Absolute Stereochemistries of the Aplysiatoxins and Oscillatoxin A

Richard E. Moore,* Adrian J. Blackman,[1] Chad E. Cheuk, and Jon S. Mynderse

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

Gayle K. Matsumoto and Jon Clardy

Department of Chemistry, Cornell University, Ithaca, New York 14853

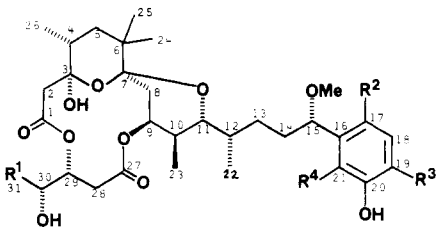
Ronald W. Woodard and J. Cymerman Craig

Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco, California 94143

Received August 30, 1982

Optical and proton NMR spectral studies of the aplysiatoxins and derivatives, degradation products of the toxins, and an X-ray crystallographic analysis of 19,21-dibromoaplysiatoxin show that the absolute configurations of the ten asymmetric carbons are 3*S*,4*R*,7*S*,9*S*,10*S*,11*R*,12*S*,15*S*,29*R*,30*R*. The 31-nor compound, oscillatoxin A, has the same absolute stereochemistry at C(3), C(4), C(7), C(9), C(10), C(11), C(12), C(15), and C(29).

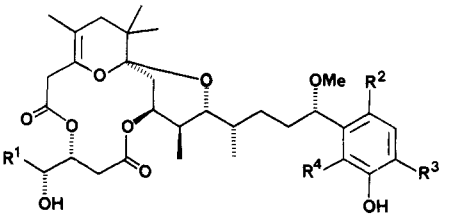
The marine blue-green alga *Lyngbya majuscula* contains two highly inflammatory agents, aplysiatoxin (1) and debromoaplysiatoxin (2), which are the causative agents of a severe contact dermatitis that sometimes affects swimmers and bathers during the summer months in Hawaii.[2,3] These two toxins were first isolated by Kato and Scheuer from the digestive gland of the sea hare *Stylocheilus longicauda*,[4] a gastropod mollusk that feeds preferentially on *L. majuscula*. Debromoaplysiatoxin is also present in other blue-green algae belonging to the Oscillatoriaceae; for example, 2 has been isolated from a mixture of two blue-green algae found on the seaward side of Enewetak Island, viz., *Schizothrix calcicola* and *Oscillatoria nigroviridis*, along with the 31-nor compound, oscillatoxin A (3), and three brominated toxins (4–6).[5]



	R^1	R^2	R^3	R^4
1	Me	Br	H	H
2	Me	H	H	H
3	H	H	H	H
4	H	Br	H	H
5	H	Br	Br	H
6	Me	Br	Br	H
7	Me	H	Br	H
8	Me	Br	Br	Br

Toxins 1 and 6 are produced when 2 is treated with bromine in aqueous methanol buffered at pH 6; also produced are 7 and 8, which to date have not been isolated as natural products. Debromoaplysiatoxin displays some antineoplastic activity[3] and recently the aplysiatoxins and oscillatoxin A have been found to be powerful tumor promoters.[6]

The aplysiatoxins and oscillatoxin A are always accompanied in blue-green algae and sea hares by the corresponding anhydrotoxins, viz., the anhydroaplysiatoxins 9, 10, 11, and 12 and anhydrooscillatoxin A (13). Even



	R^1	R^2	R^3	R^4
9	Me	H	H	H
10	Me	Br	H	H
11	Me	Br	Br	H
12	Me	Br	Br	Br
13	H	H	H	H

though the aplysiatoxins are quite labile, dehydrating readily to anhydrotoxins if treated with very mild acid, the anhydrotoxins are not artifacts generated during the isolation. Dehydration can be prevented entirely if reasonable care is exercised during the isolation procedure to avoid exposure to acidic solvents such as chloroform or contact with chromatographic adsorbents such as silica gel or reverse-phase materials that have not been properly endcapped with silylating reagents. Unlike 1–8, the anhydrotoxins (9–13) are relatively nontoxic.

In this paper evidence is presented which shows that the aplysiatoxins, oscillatoxin A, and the corresponding anhydrotoxins have relative and absolute stereochemistries depicted in the structural formulae.

Relative Stereochemistry from ^{1}H NMR Studies. The gross structures of 1 and 2 were elucidated in 1974 by Kato and Scheuer[4a,b] who were also able to propose the relative stereochemistries of the two oxane rings in both compounds, shown in a and b, on the basis of chemical reactivities and spectral properties.[4c] To explain the facile

(1) Work performed at the University of Hawaii while on sabbatical leave from the Department of Chemistry, University of Tasmania, Hobart, Tasmania, Australia in 1981–1982.

(2) Serdula, M.; Bartolini, G.; Moore, R. E.; Gooch, J.; Wiebenga, N. *Hawaii Med. J.* **1982**, *41*, 200.

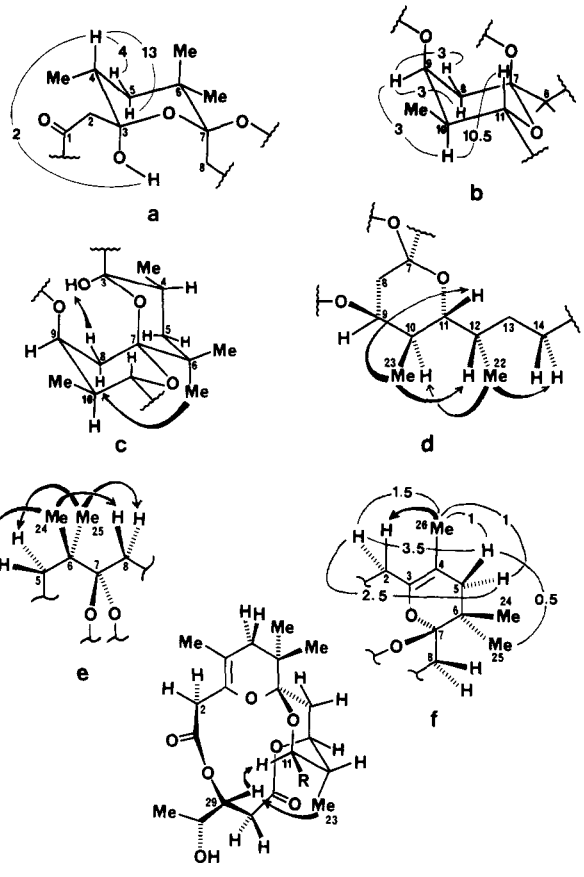
(3) Mynderse, J. S.; Moore, R. E.; Kashiwagi, M.; Norton, T. R. *Science* **1977**, *196*, 538.

