JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Subscriber access provided by ISTANBUL TEKNIK UNIV

Article

The Total Synthesis of Roquefortine C and a Rationale for the Thermodynamic Stability of Isoroquefortine C over Roquefortine C

Ning Shangguan, Warren J. Hehre, William S. Ohlinger, Mary Pat Beavers, and Madeleine M. Joullié

J. Am. Chem. Soc., **2008**, 130 (19), 6281-6287 • DOI: 10.1021/ja800067q • Publication Date (Web): 16 April 2008

Downloaded from http://pubs.acs.org on February 8, 2009

More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 2 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

ACS Publications
High quality. High impact.

Journal of the American Chemical Society is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

J|A|C|S
ARTICLES

Published on Web 04/16/2008

The Total Synthesis of Roquefortine C and a Rationale for the Thermodynamic Stability of Isoroquefortine C over Roquefortine C

Ning Shangguan,[§] Warren J. Hehre,[†] William S. Ohlinger,[†] Mary Pat Beavers,[‡] and Madeleine M. Joullié*[,§]

Wavefunction, Inc., 18401 Von Karman Avenue, Suite 370, Irvine, California 92612, Institute for Medicine and Engineering, University of Pennsylvania, 1026 Vagelos Research Labs, Philadelphia, Pennsylvania 19104-6383, and Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19104-6323

Received January 4, 2008; E-mail: mjoullie@sas.upenn.edu

Abstract: The first total synthesis of roquefortine C is achieved by implementation of a novel elimination strategy to construct the thermodynamically unstable *E*-dehydrohistidine moiety. Molecular modeling studies are presented which explain the instability of the roquefortine C structure compared to that of isoroquefortine C.

Introduction

The roquefortines are a class of biologically active indole alkaloids featuring a hexahydro[2,3-*b*]indole nucleus substituted at the benzylic ring junction with a 1,1-dimethylallyl group. A number of structurally similar indole alkaloids that belong to the amauromine, ardeemin, or flustramine families have received significant attention from synthetic chemists.[1–5] The roquefortine family of fungal metabolites was initially isolated from cultures of *Penicillium roqueforti* obtained from soil samples, though these compounds are also produced by other *Penicillium* species.[6–10] Roquefortine C was found in a variety of food products, due to both natural occurrence and contamination.[7,11–13] Roquefortine D (**1**) is the dihydro derivative of roquefortine C (**2**) and the biosynthetic precursor to **2**. More recently, both were isolated from *P. aurantiogriseum* strains obtained from Artic and Antartic sediments.[14] A new member of this family, roquefortine E (**3**), has been isolated from an Australian soil isolate of the ascomycete *Gymnoascus reessii* (Figure 1).[15]

Roquefortine E (**3**) is the first roquefortine to be isolated from a fungus other than *Penicillium.* Roquefortine C possesses bacteriostatic activity against Gram-positive bacteria and also interacts with cytochrome P450 by binding to the heme. The effect of roquefortine on the growth of gram-positive organisms only occurs in those containing hemins.[16–19] However, the mechanisms of toxicity and metabolic pathway of roquefortine are still unclear.[12,20–22]

An important structural feature of **2** is the configuration of the attachment to the imidazole ring. The *E*-configuration of the 3–17 double bond of roquefortine was established by comparison of its spectral properties with those of its photoisomer, isoroquefortine C (**4**, Figure 2) that has the *Z*-configuration. The unusual *E*-dehydrohistidine moiety typically undergoes facile isomerization under acidic, basic, or photochemical conditions.[23–27] Isoroquefortine C (**4**) is not a natural product. In contrast to roquefortine C, isoroquefortine C does not bind to iron.

The interest in roquefortine C (**2**) is due to its presence in all blue cheeses and the health implications of its existence in

[§] Department of Chemistry, University of Pennsylvania.
[†] Wavefunction, Inc.
[‡] Institute for Medicine and Engineering, University of Pennsylvania.

