Scytophycins, Cytotoxic and Antimycotic Agents from the Cyanophyte *Scytonema pseudohofmanni*

Masami Ishibashi, Richard E. Moore,* and Gregory M. L. Patterson

Department of Chemistry, University of Hawaii, Honolulu, Hawaii 96822

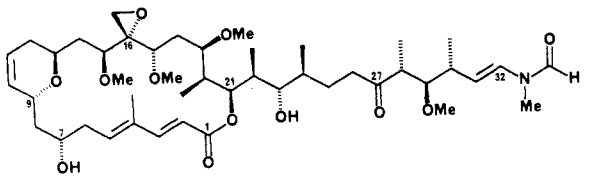
Changfu Xu and Jon Clardy*

Department of Chemistry, Baker Laboratory, Cornell University, Ithaca, New York 14650

Received June 23, 1986

The lipophilic extract of the cultured terrestrial blue-green alga *Scytonema pseudohofmanni* Bharadwaja contains five novel macrolides, scytophycins A (1), B (2), C (3), D (4), and E (5), which exhibit cytotoxicity and antifungal activity. The structures of 1–5 have been determined mainly from spectroscopic data. The relative stereochemistries of the scytophycins are based on an X-ray crystallographic analysis of a transformation product of scytophycin C (8) and comparison of the ^1H and ^{13}C NMR spectra of 1–5. The absolute stereochemistries of the scytophycins have been deduced from a circular dichroism study of the dibenzoate ester 16, obtained in five steps from scytophycin B, and comparison of the CD curves of 1–5. These novel secondary metabolites appear to be biogenetically related to the marine natural products swinholide A, ulapualides A and B, and kabiramide C.

Scytophycins are potent cytotoxins and broad-spectrum fungicides associated with the terrestrial blue-green alga *Scytonema pseudohofmanni* Bharadwaja.[1] In a previous report, we described the gross structures of scytophycins A (1) and B (2).[2] This paper deals with the determination of the relative and absolute stereochemistry of the scytophycins and the isolation and identification of three related macrolides in this alga, viz., scytophycins C (3), D (4), and E (5).



2

1 Same as 2 except at C27:

3 Same as 2 except at C16:

4 Same as 2 except at C16:

5 Same as 2 except at C16:

Isolation and Gross Structure Determination. The scytophycins were isolated from cultured *S. pseudohofmanni* by extracting the freeze-dried alga with 1:1 dichloromethane/2-propanol and subjecting the extract to solvent partitioning, gel filtration, and reverse-phase high-performance liquid chromatography.

The molecular weights and compositions of all of the scytophycins were determined by fast-atom-bombardment mass spectroscopy, and the elemental compositions were confirmed by detailed analyses of the ^1H and ^{13}C NMR spectra (Tables I and II). All of the compounds exhibited ultraviolet spectra typical of a dienoate ester.

Two-dimensional NMR techniques, in particular relayed coherence transfer (RCT) spectroscopy[3-5] coupled with conventional homonuclear correlation spectroscopy (COSY) and heteronuclear correlation spectroscopy (HETCOR or CSCM), and difference decoupling and difference NOE experiments were very useful for determining partial structures in the scytophycins. The connectivities of C-9 to C-13 via an ether oxygen, C-1 to C-21 via an ester oxygen, and C-15 and C-17 to methoxyl groups were obtained by selective polarization transfer spectroscopy (selective INEPT).[6,7]

Comparison of the ^1H NMR spectra (Table I) of the new compounds 3, 4, and 5 with those of 1 and 2 indicated that the same C-1–C-15, C-17–C-26, and C-28–C-32 segments were present. Scytophycins C, D, and E possessed a ketone group at C-27 since each compound showed a ^{13}C signal around 214 ppm and the chemical shifts for the protons on C-26 and C-28 were similar to those observed for scytophycin B. The structural differences were at C-16. The COSY16 spectrum and proton–proton decoupling studies indicated that a hydrogen and a methyl group were on C-16 in scytophycin C (3) and further that a hydrogen and a nonequivalent methylene group bearing a hydroxy group were on C-16 in scytophycin E (5). Inspection of the ^1H NMR spectrum of scytophycin D (4) showed that C-16 was a quaternary carbon since the H-15 and H-17 signals were doublets of doublets. Methyl and hydroxy groups had to be on C-16 in 4 to account for the additional methyl signal (a singlet at 1.00 ppm) and the molecular weight indicated by mass spectrometry.

Stereochemistry. Since each scytophycin existed as a mixture of two slowly interconverting conformational isomers, the preparation of a crystalline derivative such as the 7-(*p*-bromobenzoate) ester for X-ray analysis was complicated. Both the ^1H and ^{13}C NMR spectra showed that restricted rotation about the *N*-methylformamide terminus was responsible for this conformational isomer-

(1) Bharadwaja, Y. *Rev. Algol. (Paris)* **1934**, *7*, 149.
(2) Moore, R. E.; Patterson, G. M. L.; Mynderse, J. S.; Barchi, J., Jr.; Norton, T. R.; Furusawa, E.; Furusawa, S. *Pure Appl. Chem.* **1986**, *58*, 263.
(3) Eich, G.; Bodenhausen, G.; Ernst, R. R. *J. Am. Chem. Soc.* **1982**, *104*, 3732.
(4) Wagner, G. *J. Magn. Reson.* **1983**, *55*, 151.
(5) Bax, A.; Drobny, G. *J. Magn. Reson.* **1985**, *61*, 306.
(6) Bax, A. *J. Magn. Reson.* **1984**, *57*, 314.
(7) Moore, R. E.; Niemczura, W. P. In *Pharmaceuticals and the Sea*; Jefford, C. W., Ed.; Technomic: Lancaster, PA, in press.

