Lactone 4: 0.4 mg; colorless oil; IR (neat) 1770, 1730 cm^{-1}; low-resolution mass spectrum (12 eV), m/z (relative intensity) 375 (M^+ - OAc, 8), 332 (16), 316 (26), 314 (100), 286 (85), 258 (26), 229 (34), 203 (21), 137 (32), 124 (58).

Lactone 5: 0.2 mg; colorless oil; IR (neat) 1770, 1730 cm^{-1}; low-resolution mass spectrum (12 eV), m/z (relative intensity) 403 (M^+ - OAc, 6), 332 (17), 314 (100), 286 (51), 258 (9), 229 (14).

Lactone 6: 0.5 mg; white crystals ($CHCl_3$); IR (neat) 3400, 1770 cm^{-1}; low-resolution mass spectrum (12 eV), m/z (relative intensity) 288 (M^+ - HCOOH, 100), 273 (13), 245 (6), 207 (8), 177 (20), 149 (18), 137 (17), 123 (10).

Lactone 7: 0.8 mg; colorless oil; IR (neat) 1760, 1710 cm^{-1}; low-resolution mass spectrum (70 eV), m/z (relative intensity) 318 (M^+, 24), 290 (17), 275 (18), 218 (100), 203 (22), 167 (14), 149 (30), 137 (37), 123 (48), 109 (31), 91 (55).

Lactone 8: 5.5 mg; colorless oil; IR (neat) 1735 cm^{-1}; low-resolution mass spectrum (12 eV), m/z (relative intensity) 449 (M^+ - OAc, 22), 405 (19), 360 (11), 345 (5), 328 (76), 300 (100), 286 (21), 241 (13), 167 (23), 149 (52).

Lactone 9: 1 mg; colorless oil; IR (neat) 1735 cm^{-1}; low-resolution mass spectrum (12 eV), m/z (relative intensity) 477 (M^+ -OAc, 8), 476 (2), 434 (3), 433 (7), 328 (100), 286 (19), 241 (14).

Lactone 10: 1.5 mg; colorless oil; IR (neat) 1735 cm^{-1}; low-resolution mass spectrum (12 eV), m/z (relative intensity) 408 (M^+ - CH_2CO, 3), 391 (M^+ - OAc, 20), 390 (M^+ - AcOH, 3) 330 (53), 302 (100), 287 (20), 178 (18), 136 (50).

Acetylation of Lactone 3. Lactone **3** (3 mg) was reacted with acetic anhydride–pyridine (1:1) (0.4 mL) at room temperature overnight. Excess reagents were evaporated under nitrogen, and the residue was chromatographed by HPLC [H_2O/MeOH (15/85); 5 μm, C-18] to give lactone **4** and lactone **11** in a 3/7 ratio.

Lactone 11: colorless oil; IR (neat) 2730, 1780, 1740, 1715 cm^{-1}; low-resolution mass spectrum (70 eV), m/z (relative intensity) 375 (M^+ - OAc, 4), 314 (M^+ - 2AcOH, 16), 286 (100), 258 (51), 229 (33), 203 (28), 175 (32), 161 (25), 137 (88), 123 (46), 109 (75), 105 (52); ^1H NMR (300 MHz, $CDCl_3$) δ 0.77 (3H, s, H-20), 0.84 (3 H, s, H-19), 1.01 (3 H, s, H-18), 1.17 (1 H, t, J = 12.4 Hz, H-7α), 1.25 (1, H, d, J = 12.4 Hz, H-5α), 2.05 (6 H, s, OAc), 2.30 (1 H, d, J = 7.8 Hz, H-14), 2.53, (1 H, br d, J = 15 Hz, H-12), 2.87 (1 H, dd, J = 12.4, 4 Hz, H-7β), 2.98 (1 H, br t, J = 7.8 Hz, H-13), 5.17 (1 H, dt, J = 4, 12.4 Hz, H-6), 6.15 (1 H, s, H-15), 9.96 (1 H, s, H-17); ^{13}C NMR ($CDCl_3$, 75.4 MHz) (multiplicities by DEPT, assignment by analogy to other compounds in this series) δ 16.5 (t, C-11), 17.0 (q, C-20), 18.4 (t, C-2), 21.0 (q, OAc), 22.0 (q, C-19), 22.2 (q, OAc), 22.2 (t, C-12), 33.2 (s, C-4), 35.0 (d, C-13), 36.2 (q, C-18), 39.0 (t, C-1), 39.5 (s, C-10), 42.0 (t, C-3), 43.7 (t,C-7), 49.7 (s, C-8), 53.4 (d, C-14), 55.8 (d, C-9), 58.0 (d, C-5), 68.6 (d, C-6), 93.6 (d, C-15), 168.5 (s, $OCOCH_3$), 169.7 (s, $OCOCH_3$), 175.9 (s, C-16), 203.0 (d, C-17).

