Antimicrobial Ambiguines from the Cyanobacterium *Fischerella* sp. Collected in Israel

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Bioassay-guided fractionation of the 7:3 MeOH/water extract of a cultured cyanobacterium strain identified as *Fischerella* sp. yielded nine isonitrile-containing alkaloids. Three of the compounds, ambiguine H isonitrile (**1**), ambiguine I isonitrile (**2**), and ambiguine J isonitrile (**3**), are new, while the other six, 12-*epi*-hapalindole H, ambiguine A isonitrile, ambiguine B isonitrile, ambiguine D isonitrile, ambiguine E isonitrile, and ambiguine F isonitrile, have been previously isolated from *Fischerella ambigua*. The structures of the compounds were determined by 1D and 2D NMR techniques and mass spectrometric data. Ambiguine H isonirile and ambiguine I isonirile possess antibacterial and antimycotic activity.

Cyanobacteria (blue green algae) are photosynthetic prokaryotes, some of which are capable of assimilating atmospheric nitrogen. They are a common occurring component of most terrestrial and aquatic ecosystems. Many cyanobacteria exhibit unique capabilities to adapt to various environmental conditions, which may result in massive blooms.[1] Cyanobacteria produce a large number of bioactive substrates with a diverse range of biological activities.[2,3] Their chemical structures include polyketide macrolides, linear and cyclic peptides, and alkaloids.[4] These natural products affect many biochemical processes within cells and display an array of activities such as hepatotoxicity, neurotoxicity, antibacterial and antifungal activity as well as protease inhibition.[3]

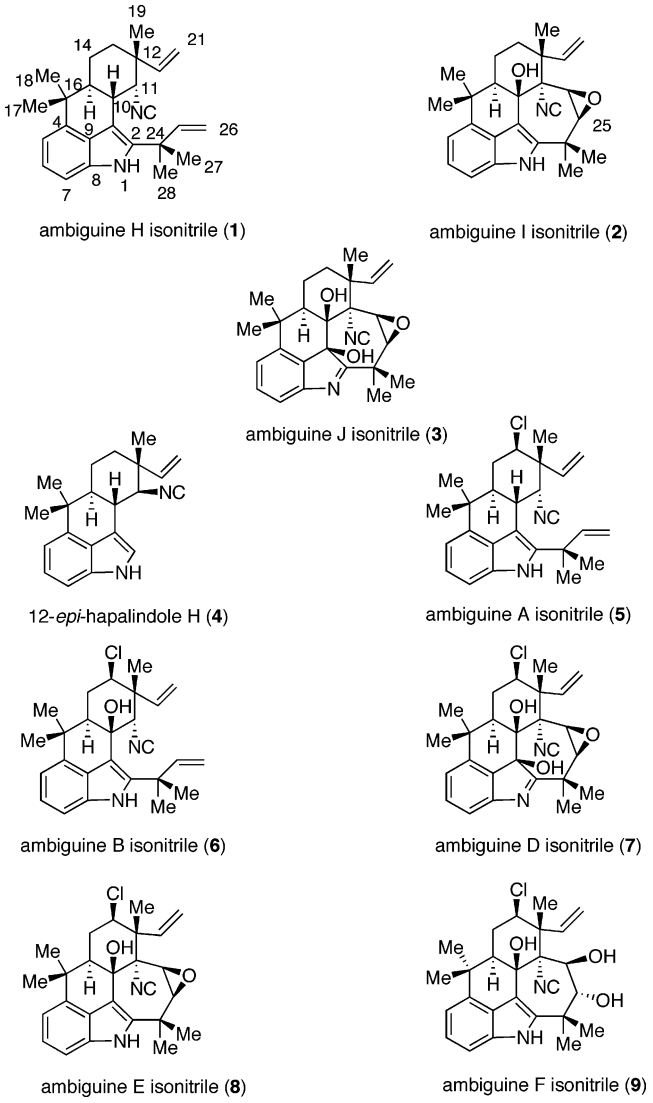
Isonitrile-containing indole alkaloids are often present in branched, filamentous cyanobacteria (blue-green algae) belonging to the Stigonemataceae. Four main classes of this type have been identified to date: hapalindoles,[5] ambiguines,[6] fischerindoles,[7] and welwitindolinones.[8] These tetra- and pentacyclic indole alkaloids appear to be biosynthesized from precursors derived from tryptophan and geranyl pyrophosphate.[8]