0022-3263/86/1951-5300$01.50/0

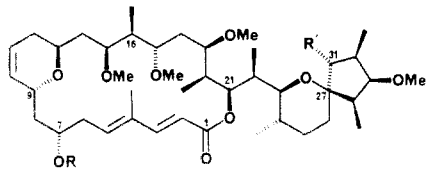
Table I. ^{1}H NMR Chemical Shift Data for Scytophycins A (1), B (2), C (3), D (4), and E (5) in Acetone-d_6

position[a]	1	2[b,c]	3	4	5
2	5.78	5.780 d	5.78	5.76	5.75
3	7.65	7.657 d	7.61	7.47	7.64
Me on 4	1.80	1.853 br s	1.82	1.84	1.82
5	6.01	6.017 br dd	6.03	5.87	6.01
6	2.43	2.483 ddd	2.46	2.50	2.52
6′	2.57	2.567 br ddd			
7	4.06	4.063 br td	4.02	4.01	4.03
8	1.2	1.262 ddd	1.28	1.22	1.34
8′	1.71	1.768 ddd	1.77	1.73	1.78
9	4.57	4.582 br d	4.53	4.47	4.57
10	5.66	5.655 ddt	5.67	5.63	5.66
11	5.81	5.807 dtd	5.77	5.76	5.78
12	1.91	1.910 m	1.89	2.08	1.95
13	3.38	3.39 m	3.34	3.53	3.28
14	1.46	1.455	1.68	1.85	1.86
14′	1.54	1.545 ddd	1.63	1.54	1.79
15	3.93	3.942 dd	3.62	3.22	3.80
MeO on 15	3.37	3.372 s	3.30	3.32	3.36
16			1.68		1.67
Me on 16			0.80 d	1.00 s	
CH_2 on 16	2.63	2.629 d			3.80 br d
	2.72	2.719 d			3.65 m
17	3.84	3.875 dd	3.26	3.46	3.47
MeO on 17	3.25	3.243 s	3.23	3.24	3.18
18	1.43	1.456 ddd	1.73	1.90	NA[d]
18′	1.94	1.955 ddd	1.83	1.94	1.97[g]
19	3.30	3.308 ddd	3.47	3.39[g]	3.74[g]
MeO on 19	3.19	3.198 s	3.17	3.18	3.19
20	2.09	2.089 m	2.04	2.16	2.10
Me on 20	0.84	0.859 d	0.89	0.87	0.90
21	5.20	5.217 br d	5.16	5.27	5.18
22	1.93	1.988 m	2.00	1.96	2.03
Me on 22	0.84	0.875 d	0.84	0.84	0.85
23	3.02	3.030 dd	3.00	3.01	3.13
OH on 23	NA	NA	NA	3.88	4.12
24	1.68	1.690 m	1.67	1.66	1.74
Me on 24	1.00	0.986 d	0.97	0.95	0.97
25	NA	1.37 m	1.38	1.42	1.35
25′	NA	1.75 m	1.76	1.74	1.71
26	2.29	2.54 m	2.55	2.53	2.54
26′	1.55				
27	3.39				
28	1.58	2.776 dq	2.77	2.76	2.76
28[e]		2.740 dq			
Me on 28	0.84	0.919 d	0.90	0.90	0.90
29	3.12	3.284 dd	3.27	3.30	3.28
MeO on 29	3.12	3.312 s	3.29	3.32	3.30
30	2.51	2.46 m	2.44	2.41	2.47
Me on 30	1.13	1.149 d	1.13	1.13	1.13
31	5.11	5.118 dd	5.12	5.10	5.10
31[e]	5.19	5.180 dd	5.17	5.16	5.17
32	6.74	6.786 d	6.77	6.77	6.77
32[e]	7.09	7.107 d	7.09	7.09	7.09
Me on N	2.96	2.990 s	2.97	2.97	2.97
Me on N[e]	3.07	3.108 s	3.09	3.09	3.09
NCHO	8.35	8.360 s	8.34	8.34	8.34
NCHO[e,f]	8.09	8.113 s	8.09	8.10	8.10

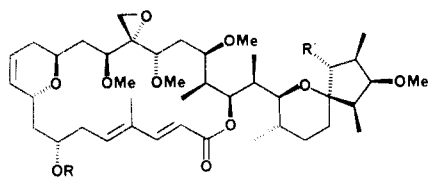
[a] 300 MHz; residual acetone-d_5 as internal reference = 2.04 ppm. [b] Concentration = 0.1 mM. [c] Coupling constants for **2** in hertz: 2,3 = 15.8; Me(4), 5 = 1.5; Me(4), 6′ = 1.3; 5,6 = 9.3; 5,6′ = 4.3; 6,6′ = −16.2; 6,7 = 10.2; 6′,7 = 3.1; 7,8 = 10.2; 7,8′ = 1.2; 8,8′ = −14.7; 8,9 = 1.8; 8′,9 = 9.8; 9,10 = 2.9; 9,11 = 1.6; 9,12 = 1.8; 10,11 = 10.4; 10,12 = 1.8; 11,12 = 4; 13,14 = 2.0; 13,14′ = 8.4; 14,14′ = −14.5; 14,15 = 7.4; 14′,15 = 3.1; gem CH_2(16) = 4.5; 17,18 = 4.0; 17,18′ = 11.4; 18,18′ = −13.6; 18,19 = 9.7; 18′,19 = 4.0; 19,20 = 1.0; 20,Me(20) = 7.0; 20,21 = 10.3; 21,22 = 1; 22,Me(22) = 6.8; 22,23 = 9.7; 23,24 = 2.0; 24,Me(24) = 6.7; 28,Me(28) = 7.0; 28,29 = 9.5; 29,30 = 2.2; 30,Me(30) = 7.0; 30,31 = 9.2 (9.0); 31,32 = 14.1 (14.8). Most of these coupling constants are essentially the same for the other scytophycins, except for ones associated with protons in the vicinity of C16 and C27. [d] Not assigned. [e] Signals for minor conformer. [f] Doublet of 1:1:1 triplets, J = 0.6 Hz, due to coupling to nitrogen. [g] Tentative assignment.

ism. Since the *N*-methylformamide group was in an acid labile enamide system, however, studies were carried out on the major scytophycins to establish conditions for converting the enamide functionality to an aldehyde group without affecting other acid-sensitive groups in the molecule, such as the epoxy group.