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Fontonamide and Anhydrohapaloxindole A, Two New Alkaloids from the Blue-Green Alga *Hapalosiphon fontinalis*

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Fontonamide (**3**) and anhydrohapaloxindole A (**4**) are two minor alkaloids that have been isolated from a cultured strain of the terrestrial blue-green alga *Hapalosiphon fontinalis*. Both compounds appear to be singlet oxygen oxidation products of hapalindole A (**1**), the major alkaloid in this cyanophyte. Hapalonamide A (**5**), the probable precursor of fontonamide, is formed along with **3** and **4** when an oxygen-aerated solution of **1** in aqueous methanol buffered at pH 8 with sodium phosphate and containing a trace of rose bengal is irradiated at room temperature. To date, however, **5** has not been identified as a constituent of *H. fontinalis*.

Hapalindole A (**1**), an unusual chlorine-containing isonitrile, is the major alkaloid in a cultured strain of the terrestrial blue-green alga *Hapalosiphon fontinalis* (Ag.) Bornet (Stigonemataceae) and is responsible in part for the antimycotic activity of this prokaryote.[1,2] Hapalindole A and the corresponding isothiocyanate **2** (hapalindole B) are isolated from a fraction resulting from flash chromatography of the lipophilic extract of *H. fontinalis* on silica gel (TLC grade) using 1:1 hexane/CH_2Cl_2 as the eluant.[1] A more polar fraction eluted with CH_2Cl_2 contains two new compounds which appear to be singlet oxygen oxidation products of hapalindole A, viz. fontonamide (**3**) and anhydrohapaloxindole A (**4**) (Chart I).

Fontonamide (**3**), mp 156–157 °C, $[\alpha]_D$ −141° (c 0.21, $CHCl_3$), is the first compound to be eluted from silica gel with dichloromethane (0.01% yield based on dried alga). The UV spectrum of **3** [λ_{max} nm (ϵ) 240 (5680), 289 (4770), 345 (1630)] indicates that the indole moiety is modified and the ^{13}C NMR spectrum shows that two carbonyls are present. One of the carbonyls has to be a conjugated ketone (singlet at δ 188.97) and the other a formamide (doublet at δ 159.75). The EI mass spectrum reveals the presence of chlorine in the molecular ion (3:1 isotopic cluster at m/z 343,345) and a high resolution mass measurement shows that the elemental composition is $C_{20}H_{22}NO_2Cl$ (obsd m/z 343.1353; calcd m/z 343.1339). The

(1) Moore, R. E.; Cheuk, C.; Patterson, G. M. L. *J. Am. Chem. Soc.* **1984**, *106*, 6456. In this preliminary communication we reported that our strain of *Hapalosiphon fontinalis* produces an extracellular substance when grown on an agar plate that inhibits the growth of other blue-green algae. This substance may not be hapalindole A.

(2) Moore, R. E.; Cheuk, C.; Yang, X.-Q. G.; Patterson, G. M. L.; Bonjouklian, R.; Smitka, T. A.; Mynderse, J. S.; Foster, R. S.; Jones, N. D.; Swartzendruber, J. K.; Deeter, J. B. *J. Org. Chem.* **1987**, *52*, 1036.

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Table I. NMR Spectral Data for Fontonamide (3) in $CDCl_3$

$^{13}C^{a,b}$	C	$^{1}H^{c}$	$^{13}C^{a,b}$	C	$^{1}H^{c}$
188.97 s	3		64.59 d	13	4.071 dd
159.75 d	2	8.483 d	44.43 d	15	2.838 ddd
153.68 s	10[d]		44.01 s	12[d]	
144.70 d	11	7.113 d	37.74 s	16[d]	
142.81 d	20	5.938 dd	30.85 t	14(eq)	2.336 ddd
140.83 s	8[d]			14(ax)	2.098 td
135.27 d	6	7.528 t	25.68 q	17	1.057 s
134.76 s	4[d]		24.25 q	18	1.468 s
133.54 s	9[d]		19.75 q	19	1.311 s
119.78 d	7	8.597 br d		1	11.96 br
118.85 d	5	7.200 br *d*			
115.07 t	21*E*	5.217 dd			
	21*Z*	5.193 dd			

J (H, H) in Hz: 1,2 = 1.8; 5.6 = 7.9; 5,7 = 0.5; 6,7 = 8.6; 11,15 = 2.5; 13,14(eq) = 3.5; 13,14(ax) = 12.8; 14(ax),14(eq) = −12.9; 14(ax),15 = 6.0; 20,21*Z* = 17.5; 20,21*E* = 10.7; 21*E*,21*Z* = 0.5

[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] Assignments based on selective INEPT experiment (ref 5, 6).

