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# TOLYTOXIN AND NEW SCYTOPHYCINS FROM THREE SPECIES OF *SCYTONEMA*

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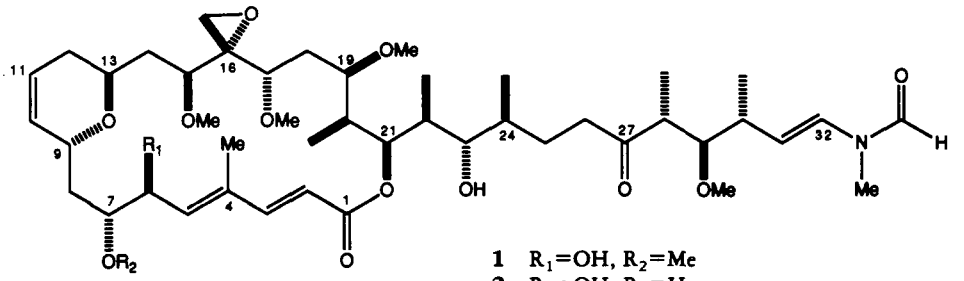
ABSTRACT.—Tolytoxin [**1**], the major cytotoxin associated with *Scytonema mirabile* strain BY-8-1, *Scytonema burmanicum* strain DO-4-1 and *Scytonema ocellatum* strains DD-8-1, FF-65-1, and FF-66-3, has been shown to be 6-hydroxy-7-*O*-methylscytophycin B. Minor amounts of three other new cytotoxic scytophycins, 6-hydroxyscytophycin B [**2**], 19-*O*-demethylscytophycin C [**3**], and 6-hydroxy-7-*O*-methylscytophycin E [**4**], have also been isolated from these cyanophytes. The gross structures and stereochemistry are based on nmr and cd analysis and on comparison with scytophycins A–E.

Tolytoxin, a potent cytotoxin and fungicide, was first isolated from a terrestrial blue-green alga *Tolypothrix conglutinata* var. *colorata* Ghose found at Fanning Island in 1977 (1). Inadequate material from the 1977 field collection and our failure to culture tolytoxin-producing *T. conglutinata* precluded its structure elucidation at that time. The closely-related scytophycins A–E, which were subsequently isolated from a cultured terrestrial blue-green alga *Scytonema pseudohofmanni* Bharadwaja (strain BC-1-2; ATCC 53141) as a result of our screening program to discover new anticancer drugs from this phylum of prokaryotic microorganisms (2,3), were the first compounds in this class of acetogenic macrolides to be fully characterized (4,5). Scytophycins A and B were strongly cytotoxic ($IC_{50}$'s against KB, a human nasopharyngeal carcinoma cell line, 1 ng/ml), but exhibited only moderate activity against intraperitoneally implanted P-388 lymphocytic leukemia and Lewis lung carcinoma and no activity against intraperitoneally implanted B16 melanoma in mice (4,6). These compounds were also strongly antifungal and proved to be effective against some phytopathogenic fungi (6). Scytophycins C–E were less cytotoxic (KB $IC_{50}$'s 10–100 ng/ml) and less fungicidal. In this paper we discuss the total structure determination of tolytoxin [**1**] and three new scytophycins **2–4** from three other cultured cytotoxic and fungicidal species of Scytonemataceae: *Scytonema mirabile* (Dillwyn) Bornet (strain BY-8-1), *Scytonema burmanicum* Skuja (strain DO-4-1), and *Scytonema ocellatum* Lyngbye ex Bornet & Flahault (strains DD-8-1, FF-65-1, and FF-66-3).

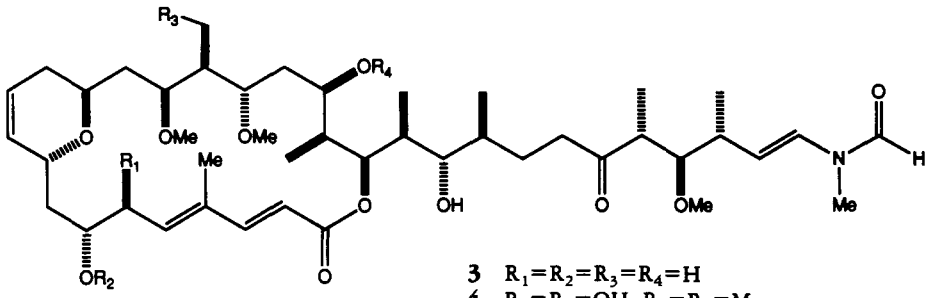
## RESULTS AND DISCUSSION

A cultured sample of each strain of alga was freeze-dried and extracted with 70% aqueous EtOH, and the extract was then subjected to reversed-phase chromatography to obtain compounds **1–4**. The molecular weights and elemental compositions of the four compounds were determined by fabms. Their uv spectra were, like those of scytophycins A–E, typical of dienoate esters ($\lambda$ max 261 nm, $\epsilon$ 22,000–28,000). In addition, their cd curves (positive peak at 268 nm and negative peaks at 297 and 226 nm) were also very similar to those of scytophycins A–E, strongly suggesting that the absolute stereochemistry of compounds **1–4** was the same as that for scytophycins A–E. Two-dimensional nmr experiments, particularly inverse-detected heteronuclear correlation spectroscopy (HMQC and HMBC), homonuclear COSY, and hypercomplex phase sensitive NOESY experiments, were very useful for determining the gross structures, including the relative stereochemistry of each compound. The $^{1}H$- and $^{13}C$-nmr

[1]G.M.L. Patterson, F.R. Caplan, L.K. Larsen, and R.E. Moore, manuscript in preparation.



**1** $R_1$=OH, $R_2$=Me
**2** $R_1$=OH, $R_2$=H
**5** $R_1$=H, $R_2$=H



**3** $R_1$=$R_2$=$R_3$=$R_4$=H
**4** $R_1$=$R_3$=OH, $R_2$=$R_4$=Me
**6** $R_1$=$R_2$=H, $R_3$=OH, $R_4$=Me

spectra of **1–4** were complex, since the signals for the protons and carbons in or around the ene-*N*-methylformamide unit were found to be doubled in a 2:1 ratio (A = major conformer and B = minor conformer), as they were for scytophycins A–E (4,5), due to restricted rotation around the *N*-formyl bond.