(4) (a) Kato, Y.; Scheuer, P. J. *J. Am. Chem. Soc.* **1974**, *96*, 2245. (b) Kato, Y.; Scheuer, P. J. *Pure Appl. Chem.* **1975**, *41*, 1. (c) Kato, Y.; Scheuer, P. J. *Ibid.* **1976**, *48*, 29.

(5) Mynderse, J. S.; Moore, R. E. *J. Org. Chem.* **1978**, *43*, 2301.

(6) (a) Fujiki, H.; Suganuma, M.; Nakayasu, M.; Hoshino H-o.; Moore, R. E.; Sugimura, T. *Gann* **1982**, *73*, 495. (b) Eliasson, L.; Kallin, B.; Patarroyo, M. Klein, G.; Fujiki, H.; Sugimura, T. *Int. J. Cancer* **1983**, *31*, 7. (c) Suganuma, M.; Fujiki, H.; Tahira, T.; Cheuk, C.; Moore, R. E.; Sugimura, T. *Carcinogenesis (N.Y)* **1984**, *5*, 315.

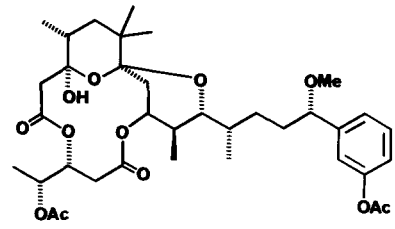
0022-3263/84/1949-2484$01.50/0



dehydration of 1 and 2 to anhydroaplysiatoxin (9) and anhydrodebromoaplysiatoxin (10), the OH on C(3) and the proton on C(4) had to be trans to each other and located in axial positions. The ^{1}H NMR spectral data for 1 and 2 (Table I) support this conclusion and the chair conformation shown in a since the proton on C(4) shows large and small couplings to the axial and equatorial protons on C(5), respectively, and the proton of the hydroxyl group on C(3) shows significant *W* coupling to the C(4) proton. Generally in six-membered rings equatorial protons resonate at lower field than axial protons. In 1 and 2, however, the axial proton on C(5) (δ 1.63) resonates at lower field than its equatorial partner (δ 1.06), presumably due to deshielding by the axial substituents on C(3) and C(7).

The ^{1}H NMR spectra of 1 and 2 indicated that the oxane ring in b was also in a chair conformation. The ester oxygen on C(9) had to be oriented axially since the proton on C(9) showed small coupling to both protons on C(8) and to the proton on C(10). The methyl group on C(10) was in an equatorial position since a large coupling, typical of axial–axial coupling, was observed between the protons on C(10) and C(11). The side chain at C(11) was therefore in an equatorial position. Kato and Scheuer suggested that C(8) was probably attached axially to C(7) as shown in a since the proton on C(9) exhibited an appreciable change in chemical shift (~0.4 ppm shift to lower field) on dehydration.[4c] Kato and Scheuer, however, did not assign relative stereochemistry at C(12), C(15), C(29), and C(30), nor did they determine the absolute configurations of any of the ten asymmetric carbons.

We confirmed the proposed relative stereochemistry (c) for the two oxane rings with difference NOE spectral studies on debromoaplysiatoxin 20,30-diacetate (14) in benzene-d_6. Irradiation of the methyl signal at δ 0.772, which we assigned to the equatorial Me on C(6), induced a positive NOE on the signal at δ 1.390 for the axial proton



14

on C(8), but no NOE on the signal at δ 2.738 for the equatorial proton on C(8). No NOEs were observed on either of the C(8) protons when the axial methyl on C(6) (δ 0.956) was irradiated. When the equatorial C(8) proton was irradiated, a strong positive NOE was seen for the axial OH on C(3) (δ 4.187). The NOE results were consistent with c and ruled out a structure in which C(8) was attached equatorially to C(7).

The most informative NOEs resulted from irradiation of the equatorial methyl on C(10) (δ 0.657). Significant positive NOEs were observed for the methine protons on C(9), C(11), C(12), and C(29) (δ 5.238, 4.037, 1.485, and 5.309, respectively) and one of the methylene protons on C(28) [δ 2.460, coupled to the C(29) proton by 2.3 Hz]. Examination of a Dreiding model indicated that the C(29) proton and a syn (to the C-29 H) proton on C(28) could only come close to the methyl group on C(10) when the relative stereochemistry was 3*S**, 4*R**, 7*S**, 9*S**, 10*S**, 11*R**, 29*R**.

A positive NOE between the Me on C(10) and the H on C(12) indicated that these two groups were spatially close (eclipsed) in the preferred conformation of 14 in solution. Irradiation of the Me group on C(12) (δ 0.805) produced appreciable positive NOEs on the C(10) H (δ 1.35) and one of the protons on C(14), as well as on the Me on C(10), possible only if the C(12)–C(13)–C(14)–C(15)··· side chain was fully extended and coplanar with C(11)–C(10)–C(9) so that the Me group on C(12) eclipsed both the C(10) and C(14) protons (see d). This conformation was further supported by a large coupling (10.5 Hz) for $J_{10,11}$ and a small coupling (2 Hz) for $J_{11,12}$. The relative stereochemistry of C(12) in 1 and 2 was therefore *S**.

Comparable ^{1}H NMR studies of 3 indicated that the relative stereochemistry of C(3), C(4), C(7), C(9), C(10), C(11), and C(12) in oscillatoxin A was the same.

The same relative stereochemical conclusions for C(7), C(9), C(10), C(11), C(12), and C(29) were reached after detailed ^{1}H NMR studies of 9–12 (Table II). Difference NOE experiments clearly showed that the two methyl groups on C(6) eclipse the methylene protons on both C(5) and C(8) as shown in e. Irradiation of the C(24) methyl group produced positive NOEs of the signals for the pseudoequatorial proton on C(5) (H_β) and the axial proton on C(8). Irradiation of the C(25) methyl group, on the other hand, induced comparable NOEs on the signals for the pseudoaxial proton on C(5) (H_α) and the equatorial proton on C(8).

