Isolation of a Nitrile-Containing Indole Alkaloid from the Terrestrial Blue-Green Alga *Hapalosiphon delicatulus*

Udo Huber, Richard E. Moore,* and Gregory M. L. Patterson

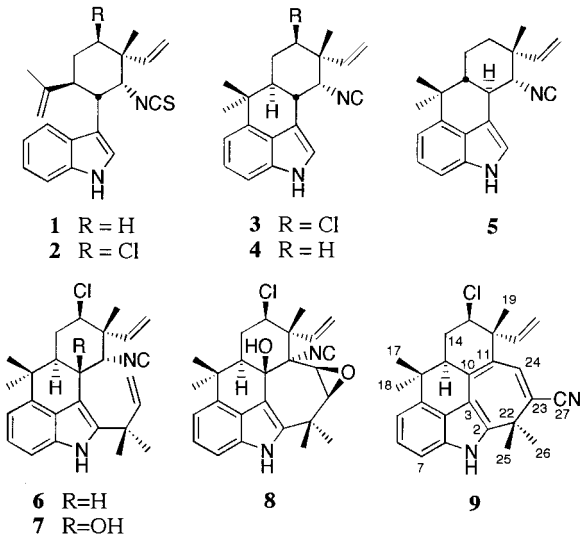
Department of Chemistry, University of Hawaii at Manoa, Honolulu, Hawaii 96822

Received April 20, 1998

Ambiguine G nitrile is a new indole alkaloid from the terrestrial blue-green alga *Hapalosiphon delicatulus* (UH isolate IC-13-1). It is the first nitrile to be found in the Stigonemataceae.

Isonitrile-containing indole alkaloids are often present in branched, filamentous blue-green algae (cyanobacteria) belonging to the Stigonemataceae.[1−9] Four main classes have been identified to date: hapalindoles,[1−5,8,9] ambiguines,[6] fischerindoles,[7,8] and wetwitindolinones.[9] Ambiguines, which are 2-(1,1-dimethylallyl)hapalindoles and modifications thereof, were first found in the terrestrial cyanophytes *Fischerella ambigua* (Nageli) Gomont (UTEX 1903), *Hapalosiphon hibernicus* W. & G. S. West (UH isolate BZ-3−1), and *Westiellopsis prolifica* Janet (UH isolate EN-3-1).[6] We report here the isolation and structure elucidation of a new ambiguine from an epidaphic cyanophyte *Hapalosiphon delicatulus* W. & G. S. West (UH isolate IC-13-1), which is the first nitrile to be identified among the indole alkaloids in the Stigonemataceae.

The cyanophyte was mass cultured in the laboratory and the CH_2Cl_2−2-propanol extract subjected to gel filtration followed by normal-phase column chromatography and HPLC. Nine alkaloids were isolated and identified on the basis of spectral analysis. Eight were known compounds: hapalindole D [isothiocyanate] (**1**), hapalindole F [isothiocyanate] (**2**), hapalindole G [isonitrile] (**3**), hapalindole H [isonitrile] (**4**), hapalindole U [isonitrile] (**5**), ambiguine A isonitrile (**6**), ambiguine B isonitrile (**7**), and ambiguine E isonitrile (**8**).[2,6] Ambiguine G nitrile (**9**) was the only new compound. Compound **8** was found to be the major ambiguine in the IC-13-1 cyanophyte, as it has been in all of the other ambiguine-producing cyanophytes studied so far.



Ambiguine G nitrile (**9**) was isolated from *H. delicatulus* in 0.0064% yield. Its ^{13}C NMR spectrum exhibited 26 signals for 12 nonprotonated, seven methine, two methylene, and five methyl carbons. Only one exchangeable hydrogen was present, as shown by the broad 1H signal at 8.06 ppm, which did not correlate with any of the carbon signals in a HMQC experiment. The EIMS displayed a 3:1 M^+ ion cluster at *m/z* 402/404, which indicated that the molecule possessed one chlorine atom. The presence of a single chlorine was supported by an intense fragment ion cluster at *m/z* 387/389 and a base peak at *m/e* 351 for successive losses of methyl and $H^{35}Cl/H^{37}Cl$ from the molecular ion. To account for a molecular weight of 402/404 and a partial elemental composition of $C_{26}H_{27}Cl$, two nitrogens had to be present in **9**. HREIMS measurement of the ^{35}Cl-containing M^+ peak confirmed the molecular formula of $C_{26}H_{27}ClN_2$ for **9**.