Results and Discussion

A cyanobacterium isolate (TAU isolate IL-199-3-1) was purified from a soil sample and identified as a *Fischerella* sp.[9] It was mass cultured in the laboratory in a modified BG-11 medium.[5] The freeze-dried bacterial mass was first extracted with 7:3 MeOH/H_2O and then with 1:1 MeOH/CH_2Cl_2 solvent systems. The polar crude extract exhibited antibacterial, antifungal, and antimalarial properties. Three new alkaloids, ambiguine H isonitrile (**1**, 5.8 mg, 0.015% of dry bacterial mass), ambiguine I isonitrile (**2**, 11.8 mg, 0.03% of dry bacterial mass), and ambiguine J isonitrile (**3**, 3.3 mg, 0.008% of dry bacterial mass), and six known alkaloids, 12-*epi*-hapalindole H (**4**, 16.9 mg, 0.042% of dry bacterial mass),[11] ambiguine A isonitrile (**5**, 11.3 mg, 0.028% of dry bacterial mass),[6] ambiguine B isonitrile (**6**, 4.7 mg, 0.012% of dry bacterial mass),[6] ambiguine D isonitrile (**7**, 28.4 mg, 0.071% of dry bacterial mass),[6] ambiguine E isonitrile (**8**, 21.8 mg, 0.055% of dry bacterial mass),[6] and ambiguine F isonitrile (**9**, 2.9 mg, 0.007% of dry bacterial mass),[6] were isolated from the polar extract and their structures elucidated using 1D and 2D NMR and MS techniques. It is interesting to note that compounds **1**, **2**, and **3** eluted from the reversed-phase column after their chlorinated analogues, **5**, **8**, and **7**, respectively.

Ambiguine H isonitrile (**1**) was isolated as an amorphous, white solid. The molecular formula of **1**, $C_{26}H_{32}N_2$, was deduced from the HREIMS molecular ion, *m*/*z* 372.2560 (calculated 372.2565).

Chart 1



Ambiguine H isonitrile (**1**) exhibited a typical indole UV spectrum [λ_{max} (log ϵ) 222 (4.38), 282 (3.72), 291 (3.62)] and ^{13}C NMR and IR peaks [δ_C 156.6 ppm and ν_{max} 2139 cm^{-1}] characteristic of an isonitrile.[5] The ^1H NMR showed signals characteristic of two monosubstituted double-bond residues (δ 5.93, 5.10, 5.13; 6.21, 5.19, 5.24), three consecutive aromatic protons (δ 6.98 brd, J = 7.0 Hz; 7.09, dd J = 7.9, 7.0 Hz; 7.11, brd, J = 7.9 Hz), and five

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Table 1. NMR Data of Ambiguine H Isonitrile (**1**) in $CDCl_3$[a]

position	δ_C, mult.[b]	δ_H, mult., *J* (Hz)	COSY correlations[d]	LR H−C correlations[c]	NOE correlations[g]
1		8.00 s		C-2, 3, 8, 9	H-7, H_3-27, 28
2	136.7 qC				
3	107.0 qC				
4	140.7 qC				
5	112.9 CH	6.98 brd 7.0	H-6	C-6, 7, 9, 16	H_3-17, 18
6	122.0 CH	7.09 dd 7.0, 7.9	H-5, 7	C-5, 7, 8	
7	107.5 CH	7.11 brd 7.9	H-6	C-8, 9	H-1
8	132.1 qC				
9	127.4 qC				
10	34.9 CH	3.15 dd 10.9, 3.6	H-11, 15	C-3, 12, 15	H-11, 14ax, H_3-17, 19
11	65.2 CH	4.49 brs	H-10	C-3, 10, 12, 13, 15, 23	H-10, 25, 26E, H_3-19, 27, 28
12	39.9 qC				
13	30.7 CH_2	1.57 m[e]	H-13ax, 14ax, 14eq	C-12, 15	H_3-19 H-20, 21Z
		1.96 dt 3.9, 14.1[f]	H-13eq, 14ax, 14eq	C-12, 15	
14	21.8 CH_2	2.00 m[e]	H-13ax, 13eq, 14ax, 15	C-13, 15	H_3-17, 18 H-10, H_3-17, 19
		1.60 m[f]	H-13ax, 13eq, 14eq, 15	C-13, 15	
15	43.9 CH	2.04 ddd 12.3, 10.9, 1.8	H-10, 14ax, 14eq	C-10, 14, 16	
16	36.3 qC				
17	24.0 CH_3	1.50 s[f]		C-4, 15, 16, 18	H-5, 10, 14ax, 14eq, H_3-18
18	24.9 CH_3	1.02 s[e]		C-4, 15, 16, 17	H-5, 14eq, H_3-17
19	21.7 CH_3	1.23 s		C-11, 12, 13, 20	H-10, 11, 13eq, 14ax, 20
20	145.8 CH	5.93 dd 17.4, 10.9	H-21E, 21Z	C-12, 21	H-13ax, 21E, H_3-19
21	112.0 CH_2	5.10 d 17.4[h]	H-20 H-20	C-12, 20 C-12, 20	H-13ax H-20
		5.13 d 10.9[i]			
23	156.6 qC				
24	38.7 qC				
25	146.3 CH	6.21 dd 17.6, 10.6	H-26E, 26Z	C-2, 26, 27, 28	H-11, 26E, H_3-27, 28
26	113.0 CH_2	5.19 d 17.6[h]	H-25	C-24, 25	H-11, 26Z
		5.24 d 10.6[i]		C-24, 25	H-25, 26Z
27	27.9 CH_3	1.57 s		C-2, 24, 25, 28	H-1, 11
28	29.2 CH_3	1.52 s		C-2, 24, 25, 27	H-1, 11

