1993.
29. G.R. Pettit, R.B. Von Dreele, G.G. Bolliger, P.M. Traxler, and P. Brown, *Experientia*, **29**, 521 (1973).
30. A.C. Stierle, J.H. Cardellina II, and F.L. Singleton, *Experientia*, **44**, 1021 (1988).
31. M. Adamczeski, E. Quiñoá, and P. Crews, *J. Am. Chem. Soc.*, **111**, 647 (1989).

32. F.J. Schmitz, D.J. Vanderah, K.H. Hollenbeak, C.E.L. Enwall, and Y. Gopichand, *J. Org. Chem.*, **48**, 3941 (1983).
33. R. Kazlauskas, P.T. Murphy, and R.J. Wells, *Tetrahedron Lett.*, 4945 (1978).
34. A. Leiberknecht and H. Greisser, *Tetrahedron Lett.*, **28**, 4275 (1987).
35. S. Omar, L. Tenenbaum, L.V. Manes, and P. Crews, *Tetrahedron Lett.*, **29**, 5489 (1988).
36. W. De Weerdt, *Beaufortia*, **35**, 61 (1985).
37. R.W.M. van Soest, "Marine Sponges from Curaçao and other Caribbean Localities," in "Studies on the Fauna of Curaçao and Other Caribbean Islands: No. 191," 1 (1980).
38. J. Fromont, *The Beagle, Records of the Northern Territory Museum of the Arts and Sciences*, **8**, 73 (1991).
39. S. Zea, in: "Esponjas del Caribe Colombiano." Editorial Catálogo Cientifico. Primera edición,1987, p. 283.
40. R.W.M. van Soest and N. Stentoft, in: "Studies on the Fauna of Curacao and Other Caribbean Islands," No. 215. Ed. by P.W. Hummelinck and L.J. vander Stean, 1988, p. 133.

Received 18 July 1994