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HAPALONAMIDES AND OTHER OXIDIZED HAPALINDOLES FROM *HAPALOSIPHON FONTINALIS*

RICHARD E. MOORE, XU-QIANG G. YANG, GREGORY M. L. PATTERSON, ROSANNE BONJOUKLIAN* and TIM A. SMITKA*

Department of Chemistry, University of Hawaii, Honolulu, HI 96822, U.S.A.; *Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, IN 46285, U.S.A.

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Key Word Index—*Hapalosiphon fontinalis*; Cyanophyta; Stigonemataceae; indole alkaloids; fungicides.

Abstract—Dechlorofontonamide, anhydrohapaloxindoles B and M, and hapalonamides G, H, and V have been isolated as minor indole alkaloids from a cultured strain of the terrestrial blue-green alga *Hapalosiphon fontinalis*.

INTRODUCTION

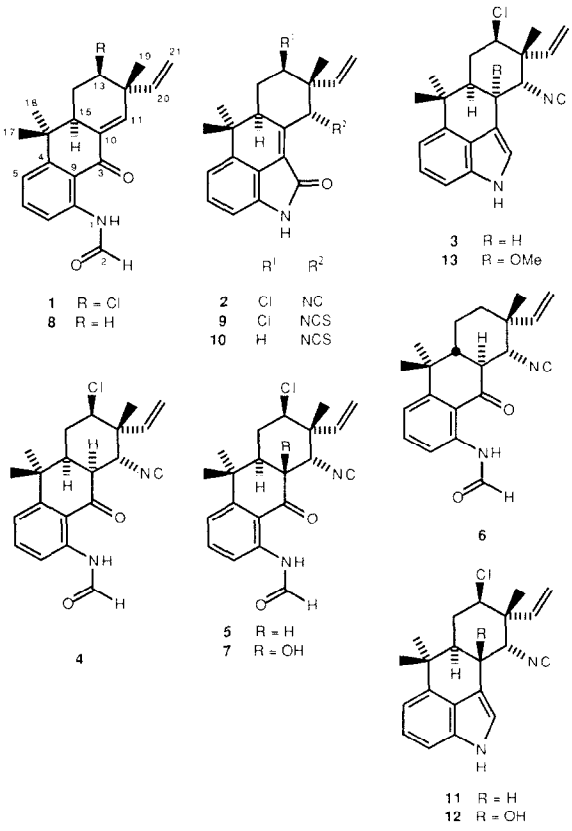
Fontonamide (**1**) and anhydrohapaloxindole A (**2**), two minor alkaloids from *Hapalosiphon fontinalis*, appear to be singlet oxygen oxidation products of hapalindole A (**3**), the major alkaloid in this terrestrial blue-green alga [1]. Hapalonamides A (**4**) and G (**5**), the proposed precursors of fontonamide [1], are formed along with **1** and **2** when hapalindole A is oxidized under singlet oxygen oxidation conditions. In our continuing studies on the minor constituents of *H. fontinalis*, we have now detected hapalonamide G in the lipophilic extract of the cyanophyte. Two other hapalonamides H (**6**) and V (**7**) have also been found, as well as dechlorofontonamide (**8**) and two new anhydrohapaloxindoles B (**9**) and M (**10**).

RESULTS AND DISCUSSION

Structure elucidation of the six new alkaloids was straightforward. The chromophore of each compound was ascertained by UV spectroscopy and the molecular composition and presence or absence of chlorine were determined by mass spectrometry. The gross structures and relative stereochemistries were solved using previously described methodology [1, 2]. Dechlorofontonamide and anhydrohapaloxindoles B and M showed ^{1}H NMR spectra, including NOE difference spectra, that were comparable with the ones for **1** and **2** [1].

The hapalonamide G from *H. fontinalis* was found to be identical in all respects with semisynthetic material, the latter formed when an oxygen-aerated solution of **3** in aqueous methanol buffered at pH 8 with sodium phosphate was irradiated at room temperature in the presence of a trace of rose bengal [1]. Hapalindole G (**11**) was also produced in the reaction mixture when **3** was incompletely oxidized. Hapalonamide G appeared to have formed by a free-radical induced transformation of **3** to **11**, followed by singlet oxygen cleavage of the indole Δ^2-double bond in **11**. Hapalonamide A [1] is presumably also present in the algal extract, but has not been detected.

Hapalonamide H gave essentially the same ^{1}H NMR data as hapalindole H [2] with respect to the chemical



shifts, coupling patterns, and NOEs for the protons on C-10 to C-19.