[a] Carried out on a Bruker Avance 400 spectrometer in acid-free $CDCl_3$.[10] [b] Multiplicity and assignment from HSQC experiment. [c] Determined from HMBC experiment, $^nJ_{CH} = 8$ Hz, recycle time 1 s. [d] By COSY experiment. [e] Equatorial. [f] Axial. [g] From NOESY experiment with 400 ms mixing delay. [h] *Z*-proton. [i] *E*-proton.

methyl groups (δ 1.02 s, 1.23 s, 1.50 s, 1.52 s, 1.57 s). A broad singlet at 8.00 ppm in the ^{1}H NMR spectrum is in accordance with a pyrole-NH. The ^{13}C NMR of **1** showed 12 signals in the 110−150 ppm region; eight were assigned to carbons of the indole skeleton, and the other four were due to the two terminal double-bond carbons. Analysis of the 1D and 2D NMR data (Table 1) revealed that **1** is the dechloro derivative of ambiguine A isonitrile (**5**; see Table 6 in the Supporting Information). A signal at 4.49 ppm (H-11) was assigned to a methine proton adjacent to the isonitrile via an HMBC correlation with the quaternary isonitrile carbon signal at 156.6 ppm. Sequential COSY correlations H-11/H-10/H-15/H-14ax, H-14eq/H-13ax, and H-13eq followed by HMBC correlations of H-10, H-11, H-13ax, H-13eq, H_3-19, H-20, H-21Z, and H-21E with C-12 established the presence of the cyclohexyl ring with methyl (C-19) and ethene (C-20) substituents at C-12 and an isonitrile substituent at C-11. The indole moiety was confirmed through HMBC correlation of H-1 with C-2, C-3, C-8, and C-9, of H-6 and H-7 with C-8, and of H-5 with C-9. HMBC correlations of H_3-17 and H_3-18 with C-4, C-15, and C-16 and of H-10 with C-3 established the tetracyclic ring system. Finally, the analyses of the HMBC correlations between H_3-27 and H_3-28 and C-2, C-24, and C-25 completed the planar structure of **1**. Overlapping of protons 13eq, 14ax, and 15 did not allow the measurement of all of the coupling constants of these protons. However, the number of large coupling constants of each of these protons could be determined from HSQC correlations and allowed their assignments as axial or equatorial in the cyclohexane ring. NOEs observed between H_3-19 and H-10, H-11, H-13eq, H-14ax, H-20, and H-21Z, between H-10 and H-11, H-14ax, and H_3-17, and between H-14eq and H_3-17 and H_3-18 suggested the 1,3-diaxial relationships between H-10 and H_3-17 and H_3-19 and the *syn* relationships between H-10 and H-11. The coupling constant observed for H-10 and H-15 (J = 10.9 Hz) indicated the *anti* relationship between these two protons. Ambiguine H isonitrile (**1**) is thus a dechloro derivative of ambiguine A isonitrile (**5**) and has the relative configuration 10*S**, 11*R**, 12*R**, and 15*S**.

Ambiguine I isonitrile (**2**) was isolated as an amorphous, transparent solid. The molecular formula of **2**, $C_{26}H_{30}N_2O_2$, was deduced from the molecular ion at *m*/*z* 402.2307 (calculated 402.2307) from HREIMS. As for **1**, the UV [λ_{max} (log ϵ) 222 (4.04), 282 (3.69), 286 (3.67)], ^{13}C NMR, and IR [δ_C 159.0 ppm and ν_{max} 2129 cm^{-1}] data indicated the presence of indole and isonitrile moieties, respectively. The ^{1}H NMR spectrum showed signals characteristic of a terminal double-bond residue (δ 6.16, 5.26 5.29), three consecutive aromatic protons (δ 6.97 brd, J = 7.1 Hz; 7.10, dd J = 7.7, 7.0 Hz; 7.05, brd, J = 7.7 Hz), and five methyl groups (δ 1.32 s, 1.42 s, 1.59 s, 1.62 s, 1.63 s). A broad singlet at 8.98 ppm in the ^{1}H NMR spectrum is in accordance with a pyrole-NH. A comparison of the ^{1}H NMR data for **2** with those for **1** revealed several differences. Compound **2** possessed one less terminal double bond than **1**. The signals for H-10 and H-11 were missing in the ^{1}H NMR spectrum of **2** and were replaced by an exchangeable proton at δ 3.62. Two additional proton signals appeared at δ 3.07 (H-25, d, J = 4.4 Hz) and 3.63 (H-26, d, J = 4.4 Hz) and were assigned as protons of a 1,2-disubstituted epoxide moiety. The ^{13}C NMR spectrum presented 10 signals between 110 and 150 ppm, eight of which belonged to an indole residue and two to a terminal double bond. In comparison with the spectrum of **1**, there were two new signals at 61.8 and 66.6 ppm attributed to the epoxide moiety as well as a tertiary oxygenated carbon at δ 75.4 (replacing a methine at δ 34.9 in **1**).