Fifteen of the ^{13}C signals had chemical shifts >100 ppm, suggesting that seven CC double bonds and one CN double or triple bond were present. One of the ^{13}C signals (δ_C 119.7), however, and an IR band at ν_{max} 2202 cm^{-1} were typical spectral features for a conjugated cyano group. Nine π-bonds were therefore present in the molecular structure, and this meant that **9** was pentacyclic to account for a total unsaturation number of 14. The exchangeable proton had to be an aryl NH (δ_H 8.06; ν_{max} 3337 cm^{-1}).

The nine π-bonds in **9** could be accounted for by a vinyl group and a conjugated 3-(4-cyanodienyl)indole chromophore. A vinyl group (δ_H 5.71/5.32/5.26) attached to a nonpronated carbon (δ_C 46.2) bearing a methyl group (δ_H 1.52) was present, as HMBC crosspeaks were observed between the quaternary carbon signal and all of the vinyl and methyl proton signals. A 2,3,4-trisubstituted indole moiety was present because the ^1H NMR spectrum showed signals with the expected chemical shifts and coupling constants for three adjacent protons on C-5, C-6, and C-7; moreover, most of the proton−carbon couplings from H-5, H-6, H-7, and the NH to the various carbons two and three bonds removed in this 2,3,4-trisubstituted indole system could be seen in the HMBC spectrum (using parameters to observe couplings around $J = 7$ Hz), but crosspeaks were missing for H-5−C-6, H-6−C-7, H-7−C-6, H-7−C-9, NH−C-8, and NH−C-22. Nevertheless, the ^{13}C chemical shifts were consistent with ones assigned to hapalindoles and other ambiguines possessing the 2,3,4-trisubstituted indole system.

Attached to C-2 of the indole (δ_C 137.4) was a nonprotonated carbon (δ_C 35.3) bearing two methyl groups (δ_H 1.04/1.91). This partial structure was deduced from the HMBC crosspeaks that were seen between the signals for

* To whom correspondence should be addressed. Tel.: (808) 956-7232. E-mail: moore@gold.chem.hawaii.edu.

10.1021/np9801561 CCC: $15.00
Published on Web 08/08/1998

the *gem*-methyl protons and C-2. Attached to C-3 of the indole (δ_C 110.1) was a nonprotonated sp^2-carbon (δ_C 135.7) bearing a sp^3-methine (δ_H 3.18; δ_C 47.9). This partial structure was deduced because the δ 3.18 proton signal showed HMBC crosspeaks to not only the carbon signals at δ 110.1 and 135.7, but also the carbon signals at δ 132.3 (assigned to a nonprotonated sp^2-carbon attached to the δ135.7 carbon), 40.1/24.7/23.2 (assigned to the quaternary and methyl carbons of a second *gem*-dimethyl group), and 30.1 (assigned to a methylene, the protons of which were vicinal to the δ3.18 proton on the basis of COSY data). All of this meant that an olefinic double bond was attached to C-3 of the indole, and a methine bearing a methylene and a *gem*-dimethyl group was connected to the α-carbon of the olefinic side chain. The latter *gem*-dimethyl group was further connected to C-4 of the indole as shown by the HMBC crosspeaks from H-5 of the indole to the δ 40.1 carbon signal and the *gem*-dimethyl protons (δ_H1.06/1.56) to C-4 (δ_C 139.5) of the indole. Thus, C-3 and C-4 of the indole had to be present together in a six-membered ring.

