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OCEANAPAMINE, A SESQUITERPENE ALKALOID FROM THE PHILIPPINE SPONGE *OCEANAPIA* SP.

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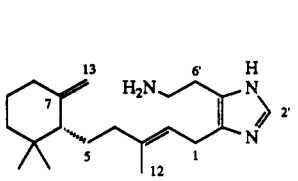
ABSTRACT.—The major metabolite of the Philippine sponge *Oceanapia* sp. is the antimicrobial alkaloid oceanapamine [**1**], which was isolated as a trifluoroacetate salt. The structure and the absolute configuration of oceanapamine, which consists of a monocyclic sesquiterpene attached to a histamine residue, were elucidated by interpretation of spectroscopic data.

Terpenoids are among the most abundant secondary metabolites of marine sponges (1) but there are relatively few examples of alkaloids that are derived from terpene and amino acid units (2,3). During routine antimicrobial screening of crude extracts of the voucher specimens from a collection of marine invertebrates from the Philippines, two specimens of the sponge *Oceanapia* sp. (order Haploscerida, family Phloeodictyiidae [=Oceanapiidae]) were found to have similar antimicrobial profiles and ^{1}H-nmr spectra. The ^{1}H-nmr spectra of the crude extracts were dominated by signals of a single major terpenoid metabolite and a bioassay-guided fractionation revealed that the antimicrobial activity was associated with this major metabolite, oceanapamine [**1**].

Specimens of *Oceanapia* sp. were collected by hand using scuba (−15 m) from the under surfaces of rocks at Inambuyod Island, Palawan, the Philippines, and were immediately frozen. The MeOH extract was partitioned between EtOAc and H_2O and both fractions showed activity against *Escherichia coli* and *Staphyloccocus aureus*. The MeOH-soluble material from the aqueous extract was purified by reversed-phase chromatography on a C_{18} Si gel column using a gradient of 70% aqueous MeOH containing 0.1% TFA to 100% MeOH (0.1% TFA) to obtain the TFA salt of oceanapamine [**1**] as the only active component. Under the same chromatographic conditions, the EtOAc extract also yielded the TFA salt of oceanapamine [**1**]. From small differences in the ^{1}H-nmr spectra, we concluded that the EtOAc extract contained oceanapamine [**1**] as a free base and the MeOH-soluble material contained a salt of oceanapamine [**1**]. No other sesquiterpene alkaloids were detected in the extracts.

The TFA salt of oceanapamine [**1**] was isolated as an oil. The molecular formula of the free base was shown to be $C_{20}H_{33}N_3$ [*m/z* 315.2689 $[M]^+$]. The ir spectrum contained a broad band from 2500–3500 cm^{-1} due to the amine salt and a band at 1675 cm^{-1} assigned to the imidazole ring. The uv spectrum contained a band at 202 nm (ϵ 14290) with a pronounced shoulder at 220 nm. The ^{13}C-nmr spectrum of oceanapamine [**1**] contained seven signals in the olefinic region: four of these were assigned to an exocyclic methylene [δ 109.7 (C-13), 150.0 (C-7)] and a trisubstituted olefin [δ 119.0 (C-2), 141.3 (C-3)], while the remaining three signals must be assigned to a 4,5-disubstituted imidazole ring [δ 134.4 (C-2′), 132.0 (C-4′), 124.9 (C-5′)]. The ^{1}H-nmr spectrum contained signals at δ 3.22 (2H, t, J=7 Hz) and 3.10 (2H, t, J=7 Hz). HMBC correlations indicated that these signals were due to a $-CH_2-CH_2-NH_2$ chain attached to C-5′ on the imidazole ring. This assignment was confirmed by reacting oceanapamine [**1**] with carbonyldiimidazole in CH_2Cl_2 containing triethylamine to neutralize the TFA salt, to obtain the cyclic urea [**2**]. The remaining signals in the ^{1}H- and ^{13}C-nmr spectra were assigned using the HMQC and HMBC



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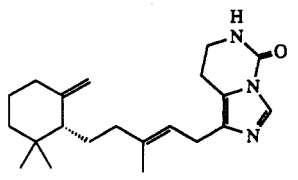

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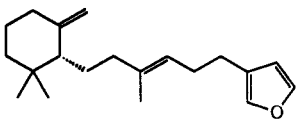

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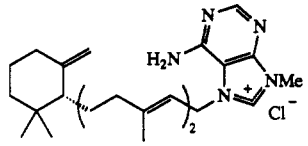

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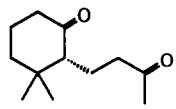

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experiments to a familiar sesquiterpene skeleton, found in such compounds as dehydroambliol-A [**3**] (4) and agelasine-E [**4**] (5), completing the structural elucidation of oceanapamine [**1**].