Analysis of the 1D and 2D NMR data (Table 2) revealed the ambiguine skeleton of this compound as follows. The indole moiety was assigned in a similar way to that of **1** (see Table 2). HMBC correlations of H_3-17 and H_3-18 with C-4, C-15, and C-16 established their connection. Sequential COSY correlations H-15/H-14ax and H-14eq/H-13ax, H-13eq followed by HMBC correlations of H-13ax, H-13eq, H_3-19, H-20, H-21Z, and H-21E with

Table 2. NMR Data of Ambiguine I Isonitrile (**2**) in $CDCl_3$[a]

position	δ_C, mult.[b]	δ_H, mult., *J* (Hz)	COSY correlations[d]	LR H−C correlations[c]	NOE correlations[g]
1		8.98 s		C-2, 3, 8, 9	H-7, H_3-27, 28
2	133.9 qC				
3	110.0 qC				
4	140.7 qC				
5	113.9 CH	6.97 d 7.1	H-6	C-6, 7, 9, 16	H_3-17, 18
6	122.6 CH	7.10 brdd 7.7, 7.1	H-5, 7	C-5, 7, 8	
7	106.8 CH	7.05 d 7.7	H-6	C-8, 9	H-1
8	133.7 qC				
9	124.2 qC				
10	75.4 qC				
10-OH		3.62 brs		C-3, 10	H-14ax, H_3-17,19
11	68.0 qC				
12	44.6 qC				
13	35.0 CH_2	1.58 brd 12.9[e]	H-13ax, 14ax, 14eq	C-12, 14	H-13ax, 14ax,eq, H_3-19
		1.94 brdd 12.9, 12.0[f]	H-13eq, 14ax, 14eq	C-12, 14	H-13eq, 14eq, 15, 20
14	17.7 CH_2	1.78 brd 10.3[e] 2.12 m[f]	H-13ax, 13eq, 14eq	C-13, 15	H-13eq,ax, H_3-17, 18
			H-13ax, 13eq, 14ax	C-13, 15	10-OH, H-13eq, H_3-17, 19
15	48.2 CH	2.12 m	H-14ax, 14eq	C-10, 14, 16	H-13ax, H_3-18
16	37.4 qC				
17	28.3 CH_3	1.42 s[f]		C-4, 15, 16, 18	H-5, 14ax, 10-OH, H_3-18
18	26.5 CH_3	1.32 s[e]		C-4, 15, 16, 17	H-5, 15, H_3-17
19	29.5 CH_3	1.62 s		C-11, 12, 13, 20	10-OH, H-13eq, 14ax, 21Z
20	144.6 CH	6.16 dd 17.3, 10.8	H-21E, 21Z	C-11, 12, 21	H-13ax, 21E
21	116.6 CH_2	5.26 d 17.3[h]	H-20	C-11, 12, 20	H_3-19
		5.29 d 10.8[i]	H-20	C-11, 12, 20	H-20, 26
23	159.0 qC				
24	36.2 qC				
25	66.4 CH	3.07 d 4.4	H-26	C-2, 11, 24, 26	H-26, H_3-27, 28
26	61.5 CH	3.63 d 4.4	H-25	C-11, 25	H-20, 21E, 25
27	18.1 CH_3	1.63 s		C-2, 24	H-1, 25
28	27.0 CH_3	1.59 s		C-2, 24	H-1, 25

[a] Carried out on a Bruker Avance 400 spectrometer in acid-free $CDCl_3$.[10] [b] Multiplicity and assignment from HMQC experiment. [c] Determined from HMBC experiment, $^nJ_{CH} = 8$ Hz, recycle time 1 s. [d] By COSY experiment. [e] Equatorial. [f] Axial. [g] From NOESY experiment with 400 ms mixing delay. [h] *Z*-proton. [i] *E*-proton.

C-12; H_3-19 and H-20 with C-11; H-15 with C-10; and 10-OH with C-10 and C-3 confirmed the presence of a tetracyclic system. HMBC correlations of H_3-27 and H_3-28 with C-2, C-24, and C-25 and of H-25 and H-26 with C-11 closed the seven-membered ring and established the planar structure of **2**. The NOEs from 10-OH to H_3-19, H-14ax, and H_3-17 and from H-14ax to H_3-17 and H_3-19 confirmed the 1,3-diaxial relationships between these substituents. The NOEs of H-15 with H-13ax and H_3-18 established their *syn* relationship and consequently the *anti* relationship of 10-OH and H-15. NOEs from H-26 to H-20, H-21E, and H-25 indicate that these four protons are on the same face of the molecule, thus indicating the *syn* relationship between H-26 and the isonitrile. Ambiguine I isonitrile (**2**) is thus a dechloro derivative of ambiguine E isonitrile (**8**) and has the relative configuration 10*R**, 11*R**, 12*R**, 15*R**, 25*S**, and 26*R**.