The *gem*-dimethyl group on C-2 of the indole was further connected to the nonprotonated carbon (δ_C 109.3) of a trisubstituted C=C (the last π-bond to be assigned), as shown by HMBC crosspeaks between the signals for the *gem*-dimethyl protons and the quaternary olefinic carbon. Attached to this δ 109.3 carbon was the nitrile group and a sp^2-methine (δ_C 142.1), since the proton (δ_H 6.76) on this sp^2-methine showed HMBC crosspeaks to the δ 109.3 carbon, the nitrile carbon, and the quaternary carbon (δ_C 35.3) of the *gem*-dimethyl group on C-2. The δ 142.1 carbon had also to be connected to the β-carbon of the C=C on C-3 of the indole, as the δ 6.76 proton signal showed HMBC crosspeaks to the δ 135.7 and 132.3 carbon signals. Therefore, this meant that a conjugated δ-cyanodienyl group was connected to C-3 of the indole where the δ-carbon was joined via a *gem*-dimethyl carbon to C-2 of the indole. The resulting seven-membered ring was a cycloheptatriene. The presence of a cycloheptatriene, the C-1=C-2 of which was coincidental with the C-2=C-3 of the indole, was further supported by the EIMS of **9**, which showed an intense 3:1 M-15 fragment ion cluster at *m*/*z* 387/389 for a tropylium ion formed from loss of one of the geminal methyl substituents on the cycloheptatriene ring.

The structure of the fifth and last ring in **9**, a cyclohexene, was established as follows: Attached to the quaternary carbon (δ_C 46.2) bearing the vinyl group and a methyl substituent was the β-carbon (δ_C 132.3) of the cyanodienyl side chain and a sp^3-methine carbon (δ_C 65.3). Both the methyl (δ_H 1.52) and vinyl methine (δ_H 5.71) proton signals showed HMBC crosspeaks (due to three-bond coupling) with the δ 132.3 and 65.3 carbon signals. All of the carbons in **9** were now assigned. By process of elimination, the chlorine had to be connected to the δ 65.3 carbon and, in turn, the δ 65.3 carbon to the δ 30.1 methylene carbon to complete the gross structure. Further proof was provided by the HMBC spectrum, which showed crosspeaks between the signal for the proton on the chlorine-bearing methine (δ_H 4.19) and the carbon signals for the δ 30.1 methylene and its attached δ 47.9 methine carbon, as well as carbon signals for the methylvinylmethylene attached to the chlorine-bearing methine.

The relative stereochemistry in the cyclohexene ring was elucidated from ^{1}H−^{1}H coupling constant data for the ClCH−CH$_2$−CH fragment in **9** and difference NOE studies. Because the ClCH proton (δ_H 4.19) showed a 12.7-Hz coupling to the axial proton and a 3.9-Hz coupling to the equatorial proton on the adjacent methylene, the ClCH proton had to be axial. The axial and equatorial methylene protons also showed vicinal couplings of 11.2 and 7.5 Hz, respectively, to the proton of the second methine (δ_H 3.18) attached to the methylene. These couplings suggested that the δ 3.18 methine proton was axial and that the dihedral angles between this proton and the axial and equatorial methylene protons should be approximately 160° and 38°, respectively. The methyl group on the vinyl-bearing carbon attached to the ClCH carbon had to be axial, as it showed a strong NOE to the axial methylene proton, but no NOE with any of the other protons in the ClCH−CH$_2$−CH fragment. Conversely, the axial methylene proton showed a NOE to the axial methyl group on the vinyl-bearing carbon. Finally, the axial methine protons showed NOEs to each other and the equatorial methylene proton. The relative stereochemistry was therefore 12*R**,13*R**,15*S** using the numbering system shown in **9**.

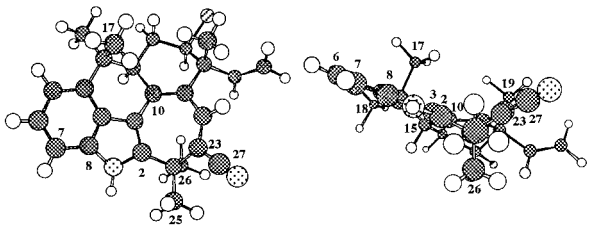


Figure 1. Energy-minimized molecular model of **9**. Topside view is shown on the left; frontal view of southern face is shown on the right. Note that the planes of the indole and C-24/H=C-23−C-27/N fragments bisect the plane of the C-10=C-11 fragment in such a way that the three fragments appear to be connected to one another in a curved manner.

