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Studies toward the Total Synthesis of the Cytotoxic Sponge Alkaloid Pyrinodemin A

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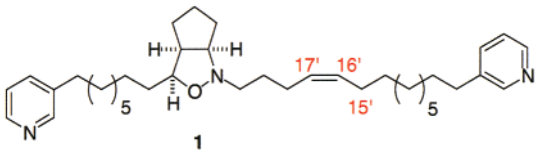
ABSTRACT



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The syntheses of the proposed structure of pyrinodemin A (1) and its *cis* double bond positional isomer (C15′−C16′) in racemic form are described. The key reaction involved an intramolecular nitrone/double bond cycloaddition. Our results suggest that neither 1 nor its double positional isomer is the correct structure of pyrinodemin A

In 1999, Kobayashi et al. reported the isolation of a structurally novel alkaloid, pyrinodemin A, from the marine sponge *Amphimedon* sp. collected off Nakijin, Okinawa.[1] On the basis of a combination of NMR and mass spectrometry studies, Kobayashi et al. proposed the structure of pyrinodemin A as **1** with the relative stereochemistry as shown. Pyrinodemin A is chiral and possesses a unique *cis*-cyclopent[*c*]isoxazolidine moiety. Subsequently, Kobayashi et al. reported a family of compounds similar to pyrinodemin A obtained from the same sponge.[2] Pyrinodemin A is cytotoxic toward murine leukaemia L1210 and KB epidermoid carcinoma cells. Our continued interest[3] in sponge alkaloid chemistry coupled with the novel structure of pyrinodemin A prompted us to investigate its synthesis. Herein we report the synthesis of racemic structures **1** and **19** and our finding that the spectroscopic data of synthetic **1** and **19** do not correspond to that of pyrinodemin A.[4]

Biosynthetically, structure **1** can be formed from an intramolecular 1,3-dipolar cycloaddition of nitrone **2**.[5] Nitrone **2**, in turn, can be derived from aldehyde **3** and hydroxylamine **4**. This biosynthetic proposal forms the basis of our biomimetic synthesis (Figure 1).

The synthesis of aldehyde **3** commenced with the protection of 5-hexyn-1-ol **5** as its *tert*-butyldiphenylsilyl ether **6** in 96% yield with *tert*-butyldiphenylsilyl chloride and imidazole in THF.[6] Deprotonation of the terminal acetylene in **6** with *n*-butyllithium followed by addition of the lithiated acetylene to excess 1,7-dibromoheptane in 1,3-dimethyl-3,4,5,6-tetrahydro-2-(1*H*)-pyrimidinone (DMPU) gave compound **7** in 68% yield.[7] Semi-hydrogenation of acetylene **7** in benzene with Lindlar catalyst and quinoline delivered alkene **8** in 94% yield.[8] Alkene **8** was treated with lithiated

(1) Tsuda, M.; Hirano, K.; Kubota, T.; Kobayashi, J. *Tetrahedron Lett.* **1999**, *40*, 4819−4820.

(2) Hirano, K.; Kubota, T.; Tsuda, M.; Mikami, Y.; Kobayashi, J. *Chem. Pharm. Bull.* **2000**, *48*, 974−977.

(3) (a) Baldwin, J. E.; Claridge, T. D. W.; Culshaw, A. J.; Heupel, F. A.; Lee, V.; Spring, D. R.; Whitehead, R. C.; Boughflower, R. J.; Mutton, I. A.; Upton, R. J. *Angew. Chem., Int. Ed.* **1998**, *37*, 2661−2663. (b) Baldwin, J. E.; Spring, D. R.; Atkinson, C. E.; Lee, V. *Tetrahedron* **1998**, *45*, 13655−13680. (c) Baldwin, J. E.; Vollmer, H. R.; Lee, V. *Tetrahedron Lett.* **1999**, *40*, 5401−5404. (d) Baldwin, J. E.; Claridge, T. D. W.; Culshaw, A. J.; Heupel, F. A.; Lee, V.; Spring, D. R.; Whitehead, R. C. *Chem. Eur. J.* **1999**, *5*, 3154−3161. (e) Baldwin, J. E.; James, D.; Lee, V. *Tetrahedron Lett.* **2000**, *41*, 733−736.