Ambiguine J isonitrile (**3**) was isolated as an amorphous, yellow solid. The molecular formula of **3**, $C_{26}H_{30}N_2O_3$, was deduced from the HREIMS molecular ion at *m*/*z* 418.2250. As for **1** and **2**, the ^{13}C NMR data (δ_C 159.0 ppm) indicated the presence of an isonitrile moiety, while the UV data [λ_{max} (log ϵ) 222 (3.68), 270 (2.97), 308 (2.78)] indicated the presence of an indolenine moiety. The ^1H NMR showed signals characteristic of one terminal monosubstituted double-bond residue, three consecutive aromatic protons, and five methyl groups. Comparison of the ^1H NMR spectra of **2** and **3** revealed they were very similar; however, the spectrum of **3** contained two broad singlets at δ 4.19 and 3.50 ppm due to hydroxyl groups and was lacking an indole NH signal. The ^{13}C NMR spectrum presented 10 signals at low field (110−190 ppm), seven of which belonged to the indolenine residue (including also the tertiary carbinol at δ 83.6), two to a terminal double bond, and one to the isonitrile carbon. A similar indolenine moiety exists in ambiguine D isonitrile (**7**). Comparison of the NMR data of **3** and **7**[6] (see Table 8 in Supporting Information) revealed that the spectra were similar except for the protons and carbons adjacent to C-13, which were substituted by an equatorial chlorine in **7** and by a proton in **3**. The assignment continued with the HMBC correlations of H_3-17 and H_3-18 with C-4, C-16, and C-15. Sequential COSY correlations H-15/H-14ax and H-14eq/H-13ax, H-13eq followed by HMBC correlations of H_2-13, H_3-19, H-20, H-21Z, and H-21E with C-12; H_3-19 with C-11; and 10-OH with C-15, C-10, and C-3 confirmed the presence of a tetracyclic system. HMBC correlations of H_3-27 and H_3-28 with C-2, C-24, and C-25 and of H-25 with C-11 established the structure of the seven-membered ring and the planar structure of **3**. NOEs from 10-OH and H-14ax to H_3-17 and H_3-19 confirmed the 1,3-diaxial relationships between these substituents. The NOEs from H-26 to H-21Z and H-25 indicated that the three protons are on the same face of the molecule, thus indicating the *syn* relationship between H-26 and the isonitrile. No NOE correlations were observed to the 3-OH, but the similar chemical shifts of carbons 2 to 10 in **3** and **7** suggested that the stereochemistry at C-3 in both compounds was identical. Ambiguine J isonitrile (**3**) is thus a dechloro derivative of ambiguine D isonitrile (**7**) and has the relative configuration 3*R**, 10*S**, 11*R**, 12*R**, 15*R**, 25*S**, and 26*R**.

Ambiguines H isonitrile (**1**) and I isonitrile (**2**) possess strong antibacterial and antifungal activities (see Table 4), comparable with clinical agents and the activity of the known ambiguines.[6] The biological activity of ambiguine J isonitrile (**3**) was not determined.

The co-occurrence of the nonchlorinated derivatives **1**−**4** with the chlorinated ambiguines **5**−**9** implies some imperfection in the biosynthesis of the ambiguines. Moore et al.[7,8,13] proposed a common biogenesis of the hapalindoles,[5] ambiguines,[6] fischerindoles,[7] and welwitindolinones[8] (see Figure 1), which may involve a chloronium ion-induced condensation of (*Z*)-β-ocimene and 3-((*Z*)-2′-isocyanoethenyl)indole (a, see Figure 1), leading to intermediate 3-cyclohexylindole derivatives with 10,15-*cis*-diequatorial stereochemistry (like b, Figure 1, in the case of the ambiguines) or 10,15-*cis*-equatorial,axial stereochemistry (as the intermediate of hapalindole A) that are converted to the final products by additional cyclization and tailoring processes. When

Table 3. NMR Data of Ambiguine J Isonitrile (**3**) in $CDCl_3$[a]

position	δ_C, mult.[b]	δ_H, mult., *J* (Hz)	COSY correlations[d]	LR H−C correlations[c]	NOE correlations[g]
1					
2	186.6 qC				
3	83.6 qC				
3-OH		3.50 s			
4	146.3 qC				
5	120.8 CH	7.12 d 7.2	H-6	C-6, 7, 9, 16	
6	130.3 CH	7.31 m	H-5, 7	C-5, 7, 8	
7	118.5 CH	7.32 m	H-6	C-8, 9	
8	151.7 qC				
9	135.6 qC				
10	76.6 qC				
10-OH		4.19 brs		C-3,10, 15	H_3-17,19
11	66.5 qC				
12	45.1 qC				
13	35.0 CH_2	1.52 brd 12.9[e]	H-13ax, 14ax, 14eq	C-12, 14	
		1.70 m[f]	H-13eq, 14ax, 14eq	C-12, 14	H-14ax
14	17.7 CH_2	1.72 m[e]	H-13ax, 13eq, 14ax	C-13, 15	
		2.05 bddd[f] 15.6, 13.7, 11.0	H-13ax, 13eq, 14eq	C-13, 15	H_3-17, 19
15	53.7 CH	1.58 m	H-14ax, 14eq	C-14, 16	
16	38.0 qC				
17	25.5 CH_3	1.39 s[f]		C-4, 15, 16, 18	H-5, 14ax, 10-OH, H_3-18
18	26.3 CH_3	1.33 s[e]		C-4, 15, 16, 17	
19	19.0 CH_3	1.63 s		C-11, 12, 13, 20	10-OH, H-13eq, 14ax, 20
20	143.7 CH	6.05 dd 17.2, 11.2	H-21Z, 21E	C-12, 21	H_3-19, 21E
21	116.6 CH_2	5.26 d 17.2[h]	H-20	C-12, 20	H-26
		5.30 d 11.2[i]	H-20	C-12, 20	H-20
23	160.0 qC				
24	38.3 qC				
25	64.8 CH	3.14 d 4.0	H-26	C-2, 11, 24, 26	H-26, H_3-27, 28
26	60.0 CH	3.50 d 4.0	H-25	C-25	H-21Z, 25
27	26.6 CH_3	1.75 s		C-2, 24	H-25
28	30.4 CH_3	1.75 s		C-2, 24	H-25