The reaction of the scytophycins with acetic acid in ethanol was found to proceed cleanly and to give a useful product. Treatment of scytophycin C (**3**) with acetic acid and ethanol (1:1) at room temperature for 1 day, for example, afforded an aldehyde (**6**). Inspection of the ^{1}H NMR spectrum showed that the signals for the *N*-methyleneformamide group had disappeared but, more importantly, that the aldehydic product was no longer a mixture of conformational isomers. The aldehyde **6** was reduced with 1 equiv of sodium borohydride to a primary alcohol (**7**), but even when a slight excess of $NaBH_4$ was used, no further reduction appeared to take place. When



6 R = H; R' = CHO
7 R = H; R' = CH_2OH
8 R = CO-(p-Br-C$_6$H$_4$); R' = CH_2OCO-(p-Br-C$_6$H$_4$)



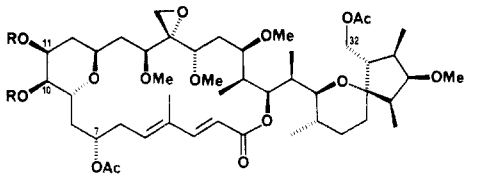
9 R = H; R' = CHO
10 R = H; R' = CH_2OH
11 R = CO-(p-Br-C$_6$H$_4$); R' = CH_2OCO-(p-Br-C$_6$H$_4$)
14 R = Ac; R' = CH_2OAc

alcohol **7** in dichloromethane was treated with *p*-bromobenzoyl chloride in the presence of triethylamine and a catalytic amount of 4-(dimethylamino)pyridine, a crystalline di-*p*-bromobenzoate (**8**), mp 124–125 °C, was formed in an overall 30% yield. Via the same procedure, scytophycin B (**2**) afforded an aldehyde (**9**), a primary alcohol (**10**), and a crystalline di-*p*-bromobenzoate (**11**), mp 105–106 °C, in an overall 36% yield and scytophycin E (**5**) led to aldehyde **12** and alcohol **13**, but scytophycin A (**1**) was recovered essentially unchanged.

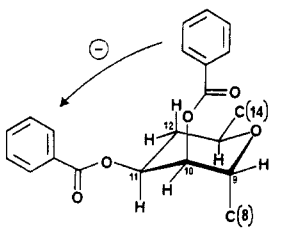
Compounds **6**, **9**, and **12** were not the aldehydic products resulting from simple hydrolysis of the enamide groups in scytophyscins C, B, and E, respectively. The ^{1}H NMR spectra (Table III) showed aldehydic signals that were doublets (J = 4.9 Hz) instead of triplets, and the fast-atom-bombardment mass spectra indicated that the molecular weights of **6**, **9**, and **12** were 18 amu lower than the anticipated products. X-ray analysis of di-*p*-bromobenzoate **8** (see below) showed that a 1-oxaspiro[5.4]decane system had been formed during the hydrolysis. Presumably condensation of the enamide group and the ketone carbonyl had occurred first, followed by hydrolytic cleavage of resulting *N*-methyl-*N*-formylimmonium ion to the aldehyde, loss of oxygen at C-27 (as a hydroxyl group) to form an intermediate carbonium ion, and ether ring closure from the hydroxyl group on C-23 to give the product. Comparison of the ^{1}H NMR spectra of **6**, **9**, and **12** strongly suggested that **9** and **12** contained a similar 1-oxaspiro-[5.4]decane system. In the three spectra, the aldehydic proton signal (9.81 ppm) was coupled (4.9 Hz) to a 1-H doublet of doublets at 2.63 ppm (H on C-31), which in turn was coupled (11.0 Hz) to a 1-H mutliplet at 2.30 ppm (H on C-30). The H-30 signal was coupled (6.8 Hz) to a 3-H doublet at 0.92 ppm (Me on C-30) and coupled (4.4 Hz) to a 1-H doublet of doublets at 3.37 ppm (H on C-29). Finally the H-29 signal was coupled (4.4 Hz) to a 1-H multiplet at 1.73 ppm (H on C-28), which in turn was coupled (7.2 Hz) to a 3-H doublet at 0.89 ppm (Me on C-28) (Table III). Comparison of the ^{1}H NMR spectra of **2** and **9** revealed that nothing had happened to the epoxide functionality during the hydrolysis. Doublets at 2.58 and 2.68 ppm (J = 4.5 Hz) could still be seen for the epoxide methylene protons. The remaining portions of the ^{1}H NMR spectra of **6**, **9**, and **12** were very similar to those of the ^{1}H NMR spectra of scytophycins C, B, and E, respectively. Some chemical shifts were different, but the coupling constants were essentially the same. Even though some unexpected chemistry had occurred during hydrolysis, we concluded from the similarity of the spectra that none of the asymmetric centers in scytophycins B, C, and E had isomerized during the reaction. This meant that the relative stereochemistry in scytophycins B, C, and E was identical with that found in di-*p*-bromobenzoate **8** by X-ray crystallography.

The stereochemistry of C-16 in scytophycin B could be deduced from the following difference NOE experiment. When the proton on C-17 was irradiated, positive NOEs were seen in the signals for the methoxyl groups on C-15 and C-17 and one of the protons (2.719 ppm) in the methylene group attached to C-16. Assuming that the solution-state conformation of the lactone ring system was identical with the solid-state conformation depicted by X-ray crystallography, the NOEs indicated that the methylene group on C-16 in **2** had to be in the same relative position as the methyl group on C-16 in **3**.

To determine the absolute stereochemistry of scytophycin B, a vicinal dibenzoate system was introduced into the dihydropyran ring. Alcohol **10** was first acetylated with acetic anhydride and pyridine to give diacetate **14**. Treatment of **14** with 1 equiv of osmium tetraoxide then gave a 10% yield of a single 10,11-diol **15** along with 20%



15 R = H
16 R = COC_6H_5



16a

yields of two 4,5-diols. On the basis of work in the literature,[8] we expected osmylation to occur exclusively on the opposite side of the dihydropyran ring from the pseudoaxially oriented C-8 methylene. As predicted, the desired dibenzoate derivative **16** was obtained by treating diol **15** in dichloromethane with benzoyl chloride and triethylamine in the presence of a catalytic amount of 4-(dimethylamino)pyridine. ^{1}H NMR analysis of **16** indicated that the relative stereochemistry in the tetrahydropyran ring was as shown in **16a**. Small couplings were observed for $J_{9,10}$ (4.5 Hz), $J_{10,11}$ (4.5 Hz), and $J_{11,12eq}$ (0 Hz), and a large coupling was seen for $J_{11,12ax}$ (9 Hz).

The CD spectrum of dibenzoate **16** showed a relatively large negative first Cotton effect at 236 nm, [θ] −62 800, implying that the absolute configuration of the tetrahydropyran ring was 9*R*,10*R*,11*S*,13*R* as shown in **16a**. The absolute stereochemistry of scytophycin B (**2**) was therefore 7*S*,9*R*,13*S*,15*S*,16*R*,17*S*,19*R*,20*S*,21*R*,22*S*,23*S*,24*S*,28*R*,-29*R*,30*R*.

(8) Cha, J. K.; Christ, W. J.; Kishi, Y. *Tetrahedron* **1984**, *40*, 2247.

