Amino Acid Derivatives from the Marine Sponge Jaspis digonoxea

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AMINO ACID DERIVATIVES FROM THE MARINE SPONGE *JASPIS DIGONOXEA*

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ABSTRACT.—Six heterocycles, bengamide A [**1**], bengamide B [**2**], cyclo(L-*trans*-(4-hydroxyprolinyl)-L-phenylalanine) [**4**], the functionalized nonene lactone **5**, the novel digonazole [**6**], and cyclo(L-prolinyl-L-tyrosine) [**7**], previously unreported from marine origin, have been isolated from the South African sponge *Jaspis digonoxea*. The structures of the known compounds and the new digonazole [**6**] were elucidated primarily by nmr spectroscopy.

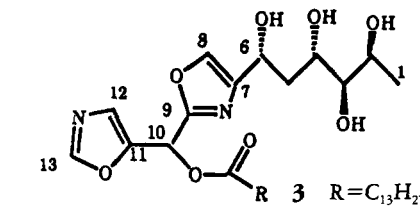
Cyclic peptides (1–3), as well as other interesting bioactive heterocyclic amino acid derivatives (4–7) have recently been reported from the sponge family Jaspidae. Thus, the bengamides (e.g., A and B, **1**, **2**, respectively), isobengamide E, the bengazoles (e.g., A, **3**) the diketopiperazine, cyclo(L-*trans*-(4-hydroxyprolinyl)-L-phenylalanine) [**4**] and *N*-acetyl-L-phenylalanine methyl ester have been isolated from a *Jaspis* sp. by Crews *et al.* (4–6). It has also been shown that isobengamide E readily cleaves, under acetylation conditions, to *N*-acetylcyclolysine and the 3,5-diacetoxy derivative of the methylnonene lactone **5** (5). Furthermore, lactone **5** has also been isolated as a constituent of the crude oil extract of the *Jaspis* sp. sponge (5).

We wish to report herein the isolation from the Indo-Pacific sponge *Jaspis digonoxea* (De Laubenfels, 1950; class Demospongiae, order Choristida, family Jaspidae) of four known Jaspidae metabolites, namely, bengamides A and B [**1** and **2**], the diketopiperazine **4**, the lactone **5**, the diketopiperazine cyclo(L-prolinyl-L-tyrosine) [**7**], and a new marine natural product designated digonazole [**6**].

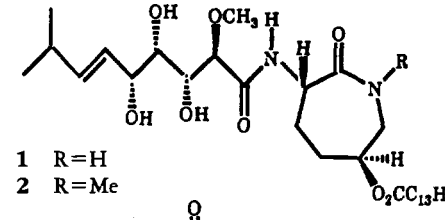
The freeze-dried sponge (32 g) was extracted with EtOAc and the derived oil (495 mg) was separated on Sephadex LH-20 and Si gel columns to afford the following compounds in order of increasing polarity (%, dry wt): lactone **5** (0.015%), bengamide B [**2**] (0.1%), bengamide A [**1**] (0.015%), digonazole [**6**] (0.1%), diketopiperazine **7** (0.015%), and diketopiperazine **4** (0.015%).

Digonazole [**6**] had the composition $C_{34}H_{58}N_2O_7$ as deduced from the hrms and ^{13}C nmr. Its ^1H-nmr and ^{13}C-nmr spectra were similar to those of bengazole A [**3**] (4). Thus, the nmr data of **6** pointed clearly to two oxazole rings (Table 1) with the same substitution pattern as in compound **3** (see below). Most characteristic for the latter oxazoles, as already pointed out by Crews *et al.* (4), were the various one- to three-bond carbon-hydrogen coupling constants (Table 1).