(4) Taken in part from Romeril, S. P. Part II Thesis, June 2000, University of Oxford.

(5) After the synthesis of compound **1** was completed, a similar proposal appeared in print. See ref 2.

(6) Hall, D. G.; Deslongchamps, P. *J. Org. Chem.* **1995**, *60*, 7796−7814.

(7) Poulain, S.; Noiret, N.; Nugier-Chavin, C.; Patin, H. *Liepigs Ann.* **1997**, 35−40.

(8) Lindlar, H.; Dubuis, R. *Organic Syntheses*; Wiley: New York, 1973; Collect. Vol. V, pp 880−883.

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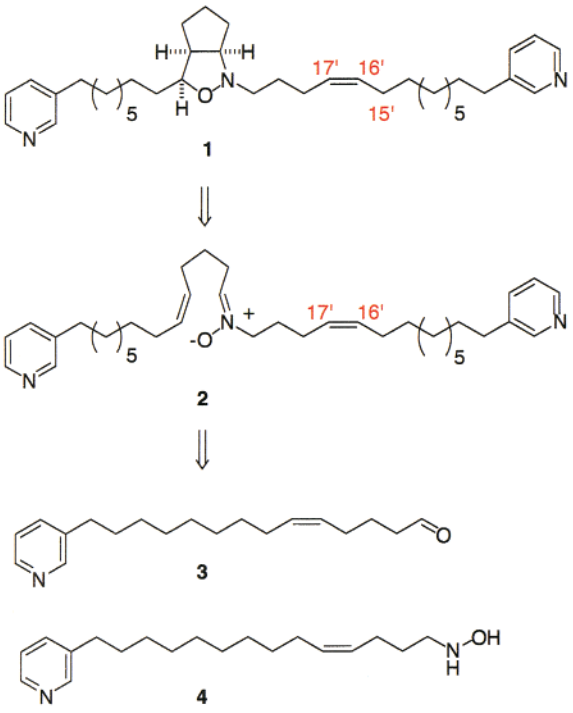
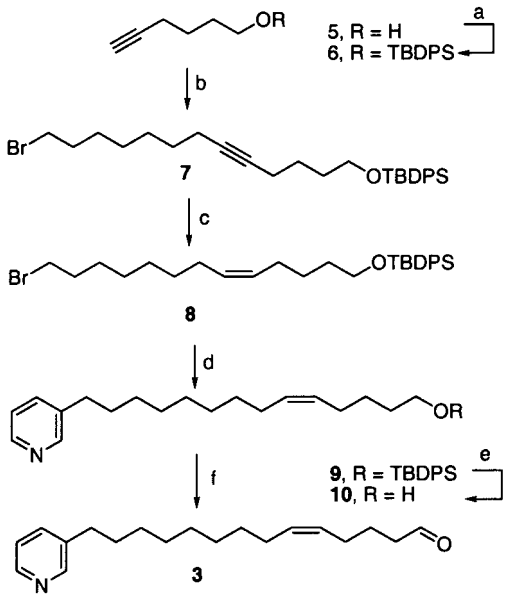


Figure 1. The proposed structure of pyrinodemin A and its retrosynthetic biosynthetic scheme.

Scheme 1[a]



[a] (a) TBDPSCI, imidazole, THF, 96%. (b) ^{n}BuLi, THF, and then $Br(CH_2)_7Br$, DMPU, 68%. (c) Lindlar catalyst, quinoline, H_2, PhH, 94%. (d) 3-Picoline, LDA, THF, DMPU, 63%. (e) NH_4F, MeOH, 97%. (f) IBX, DMSO, THF, 92%.