Hapalonamide V was found to possess a third oxygen by high resolution mass spectrometry which was assigned to a hydroxyl group on C-10. Spin–spin decoupling studies indicated that a $CH_{ax}CH_2CH_{ax}$ unit was present from C-13 to C-15 and NOE difference spectroscopy showed that the methyl group on C-12 was axial and H-11 was equatorial since irradiation of the H_3-19 signal showed positive NOEs in the H-11 and axial H-14 signals. The hydroxy group on C-10 had to be axial since the chemical shift for the methyl group on C-12 (H_3-19) was 1.50 ppm, essentially identical with the one for hapalindole V (**12**) and only slightly lower field than the ones for **11** and **5** (Table 1). If the hydroxyl on C-10 was equatorial, the signal for H_3-19 would have been expected at much higher field since the methyl group on C-12 lies over the aromatic system in this isomer. Hapalindole A, 10-methoxyhapalindole A (**13**) [1], and **4** show this signal at 0.66–0.88 ppm (Table 2). Moreover, the signals for H-14_{ax}, H_3-17 and H_3-19 in **7** and **12** appeared at lower field than the ones in **5** and **11**, indicating 1,3-diaxial relationships between the hydroxy group and these three neighbours.

EXPERIMENTAL

Isolation. Freeze-dried *Hapalosiphon fontinalis* (strain V-3-1) was extracted with CH_2Cl_2–2-PrOH (1:1) as previously described [2]. The extract (4.3 g) was subjected to gel filtration [3] on a column (175 × 2.7 cm diameter) of Sephadex LH-20 with 600 ml CH_2Cl_2–hexane (1:4), 880 ml of Me_2CO–CH_2Cl_2 (2:3), and 880 ml of Me_2CO–CH_2Cl_2 (4:1). NMR analysis indicated that the fraction (154 mg) eluting at 500–550 ml contained fontonamides and hapalonamides. Further purification was achieved by HPLC on a Zorbax CN column (25 cm × 9.4 mm) with 5:1 isooctane–THF (flow rate 3 ml/min; 5–8 mg per injection) to give 4.3 mg of dechlorofontonamide (**8**) (16 min), 38.2 mg of fontonamide [1] (18 min), 6.1 mg of hapalonamide G [1] (**5**) (36 min), and 7 mg of hapalonamide H (**6**) (38 min). Traces of pigment were removed by passing each compound through a 2 × 0.9 cm column of BondElut C-18 (Analytichem International) with EtOH–H_2O (3:1).

Dechlorofontomide (**8**). $[\alpha]_D$ −100° ($CHCl_3$, *c* 0.2); UV λ_{max}^{MeOH} nm: 209 (ε 3400), 241 (8000), 290 (6700), 339 (2500); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3350, 1693, 1655, 1599, 1248; FDMS *m/z* 309; high resolution EIMS *m/z* 309.1726 (calcd for $C_{20}H_{23}NO$, +0.3 mmu). ^1H NMR (300 MHz, $CDCl_3$): δ11.99 (1H, *br*, N-1), 8.557 (1H, *d*, C-7), 8.465 (1H, *d*, C-2), 7.490 (1H, *t*, C-6), 7.189 (1H, *d*, C-5), 7.150 (1H, *dd*, C-11), 5.854 (1H, *dd*, C-20), 5.052 (1H, *d*, *E* H on C-21), 5.005 (1H, *d*, *Z* H on C-21), 2.549 (1H, *ddd*, C-15), 1.955 (1H, *ddd*, *eq* H on C-14), 1.726 (1H, *m*, *eq* H on C-13), 1.688 (1H, *m*, *ax* H on C-14), 1.644 (1H, *m*, *ax* H on C-13), 1.454 (3H, *s*, C-18), 1.186 (3H, *s*, C-19), 1.012 (3H, *s*, C-17); $J_{1,2}$ = 1.3 Hz, $J_{5,6}$ = 7.8, $J_{6,7}$ = 8.3, $J_{11,13}$ = 1.5, $J_{11,15}$ = 2.5, $J_{13,13}$ = −14.5, $J_{13ax,14eq}$ = 3.5, $J_{13eq,14eq}$ = 3.5, $J_{13eq,14ax}$ = 3.0, $J_{14,14}$ = −14.5, $J_{14eq,15}$ = 5.7, $J_{14ax,15}$ = 11.1, $J_{20,21Z}$ = 17.5, $J_{20,21E}$ = 10.7, $J_{21E,21Z}$ = 0.5. ^1H(irr) → ^1H(+ NOE): 1.454 → 2.549; 1.186 → 7.150, 5.854, 5.052, 5.005; 1.012 → 7.189, 1.688.

