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Fischerindole L, a New Isonitrile from the Terrestrial Blue-Green Alga *Fischerella muscicola*

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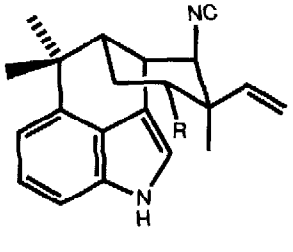
Abstract. Fischerindole L (**3**) is a novel octahydroindeno[2,1-*b*]indole isonitrile from the terrestrial cyanophyte *Fischerella muscicola* that possesses the same relative stereochemistry as hapalindole L (**4**).

In evaluating hundreds of laboratory-cultured blue-green algae (cyanobacteria) for antifungal activity, the extract (70% ethanol) of *Fischerella muscicola* (Thuret) Gomont (UTEX 1829) was found to inhibit the growth of four test fungi, viz. *Aspergillus oryzae*, *Penicillium notatum*, *Saccharomyces cerevisiae*, and *Trichophyton mentagrophytes*, in a soft-agar disc-diffusion assay (250 μg, 10-17 mm zones). Using a bioassay-guided isolation scheme,[1] most of the antifungal activity was associated with an indole alkaloid fraction. Hapalindoles A (**1**)[2] and J (**2**)[3] and fontonamide[4] were the major components in this mixture by HPLC analysis. In addition a new antifungal[5] tetracyclic alkaloid, fischerindole L (**3**), possessing a hexahydroindeno[2,1-*b*]indole ring system and an isonitrile functionality, and having the same relative stereochemistry as hapalindole L (**4**),[3] was present. We present here the isolation and structure determination of **3**.

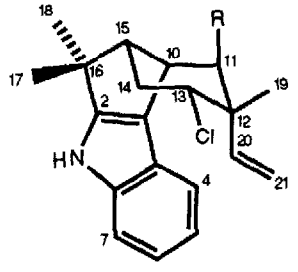
The UV spectrum of **3** was typical of an indole and the EI mass spectrum, coupled with NMR data, established that **3** had the same elemental composition ($C_{21}H_{23}ClN_2$) as **1** and **4**.[6] Inspection of the ^1H and ^{13}C NMR spectra in acetone-d_6[6] indicated that an *o*-disubstituted benzenoid ring, a vinyl and three methyl groups attached to quarternary carbons, and a CHeqX-CHeq-CHax-CH_2-CHaxY unit in a six-membered ring were present. Since the H-11 (4.36 ppm) and C-11 (63.5 ppm) signals showed a 1:1:1 coupling (to ^{14}N) pattern and a HMBC cross peak between the H-11 and isonitrile carbon (160.2 ppm) signals, X had to be the isocyano group and this meant that Y was the chloro group. The HMBC experiment also showed that a quaternary carbon (C-12) bearing methyl and vinyl substituents was between CHeqNC and CHaxCl as ^2J and ^3J cross peaks were clearly visible between the H-11 and C-3/C-10/C-12/C-13/C-15/C-19/C-20 signals and the H-13 and C-12/C-14/C-15/C-19/C-20 signals. The cyclohexane ring was connected to the indole C-3 via C-10 on the basis of HMBC couplings between the H-10 and C-2/C-3/C-11/C-12/C-14/C-15 signals. Long-range zig-zag coupling between H-21*E* and H-13 (0.7 Hz) strongly suggested that C-12 had the *S** configuration (same as in **4**[3]), not *R* as in **1**.[3]

Since the signal for H-2 was missing, the molecular skeleton for **3** had to differ from **1** in having C-16, the *gem*-dimethyl carbon, attached to C-2 instead of to C-4. This connection was supported by ^{3}J-couplings (HMBC cross peaks) between the *gem*-dimethyl protons and C-2/C-15/C-16. In **3** H-14ax was no longer located over the aromatic system as close as in **1**, resulting in a significant paramagnetic shift of the H-14ax signal with virtually no effect on the C-14 chemical shift. Irradiation of the C-19 methyl protons in a difference NOE experiment induced strong positive NOEs in the H-11, H-13 and H-21*Z* signals, but not in the H-4 and H-14ax signals, and irradiation of H-11 produced significant NOEs in the H-4 and H-20 signals. Strong NOEs between the H_3-17 and H_2-14 signals and between the H_3-18 and H-15 signals provided further proof for the stereochemistry depicted in **3**.