[a] Carried out on a Bruker Avance 400 spectrometer in acid-free $CDCl_3$.[10] [b]Multiplicity and assignment from HMQC experiment. [c]Determined from HMBC experiment, $^nJ_{CH} = 8$ Hz, recycle time 1 s. [d]By COSY experiment. [e]Equatorial. [f]Axial. [g]From NOESY experiment with 400 ms mixing delay. [h]Z-proton. [i]*E*-proton.

Table 4. MIC Values of Ambiguine H Isonitrile (**1**) and Ambiguine I Isonitrile (**2**) against Test Organisms *in Vitro*

	MIC values (μg/mL)			
organism	**1**	**2**	sterptomycin	puramycin/amphotericin B
Escherichia coli ESS K-12[a]	10	2.5	0.312	
Staphyloccocus albus[a]	0.625	0.078	0.156	
Bacillus subtilis[a]	1.25	0.312	2.5	
Saccharomyces cerevisiae[b]	5	0.312		0.312[d]
Candida albicans ATCC 90028[b]	6.25[c]	0.39[c]		1.56[e]

[a] Tested in LB broth. [b]Tested in YPD broth. [c]Fungistatic. [d]Puramycin. [e]Amphotiricin B, fungicidic.

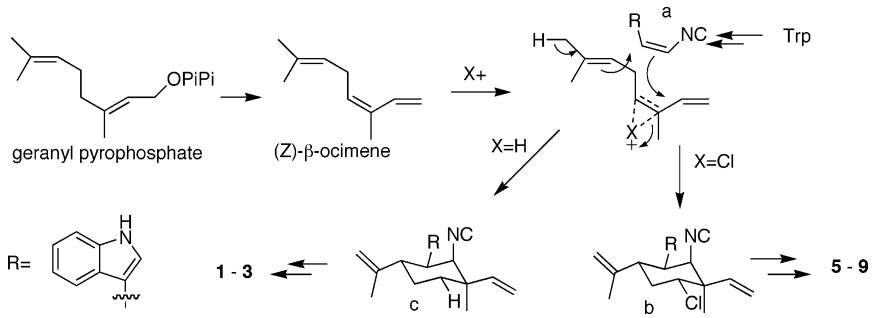

Figure 1.

isolated from the cyanobacteria extracts, all four groups of these indole alkaloids contain mixtures of (i) chlorinated (major, type a) and (ii) nonchlorinated (minor constituents, type b) as well as (iii) minor stereoisomers of the isonitrile or isothiocyanate bearing a cyclohexyl moiety (type c). e.g., in the case of the hapalindoles[13] from *Hapalosiphon fontialis*, hapalindoles A and B (type a), hapalindoles J and M (type b), and hapalindoles C−H (type c) and in the case of the welwitindolinones and fischerindoles[14] from *H. welwitschii*, *N*-methylwelwitindolinones C isothiocyanate and C isonitrile and 12-*epi*-fisherindole G isonitrile (type a), 12-*epi*-fisherindole U isonitrile and 12-*epi*-hapalindole C isonitrile (type b), and 12-*epi*-hapalindole E isonitrile and 12-*epi*-hapalindole F isonitrile (type c). Ambiguines D isonitrile (**7**) and E isonitrile (**8**) (type a), ambiguine C isonitrile (type b), and hapalindole H (type c) were isolated from *Fischerella ambigua*.[14] In the present study, ambiguines D isonitrile and E isonitrile (**8**) (type a), ambiguines H isonitrile (**1**), I isonitrile (**2**), and J isonitrile (**3**) (type b), and 12-*epi*-hapalindole H (**4**) (type c) are observed. Synthesis of such a

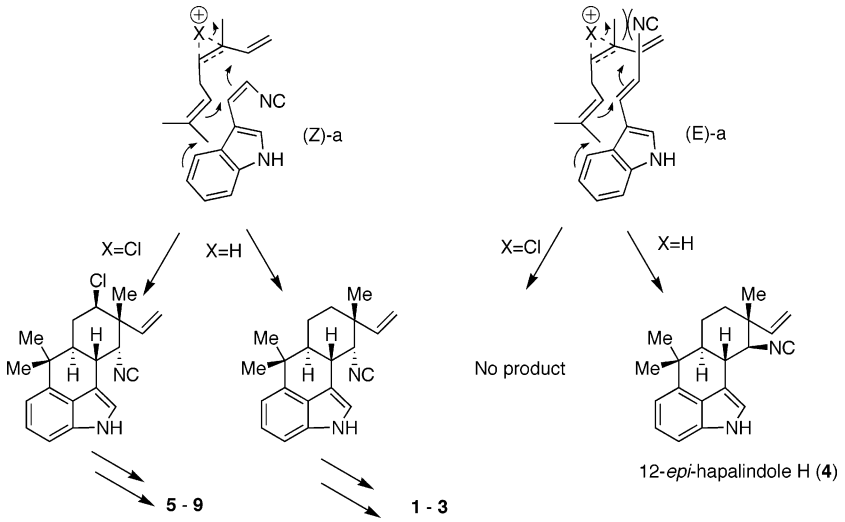

Figure 2.