^1H NMR spectrum of **3** indicates that three adjacent protons exist on the benzenoid ring and that three quaternary methyls, a vinyl group, and a CH(ax)–CH_2–CH(ax) segment are present like in hapalindole A. The isonitrile group, however, is clearly missing. The most logical structure for fontonamide appears to be **3** where the C2–C3 bond of hapalindole A has been cleaved oxidatively and a double bond has been formed between C10 and C11 by β-elimination of HCN from an intermediate of structure **5**.

The stereochemistry of **3** is supported by the following ^1H NMR data. The proton on C15 shows a 2.5-Hz allylic coupling to the proton on C11, since the C(15)–H bond is essentially orthogonal to the C(15)—C(10)═C(11)H plane when examined in a Dreiding model. Difference NOE spectra exhibit positive NOEs for the H5 and H(ax)14 signals when the peak at δ 1.057, which has to be assigned to the C17 methyl group, is irradiated, positive NOEs for the H(eq)14 and H15 signals when the peak at δ 1.468, which has to be due to the C18 methyl group, is irradiated, and positive NOEs for the H11, H(ax)14, H20, and *Z* H21 signals when the signal at δ 1.311, which is attributed to the C19 methyl group, is irradiated. C19 has to be attached axially to C12, a carbon which also bears the vinyl group, to account for the NOEs on H(ax) 14, H20, and *Z* H21.

Anhydrohapaloxindole A (**4**), mp 123 °C dec, $[\alpha]_D$ + 150° (EtOH, *c* 0.4), is eluted next from silica gel with CH_2Cl_2 (0.01% yield). The UV spectrum of **4** [λ_{max} nm (ϵ) 221 (11 800), 262 (13 100), 268 (14 200), 320 (4900)] again shows that the indole system is modified. A 3:1 molecular ion cluster at *m/z* 352/354 in the EI mass spectrum reveals the presence of one chlorine atom in **4** and a high resolution mass measurement indicates that the molecular formula is $C_{21}H_{21}N_2OCl$ (obsd *m/z* 352.1418; calcd *m/z* 352.1342). The proton noise-decoupled ^{13}C NMR spectrum shows signals for an amide carbonyl (singlet at δ 167.26) and an isonitrile group (broad 1:1:1 triplet at δ 159.02, $J_{13C,14N}$ = 5 Hz). The ^1H NMR spectrum reflects the presence of three adjacent aromatic protons, an amide NH, a CH(ax)–CH_2–CH(ax) unit in a six-membered ring, a vinyl group, three quaternary Me groups, and an isolated methine bearing the isonitrile group. These data are consistent with structure **4**.

The relative stereochemistry depicted in **4** is supported by NOE studies. Strong positive NOEs are observed in the H11, H(ax)14, H20, and *Z* H21 signals when the C19 methyl group is irradiated, proving that C19 is axial and H11 is pseudoequatorial and that the vinyl group is attached to C12. Strong positive NOEs are also observed in the H5, H(eq) 14, and H15 signals when the C17 Me group is irradiated and in the H15 signal when the C18 Me group is irradiated.

Hapalindole A is readily converted into fontonamide and anhydrohapaloxindole A under $^1O_2$ oxidation conditions.