Table II. ^{13}C NMR Chemical Shift Data for Scytophycins A (1), B (2), C (3), D (4), and E (5) in Acetone-d_6

position[a]	1	2[b]	3	4	5
1	169.57	169.63	169.38	168.83	169.61
2	115.63	115.58	115.71	116.38	115.68
3	151.81	151.85	151.33	151.12	151.70
4	134.77	134.76	134.69	135.03	134.76
Me on 4	12.30	12.29	12.09	12.27	12.24
5	139.90	139.95	139.73	139.55	139.93
6	41.54	42.08	41.92	42.10	42.11
7	68.91	68.86	68.54	68.02	68.82
8	41.06	41.51	41.19	40.48	41.61
9	70.79	70.91	70.76	70.39	70.98
10	131.52	131.51	131.48	131.32	131.67
11	125.02	124.90	124.48	124.85	124.76
12	31.85	31.84	32.19	31.18	31.48
13	66.86	66.87	65.75	65.94	66.04
14	34.76	35.63	32.61	32.50	32.21
15	78.42	78.44	79.79	78.46	79.94
MeO on 15	57.46	57.46	56.46	57.19	57.12
16	61.69	61.12	40.93	82.73	46.95
CH_2 on 16	45.66	45.56			59.58
Me on 16			9.25	18.08	
17	75.27	75.20	76.32	76.25	76.70
MeO on 17	52.89	52.83	53.55	58.25	51.87
18	27.49	27.45	27.31	27.92	27.18
19	77.47	77.49	77.93	77.35	77.44
MeO on 19	57.78	57.78	58.03	58.25	58.12
20	38.14	38.15	40.29	40.36	40.02
Me on 20	9.36	9.23	9.05	9.23	9.37
21	76.51	76.51	76.56	76.52	76.52
22	37.90	38.15	38.45	38.19	38.80
Me on 22	9.27	9.23	8.79	9.23	9.27
23	76.51	76.51	77.18	76.44	76.30
24	34.29	33.78	33.69	33.87	33.85
Me on 24	18.63	18.27	18.13	18.25	18.31
25	25.74	22.71	22.55	22.74	22.71
26	39.39	39.36	39.17	39.48	39.43
27	71.00	214.07	213.84	213.98	213.93
28	35.72	49.48	49.33	49.50	49.50
Me on 28	9.84	13.65	13.47	13.65	13.64
29	88.66	88.26	88.11	88.28	88.27
MeO on 29	61.13	61.12	60.96	61.13	61.14
30	38.53	37.98	38.03	36.83	38.21
30[c]	38.71	38.35	38.18	38.36	38.36
Me on 30	20.06	19.62	19.45	19.62	19.63
31	112.27	111.18	110.97	111.10	111.09
31[c]	114.20	113.25	113.05	113.18	113.16
32	129.65	130.15	129.98	130.17	130.17
32[c]	124.76	125.44	125.46	125.46	125.45
Me on N	27.22	27.22	27.03	27.19	27.18
Me on N[c]	33.02	33.03	32.85	33.00	33.01
CHO	162.81	162.89	162.71	162.83	162.84
CHO[c]	161.52	161.6	161.62	161.58	161.58

[a] 75 MHz; acetone-d_6 as internal reference = 29.80 ppm. [b] ^1H–^{13}C connectivities determined by using a phase-cycled 16-step heteronuclear chemical shift correlation map (CSCM) experiment. [c] Signals for the minor conformer.

Since all of the scytophycins showed similar optical properties, exhibiting optical rotations ranging from $[\alpha]_D$ −23° to $[\alpha]_D$ −38°, and similar CD curves with $[\theta]_{265-268}$ values ranging from +10 600 to +27 000, the absolute configurations at C-7, C-9, C-13, C-15, C-17, C-19, C-20, C-21, C-22, C-23, C-24, C-28, C-29, andC-30 for **1–5** were probably the same.

The stereochemistry of C-16 in scytophycins D and E is unknown at this writing, as is that of C-27 in scytophycin A.

X-ray Crystallography. A computer-generated perspective drawing of the final X-ray model for **8** is given in Figure 1. The X-ray experiment did not establish the absolute configuration, but the enantiomer shown was selected to agree with the chiroptical measurements on dibenzoate ester **16**. While several transformations separate **8** from scytophycin C, the stereochemical information has been faithfully preserved, and thus structure **8** estab-

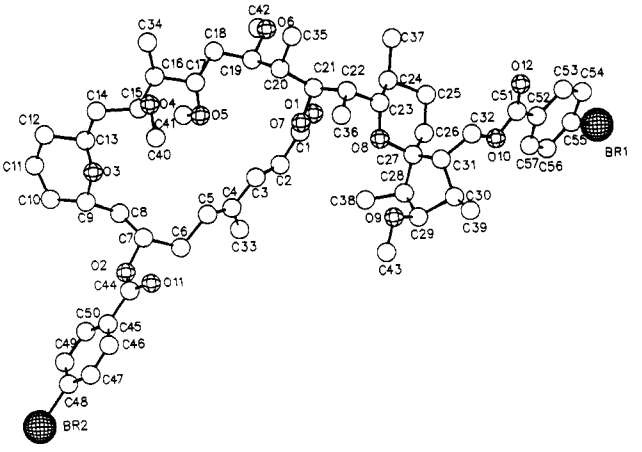


Figure 1. A computer-generated perspective drawing of scytophycin C transformation product **8**. Hydrogens are omitted for clarity.

Table III. ^{1}H NMR Spectral Data for Aldehydes and Primary Alcohols Derived from Scytophycins B (2), C (3), and E (5) in Acetone-d_6 (Incomplete)

