Hapalindoles, Antibacterial and Antimycotic Alkaloids from the Cyanophyte *Hapalosiphon fontinalis*

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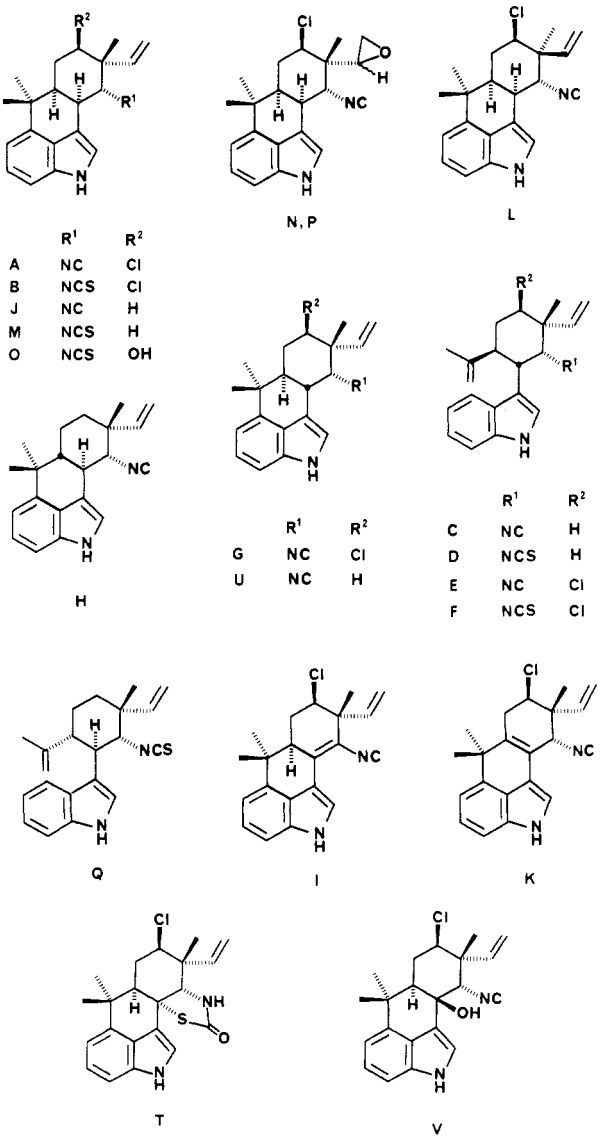
Eighteen new indole alkaloids, hapalindoles C–Q and T–V, have been isolated from the cultured cyanophyte *Hapalosiphon fontinalis*. The gross structures and relative stereochemistries of these 18 minor hapalindoles have been determined by using spectral methods. The relative stereochemistries of hapalindoles A, D, and K have been confirmed and the absolute configuration of hapalindole K has been established as 11*R*,12*R*,13*R* by X-ray crystallography. Assuming that all of the alkaloids have the same absolute stereochemistry as K, all of the hapalindoles isolated to date are 11*R* and all of the chlorine-containing ones are 13*R*. With the exception of hapalindole L, all of the hapalindoles are 12*R*. Hapalindoles A and B, the major alkaloids in the blue-green alga, and several of the minor hapalindoles are 10*R*,15*S*. Some of the alkaloids, however, viz. C–G and U, are 10*S*,15*S* and two, viz. H and Q, are 10*R*,15*R*. Most of the hapalindoles are tetracyclic, but C–F and Q are tricyclic; none of the tricyclic hapalindoles isolated to date, however, are 10*R*,15*S*. Hapalindole T is an unusual thiocarbamate and hapalindole V is 10-hydroxyhapalindole G.

Recently we have described the structure of hapalindole A, a novel chlorine- and isonitrile-containing indole alkaloid which is responsible for most of the antibacterial and antimycotic activity associated with the blue-green alga *Hapalosiphon fontinalis*.[1] Minor amounts of several other active indoles are present in this cyanophyte, such as the corresponding isothiocyanate hapalindole B.[1] In this paper we report the structures of 18 more minor indoles, viz. hapalindoles C–Q and T–V.

Isolation and Characterization. *Hapalosiphon fontinalis* (Ag.) Bornet (Stigonemataceae) was isolated from a soil sample collected in the Marshall Islands in 1982 and mass cultured in the laboratory. The freeze-dried alga was extracted with 1:1 dichloromethane/2-propanol and the extract was subjected to rapid chromatography on silica gel with 1:1 heptane/CH_2Cl_2, chloroform, and CH_2Cl_2/ethyl acetate. The resulting fractions were then separated by HPLC on silica gel into the hapalindoles. The hapalindoles were eluted in the following order by using 5:1 to 5:2 isooctane/tetrahydrofuran: D, F, H, I, C, E, Q, M, K, J, L, U, B, G, A, O, V, T, N, and P.

The EI mass spectra exhibit intense molecular ions or 3:1 chlorine-containing ion clusters at m/z 304 ($C_{21}H_{24}N_2$) for hapalindoles C, H, J, and U; m/z 336 ($C_{21}H_{24}N_2S$) for hapalindoles D, M, and Q; m/z 336, 338 ($C_{21}H_{21}N_2Cl$) for hapalindoles I and K; m/z 338, 340 ($C_{21}H_{23}N_2Cl$) for hapalindoles A, E, G, and L; m/z 352 ($C_{21}H_{24}N_2SO$) for hapalindole O; m/z 354, 356 ($C_{21}H_{23}N_2OCl$) for hapalindoles N, P, and V; m/z 370, 372 ($C_{21}H_{23}N_2ClS$) for hapalindoles B and F; and m/z 386, 388 ($C_{21}H_{23}N_2SOCl$) for hapalindole T. In the IR spectra all of the hapalindoles show a sharp band at 3480 cm^{-1} for the indole NH. Thirteen of the new alkaloids, viz. hapalindoles C, E, G–L, N, P, Q, U, and V, also show a single, sharp band at 2140 cm^{-1} for an isonitrile group, whereas four other compounds, viz. hapalindoles D, F, M, and O, show two strong peaks at 2080, 2160 cm^{-1} for an isothiocyanate group. Hapalindole T, however, shows neither of these IR absorptions but instead another NH peak at 3390 cm^{-1} and an intense carbonyl band at 1679 cm^{-1}. All of the compounds, except hapalindoles I and K, exhibit UV spectra typical of indoles: for example, λ_{max} nm (ϵ) are observed at 222 (38 700), 280 (7000), and 291 (5700) for hapalindole J, one of the isonitriles, and at 223 (41 400), 281 (7300), and 291 (5900) for hapalindole M,

Chart I



(1) Moore, R. E.; Cheuk, C.; Patterson, G. M. L. *J. Am. Chem. Soc.* **1984**, *106*, 6456.