TOLYTOXIN [**1**].—On the basis of data available at the time the structures of scytophycins A–E were determined, tolytoxin initially appeared to be 6-methoxyscytophycin B (5). An HMBC experiment, however, subsequently showed that tolytoxin was 6-hydroxy-7-*O*-methylscytophycin B. Three-bond correlations of H-7 (3.48 ppm) with the carbon of the methoxyl on C-7 (60.33 ppm) and the methoxy protons (3.56 ppm) with C-7 (82.67 ppm) were consistent only with **1** for the gross structure of tolytoxin. Comparison of the coupling constants and nOe's between various protons in the C-4 to C-10 segments of tolytoxin and scytophycin B [**5**] indicated that the relative stereochemistry in this region was the same for both compounds. The proton on C-6 in tolytoxin (2.48 ppm) showed similar coupling (9.4 Hz) to H-5 and H-7 and also an nOe to the methyl group on C-4 (1.90 ppm), as did H-6 in scytophycin B, which showed 9.3 and 10.2 Hz coupling to H-5 and H-7, respectively, and an nOe to the Me on C-4. Furthermore, H-5 showed an nOe to H-7 in both compounds. These results clearly pointed to similar conformations for the C-4 to C-7 segments in **1** and **5** and thus to an anti relationship of H-6 to both H-5 and H-7 in tolytoxin. The two compounds exhibited similar coupling constants and nOe connectivities between other protons in the C-4 to C-10 segment (Table 1). The dihydropyran ring (C-9 to C-13) in both compounds appeared to be identical by nmr analysis. Moreover, comparable scalar and nonscalar couplings between various protons in the C-13 to C-20 segments strongly suggested that their stereochemistries and conformations were the same. The conformation implied by the nmr data for the C-13 to C-20 segment was the one where H-13 and H-14, H-14′ and H-15, H-15 and the $CH_2$ of the epoxide, the O on C-16 and H-17, H-17 and H-18′, H-18 and H-19, H-19 and the Me on 20, and H-20 and H-21 all have transoid relationships (dihedral angles of about 180°); this is essentially identical

TABLE 1. Comparison of $^{1}H$ and $^{13}C$ Data for Tolytoxin [**1**] and Scytophycin B [**5**].

| Position | Compound | | | | | |
|---|---|---|---|---|---|---|
| | **5** | | | **1** | | |
| | $\delta_C$ mult. | $\delta_H$ mult., *J* in Hz | $^{1}H$-$^{1}H$ NOE | $\delta_C$ mult. | $\delta_H$ mult., *J* in Hz | $^{1}H$-$^{1}H$ NOE |
| 1 | 169.63 s | | | 169.10 s | | |
| 2 | 115.58 d | 5.78 d 15.8 | 4-Me | 117.64 d | 5.87 d 15.8 | Me on 4 |
| 3 | 151.85 d | 7.66 d 15.8 | H-5, 17-OMe | 151.54 d | 7.63 d 15.8 | H-5, 19 |
| 4 | 134.76 s | | | 135.63 s | | |
| 4-Me | 12.29 q | 1.85 brd 1.5 | H-2 | 12.40 q | 1.90 brd 1.0 | H-2, 6 |
| 5 | 139.95 d | 6.01 brdd 9.3, 4.3 | H-3, 6′, 7 | 141.66 d | 5.96 brd 9.4 | H-3, 6, 7, 15-OMe |
| 6 | 39.36 t | 2.48 ddd − 16.2, 10.2, 9.3 | | 71.97 d | 4.38 t 9.4 | H-5, 8′, 4-Me, 7-OMe |
| | | 2.56 ddd − 16.2, 4.3, 3.1 | H-5 | | | |
| 6-OH | | | | | 4.16 brs | |
| 7 | 68.86 d | 4.06 ddt 3.1, 1.2, 10.2 | H-5, 9 | 82.67 d | 3.48 ddd 10.9, 9.4, 1.7 | H-5, 9 |
| 7-OMe | | | | 60.33 q | 3.56 s | |
| 8 | 40.51 t | 1.26 ddd − 14.7, 10.2, 1.8 | H-9, 10 | 36.27 t | 1.30 ddd − 13.8, 10.9, 2.4 | H-8′, 9 (10) |
| | | 1.76 ddd − 14.7, 9.8, 1.2 | H-13 | | 1.59 ddd − 13.8, 9.6, 1.7 | H-6, 8, 13 |
| 9 | 70.91 d | 4.58 dddt 9.8, 2.9, 2.1, 1.8 | H-8, 10 | 70.62 d | 4.39 br d 9.6 | H-7, 8, 10 |
| 10 | 131.51 d | 5.66 ddt 10.5, 2.9, 1.7 | H-8, 9, 11 | 130.78 d | 5.64 ddt 10.3, 2.8, 1.7 | H-9, 11 (8) |
| 11 | 124.90 d | 5.81 ddt 10.5, 2.1, 4.0 | H-10, 12, 12′ | 124.98 d | 5.79 dddd 10.3, 5.8, 4.0, 3.0 | H-10, 12, 12′ |
| 12 | 31.84 t | 1.89 m | H-11, 13 | 31.75 t | 1.90 m | H-11, 13 |
| | | 1.91 m | H-11 | | 1.91 m | H-11 |
| 13 | 66.87 d | 3.39 dddd 10.2, 8.8, 3.1, 2.0 | H-8′, 12, 15 | 67.03 d | 3.42 dddd 10.1, 9.0, 2.1, 1.5 | H-8′, 12, 15 |
| 14 | 35.63 t | 1.45 ddd − 14.5, 7.4, 2.0 | CH on 16, 15-OMe | 37.01 t | 1.57 ddd − 14.4, 6.4, 1.5 | CH on 16 |
| | | 1.55 ddd − 14.5, 8.8, 3.0 | CH on 16, H-15 | | 1.63 ddd − 14.4, 9.0, 3.2 | CH on 16, H-15 |
| 15 | 78.44 d | 3.94 dd 7.4, 3.0 | H-13, 14′, 17 | 77.77 d | 3.90 dd 6.4, 3.2 | H-13, 17 |
| 15-OMe | 57.46 q | 3.37 s | CH on 16 | 57.37 q | 3.40 s | CH′on 16 |
| 16 | 61.12 s | | | 61.21 d | | |
| $CH_2$ on 16 | 45.56 t | 2.63 d − 4.5 | CH′on 16, 15-OMe | 48.42 t | 2.77 d − 4.5 | H-17 |
| | | 2.72 d − 4.5 | CH on 16 | | 2.78 d − 4.5 | H-14, 14′, 15-OMe |
| 17 | 75.20 d | 3.87 dd 11.3, 4.0 | H-15, 18, 18′ | 77.21 d | 3.64 dd 11.0, 4.1 | H-15, 18, 20, CH on 16 |

TABLE 1. (Continued).