Hapalonamide H (**6**). $[\alpha]_D$ +13.7° ($CHCl_3$; *c* 0.15); UV λ_{max}^{MeOH} nm: 235 (ε 8800), 264 (4600), 325 (2300); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3300, 2147, 1697, 1666; FDMS *m/z* 336; high resolution EIMS *m/z* 336.1831 (calcd for $C_{21}H_{24}N_2O_2$, −0.6 mmu). ^1H NMR

Table 1. Comparison of ^1H NMR chemical shifts (300 MHz, $CDCl_3$)

H	Hapalindole G (**11**)	Hapalindole V (**12**)	Hapalonamide G (**5**)	Hapalonamide V (**7**)
5	7.04	7.10	7.27	7.30
11	4.24	4.11	4.49	4.17
13	4.43	4.40	4.33	4.37
14 ax	2.01	2.51	1.47	2.43
14 eq	2.41	2.34	2.22	2.30
15	2.11	2.24	2.61	2.52
17	1.17	1.36	1.25	1.40
18	1.52	1.52	1.51	1.50
19	1.39	1.57	1.25	1.50

Table 2. Comparison of ^{1}H NMR chemical shifts (300 MHz, $CDCl_3$)

H	Hapalindole A (3)	10-Methoxyhapal indole A (13)	Hapalonamide A (4)
5	6.97	7.01	7.13
11	4.37	4.31	4.72
13	4.36	4.47	4.30
14 ax	1.47	1.38	1.47
14 eq	2.14	2.26	2.22
15	2.32	2.42	2.42
17	1.55	1.40	1.54
18	1.19	1.53	1.33
19	0.88	0.66	0.83

(300 MHz, $CDCl_3$): δ11.33 (1H, *br*, N-1), 8.537 (1H, *br d*, C-7), 8.491 (1H, *d*, C-2), 7.528 (1H, *t*, C-6), 7.205 (1H, *d br d*, C-5), 6.033 (1H, *dd br s*, C-20), 5.328 (1H, *d br s*, *Z*, H on C-21), 5.242 (1H, *dd*, *E* H on C-21), 3.716 (1H, *d br s*, C-11), 2.925 (1H, *dd*, C-10), 1.948 (1H, *dt*, *eq* H on C-13), 1.825 (1H, *m*, C-15), 1.72 (2H, *m*, C-14), 1.364 (3H, *s*, C-17), 1.334 (3H, *s*, C-18), 1.308 (3H, *s*, C-19); $J_{1,2}$ = 1.8 Hz, $J_{5,6}$ = 8.0, $J_{5,7}$ = 0.8, $J_{6,7}$ = 8.2, $J_{10,11}$ = 10.4, $J_{10,15}$ = 13.1, $J_{13eq,14ax}$ = 3.2, $J_{13eq,14eq}$ = 3.2, $J_{13eq,13ax}$ = −14.0, $J_{20,21E}$ = 11.1, $J_{20,21Z}$ = 17.6, $J_{21E,21Z}$ = 0.6. ^{1}H(irr) → ^{1}H (+NOE): 1.364 → 7.205, 1.72; 1.334 → 7.205, 2.925, 1.72; 1.308 → 5.328, 5.242, 3.716.

Another sample of the CH_2Cl_2–2-PrOH (1:1) extract (15.7 g) in 50 ml of THF–MeOH (3:2) was chromatographed on a column (100 × 5.5 cm diameter) of HP-20 (Mitsubishi) with THF–H_2O–MeOH (6:5:5) to give 371 mg of fraction A eluting at 2760–3360 ml. Fraction A (280 mg) was further separated by HPLC on a 2.5 cm × 50 cm column of Whatman LPS-1 silica gel with heptane–THF (7:1) (3.5 ml/min); after 1 l of solvent had passed, subfractions which contained material having an R_f value of 0.2 on silica gel with heptane–THF (2:1) were combined and evapd to give 23 mg of solid. Prep. TLC on silica gel with CH_2Cl_2–MeOH (100:3) gave 12.3 mg of hapalindole 0 [2] (R_f 0.44) and 3.6 mg of hapalonamide V (7) (R_f 0.22).