0022-3263/87/1952-1036$01.50/0

Table I. ^{1}H NMR Data for Hapalindoles A, B, J, L, M, and O in $CDCl_3$[a]

	A	B	J	M	O	L
1	8.09 br	8.06	8.00 br	7.99	8.06	8.04
2	6.88 t	6.88	6.88 br t	6.88	6.86	6.69
5	6.97 m	6.96	6.92 m	6.96	6.96	6.96
6	7.19 m	7.18	7.17 m	7.17	7.17	7.18
7	7.20 m	7.20	7.18 m	7.18	7.18	7.20
10eq	3.88 br m	3.87	3.85 br m	3.83	3.84	3.85
11eq	4.37 br d	4.53	4.17 br d	4.28	4.45	4.40
13ax	4.36 dd	4.32	1.88 td	1.83	3.99	4.19 ddd
13eq			1.37 br dt	1.40		
14ax	1.47 q	1.49	1.11 br q	1.13	1.12	1.52
14eq	2.14 dtd	2.15	1.72 br dq	1.74	1.91	2.09
15ax	2.32 ddd	2.22	2.10 dt	2.01	2.21	2.30
17	1.55 s	1.56	1.51 s	1.52	1.55	1.55
18	1.19 s	1.20	1.21 s	1.22	1.21	1.21
19	0.88 s	0.87	0.80 s	0.79	0.74	1.50
20	6.10 dd	6.02	6.04 dd	5.99	6.08	5.46
21*E*	5.35 dd	5.32	5.13 br d	5.12	5.36	5.13
21*Z*	5.24 dd	5.12	5.07 br d	5.05	5.26	4.94

[a] J(H,H) in Hz for A: 1,2 = 2; 2,10 = 2; 5,6 = 7.2; 5,7 = 0.6; 6,7 = 8.2; 10,11 = 1.6; 10,14eq = 1.2; 10,15 = 4.6; 13,14ax = 12.4; 13,14eq = 4.0; 14ax,14eq = −13.5; 14ax,15 = 13.0; 14eq,15 = 3.8; 20,21*Z* = 17.4; 20,21*E* = 10.9; 21*E*,21*Z* = 0.5. Most coupling constants for B, L, and O are within ±0.2 Hz of the values reported for A and none deviate more than ±0.7 Hz. Additional J(H,H) for L: 13ax,21*E* = 1. Additional J(H,H) for J: 11,13eq = 1; 13ax,13eq = −13.9; 13ax,14ax = 12.7; 13ax,14eq = 3.9; 13eq,14ax = 3.3; 13eq,14eq = 3.9; 14ax,14eq = −14.0; 14ax,15 = 12.1; 14eq,15 = 3.9. All other coupling constants for J are essentially identical with those for A; similarly coupling constants for M are comparable with values for J and A.

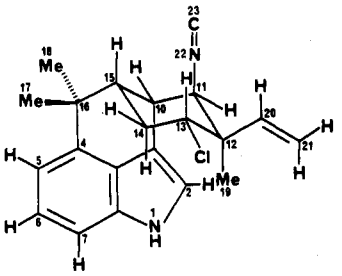
Table II. ^{1}H NMR Data for Hapalindoles N and P in Acetone-d_6[a]

	N	P		N	P
1	8.03 br	8.03	14ax	1.39 q	1.37
2	7.29 br t	7.32	14eq	2.15 dtd	2.15
5	6.93 br dd	6.93	15ax	2.37 ddd	2.39
6	7.10 dd	7.11	17	1.59 s	1.59
7	7.21 br dd	7.21	18	1.20 s	1.20
10eq	3.85 br m	3.92	19	0.57 s	0.56
11eq	4.92 br d	4.81	20	3.10 dd	3.19 br t
13ax	4.56 dd	4.51	21*Z*	2.78 dd	2.79 m
			21*E*	2.70 dd	2.78 m

[a] J(H,H) in Hz for N: 1,2 = 2; 2,10 = 2; 5,6 = 7.1; 5,7 = 0.7; 6,7 = 8.1; 10,11 = 1; 10,14eq = 1.2; 10,15 = 4.8; 13ax,14ax = 12.5; 13ax,14eq = 4.0; 14ax,14eq = −13.2; 14ax,15 = 12.9; 14eq,15 = 12.9; 14eq,15 = 3.6; 20,21*Z* = 2.7; 20,21*E* = 4.2; 21*E*,21*Z* = 4.9. Most coupling constants for P are within ±0.1 Hz of the values reported for N and none deviate more than ±0.5 Hz.

the corresponding isothiocyanate. Hapalindole I, on the other hand, exhibits λ_{max} nm (ϵ) at 224 (25 800), 274 (6700), and 322 (13 400) with a shoulder at 240 (13 000) and hapalindole K shows atypical maxima at 226 (19 300) and 301 (9600).

Structure Determination of Hapalindoles J, L, M, N, O, P, and T. Hapalindole A has the relative and absolute stereochemistry shown in A. We reported its gross



A

structure and relative stereochemistry in a preliminary communication based mainly on ^{1}H NMR studies. Difference NOE spectroscopy was particularly useful in this structure determination. We have now confirmed the relative stereochemistry of hapalindole A by X-ray crystallography (Figure 1). Attempts to determine its absolute stereochemistry through use of the anomalous dispersion technique led to unacceptable results. The absolute stereochemistry of 1, which is depicted in Figure 1, is

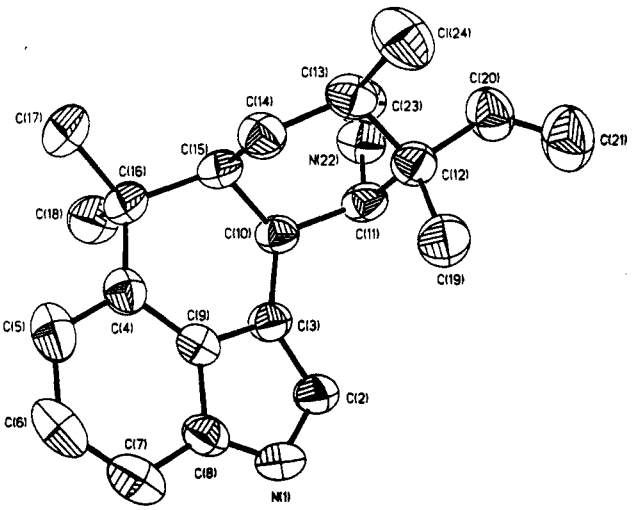


Figure 1. ORTEP stereodrawing of hapalindole A. Hydrogens are omitted for clarity.

10*R*,11*R*,12*R*,13*R*,15*S* since 1 can be converted to hapalindole K (unpublished results), the absolute configuration of which has been solved by X-ray crystallography (see below). Similar NMR arguments were used to arrive at the gross structure and relative stereochemistry of hapalindole B.

Hapalindoles J, M, N, O, and P exhibit ^{1}H NMR spectra that are comparable to those for hapalindoles A and B

Table III. NMR Data for Hapalindole T in $CDCl_3$

$^{13}C^{a,b}$		$^{1}H^{c,d}$	$^{13}C^{a,b}$		$^{1}H^{c,d}$
173.5 s	23		71.3 d	11	4.55 br
142.8 d	20	5.87 dd	61.5 d	13	4.50 dd
137.9 s	8		56.7 s	10	
133.4 s	4		53.1 d	15	2.74 dd
123.9 d	6	7.20 m	44.9 s	12	
123.4 s	9		39.9 s	16	
120.1 d	2	7.25 d	35.1 q	18	1.43 s
116.9 t	21*E*	5.37 br d	33.2 t	14ax	1.53 q
	21*Z*	5.32 br d		14eq	2.31 dt
114.6 d	5	6.99 m	26.2 q	17	1.59 s
111.6 s	3		19.8 q	19	0.69 s
108.8 d	7	7.21 m		1	8.33 br
				22	5.63 br

[a] 75 MHz; $CDCl_3$ as internal reference = 76.9 ppm. [b] Proton–carbon connectivities determined by using a phase-cycled 16-step heteronuclear chemical shift correlation map (CSCM) experiment. [c] 300 MHz; residual $CHCl_3$ as internal reference = 7.25 ppm. [d] J(H,H) in Hz: 1,2 = 2; 5,6 = 7.2; 5,7 = 0.6; 6,7 = 8.2; 11,22 = 1; 13ax,14ax = 12.6; 13ax,14eq = 4.1; 14ax,14eq = −14.0; 14ax,15 = 13.2; 14eq,15 = 4.4; 20,21*Z* = 17.8; 20,21*E* = 11.1; 21*E*,21*Z* = <0.5.