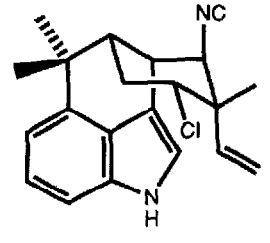
On standing in chloroform-*d* for a few days, **3** was converted to the corresponding fischerindole L formamide (**5**).[7] The ^{1}H NMR spectrum indicated that **5** existed as two conformational isomers in solution, the major conformer being the *E*-formamide ($J_{22,23}$ = 11.5 Hz) and the minor conformer being the *Z*-formamide ($J_{22,23}$ = 1.5 Hz).



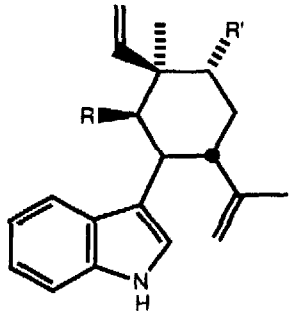
1 R = Cl
2 R = H



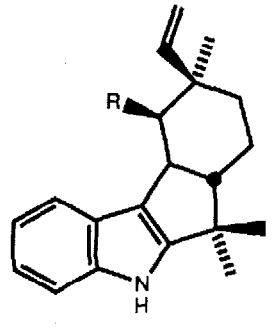
3 R = NC
5 R = NHCHO



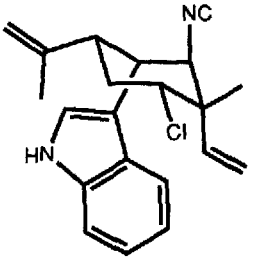
4



6 R = NHCHO; R' = H
9 R = NH_2; R' = H
10 R = NHCHO; R' = Cl
11 R = NH_2; R' = Cl



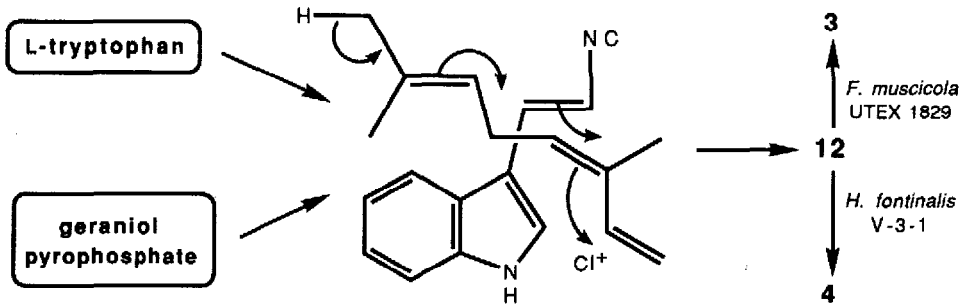
7 R = NHCHO
8 R = NH_2



12

Fischerindole L is the first octahydroindeno[2,1-*b*]indole[8] to be isolated from a blue-green alga. Interestingly we had found earlier[9] that hapalindole C formamide (**6**) from *Hapalosiphon fontinalis* V-3-1 could be transformed into a 2:1:1 mixture of octahydroindeno[2,1-*b*]indoles **7** and **8** and hapalindole C amine (**9**) in the presence of strong acid. Hapalindole E formamide (**10**), however, did not cyclize under similar conditions and only hapalindole E amine (**11**) was formed. Neither **9** nor **11** could be coverted into a octahydroindeno[2,1-*b*]indole on further treatment with acid. Fischerindole-type compounds could not be detected in *H. fontinalis*.[9]

Schwartz et al.[10] have reported the isolation of a new tricyclic hapalindole (**12**) from *Fischerella* sp. ATCC 53558 which could be the biosynthetic precursor of both **3** and **4** (Scheme 1).