One of the protons on C-2 (H_α) showed homoallylic coupling to both protons on C(5) and allylic coupling to the C(26) methyl group whereas the other one (H_β) showed no coupling to these protons. These data indicated that the β proton on C-2 was essentially in the same plane as C(2)–C(3)–C(4)(C(26))C(5) and moreover that the α proton on C(2) and the protons on C(5) were oriented above and below this plane. A positive NOE on H-2β (but no NOE on H-2α) from irradiation of the C(26) methyl group supported this stereostructure as shown in f.

When the C(23) methyl group was irradiated, positive NOEs were detected in the signals for the methine protons

Table I. ^{1}H NMR Data for Aplysiatoxins and Oscillatoxin A in Acetone-d_6

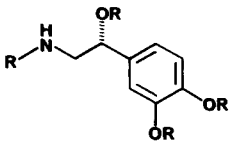
	1[a]	2[b]	6[a]	7[a]	8[a]	3[b]
H-2	2.628 (d)	2.53	2.54	2.480	2.433	2.53
H-2′	2.744 (d)	2.77	2.75	2.778	2.717	2.74
OH on C(3)	4.292 (d)	4.30	4.28	4.283	4.288	4.35
H-4	1.88 (m)	1.86	1.86	1.84	1.888	1.84
H-5 ax	1.625 (t)	1.63	1.630	1.618	1.644	1.62
H-5 eq	1.064 (dd)	1.06	1.08	1.062	1.104	1.06
H-8 ax	1.724 (dd)	1.71	1.73	1.710	1.743	1.72
H-8 eq	2.699 (dd)	2.70	2.70	2.685	2.706	2.72
H-9	5.241 (m)	5.24	5.24	5.232	5.237	5.21
H-10	1.742 (m)	1.70	1.75	1.722	1.745	1.70
H-11	3.941 (dd)	3.94	3.93	3.909	3.914	3.92
H-12	1.54 (m)	1.53	1.55	1.525	1.588	1.53
H-13	1.50 (m)	1.4	1.5	1.37	1.5	1.40
H-13′	1.43 (m)	1.3	1.4	1.30	1.4	1.32
H-14	2.00 (m)	1.98	1.97	1.937	1.98	1.95
H-14′	1.6 (m)	1.60	1.6	1.600	1.6	1.60
H-15	4.485 (t)	4.02	4.435	4.003	4.95	3.99
H-17		6.86		6.831[d]		6.84
H-18	7.328 (d)	7.16	7.647[c]	7.442[e]	7.796[c]	7.13
H-19	6.702 (dd)	6.74				6.72
OH on C(20)[f]		8.21				8.25
H-21	7.106 (d)	6.95	7.229[c]	7.067[d]		6.93
3H-22	0.826 (d)	0.80	0.831	0.798	0.863	0.80
3H-23	0.740 (d)	0.72	0.735	0.714	0.734	0.73
3H-24	0.850 (s)	0.82	0.862	0.783	0.886	0.81
3H-25	0.861 (s)	0.85	0.881	0.840	1.018	0.84
3H-26	0.860 (d)	0.88	0.870	0.870	0.869	0.86
H-28	2.907 (m)	2.93	2.91	2.922	2.898	2.93
H-28′	2.895 (m)	2.91	2.87	2.87	2.868	2.96
H-29	5.208 (m)	5.25	5.20	5.226	5.153	5.22
H-30	3.999 (dq)	4.07	3.99	4.035	3.945	3.67, 3.69
OH on C(30)[g]		4.23				4.14
3H-31	1.111 (d)	1.14	1.111	1.132	1.076	
OCH_3	3.194 (s)	3.19	3.210	3.189	3.176	3.17

[a] J (Hz) for 1: 2,2′ = −12.6; 3(OH),4 = 2.0; 4,5(ax) = 13.0; 4,5(eq) = 3.7; 4,26 = 6.4; 5,5 = −13.3; 8,8 = −14.6; 8(ax),9 = 3.6; 8(eq),9 = 3.0; 9,10 = 3.3; 10,11 = 10.7; 10,23 = 6.9; 11,12 = 2.0; 12,22 = 6.6; 14,15 = 14′,15 = 6.5; 18,19 = 8.5; 19,21 = 3.0; 28,28′ = −18.1; 28,29 = 11.6; 28′,29 = 2.3; 29,30 = 4.1; 30,31 = 6.5. Coupling constants for **6**, **7**, and **8** are essentially the same. [b] J (Hz) for **2** and **3**: see ref 5. [c] Singlet. [d] Doublet. [e] Doublet of doublets. [f] Broad singlet. [g] OH signals for **1**, **6**, **7**, and **8** not observed due to the presence of a small amount of D_2O in the solvent.

on C(9), C(10), C(11), C(29), and Hβ on C(28). Interestingly, irradiation of the C(29) proton produced a positive NOE on the signal for the C(11) proton. A Dreiding model examination showed that the tricyclic bis lactone system could only have the conformation and stereochemistry shown in g to produce all of the observed NOEs.

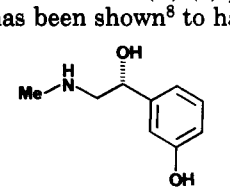
Absolute Stereochemistry of the Toxins. Optical and chemical degradative data indicate that the aplysiatoxins and oscillatoxins have the absolute stereochemistries depicted in **1**–**8**. The anhydrotoxins therefore have the absolute stereochemistries shown in **9**–**13**.

The circular dichroism (CD) spectrum of **2** in ethanol shows a positive Cotton effect (CE) with $[\theta]_{269}$ +902° and $[\theta]_{286}$ +1031° (Figure 1). An almost identical CD spectrum is given by (*R*)-(−)-noradrenaline hydrochloride (**15**).