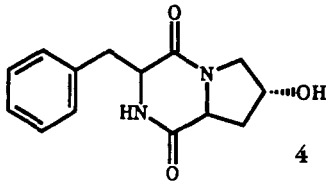
From the HMBC nmr experiment it was clear that the two oxazoles of **6** are connected to each other through a methylene [δ_C 25.2 t, δ_H 4.2 s (2H-10)] rather than a methinoxy group as in **3**. The latter methylene bridge was established by CH correlations between CH_2-10 and C-9, -11, and -12. Furthermore, homo and hetero COSY nmr correlations confirmed unequivocally a 2,3,4,6-tetraoxygenated hexane chain [δ_H 6.02 (H-6), 4.03 (H-2), 3.80 (H-4), 3.30 (H-3), 2.30 (H-5), 2.10 (H-5′), 1.24 (Me-1)] (see Experimental).



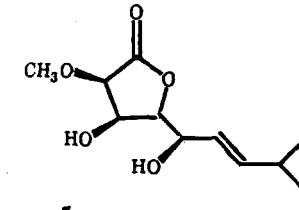
3 R=$C_{13}H_{27}$



1 R=H
2 R=Me



4



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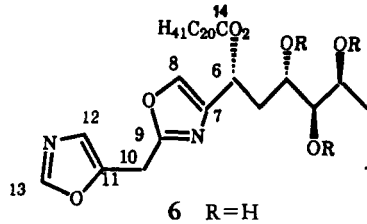
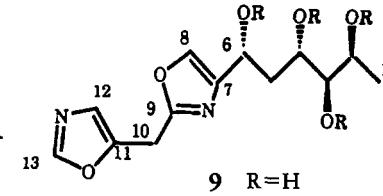
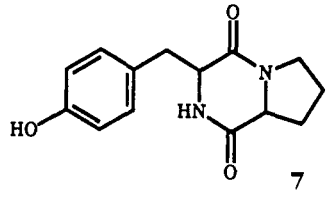


6 R=H
8 R=Ac



9 R=H
10 R=Ac



7

The lower-field resonance of H-6, in **6**, in comparison with the corresponding proton in **3**, suggested the C-6 methinoxy, rather than the C-10 in **3**, to be esterified by a long-chain carboxylic acid. The location of the ester group, at C-6, was fully confirmed by CH correlations between H-6 (δ_H 6.02) and the ester CO group (δ_C 173.1). A C_{21}-fatty acid chain was evident from the *m/z* 325 ($C_{21}H_{41}O_2^+$, 100%) fragment in the mass spectrum. The functionalization mode of the six-carbon

TABLE 1. Comparison of the Nmr Data of **3** and **6** in $CDCl_3$.

Position	6			3[a]		
	δ_C[b]	δ_H	J_{CH} in Hz (with H#)	δ_C[b]	δ_H	J_{CH} in Hz (with H#)
7	138.9 s	—	10.8 (H-8)	144.2 s	—	15.8 (H-8)
8	137.7 d (J=209 Hz)	7.65 s	—	136.3 d (J=211.7 Hz)	7.66 s	—
9	159.5 s	—	8.0 (H-8), 8.0 (H-10)	158.0 s	—	7.2 (H-8)
11	145.6 s	—	multiplet	147.6 s	—	18.4 (H-12) 4.4 (H-13)
12	124.4 d (J=195 Hz)	7.00 s	5.8 (H-13)	127.0 d (J=197.8 Hz)	7.21 s	7.3 (H-13)
13	151.1 d (J=222 Hz)	7.85 s	8.2 (H-12)	152.3 d (J=233.6 Hz)	7.94 s	10.5 (H-12)

[a]Data taken from Adamczeski *et al* (4).

chain was further confirmed by a homo COSY nmr experiment, which was run on the 2,3,4-triacetate derivative of **6**, i.e., compound **8** (Experimental). In the ^{1}H-nmr spectrum of triacetate **8** the doublet of Me-1 moved upfield, from δ 1.25 in **6**, to δ 1.10. As a result, its correlation with H-2 was observed clearly. Mild basic hydrolysis of digonazole (1% KOH in MeOH, at room temperature) afforded the C_{21}-carboxylic acid and the tetrahydroxyhexyl dioxazole **9**. The spectral data of **9**, (except for the 10-methylene instead of a 10-methinoxy group) and the hydrolysis product of **3** (4) were essentially the same. For comparison purposes, compound **9** was acetylated to the corresponding tetraacetate, **10**.