3-picoline (prepared from 3-picoline and LDA in THF/DMPU) to deliver alkylpyridine **9** in 63% yield.[9] The deprotection of **9** was effected with ammonium fluoride in methanol to afford alcohol **10** in 97% yield.[10] Aldehyde **3** was obtained in 92% yield by oxidation of alcohol **10** with 2-iodoxybenzoic acid (IBX) in DMSO and THF[11] (Scheme 1).

To prepare hydroxylamine **4**, 4-pentyn-1-ol **11** was protected as its *tert*-butyldiphenylsilyl ether **12** in 93% yield.[6] Alkylation of 1,8-dibromooctane with the acetylide anion generated from **12** gave compound **13** in 77% yield.[7] Lindlar hydrogenation[8] of **13** gave alkene **14** in 97% yield which was treated with lithiated 3-picoline[9] to afford the protected pyridine **15** in 59% yield. Alcohol **16** was obtained in 97% yield from fluoride deprotection[10] of **15**. **16** was subjected to IBX oxidation to give aldehyde **17** in 92% yield.[11] Treatment of aldehyde **17** with hydroxylamine hydrochloride and sodium acetate in methanol delivered oxime **18** as a mixture of *cis* and *trans* isomers in 93% yield.[12] Oxime **18** was reduced with sodium cyanoborohydride in methanol at pH 3 to give hydroxylamine **4** (Scheme 2).

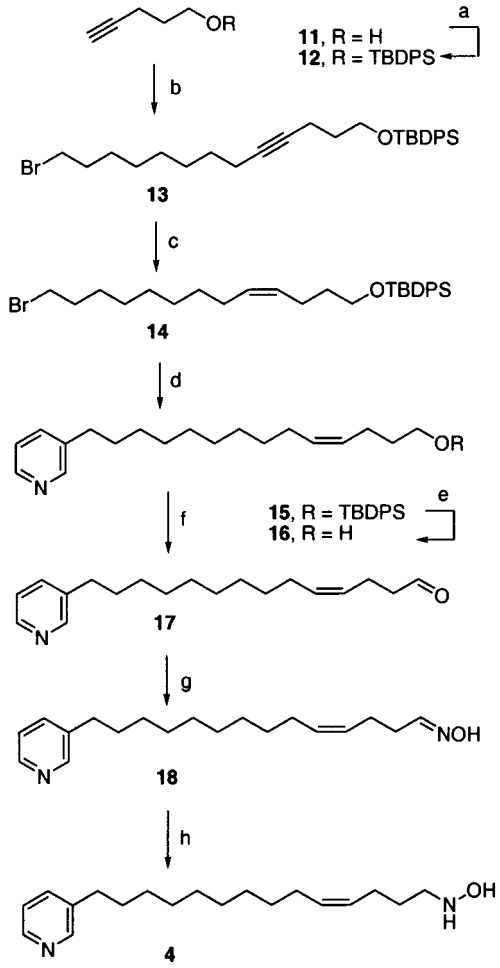
Unpurified **4** was immediately condensed with aldehyde **3** in dichloromethane in the presence of anhydrous sodium sulfate[12] to afford nitrone **2** in 89% (over two steps). Thermal cyclization of nitrone **2** in anhydrous benzene under high dilution condition afford the desired product **1** in 41% yield after chromatographic purification (Scheme 3).

The structure of **1** was unambiguously established by HSQC, HSQC-TOCSY[13] (τ_m = 80 ms), DQF-COSY, and 1D DPFGSE-NOESY[14] (τ_m = 400 ms) spectroscopy.[15] In the HSQC-TOCSY experiment, C-20′ in **1** correlates with H19′, H18′, and H17′; therefore the double bond is between C17′ and C16′.[16] When the spectroscopic data of **1** were compared with those of the natural pyrinodemin A, subtle differences were noticed. Kobayashi assigned the position of the double bond in natural pyrinodemin A between C16′−C17′. This conclusion was based on the fragments *m*/*z* 204 (interpreted as cleavage of C15′−C16′ bond) and *m*/*z* 231 (interpreted as cleavage of C17′−C18′ bond plus gaining a hydrogen atom) observed in electron impact mass spectrometry (EIMS) of the natural product.