position[a,b]	6	7	9	10	12	13
2	5.69	5.69	5.71	5.70	5.680 d	5.66
3	7.44	7.44	7.43	7.42	7.465 d	7.47
Me on 4	1.79	1.78	1.79	1.80	1.784 br t	1.77
5	5.89	5.88	5.86	5.88	5.884 br dd	5.88
6			2.42		2.41	
6′			2.51		2.48	
7	3.98	3.98	3.96	3.97	4.013 br tm	3.99
8			1.25			
8′			1.78			
9	4.49	4.47	4.51	4.50	4.540 br dm	4.52
10	5.69	5.69	5.62	5.64	5.673 dm	5.68
11	5.74	5.75	5.76	5.77	5.777 m	5.75
12			1.88			
13			3.40			
14			1.44			
14′			1.55			
15	3.55	3.55	3.84 dd	3.81		3.76
16						
Me on 16	0.78	0.77				
CH_2 on 16			2.65 d			
			2.55 d			
17	3.36	3.37	3.77 dd			
18			1.44			
18′			1.90			
19			3.23			
20			1.98		1.988	
Me on 20	0.90	0.89	0.85	0.89	0.903 d	0.89
21	5.16	5.12	5.20	5.20	5.200 dd	5.14
22			2.13		2.12	
Me on 22	0.78	0.77	0.77	0.78	0.776 d	0.77
23			3.08		3.098 dd	
24			2.13		2.13	
Me on 24	1.06	1.06	1.06	1.06	1.058 d	1.06
25ax			1.12[c]			
25eq			1.25[c]			
26ax			0.90[c]			
25eq			1.77[c]			
28			1.73		1.732 dq	
Me on 28	0.90	1.04	0.87	1.04	0.894 d	1.05
29			3.37		3.374 t	
30			2.31		2.307	
Me on 30	0.90	0.91	0.92	0.91	0.917 d	0.91
31			2.58		2.628 dd	
32	9.80	3.65	9.81	3.64	9.812 d	3.63
32′		3.53		3.54		3.54
OMe	3.29	3.35	3.36	3.38	3.372 s	3.32
OMe	3.27	3.29	3.31	3.27	3.312 s	3.25
OMe	3.16	3.19	3.19	3.19	3.243 s	3.16
OMe	3.16	3.16	3.19	3.19	3.198 s	3.16

[a] 300 MHz; residual acetone-d_5 as internal reference = 2.04 ppm. [b] Coupling constants for **12** in hertz: 2,3 = 15.8; Me(4), 5 = 1.5; Me(4),6′ = 1.3; 5,6 = 4.3; 5,6′ = 9.5; 6,6′ = −16.2; 6,7 = 3.1; 6′,7 = 10.2; 7,8 = 10.2; 7,8′ = 1.2; 8,8′ = −14.7; 8,9 = 1.8; 8′,9 = 9.8; 9,10 = 2.9; 9,11 = 1.6; 9,12 = 1.8; 10,11 = 10.4; 10,12 = 1.8; 11,12 = 4; 13,14 = 2.0; 13,14′ = 8.4; 14,14′ = −14.5; 14,15 = 7.4; 14′,15 = 3.1; gem CH_2(16) = 4.5; 17,18 = 4.0; 17,18′ = 11.4; 18,18′ = −13.6; 18,19 = 9.8; 18′,19 = 4.0; 19,20 = 1.0; 20,Me(20) = 7.1; 20,21 = 10.0; 21,22 = 1; 22,Me(22) = 7.2; 22,23 = 9.7; 23,24 = 2.0; 24,Me(24) = 6.6; 28,Me(28) = 7.1; 28,29 = 4.4; 29,30 = 4.4; 30,Me(30) = 6.8; 30,31 = 11.0; 31,32 = 4.8. Coupling constants for **9** in Hz: 14,15 = 7.2; 14′,15 = 3.7; 17,18 = 4.4; 17,18′ = 10.6; gem CH_2(16) = 4.5. [c] Tentative assignment.

lishes the stereochemistry of **3**. Figure 2 is also derived from the X-ray model. The bromobenzoates have been eliminated, and the hydrogens at stereocenters have been included.

The conformation of the macrocyclic ring, C-1–C-21, is of interest. The dienoic ester portion, O-7–C-6, is planar; the dihydropyran ring, C-9–O-3, is in the half-chair conformation; and C-15–C-19 are in an extended antiperiplanar conformation.

Related Natural Products. Tolytoxin, an apparent antineoplastic and antifungal agent isolated from the blue-green alga *Tolypothrix conglutinata* var. *colorata*, has a structure that is closely related to that of scytophycin B (**2**).[9] The ^{1}H NMR spectra of the two compounds are virtually identical except for signals that strongly suggest that tolytoxin differs from scytophycin B in possessing a methoxyl group on C-6. This is probably the only dif-

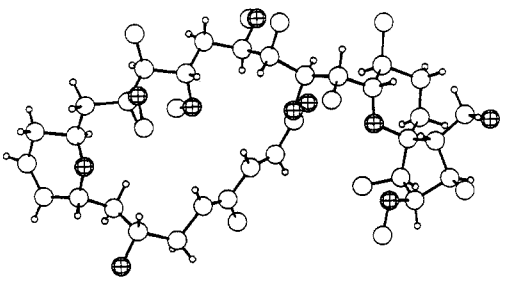
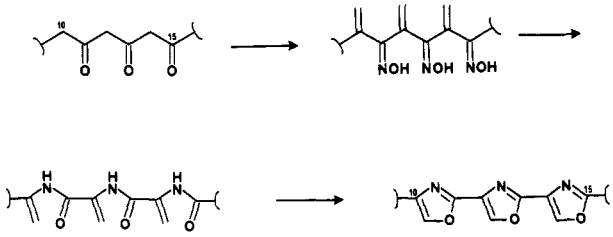


Figure 2. A computer-generated perspective drawing of **8**. The *p*-bromobenzoate groups have been eliminated, and the hydrogens at stereocenters are shown.

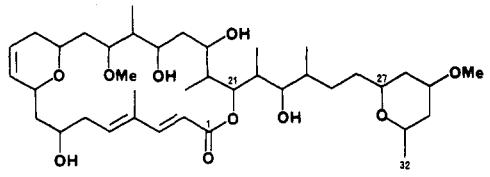
(9) Moore, R. E. In *Marine Natural Products*; Scheuer, P. J., Ed.; Academic: New York, 1981; Vol. 4, pp 1–52.

Scheme I

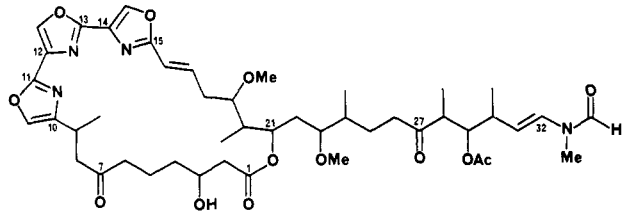


ference between the two compounds.

The scytophycins are also structurally related to the marine natural products swinholide A (17),[10] ulapualides A (18) and B,[11] and kabiramide C.[12] Swinholide A is an



17



18

antifungal compound that has been isolated from *Theonella swinhoei*, a Red Sea sponge that has a symbiotic unicellular blue-green alga associated with it. The ulapualides and kabiramides are cytotoxic and antifungal macrolides that have been isolated from the egg masses of certain nudibranches. The extraordinary feature of the ulapualides and kabiramides is the presence of three contiguous oxazole rings within the macrolide ring. The biogenesis of these oxazole rings is intriguing and may involve introduction of nitrogens into a polyketide intermediate via a naturally occurring Beckmann rearrangement (Scheme I).