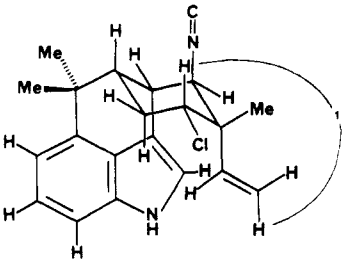
(Tables I and II). Essentially the same ^{1}H NMR signals are seen for the aromatic protons. The signal for H-2 is a broad triplet in all of the spectra, reflecting vicinal coupling to the adjacent NH and long-range coupling to H-10. Spin–spin decoupling studies indicate that H-10 is in either a $CH_{eq}C(10)H_{eq}CH_{ax}CH_2CH_{ax}$ or $CH_{eq}C(10)H_{eq}CH_{ax}CH_2CH_2$ unit located in a six-membered ring. In all of the spectra W-coupling (1.2 Hz) is observed between the equatorial protons on C-10 and C-14. In the spectra of hapalindoles J and M (chloro substituent at C-13 is missing), W-coupling (1 Hz) is also seen between the equatorial protons on C-11 and C-13. The signal for H-11 is broadened for hapalindoles J, N, and P due to small coupling to the isonitrile nitrogen; this signal, however, is much sharper for hapalindoles M and O which are isothiocyanates. Chemical shifts and coupling constants characteristic of a vinyl group are seen in the spectra of hapalindoles J, M, and O, but these are replaced with ones characteristic of a monosubstituted epoxide in the spectra of hapalindoles N and P.

The relative stereochemistry at C-12 for hapalindoles J, M, N, O, and P is indicated from the following difference NOE experiment. Irradiation of the signal for the methyl group on C-12 produces strong positive NOEs in the H-2, H-11, H_{ax}-14, and *Z* H-21 signals. This means that the methyl group on C-12 has to be axially disposed for these five compounds. Furthermore the NOEs on H-2 and H-11 confirm that C-3 is attached axially to C-10 and that N-22 is connected axially to C-11. Positive NOEs similar to the ones observed for hapalindoles A and B are obtained when the methyl groups on C-16 are irradiated. Positive NOEs are seen in the H-5, H_{eq}-14, and H-15 signals when 3H-17 is irradiated and in the H-10 and H-15 signals when 3H-18 is irradiated.[2] The data above show that hapalindoles J, M, N, O, and P have the same relative stereochemistry at C-10, C-11, C-12, C-13, and C-15 as hapalindoles A and B.

Hapalindoles N and P are epimeric at C-20, but at this writing the stereochemistry of the two isomeric epoxides has not been established.

The absolute configurations of C-10, C-11, C-12, C-13, and C-15 in hapalindoles B, J, M, N, O, and P are probably the same as in hapalindole A, viz. 10*R*,11*R*,12*R*,13*R*,15*S* for hapalindoles B, N, O, and P and 10*R*,11*R*,12*R*,15*S* for hapalindoles J and M. All of these compounds, except hapalindole J, are levorotatory. The optical rotations of hapalindoles A and B are −78° and −194°, respectively, whereas the $[\alpha]_D$ for hapalindoles J and M are +54° and −83°, respectively. Interestingly, there appears to be a 125 ± 15° difference between the $[\alpha]_D$ of the isonitrile and the corresponding isothiocyanate.

Hapalindole L differs from hapalindole A only at C-12. Except for chemical shift differences (Table I), the ^{1}H NMR spectra of hapalindoles L and A are very similar.



L

The H-13 signal, however, shows an additional coupling, viz. a long-range zig–zag coupling (1 Hz) to the *E* proton on C-21. As expected, irradiation of the Me group on C-12 produces positive NOEs in the H-11, H_{ax}-13, H-20, and *Z* H-21 signals but none in the H-2 and H_{ax}-14 signals. NOEs are not observed in the H-2 and H_{ax}-14 signals when H-20 is irradiated, but a small positive NOE is observed in the H-2 signal when H-11 is irradiated and conversely in the H-11 signal when H-2 is irradiated. As for hapalindole A, positive NOEs are seen in the H-5 and H_{eq}-14 signals when 3H-17 is irradiated and in the H-10 signal when 3H-18 is irradiated. Hapalindole L is levorotatory, $[\alpha]_D$ −74°, and exhibits essentially the same optical rotation as hapalindole A, suggesting that its absolute stereochemistry is 10*R*,11*R*,12*S*,13*R*,15*S*.

The ^{1}H NMR spectrum of hapalindole T (Table III) shows signals for all of the protons found in hapalindole A but lacks one for H-10. The H-2 signal is a doublet, showing coupling (2 Hz) to the indole NH only, and the H-15 signal is a doublet of doublets, reflecting axial–axial coupling (13.2 Hz) and axial–equatorial coupling (4.4 Hz) to the C-14 methylene protons. In addition to the indole NH peak, another exchangeable proton signal is present at δ 5.63 which is attributed to an NH attached to C-11 since coupling (1 Hz) is observed between this signal and the one for H-11 and positive NOEs are seen in the H-11, H-13, and H-20 signals when this NH is irradiated in a difference NOE experiment. The elements of COS have to bridge C-10 and the NH on C-11 to account for the molecular composition. The most logical structure is a thiocarbamate ester C(10)SCONHC(11) where sulfur (not oxygen) is connected to C-10. The IR data support this

(2) 3H-17 and 3H-18 refer to the two methyl groups on C-16 and 3H-19 refers to the methyl group on C-12.

Table IV. ^{1}H NMR Data for Hapalindoles G, U, V, and H in $CDCl_3$

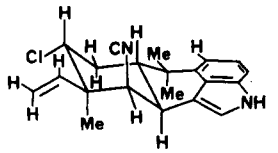
	G[a]	U[a]	V[a]	H[b]
1	8.04 br	8.02 br	8.14 br	8.04 br
2	6.89 dd	6.90 t	7.07 d	7.62 dd
5	7.04 m	7.02 m	7.10 dd	7.02 m
6	7.18 m	7.17 m	7.24 t	7.18 m
7	7.20 m	7.18 m	7.18 dd	7.19 m
10ax	3.32 br dm	3.27 br dm		3.17 td
11ax				3.51 br dtd
11eq	4.24 br d	4.09 br d	4.11 br s	
13ax	4.43 dd	1.56–1.71 m	4.40 dd	1.38 td
13eq		1.56–1.71 m		2.07 dt
14ax	2.01 dt	1.60 m	2.51 m	1.66 qd
14 eq	2.41 ddd	1.92–2.05 m	2.24–2.34 m	1.79 dq
15ax	2.11 ddd	1.91 td	2.24–2.34 m	1.52 ddd
17	1.17 s	1.14 s	1.36 s	1.45 s
18	1.52 s	1.49 s	1.52 s	1.11 s
19	1.39 s	1.27 s	1.57 s	1.28 s
20	6.14 dd	6.04 dd	6.09 dd	6.29 ddd
21*E*	5.39 dd	5.18 br d	5.36 br d	5.36 ddd
21*Z*	5.34 dd	5.16 br d	5.33 br d	5.29 dd

[a] J(H,H) in Hz for G: 1,2 = 2.2; 2,10 = 1.6; 5,6 = 7.2; 5,7 = 0.6; 6,7 = 8.2; 10,11 = 3.1; 10,15 = 10.4; 13,14ax = 11.9; 13,14eq = 4.5; 14ax,14eq = −12.5; 14ax,15 = 11.9; 14eq,15 = 3.0; 20,21*E* = 10.9; 20,21*Z* = 17.4; 21*E*,21*Z* = 0.5. Most coupling constants for U and V are within ±0.2 Hz of the values for G. The OH on C10 signal for V was not observed. [b] J(H,H) for H: 1,2 = 2.2; 2,10 = 1.6; 5,6 = 7.2; 5,7 = 0.6; 6,7 = 8.2; 10,11 = 10.8; 10,15 = 11.0; 11,21*E* = 0.4; 13ax,13eq = −13.9; 13ax,14ax = 13; 13ax,14eq = 3.5; 13eq,14ax = 3.2; 13eq,14eq = 3.5; 13ax,20 = 0.5; 14ax,14eq = −13.2; 14ax,15 = 12.0; 14eq,15 = 3.5; 20,21*E* = 11.1; 20,21*Z* = 17.5; 21*E*,21*Z* = 1.1.

structure and rules out the C(10)OCSNHC(11) possibility.[3] The ^{13}C NMR spectrum (Table III) is also consistent with the C(10)SCONHC(11) structure since an amide carbonyl signal is exhibited at δ 173.5 and the quaternary C-10 signal is found at δ 56.7.