| Position | Compound | | | | | |
|---|---|---|---|---|---|---|
| | **5** | | | **1** | | |
| | $\delta_C$ mult. | $\delta_H$ mult., *J* in Hz | $^1H$-$^1H$ NOE | $\delta_C$ mult. | $\delta_H$ mult., *J* in Hz | $^1H$-$^1H$ NOE |
| 17-OMe | 52.83 q | 3.24 s | H-3 | 54.73 q | 3.35 s | H-14 |
| 18 | 27.45 t | 1.50 ddd − 13.6, 9.7, 4.0 | H-17, 20-Me | 28.82 t | 1.50 ddd − 14.3, 9.6, 4.1 | H-17, 18′, 20-Me |
| | | 1.95 ddd − 13.6, 11.4, 4.0 | H-17, 19 | | 1.93 ddd − 14.3, 11.0, 4.0 | H-19, 19-OMe |
| 19 | 77.49 d | 3.31 ddd 9.7, 4.0, 1.0 | H-18′, 21 | 77.21 d | 3.37 ddd 9.6, 4.0, 1.0 | H-18′, 20, 21 |
| 19-OMe | 57.78 q | 3.20 s | H-21 | 57.37 q | 3.14 s | H-18′ |
| 20 | 38.15 d | 2.09 ddq 10.3, 1.0, 7.0 | H-17, 19, 20-Me, 22-Me | 37.66 d | 2.08 ddq 9.9, 1.0, 7.0 | H-17, 19, 20-Me, 22-Me |
| 20-Me | 9.23 q | 0.86 d 7.0 | H-18, 20, 22 | 9.07 q | 0.83 d 7.0 | H-18, 20, 21, 22 |
| 21 | 76.51 d | 5.22 brdd 10.3, 1.0 | H-19, 22, 23, 19-OMe $H_2O$, 23-OH | 76.86 d | 5.19 dd 9.9, 0.9 | H-19, 22, 23, 23-OH, 19-OMe, $H_2O$, 20-Me |
| 22 | 38.15 d | 1.99 ddq 9.9, 1.0, 6.8 | H-21, 24, 20-Me, 22-Me | 37.79 d | 1.92 ddq 9.9, 0.9, 6.8 | H-21, 24, 20-Me, 22-Me |
| 22-Me | 9.23 q | 0.87 d 6.8 | H-22, 23, 24 | 9.07 q | 0.87 d 6.8 | H-20, 22, 23, 24 |
| 23 | 76.51 d | 3.03 ddd 9.5, 4.5, 2.0 | H-21, 24, 23-OH | 76.36 d | 3.04 ddd 9.9, 4.3, 2.0 | H-21, 24, 22-Me, 24-Me |
| 23-OH | | 4.00 d 4.5 | H-21, 23, 20-Me | | 4.04 d 4.3 | H-21 |
| 24 | 33.78 d | 1.69 m | H-22, 23 | 33.72 d | 1.69 dddq 9.6, 3.7, 2.0, 6.7 | H-22, 23, 26′, 22-Me, 24-Me |
| 24-Me | 18.27 q | 0.99 d 6.7 | 23-OH | 18.06 q | 0.97 d 6.7 | H-23, 24, 26 |
| 25 | 22.71 t | 1.37 m | | 22.61 t | 1.38 m | H-25′, 26′ |
| | | 1.75 m | | | 1.76 m | H-25 |
| 26 | 42.08 t | 2.54 m | | 41.92 t | 2.50 m | 24-Me, 28-Me |
| | | 2.54 m | | | 2.55 m | H-24, 25, 28 |
| 27 | 214.07 s | | | 213.88 s | | |
| 28 | 49.48 d | 2.78 dq 9.5, 7.0 | 29-OMe | 49.30 d | 2.76 dq 9.5, 7.0 | H-26′, 29-OMe |
| 28-Me | 13.65 q | 0.96 d 7.0 | | 13.47 q | 0.90 d 7.0 | H-26, 28, 30, 29-OMe |
| 29 | 88.26 d | 3.28 dd 9.5, 2.7 | H-30 (A,B) | 88.14 d | 3.28 dd 9.5, 2.7 | H-30 (A,B) |
| 29-OMe | 61.12 q | 3.31 s | H-28 | 60.94 q | 3.30 s | H-28, 31, 28-Me, 30-Me |
| 30(A) | 37.98 d | 2.46 ddq 9.2, 2.7, 7.0 | H-29 | 37.99 d | 2.45 ddq 9.2, 2.7, 7.0 | H-29, 31(A), 28-Me, 30-Me |

TABLE 1. (Continued).

| Position | Compound | | | | | |
|---|---|---|---|---|---|---|
| | **5** | | | **1** | | |
| | $\delta_C$ mult. | $\delta_H$ mult., *J* in Hz | $^1H$-$^1H$ NOE | $\delta_C$ mult. | $\delta_H$ mult., *J* in Hz | $^1H$-$^1H$ NOE |
| 30(B)[a] | 38.35 d | 2.50 ddq 9.2, 2.7, 7.0 | H-29 | 38.18 d | 2.50 ddq 9.2, 2.7, 7.0 | H-29, 31(B), 28-Me, 30-Me |
| 30-Me | 19.62 q | 1.15 d 7.0 | | 19.41 q | 1.13 d 7.0 | H-30(A,B), 29-OMe |
| 31(A)[a] | 111.18 d | 5.12 dd 14.1, 9.2 | NMe(A) | 111.03 d | 5.10 dd 14.0, 9.2 | H-30(A), 29-OMe, NMe(A) |
| 31(B)[a] | 113.25 d | 5.16 dd 14.1, 9.2 | NMe(B) | 113.09 d | 5.16 dd 14.0, 9.2 | H-30(B), 29-OMe, NMe(B) |
| 32(A)[a] | 130.15 d | 6.79 d 14.1 | NCHO(A) | 130.10 d | 6.77 d 14.0 | NCHO(A) |
| 32(B)[a] | 125.44 d | 7.09 d 14.1 | | 125.31 d | 7.09 d 14.0 | |
| NMe(A)[a] | 27.22 q | 2.99 s | H-31(A) | 27.03 q | 2.98 s | H-31(A) |
| NMe(B)[a] | 33.03 q | 3.09 s | H-31(B), NCHO(A) | 33.00 q | 3.05 s | H-31(B), NCHO(B) |
| NCHO | 162.89 d | 8.36 s | H-32(A) | 163.71 d | 8.34 s | H-32(A) |
| NCHO | 161.60 d | 8.10 s | NMe(B) | 161.47 d | 8.09 s | NMe(B) |

[a]The $^1H$ and $^{13}C$ signals for the ene-*N*-methylformamide group are doubled due to restricted rotation of the *N*-formyl bond; (A) and (B) refer to the resulting major and minor conformers.

with the conformation suggested for the C-13 to C-20 segment in scytophycin C by X-ray analysis (5).

6-HYDROXYSCYTOPHYCIN B [**2**].—This compound was slightly more polar than tolytoxin. When the $^{1}H$- and $^{13}C$-nmr data for **2** and tolytoxin were compared (Table 1, Experimental), the lack of the 7-OMe signals was the only appreciable difference in the spectra of **2**. Comparison of the coupling constants between the various protons in the C-4 to C-9 segments in **1** and **2** suggested that H-6 and H-7 have the same anti relationship in the two compounds. The $^{1}H$ and $^{13}C$ chemical shifts and the $^{1}H$-$^{1}H$ nOe's for the rest of the molecule essentially matched those of scytophycin B and tolytoxin, suggesting that **2** had the same overall stereochemistry and conformation.