Experimental Section

Isolation. A clonal culture of *Scytonema pseudohofmanni* Bharadwaja (Scytonemataceae), which was collected in a moist, heavily forested region of the Koolauloa District (near Pupukea) on the island of Oahu, HI, was isolated by repeated subculture on solidified media[13] and designated strain number BC-1-2. Unialgal, nonaxenic cultures of BC-1-2 were grown in 25-L glass bottles containing an aqueous inorganic medium adjusted to pH 7.[14] The cultures were illuminated continuously at an incident intensity of 330 μeinsteins m^{-2} sec^{-1} from banks of cool-white fluorescent tubes and were vigorously aerated with 0.5% carbon dioxide in air. Following incubation at 24 ± 1 °C for periods ranging from 29 to 33 days, the alga was harvested by filtration or centrifugation at 5000*g* in a refrigerated continuous-flow centrifuge and freeze-dried. Yields of the lyophilized algal cells were typically 0.3–0.4 g/L of culture.

The freeze-dried alga (176 g) was steeped in 6 L of 1:1 dichloromethane/2-propanol for 24 h at room temperature with stirring, and the filtered extract was evaporated to give a semisolid residue. The residue was dissolved in 2 L of 90% methanol, particulates were removed by filtration, and the filtrate was partitioned with 2 L of hexane. The 90% methanol layer was adjusted in concentration to 65% methanol with water and extracted twice with 2-L portions of dichloromethane. Evaporation of the CH_2Cl_2 layer gave a residue, which was then subjected to gel filtration on an 83 cm × 9 cm column of Sephadex LH-20 with 1:4 hexane/dichloromethane as the eluant. The fraction eluting from 2100 mL to 2600 mL contained mostly scytophycins B and C whereas the fraction eluting from 2600 mL to 3100 mL comprised mostly scytophycin A with small amounts of scytophycins D and E. Each fraction was dissolved in a minimum amount of absolute ethanol and applied to a 2 cm × 0.9 cm column of BondElut C-18 (Analytichem International, Harbor City, CA) that has been previously equilibrated with 70% ethanol; the scytophycins were separated from most of the pigments by elution with 15 mL of 70% ethanol. Final purification of the fraction containing scytophycins B and C was achieved by reverse-phase HPLC on ODS-2 (Whatman M9 10/50 column) with either 70% acetonitrile/30% aqueous triethylammonium acetate buffer (0.1 M triethylamine, 0.122 M acetic acid) or 85% MeOH as the eluant; scytophycin B (161 mg) eluted prior to scytophycin C (61 mg) on this column. The fraction containing scytophycins A, D, and E was further purified by normal-phase HPLC on silica with 3% methanol/dichloromethane as the eluant; a small amount of scytophycin B was eluted first, and this was followed by scytophycins D (6 mg), E (6 mg), and A (90 mg).

Scytophycin A (1) had the following properties: white amorphous solid; $[\alpha]_D$ −35° (*c* 1.3, MeOH); CD (MeOH) $[\theta]_{266}$ +12 200; UV (EtOH) 264 nm (ϵ 35 000); IR ($CHCl_3$) 1690, 1660, 1120 cm^{-1}; ^1H NMR (see Table I); ^{13}C NMR (see Table II); FAB MS, *m/z* 860 $(M + K)^+$, 844 $(M + Na)^+$, 822 $(M + H)^+$; high-resolution FAB MS, *m/z* 822.5407 (calcd for $C_{45}H_{76}NO_{12}$, 822.5368).

Scytophycin B (2): white amorphous solid; $[\alpha]_D$ −24° (*c* 0.63, MeOH); CD (MeOH) $[\theta]_{266}$ +16 000; UV (EtOH) 264 nm (ϵ 28 500); IR ($CHCl_3$) 1690, 1660, 1125 cm^{-1}; ^1H NMR (Table I); ^{13}C NMR (Table II); FAB MS, *m/z* 858 $(M + K)^+$, 842 $(M + Na)^+$, 820 $(M + H)^+$; high-resolution FAB MS, *m/z* 802.5123 $(M - H_2O + H)$; calcd for $C_{45}H_{72}NO_{11}$, 802.5105).

Scytophycin C (3): white amorphous solid; $[\alpha]_D$ −23° (*c* 1.2, MeOH); CD (MeOH) $[\theta]_{265}$ +10 600; UV (MeOH) 262 nm (ϵ 48 000); IR ($CHCl_3$) 1690, 1660, 1115 cm^{-1}; ^1H NMR (Table I); ^{13}C NMR (Table II); FAB MS, *m/z* 828 $(M + Na)^+$, 788 $(M - H_2O + H)^+$, 156 $(MeO^+{=}CHCHMeCH{=}CHNMeCHO)$.

Scytophycin D (4): white amorphous solid; $[\alpha]_D$ −32° (*c* 0.9, MeOH); CD (MeOH) $[\theta]_{265}$ +13 400; UV (MeOH) 264 nm (ϵ 47 000); IR ($CHCl_3$) 1690, 1660, 1120 cm^{-1}; ^1H NMR (Table I); ^{13}C NMR (Table II); FAB MS, *m/z* 844 $(M + Na)^+$.

Scytophycin E (5): white amorphous solid; $[\alpha]_D$ −38° (*c* 1.0, MeOH); CD (MeOH) $[\theta]_{268}$ +27 000; UV (MeOH) 265 nm (ϵ 39 000); IR ($CHCl_3$) 1690, 1660, 1090 cm^{-1}; ^1H NMR (Table I); ^{13}C NMR (Table II); FAB MS, *m/z* 844 $(M + Na)^+$; high-resolution FAB MS, *m/z* 844.5190 (calcd for $C_{45}H_{75}NO_{12}Na$, 844.5187).

Scytophycin B 7-Acetate. Compound **2** was reacted with 1.2 equiv of acetic anhydride, 1.2 equiv of triethylamine, and a catalytic amount of 4-(dimethylamino)pyridine in methylene chloride at 25 °C for 5 h to give the 7-acetate.