15 R = H
16 R = Me

The cactus alkaloid (*R*)-(−)-calipamine hydrochloride[7] (**16**), with an identical carbon skeleton and nitrogen and oxygen substitution pattern as **15**, has a similar CD spectrum, showing a positive maximum at 281 nm, and the closely related *m*-hydroxy-substituted (*R*)-(−)-phenylephrine hydrochloride (**17**) has been shown[8] to have an ORD spectrum superimposable on that of **15** and to possess the same absolute configuration. As usual when oxygen substitution is present in positions 3 and/or 4 of the ring, the ^1L_b transition of benzene is shifted from 260 to 280 nm, accompanied by a loss of most or all of the fine structure normally present for this transition.[9,10] The CD data indicate that debromoaplysiatoxin has the *S* configuration at C(15). Since **1** and **6** can be formed from **2**, the absolute stereochemistry of C(15) in **1** and **6** is also *S*.



17

The closely related oscillatoxin A (**3**) has a CD spectrum, $[\theta_{269}$ +1022° and $[\theta]_{282}$ +1255° (EtOH), that is essentially

(7) Bruhn, J. G.; Woodard, R. W.; Craig, J. C., unpublished results.

(8) Lyle, G. L. *J. Org. Chem.* **1960**, *25*, 1779.

(9) (a) Moffit, W. *J. Chem. Phys.* **1954**, *22*, 320. (b) Petruska, J. *Ibid.* **1961**, *34*, 1111.

(10) This is confirmed by the CDs of (*S*)-(−)-1-phenylethanol, (*S*)-(−)-1-methoxy-1-phenylethane, and (*R*)-(−)-1-phenylethane-1,2-diol which show identical superimposable maxima (positive CE) centered at 268 nm, with the normal fine structure for the ^1L_b transition and no effect due to the change OH → OMe at the asymmetric center or the presence of an additional OH group along the aliphatic chain.

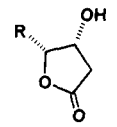
Table II. ^{1}H NMR Data for Anhydrotoxins

	9	10[a]	11	12
H-2α	3.060	3.070 (br dm)	3.073	3.063
H-2β	3.327	3.342 (d)	3.326	3.325
H-5α	1.354	1.360 (br dm)	1.355	1.370
H-5β	2.203	2.185 (br dm)	2.168	2.195
H-8ax	1.737	1.746 (dd)	1.746	1.750
H-8eq	2.228	2.232 (dd)	2.227	2.227
H-9	4.844	4.856 (br q)	4.853	4.846
H-10	0.23	0.23 (m)	0.23	0.23
H-11	3.770	3.805 (dd)	3.793	3.780
H-12	1.50	1.58 (m)	1.63	1.62
H-13, H-13′	1.50	1.53 (m)	1.54	1.51
H-14	1.74	1.75 (m)	1.72	2.17
H-14′		1.64 (m)	1.63	1.85
H-15	3.995	4.447 (dd)	4.417	4.968[b]
H-17	6.816			
H-18	7.152	7.357 (dd)	7.662	7.814
H-19	6.721	6.715 (d)		
OH on C(20)[c]	8.32	8.69 (br s)	8.41	
H-21	6.856	7.008 (d)	7.160	
3H-22	0.833	0.845 (d)	0.840	0.869
3H-23	0.822	0.856 (d)	0.850	0.842
3H-24	0.956	0.956 (s)	0.955	0.962
3H-25	0.824	0.829 (s)	0.829	0.830
3H-26	1.596	1.578 (br s)	1.558	1.595
H-28α	2.73	2.739 (dd)	2.736	2.73
H-28β	2.77	2.781 (dd)	2.780	2.77
H-29	5.340	5.357 (dt)	5.349	5.320
H-30	3.839	3.854 (qd)	3.856	3.835
OH on C(30)[c]	4.08	4.07 (d)	4.13	4.021
3H-31	1.110	1.116 (d)	1.117	1.103
OCH_3	3.170	3.210 (s)	3.210	3.169

[a] J (Hz) for **10**: 2α,2β = −13.3; 2α,5β = 3.5; 2α,5α = 2.5; 2α,26 = 1.5; 5α,5β = −15.1; 5β,25 ~ 0.5; 5α,26 and 5β,26 ~ 1.0–1.5; 8ax,8eq = −14.7; 8eq,9 = 2.7; 8ax,9 = 3.4; 9,10 = 2.7; 10,11 = 10.6; 10,23 = 6.9; 11,12 = 1.8; 12,22 = 6.5; 14,15 = 7.8; 14′,15 = 4.3; 18,19 = 8.6; 19,21 = 3.1; 28α,28β = −17.6; 28α,29 = 12.0; 28β,29 = 2.2; 29,30 = 4.2; 30,31 = 6.5; 30,OH = 5.5. Coupling constants and signal multiplicities for **9**, **11**, and **12** are essentially the same. [b] Broad signal. [c] Concentration dependent.

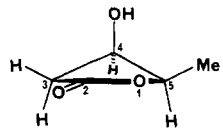
(Figure 1) the same as that of **2**. The absolute stereochemistries of oscillatoxin A and debromoaplysiatoxin are therefore identical at C(15).

Acid hydrolysis of **2** (or **9**) and **3** (or **13**) with 0.5 N HCl in aqueous MeOH produces **18** and **19**, respectively. The



18 R = Me
19 R = H

relative stereochemistry of the OH on C-4 and the methyl group on C-5 in γ-lactone **18** was concluded to be syn since the proton on C-3 which showed small coupling to the proton on C-4 did not show *W* coupling to the H on C-5 in the ^{1}H NMR spectrum (see Experimental Section and compare ^{1}H NMR spectrum of **19**). γ-Lactone **18**, $[\alpha]^{EtOH}_D$ +75°, exhibits a positive CD spectrum, $[\Theta]^{EtOH}_{220}$ +950°, indicating that C(4) is above the C(3)–C(2)–O(1)–C(5) plane of the lactone ring as shown in **18a**;[11] C(4) and C(5)