Hapalonamide V (7). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3300 (*br d*), 2090, 1698, 1650; FDMS *m/z* 386, 388; high resolution FABMS *m/z* 387.1492 (calcd for $C_{21}H_{24}N_2O_3Cl$, 1.7 mmu). ^{1}H NMR (330 MHz, Me_2CO–d_6): δ11.87 (1H, *br*, N-1), 8.6–8.7 (2H, *br*, C-7 and C-2), 7.674 (1H, *t*, C-6), 7.467 (1H, *d br d*, C-5), 6.053 (1H, *dd*, C-20), 5.331 (1H, *d*, *E* H on C-21), 5.320 (1H, *dd*, *Z* H on C-21), 4.518 (1H, *dd*, C-13), 4.289 (1H, *br s*, C-11), 2.594 (1H, *dd*, C-15), 2.468 (1H, *q*, *ax* H on C-14), 2.363 (1H, *ddd*, *eq* H on C-14), 1.536 (3H, *s*, C-19), 1.524 (3H, *s*, C-17 or 18), 1.500 (3H, *s*, C-17 or 18); $J_{5,6}$ = 8.1 Hz, $J_{5,7}$ = 0.8, $J_{6,7}$ = 8.1, $J_{13,14ax}$ = 11.6, $J_{13,14eq}$ = 4.4, $J_{14ax,14eq}$ = 12.7, $J_{14ax,15}$ = 12.0, $J_{14eq,15}$ = 3.3, $J_{20,21E}$ = 10.7, $J_{20,21Z}$ = 17.7. ^{1}H (irr) → ^{1}H(+NOE): 1.536 → 6.053, 5.320, 4.289, 2.468; 1.524 → 7.467; 1.500 → 7.467.

Fontonamide, dechlorofontonamide, anhydrohapaloxindole A, and hapalonamides H and V had R_f values of 0.55, 0.49, 0.32, 0.27, and 0.1, respectively, on a 5 × 10 cm plate of Merck F254 silica gel 60 in CH_2Cl_2–MeOH (100:1) and HPLC retention times of 5.40, 5.98, 3.93, 2.97, and 3.06 min on a 4.6 mm × 25 cm column of DuPont Zorbax ODS using MeOH–H_2O (9:1) as the eluant (flow rate 1.5 ml/min).

Anhydrohapaloxindoles B (**9**) and M (**10**) were isolated from fractions 20–29 in a previously described [1] silica gel chromatography; fractions 14–19 contained fontonamide (**1**) and fractions 30–33 contained anhydrohapaloxindole (**2**).

Anhydrohapaloxindole B (**9**): EIMS *m/z* 384, 386 (rel. int. 3:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ7.52 (1H, *br*, N-1), 7.22 (1H, *t*, C-6), 6.91 (1H, *br dd*, C-5), 6.69 (1H, *br dd*, C-7), 6.01 (1H, *dd*, C-20), 5.81 (1H, *s*, C-11), 5.40 (1H, *d br s*, *E* H on C-21), 5.33 (1H, *d br s*, *Z* H on C-21), 4.39 (1H, *dd*, C-13), 2.87 (1H, *dd*, C-15), 2.87 (1H, *dd*, C-15), 2.35 (1H, *dt*, *eq* H on C-14), 1.80 (1H, *td*, *ax* H on C-14), 1.41 (3H, *s*, C-17), 1.27 (3H, *s*, C-18), 1.07 (3H, *s*, C-19); $J_{5,6}$ = 7.9 Hz, $J_{5,7}$ = 0.5, $J_{6,7}$ = 7.7, $J_{13ax,14ax}$ = 12.2, $J_{13ax,14eq}$ = 4.4, $J_{14ax,14eq}$ = −12.9, $J_{14ax,15}$ = 13.2, $J_{14eq,15}$ = 4.5, $J_{20,21E}$ = 10.9, $J_{20,21Z}$ = 17.4. ^{1}H(irr) → ^{1}H(+NOE): 1.41 → 6.91, 2.87; 1.27 → 2.87; 1.07 → 6.01, 5.81, 5.33, 1.80.

Anhydrohapaloxindole M (**10**): EIMS *m/z* 318. ^{1}H NMR (300 MHz, $CDCl_3$): δ7.48 (1H, *br*, N-1), 7.18 (1H, *t*, C-6), 6.89 (1H, *dd*, C-5), 6.66 (1H, *dd*, C-7), 5.95 (1H, *dd*, C-20), 5.63 (1H, *d*, C-11), 5.21 (1H, *dd*, *E* H on C-21), 5.15 (1H, *dd*, *Z* H on C-21), 2.72 (1H, *dd*, C-15), 2.03 (1H, *td*, *ax* H on C-13), 1.94 (*m*), 1.38 (3H, *s*, C-17), 1.23 (3H, *s*, C-18), 0.97 (3H, *s*, C-19); $J_{5,6}$ = 7.9 Hz; $J_{5,7}$ = 0.5, $J_{6,7}$ = 7.7, $J_{11eq,13eq}$ = 1.2, $J_{13ax,14ax}$ = 14, $J_{13ax,14eq}$ = 4, $J_{13ax,13eq}$ = −14, $J_{14ax,15}$ = 12.9, $J_{14eq,15}$ = 4.5, $J_{20,21E}$ = 10.9, $J_{20,21Z}$ = 17.4, $J_{21E,21Z}$ = 0.6. ^{1}H(irr) → ^{1}H(+NOE): 1.38 → 6.89, 2.72; 1.23 → 6.89; 0.97 → 5.95. 5.63, 5.15.

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