Chart I

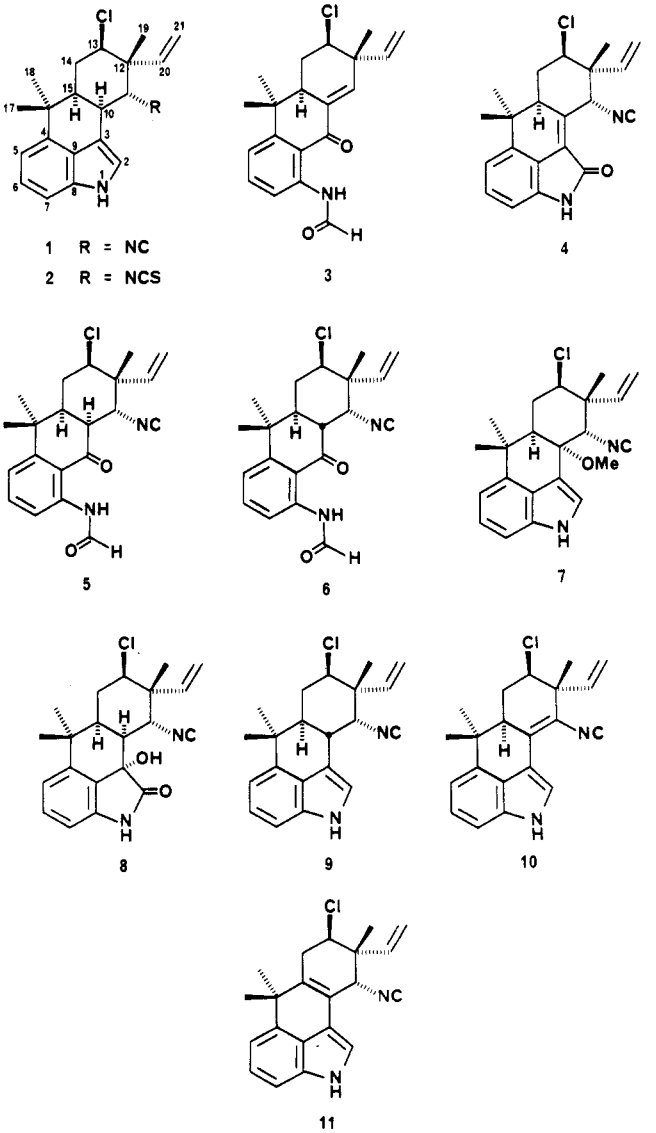


Table II. NMR Spectral Data for Anhydrohapaloxindole A (4) in $CDCl_3$

$^{13}C^{a,b}$	C	$^{1}H^{c}$	$^{13}C^{a,b}$	C	$^{1}H^{c}$
167.26 s	2		107.71 d	7	6.694 dd
159.02 s	23		60.88 d	13	4.416 dd
139.97 s			59.24 d	11	5.700 br s
139.79 d	20	6.058 dd	48.67 s	12	
138.58 s			48.56 d	15	2.915 dd
137.00 s			36.53 s	16	
130.98 d	6	7.223 t	34.96 t	14(eq)	2.331 dt
123.90 s				14(ax)	1.756 td
118.53 s			31.59 q	18	1.242 s
117.35 t	21*E*	5.413 d	22.22 q	17	1.404 s
	21*Z*	5.332 d	14.50 q	19	1.065 s
117.28 d	5	6.914 dd		1	7.62 br

J(*H*,*H*) in Hz: 5,6 = 7.9; 5,7 = 0.5; 6,7 = 7.7; 13,14(eq) = 4.4; 13,14(ax) = 12.2; 14(ax),14(eq) = −12.9; 14(eq),15 = 4.5; 14(ax),15 = 13.2; 20,21*Z* = 17.4; 20,21*E* = 10.9; 21*E*,21*Z* = 0

[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] Assignments based on selective INEPT experiment (ref 5, 6).

When an oxygen-aerated solution of **1** in methanol containing a trace of the sensitizer rose bengal is irradiated at room temperature,[3] **4** and hapalonamide A (**5**), the apparent precursor of **3**, are formed as major products. When the oxidation is carried out in aqueous MeOH buffered at pH 8.0 with 0.5 M sodium phosphate, however, **3** and **4** are major products.

In methanol solution hapalonamide G (**6**), 10-methoxyhapalindole A (**7**), and hapaloxindole A (**8**) are also major oxidation products and hapalindoles G (**9**), I (**10**), and K (**11**), which are minor alkaloids in *H. fontinalis*,[2] are minor oxidation products. In the pH 8 buffered aqueous MeOH solution, however, **8** is also a major oxidation product, but **5** and **6** are minor products along with **9**, **10**, and **11**. Hapalonamide G also appears to be a precursor of **3**. Hapaloxindole A and hapalonamides A and G, however, have not been detected yet in extracts of *H. fontinalis*.

The ^{1}H NMR spectra of hapalonamides A and G are consistent with the structures shown for these two oxidation products. Both compounds can be readily converted into **3**.

Compound **7** has the molecular composition $C_{22}H_{25}N_2OCl$ by mass spectrometry and exhibits a UV spectrum typical of an indole. The ^{1}H NMR spectrum is similar to that of **1** but lacks a signal for H10 and the couplings to this proton in the H2, H11, H(eq)14, and H15 signals. The spectrum does show a signal for a methoxyl group which has to be on C10. The difference NOE spectrum shows the same strong positive NOEs in the H2, H11, H(ax) 14, H20, and *Z* H21 signals as hapalindole A when the C19 methyl group (δ 0.659) is irradiated, proving that C19 is attached axially to C12, but more importantly that the methoxyl group on C10 is equatorial. Compound **7** is therefore 10-methoxyhapalindole A.

Hapaloxindole A (**8**) exhibits a UV spectrum typical of an oxindole. The ^{1}H NMR spectrum is essentially identical with that of **1** except that the signal for H2 is missing. The oxindole NH signal, however, is found at much lower field (9.41 ppm) than the one for anhydrohapaloxindole A (7.62 ppm). NOEs similar to the ones observed with **1** are observed when the C17, C18, and C19 methyl groups are irradiated. In **8** the stereochemistry of C3 is probably *S* since $^{1}O_2$ would be expected to add to the unhindered side of C2–C3.