(1) Depew, K. M.; Marsden, S. P.; Zatorska, D.; Zatorski, A.; Bornmann, W. G.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1999**, *121*, 11953.
(2) Lindel, T.; Braeuchle, L.; Golz, G.; Boehrer, P. *Org. Lett.* **2007**, *9*, 283.
(3) Marsden, S. P.; Depew, K. M.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1994**, *116*, 11143.
(4) Suarez-Castillo, O. R.; Sanchez-Zavala, M.; Melendez-Rodriguez, M.; Aquino-Torres, E.; Morales-Rio, M. S.; Joseph-Nathan, P *Heterocycles* **2007**, *71*, 1539.
(5) Takase, S.; Itoh, Y.; Uchida, I.; Tanaka, H.; Aoki, H. *Tetrahedron Lett.* **1985**, *26*, 847.
(6) Ohmomo, S.; Kitamoto, H. K.; Nakajima, T. *J. Sci. Food Agric.* **1994**, 211.
(7) Ohmomo, S.; Oguma, K.; Ohashi, T.; Abe, M. *Agric. Biol. Chem.* **1978**, *42*, 2387.
(8) Ohmomo, S.; Sato, T.; Utagawa, T.; Abe, M. *Agric. Biol. Chem.* **1975**, *39*, 1333.
(9) Scott, P. M.; Kennedy, B. P. C. *J. Agric. Food Chem.* **1976**, *24*, 865.
(10) Scott, P. M.; Merrien, M.-A.; Polonsky, J. *Experientia* **1976**, *32*, 140.
(11) Cole, R. J.; Dorner, J. W.; Cox, R. H.; Raymond, L. W. *J. Agric. Food Chem.* **1983**, *31*, 655.
(12) Häggblom, P. *Appl. Environ. Microbiol.* **1990**, *56*, 2924.
(13) Möller, T.; Åkerstrand, K.; Massoud, T. *Nat. Toxins* **1997**, *5*, 86.
(14) Kozlovsky, A. G.; Zhelifonova, V. P.; Adanin, V. M.; Antipova, T. V.; Overskaya, S. M.; Ivanushkina, N. E.; Grafe, U. *Appl. Biochem. Microbiol.* **2003**, *39*, 393.
(15) Clark, B.; Capon, R. J.; Lacey, E.; Tennant, S.; Gill, J. H. *J. Nat. Prod.* **2005**, *68*, 1661.
(16) Aninat, C.; Andre, F.; Delaforge, M. *Food Addit. Contam.* **2005**, *22*, 361.
(17) Aninat, C.; Delaforge, M. *Adv. Exp. Med. Biol.* **2001**, *500*, 331.
(18) Aninat, C.; Hayashi, Y.; Andre, F.; Delaforge, M. *Chem. Res. Toxicol.* **2001**, *14*, 1259.
(19) Delaforge, M.; Bouille, G.; Jaouen, M.; Jankowski, C. K.; Lamouroux, C.; Bensoussan, C. *Peptides* **2001**, *22*, 557.
(20) Arnold, D. L.; Scott, P. M.; MCGuire, P. F.; Hartwig, J.; Nera, E. A. *Food Cosmet. Toxicol.* **1978**, *16*, 369.
(21) Ohmomo, S. J. *Antibac. Antifung. Agents* **1982**, *10*, 253.
(22) Wagener, R. E.; Davis, N. D.; Diener, U. L. *Appl. Environ. Microbiol.* **1980**, *39*, 882.

 © 2008 American Chemical Society

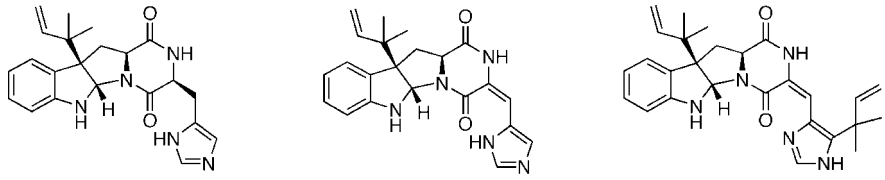


Figure 1. The roquefortine family of fungal metabolites.

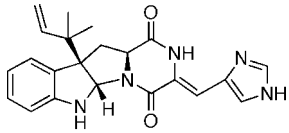


Figure 2. Isoroquefortine C (**4**).

human and animal food products. A report that intraperitoneal injection of 50–100 mg per kg in male mice caused convulsive seizures was followed by conflicting reports, but all these tests were performed on isolated roquefortine obtained from a variety of isolation procedures.[10,12,20–22] The variability of responses to different toxicity tests clearly indicates that the effects exerted were not due to the presence of a single toxin but to several substances present in variable quantities in the extracts of different strains. In view of these results, **2** might be contaminated with other mycotoxins, and it was imperative that toxicity tests should be carried out on a pure synthetic sample.