Kobayashi also reported the ^{13}C chemical shift of the olefinic carbons (in $CDCl_3$) as a singlet at 129.3 ppm. In the ^{13}C NMR (in $CDCl_3$) of synthetic product **1**, the olefinic carbons (C16′ and C17′) appeared as two separated signals at 129.2 and 130.3 ppm ($\Delta\delta$ 1.1). Small differences were also observed in the ^{1}H NMR and EIMS fragmentation

(9) Davies-Coleman, M. T.; Faulkner, D. J.; Dubouwchik, G. M.; Roth, G. P.; Polson, C.; Fairchild, C. *J. Org. Chem.* **1993**, *58*, 5925−5930.
(10) Zhang, W.; Robins, M. J. *Tetrahedron Lett.* **1992**, *33*, 1177−1180.
(11) (a) Frigerio, M.; Santagostino, M.; *Tetrahedron Lett.* **1994**, *35*, 8019−8022. (b) Frigerio, M.; Santagostino, M.; Sputore, S.; Palmisano, G. *J. Org. Chem.* **1995**, *60*, 7272−7276. (c) Frigerio, M.; Santagostino, M.; Sputore, S. *J. Org. Chem.* **1999**, *64*, 4537−4538.
(12) Holmes, A. B.; Smith, A. L.; Williams, S. F.; Hughes, L. R.; Lidert, Z.; Switenbank, C. *J. Org. Chem.* **1991**, *56*, 1393−1405.
(13) John, B. K.; Plant, D.; Heald, S. L.; Hurd, R. E. *J. Magn. Reson.* **1991**, *94*, 664−669.
(14) Stott, K.; Keeler, J.; Van, Q. N.; Shaka, A. J. *J. Magn. Reson.* **1997**, *125*, 302−324.
(15) The HSQC, HSQC-TOCSY, DQF-COSY, and 1D DPFGSE-NOESY experiments were conducted in CD_3OD.
(16) Kobayashi's system of numbering is adopted throughout this Letter, see ref 1.

Scheme 2[a]



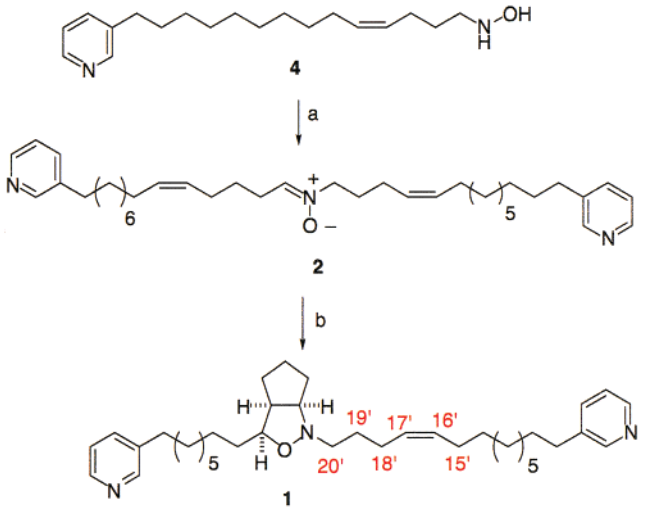
[a] (a) TBDPSCI, imidazole, THF, 93%. (b) ^{n}BuLi, THF, and then $Br(CH_2)_8Br$, DMPU, 77% (c) Lindlar catalyst, quinoline, H_2, PhH, 97%. (d) 3-Picoline, LDA, THF, DMPU, 59%. (e) NH_4F, MeOH, 97%. (f) IBX, DMSO, THF, 92%. (g) $NH_2OH{\cdot}HCl$, MeOH, NaOAc, 93%. (h) $NaCNBH_3$, MeOH, HCl.

pattern between synthetic product **1** and that reported for pyrinodemin A. If **1** was the correct structure of pyrinodemin A, it would be unlikely that the ^{13}C resonance of C16′ and C17′ would converge into one signal due to the unsymmetrical nature of the double bond. In addition, Kobayashi also conducted an HSQC-TOCSY experiment on natural pyrinodemin A but did not report any correlation observed between C-20′ and the olefinic H17′ and H16′.