The hapalindole K obtained from oxidation of hapalindole A has the same optical rotation as the natural product, viz. $[\alpha]_D$–12.5°. Since X-ray crystallography indicates that the absolute stereochemistry of natural hapalindole K is 11*R*,12*R*,13*R*[2], the absolute stereochemistry of hapalindole A must be 10*R*,11*R*,12*R*,13*R*,15*S*. Furthermore since the optical rotations of semisynthetic and natural **3**, **4**, **9**, and **10** are identical, viz. $[\alpha]_D$ −141°, +150°, −43.9°, and −12°, respectively, the absolute stereochemistries of semisynthetic and natural fontonamide, anhydrohapaloxindole A, and hapalindoles G and I have to be the same. The absolute configurations of C10, C11, C12, C13, and C15 in these compounds are therefore identical with those in hapalindole A, except in **9** where C10 is *S*, presumably the result of a free radical induced epimerization at this position in **1** during the oxidation. Hapalindoles I and K probably also arise from a free radical induced oxidation of **1**.

Fontonamide and anhydrohapaloxindole, however, appear to be singlet oxygen oxidation products of hapalindole A. According to the literature,[4] the indole and singlet oxygen react initially to form a labile intermediate which cyclizes to either a dioxetane or a perepoxide. The perepoxide then rapidly rearranges to an allylic hydroperoxide (Scheme I). At room temperature both the dioxetane and the allylic hydroperoxide are unstable. The dioxetane fragments to form a ketoformamide (**5**) which then loses the elements of hydrogen cyanide in aqueous medium to give fontonamide (**3**). The allylic hydroperoxide, on the other hand, loses water to form anhydrohapaloxindole A (**4**). The origin of hapaloxindole A (**8**) is unknown at this writing.

Experimental Section

Isolation. *Hapalosiphon fontinalis* (strain V-3-1) was mass cultured in the laboratory as previously described.[1,2] The freeze-dried alga (360 g) was extracted with 1:1 2-propanol/dichloromethane. Gel filtration of the oily extract (15.1 g) on Sephadex LH-20 with 1:1 CH_2Cl_2/2-propanol gave an antimycotic[2] fraction (10.7 g) which was subjected in 2-g portions to rapid chromatography on 30 g of silica gel (TLC grade) with hexane, 1:1 hexane/CH_2Cl_2, CH_2Cl_2, CH_2Cl_2/EtOAc, EtOAc, and EtOAc/EtOH.

The material that was eluted with CH_2Cl_2 (1.2 g) was rechromatographed on a 27 × 1.8 cm column of silica gel (TLC grade) with 1:1 hexane/CH_2Cl_2. Ten-milliliter fractions were collected. Fractions 14–19 were combined to give 32 mg of crude fontonamide and fractions 30–33 were combined to give 44 mg of crude anhydrohapaloxindole A. Final purification of these compounds

(3) Saito, I.; Imuta, M.; Matsugo, S. *Synthesis* **1976**, 255.

(4) (a) Shimizu, N.; Bartlett, P. D. *J. Am. Chem. Soc.* **1976**, *98*, 4193. (b) Frimer, A. A.; Bartlett, P. D.; Boschung, A. F.; Jewett, J. G. *J. Am. Chem. Soc.* **1977**, *99*, 7977.

(5) Bax, A. *J. Magn. Reson.* **1984**, *57*, 314.

(6) Moore, R. E.; Niemczura, W. P. In *Pharmaceuticals and the Sea*; Jefford, C. W., Ed.; Technomic: Lancaster, PA, in press.

Table III. ^{1}H NMR Spectral Data for Hapalonamides A (5) and G (6) in $CDCl_3$[a]