Difference NOE experiments indicate hapalindole T has the same relative stereochemistry as hapalindole A. Strong positive NOEs are observed in the H-2, H-11, H_{ax}-14, H-20, and *Z* H-21 signals when 3H-19 is irradiated, proving that the methyl group on C-12 is axial and furthermore that the vinyl group is attached to C-12. Similarly NOEs are seen in the H-5, H_{eq}-14, and H-15 signals when 3H-17 is irradiated and in the H-15 signal when 3H-18 is irradiated.

Hapalindoles G, H, U, and V. Hapalindoles G and U show proton NMR signals for the aromatic protons that have chemical shifts virtually identical with those for hapalindole A (Table IV). Further inspection of the spectrum of hapalindole G coupled with difference NOE spectroscopy shows that the relative stereochemistry of C-11, C-12, C-13, and C-15 is the same as that shown by hapalindole A. Vicinal coupling constants indicate that the protons on C-10, C-13, and C-15 are axial ($J_{10,15}$ = 10.4; $J_{13,14ax}$ = $J_{14ax,15}$ = 11.9 Hz) and the proton on C-11 is equatorial ($J_{10,11}$ = 3.1 Hz). Irradiation of the signal for the methyl group on C-12 shows appreciable NOEs in the H-10, H-11, H_{ax}-14, H-20, and *Z* H-21 signals but none in the C-2 signal, indicating that this methyl group is axial and furthermore that the vinyl group is also on C-12 and equatorially disposed. Irradiation of the 3H-17 signal shows prominent NOEs in the H-10 and H_{ax}-14 signals, whereas irradiation of the 3H-18 signal produces strong NOEs in the H-5, H_{eq}-14, and H-15 signals. The relative stereochemistry of hapalindole G is therefore 10*S**,11*R**,12*R**,13*R**,15*S**.

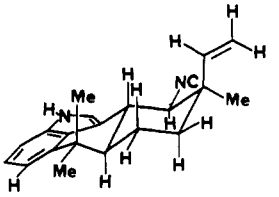


G

Analysis of the ^{1}H NMR spectrum of hapalindole U (Table IV) indicates that H-10 and H-15 are axial and H-11 is equatorial. Irradiation of the signal for the methyl group on C-12 produces prominent NOEs in the H-10, H-11, H_{ax}-14, H-20, and *Z* H-21 signals, again indicating that the C-19 methyl group is axial. Irradiation of the 3H-17 signal induces an appreciable NOE in the H-10 signal and irradiation of the 3H-18 signal causes NOEs in the H-5 and H-15 signals. Hapalindole U is therefore the dechloro analogue of hapalindole G.

Inspection of the ^{1}H NMR spectrum of hapalindole V (Table IV) indicates that the H-10 signal is missing. The H-2 signal is a doublet and the H-11 signal is a broad singlet, consistent with the lack of a proton on H-10. The rest of the NMR spectrum is essentially the same as the one for hapalindole G, except for the appreciable differences in chemical shift. Since the IR spectrum shows that a hydroxyl group is present, an OH must be present on C-10. Hapalindole V has to be 10-hydroxyhapalindole G since this stereochemistry explains the chemical shifts and the G-like NOEs that are observed when the three methyl groups are irradiated. The signals for H_{ax}-14 and the axial methyl groups on C-12 and C-16, for example, are shifted downfield 0.2–0.5 ppm by the axial OH on C-10, but the signal positions of H_{eq}-14, the equatorial vinyl on C-12, and the equatorial methyl on C-16 are paramagnetically displaced only 0.01–0.1 ppm.

In the ^{1}H NMR spectrum of hapalindole H (Table IV), vicinal coupling constants indicate that H-10, H-11, and H-15 are axial ($J_{10,11}$ = 10.8; $J_{10,15}$ = 11.0 Hz). The chemical shifts of the protons on the benzenoid portion



H

(3) Pretsch, E.; Clerc, T.; Seibl, J.; Simon, W. *Tables of Spectral Data for Structure Determination of Organic Compounds*; Springer-Verlag: Berlin, 1983; p I160.

Table V. ^{1}H NMR Data for Hapalindoles C, D, E, F, and Q in $CDCl_3$[a]

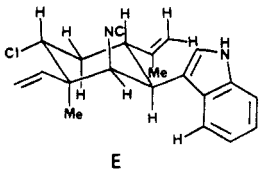
	C	D	E	F	Q
1	8.12 br	8.11	8.12 br	8.15	7.99 br
2	7.19 br d	7.10	7.17 br	7.08	6.99 d
4	7.47 dm	7.48	7.44 ddt	7.47	7.63 br d
5	7.12 ddd	7.13	7.13 ddd	7.14	7.16 td
6	7.19 ddd	7.20	7.20 ddd	7.21	7.08 td
7	7.37 ddd	7.37	7.38 ddd	7.38	7.34 br d
10ax	3.55 dd	3.58	3.61 br dd	3.65	3.12 br t
11eq	3.66 br m	3.82	3.81 br d	4.03	
11ax					3.86 br d
13ax	1.55 m	1.58	4.46 dd	4.41	1.5–1.62 m
13eq	2.06 m	2.00			1.99 dt
14ax	1.83 m	1.84	2.15 dt	2.17	1.82 qd
14eq	1.78 m	1.80	2.25 ddd	2.26	1.5–1.62 m
15ax	2.87 m	2.82	3.06 td	3.02	2.76 br m
17	1.54 dd	1.54	1.55 dd	1.55	1.49 br s
18*E*	4.66 p	4.67	4.72 p	4.72	4.49 br
18*Z*	4.81 br dq	4.83	4.85 dq	4.87	4.50 br
19	1.37 s	1.35	1.48 s	1.47	1.22 s
20	5.92 dd	5.87	6.05 dd	5.98	6.21 dd
21*E*	5.12 m	5.11	5.30 dd	5.28	5.36 br d
21*Z*	5.11 m	5.10	5.25 dd	5.23	5.27 dd

[a] J(H,H) in Hz for E: 1,2 = 2.2; 2,10 = 0.3; 4,5 = 7.7; 4,6 = 1.2; 4.7 = 0.8; 5,6 = 7.1; 5,7 = 1.1; 6,7 = 8.1; 10,11 = 2.9; 10,15 = 12.1; 13,14ax = 12.1; 13,14eq = 5.0; 14ax,14eq = −13.7; 14ax,15 = 12.1; 14eq,15 = 4.3; 17,18*Z* = 0.8; 17,18*E* = 1.5; 18*E*,18*Z* = 1.5; 20,21*Z* = 17.5; 20,21*E* = 10.9; 21*E*,21*Z* = 0.3. Most coupling constants for F are within ±0.2 Hz of the values reported for E and none deviate more than ±0.7 Hz. Additional J(H,H) for C: 11,13eq = 1.5; 13ax,13eq = −13.9; 13eq,14ax = 3.8; 14ax,14eq = −13.9; 14ax,15 = 12.0; 14eq,15 = 3.3. All other coupling constants for C are essentially identical with those for E; similarly coupling constants for D and Q are comparable with values for C and E. Additional J(H,H) for Q: 10,11 = 11.0; 10,15 = 11.0; 11,21*E* = 0.5; 13ax,14ax = 13; 13ax,14eq = 3; 13eq,14eq = 3; 13ax,20 = 0.5.

of the indole system are essentially the same as those in all of the other hapalindoles; H-2, however, resonates at much lower field due to the close proximity of the equatorial isocyano group on C-11. On C-12 the methyl group is equatorial and the vinyl group is axial. In support of this stereochemistry, which is similar to that in hapalindole L, long-range zig–zag coupling (0.4 Hz) is observed between H-11 and *E* H-21 and W-coupling (0.5 Hz) is seen between H_{ax}-13 and H-20. These small couplings presumably result from a conformation of the vinyl group in which H-20 is oriented toward C-11 and C-21 points toward C-13. The relative stereochemistry of hapalindole H therefore appears to be 10*R**,11*S**,12*R**,15*S**. This stereochemistry is further supported by difference NOE spectroscopy. Irradiation of the 3H-17 and 3H-18 signals show strong positive NOEs in the H-5 and H-10 signals, respectively, whereas irradiation of the 3H-19 signal produces strong NOEs in the H-11, H-20, and *Z* H-21 signals.