Compound 6. Scytophycin C (**3**, 24 mg) was dissolved in acetic acid (24 mL) and ethanol (24 mL). After standing at room temperature for 1 day, the reaction mixture was evaporated under reduced pressure. The residue was dissolved in a minimum amount of ethanol and introduced onto a 2 cm × 0.9 cm column of BondElut C-18. Elution with 30 mL of 70% ethanol afforded aldehyde **6**: UV (MeOH) 263 nm; ^1H NMR (Table III); FAB MS, *m/z* 769 $(M + Na)^+$, 747 $(M + H)^+$, 715 $(M - CH_3OH + H)^+$.

Compound 7. Aldehyde **6** was dissolved in ethanol (10 mL). Sodium borohydride (1 mg, 1.1 equiv) in ethanol (0.5 mL) was

(10) Carmely, S.; Kashman, Y. *Tetrahedron Lett.* **1985**, *26*, 511. A related antitumor and antimycotic macrolide, misakinolide A, which lacks the C2–C3 ethene unit in swinholide A, has recently been isolated from an Okinawan *Theonella* Sp. (Sakai, R.; Higa, T.; Kashman, Y. *Chem. Lett.*, in press).

(11) Roesener, J. A.; Scheuer, P. J. *J. Am. Chem. Soc.* **1986**, *108*, 846.

(12) Matsunaga, S.; Fusetani, N.; Hashimoto, K.; Koseki, K.; Noma, M. *J. Am. Chem. Soc.* **1986**, *108*, 847.

(13) Allen, M. M. *J. Phycol.* **1968**, *4*, 1.

(14) Moore, R. E.; Cheuk, C.; Yang, X.-Q. G.; Patterson, G. M. L.; Bonjouklian, R.; Smitka, T. A.; Mynderse, J. S.; Foster, R. S.; Jones, N. D.; Swartzendruber, J. K.; Deeter, J. B. *J. Org. Chem.*, in press.

added, and the mixture was stirred at 0 °C for 30 min. Acetone (2 mL) was added, and the reaction mixture was evaporated to about 1 mL. Saturated brine was added and the mixture extracted with ethyl acetate to give primary alcohol **7**: UV (MeOH) 265 nm; ^{1}H NMR (Table III); FAB MS, m/z 771 $(M + Na)^+$, 749 $(M + H)^+$, 717 $(M - CH_3OH + H)^+$; high-resolution FAB MS, m/z 771.5030 (calcd for $C_{43}H_{72}O_{10}Na$, 771.5024).

Compound 8. The alcohol **7** obtained above and a catalytic amount of 4-(dimethylamino)pyridine were dissolved in dichloromethane (6 mL). To this solution were added successively 50 μL of triethylamine and 30 mg of *p*-bromobenzoyl chloride. After the reaction mixture was stirred at room temperature for 1 day, it was poured onto a 7 × 0.8 cm column of silica gel packed in CH_2Cl_2. The column was washed with 20 mL of CH_2Cl_2 to remove unreacted acid chloride followed by 1:1 dichloromethane/ethyl acetate to give the crude di-*p*-bromobenzoate. Rechromatography on silica gel with 4:1 hexane/ethyl acetate afforded 10 mg of pure **8**: mp 124–125 °C after crystallization from ethanol/dichloromethane; ^{1}H NMR (acetone-d_6) δ 7.95 (m, 4 H), 7.73 (m, 4 H), 7.53 (d, H-3), 6.06 (m, H-5), 5.82 (m, H-11), 5.75 (d, H-2), 5.75 (m, H-10), 5.42 (m, H-7), 5.18 (dd, H-21), 4.32 (m, H-9), 4.54 (dd, H-32), 4.27 (dd, H-32), 3.62 (m, H-15), 3.40 (m, H-17), 3.33 (s, OMe), 3.29 (s, OMe), 3.27 (s, OMe), 3.18 (s, OMe), 1.84 (br s, Me on C-4), 1.12 (d, Me), 1.09 (d, Me), 0.95 (d, 2 Me), 0.81 (d, Me), 0.79 (d, Me).

Compounds 9, 10, and 11. Via the procedures described above, scytophycin B (**2**, 18 mg) was converted to aldehyde **9**, alcohol **10**, and di-*p*-bromobenzoate **11** (9 mg).

Compound 9: UV (MeOH) 265 nm; ^{1}H NMR (see Table III); FAB MS, m/z 783 $(M + Na)^+$, 761 $(M + H)^+$, 729 $(M - CH_3OH + H)^+$.

Compound 10: UV (MeOH) 263 nm; ^{1}H NMR (see Table III); FAB MS, m/z 785 $(M + Na)^+$, 763 $(M + H)^+$.

Compound 11: mp 105–106 °C; ^{1}H NMR (acetone-d_6) δ 7.98 (m, 4 H), 7.74 (m, 4 H), 7.49 (d, H-3), 6.01 (m, H-5), 5.85 (m, H-11), 5.78 (d, H-2), 5.68 (m, H-10), 5.36 (br t, H-7), 5.25 (dd, H-21), 4.31 (m, H-9), 4.54 (dd, H-32), 4.27 (dd, H-32), 3.82 (dd, H-15), 3.75 (dd, H-17), 3.42 (s, OMe), 3.31 (s, OMe), 3.29 (s, OMe), 3.23 (s, OMe), 2.70 and 2.60 (AB quartet, CH_2 on C-16), 1.88 (br s, Me on C-4), 1.12 (d, Me), 1.09 (d, Me), 0.92 (d, Me), 0.89 (d, Me), 0.79 (d, Me).

Compounds 12 and 13. Via the procedures described above, scytophycin E (**5**) was converted into aldehyde **12** and alcohol **13**.

Compound 12: UV (MeOH) 265 nm; ^{1}H NMR (see Table III); FAB MS, m/z 785 $(M + Na)^+$, 763 $(M + H)^+$, 731 $(M - CH_3OH + H)^+$; high-resolution FAB MS, m/z 785.4837 (calcd for $C_{43}H_{70}O_{11}Na$, 785.4816).

Compound 13: UV (MeOH) 265 nm; ^{1}H NMR (see Table III); FAB MS, m/z 787 $(M + Na)^+$, 765 $(M + H)^+$, 733 $(M - CH_3OH + H)^+$.

Compound 14. Compound **10** was acetylated with acetic anhydride in pyridine (1:4) at room temperature for 1 day. Standard workup gave the 7,32-diacetate: ^{1}H NMR (acetone-d_6) δ 7.45 (d, H-3), 5.92 (m, H-5), 5.83 (m, H-11), 5.76 (d, H-2), 5.64 (m, H-10), 5.23 (dd, H-21), 5.08 (tm, H-7), 4.23 (m, H-9), 3.79 (dd, H-15), 3.73 (dd, H-17), 3.38 (s, OMe), 3.27 (s, OMe), 3.24 (s, OMe), 3.20 (s, OMe), 2.67 and 2.57 (AB quartet, CH_2 on C-16), 1.85 (br, Me on C-4), 1.07 (d, 2 Me), 0.89 (d, 2 Me), 0.82 (d, Me).