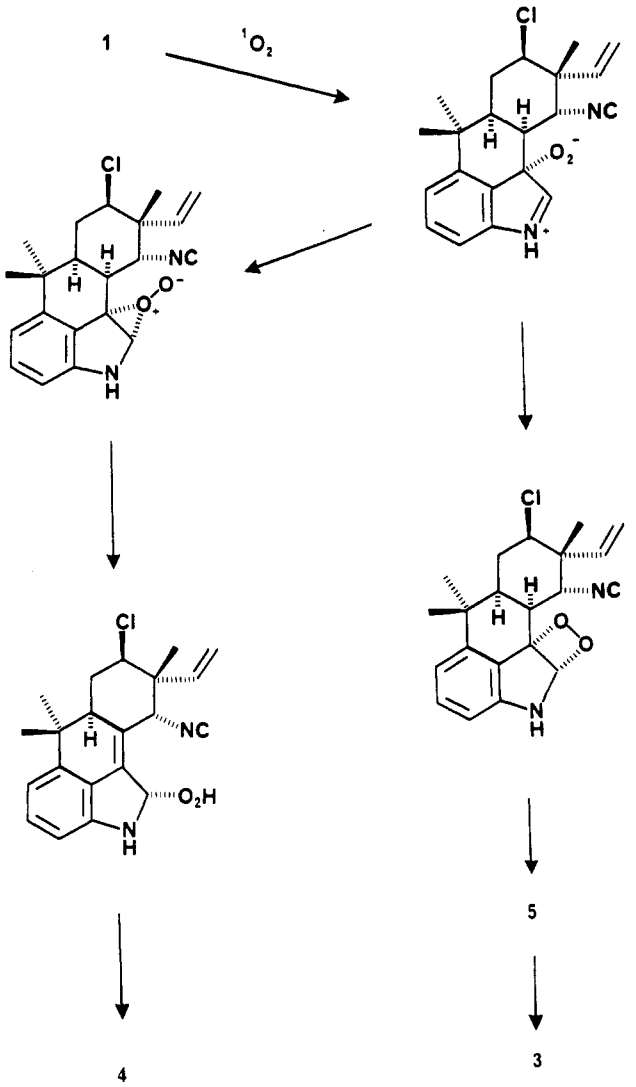
C	5	6	C	5	6
1	11.77 br	12.02	14(ax)	1.473 q	1.91
2	8.51 br	8.51	14(eq)	2.217 td	2.39
5	7.133 br d	7.27	15(ax)	2.422 dt	2.61
6	7.588 t	7.58	17	1.539 s	1.51
7	8.670 br d	8.68	18	1.328 s	1.25
10(eq)	3.353 br d		19	0.826 s	1.25
10(ax)		2.89	20	6.005 dd	6.05
11(eq)	4.718 br d	4.49	21*E*	5.664 d	5.33
13(ax)	4.303 dd	4.33	21*Z*	5.254 d	5.40

J(H,H) for **5** in Hz: 5,6 = 7.8; 6,7 = 8.1; 10,11 = 0.5; 10,15 = 5; 10,14(eq) = 1; 13,14(ax) = 12.4; 13,14(eq) = 4.3; 14(ax),14(eq) = −13.1; 14(ax),15 = 13.1; 14(eq),15 = 4.5; 20,21*E* = 10.9; 21,22*Z* = 17.5; 21*E*,21*Z* = 0

J(H,H) for **6** in Hz: 5,6 = 8.1; 5,7 = 0.7; 6,7 = 8.2; 10,11 = 3; 10,15 = 13; 13,14(ax) = 12.4; 13,14(eq) = 4.5; 14(ax),14(eq) = −13; 14(ax),15 = 12.6; 14(eq),15 = 3.7; 20,21*Z* = 17.5; 20,21*E* = 11.0; 21*E*,21*Z* = 0

[a] 300 MHz; residual $CHCl_3$ as internal reference = 7.25 ppm.

Scheme I



was achieved by HPLC on Whatman Partisil with CH_2Cl_2.

$^1O_2$ Oxidation of 1. A solution of hapalindole A (40 mg) in 5 mL of methanol containing a trace of rose bengal was irradiated at 25 °C with a slide projector lamp and oxygen was passed continuously through this mixture. Progress of the oxidation was monitored by TLC. After 5 h the MeOH was evaporated and the crude mixture was passed through a short (2 × 0.9 cm) silica gel column (Bond Elut Si, Analytichem International) with 20 mL of CH_2Cl_2, followed by 20 mL of EtOAc. The material eluted with CH_2Cl_2 (24.5 mg) was subjected to normal-phase HPLC on a Whatman Partisil M9 10/50 column with CH_2Cl_2 to give 2.5 mg of recovered **1**, 0.5 mg of hapalindole G (**9**), 0.5 mg of hapalindole K (**11**), and 1.3 mg of hapalindole I (**10**). The EtOAc flush (15.5 mg) from the M9 column was rechromatographed on a Whatman Partisil 5 column with 1:12 CH_2Cl_2/EtOAc to give 1.5 mg of 10-methoxyhapalindole A (**7**), 1.8 mg of hapalonamide A (**5**), 1.5 mg of hapalonamide G (**6**), and 3.1 mg of anhydrohapaloxindole A (**4**).

In a second experiment the oxidation was carried out on 44 mg of **1** in 10 mL of 80% EtOH/H_2O buffered at pH 8 with 0.01 M sodium phosphate for 1.3 h. A reverse-phase chromatography of the reaction mixture on a 2 × 0.9 cm column of BondElut C-18 (Analytichem International, Harbor City, CA) was carried out first to remove the inorganic salts. Two fractions were obtained. Fraction 1 was subjected to flash chromatography on silica gel (TLC grade) with CH_2Cl_2 to give 7 mg of **5**, 2 mg of **6**, and traces of **9**, **10**, and **11**. Fraction 2 was subjected to preparative TLC on silica gel with 5% MeOH/CH_2Cl_2 to give 4 mg of **3**, 5 mg of **4**, and 3 mg of **8**.