19-*O*-DEMETHYLSCYTOPHYCIN C [**3**].—The chemical shifts and *J* values for the C-1 to C-13 and the C-21 to NCHO segments of **3** were similar to those for scytophycin B. The C-13 to C-21 spin system showed chemical shifts and coupling constants which were consistent with a scytophycin-C-type structure. The H-14 signal showed a large coupling (10.0 Hz) with H-13 and a small coupling (1.5 Hz) with H-15; however, H-14′ showed a small coupling (2.0 Hz) with H-13 and a larger one (8.2 Hz) with H-15. In turn H-15 showed small coupling (1.7 Hz) to H-16. The H-16 signal was coupled also with the methyl signal at 0.83 ppm (6.7 Hz) and H-17 (8.0 Hz). Both C-15 and C-17 showed three-bond correlations (HMBC) with methoxyl protons (3.28 and 3.34 ppm, respectively), suggesting that the C-1 to C-17 segment of **3** was the same as that for scytophycin C. Further inspection indicated that H-18 showed large coupling to H-17 (10.2 Hz) and small coupling with H-19 and that H-18′ showed small coupling with H-17 (2.7 Hz) and medium-sized coupling with H-19 (6.0 Hz). Because H-19 was coupled (2.0 Hz) to an exchangeable proton at 3.35 ppm and also coupled to H-20 (<1 Hz), a hydroxyl had to be on C-19 instead of a methoxyl. H-20 was coupled to a methyl signal at 0.92 ppm (7.3 Hz) and to H-21 (10.4 Hz). Compound **3** was therefore 19-*O*-demethylscytophycin C.

6-HYDROXY-7-*O*-METHYLSCYTOPHYCIN E [**4**].—The nmr data ($^{13}C$ chemical shifts, $^{1}H$ chemical shifts and coupling constants, and $^{1}H$-$^{1}H$ nOe's) for the C-1 to C-13 segment of 6-hydroxy-7-*O*-methylscytophycin E and tolytoxin were found to be virtually identical, and this allowed us to assign the same gross structure and stereochemistry for the C-1 to C-13 unit in the two compounds. The same argument could be applied to the side chain (C-21 to N-CHO). The C-14 to C-20 segment, on the other hand, was found to be different in both structure and conformation from that of tolytoxin. The substituent on C-16 was found to be a hydroxymethyl group, the same one that is on C-16 in scytophycin E [**6**]. Compound **6** was isolated as a minor constituent along with **4**. The same arguments used below to assign the relative stereochemistry in the C-14 to C-20 segment of **4** could be applied to the same unit in **6**, as both compounds showed the same $^{1}H$ and $^{13}C$ chemical shifts and $^{1}H$-$^{1}H$ coupling constants. A 10.3 Hz coupling constant and nOe between H-13 and H-14 suggested that a small dihedral angle (at or near 0°) exists between these protons. Because H-15 showed a large coupling (8.9 Hz) with H-14′ and small couplings (<1 Hz) with H-14 and H-16, along with nOe's to H-13, H-16 and the methoxyl groups on C-15 and C-17, the resulting pattern of scalar and nonscalar couplings suggested that a large dihedral angle (around 180°) was present between H-14′ and H-15, and that dihedral angles of about 60–80° existed between H-14 and H-15 and between H-15 and H-16. The proposed stereochemistry was further supported by an nOe between H-14′ and CH′ on 16, suggesting that both atoms point to the β-face of the molecule. The large coupling (9.8 Hz) between H-16 and H-17 and the nOe between H-16 and the OMe on

C-17 indicated an anti relationship between H-16 and H-17. The medium-sized coupling constants (4.4–4.9 Hz) between H-17 and the protons on C-18 and between the latter protons and H-19 suggested a conformation in which H-17 and H-19 were each gauche to both H-18 and -18′. Finally H-20 showed small (<1.0 Hz) and large (9.9 Hz) couplings with H-19 and H-21, respectively, as well as significant nOe's with the methoxyls on C-17 and C-19. Moreover, the methyl protons on C-20 showed appreciable nOe's with H-21 and the OMe on C-19. These data suggested the presence of a skew conformation around the C-19 to C-20 bond, where a dihedral angle of about 90° existed between H-19 and H-20 and dihedral angles of about 30° existed between C-18 and H-20, between the methoxyl group on C-19 and the methyl group on C-20, and between H-19 and C-21. Furthermore the C-20 to C-21 bond appeared to have a staggered conformation where H-20 was anti to H-21, C-19 was anti to C-22, and the methyl group on C-20 was gauche to H-21. This analysis indicated that the relative stereochemistry of the C-14 to C-21 segment was the same as that for scytophycin C but that the conformation of C-17 to C-19 was different.

## EXPERIMENTAL

SPECTRAL ANALYSIS.—Nmr spectra were determined on a GN-OMEGA instrument operating at 500 MHz for $^1H$ and 125 MHz for $^{13}C$. $^1H$ chemical shifts are referenced in $Me_2CO$-$d_6$ to the residual $Me_2CO$-$d_5$ signal (2.04 ppm), and $^{13}C$ chemical shifts are referenced in $Me_2CO$-$d_6$ to the solvent signal (206.0 ppm). Homonuclear $^1H$ connectivities were determined by using the COSY experiment. Homonuclear $^1H$ nOe's were obtained by hypercomplex phase sensitive NOESY experiments using a 3 sec recycling delay and 500 msec mixing period. Heteronuclear $^1H$-$^{13}C$ connectivities were determined by HMQC and HMBC experiments (7,8). Ir spectra were measured in $CH_2Cl_2$. Uv and cd spectra were recorded in MeOH at 25°. Mass spectra were determined in either the ei or fab mode with a VG Analytical 70 SE instrument; high resolution mass measurements were obtained in the ei mode.

CULTURE CONDITIONS.—An aerial form of *S. mirabile* was isolated from an algal sample collected from a shingled roof of an abandoned home on the slopes of Mt. Tantalus, Oahu, Hawaii, and designated strain number BY-8-1. An epidaphic form of *S. burmanicum* was isolated from an algal sample collected at Moon Beach, Okinawa (strain DO-4-1). Epidaphic forms of *S. ocellatum* were isolated from algal samples collected at the University of Guam Marine Laboratory (strain DD-8-1), Columbia, Missouri (strain FF-65-1), and South Pasture Pond, Shawnee, Illinois (strain FF-66-3). Clonal cultures were prepared by repeated subculture on solidified media. Each alga was cultured in 20 liter glass bottles containing a modified inorganic medium, designated $A_3M_7$ as previously described for *Hapalosiphon fontinalis* (9). Prior to autoclaving, the pH of the medium was adjusted to 7.0 with NaOH. Cultures were illuminated continuously at an incident intensity of 300 $\mu einstein \cdot m^{-2} \cdot s^{-1}$ from banks of cool-white fluorescent tubes, aerated at a rate of 1 liter per min with a mixture of 0.5% $CO_2$ in air, and maintained at an incubation temperature of 24 ± 1°. Each alga was harvested by filtration, after 30–45 days for BY-8-1, 37–39 days for DD-8-1, 28–32 days for DO-4-1, 28–35 days for FF-65-1, and 30 days for FF-66-3. Yields of lyophilized cells averaged 0.125 g/liter of culture for BY-8-1, 0.059 g/liter for DD-8-1, 0.220 g/liter for DO-4-1, 0.167 g/liter for FF-65-1, and 0.374 g/liter for FF-66-3.