From a synthetic chemistry standpoint, roquefortine C (**2**) is an attractive target for total synthesis because of its unique structure. Therefore its synthesis was investigated in our laboratory. Earlier efforts resulted only in preparations of isoroquefortine C.[28,29] Now for the first time, we have achieved a total synthesis of this molecule, paving the way for a clear toxicological assessment of this compound. Curious about the reluctance of this compound to yield to synthesis, we also present molecular modeling studies that confirm the stability of the isoroquefortine C (**4**) structure compared to that of roquefortine C (**2**), accounting for the difficulties encountered in generating this compound. What is still unexplained is the ubiquitous presence of roquefortine C in nature and the absence of isoroquefortine C.

The two synthetic challenges presented by roquefortine C (**2**) are the construction of the prenylated pyrroloindole tricyclic structure and the *E*-dehydrohistidine moiety. As shown in the retrosynthetic analysis, the pyrroloindole **5** will be synthesized from L-tryptophan methyl ester **6** and the imidazole derivative **7** will be obtained from the methyl ester of urocanic acid **8**.

The synthesis of acid **5** was accomplished according to the method developed by the Danishefsky group.[1,3] Starting from L-tryptophan methyl ester hydrochloride **6** (Scheme 1), Boc-protection of both nitrogens afforded **9**, which was treated with *N*-phenylselenyl phthalimide (NPSP)[30] to form the selenenylated pyrroloindole **10**. The reaction of tryptophan derivatives with NPSP was extensively investigated by Crich.[31] Compound **10** was treated with methyl triflate, prenyl tributyl stannane and

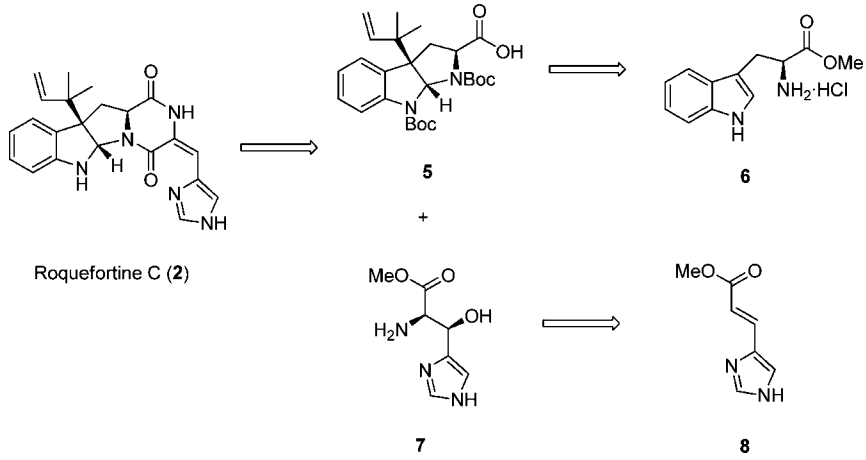


Figure 3. Retrosynthetic analysis of roquefortine C (**2**).