Hapalindoles C, D, E, F, and O. The ^{1}H NMR spectra of hapalindoles C–F and Q (Table V) show aromatic signals for four adjacent protons. Only two methyl signals are present in each spectrum, but there are two additional 1H quintets for a terminal methylene group. The multiplicities reflect geminal coupling between *E* H-18 and *Z* H-18 (1.5 Hz) and allylic coupling of *E* H-18 and *Z* H-18 to the C-17 methyl group (1.5 and 0.8 Hz, respectively).

Vicinal coupling constants for hapalindoles C and D indicate that the protons on C-10, C-13, and C-15 are axial ($J_{10,15}$ = 12.1; $J_{13,14ax}$ = $J_{14ax,15}$ = 12.1 Hz) and the proton on C-11 is equatorial ($J_{10,11}$ = 2.9 Hz). Similarly for hapalindoles E and F the protons on C-10 and C-15 are axial and the proton on C-11 is equatorial. As in the spectra



E

for all of the other isonitrile-containing hapalindoles, the H-11 signal is broad for hapalindoles C and E due to small coupling to the isonitrile nitrogen. The H-11 signal for hapalindoles D and F, however, is much sharper and is shifted downfield about 0.2 ppm. The methyl group on C-12 in hapalindoles C–F is axial since irradiation causes strong positive NOEs in the H-10, H-11, H_{ax}-14, and *Z* H-21 signals.

The relative stereochemistry of hapalindoles C–F is supported by other difference NOE experiments. Irradiation of 3H-17 induces strong positive NOEs in the H-10, H_{ax}-14, and *E* H-18 signals, but none in the H-15 and aromatic signals. Furthermore irradiation of H-15 produces strong positive NOEs in the H_{ax}-13 and *Z* H-18 signals but not in the 3H-17 signal. These results suggest that the preferred conformation of the isopropenyl group is one in which the C-17 methyl group is syn to H-10 and the C-18 methylene is syn to H-15. Finally irradiation of H-10 shows strong positive NOEs in the H-4 and H-11 signals, suggesting that the indole system is coplanar with the C(10)–H bond where H-4 is predominately syn to H-10. Hapalindoles C–F therefore have the same relative stereochemistry as hapalindoles G and U.

The gross structure and relative stereochemistry of hapalindole D is confirmed by X-ray crystallography (Figure 2).

Vicinal coupling constants for hapalindole Q show that H-10, H-11, and H-15 are axial ($J_{10,11}$ = $J_{10,15}$ = 11.0 Hz). Long-range zig–zag coupling (0.5 Hz) is observed between H-11 and *E* H-21 and W-coupling (0.5 Hz) is seen between H_{ax}-13 and H-20, indicating that the vinyl group on C-12 is axial. The methyl group on C-12 is therefore equatorial. The stereochemistry at C-12 is confirmed by the following difference NOE experiments: Irradiation of 3H-19 induces positive NOEs in the H-11, H-13, H-20, and *Z* H-21 signals and irradiation of H-10 causes positive NOEs in the H-20 signal as well as the H-2 and H-4 signals. As for hapalindoles C–F, irradiation of 3H-17 produces positive NOEs in the H-10 and *E* H-17 signals. Hapalindole Q therefore has the same relative stereochemistry as hapalindole H.

Hapalindoles I and K. ^{1}H NMR analysis of hapalindoles I and K (Table VI) indicates the presence of three adjacent protons on the benzenoid portion of the indole

Table VI. ^{1}H NMR Data for Hapalindoles I and K in $CDCl_3$

	I[a]	K[b]		I	K
1	8.43 br	7.92 br	14ax	2.23 td	2.70 br dd
2	7.90 d	7.16 d	14eq	2.41 ddd	3.08 ddd
5	7.06 m	7.00 dd	15ax	2.86 dd	
6	7.24 m	7.24 dd	17	1.54 s	1.48 s
7	7.23 m	7.13 dd	18	1.06 s	1.50 s
11ax		4.49 br	19	1.47 s	1.32 s
13ax	4.14 dd		20	5.85 dd	6.15 dd
13eq		4.43 dd	21*Z*	5.46 dd	5.42 dd
			21*E*	5.36 dd	5.38 dd

[a] J(H,H) in Hz for I: 1,2 = 2.6; 5,6 = 7.2; 5,7 = 0.6; 6,7 = 8.2; 13,14ax = 12.9; 13,14eq = 3.6; 14ax,14eq = −13.2; 14ax,15 = 11.6; 14eq,15 = 6.2; 20,21*E* = 10.7; 20,21*Z* = 17.3; 21*E*,21*Z* = 0.5. [b] J(H,H) for K: 1,2 = 2.2; 5,6 = 7.3; 5,7 = 0.5; 6,7 = 8.1; 11,14eq = 1.2; 11,14ax = 0.3; 13,14ax = 7.8; 13,14eq = 5.3; 14ax,14eq = −18.5; 20,21*E* = 10.9; 20,21*Z* = 17.5; 21*E*,21*Z* = 0.4.

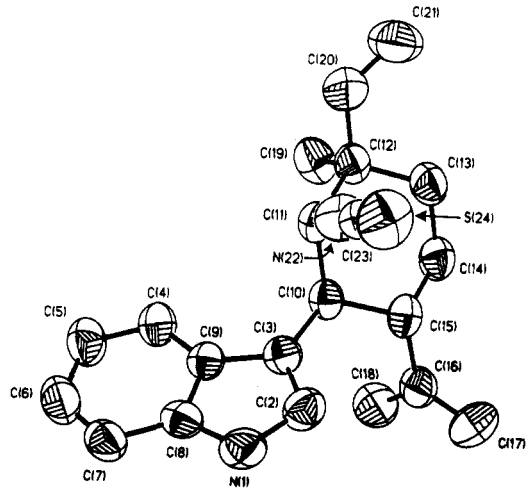


Figure 2. ORTEP stereodrawing of hapalindole D. Hydrogens are omitted for clarity.

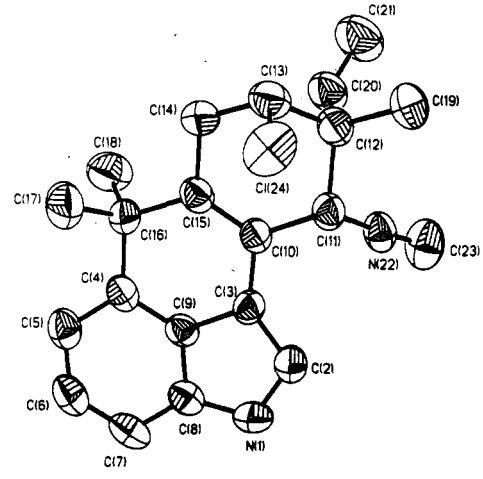
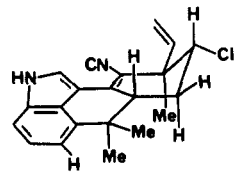


Figure 3. ORTEP stereodrawing of hapalindole K. Hydrogens are omitted for clarity. The absolute stereochemistry is as shown.

system. A proton on C-2 is present in both compounds. The H-2 signal for hapalindole I, however, is at almost 1



I

ppm lower field than the one for hapalindole K or any of the other hapalindoles; undoubtedly this appreciable difference in chemical shift is due to the conjugation of the isonitrile to the indole system via the C(10)–C(11) double bond and the close proximity of the isonitrile group to H-2.