18a

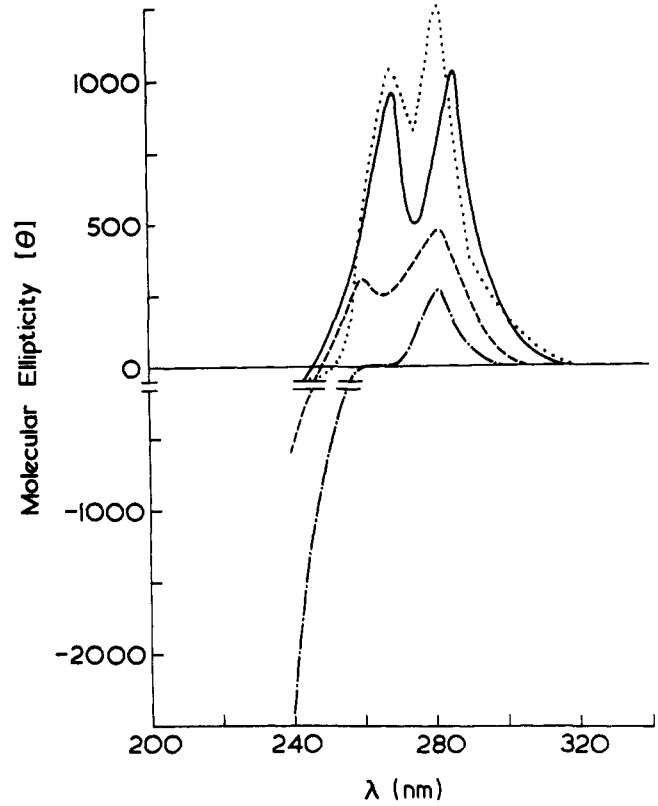


Figure 1. Circular dichroism spectra of debromoaplysiatoxin (—), oscillatoxin A (···), (*R*)-(−)-noradrenaline hydrochloride (– –), and (*R*)-(−)-calipamine hydrochloride (–·).

in **18** and C(29) and C(30) in **2** and **9** are therefore all *R*. γ-Lactone **19** has an optical rotation, $[\alpha]^{EtOH}_D$ +44°, that is opposite in sign to that of (*S*)-(−)-3,4-dihydroxybutanoic acid lactone, $[\alpha]^{EtOH}_D$ −72°, synthesized from (*S*)-(−)-malic acid;[12] C(4) in **19** and C(29) in **3** and **13** are therefore both *R*. The 29*R*,30*R* assignment in **2** and **9** was further supported by converting anhydrodebromoaplysiatoxin ($LiAlH_4$ reduction followed by acetylation with Ac_2O/pyridine) to (3*R*,4*R*)-1,3,4-triacetoxypentane, $[\alpha]^{EtOH}_D$ +26° and $[\Theta]^{EtOH}_{208}$ +95°, which had optical properties that were close to those of (2*R*,3*R*)-1,2,3,5-tetraacetoxypentane,[13] $[\alpha]^{EtOH}_D$ +22° $[\Theta]^{EtOH}_{221}$ +72°.

The foregoing optical and proton NMR spectral data of various aplysiatoxins and their derivatives and degradation products show that the absolute configurations of the ten asymmetric carbons are 3*S*,4*R*,7*S*,9*S*,10*S*,11*R*,12*S*,15*S*,-29*R*,30*R*. The 31-nor compound, oscillatoxin A, has the same absolute stereochemistry.

The stereochemistry of these toxins is further supported by X-ray analysis of 19,21-dibromoaplysiatoxin (**8**). A computer generated perspective drawing of the X-ray model of **8** is shown in Figure 1. In general the molecular parameters agree well with anticipated values, although they are subject to relatively high errors due to the dominating effect of the bromine scattering (50%).

Experimental Section

Spectral Analysis. NMR spectra were determined at 300 MHz for proton and 75 MHz for carbon-13 on a Nicolet NT-300 spectrometer. Proton chemical shifts were referenced in acetone-d_6 to the residual acetone-d_5 signal (2.06 ppm) and in benzene-d_6

(11) Beecham, A. F. *Tetrahedron Lett.* **1968**, 2355.
(12) Mori, K.; Takigawa, T.; Matsuo, T. *Tetrahedron* **1979**, *35*, 933.
(13) We thank Professor Satoru Masamune for a sample of this compound. (2*R*,3*S*)-1,2,3,5-tetraacetoxypentane had $[\alpha]_D$ −8° (EtOH).

to the residual benzene-d_5 (7.15). Homonuclear ^1H connectivities were determined by using a phase-cycled 16-step COSY experiment described by Bax.[14] Conformatory information was obtained from difference double resonance experiments when necessary. Qualitative homonuclear ^1H NOEs were obtained by selective continuous irradiation (decoupler on, hetero mode) for 3 s, followed by data acquisition and a recycling delay (decoupler off) for 3 s; off-resonance experiments were also performed in a similar manner and the NOE enhancements were observed in difference spectra produced by subtracting on-resonance spectra from off-resonance spectra.

EI mass spectra, including high-resolution mass measurements, were determined on a Varian MAT-311 instrument. CD curves were measured on a Jouan Mark II spectropolarimeter at 25 °C in 95% ethanol and were recorded in terms of molecular ellipticity units [θ].

Isolation. A. Debromoaplysiatoxin and Anhydrodebromoaplysiatoxin. *Lyngbya majuscula* was collected in the lagoon of Enewetak Atoll in the Marshall Islands. One collection was made from Reefer 8 and South Medren Pinnacles in early December, 1981, at depths ranging from 50 to 75 feet. The freeze-dried alga (10.5 kg) was extracted with isopropanol/dichloromethane (1:1) to give 210 g of extract. Gel filtration of the extract in 14 × 15 g portions on a 9 cm × 101 cm column of Sephadex LH-20 with isopropanol/dichloromethane (1:1) yielded in the combined 2700–3500 mL fractions a total of 70 g of material which was further fractionated by reverse-phase chromatography in 3-g portions on a 2.5 cm × 75 cm column of phenylporasil B with a 60–100% methanol/water gradient. Several fractions were collected and analyzed by the irritation on mouse ear test[15] and ^1H NMR spectroscopy to detect the mixture of **2** and **9**. Separation of this mixture was achieved by reverse-phase HPLC with a Whatman Partisil M9 10/50 ODS-3 column with a methanol/water (4:1) mobile phase (80 mg per injection); anhydrodebromoaplysiatoxin (**9**, 1.35 g) was eluted before debromoaplysiatoxin (**2**, 1.65 g). Debromoaplysiatoxin was obtained as colorless needles, mp 105.2–107.0 °C; $[\alpha]^{25}_D$ +60.6° (EtOH, *c* 0.66), by crystallization from diethyl ether/*n*-pentane (1:1) at −10 °C and recrystallization from aqueous methanol. Anhydrodebromoaplysiatoxin crystallized from diethyl ether/*n*-pentane, mp 116.0–117.5 °C.