library of biosynthetic products may suggest a need for an array of related compounds so as to obtain an ecological advantage in a highly diverse ecosystem; however, it may also suggest an imperfection in the biosynthetic machinery. In the case of these four groups of indole alkaloids, both issues may apply. In the present study, we isolated an array of five chlorinated ambiguines (major components), which may be the major products that give the cynobacterium its ecological advantage, but at the same time we isolated three nonchlorinated ambiguines (minor components), which present antimicrobial properties similar to those of the chlorinated counterparts, and a hapalindole with the "inverted stereochemistry" on the isonitrile-bearing carbon, 12-*epi*-hapalindole H (**4**). While the chlorinated and nonchlorinated ambiguines may derive from a competition between chloronium and proton ions in the active site of the condensing enzyme (see Figure 1 and 2), the presence of 12-*epi*-hapalindole H (**4**) likely represents an alternative biosynthetic manifold, which may derive from the reaction of 3-((*E*)-2′-isocyanoethenyl)indole or rearrangement of intermidiate *a* in the catalytic process. Utilization of the *E*-isomer over the *Z*-isomer could result in a larger steric hindrance due to the bulky isonitrile group, which prevents the insertion of the larger chloronium ion into the active site of the enzyme, thus resulting in the production of only 12-*epi*-hapalindole H (**4**) but not its chloro derivative. The equatorial isonitrile group in **4** most probably prevents its further processing to ambiguine. The presence of only *tetra*- and *penta*cyclic hapalinoles and ambiguines in *Fisherella* sp. (this study), *F. ambigua*, and *H. hibernicus*[14] suggests that, at least in the case of the biosynthesis of the ambiguines, the tetracyclic product is synthesized in one step, as illustrated in Figure 2.

Experimental Section

General Experimental Procedures. Optical rotations were determined on a JASCO P-1010 polarimeter. UV spectra were obtained on a Varian Cary 5000 UV−vis−NIR spectrophotometer. IR spectra were obtained on a Bruker Vector 22 spectrometer. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker Avance 400 spectrometer, using tetramethylsilane as the internal standard. Low- and high-resolution electron impact and chemical ionization mass spectra were obtained on a VG AutoSpecQ M 250 spectrometer.

Culture Conditions. An edapic form of *Fischerella* sp.[9] designated Tel Aviv University (TAU) strain number IL-199-3-1 was isolated from a soil sample collected in "The Cactus Nursery", Herzliya, Israel, in July 1996. A clonal strain was purified on a BG-11 agar medium.[12] The cyanobacterium was cultured in 20 L glass bottles containing a modified BG-11 medium.[5] Cultures were continuously illuminated at an intensity of 100 μmol photons/M^2/s from fluorescent tubes and aerated with 0.5% CO_2 in air (1 L/min) at an incubation temperature of 25 °C for 30−35 days. Yield of lyophilized cells was about 0.2 g/L of culture.

Extraction and Isolation. The freeze-dried cells (40 g from 200 L of culture) were extracted with 7:3 MeOH/H_2O (5 × 700 mL). The crude extract exhibited antibacterial, antifungal, and antimalarial properties. The crude extract (7.3 g) was separated on an ODS (YMC-GEL, 120A, 4.5 × 6.5 cm) flash column with increasing amounts of MeOH in H_2O. The bioassay-guided (antibacterial) separation of the active fractions, 9 and 10 (8:2 and 9:1 MeOH/H_2O), followed. The combined fractions (670 mg) were separated on a Sephadex LH-20 gel-filtration column with 1:1 CH_2Cl_2/MeOH. Twenty-two fractions were collected and combined to five groups on the basis of proton NMR data. The combined active fraction b (fractions 6−14, 400 mg) from the Sephadex LH-20 column was subjected to RP HPLC (Merck HiBar LiChrospher 60 RP-Select B, 5 μm, 250 mm × 25 mm at 21 °C, DAD at 238 nm, flow rate 5.0 mL/min) in 9:1 MeOH/H_2O. From this crude separation five fractions were collected; fractions 2, 3, and 4 were further separated. Fraction 4 (103 mg) was separated on a HiBar Select B column with 85:15 CH_3CN/H_2O to give eight fractions (4a−4h). Fraction 4c (t_R 54.8 min, 14.4 mg) was shown to be the known 12-*epi*-hapalindole H (**4**). The structure of fraction 4d (t_R 58.7 min, 11.8 mg) was established to be the new ambiguine I isonitrile (**2**). Fraction 4e (t_R 63.9 min, 21.8 mg) was identified as the known compound ambiguine E isonitrile (**8**). Fraction 4f (t_R 69.3 min, 5.8 mg) was found to be a new natural product designated ambiguine H isonitrile (**1**). Fraction 4g (t_R 72.3 min, 11.3 mg) was recognized as the known compound ambiguine A isonitrile (**5**). Fraction 3 (131.2 mg) was separated once again under the same conditions to give nine fractions (3a−3j). Fraction 3b (t_R 13.6 min, 28.4 mg) was identified as the known ambiguine D isonitrile (**7**). Fractions 3a, 3d, and 3e were further separated. Fraction 3a (14.4 mg) was separated on HiBar Select B column, using 75:25 CH_3CN/H_2O as eluant, to give three fractions (3a1−3a3). Fraction 3a1 (t_R 78.5 min, 3.5 mg) was established to be the new ambiguine J isonitrile (**3**). Fraction 3d (13.8 mg) was separated again on the HiBar Select B column using 8:2 CH_3CN/H_2O as eluant to give six fractions (3d1−3d6). Fraction 3d5 (t_R 41.4 min, 2.6 mg) was identified as the known ambiguine B isonitrile (**6**). Fraction 3e (19.7 mg) was separated on the HiBar Select B column with 7:3 CH_3CN/H_2O to give nine fractions (3e1−3e9). Fraction 3e7 (t_R 51.2 min, 2.5 mg) was found to be the known compound 12-*epi*-hapalindole H (**4**). Fraction 3e9 (t_R 41.3 min, 2.1 mg) was identified as ambiguine B isonitrile (**6**). Fraction 2 was separated on the HiBar Select B column eluted with 55:45 CH_3CN/H_2O to yield five fractions (2a−2e). Fraction 2e (t_R 63.5 min, 2.9 mg) was identified as the known ambiguine F isonitrile (**9**).