Molecular models of **9** were generated from CSC Chem3D Plus. For the conformer of lowest energy (Figure 1), the dihedral angles for H-15−C-15−C-14−H-14ax and H-15−C-15−C-14−H-14eq were found to be 154° and 38°, respectively, in good agreement with the ones predicted from the coupling constants (see above). Also found were the following conformational features: (a) One of the methyl groups on C-16 was axial and situated above (essentially perpendicular to) the plane of the indole system (H_3-17, 1.06 ppm), whereas its geminal neighbor was equatorial and almost coplanar with the indole system (H_3-18, 1.56 ppm). The ^{1}H chemical shifts and NOEs between the H_3-17 andH-14ax signals and between the H_3-18 and H-5 signals agreed with this conformation. (b) One of the methyl groups on C-22 was pseudoequatorial, coplanar with the indole system, and juxtapositioned with the NH proton (H_3-25, 1.91 ppm), whereas its geminal partner was pseudoaxial and oriented below the plane of the indole system (H_3-26, 1.04 ppm). The ^{1}H chemical shift data and a NOE between H_3-25 and the indole NH supported this conformation. (c) The δ-cyanodienylindole system was not planar, but the indolyl-C-10 and C-11−C-24=C-23−CN sections were planar, where all of the dihedral angles within each section were at or very near to 0° or 180°. The indolyl−C-10=C-11 and C-10=C-11/C-24=C-23−CN sections deviated significantly from planarity, and this meant that the indole, C-10=C-11, and C-24=C-23−CN chromophores were not conjugated to the maximal extent in the generated model. The dihedral angles for C-2−C-3−C-10−C-11, C-9−C-3−C-10−C-11, C-3−C-10−C-11−C-24, and C-23−C-24−C-11−C-10 were −25°, 159°, −11°, and 38°, respectively. This distortion from planarity, however, did not appear to be severe enough to affect markedly the probability of an electronic excitation such as :NHC-2=C-3−C-10=C-11−C-24=C-23−C≡N → N$^+$H=C-2−C-3=C-10−C-11=C-24−C-23=C=N:$^-$, as suggested by the position

and intensity of the absorption maximum at 390 nm (ϵ 6750) in the ultraviolet spectrum of **9**.

A structure where C-23═C-24 is *Z* instead of *E* (i.e., H-23 anti to the cyano group) is an unlikely one for **9**. Not only is the physical data for **9** in disagreement with a *Z*-structure for C-23═C-24, the *Z*-structure is at least 25 kcal/mol higher in energy than the *E*-structure.

The biogenesis of alkaloid **9** probably involves a rearrangement of an ambiguine isonitrile, possibly **8**, or a related compound having a hydrogen rather than a hydroxyl group on C-10.

Experimental Section

Spectral Analysis. NMR spectra were determined on a 11.75-T instrument operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C. ^{1}H chemical shifts are referenced in $CDCl_3$ to residual $CHCl_3$ (7.24 ppm); ^{13}C chemical shifts are referenced to the solvent ($CDCl_3$, 77.0 ppm). Homonuclear connectivities were determined by using 2D double-quantum filtered COSY and 1D decoupling experiments. Homonuclear ^{1}H NOEs were obtained by difference NOE experiments using a 2-s irradiation period. One-bond heteronuclear ^{1}H−^{13}C connectivities were determined by 2D proton-detected HMQC experiments; two- and three-bond ^{1}H−^{13}C connectivities were determined by 2D proton-detected HMBC experiments. UV spectra and optical rotations were measured in MeOH at 20 °C.

Isolation and Cultivation of Alga. A nonaxenic, unialgal strain of *Hapalosiphon delicatulus* W. & G. S. West, designated UH strain IC-13-1, was isolated from a soil sample collected in March 1990, on the grounds of the Australian Institute of Marine Sciences, Australia, and purified by repeated subculture on solidified media. The cyanophyte was cultured in autoclaved 20-L glass carboys containing an inorganic medium (modified BG-11) adjusted to pH 7.0 with MOPS. Cultures were continuously illuminated at an incident intensity of 80−100 μmol photos m^{-2} s^{-1} (photosynthetically active radiation) from banks of cool-white fluorescent tubes and sparged with 0.5% carbon dioxide in air at a rate of 5 L/min. The temperature was maintained at 24 ± 1 °C. After 38 d the alga was harvested by filtration on Whatman no. 4 paper and freeze-dried. The yield of lyophilized cells was 0.34 to 0.47 g/L.