B. Aplysiatoxin and Debromoaplysiatoxin. A 1.5-kg sample of *L. majuscula*, which was floating abundantly either freely or entangled with other seaweeds in waist-deep water, was collected at Kailua Beach on August 26, 1980, during an outbreak of seaweed dermatitis on the windward side of Oahu, Hawaii. The freeze-dried alga (309 g) was extracted exhaustively with a 1:1 mixture of methylene chloride and isopropanol to give 4.62 g of a dark gum, which was partitioned between 400 mL of hexane and 400 mL of 10% aqueous methanol. The hexane layer was discarded and the water content in the toxic aqueous methanol layer was adjusted to 35% by adding 115 mL of water. Extraction of the 35% aqueous methanol portion with 500 mL of methylene chloride gave 2.29 g of a gum, which was subjected to gel filtration on a column (2.5 cm × 180 cm) of Sephadex LH-20 with 1:1 methylene chloride–isopropanol. The inflammatory activity, which was monitored by a mouse ear test, appeared in a fraction that was eluted between 545 and 745 mL. Further purification of the 1.38 g of material in this fraction was achieved by reverse-phase low-pressure acetonitrile–water chromatography (LC) on a C-18 column (Partisil-10 ODS) with acetonitrile–water (7:3), followed by LC on a CN column (Bondapak-CN) with 8% ethyl acetate in water to give 100 mg of debromoaplysiatoxin and 25 mg of aplysiatoxin.

C. Oscillatoxin A and Other Toxins. A mixture of cyanophytes, identified as *Schizothrix calcicola* and *Oscillatoria nigroviridis*, was collected on the seaweed side of Enewetak Island, Enewetak Atoll in September, 1975. The wet seaweed (8 kg) was extracted with CH_2Cl_2/MeOH to give 14.5 g of extract. Chromatography of the extract (13.9 g) on a 20 cm × 3.7 cm column of Florisil with 75% hexane/$CHCl_3$, 50% hexane/$CHCl_3$, and $CHCl_3$ resulted in two toxic fractions A (1.30 g) and B (0.77 g). Gel filtration of fraction A (1.18 g) on Sephadex LH-20 with 1:1 MeOH/$CHCl_3$ gave 0.48 g of debromoaplysiatoxin followed by a mixture of toxins which could be separated by HPLC on Porasil A with 15% CH_3CN in $CHCl_3$ into 3 mg of 19-bromoaplysiatoxin (**6**), 7 mg of a 1:1 mixture of 17-bromooscillatoxin A (**4**)[5] and 17,19-dibromooscillatoxin A (**5**),[5] and 86 mg of oscillatoxin A (**3**). Gel filtration of fraction B (0.67 g) on a 2.5 cm × 1.9 cm column of Sephadex LH-20 with 1:1 MeOH/$CHCl_3$ gave 440 mg of a toxic fraction (eluted from 241 mL to 353 mL after introduction onto the column) which was subjected to HPLC on Porasil A with 30% CH_3CN in $CHCl_3$ to give 220 mg of oscillatoxin A (**3**), $[\alpha]_D$ +67±10° (EtOH, *c* 0.12).

Bromination of Debromoaplysiatoxin (2). To a solution of 50 mg (0.086 mmol) of debromoaplysiatoxin in 1 mL of methanol at room temperature was added 50 mL of 0.025 M sodium phosphate in 3:2 H_2O/MeOH buffered at pH 6 followed by 200 μL of 0.86 M bromine in saturated aqueous sodium bromide. The pH was adjusted to 8 and the mixture was extracted with methylene chloride. ^1H NMR analysis of the reaction product indicated that the major components were **1** and **8** with minor amounts of **6**, **7**, and unreacted **2**. Separation was achieved on a reverse-phase HPLC on Whatman Partisil M9 10/5 ODS-3 column with 4:1 MeOH/H_2O. Recovered **2** (5 mg) was eluted first, followed by 19-bromodebromoaplysiatoxin (**7**, 3 mg), aplysiatoxin (**1**, 16 mg), and 19,21-dibromoaplysiatoxin (**8**, 20 mg). 19-Bromoaplysiatoxin (**6**, 2.5 mg) was then eluted in the methanol flush.

The yield of **6** increased significantly if the bromination was carried out at pH 5.5. It was important to adjust the pH to 8 before workup of the reaction mixture. When the bromination was carried out at pH 5 on 35 mg of **2** with 140 μL of 0.86 M bromine and the reaction mixture worked up without adjusting the pH to 8, anhydrotoxins were produced. Separation by HPLC on a Whatman Partisil M9 10/50 column with 3:1 dichloromethane/ethyl acetate gave 19,21-dibromoanhydroaplysiatoxin (**12**, 11 mg), which was eluted first, followed by 19-bromoanhydroaplysiatoxin (**11**, 7 mg), anhydroaplysiatoxin (**10**, 1 mg), and anhydrodebromoaplysiatoxin (**9**, 10 mg).

When 300 μL of the bromine solution was added, **8** was obtained as the sole product (21 mg from 30 mg of **2** after HPLC). Compound **8** crystallized slowly from aqueous methanol as mica-like plates, mp 113–116 °C.

Methylation of Debromoaplysiatoxin. An ethereal solution of **2** was treated with excess diazomethane at 0 °C overnight. The monomethyl ether was purified by HPLC on a Bondapak CN column with 8% EtOAc in hexane. ^1H NMR (acetone-d_6) δ 0.722 (d, *J* = 6.8 Hz, Me on C(10)), 0.765 (s, Me on C(6)), 0.800 (d, *J* = 6.7 Hz, Me on C(12)), 0.835 (s, Me on C(6)), 0.876 (d, *J* = 6.6 Hz, Me on C(4)), 1.129 (d, *J* = 6.5 Hz, Me on C(30)), 3.176 (s, OMe on C(15)), 3.804 (s, OMe on C(20)).