Scheme 1. Possible biogenesis of fischerindole L and hapalindole L.[11]

In addition to **12**, cyclopropane-containing hapalindolinones have been isolated from *Fischerella* sp. ATCC 53558.[12] Ambiguine isonitriles, which possess an additional isoprene unit, have been isolated from *F. ambigua* UTEX 1903.[13]

Acknowledgement. This research was supported by NSF Grants CHE88-00527 and CHE90-24748. We thank Wesley Y. Yoshida for technical assistance in the NMR studies.

References and Notes

1. *Fischerella muscicola* (UTEX 1829) was purchased from the University of Texas Collection. Mass cultivation of the axenic alga was carried out in 20-L glass bottles using the procedure described for *Hapalosiphon fontinalis*.[3] After 12 to 14 days, the alga was harvested by filtration and freeze-dried. Yields of lyophilized alga were typically 0.35 g/L. The freeze-dried alga (20 g) was extracted x 3 with 1 L portions of 70% EtOH and the filtered extract was evaporated to give a green solid (3 g). The crude extract was chromatographed on a 5.2 x 9.5 cm column of silica gel with 1:4 CH_2Cl_2/hexane (200 mL), 3:1 CH_2Cl_2/hexane (400 mL), CH_2Cl_2,(400 mL), and 1:1 MeOH/CH_2Cl_2 (400 mL). The material in the 3:1 CH_2Cl_2/hexane fraction (0.31 g) was further chromatographed on a 2.7 x 3.2 cm column of C18 with 50 mL portions of 1:1 MeOH/H_2O, 3:1 MeOH/H_2O, 9:1 MeOH/H_2O, and MeOH. Gradient HPLC of the 3:1 MeOH/H_2O fraction (50 mg) on silica (Whatman Partisil) with 17:3 to 1:1 hexane/EtOAc gave a 1:1 mixture (5 mg) of fischerindole L (**4**) and an unidentified indole followed by fontonamide (6 mg),

hapalindole J (7 mg), and hapalindole A (**1**, 14 mg). Pure **4** was obtained by further HPLC on silica with 7:3 CH_2Cl_2/hexane.