Inspection of the spectrum for hapalindole I shows the familiar $CH_{ax}CH_2CH_{ax}$ signals for the protons on C-13, C-14, and C-15, but signals for H-10 and H-11 are missing. A double bond must therefore be between C-10 and C-11 to account for the molecular weight and composition and the markedly different ultraviolet spectrum. Axial methyl and equatorial vinyl groups are present on C-12 since irradiation of 3H-19 induces strong positive NOEs in the H_{ax}-14, H-20, and *Z* H-21 signals. Strong positive NOEs are observed in the H-5, H_{eq}-14, and H-15 signals when 3H-17 is irradiated and in the H_{ax}-14 signal when 3H-18 is irradiated, providing further support for the structure. The relative stereochemistry of hapalindole I is therefore 12*R**,13*R**,15*S**.

The ^{1}H NMR spectrum of hapalindole K shows signals for a $CHClCH_2$ unit, the methylene of which must be connected to a sp^2 carbon since the geminal coupling is −18.5 Hz. A double bond is therefore between C-10 and C-15 and this explains the appreciable difference in the ultraviolet spectrum. The signal assigned to H-11 shows small homoallylic coupling to both protons on C-14 (0.3 and 1.2 Hz). Unexpectedly the H-13 signal for hapalindole K shows couplings to the two protons on C-14 (7.8 and 5.3 Hz) that are quite different from those exhibited for all the other chlorine-containing hapalindoles. Furthermore the high-field H-14 signal (2.70 ppm) is very broad and devoid of multiplicity, suggesting that the cyclohexene ring is in dynamic equilibrium between the two possible ring conformations. Irradiation of 3H-19 induces positive NOEs in both the H-13 and high-field H-14 signals, as well as in the H-11, H-20, and *Z* H-21 signals, which is consistent with a rapid interconversion of the methyl group on C-12 from an axial position to an equatorial position.

Since it was not possible to unambiguously assign the relative stereochemistry of hapalindole K on the basis of the NMR data described above, an X-ray crystallographic analysis was carried out. The X-ray structure is shown in Figure 3. The data establish the relative stereochemistry. The absolute stereochemistry of hapalindole K has been shown to be 11*R*,12*R*,13*R* by using the anomalous dispersion technique.

Experimental Section

Spectral Analysis. Spectra were determined at 300 MHz for proton NMR and 75 MHz for carbon-13 NMR. Proton chemical shifts are referenced in chloroform-*d* to the residual chloroform signal (7.25 ppm) and in acetone-d_6 to the residual acetone-d_5 signal (2.04 ppm). Carbon-13 chemical shifts are referenced in chloroform-*d* to the solvent (77.0 ppm). Homonuclear ^{1}H connectivities were determined from double resonance experiments and in a few cases by using a phase-cycled 16-step COSY experiment described by Bax.[4] Qualitative homonuclear ^{1}H NOEs

were obtained by selective continuous irradiation (decoupler on, hetero mode) for 2 s, followed by data acquistion with the decoupler off; off-resonance experiments were performed in a similar manner and the NOEs were observed in difference spectra produced by subtracting off-resonance spectra from on-resonance spectra. Mass spectra, including high resolution mass measurements, were determined in either the EI, FD, or FAB mode. UV and CD spectra were measured in MeOH at 25 °C and are reported in terms of molar extinction coefficient (ϵ) and molecular ellipticity [θ], respectively. Optical rotations were measured in $CHCl_3$ or CH_2Cl_2 at 25 °C at the sodium D line.

Culture Conditions. An edaphic form of *Hapalosiphon fontinalis* (Ag.) Bornet (Stigonemataceae), designated strain number V-3-1, was isolated from a soil sample collected in the Marshall Islands. Clonal cultures were prepared by repeated subculture on solidified media. The alga was cultured in 25-L glass bottles containing a modified (citrate buffer replaced by MOPS) inorganic medium, designated A3M7, in which the major salt concentrations corresponded to those in Allen's medium, viz. in g/L for $NaNO_3$, 0.2; NH_4Cl, 0.01; $K_2HPO_4 \cdot 3H_2O$, 0.065; $MgSO_4 \cdot 7H_2O$, 0.05; $CaCl_2 \cdot 2H_2O$, 0.013; MOPS (3-morpholinopropanesulfonic acid), 0.627; minor elements solution, 1 mL/L; trace elements solution, 0.12 mL/L. The minor elements solution, designated ASMT, was composed of, in g/L, $FeCl_3 \cdot 6H_2O$, 0.54; Na_2EDTA, 3.0; H_3BO_3, 0.62; $MnCl_2 \cdot 4H_2O$, 1.40; $ZnCl_2$, 0.10; $CoCl_2 \cdot 6H_2O$, 0.005; $CuCl_2 \cdot 2H_2O$, 3.4×10^{-5}. The trace elements solution consisted of, in mg/L, MoO_3 (85%), 17.64; NH_4VO_3, 22.96; $Cr_2K_2(SO_4)_4 \cdot 24H_2O$, 96.02; $NiSO_4 \cdot H_2O$, 44.78; $Co(NO_3)_2 \cdot 6H_2O$, 49.38; $Na_2WO_4 \cdot H_2O$, 17.94; $Al_2(SO_4)_3$, 31.71; As_2O_3, 6.61; $CdCl_2$, 8.15; $SrSO_4$, 10.49; $HgCl_2$, 6.77; $PbCl_2$, 6.71; LiCl, 30.55; Rb_2SO_4, 7.81; NaBr, 6.44; KI, 6.54; NaF, 11.05; Na_2SeO_4, 11.94; $Be(NO_3)_2 \cdot 3H_2O$, 103.70. Prior to autoclaving, the pH of the medium was adjusted to 7.0 with sodium hydroxide. Cultures were illuminated continuously at an incident intensity of 330 μeinstein m^{-2} s^{-1} from banks of cool-white fluorescent tubes and vigorously aerated with approximately 1% CO_2 in air at an incubation temperature of 24 ± 1 °C. After 24 days the alga was harvested by filtration. Yields of lyophilized cells typically ranged from 0.4 to 0.5 g/L of culture.

Isolation. Freeze-dried alga (149 g) was extracted with 1:1 CH_2Cl_2/2-propanol (2 × 4.5 L) overnight under refrigeration with stirring. The filtered extracts were combined and concentrated under reduced pressure to a dry green solid (21.9 g).

Extract (11 g) was dissolved in 1 L of CH_2Cl_2 and applied to a column (4.5 cm × 10 cm diameter) of silica gel (EM Science Kieselgel 60; 230–400 mesh) equilibrated in CH_2Cl_2. The first 400 mL of effluent was discarded; then a 600-mL fraction of column eluate was collected and evaporated under reduced pressure to give a solid (1.15 g). The column was then eluted successively with 1:1 CH_2Cl_2/heptane (1 L), $CHCl_3$ (600 mL), and 1:1 CH_2Cl_2/EtOAc (2 L) to give solids which after evaporation weighed 4.8, 0.60, and 1.72 g, respectively. The remaining extract (10.9 g) was subjected to silica gel chromatography as described above to give fractions weighing 2.36, 3.07, 0.85, and 2.85 g, respectively.