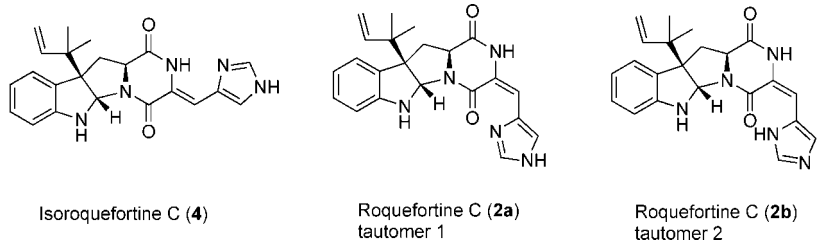
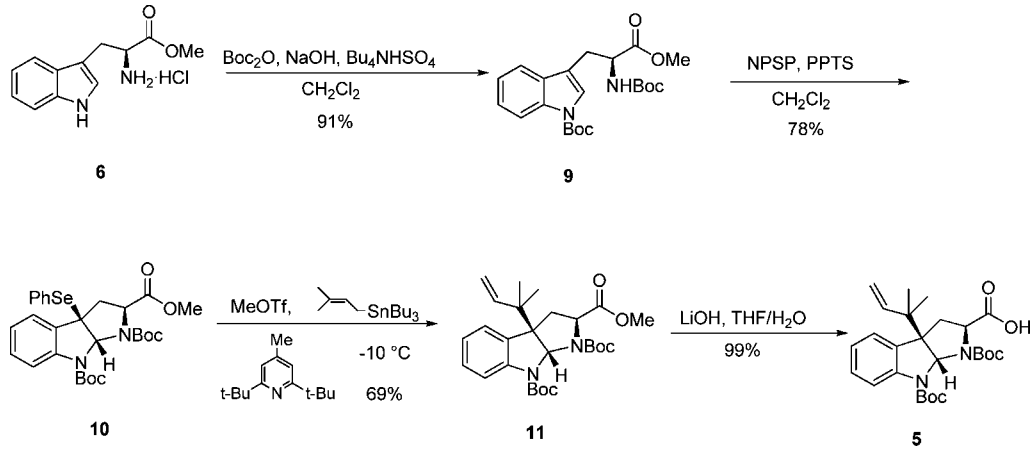
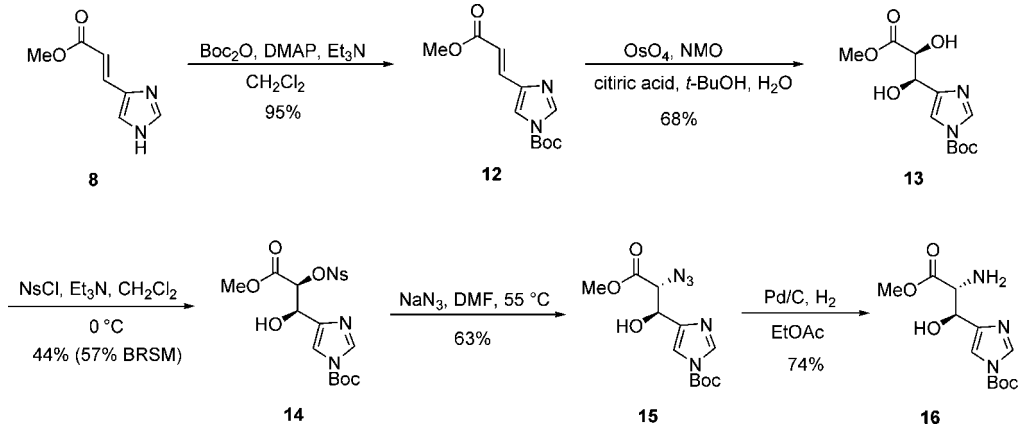


Figure 4. Isoroquefortine C **4** and two tautomers of roquefortine C (**2a** and **2b**) were the low energy tautomers selected for Hartree–Fock/6-31G* calculations in Spartan 06.

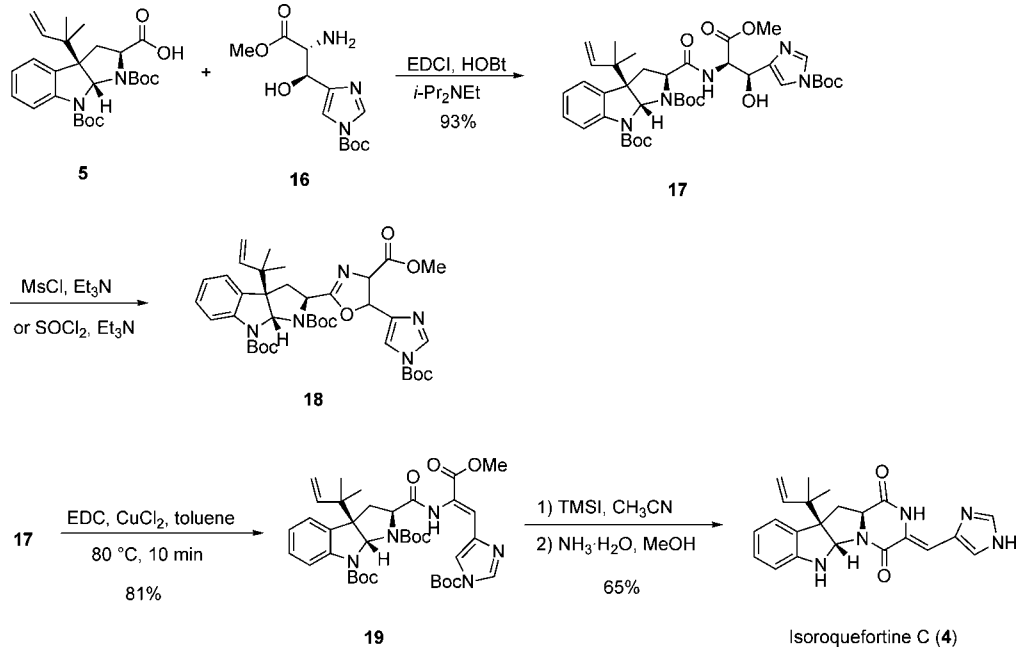
Scheme 1. Construction of the Pyrroloindole Segment (**5**)