Compound **5**: $[\alpha]_D$ −24° ($CHCl_3$, *c* 0.8); IR ($CHCl_3$) ν_{max} 3501, 2135, 1709 cm^{-1}; EIMS, *m/z* 370/372 (3:1); high resolution EIMS, *m/z* 370.1417 (calcd for $C_{21}H_{23}N_2{}^{35}ClO_2$, 370.1448); ^{1}H NMR ($CDCl_3$) see Table III. ^{1}H(irr) → ^{1}H(+NOE): δ 1.539 → 7.133, 2.422, 1.473; 1.328 → 3.353; 0.826 → 6.005, 5.254, 4.718, 1.473. After completion of the NMR analysis, the chloroform-*d* solution of **5** was evaporated with a stream of nitrogen and the residual, oily **5** stored at −20 °C; when the sample was examined several weeks later, it was found that **5** had been converted cleanly and quantitatively to fontonamide (**3**).

Compound **6**: EIMS, *m/z* 370/372 (3:1); high resolution EIMS, *m/z* 370.1413 (calcd for $C_{21}H_{23}N_2{}^{35}ClO_2$, 370.1448); ^{1}H NMR data (see Table II).

Compound **7**: $[\alpha]_D$ +4° ($CHCl_3$, *c* 0.2); IR (CCl_4) ν_{max} 3482, 2135 cm^{-1}; UV (MeOH) λ_{max} nm (ε) 220 (37 200), 273 (6200), 279 (6300), 290 (5200); EIMS, *m/z* 368/370 (3:1); high resolution EIMS, *m/z* 368.1630 (calcd for $C_{22}H_{25}N_2O^{35}Cl$, 368.1655); ^{1}H NMR ($CDCl_3$) δ 8.256 (br, H on N-1), 7.23–7.22 (m, C-6 H and C-7 H), 7.038 (d, C-2 H), 7.005 (dd, C-5 H), 6.122 (dd, C-20 H), 5.310 (d, *E* H on C-21), 5.189 (dd, *Z* H on C-21), 4.467 (dd, C-13 H), 4.310 (br s, C-11 H), 3.116 (s, OMe on C-10), 2.419 (dd, C-15 H), 2.262 (dt, eq H on C-14), 1.525 (s, 3 H on C-18), 1.404 (s, 3 H on C-17), 1.38 (q, ax H on C-14), 0.659 (s, 3 H on C-19); *J* in Hz: 1,2 = 2.4; 13,14(ax) = 12.8; 13,14(eq) = 4.3; 14(ax),14(eq) = −12.8; 14(ax),15 = 13.0; 14(eq),15 = 4.4; 20,21*E* = 10.8; 20,21*Z* = 17.5; 21*E*,21*Z* = <0.5, ^{1}H(irr) → ^{1}H(+NOE): δ 3.116 → 4.310; 1.404 → 2.419; 0.659 → 7.038, 6.122, 5.189, 4.310, 1.38.

Compound **8**: $[\alpha]_D$ −48° (CH_2Cl_2); UV (MeOH) λ_{max} nm (ε) 217 (77 100), 253 (3200), sh 300 (1500); EIMS, *m/z* 370/372 (3:1); ^{1}H NMR ($CDCl_3$) δ 9.41 (br, H on N-1), 7.299 (t, C-6 H), 6.961 (br d, C-5 H), 6.784 (br d, C-7 H), 5.951 (dd, C-20 H), 5.284 (br d, *E* H on C-21), 5.164 (br d, *Z* H on C-21), 4.250 (dd, C-13 H), 5.030 (br s, C-11 H), 3.443 (br dd, C-10 H), 2.435 (ddd, C-15 H), 1.910 (dt, eq H on C-14), 1.624 (s, 3 H on C-17), 1.419 (s, 3 H on C-18, 0.701 (q, ax H on C-14), 0.623 (s, 3 H on C-19); *J* in Hz: 5,6 = 7.9, 6,7 = 7.7, 5,7 = <0.5, 10,11 = 2, 10,15 = 6.6, 13,14(ax) = 13, 13,14(eq) = 4.5, 14(ax), 14(eq) = −13.9, 14(ax),15 = 13.9, 14(eq),15 = 4.5, 20,21*E* = 10.9, 20,21*Z* = 17.5, 21*E*,21*Z* = <0.5. ^{13}C NMR ($CDCl_3$) δ 141.6 (C20), 139.5, 136.6, 131.2 (C6), 129.7, 119.8 (C5), 116.8 (C21), 108.6 (C7), 70.6 (C3), 61.7 (C13), 58.8 (C11), 47.4, 46.5, 42.6 (C12), 39.1 (C16), 31.0, 29.7, 29.2, 25.6; chemical shifts for C2 and C23 not determined. ^{1}H(irr) → ^{1}H(+NOE): δ 1.624

→ 3.443, 2.435; 1.419 → 6.961, 2.435, 1.910; 0.623 → 5.951, 5.164, 5.030.