The first two fractions from each silica gel chromatography were treated with 1:1 cyclohexane/CH_2Cl_2 to give 3.36 g of crystalline hapalindole A. The mother liquors were combined and a portion (2.3 g) was dissolved in EtOAc (25 mL) and applied to a Prep-Pak-500 silica cartridge in a Water Prep LC 500A system. Material was eluted by using 8 L of a hexane to 85:15 hexane/EtOAc gradient. Fractions (400 mL) were collected and pools were generated on the basis of TLC analysis (4:1 hexane/EtOAc). Fractions were then individually chromatographed on a 1 in. × 50 cm stainless steel HPLC column containing Whatman LPS-1 silica gel (13–24 μM) at 2.5 mL/min using the solvent systems indicated. Thus, pool 1 (37 mg) afforded hapalindoles Q (20 mg), D (5 mg), and F (8 mg) using 1:1 CH_2Cl_2/heptane. Pool 2 (1.07 g) yielded hapalindoles M (60 mg), B (22 mg), U (10 mg), Q (3 mg), C and E (460 mg, 1:1 mixture), H (150 mg), and I (10 mg) using 1:1 CH_2Cl_2/heptane. Pool 3 (273 mg) gave hapalindole B (200 mg) using 2:1 CH_2Cl_2/heptane. Pool 4 (452 mg) led to hapalindoles B (55 mg), J (70 mg), and K (35 mg) using 4:1 CH_2Cl_2/heptane. Pool 5 (124 mg) yielded hapalindoles G (14 mg), L (11 mg), J (13 mg), and K (7 mg) using 3:1 CH_2Cl_2/heptane. Pool 6 (162 mg) gave hapalindole A (160 mg) using 2:1 CH_2Cl_2/heptane.

The CH_2Cl_2/EtOAc fraction (1.8 g) from silica gel chromatography was rechromatographed on a gravity-flow column (5 × 12 cm) of silica gel (Merck; 200–400 mesh) equilibrated with 2:1 CH_2Cl_2/cyclohexane. A gradient from 2:1 CH_2Cl_2/cyclohexane to 100:1 CH_2Cl_2/MeOH was used. Pools were generated by using analytical TLC profiles (50:1 CH_2Cl_2/MeOH) of the eluted fractions. Fractions with R_f 0.4–0.5 (290 mg) were further purified by HPLC on the LPS-1 silica column described above with 7:1 heptane/THF to give hapalindoles N (7 mg), O (35 mg), P (4 mg), T (45 mg), and V (27 mg).

The hapalindoles had the following R_f values on Merck silica gel 60 (F254) plates where the solvent systems were 2:1 hexane/EtOAc (A), 4:1 CH_2Cl_2/heptane (B), 4:1 hexane/EtOAc (C), 2:1 CH_2Cl_2/heptane (D), and 2:1 heptane/THF (E): hapalindole A, 0.45 (A); B, 0.51 (A); C, 0.64 (A); D, 0.76 (A); E, 0.61 (A); F, 0.58 (A); G, 0.46 (A); H, 0.67 (A); I, 0.58 (A); J, 0.44 (B); K, 0.36 (B); L, 0.26 (C); M, 0.53 (D); N, 0.12 (E); O, 0.2 (E); P, 0.15 (E); Q, 0.55 (C); T, 0.11 (E); U, 0.47 (A); V, 0.29 (E).

The hapalindoles had the following HPLC retention times in minutes on a Du Pont Zorbax silica column (4.6 mm × 25 cm) using 5:1 isooctane/THF (flow rate 1 mL/min, detection at 235 nm): hapalindole D (6.5), F (7.0), H (7.3), I (9.0), C (9.1), E (10.1), Q (10.9), M (12.9), K (13), J (14.1), L (15.7), U (15.8), B (16.3), G (19), A (19.5). Retention times in minutes using 5:2 isooctane/THF: hapalindole G (7.2), A (7.4), O (9.9), V (11.4), T (13.2), N (13.9), P (14.6).

Hapalindole A: mp 160–167 °C dec; $[\alpha]_D$ −78° (CH_2Cl_2, *c* 1.2); high resolution EIMS, *m/z* 338.1595 (calcd for $C_{21}H_{23}N_2{}^{35}Cl$, mmu error 4.5). Anal. Calcd for $C_{21}H_{23}N_2Cl$: C, 74.43; H, 6.84; N, 8.27. Found: C, 74.65; H, 6.63; N, 8.17.

Hapalindole B: $[\alpha]_D$ −194° (CH_2Cl_2, *c* 5.1); high resolution EIMS, *m/z* 370.1243 (calcd for $C_{21}H_{23}N_2{}^{35}ClS$, mmu error 2.8).

Hapalindole C: mp 138–143 °C; $[\alpha]_D$ +76.5° (CH_2Cl_2, *c* 0.41); high resolution EIMS, *m/z* 304.1950 (calcd for $C_{21}H_{24}N_2$, mmu error 1.1).

Hapalindole D: mp 105–107 °C; $[\alpha]_D$ +239° (CH_2Cl_2, *c* 3.1); high resolution EIMS, *m/z* 336.1650 (calcd for $C_{21}H_{24}N_2S$, mmu error −1.0).

Hapalindole E: mp 88–90 °C; $[\alpha]_D$ +25.2° (CH_2Cl_2, *c* 3.1); high resolution EIMS, *m/z* 338.1532 (calcd for $C_{21}H_{23}N_2{}^{35}Cl$, mmu error −1.8); ^{13}C NMR ($CDCl_3$) δ 123.5 (C-2), 111.7 (C-3), 116.8 (C-4), 119.5 (C-5), 122.1 (C-6), 111.4 (C-7), 135.5 (C-8), 126.2 (C-9), 34.7 (C-10), 67.0 (C-11), 44.5 (C-12), 60.8 (C-13), 38.1 (C-14), 43.9 (C-15), 145.1 (C-16), 113.4 (C-17), 18.6 (C-18), 16.0 (C-19), 141.7 (C-20), 116.1 (C-21), 158.5 (C-23).

Hapalindole F: mp 176–179 °C; $[\alpha]_D$ +93.2° (CH_2Cl_2, *c* 0.22); high resolution EIMS, *m/z* 370.1255 (calcd for $C_{21}H_{23}N_2{}^{35}ClS$, mmu error −1.6).

Hapalindole G: mp >185 °C dec; $[\alpha]_D$ −43.9° (CH_2Cl_2, *c* 0.28); high resolution EIMS, *m/z* 338.1530 (calcd for $C_{21}H_{23}N_2{}^{35}Cl$, mmu error −2.0).

Hapalindole H: mp 190–193 °C; $[\alpha]_D$ +152° (CH_2Cl_2, *c* 4.1); high resolution EIMS, *m/z* 304.1945 (calcd for $C_{21}H_{24}N_2$, mmu error 0.6); ^{13}C NMR ($CDCl_3$) δ 118.3 (C-2), 113.0 (C-3), 140.5 (C-4), 112.5 (C-5), 108.1 (C-6), 122.6 (C-7), 133.2 (C-8), 124.9 (C-9), 36.2 (C-10), 67.8 (C-11), 37.3 (C-12), 36.1 (C-13), 20.8 (C-14), 49.7 (C-15), 40.5 (C-16), 24.5 (C-17), 24.8 (C-18), 27.2 (C-19), 138.5 (C-20), 115.8 (C-21), 157.5 (C-23).

Hapalindole I: mp 180 °C dec; $[\alpha]_D$ −12° (CH_2Cl_2, *c* 0.2); high resolution EIMS, *m/z* 336.1366 (calcd for $C_{21}H_{21}N_2{}^{35}Cl$, mmu error −2.7).

Hapalindole J: $[\alpha]_D$ +54.4° ($CHCl_3$, *c* 0.9); high resolution EIMS, *m/z* 304.1949 (calcd for $C_{21}H_{24}N_2$, mmu error 1.0).