Ambiguine H isonitrile (1): white solid; $[\alpha]^{25}_D$ −65 (*c* 0.51, MeOH); UV (MeOH) λ_{max} (log ϵ) 222 (4.38), 282 (3.72), 291 (3.62) nm; IR ($CHCl_3$) ν_{max} 1018, 1246, 1265, 1418, 1601, 2139, 2976, 3477, 3627 cm^{-1}; ^{1}H and ^{13}C NMR (see Table 1); HREIMS *m*/*z* 372.2560 (calcd for $C_{26}H_{32}N_2$, 372.2565).

Ambiguine I isonitrile (2): white solid; $[\alpha]^{25}_D$ −39 (*c* 0.29, MeOH); UV (MeOH) λ_{max} (log ϵ) 222 (4.04), 282 (3.69), 286 (3.67) nm; IR ($CHCl_3$) ν_{max} 941, 1262, 1448, 1473, 1600, 2129, 2970, 3466 cm^{-1}; ^1H and ^{13}C NMR (see Table 2); HREIMS *m*/*z* 402.2307 (calcd for $C_{26}H_{30}N_2O_2$, 402.2307).

Ambiguine J isonitrile (3): transparent solid; $[\alpha]^{25}_D$ −32 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 222 (3.68), 270 (2.97), 308 (2.78) nm; ^1H and ^{13}C NMR (see Table 3); HREIMS *m*/*z* 418.2250 (calcd for $C_{26}H_{30}N_2O_3$, 418.2256).

12-*epi*-Hapalindole H (4): white solid; $[\alpha]^{25}_D$ +89 (*c* 0.13, MeOH); ^1H and ^{13}C NMR (see Table 5 in Supporting Information); EIMS *m*/*z* 304 $[M]^+$ (100), 289 (50).

Ambiguine A isonitrile (5): white solid; $[\alpha]^{25}_D$ −39 (*c* 0.37, MeOH); ^1H and ^{13}C NMR (see Table 6 in Supporting Information); EIMS *m*/*z* 406/408 $[M]^+$ (3:1, 100), 391/393 (3:1, 70), 236 (90).

Ambiguine B isonitrile (6): white solid; $[\alpha]^{25}_D$ −24 (*c* 0.37, MeOH); ^1H and ^{13}C NMR (see Table 7 in Supporting Information); EIMS *m*/*z* 422/424 $[M]^+$ (3:1, 25), 360.3 (35), 252 (100).

Ambiguine D isonitrile (7): white solid; $[\alpha]^{25}_D$ −48 (*c* 0.16, MeOH); ^1H and ^{13}C NMR (see Table 8 in Supporting Information); EIMS *m*/*z* 452/454 $[M]^+$ (3:1, 5), 434/436 (3:1, 100), 419 (35), 399 (65).

Ambiguine E isonitrile (8): white solid; $[\alpha]^{25}_D$ −100 (*c* 0.09, MeOH); ^1H and ^{13}C NMR (see Table 9 in Supporting Information); EIMS *m*/*z* 436/438 $[M]^+$ (3:1, 100).

Ambiguine F isonitrile (9): white solid; $[\alpha]^{25}_D$ −63 (*c* 0.13, MeOH); ^1H and ^{13}C NMR (see Table 10 in Supporting Information); EIMS *m*/*z* 454/456 $[M]^+$ (3:1, 100).

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Supporting Information Available: Photograph of the cyanobacterium, 1D and 2D NMR spectra of **1**, **2**, and **3**, and full NMR data of compounds **4**−**9**. This material is available free of charge via the Internet at http://pubs.acs.org.

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