ISOLATION.—Freeze-dried algae (BY-8-1, 82 g for batch 1 and 40 g for batch 2; DO-4-1, 49 g; FF-66-3, 50 g; FF-65-1, 1 g) were extracted with 3 × 3-liter portions of EtOH-$H_2O$ (7:3) (24 h for each extraction). The total extract (BY-8-1, 38.4 g for batch 1 and 14.0 g for batch 2; DO-4-1, 6.2 g; FF-66-3, 9.4 g; FF-65-1, 0.164 g) was flash chromatographed on an RP-18 column (30 ml, YMC-GEL, ODS 120A); batch 1 of BY-8-1, however, was chromatographed in five portions. The chromatogram of each algal extract was developed with 100 ml of each of the following solvents: $H_2O$, $H_2O$-MeOH (1:1, 1:3, and 1:9), MeOH, MeCN, and EtOAc. Seven fractions (100 ml) were collected.

*BY-8-1.*—Fraction 3 from flash chromatography of the batch 1 extract on the RP-18 column was subjected to gel filtration on Sephadex LH-20 (150 ml dry gel) using $CH_2Cl_2$-MeOH (1:1). Tolytoxin emerged from the column in the 200–275 ml fraction and was further purified on a preparative C-18 hplc column (YMC AM-343-5 ODS, 120A, 20 × 300 mm) using MeOH-$H_2O$ (3:1) as the eluent (6 ml/min). The separation was monitored by uv at 254 nm. Tolytoxin [**1**] (42.3 mg, 0.05% of dry cells) had an Rt of 54 min.

Batch 2 was fractionated in a different way. Fractions 3 and 4 from the RP-18 column [$H_2O$-MeOH (1:3 and 1:9) 713 mg] were separated on a preparative C-18 hplc column (Alltech Econosphere RP-18,

10μ, 22 × 250 mm) using $H_2O$-MeOH (1:3) as the eluent (5 ml/min). The separation was monitored by uv at 254 nm. Fourteen fractions were collected. $^1$H-nmr analysis of four of the fractions indicated the presence of scytophycin-like compounds: Rt 37.5 min (20.4 mg), Rt 41.5 min (30.5 mg), Rt 48.0 min (94.2 mg), and Rt 61.0 min (13.1 mg). Further purification of these fractions on a preparative C-18 hplc column (YMC AM-343-5 ODS, 120A, 20 × 300 mm) using MeCN-MeOH-$H_2O$ (2:1:1) as the eluent (6 ml/min) afforded 6-hydroxyscytophycin B [**2**] (0.5 mg, 27.5 min), 6-hydroxy-7-*O*-methylscytophycin E [**3**] (3.6 mg, 32.0 min), tolytoxin [**1**] (30.5 mg, 38.0 min), and scytophycin B [**5**] (0.6 mg, 41.5 min), respectively.

*DO-4-1*.—Fraction 3 from the RP-18 column [$H_2O$-MeOH (1:3) 151.1 mg] was further fractionated by preparative reversed-phase hplc with $H_2O$-MeOH (1:3) as described above. Seven fractions were collected: Rt 30.5 min (2.7 mg), 34.5 (2.4), 37.5 (6.7), 41.5 (16.6), 48.0 (73.1), 61.5 (10.0), and 92.5 (3.5). These fractions were further purified by reversed-phase hplc with MeCN-MeOH-$H_2O$ (2:1:1) as described above to give 6-hydroxyscytophycin B [**2**] (2.9 mg) from fraction 3, 6-hydroxy-7-*O*-methylscytophycin E [**3**] (13.0 mg) from fraction 4, scytophycin E [**6**] 4.3 mg, Rt 35.0 min), and tolytoxin [**1**] (50.0 mg) from fraction five, and 19-*O*-demethylscytophycin B [**4**] (0.7 mg, Rt 39.8 min) and scytophycin B [**5**] (1.0 mg) from fraction 6.

*FF-66-3*.—Fraction 3 from the RP-18 column [$H_2O$-MeOH (1:3) 421.4 mg] was separated by preparative reversed-phase hplc as described above to give 6-hydroxy-7-*O*-methylscytophycin E [**3**] (18.0 mg), tolytoxin [**1**] (161 mg), and 19-*O*-demethylscytophycin B [**4**] (4.2 mg).

*FF-65-1*.—Fractions 3 and 4 from the RP-18 column [$H_2O$-MeOH (1:3 and 1:9) 30.5 mg] were separated by preparative reversed-phase hplc as described above to give tolytoxin [**1**] (3.8 mg). The hplc profile was identical with that of FF-66-3.

Freeze-dried DD-8-1 (790 mg) was extracted with EtOH-$H_2O$ (7:3) to give 135 mg of extract. A suspension of 92.3 mg of the extract in $H_2O$ was introduced onto a reversed-phase BondElut C-18 column, and the column was washed with 5-ml portions of $H_2O$, $H_2O$-MeOH (1:1), MeOH, and MeOH-$CH_2Cl_2$ (1:1). Analysis indicated that all of the cytotoxic activity was in the MeOH fraction (5.0 mg). Nmr and hplc analysis indicated that tolytoxin was the major component in the MeOH fraction.

TOLYTOXIN [**1**].—Amorphous solid: cd (EtOH) $[\theta]_{297}$ −400, $[\theta]_{268}$ +1700, $[\theta]_{226}$ −3000; uv (EtOH) 261 nm (ε 27,000); ir ($CH_2Cl_2$) 3450, 3130, 1692, 1660, 1240, 1115 cm$^{-1}$; fabms (glycerol) *m/z* $[M+K]^+$ 888.6, $[M+Na]^+$ 872.6, $[MH-H_2O]^+$ 832.6; eims *m/z* (rel. int.) $[M-MeOH]^+$ 817 (0.1), $[M-MeOH-H_2O]^+$ 799 (0.3), 93 (100); hreims *m/z* 817.4878 ($C_{45}H_{71}NO_{12}$, 9.8 mmu error), 799.4792 ($C_{45}H_{69}NO_{11}$, 7.9 mmu error).