Acetylation of Debromoaplysiatoxin. A solution of 19 mg of **2** and 4 μL of acetic anhydride in 1 mL of dry pyridine was allowed to stand at room temperature overnight. The volatile components were removed by evaporation in vacuo and the residual gum was subjected to HPLC on Bondapak CN with 6% EtOAc in hexane to give 4.6 mg of debromoaplysiatoxin 20,30-diacetate (**14**) and 7.0 mg of debromoaplysiatoxin 20-acetate. **14**: ^1H NMR (benzene-d_6) δ 0.657 (d, *J* = 6.9 Hz, Me on C(10)), 0.772 (s, equatorial Me on C(6)), 0.805 (d, *J* = 6.8 Hz, Me on C(12)), 0.830 (d, *J* = 6.3 Hz, Me on C(4)), 0.956 (s, axial Me on C(6)), 0.963 (d, *J* = 6.5 Hz, Me on C(30)), 1.35 (m, C(10) H), 1.390 (dd, *J* = −14.7 and 3.5 Hz, axial H on C(8)), 1.485 (m, C(12) H), 1.503 (s, OAc on C(30)), 1.58–1.72 (complex multiplet, C(4) H, 2 H on C(5) and 2 H on C(13)), 1.773 (s, OAc on C(20)), 1.895 (m, C(14) H), 2.11 (m, C(14) H), 2.287 (d, *J* = −12.2 Hz, C(2) H), 2.452 (d, *J* = −12.2 Hz, C(2) H), 2.460 (dd, *J* = −18.0 and 2.3 Hz, C(28) H), 2.645 (dd, *J* = −18.0 and 11.6 Hz, C(28) H), 2.738 (dd, *J* = −14.7 and 2.9 Hz, equatorial H on C(8)), 3.12 (s, OCH_3), 4.037 (dd, *J* = 10.8 and 2.2 Hz, C(11) H), 4.064 (dd, *J* = 7.4 and 5.0 Hz, C(15) H), 4.187 (d, *J* = 1.4 Hz, OH on C(3)), 4.973 (qd, *J* = 6.5 and 3.6 Hz, C(30) H), 5.238 (br q, *J* ~ 3.1 Hz, C(9) H), 5.309 (ddd, *J* = 11.6, 3.6, and 2.3 Hz, C(29) H), 7.008 (ddd, *J* = 7.9, 2.4, and 1.3 Hz, C(17) H), 7.149 (t, *J* ~ 7.7 Hz, C(18) H), 7.230 (dt, *J* = 7.6 and ~1.4 Hz, C(19) H), 7.387 (dd, *J* = 2.4 and 1.5 Hz, C(21) H). ^1H NMR of debromoaplysiatoxin 20-acetate (acetone-d_6): δ 0.725

(14) Bax, A. "Two-Dimensional Nuclear Magnetic Resonance in Liquids"; Delft University Press: Delft, Holland, 1982.
(15) Hecker, E. In "Methods in Cancer Research"; Busch, H., Ed.; Academic Press: New York, 1971; Vol. 6, p 439.

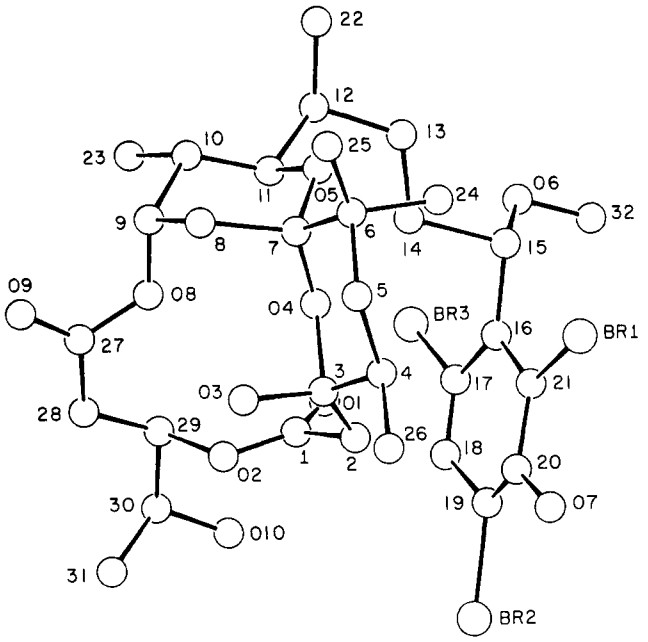


Figure 2. A computer generated perspective drawing of the current X-ray model of 19,21-dibromoaplysiatoxin (**8**). Hydrogens are omitted for clarity.

(d, J = 7.0 Hz, Me on C(10)), 0.773 (s, Me on C(6)), 0.805 (d, J = 7.0 Hz, Me on C(12)), 0.840 (s, Me on C(6)), 0.886 (d, J = 6.3 Hz, Me on C(4)), 1.130 (d, J = 6.6 Hz, Me on C(30)), 2.262 (s, OAc on C(20)), 3.194 (s, OMe on C(15)).

Acid Hydrolysis of 9 and 13. A solution of 36 mg of anhydrodebromoaplysiatoxin (**9**) in 5 mL of 0.5 M HCl containing sufficient ethanol to give a homogeneous soltuion was refluxed for 1 h. The ethanol was removed in vacuo and the concentrate was freeze-dried. The residue was subjected to short-path distillation (150 °C (0.1 mm)) to give 5 mg of **18** as a colorless oil. Further purification of **18** was accomplished by HPLC on Whatman partisil with EtOAc: $[\alpha]_D$ +75° (EtOH, c 0.25); $[\Theta]_{220}$ +950° (EtOH); ^{1}H NMR ($CDCl_3$) δ 4.561 (dq, J = 6.5 and 3.7 Hz, H on C(5)), 4.440 (ddd, H on C(4)), 2.795 (dd, J = −17.8 and 5.3 Hz, H on C(3)), 2.566 (dd, J = −17.8 and 0.7 Hz, H on C(3)), 1.433 (d, J = 6.5 Hz, Me on C(5)).

Similar acid hydrolysis of anhydrooscillatoxin A (**13**) and workup by short-path distillation gave 3.6 mg of **19**. Without HPLC purification the crude **18**, which appeared clean by ^{1}H NMR analysis, had the following results: $[\alpha]_D$ +44° (EtOH, c 0.36); ^{1}H NMR ($CDCl_3$) δ 4.687 (m, H on C(4)), 4.410 (dd, J = −10.2 and 4.5 Hz, H on C(5)), 4.292 (dt, J = −10.2 and 1 Hz, H on C(5)), 2.745 (dd, J = −18.0 and 6.0 Hz, H on C(3)), 2.524 (dt, J = −18.0, 2, and 1 Hz W coupling to H on C(5), H on C(3)).