Isolation of Alkaloids from *Hapalosiphon delicatulus* IC-13-1. Lyophilized alga (37.5 g) was extracted twice with 1-L portions of CH_2Cl_2−2-propanol overnight while stirring. The dark green crude product (2.6 g) was dissolved in MeOH, filtered, and applied to a column of Sephadex LH20−120 (Fluka, 85 cm × 4.5 cm diameter, flow rate 7 mL/min) equilibrated in MeOH. Five fractions (1−5) were collected based on Si-TLC analysis. Fractions 4 (1050−1175 mL, 196 mg) and 5 (1175−1500 mL, 311 mg) contained indole alkaloids according to NMR analysis. Fraction 4 was further separated into 10 fractions (4.1−4.10) by normal-phase column chromatography (Si gel, 30 cm × 2.5 cm diam) using successive mixtures of 50:50, 60:40, 70:30, 80:20, and 90:10 CH_2Cl_2−isooctane followed by neat CH_2Cl_2, EtOAc, and MeOH (200 mL each). Fraction 4.3 gave pure hapalindole D (**1**) (6.1 mg) after further purification by HPLC (Econosil Si 250 mm × 10 mm, 10 μm, 1:1 hexane−EtOAc, flow rate 2 mL/min). Purification of fraction 4.6 by column chromatography (Si, 18 cm × 0.8 cm diam, 9:1 hexane−EtOAc) gave ambiguine A isonitrile (**6**) (11.3 mg). Further separation of fraction 4.7 by column chromatography (Si, 20 cm × 0.8 cm diam, 9:1 hexane−EtOAc) gave additional **6** (5.8 mg) and ambiguine B isonitrile (**7**) (20.0 mg). Fraction 5 from the Sephadex column was further separated by column chromatography (Si, 45 cm × 2.5 cm diam) using successive 200-mL portions of 90:10, 85:15, 80:20, 75:25, 70:30, 65:35, 60:40, 50:50, 40:60, 30:70, 20:80, and 10:90 hexane−EtOAc followed by EtOAc, and finally MeOH. Eight fractions (5.1−5.8) were collected. After column chromatography (Si gel, 20 cm × 0.8 cm diam, 4:1 CH_2Cl_2−isooctane) fraction 5.2 gave a 3:1 mixture of hapalindoles D (**1**) and F (**2**) (5.2 mg), fraction 5.3 gave hapalindole H (**4**) (21.4 mg), fraction 5.4 gave a 3:1 mixture of hapalindoles G (**3**) and U (**5**) (55.3 mg), and fraction 5.5 gave pure ambiguine G nitrile (**9**) (2.4 mg). Fraction 5.6 led to ambiguine E isonitrile (**8**) (99.1 mg) after Si chromatography (20 cm × 2 cm diam, 9:1 CH_2Cl_2−isooctane).