6-HYDROXYSCYTOPHYCIN B [**2**].—White amorphous solid: cd (EtOH) $[\theta]_{297}$ −550, $[\theta]_{268}$ +1500, $[\theta]_{226}$ −2920; uv (EtOH) 261 nm (ε 23,800); ir ($CH_2Cl_2$) 3400, 3170, 1690, 1660, 1240, 1115 cm$^{-1}$; fabms (thioglycerol/TFA) *m/z* $[M+K]^+$ 858.5, $[M+Li]^+$ 842.4, $[MH-H_2O]^+$ 818.4; $^1$H nmr δ (multiplicity, *J* in Hz, assignment; *nOe's*): 5.87 (d, 15.9, H-2; *4-Me*), 7.64 (d, 15.9, H-3; *H-5* and *17-OMe*), 1.93 (br d, 1.0, *4-Me*; on *H-3*, 6), 5.93 (br d, 10.0, H-5; *H-3*, *H-7*, and *17-OMe*), 4.17 (dd, 10.0 and 8.9, H-6; *4-Me* and *H-8*), 3.77 (ddd, 8.9, 10.9, and 1.0, H-7; *H-5*, *H-8*, and *H-9*), 1.26 (ddd, −13.9, 10.9, and 1.5, H-8; *H-8'*), 1.61 (ddd, −13.9, 9.9, and 1.0, H-8'; *H-7*, *H-8*, and *H-13*), 4.57 (dm, 9.9, H-9; *H-7*, *H-8*, *H-10*, and *17-OMe*), 5.64 (dddd, 10.5, 2.7, 2.3, and 1.5, H-10; *H-8*, *H-9*, and *H-11*), 5.79 (ddt, 10.5, 5.3, and 2.2, H-11; *H-10*, *H-12*, and *H-12'*), 1.90 (m, H-12 and H-12'; *H-11* and *H-13*), 3.36 (m, H-13; *H-8'*, *H-12*, *H-12'*, *H-14*, *H-14'*, *H-15*, and *H-17*), 1.53 (dd, 5.5 and 5.0, H-14 and H-14'; *H-13*, *H-15*, and *H-16'*), 3.89 (t, 5.0, H-15; *H-14*, *H-14'*, and *H-19*), 3.35 (s, 15-OMe), 2.68 (d, 5.0, H of CHH' on C-16; *H-18*), 2.70 (d, 5.0, H' of CHH' on C-16; *H-14*), 3.76 (dd, 10.3 and 4.0, H-17; *H-13*, *H-18*, *H-19*, and *H-20*), 3.27 (s, 17-OMe; *H-5*, *H-9*, and *H-18'*), 1.46 (ddd, −13.9, 9.9, and 4.0, H-18; *H-16*, *H-17*, and *H-18'*), 1.94 (m, H-18'; *H-18* and *17-OMe*), 3.30 (ddd, 9.9, 4.0, and 1.0, H-19; *H-15*, *H-17*, and *H-21*), 3.17 (s, 19-OMe), 2.08 (ddq, 10.0, 7.0, and 1.0, H-20; *H-17*, *20-OMe*, and *17-OMe*), 0.85 (d, 7.0, 20-Me; *H-20*), 5.21 (dd, 10.0 and 1.0, H-21; *H-19*, *H-22*, *19-OMe*, *20-Me*, *23-OH*, and *H$_2$0*), 1.95 (ddq, 9.9, 6.9, and 1.0, H-22; *H-21*), 0.87 (d, 6.9, 22-Me; *H-24*), 3.03 (ddd, 9.9, 4.4, and 2.0, H-23; *H-22*, *22-Me*, and *24-Me*), 4.00 (d, 4.4, 23-OH; *H-21*), 1.69 (m, H-24; *H-26'*, *22-Me*, and *24-Me*), 0.97 (d, 6.4, 24-Me; *H-24* and *H-26*), 1.38 (m, H-25; *H-24*, *H-25*, and *H-26*), 1.74 (m, H-25'; *H-24*, *H-25*, and *H-26*), 2.55 and 2.57 (m, H-26 and H-26'; *H-25*, *H-25'*, *H-28*, *24-Me*, and *28-Me*), 2.75 (dq, 9.5 and 6.5, H-28; *H-26*, *H-26'*, and *28-Me*), 0.90 (d, 6.5, 28-Me; *H-26*, *H-28*, and *H-31A*), 3.27 (dd, 9.5 and 2.5, H-29; *H-30A* and *30-Me*), 3.29 (s, 29-OMe), 2.46 (ddq, 8.9, 2.5, and 7.0, H-30A; *H-29*, *28-Me*, and *30-Me*), 2.52 (m, H-30B), 1.13 (d, 7.0, 30-Me; *H-29* and *H-30A*), 5.10 (dd, 13.9 and 8.9, H-31A; *N-Me A*), 5.16 (dd, 14.4 and 8.9, H-31B; *N-Me B*), 6.77 (d, 13.9, H-32A; *N-CHO A*), 7.09 (d, 14.4, H-32B), 2.97 (br s, N-Me A; *H-31 A*), 3.09 (br s, N-Me B; *H-31 B* and *N-CHO B*), 8.34 (s, N-CHO A; *H-32 A*), 8.10 (s, N-CHO B; *Me on N B*); $^{13}$C nmr $\delta_C$ (multiplicity, position) 169.27 (s, C-1), 117.84 (d, C-2), 151.65 (d, C-3), 136.91 (s, C-4), 12.64 (q, 4-

Me), 141.36 (d, C-5), 72.21 (d, C-6), 72.67 (d, C-7), 37.45 (t, C-8), 70.64 (d, C-9), 131.36 (d, C-10), 125.27 (d, C-11), 31.98 (t, C-12), 66.98 (d, C-13), 36.45 (t, C-14), 78.32 (d, C-15), 57.55 (q, 15-OMe), 61.35 (s, C-16), 46.96 (t, $CH_2$ on C-16), 76.27 (d, C-17), 53.80 (q, 17-OMe), 28.21 (t, C-18), 77.56 (d, C-19), 57.74 (q, 19-OMe), 38.23 (d, C-20), 9.30 (q, 20-Me), 76.95 (d, C-21), 38.04 (d, C-22), 9.30 (q, 22-Me), 76.61 (d, C-23), 33.93 (d, C-24), 18.29 (q, 24-Me), 22.83 (t, C-25), 42.15 (t, C-26), 213.97 (s, C-27), 49.53 (d, C-28), 13.68 (q, 28-Me), 88.38 (d, C-29), 61.19 (q, 29-OMe), 38.42 (d, C-30A), 38.07 (d, C-30B), 19.65 (q, 30-Me), 111.21 (d, C-31A), 113.25 (d, C-31B), 130.25 (d, C-32A), 125.56 (d, C-32B), 27.24 (q, N-Me A), 33.04 (q, N-Me B), 162.90 (d, N-CHO A), 161.65 (d, N-CHO B).