Comparing the chemical shifts and the coupling constants of the hexyl chain of **6** with the corresponding values in bengazole A [**3**] revealed a high degree of similarity in all values except for the chemical shift of H-6 and one of the two coupling constants between H-6 and the neighboring C-5 methylene protons. The latter difference can be explained by a different conformation of the aliphatic six-carbon chain of **6**, in comparison to **3**, due to the long-chain acid at C-6. Indeed, after hydrolysis of the ester group of **6** to tetraol **9**, and acetylation of **9** to the tetraacetate derivative, **10**, the J_{5-6} value of **10** was ca. 9 Hz, in good agreement with the reported 8.5 Hz value for the corresponding derivative of bengazole A [**3**] (5). This experiment thus confirmed that compound **6** and bengazole A have the same relative configuration at their four chiral centers.

The sixth isolated compound [**7**], $C_{14}H_{16}N_2O_3$, *m/z* 260, was suggested by its spectral data to be a diketopiperazine and more specifically was found to be cyclo(L-prolinyl-L-tyrosine). The spectral data of **7** are in full agreement with the data reported for cyclo(L-prolinyl-L-tyrosine) isolated from *Bacillus licheniformis* (8). To the best of our knowledge, **7** has not been previously reported from a marine source, although diketopiperazines are not rare in sponges. Besides compound **4**, Schmitz *et al.* (9) reported three other diketopiperazines, and during our work with MeOH extracts of sponges, we have also encountered a mixture of others.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra were recorded on a Nicolet 205 Ft-ir spectrophotometer. Low-resolution mass spectra were recorded on a Finnigan-4021 mass spectrometer. Hrms were taken on a VG70 VSEQ instrument. ^{1}H- and ^{13}C-nmr spectra were recorded on Bruker AMX-360 and ARX-500 spectrometers. All chemical shifts are reported with respect to TMS (δ=0). Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter using a 1-cm microcell.

COLLECTION AND ISOLATION.—The sponge *Jaspis digonoxea* (family Jaspidae) was collected in Sodwana Bay, South Africa, in July 1992 by divers using scuba. A voucher (TASA 110) is deposited in the zoological department at Tel-Aviv University. The freshly collected sponge was immediately frozen at −25°. The freeze-dried sponge (32 g) was then extracted with EtOAc to give a brown gum (495 mg). The gum was chromatographed first over a Sephadex LH-20 column eluted with MeOH-$CHCl_3$-hexane (1:1:2) and then several times over Si gel columns eluted with hexane-EtOAc mixtures (9:1 to 1:20) to afford **1** (5 mg), **2** (31 mg), **4** (5 mg), **5** (5 mg), **6** (32 mg), and **7** (5 mg). R_f values (Si gel, EtOAc): **1** (0.30), **2** (0.32), **4** (0.20), **5** (0.95), **6** (0.25), **7** (0.22). Compounds **1**, **2**, **4**, and **5** possess the same spectral data as previously reported (5,6).