Further inspection of Kobayashi's electron impact mass spectrometry data led us to propose **19**, in which the double bond is located between C15′ and C16′, as the alternative structure of pyrinodemin A.[4] It is possible that **19**, upon electron impact ionization, may undergo a McLafferty rearrangement to give a fragment of *m*/*z* 231 via rearrangement of the alkene ion (cleavage of C17′−C18′ bond with hydrogen atom transfer from C19′ to C15′).

In addition, the local environment of the double bond in **19** is relatively more symmetrical than that of **1** because it is further away from the isoxazolidine moiety. We envisaged

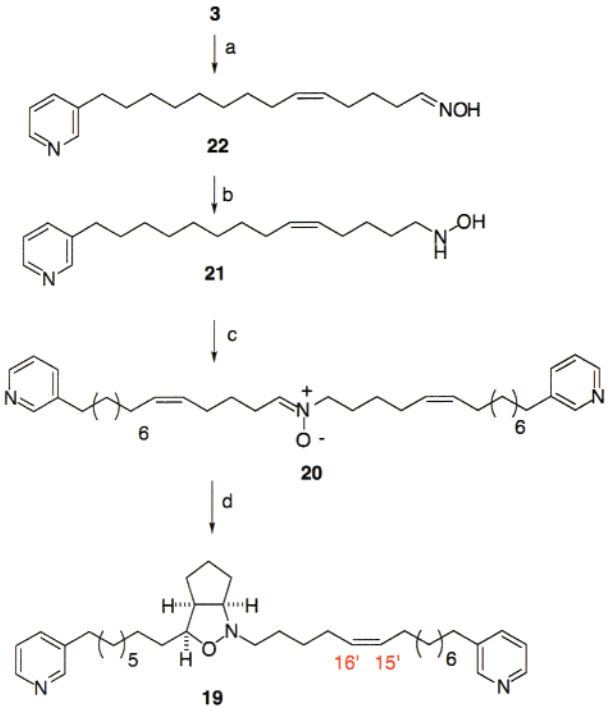
Scheme 3[a]



[a] (a) **3**, anhydrous Na_2SO_4, CH_2Cl_2, 89%. (b) PhH, heat, 41%.

that **19** could be derived from nitrone **20**. Nitrone **20** could be derived form aldehyde **3** and hydroxylamine **21**. In principle, hydroxylamine **21** could itself originate from aldehyde **3**. Therefore, **19** could be biosynthesized from the common precursor **3**. We were further encouraged by the subsequent isolation of oxime **22** (see Scheme 4) as a natural product from the same sponge together with other pyrinodemins.[2] We speculated that oxime **21** might be the biosynthetic precursor of pyrinodemin A.

Scheme 4[a]



[a] (a) $NH_2OH{\cdot}HCl$, MeOH, NaOAc, 84%. (b) $NaCNBH_3$, MeOH, HCl. (c) **3**, anhydrous Na_2SO_4, CH_2Cl_2, 71%. (d) PhH, heat, 63%.

This proposal was reduced to practice. Oxime **22** was prepared in 84% yield from aldehyde **3** and hydroxylamine hydrochloride.[12] Reduction of oxime **22** with sodium cyanoborohydride gave hydroxyamine **21**, which was condensed immediately with aldehyde **3** to afford nitrone **20** in 71% yield over two steps. Thermal cyclization of **20** gave **19** as the desired product in 63% yield (Scheme 4).