19-*O*-DEMETHYLSCYTOPHYCIN C [**3**].—White amorphous solid: cd (EtOH) $[\theta]_{297}$ −380, $[\theta]_{268}$ +1500, $[\theta]_{226}$ −2600; uv (EtOH) 261 nm (ε 21,900); ir ($CH_2Cl_2$) 3400, 3170, 1690, 1660, 1240, 1115 $cm^{-1}$; fabms (thioglycerol/TFA) *m/z* $[M+K]^+$ 830.5, $[M+Na]^+$ 814.5, $[M+Li]^+$ 798.6, $[MH-H_2O]^+$ 774.5; $^1H$ nmr δ (multiplicity, *J* in Hz, assignment) 5.73 (d, 15.8, H-2), 7.47 (d, 15.8, H-3), 1.82 (br s, 4-Me), 6.07 (dd, 10.2 and 5.0, H-5), 2.48 (ddd, −16.2, 10.2, and 10.0, H-6), 2.52 (ddd, −16.2, 5.0, and 3.1, H-6′), 4.04 (ddt, 3.1, 1.2, and 10.0, H-7), 1.20 (ddd, −14.7, 10.0, and 1.8, H-8), 1.74 (ddd, −14.7, 9.8, and 1.2, H-8′), 4.50 (dm, 9.8, H-9), 5.65 (ddt, 10.5, 2.9, and 1.7, H-10), 5.74 (ddt, 10.5, 2.1, and 4.0, H-11), 1.85 (m, H-12 and H-12′), 3.29 (m, H-13), 1.55 (ddd, −14.7, 10.0, and 1.5, H-14), 1.66 (ddd, −14.7, 8.2, and 2.0, H-14′), 3.60 (dt, 8.2 and 1.7, H-15), 3.28 (s, 15-OMe), 1.85 (ddq, 8.0, 1.7, and 6.7, H-16), 0.83 (d, 6.7, 16-Me), 3.36 (ddd, 10.2, 8.0, and 2.7, H-17), 3.34 (s, 17-Ome), 1.75 (ddd, −14.2, 10.2, and 1.5, H-18), 1.85 (ddd, −14.2, 6.0, and 2.7, H-18′), 4.19 (br ddd, 6.0, 2.0, and 1.5, H-19), 3.35 (br d, 2.0, 19-OH), 1.90 (ddq, 10.4, 1.0, and 7.3, H-20), 0.92 (d, 7.3, 20-Me), 5.12 (br d, 10.4, H-21), 1.95 (ddq, 9.9, 1.0, and 6.9, H-22), 0.84 (d, 6.9, 22-Me), 3.03 (ddd, 9.9, 4.4, and 2.0, H-23), 4.35 (d, 4.4, 23-OH), 1.69 (m, H-24), 0.97 (d, 6.4, 24-Me), 1.38 (m, H-25), 1.76 (m, H-25′), 2.50 (m, H-26), 2.55 (m, H-26′), 2.75 (dq, 9.5 and 6.5, H-28), 0.92 (d, 6.5, 28-Me), 3.27 (dd, 9.5 and 2.5, H-29), 3.30 (s, 29-OMe), 2.46 (ddq, 8.9, 2.5, and 7.0, H-30A), 2.25 (m, H-30B), 1.13 (d, 7.0, 30-Me), 5.11 (dd, 13.9 and 8.9, H-31A), 5.18 (dd, 14.4 and 8.9, H-31B), 6.78 (d, 13.9, H-32A), 7.09 (d, 14.4, H-32B), 2.98 (br s, N-Me A), 3.10 (br s, N-Me B), 8.35 (s, N-CHO A), 8.09 (s, N-CHO B); $^{13}C$ nmr $\delta_C$ (multiplicity, position) 169.89 (s, C-1); 116.54 (d, C-2), 150.83 (d, C-3), 135.01 (s, C-4), 12.31 (q, 4-Me), 139.37 (d, C-5), 39.46 (t, C-6), 68.17 (d, C-7), 40.63 (t, C-8), 70.33 (d, C-9), 131.67 (d, C-10), 125.50 (d, C-11), 32.43 (t, C-12), 65.03 (d, C-13), 39.25 (t, C-14), 77.92 (d, C-15), 56.58 (q, 15-OMe), 40.28 (d, C-16), 9.27 (q, 16-Me), 82.50 (d, C-17), 56.08 (q, 17-OMe), 35.78 (t, C-18), 65.88 (d, C-19), 43.14 (d, C-20), 9.41 (q, 20-Me), 76.97 (d, C-21), 38.07 (d, C-22), 9.32 (q, 22-Me), 76.49 (d, C-23), 33.97 (d, C-24), 18.39 (q, 24-Me), 22.78 (t, C-25), 42.18 (t, C-26), 214.07 (s, C-27), 49.54 (d, C-28), 13.67 (q, 28-Me), 88.34 (d, C-29), 61.17 (q, 29-OMe), 38.42 (d, C-30A), 38.23 (d, C-30B), 19.67 (q, 30-Me), 111.14 (d, C-31A), 113.21 (d, C-31B), 130.24 (d, C-32A), 125.52 (d, C-32B), 27.22 (q, N-Me A), 33.04 (q, N-Me B), 162.88 (d, N-CHO A), 161.64 (d, N-CHO B).