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4. Moore, R. E., Yang, X-Q. G., and Patterson, G. M. L. *J. Org. Chem.* **1987**, *52*, 3773.
5. The antifungal activity of **3** was not studied in detail since it appeared to be similar to **1** and other hapalindoles in a soft-agar disc-diffusion assay.
6. *Fischerindole L isonitrile* (**3**). EIMS *m/z* (rel int, composition) 338/340 (70/22, $C_{21}H_{23}{}^{35}ClN_2$/ $C_{21}H_{23}{}^{37}ClN_2$), 323/325 (30/10, $C_{20}H_{20}{}^{35}ClN_2$/ $C_{20}H_{20}{}^{37}ClN_2$), 303 (27, $C_{21}H_{23}N_2$), 183 (100, $C_{13}H_{13}N$ resulting from cleavage of C10-C11 and C14-C15 bonds); HREIMS *m/z* 338.1545 (Δ -0.5 mmu); UV (MeOH) λ_{max} 220 nm (ε 38000), 278 (6800), sh 290 (5000); ^1H NMR (500 MHz, acetone-d_6) δ 10.20 (br s, indole NH), 7.54 (dbrm, J = 8.0 Hz, H-4), 6.95 (ddd, J = 8.0, 7.0 and 1.2 Hz, H-5), 7.00 (ddd, J = 8.0, 7.0 and 1.2 Hz, H-6), 7.29 (dbrm, J = 8.0 Hz, H-7), 3.74 (t, J = 6.7 Hz, H-10), 4.36 (dbrt, $J_{10,11}$ = 6.7 Hz, $J_{H,N}$ ≈ 2 Hz, H-11), 4.29 (dd, J = 12.2 and 4.6 Hz, H-13), 2.15 (dt, J = -13.2 and 12.2 Hz, H-14ax), 2.12 (ddd, J = -13.2, 7.6 and 4.6 Hz, H-14eq), 2.92 (m, H-15), 1.39 (s, H_3-17), 1.31 (s, H_3-18), 1.47 (s, H_3-19), 5.92 (dd, J = 17.4 and 11.0 Hz, H-20), 5.28 (dd, J = 17.4 and 0.7 Hz, H-21*Z*), 4.96 (dbrt, J = 11.0 and 0.7 Hz, H-21*E*); ^{13}C NMR (125 MHz, acetone-d_6) δ (^1J multiplicity, carbon position) 152.0 (s, C-2), 116.7 (s, C-3), 120.4 (d, C-4), 119.8 (d, C-5), 121.2 (d, C-6), 112.5 (d, C-7), 141.8 (s, C-8), 125.3 (s, C-9), 42.9 (d, C-10), 63.5 (d of 1:1:1 t, $J_{C,N}$ = 6 Hz, C-11), 44.9 (s, C-12), 65.0 (d, C-13), 31.9 (t, C-14), 53.7 (d, C-15), 42.3 (s, C-16), 23.5 (q, C-17), 27.3 (q, C-18), 21.9 (q, C-19), 138.2 (d, C-20), 116.6 (t, C-21), 160.2 (s of vbr 1:1:1 t, C-23).
7. *Fischerindole L formamide* (**5**). EIMS m/z (rel int, composition) 356/358 (3/1, $C_{21}H_{25}{}^{35}ClN_2O$/ $C_{21}H_{25}{}^{37}ClN_2O$), 311/313 (42/12, $C_{20}H_{22}{}^{35}ClN$/$C_{20}H_{22}{}^{37}ClN$), 276 (100, $C_{20}H_{22}N$); UV (MeOH) λ_{max} 224, 276; HREIMS *m/z* 356.1650 ($C_{21}H_{25}{}^{35}ClN_2O$, Δ -0.5 mmu). ^1H NMR (500 MHz, $CDCl_3$) δ_E/δ_Z 7.86/7.80 (br s, indole NH), 7.36/7:41 (dbrm, J = 7.8 Hz, H-4), 7.10/6.94 (ddd, J = 7.8, 7.2 and 1.2 Hz, H-5), 7.10/6.98 (ddd, J = 8.0, 7.2 and 1.2 Hz, H-6), 7.30/7.28 (dbrm, J = 8.0 Hz, H-7), 3.30/3.33 (dd/t, J = 10.5,8.3/7.3 Hz, H-10), 3.42/4.77 (t/dd, J = 10.5,7.0/10.5 Hz, H-11), 4.003/4.000 (dd, J = 12.7 and 3.3 Hz, H-13), 2.23/2.28 (q/q, J = -13.0,12.7,12.0/-13.0,12.7,12.3 Hz, H-14ax), 2.10/2.08 (m, H-14eq), 2.89/2.72 (dt/dt, J = 12.0,7.9/12.3,6.8 Hz, H-15), 1.35/1.39 (s, H_3-17), 1.37/1.29 (s, H_3-18), 1.32/1.33 (s, H_3-19), 5.95/5.98 (dd, J = 17.3 and 11.0 Hz, H-20), 5.10/5.185 (brdd, J = 17.3 and 0.7 Hz, H-21*Z*), 5.180/5.01 (dbrt, J = 11.0 and 0.7 Hz, H-21*E*), 5.90/5.64 (brt/brd, formamide NH ≡ H-22), 7.62/8.17 (d/d, J = 1.5/11.5 Hz, H-23); NOE correlations δ_E/δ_Z 1.35/1.39 (2.23/2.28), 1.37/1.29 (2.89/2.72), 1.32/1.33 (5.10/5.185, 3.42/4.77, 4.003/4.000), 4.77 (7.41), 3.42 (7.62).
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9. Bonjouklian, R.; Moore, R. E.; Patterson, G. M. L. *J. Org. Chem.* **1988**, *53*, 5866. In this paper (1) the structures for **9** and **11** have been drawn incorrectly and (2) indeno[2,1-*b*]indoles **7** and **8** are referred to inappropriately as hexahydro derivatives. Compounds **7** and **8** are 5,6,6a,7,8,9,10,10a-octahydro-indeno[2,1-*b*]indoles.
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11. The *Z*-isomer of the proposed tryptophan-derived intermediate, *E*-β-isocyanostyrene, has been isolated from a *Pseudomonas* sp. (antibiotic B371) [Evans, J. R.; Napier, E. J.; Yates, P. *J. Antibiotics* **1976**, *29*, 850].
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