Hapalindole K: mp 110–240 °C dec; $[\alpha]_D$ −12.5° ($CHCl_3$, *c* 1.8); high resolution EIMS, *m/z* 336.1402 (calcd for $C_{21}H_{21}N_2{}^{35}Cl$, mmu error 0.9).

Hapalindole L: $[\alpha]_D$ −74.0° ($CHCl_3$, *c* 1.1); high resolution EIMS, *m/z* 338.1559 (calcd for $C_{21}H_{23}N_2{}^{35}Cl$, mmu error 0.9).

Hapalindole M: $[\alpha]_D$ −83.1° ($CHCl_3$, *c* 1.8); high resolution EIMS, *m/z* 336.1657 (calcd for $C_{21}H_{24}N_2S$, mmu error −0.3).

Hapalindole N: $[\alpha]_D$ −31.5° ($CHCl_3$, *c* 1.7); FDMS, *m/z* 354,356 (relative intensity 3:1).

(4) Bax, A. *Two-Dimensional Nuclear Magnetic Resonance in Liquids*; Delft University Press: Delft, Holland, 1982.

Hapalindole O: $[\alpha]_D$ -106.0° ($CHCl_3$, c 2.4); high resolution EIMS, m/z 352.1609 (calcd for $C_{21}H_{24}N_2OS$, mmu error 0.0).

Hapalindole P: $[\alpha]_D$ -16.3° ($CHCl_3$, c 0.8); FDMS, m/z 354,356 (relative intensity 3:1).

Hapalindole Q: $[\alpha]_D$ +24.1° (CH_2Cl_2, c 1.1); high resolution EIMS, m/z 336.1659 (calcd for $C_{21}H_{24}N_2S$, mmu error -0.1).

Hapalindole T: $[\alpha]_D$ -137° (CH_2Cl_2, c 1.5); high resolution FABMS, m/z 387.1299 (calcd for $C_{21}H_{24}N_2SO^{35}Cl$, mmu error +0.1, MH ion); UV (MeOH) λ_{max} nm (ϵ) 222 (35 400), 283 (7100); IR ($CHCl_3$) ν_{max} 3473, 3400, 1679 cm^{-1}.

Hapalindole U: $[\alpha]_D$ +12° (CH_2Cl_2, c 0.6); high resolution FABMS, m/z 305.1996 (calcd for $C_{21}H_{25}N_2$, mmu error -2.0, MH ion).

Hapalindole V: high resolution FABMS, m/z 355.1599 (calcd for $C_{21}H_{24}N_2O^{35}Cl$, mmu error +2.2, MH ion); UV (MeOH) λ_{max} nm (ϵ) 220 (34 000), 273 (5700), 279 (5800), 290 (4500); IR ($CHCl_3$) ν_{max} 3600, 3480, 2140 cm^{-1}; ^{13}C NMR ($CDCl_3$) δ 117.3 (C-2), 116.8 (C-3), 133.9 (C-4), 115.0 (C-5), 123.9 (C-6), 108.4 (C-7), 139.7 (C-8), 123.5 (C-9), 72.4 (C-10), 67.8 (C-11), 44.8 (C-12), 63.8 (C-13), 29.3 (C-14), 47.4 (C-15), 37.7 (C-16), 26.6 (C-17), 28.0 (C-18), 17.9 (C-19), 143.9 (C-20), 116.0 (C-21), 159.4 (C-23).

X-ray Structure Analysis. Hapalindole A crystallized from CH_2Cl_2/heptane as yellow plates in the space group $P2.2.2.$, $Z = 4$, with unit cell dimensions $a = 7.926$ (2), $b = 10.118$ (4), and $c = 23.302$ (8) Å. The calculated density was found to be 1.200 g cm^{-3}. A total of 1515 unique reflections with $2[\theta]$ less than 116.0 were measured on an automated four-circle diffractometer using monochromatic copper radiation. The structure was solved by the direct methods routines of the SHELXTL program library (G. M. Sheldrick, 1981) and was refined by the least-squares method with anisotropic temperature factors for all atoms except hydrogen. Hydrogen atoms were included with isotropic temperature factors at calculated positions. The final R-factor was 0.0386 for 1393 observed reflections. Figure 1 shows an ORTEP plot of the molecule and tables in the supplementary material section give atom coordinates, anisotropic temperature factors, bond lengths, bond angles, and hydrogen coordinates.

Hapalindole D crystallized from CH_2Cl_2 as yellow-orange prisms in the space group $P222$, $Z = 4$, with unit cell dimensions $a = 9.847$ (4), $b = 9.990$ (3), and $c = 19.439$ (5) Å. The calculated density was 1.169 g cm^{-3}. A total of 1539 unique reflections with $2[\theta]$ less than 116.0 were measured and the structure was solved as described above. The final R-factor was 0.0610 for 1457 observed reflections. Figure 2 shows an ORTEP plot of the molecule and tables in the supplementary material section give atom coordinates, anisotropic temperature factors, bond lengths, bond angles, and hydrogen coordinates.

Hapalindole K crystallized from CH_2Cl_2/heptane as thick yellow plates in the space group $P222$, $Z = 4$, with unit cell dimensions $a = 8.447$ (4), $b = 13.487$ (7), and $c = 15.497$ (9) Å. The calculated density was 1.263 g cm^{-3}. A total of 1429 unique observed reflections with $2[\theta]$ less than 116.0 were measured and the structure was solved as described above. The final R-factor was 0.0559 for 1260 observed reflections. The absolute configuration was determined through use of the anomalous dispersion technique as suggested by Rogers.[5] Figure 3 shows an ORTEP plot of the molecule and tables in the supplementary material section give atom coordinates, anisotropic temperature factors, bond lengths, bond angles, and hydrogen coordinates.

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Supplementary Material Available: ORTEP stereodrawings showing hydrogens; tables for atom coordinates and temperature factors, bond lengths, bond angles, anisotropic temperature factors, and hydrogen coordinates and temperature factors for hapalindoles A, D, and K; tables for in vitro antibacterial and antifungal activity (13 pages). Ordering information is given on any current masthead page.

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Reduction of Heterocycles with Nickel-Aluminum Alloy†

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Pyrazines, pyridazines, isoxazoles, oxazole, 4-methylpyrimidine, and indole are reduced by nickel-aluminum alloy in potassium hydroxide solution. The reaction is simple to carry out and does not require special apparatus or hydrogen atmospheres. The products were the fully hydrogenated species although benzene rings were not attacked. 4-Methylpyrimidine gave 1,3-diaminobutane and oxazole gave 2-(methylamino)ethanol. It was found that the reaction frequently exhibited an induction period.

Introduction

We recently discussed[1] the use of nickel-aluminum alloy in dilute base to reduce pyridines to the corresponding piperidines. The reaction is particularly easy to perform and high temperatures, high pressures, and hydrogen atmosphere and special apparatus are not required. We now wish to report that this process may be used to reduce a variety of other heterocycles. Specifically, we have reduced pyrazines, pyridazines, isoxazoles, oxazole, 4-methylpyrimidine, and indole; these results are summarized in Table I. We also found that imidazole, 3,5-dimethylpyrazole, and 2,3,5,6-tetramethylpyrazine were essentially not reduced in 24 h under these conditions (lack of reduction was confirmed by comparing ^{13}C NMR spectra of the reaction mixtures before and after the reduction procedure). Pyrrole was reduced only slowly to pyrrolidine (24% after 24 h; 58% after 4 days). The compounds listed in Table I were completely reduced after an overnight reaction, with the longest reaction taking about 30 h. In a number of instances, much more rapid reaction was observed; in fact, in some cases it was not necessary to add the usual amount of nickel-aluminum alloy. It is useful to monitor the reaction by gas chromatography and thus

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