The optical rotation of oceanapamine [**1**], $[\alpha]_D$ −6.4° (c=3.1, MeOH), had the same sign as (−)-dehydroambliol-A [**3**] (4) and (−)-agelasine-E [**4**] (5), to which the *R* absolute configuration had been assigned,[1] and the opposite sign to that of luffarin-P and luffarin-W (6). In order to confirm this assignment, oceanapamine [**1**] was oxidized with osmium tetroxide and sodium periodate to obtain the known diketone **5**, $[\alpha]_D$ −10.0° (c=0.28, $CHCl_3$) [literature values: from (+)-trixagol (7), $[\alpha]_D$ +5.5° (c=1.5, $CHCl_3$); from (−)-ambrein (8), $[\alpha]_D$ +6.19° (c=1.5, $CHCl_3$)].

The TFA salt of oceanapamine [**1**] was screened quite broadly but showed only antimicrobial activity. In the standard disk (6-mm) assay, oceanapamine inhibited *B. subtilis* and *E. coli* at 25 μg/disk, *S. aureus* and *C. albicans* at 50 μg/disk, and *P. aeruginosa* at 100 μg/disk. The cyclic urea **2** was inactive in these assays. Oceanapamine [**1**] is presumed to be derived biosynthetically from histidine, a common α-amino acid, and a monocyclic sesquiterpene.

EXPERIMENTAL

ANIMAL MATERIAL.—*Oceanapia* sp. (collection number NCI-1384) was collected by hand using scuba (−15 m) from the undersides of rock overhangs at Inambuyod Island, Palawan, the Philippines,[2] and was immediately frozen. The off-white sponge forms rounded masses (10–15 cm length, 4–6 cm thick); without fistules. The con-

[1]There is some confusion in the literature concerning the assignment of *R* or *S* configuration to compounds in this series. The confusion has been caused by misinterpretation of the Cahn-Ingold-Prelog rules, because the absolute configurations as drawn in the structural diagrams are all consistent with the sign of the optical rotation.

[2]This sponge appears to have a restricted distribution; we have made extensive collections in the Philippines and have only encountered this sponge in cyptic habitats in the western province of Palawan. We have examined five specimens of this *Oceanapia* sp.; all have the same morphology and chemical composition. A voucher specimen is on deposit at the Scripps Institution of Oceanography Benthic Invertebrate Collection (registry #P1153).

sistency is firm and incompressible and the surface is rough. The spicules are oxea and abundant toxas and sigmas. The ectosomal skeleton is tangential, unispicular, and easily detachable; the endosomal skeleton is a multispicular reticulation with circular meshes. This non-fistulose *Oceanapia* most likely represents an undescribed species.

EXTRACTION AND ISOLATION.—The frozen sponge (99.3 g dry wt) was chopped into small pieces and extracted with MeOH (2×1000 ml) over a period of four days. The combined MeOH extracts were concentrated under vacuum to give an aqueous suspension which was diluted with H_2O (150 ml) and extracted with EtOAc (4×300 ml). The combined EtOAc extracts were dried over Na_2SO_4 and evaporated to give a light-brown foam (1.46 g). The aqueous extract was evaporated to dryness and the residue was triturated with MeOH (100 ml). Filtration and evaporation gave a light-brown foam (8.3 g). Both extracts showed antimicrobial activity against *E. coli* and *S. aureus*. A portion of the MeOH extract (300 mg) was subjected to chromatography on a reversed-phase C_{18} Si Sep-Pak (35 ml) column, eluting with a gradient of 30–100% MeOH (containing 0.1% TFA) in H_2O, to give the TFA salt of oceanapamine [**1**] (37 mg, 1.03% dry wt) as the only active compound. A portion of the EtOAc extract (50 mg) was treated in a similar manner to obtain the same TFA salt of oceanapamine [**1**] (10.3 mg, 0.3% dry wt). The two isolates showed identical activities in the antimicrobial assays and were judged to be identical by ^{1}H-nmr and tlc analysis.

Oceanapamine trifluoroacetate [**1**].—Oil, $[\alpha]^{22}D$ −6.4° (*c*=3.1, MeOH); uv (MeOH) 202 nm (ε 14290), 220 (sh, ε 6670); ir (film) ν max 2939 (br), 1675 (br) cm^{-1}; ^{1}H-nmr data (CD_3OD, 500 MHz), see Table 1; ^{1}H nmr (200 MHz, DMSO-d_6) δ 14.48 (1H, br s), 8.96 (1H, s), 8.09 (3H, br s), 5.16 (1H, t, *J*=6.5 Hz), 4.73 (1H, s), 4.48 (1H, s), 3.33 (2H, d, *J*=7 Hz), 3.05 (2H, br s), 2.95 (2H, m), 2.00–1.5 (11H, m), 1.66 (3H, s), 0.85 (3H, s), 0.78 (3H, s); ^{13}C-nmr data (CD_3OD, 50 MHz), see Table 1; eims (70 ev) *m/z* 315 (14), 271 (14), 192 (18), 178 (42), 135 (52), 95 (100); hreims, *m/z* 315.2689 ($C_{20}H_{33}N_3$ requires 315.2674).