Digonazole [**6**].—Viscous oil; $[\alpha]^{20}$D −8.9° (*c*=2.2, $CHCl_3$); ir (neat) ν max 3600, 1750, 1500 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 7.85 (1H, s, H-13), 7.65 (1H, s, H-8), 7.00 (1H, s, H-12), 6.02 (1H, dd, *J*=9.6 and 5.1 Hz, H-6), 4.20 (2H, s, H-10), 4.03 (1H, dq, *J*=3.2 and 6.6 Hz, H-2), 3.80 (1H, m, H-4), 3.30 (1H, t, *J*=3.0 Hz, H-3), 2.32 (2H, t, *J*=7.0 Hz, H-15), 2.30 (1H, ddd, *J*=15.0, 9.6, and 2.1 Hz, H-5), 2.10 (1H, ddd, *J*=15.0, 9.7, and 5.1 Hz, H-5′), 1.60 (2H, m, H-16), 1.25 (32H, m), 1.24 (3H, d, *J*=6.6 Hz, Me-1), 0.90 (3H, t, *J*=6.6 Hz, Me-34), 0.88 (2H, m); ^{1}H nmr (MeOH-d_4) δ 8.00 (1H, s, H-13), 7.77 (1H, s, H-8), 6.98 (1H, s, H-12), 6.02 (1H, dd, *J*=9.6 and 5.1 Hz, H-6), 4.21 (2H, s, H-10), 3.82 (1H, dq, *J*=3.1 and 6.5 Hz, H-2), 3.36 (1H, ddd, *J*=2.1, 6.7, and 10.4 Hz, H-4), 3.05 (1H, dd, *J*=3.3 and 6.7 Hz, H-3), 2.35 (1H, ddd, *J*=15.4, 9.6, and 2.1 Hz, H-5), 1.87 (1H, ddd, *J*=15.4, 10.1, and 5.1 Hz, H-5′), 1.08 (3H, d, *J*=6.6 Hz, Me-34); ^{13}C

nmr ($CDCl_3$) δ 173.1 (s, C-14), 159.5 (s, C-9), 151.1 (d, C-13), 145.6 (s, C-11), 138.9 (s, C-7), 137.7 (d, C-8), 124.4 (d, C-12), 77.0 (d, C-3), 69.9 (d, C-4), 67.0 (d, C-2), 65.7 (d, C-6), 36.2 (t, C-5), 34.4 (t, C-15), 31.9t, 29.7 (t, 10 CH_2), 29.4t, 29.2t, 29.1t, 29.0t, 25.2 (t, C-10), 24.8 (t, C-16), 22.7t, 19.5t, 14.0 (q, C-34).

HMBC Correlations, C to H: C-2/H-1; C-3/H-1, H-5; C-4/H-5′, H-6; C-5/H-6; C-6/H-5; C-7/H-5, H-8; C-8/H-6; C-9/H-8, H-10; C-11/H-10, H-13; C-12/H-10, H-13; C-13/H-12; C-14/H-6, H-15, H-15′, H-16, H-16′. Hreims 607.4332 (MH^+, $C_{34}H_{59}N_2O_7$) (calcd 607.4325).

Acetylation of **6** *to form* **8**.—A solution of **6** (8 mg) in dry pyridine (0.5 ml) and Ac_2O (0.5 ml) at room temperature was placed in the dark for 24 h. The reaction mixture was concentrated *in vacuo* and the residue was chromatographed on Si gel [EtOAc-hexane (1:1)] to afford triacetate **8** in 90% yield; $[\alpha]^{20}D$ −8.0° (*c*=0.9, $CHCl_3$); ^1H nmr ($CDCl_3$) δ 7.96 (1H, s), 7.68 (1H, s), 7.13 (1H, s), 5.85 (1H, dd, *J*=6.0 and 9.6 Hz), 5.12 (2H, m), 4.95 (1H, dq, *J*=3.1 and 6.6 Hz), 4.30 (2H, s), 2.24 (3H, s), 2.16 (3H, s), 2.11 (3H, s), 1.25 (3H, d, *J*=6.6 Hz), 0.99 (3H, t, *J*=6.6 Hz). The following H to H correlations have been observed in a COSY-45 spectrum (H/H): Me-1/H-2; H-3/H-4; H-4/H-5, H-5′; H-6/H-5, H-5′.