Synthesis of (4*S*)-Hydroxy-2(3*H*)-dihydrofuranone. Methyl malate (6.1 g) was partially reduced with lithium aluminum hydride (0.9 g) in ether. The mixture was acidified to pH 6 with HCl and the ether extract was separated by HPLC on Whatman partisil with 4% MeOH in EtOAc to give methyl 3,4-dihydroxybutanoate which was hydrolyzed in 0.5 M HCl after a 10 min reflux to 3,4-dihydroxybutanoic acid. The dihydroxy acid was subjected to a short-path distillation (120 °C (0.1 mm)) to give the γ-lactone, $[\alpha]_D$ −72° (EtOH, c 0.8).

Reduction of 9 to (3*R*,4*R*)-1,3,4-Triacetoxypentane. Compound **9** (6 mg) in dry ether (5 mL) was treated with excess lithium aluminum hydride. Normal workup and acetylation with acetic anhydride/pyridine gave an oil which was subjected to short-path distillation (140 °C (0.1 mm)) to give 9.8 mg of the triacetate, $[\alpha]_D$ +22° (EtOH, c 1.0); $[\Theta]_{208}$ +95° (EtOH).

Single-Crystal X-ray Diffraction Analysis of 19,21-Dibromoaplysiatoxin (8). Large crystals of 19,21-dibromoaplysiatoxin (**8**) were grown slowly from methanol–water solutions at 20–25 °C (Figure 2). A single crystal and some mother liquor were carefully transferred to a Lindemann capillary which was subsequently sealed. A variety of crystals and experimental conditions were employed, but the crystal ultimately used was roughly rectangular with dimensions 0.6 × 0.5 × 0.3 mm. Preliminary X-ray photographs showed only triclinic symmetry. Accurated lattice constants of a = 9.859 (5) Å, b = 9.929 (5) Å, c = 11.351 (7) Å, α = 101.132 (44)°, β = 112.072 (41)°, and γ = 74.941 (39)°. This would be consistent with space group $P1$, the only possible space group for an optically active material in the triclinic system, with one molecule of composition $C_{32}H_{45}Br_3O_{10}$ forming the asymmetric unit. All unique diffraction maxima with $2\Theta \leq 114°$ were collected on a computer-controlled four-circle diffractometer using graphite monochromated Cu Kα radiation (1.54178 Å) and a variable speed 1° ω scan. The minimum scan rate was 1.5°/min and backgrounds were collected for 75% of the total scan time. There was a deterioration of periodically monitored check reflections of approximately 10% which was not corrected for. Of the 1717 reflections surveyed in this fashion, 1417 (83%) were judged observed after correction for Lorentz, polarization, and background effects. A phasing model was achieved by heavy atom methods.[16] An $E^2 - 1$ Patterson synthesis was deconvoluted to yield the three bromine positions and a Br-phased $2F_o - F_c$ synthesis revealed 42 of the 45 non-hydrogen atoms. The remaining heavy atoms were located in a subsequent synthesis. Hydrogen atoms were generally not visible in $F_o - F_c$ syntheses and have been included at calculated positions. Block-diagonal, least-squares refinements with anisotropic non-hydrogen atoms have converged to a current crystallographic residual of 0.0965 for the observed reflections. Further attempts at refinement by using geometrical constraints are currently underway. Additional crystallographic details are available and are described in the supplementary material paragraph.

Acknowledgment. This research was supported by PHS Grant CA12623-10, awarded by the National Cancer Institute, Department of Health and Human Services. ^{1}H NMR studies at 300 MHz were made possible by Grant CHE81-00240 from the National Science Foundation.

Registry No. **1**, 52659-57-1; **2**, 52423-28-6; **2** (20-acetate), 90359-05-0; **3**, 66671-95-2; **4**, 66648-16-6; **5**, 66648-17-7; **6**, 66648-18-8; **7**, 90414-33-8; **8**, 90359-02-7; **9**, 63543-22-6; **10**, 90359-03-8; **11**, 90414-34-9; **12**, 90414-35-0; **13**, 90359-04-9; **14**, 58077-31-9; **15**·HCl, 329-56-6; **16**·HCl, 69787-04-8; **18**, 38996-24-6; **19**, 58081-05-3; (*S*)-1-phenylethanol, 1445-91-6; (*S*)-1-methoxy-1-phenylethane, 2511-06-0; (*R*)-phenylethane-1,2-diol, 16355-00-3; methyl (*S*)-malate, 66178-02-7; methyl (*S*)-3,4-dihydroxybutanoate, 90414-36-1; (*S*)-3,4-dihydroxybutanoic acid, 51267-44-8; (3*R*,4*R*)-1,3,4-triacetoxypentane, 90414-37-2.

Supplementary Material Available: 300-MHz ^{1}H NMR spectra of aplysiatoxin in acetone-d_6; a table of circular dichroism spectral data for compounds **15**, **16**, (*S*)-(−)-1-phenylethanol, (*S*)-(−)-1-methoxy-1-phenylethane, and (*R*)-(−)-phenylethane-1,2-diol; a figure of the CD spectra for the latter three compounds; tables of fractional coordinates and thermal parameters for 19,21-dibromoaplysiatoxin (**8**) (4 pages). Ordering information is given on any current masthead page.

(16) All crystallographic calculations were done on a Prime 850 computer operated by the Cornell Chemistry Computing Facility. Principal programs employed were as follows: REDUCE and UNIQUE, data reduction programs by M. E. Leonowicz, Cornell University, 1978; MULTAN 78, a system 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, 1978; BLS78A, an anisotropic block-diagonal least-squares refinement written by K. Hirotsu and E. Arnold, Cornell University, 1980; CRYSTALS, a crystallographic system written by D. J. Watkin and J. R. Carruthers, Chemical Crystallography Laboratory, University of Oxford, 1981; PLUTO78, a crystallographic illustration program by W. D. S. Motherwell, Cambridge Crystallographic Data Centre, 1978; BOND, a program to calculate molecular parameters and prepare tables written by K. Hirotsu, Cornell University, 1978.