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Synthesis of C-Glycosides of 3-Deoxy-D-*manno*-2-octulosonic Acid. Stereoselectivity in an Enolate Reaction

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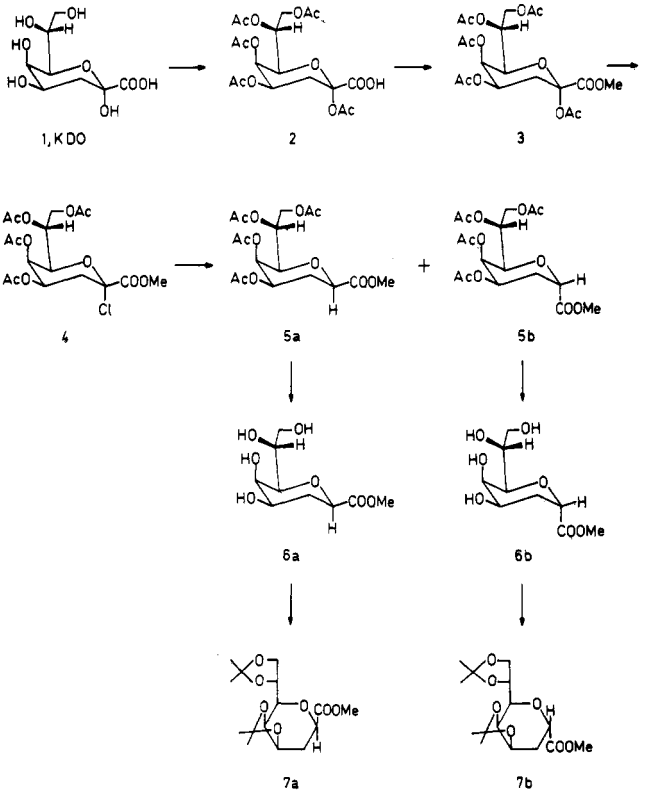
Eight different C-glycosidic derivatives of 3-deoxy-D-*manno*-2-octulosonic acid (KDO) were prepared by reacting the enolate of methyl or ethyl 2,6-anhydro-3-deoxy-4,5:7,8-di-*O*-isopropylidene-D-*glycero*-D-*talo*-(or *galacto*)octonate (**7a,b** and **22**) with the electrophiles cyanogen, formaldehyde, carbon dioxide, acetic anhydride, acetyl chloride, phenyl acetate, iodomethane, 3-bromopropyne, benzyl bromide, *tert*-butyl 2-bromoacetate, and methyl acrylate. All C-glycosides were formed with the β-configuration predominating; the β to α ratio varied from 70:30 (formaldehyde, phenyl acetate) to ≥95:5 (alkyl halides). Total yields varied from 30% to 67%. The key intermediates in the synthesis, i.e., the acetonide-protected 2-deoxy-KDO derivatives **7a,b**, were prepared by hydrogenolysis of 4,5,7,8-tetra-*O*-acetyl-2-chloro-2-deoxy-KDO (**4**) followed by deacetylation and acetonide formation. α,β-Configurations were assigned on the basis of chemical correlation with the nitrile **9b**, which has been studied by X-ray crystallography, and of the three-bond coupling constants between the C-glycosidic carbon and the deoxyprotons at C-3. Labeling with $^{13}CO_2$ was used in one instance.

Introduction

The biosynthesis of the lipopolysaccharide (LPS) of Gram-negative bacteria has recently attracted interest in connection with developing novel antibacterial agents with specificity for Gram-negative bacteria.[2] Our work has focused on the inhibition[3] of the enzyme CTP:CMP-3-deoxy-2-octulosonate cytidylyl-transferase (CMP-KDO synthetase) which catalyzes the formation of the nucleotide derivative CMP-KDO from KDO (**1**)[4] and cytidine triphosphate. It has recently been shown by ^{13}C NMR spectroscopy that the enzyme utilizes the β-pyranose form of KDO as a substrate.[5] This was also indicated by our earlier observation that only the β-2-deoxy and not the α-2-deoxy analogue of KDO is an inhibitor of the enzyme.[3a]

In order to further investigate the inhibitory activity of structural analogues of KDO, we have synthesized C-glycosides of KDO, a class of compounds that are unknown in the literature.[3c] The new carbon–carbon bond was formed in a straightforward way by an enolate reaction (eq 1) of compounds **7a** and **7b** separately or as a mixture or of a mixture of the corresponding ethyl esters (**22**).

Scheme I



Knowledge of the stereochemistry of the enolate reaction[7] was of crucial importance for the applicability of the

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