Ambiguine G nitrile (9): $[\alpha]_D$ +138.3° (0.6, $CHCl_3$); UV (MeOH) λ_{max} (ϵ) 230 (32 400), 274 (13 900), 390 (6750) nm; IR (neat) υ_{max} 3337, 2970, 2202, 1534, 1470, 1364, 1315 cm^{-1}; ^{1}H NMR δ (assignment; multiplicity, *J* in Hz; *^{1}H NOE*) 8.06 (NH; s), 7.24 (H-6; A part of ABX spectrum, $J_{6,7} = 7.5/J_{5,6} = 8.2$), 7.22 (H-7; B part of ABX spectrum, $J_{6,7} = 7.5/J_{5,7} = 0.6$), 7.07 (H-5; X part of ABX spectrum, 8.2/0.6), 6.76 (H-24; s), 5.71 (H-20; dd, 17.4/10.8), 5.32 (H-21*E*; d, 10.8), 5.26 (H-21*Z*; d, 17.4), 4.19 (H-13; dd, 12.7/3.9; *20*, *15*, *14eq*), 3.18 (H-15; dd, 11.2/7.5; *13*, *14eq*,*18*), 2.44 (H-14eq; ddd, −13.3/7.5/3.9; *13*,*15*,*18*), 2.34 (H-14ax; 1:3:3:1 q, −13.3/12.7/11.2; *17*, *19*), 1.91 (H_3-25; s; *NH*,*26*), 1.56 (H_3-18; s; *5*), 1.52 (H_3-19; s; *14ax*, *21Z*, *24*), 1.06 (H_3-17; s; *14ax*), 1.04 (H_3-26; s); ^{13}C NMR δ (assignment, $^1J_{CH}$-multiplicity; *^{1}H−HMBC*) 143.8 (C-20, d; *13*, *19*, *21E*, *21Z*), 142.1 (C-24, d), 139.5 (C-4, s; *5*, *6*, *17*, *18*), 137.4 (C-2, s; *1*, *25*, *26*), 135.7 (C-10, s; *14eq*, *15*, *24*), 132.9 (C-8, s; *5*, *6*), 132.3 (C-11, s; *13*, *20*, *21E*, *21Z*, *24*), 124.5 (C-9, s; *5*, *7*), 124.1 (C-6, d; *1*), 119.7 (CN, s; *24*), 116.1 (C-21, t), 114.4 (C-5, d; *7*), 110.1 (C-3, s; *1*, *15*), 109.3 (C-23, s; *24*, *25*, *26*), 108.8 (C-7, d; *5*), 65.3 (C-13, d; *14ax*, *14eq*, *19*, *20*, *21E*, *21Z*), 47.9 (C-15, d; *13*, *14ax*, *14eq*, *17*, *18*), 46.2 (C-12, s; *13*, *14ax*, *14eq*, *19*, *20*, *21E*, *21Z*, *24*), 40.1 (C-16, s; *5*, *14eq*, *15*, *17*, *18*), 35.3 (C-22, s; *24*, *25*, *26*), 30.1 (C-14, t; *13*, *15*), 24.9 (C-26, q; *25*), 24.7 (C-17, q, *15*, *18*), 24.6 (C-25, q; *26*), 23.2 (C-18, q; *15*, *17*), 18.6 (C-19, q; *13*, *20*); EIMS *m*/*z* (rel int, assignment) 404/402 (6/2, M$^+$), 389/387 (40/14, M−CH_3), 351 (100, M−CH_3−HCl), 334 (23), 321 (16), 281 (24), 111 (20), 97 (25), 85 (55), 71, (99), 69 (66); HREIMS *m*/*z* 402.1862 (calcd for $C_{26}H_{27}{}^{35}ClN_2$, 402.1862).

Acknowledgment. This research was supported by a grant from the National Science Foundation (CHE95-30794). One of us (U.H.) thanks the Deutsche Forschungsgemeinschaft for a postdoctoral fellowship.

References and Notes

(1) Moore, R. E.; Cheuk, C.; Patterson, G. M. L. *J. Am. Chem. Soc.* **1984**, *106*, 6456−6457.
(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*, 1036−1043.
(3) Schwartz, R. E.; Hirsch, C. F.; Springer, J. P.; Pettibone, J. P.; Zink, D. L. *J. Org. Chem.* **1987**, *52*, 3706−3708.
(4) Moore, R. E.; Yang, X.-Q. G.; Patterson, G. M. L. *J. Org. Chem.* **1987**, *52*, 3773−3777.
(5) Moore, R. E.; Yang, X.-Q. G.; Patterson, G. M. L.; Bonjouklian, R.; Smitka, T. A. *Phytochemistry* **1989**, *28*, 1565−1567.
(6) Smitka, T. A.; Bonjouklian, R.; Doolin, L.; Jones, N. D.; Deeter, J. B.; Prinsep, M. R.; Yoshida, W.; Moore, R. E.; Patterson, G. M. L. *J. Org. Chem.* **1992**, *57*, 857−861.
(7) Park, A.; Moore, R. E.; Patterson, G. M. L. *Tetrahedron Lett.* **1992**, *33*, 3257−3260.
(8) Stratmann, K.; Moore, R. E.; Bonjouklian, R.; Deeter, J. B.; Patterson, G. M. L.; Shaffer, S.; Smith, C. D.; Smitka, T. A. *J. Am. Chem. Soc.* **1994**, *116*, 9935−9942.
(9) Klein, D.; Daloze, D.; Braekman, J. C.; Hoffmann, L.; Demoulin, V. *J. Nat. Prod.* **1995**, *58*, 1781−1785.
(10) Hapalindole I is a minor $^1O_2$-oxidation product of hapalindole A and **3**.[4]

NP9801561