Reaction of oceanapamine [**1**] *with carbonyldiimidazole.*—To a stirred suspension of **1** (10 mg, 0.023 mmol) in CH_2Cl_2 (3 ml) was added a few drops of triethylamine. Carbonyldiimidazole (5.9 mg, 0.030 mmol) was added to the resulting solution which was stirred at room temperature for three days. The reaction mixture was diluted with CH_2Cl_2 (20 ml) and washed with H_2O (3×20 ml).

TABLE 1. ^{1}H- and ^{13}C-Nmr Data for the Trifluoroacetate Salt of Oceanapamine [**1**].

Position	δ_H	[int., mult., *J* (Hz)]	δ_C	HMBC correlations
1	3.44	(2H, d, 7)	23.3	C-2, C-3, C-4′, C-5′
2	5.21	(t, 7)	119.0	C-1, C-4, C-12, C-4′
3			141.3	
4	2.04	(m)	39.3	C-6
	1.82	(m)		C-2, C-3, C-5, C-6, C-12
5	1.62	(m)	25.7	C-4, C-6
	1.52	(m)		C-3, C-4, C-7
6	1.71	(dd, 11, 2)	55.0	C-4, C-5, C-7, C-8, C-11
				C-13, C-15
7			150.0	
8	2.10	(m)	33.3	C-7, C-9, C-10, C-13
	2.00	(m)		C-6, C-7, C-9, C-10, C-13
9	1.56	(2H, m)	24.7	C-7, C-8, C-10, C-11
10	1.54	(m)	37.2	C-6, C-8, C-9, C-11, C-14, C-15
	1.24	(m)		C-9
11			35.7	
12	1.76	(3H, br s)	16.5	C-2, C-3, C-4
13	4.75	(br s)	109.7	C-6, C-8
	4.52	(br s)		C-6, C-8
14	0.91	(3H, s)	28.8	C-6, C-10, C-11, C-15
15	0.83	(3H, s)	26.8	C-6, C-10, C-11, C-14
2′	8.78	(s)	134.4	C-4′, C-5′
4′			132.0	
5′			124.9	
6′	3.10	(2H, t, 7)	22.7	C-4′, C-5′, C-7′
7′	3.22	(2H, t, 7)	39.7	C-5′, C-6′

The organic layer was dried over Na_2SO_4 and evaporated to dryness. Si gel cc (100% EtOAc) gave the cyclic urea **2** (4.2 mg, 0.012 mmol, 52%): $[\alpha]^{22}D$ −7.1° (*c*=0.28, $CHCl_3$); uv ($CHCl_3$) λ max 248 nm (ε 6980); ir ($CHCl_3$) ν max 1727 cm^{-1}; ^1H nmr ($CDCl_3$, 200 MHz) δ 8.08 (1H, s), 5.46 (1H, br s), 5.29 (1H, t, *J*=8 Hz), 4.71 (1H, s), 4.50 (1H, s), 3.50 (2H, td, *J*=6.5 and 3 Hz), 3.24 (2H, d, *J*=7 Hz), 2.90 (2H, t, *J*=6.5 Hz), 1.1–2.1 (11H, m), 1.70 (3H, s), 0.88 (3H, s), 0.80 (3H, s); eims (70 ev) *m/z* 341 (48), 326 (40), 204 (70), 151 (100); hreims, *m/z* 341.2481 ($C_{21}H_{31}NO_3$ requires 341.2467).

Oxidative degradation of oceanapamine [**1**].—A 2.5% solution of osmium tetroxide in *i*-PrOH (0.5 ml, 0.040 mmol) was added to a solution of oceanapamine trifluoroacetate [**1**] (20 mg, 0.047 mmol) and sodium periodate (200 mg, 0.94 mmol) in H_2O (5 ml), and the reaction mixture was stirred at room temperature for 20 h. The reaction mixture was extracted with CH_2Cl_2 (5×10 ml) and the combined organic extracts dried over Na_2SO_4. Evaporation gave a dark oil which was purified by Si gel cc using EtOAc in hexane as eluent and followed by hplc (EtOAc-hexane, 3:7) to obtain the ketone **5** as a clear oil (2.1 mg, 0.011 mmol, 24%): $[\alpha]^{22}D$ −10.0° (*c*=0.08, $CHCl_3$); ir ($CHCl_3$) ν max 1708 cm^{-1}; ^1H nmr (200 MHz, $CDCl_3$) δ 0.75 (3H, s), 1.06 (3H, s), 2.09 (3H, s), 1.50–2.65 (11H, m); eims, *m/z* 196 (19), 181 (58), 121 (28), 83 (100).

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