6-HYDROXY-7-*O*-METHYLSCYTOPHYCIN E [**4**].—White amorphous solid: cd (EtOH) $[\theta]_{297}$ −350, $[\theta]_{268}$ +2400, $[\theta]_{226}$ −3200; uv (EtOH) 261 nm (ε 27,900); ir ($CH_2Cl_2$) 3400, 3180, 1690, 1665, 1240, 1125 $cm^{-1}$; fabms (glycerol) *m/z* $[M+K]^+$ 890.6, $[M+Na]^+$ 874.6, $[MH-H_2O]^+$ 834.5 $[MH-2H_2O]^+$ 816.6; $^1H$ nmr δ (multiplicity, *J* in Hz, assignment; *nOe's*) 5.83 (d, 15.8, H-2; *4-Me* and *17-OMe*), 7.57 (d, 15.8, H-3; *H-5, H-19*, and *17-OMe*), 1.86 (br d, 1.0, 4-Me; *H-2, H-6*, and *17-OMe*), 6.02 (br d, 8.9, H-5; *H-3, H-6*, and *H-7*), 4.39 (t, 8.9, H-6; *H-5, 4-Me*, and *7-OMe*), 4.16 (brs, OH on C-6), 3.42 (ddd, 11.0, 8.9, and 2.0, H-7; *H-5, H-8′*, and *H-9*), 3.57 (s, 7-OMe; *H-6* and *4-Me*), 1.30 (ddd, −13.8, 11.0, and 3.0, H-8; *H-8′* and *H-9*), 1.57 (ddd, −13.8, 9.5, and 2.0, H-8′; *H-7, H-8*, and *H-13*), 4.40 (dm, 9.5, H-9; *H-7* and *H-8*), 5.66 (ddt, 10.3, 2.8, and 1.7, H-10; *H-9* and *H-11*), 5.76 (dddd, 10.3, 5.4, 2.9, and 2.5, H-11; *H-10*), 1.87 (m, H-12; *CH′ on 16* and *H-13*), 1.91 (m, H-12′), 3.26 (br ddd, 10.3, 8.9, and 3.4, H-13; *H-8′* and *H-12*), 1.75 (br dd, −13.8 and 10.3, H-14; *15-OMe*), 1.88 (m, H-14′), 3.87 (br d, 8.9, H-15; *H-13, H-16, 15-OMe*, and *17-OMe*), 3.36 (s, 15-OMe; *H-14* and *H-15*), 1.51 (br dd, 9.8 and 2.4, H-16; *H-15, 17-OMe*, and *19-OMe*), 3.70 (br d, 11.7, CH on C-16; *H-12, H-16*, and *CH′ on 16*), 3.77 (dd, 11.7 and 2.4, CH′ on C-16; *H-16* and *CH on 16*), 3.67 (ddd, 9.8, 4.9, and 4.4, H-17; *H-18* and *H-18′*), 3.39 (s, 17-OMe; *H-3, H-15, H-16*, and *H-20*), 1.98 (m, H-18 and H-18′; *H-17*), 3.72 (br t, 4.9, H-19; *H-3, H-21, 17-OMe*, and *19-OMe*), 3.14 (s, 19-OMe; *H-16, H-19, H-20, H-21, 20-Me*), 2.04 (ddq, 9.9, 1.0, and 6.9, H-20; *17-OMe, 19-OMe*, and *20-Me*), 0.94 (d, 6.9, 20-Me; *H-20, H-21*, and *19-OMe*), 5.15 (dd, 9.9 and 0.9, H-21; *H-19, H-22, H-23, 19-OMe, 23-OH, 20-Me*, and *$H_2O$*), 1.97 (ddq, 9.9, 0.9, and 6.9, H-22; *H-21* and *22-Me*), 0.84 (d, 6.9, 22-Me; *H-22* and *H-23*), 3.07 (ddd, 9.9, 4.0, and 1.9, H-23; *H-21* and *H-25′*), 4.09 (d, 4.0, 23-OH; *H-21, 22-Me*, and *24-Me*), 1.68 (dddq, 9.9, 4.0, 1.9, and 6.9, H-24), 0.97 (d, 6.9, 24-Me; *H-23* and *H-26*), 1.37 (m, H-25; *H-25′* and *24-Me*), 1.75 (m, H-25′; *H-23, H-25, H-26, 22-Me*, and *24-Me*), 2.50 and 2.55 (m, H-26 and H-26′; *H-25, H-28*, and *24-Me*), 2.76 (dq, 9.4 and 6.9, H-28; *H-26′, 29-OMe*, and *28-Me*), 0.90

(d, 6.9, 28-Me; *H-28, H-29, H-30A*, and *H-30B*), 3.28 (dd, 9.4, and 2.9, H-29; *H-28, H-30A, H-30B, 28-Me*, and *30-Me*), 3.30 (s, 29-OMe; *H-29*), 2.46 (dq, 2.9 and 7.4, H-30A; *H-29, 28-Me*, and *30-Me*), 2.47 (dq, 2.9 and 7.4, H-30B; *28-Me* and *30-Me*), 1.13 (d, 7.4, 30-Me; *H-29, H-30A*, and *H-30B*), 5.10 (dd, 14.3 and 9.3, H-31A; *N-Me A*), 5.15 (dd, 14.7 and 9.3, H-31B), 6.76 (d, 14.3, H-32A; *N-CHOA*), 7.09 (d, 14.7, H-32B), 2.97 (s, N-Me A; *H-31 A*), 3.09 (s, N-Me B; *N-CHO B*), 8.35 (s, N-CHO A; *H-32 A*), 8.10 (s, N-CHO B; *Me on N B*); $^{13}$C nmr $\delta_C$ (multiplicity, position) 169.06 (s, C-1), 117.77 (d, C-2), 151.37 (d, C-3), 135.05 (s, C-4), 12.72 (q, 4-Me), 142.70 (d, C-5), 72.38 (d, C-6), 82.62 (d, C-7), 60.51 (q, 7-OMe), 36.33 (t, C-8), 70.41 (d, C-9), 130.98 (d, C-10), 124.86 (d, C-11), 32.27 (t, C-12), 65.45 (d, C-13), 38.91 (t, C-14), 79.51 (d, C-15), 57.01 (q, 15-OMe), 47.61 (d, C-16), 59.67 (t, $CH_2OH$ on C-16), 78.28 (d, C-17), 55.76 (q, 17-OMe), 33.93 (t, C-18), 76.80 (d, C-19), 58.77 (q, 19-OMe), 42.60 (d, C-20), 9.76 (q, 20-Me), 77.19 (d, C-21), 38.56 (d, C-22), 9.22 (q, 22-Me), 76.51 (d, C-23), 34.05 (d, C-24), 18.28 (q, 24-Me), 22.76 (t, C-25), 42.11 (t, C-26), 213.97 (s, C-27), 49.51 (d, C-28), 13.65 (q, 28-Me), 88.33 (d, C-29), 61.12 (q, 29-OMe), 38.20 (d, C-30A), 38.42 (d, C-30B), 19.60 (q, 30-Me), 111.17 (d, C-31A), 113.22 (d, C-31B), 130.19 (d, C-32A), 125.51 (d, C-32B), 27.21 (q, N-Me A), 33.00 (q, N-Me B), 162.82 (d, N-CHO A), 161.57 (d, N-CHO B).

DETERMINATION OF BIOLOGICAL ACTIVITY.—Antitumor activity was determined in vitro using the KB (ATCC CCL 17; human epidermoid carcinoma of the nasopharynx) and LoVo (ATCC CCL 229; human colon adenocarcinoma) cell lines (10).[1] The algal extracts [EtOH-$H_2O$ (7:3)] showed MICs of 0.05–1 μg/ml and 0.5–10 μg/ml against KB and LoVo, respectively. The pure compounds **1–4** and **6** showed MICs of 1–5 ng/ml and 10–50 ng/ml against KB and LoVo, respectively. Antifungal activity was determined using an agar diffusion assay (11) and the test organisms *Aspergillus oryzae* (Ao), *Candida albicans* (Ca), *Penicillium notatum* (Pn), *Saccharomyces cerevisiae* (Sc), and *Trichophyton mentagrophytes* (Tm) (strains from the teaching collections held by the Botany and Microbiology Departments, University of Hawaii). The algal extracts generally showed 20–30 mm zones of inhibition against Ao, Ca, Pn, and Sc at 250 μg/disk, but only trace activity against Tm at this concentration. Compounds **1–4** exhibited antifungal activity against Ao, Ca, Pn, and Sc, but MICs were not determined; it is expected that the activities will be comparable with those determined for scytophycins A and B (5,6).

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