Compound 15. To a solution of **14** (5.5 mg) in tetrahydrofuran (2 mL) and pyridine (0.5 mL) was added osmium tetraoxide (1.65 mg, 1.0 equiv) in tetrahydrofuran (16.5 μL). The mixture was stirred at room temperature for 17 h, and aqueous sodium bisulfite solution was added to decompose the osmate ester. After the mixture was stirred for 1 h, the products were separated by silica gel chromatography with a gradient of 1:1 hexane/ethyl acetate to 100% ethyl acetate to give successively 3 mg of starting material, 2 mg of a 1:1 mixture of 4,5-diols, and 0.5 mg of **15**: ^{1}H NMR δ 7.45 (d, H-3), 5.97 (m, H-5), 5.78 (d, H-2), 5.23 (br d, H-21), 5.03 (m, H-7), 4.22 (dd, H-32), 3.98 (dd, H-32), 3.38 (s, OMe), 3.34 (s, OMe), 3.29 (s, OMe), 3.22 (s, OMe), 1.87 (br s, Me on C-4), 1.09 (d, 2 Me), 0.90 (d, Me), 0.89 (d, Me), 0.83 (d, Me).

Compound 16. The 10,11-diol **15** (1 mg) and a catalytic amount of 4-(dimethylamino)pyridine were dissolved in CH_2Cl_2 (1 mL). To this solution was added triethylamine (100 μL) followed by benzoyl chloride (50 μL). After the reaction mixture was stirred at room temperature for 1 day, it was introduced onto a silica gel column (7 × 0.8 cm) packed in CH_2Cl_2. Unreacted acid chloride was removed with 15 mL of CH_2Cl_2, and the 10,11-dibenzoate was eluted with 10% methanol/dichloromethane. Purification by HPLC on silica with 60:40 hexane/ethyl acetate afforded 0.13 mg of **16**: UV (MeOH) 231 nm (ε 60 000), 259 (28 000); CD (MeOH) $[\theta]_{272}$ +8700, $[\theta]_{236}$ −63 000, $[\theta]_{221}$ +39 000; ^{1}H NMR (CD_2Cl_2) δ 8.00 (m, aromatic H's), 7.50 (m, aromatic H's), 5.87 (m, H-5), 5.84 (d, 15 Hz, H-2), 5.21 (br d, 11 Hz, H-21), 5.05 (m, H-7), 4.18 (m, H-32), 3.99 (m, H-32), 3.42 (s, OMe), 3.30 (s, OMe), 3.26 (s, OMe), 3.24 (s, OMe), 2.68 and 2.60 (AB quartet, 4.5 Hz, epoxide CH_2), 2.09 (s, OAc), 2.03 (s, OAc), 1.86 (br s, Me on C-4), 1.08 (d, 2 Me), 0.92 (d, Me), 0.89 (d, Me), 0.86 (d, Me).

Single-Crystal X-ray Diffraction Analysis of 8. Crystals of **8** suitable for X-ray analysis were grown by slow evaporation of a methanol solution. The crystals, which appeared to be solvated, were mounted in thin-walled glass capillaries. Preliminary X-ray photographs displayed monoclinic symmetry, and accurate lattice constants of a = 15.258 (4), b = 16.765 (7), and c = 14.588 (3) Å and β = 111.54 (2)° were determined from a least-squares fit of 15 moderate 2θ values, which were obtained from a diffractometer. An estimated crystal density, systematic extinctions, and optical activity were uniquely consistent with space group $P2_1$ with one molecule of $C_{57}H_{78}Br_2O_{12}$·(3/4)CH_3OH forming the asymmetric unit. All unique diffraction maxima with $2\theta < 114°$ were examined on a computer-controlled four-circle diffractometer using graphite-monochromated Cu K$\bar{\alpha}$ radiation (1.541 78 Å) and variable-speed, 1° ω scans. Of the 3739 reflections examined in this fashion, 2347 (63%) were judged observed ($F_o > 3\sigma(F_o)$) after correction for Lorentz, polarization, and background effects.[15] A phasing model was found by locating bromine positions from the Patterson synthesis, and this model was extended by weighted Fourier syntheses. Hydrogens were located in a difference synthesis following partial refinement of the heavy atoms or placed at calculated positions. Block diagonal least-squares refinements with anisotropic non-hydrogen atoms and heavily damped, isotropic hydrogens have converged to a current crystallographic residual of 0.0816 for the observed reflections. The difference electron density synthesis shows evidence for four methanols of solvation in the asymmetric unit, but these appear to be badly disordered or partially occupied. Additional experimental details are available as supplementary material.

Acknowledgment. The research in Hawaii was supported by PHS Grant CA12623-12, awarded by the National Cancer Institute, Department of Health and Human Services. We thank John Occolowitz at Lilly Research Laboratories for determining the FAB mass spectra. The X-ray crystallographic studies at Cornell were financially supported by the New York State Sea Grant, NSF Grant INT14133, and PHS Grant CA24487.

Supplementary Material Available: Tables of fractional coordinates, thermal parameters, interatomic distances, interatomic angles, and torsional angles for compound **8** and tables for in vitro agar diffusion activity of the scytophycins against fungal pathogens (12 pages). Ordering information is given on any current masthead page.

(15) All crystallographic calculations were done on a PRIME 9950 computer operated by the Cornell Chemistry Computing Facility. Principal programs employed were the following: REDUCE and UNIQUE, data reduction programs by M. E. Leonowicz, Cornell University, 1978; MULTAN 80 and RANTAN 80, systems of computer programs for the automatic solution of crystal structures from X-ray diffraction data (locally modified to perform all Fourier calculations, including Patterson syntheses), written by P. Main, S. E. Hull, L. Lessinger, G. Germain, J. P. Declercq, and M. M. Woolfson, University of York, England, 1980; BLS78A, an anisotropic block diagonal least-squares refinement written by K. Hirotsu and E. Arnold, Cornell University, 1980; PLUTO78, a locally modified crystallographic illustration program by W. D. S. Motherwell, Cambridge Crystallographic Data Centre, 1978; and BOND, a program to calculate molecular parameters and prepare tables, written by K. Hirotsu and G. Van Duyne, Cornell University, 1985.