Compound **9**.—A solution of **6** (10 mg) in 1% MeOH-KOH (1 ml) was placed in the dark for 24 h. After being neutralized with a 1% solution of HCl, the mixture was partitioned between H_2O (5 ml) and CH_2Cl_2 (3×5 ml). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated to dryness to obtain the *n*-C_{21}-carboxylic acid [identified by characteristic nmr data and the eims of its methyl ester, *m/z* 340 (M^+)].

The aqueous extract was concentrated *in vacuo* and the residue extracted with a 50% mixture of MeOH and CH_3CN to yield after filtration and concentration, an 80% yield of pure hydrolyzed product **9**; $[\alpha]^{20}D$ −1° (*c*=0.1, MeOH); an oil; ^1H nmr [MeOH-d_4-CD_3CN (1:1)] δ 8.15 (1H, s), 7.74 (1H, s), 7.09 (1H, s), 4.86 (1H, m), 4.28 (2H, s), 3.99 (1H, dd, *J*=5.0 and 2.2 Hz), 3.72 (1H, m), 3.12 (1H, m), 2.12 (1H, ddd, *J*=15.0, 9.7, and 2.3 Hz), 1.88 (1H, ddd, *J*=15.0, 10.2, and 5.1 Hz), 1.15 (3H, d, *J*=6.6 Hz); (eims was performed on the tetraacetate derivative, **10**).

Compound **10**.—In the same procedure as described for **6**, compound **9** (4 mg) was acetylated to afford tetraacetate **10** in 90% yield; an oil; ^1H nmr ($CDCl_3$) δ 7.75 (1H, s), 7.54 (1H, s), 6.92 (1H, s), 5.66 (1H, dd, *J*=5.3 and 9.3 Hz), 5.01 (2H, m), 4.77 (1H, ddd, *J*=9.9, 4.3, and 2.3 Hz), 4.12 (2H, s), 2.30 (1H, ddd, *J*=14.3, 9.2, and 2.4 Hz), 2.15 (1H, ddd, *J*=14.3, 9.8, and 5.0 Hz), 2.04 (3H, s), 1.96 (3H, s), 1.93 (3H, s), 1.91 (3H, s), 1.06 (3H, d, *J*=6.6 Hz); eims 466 (M^+, $C_{21}H_{26}O_{10}N_2$, 7), 406 (M^+ −60, 11), 346 (17), 304 (23), 243 (50).

Cyclo(L-*prolinyl*-L-*tyrosine)* [**7**].—Viscous oil; $[\alpha]^{20}D$ −5° (*c*=0.6, $CHCl_3$); ir (neat) ν max 3500, 1750, 1650 cm^{-1}; ^1H nmr ($CDCl_3$) δ 6.97 (2H, d, *J*=8.2 Hz, H-12, 16), 6.73 (2H, d, *J*=8.2 Hz, H-13, 15), 4.16 (1H, dd, *J*=10.4 and 7.4 Hz, H-3), 3.95 (1H, t, *J*=8.3 Hz, H-6), 3.55 (1H, m, H-9), 3.50 (1H, m, H-9′), 3.30 (1H, dd, *J*=7.4 and 14.4 Hz, H-10), 2.85 (1H, dd, *J*=10.4 and 14.4 Hz, H-10′), 2.10 (1H, m, H-7), 1.75 (1H, m, H-7′), 1.85 (1H, m, H-8), 1.80 (1H, m, H-8′); ^{13}C nmr ($CDCl_3$) δ 169.0 (s, C-2), 165.0 (s, C-5), 156.0 (s, C-14), 130.2 (d, C-12, 16), 126.0 (s, C-11), 116.0 (d, C-13, 15), 59.5 (d, C-6), 59.3 (d, C-3), 45.2 (t, C-9), 36.2 (t, C-10), 28.3 (t, C-7), 22.2 (t, C-8); hrcims *m/z* 261.1246 ($C_{14}H_{17}N_2O_3$) (calcd 261.1240).

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