The structure of **19** was established by NMR spectroscopy[17] and mass spectrometry. When the spectral data of **19** were compared with those of pyrinodemin A, once again subtle differences were observed. In the ^{13}C NMR of **19** (in $CDCl_3$), the olefinic signals appeared at 129.5 and 129.9 ppm ($\Delta\delta$ 0.4). Therefore our data suggested that **19** is not the correct structure of pyrinodemin A. We stress that it is possibile that the small discrepancies between ours and Kobayashi's NMR data may be due to the effects of concentration, temperature, and purity of the samples. However, the small differences in the EIMS fragmentation pattern between **19** and pyrinodemin A suggest that they are different compounds.

In conclusion, we report the synthesis of the proposed structure of pyrinodemin A and its double bond positional isomer in racemic form based on a biosynthetic hypothesis. We are confident that the core *cis*-cyclopent[*c*]isoxazolidine structure of pyrinodemin A was correctly proposed by Kobayashi. However, we believe that the EIMS method used by Kobayashi to locate the position of the double bond in pyrinodemin A is unreliable. It is known that alkene ions show a strong tendency to isomerize through the migration of double bond in EIMS.[18] The normal practice to locate a double bond in an aliphatic compound by mass spectrometry is to chemically modify the double bond and analyze the derivative with soft ionization technique.[19] Alternatively, collision activation decomposition in tandem mass spectrometry had been applied directly to natural products for the same purpose without the need of derivation.[20] These facts therefore cast doubt on Kobayashi's structural assignment.[21] Our results clearly show that the difference in chemical shifts between the two olefinic carbons diminishes as the double bond is moved farther away from the central bicyclic core. Further work in establishing the true identity of pyrinodemin A is currently underway and will be reported in due course.

Acknowledgment. We thank the States of Jersy Education Committee for a studentship (S.P.R.), Prof. Jun'ichi Kobayashi for spectral data of natural pyrinodemin A, and Dr. Robin Aplin for helpful discussion.

Note added in proof: During the process of publishing this Letter, Snider and Shi reported the syntheses of racemic **1** and **19**.[22] The spectral data (^1H and ^{13}C) of **1** and **19** presented in this Letter matched those for the corresponding structures reported by Snider and Shi. Although they too observed the same inconsistencies between the spectral data of **19** and natural pyrinodemin A, Snider and Shi concluded that **19** is probably the correct structure of pyrinodemin A.

Supporting Information Available: Experimental procedures, spectral data, and full spectroscopic data of natural pyrinodemin A, **1**, and **19**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(17) All NMR experiments for **18** were conducted in $CDCl_3$.

(18) McLafferty, F. W.; Turecek, F. *Interpretation of Mass Spectra*, 4th ed.; University Science Books: Mill Valley, CA, 1993; p 230.

(19) Schmitz, B.; Klein, R. A. *Chem. Phys. Lipids* **1986**, *39*, 285−311. For a recent example, see: Devijver, C.; Salmoun, M.; Daloze, D.; Braekman, J. C.; De Weerdt, W. H.; De Kluijver, M. J.; Gomez, R. *J. Nat. Prod.* **2000**, *63*, 978−980.

(20) Tomer, K. B.; Crow, F. W.; Gross, M. L. *J. Am. Chem. Soc.* **1983**, *105*, 5487−5488. For a recent example, see: Watanabe, K.; Tsuda, Y.; Yamane, Y.; Takahashi, H.; Iguchi, K.; Naoki, H.; Fujita, T.; Van Soest, R. W. M. *Tetrahedron Lett.* **2000**, *41*, 9271−9276.

(21) Kobayashi applied the same method to determine the position of the double bond in pyrinodemin C and natural oxime **21**, see ref 2.

(22) Snider, B. B.; Shi, B. *Tetrahedron Lett.* **2001**, *42*, 1639−1642.