Scheme 2. Synthesis of the Imidazole Derivative (**7**)



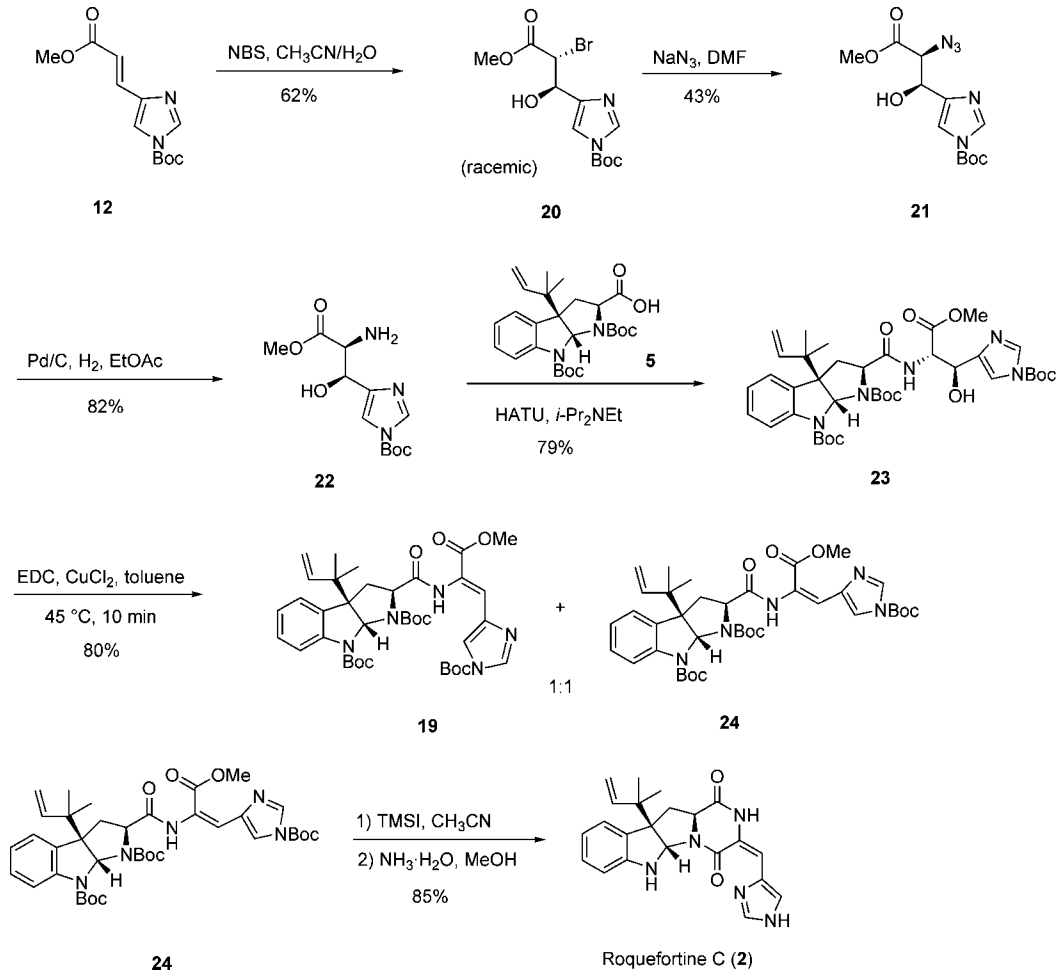
Scheme 3. Route to Isoroquefortine C (**4**)



2,6-di-*tert*-butyl-4-methylpyridine to provide the alkylated compound **11**. Hydrolysis of the methyl ester afforded the acid **5**.

The next step was the construction of the dehydrohistidine fragment (Scheme 2) that began with the methyl ester of urocanic acid **8**.

Scheme 4. Completion of the Synthesis of Roquefortine C (**2**)



Boc protection of the imidazole ring **12** was followed by dihydroxylation to afford a racemic *syn* diol **13**. Selective nosylation of the more acidic α-hydroxyl group in **14** and subsequent azide displacement of the nosylate afforded azide **15**.[32] Hydrogenation of the azide gave the amine **16**.

Coupling of acid **5** and amine **16** under conventional conditions using EDCI and HOBt afforded **17** in 93% yield (Scheme 3). It was expected that an anti E2 elimination on substrate **17** would give the desired *E* double bond. However, treatment of **17** with mesyl chloride or thionyl chloride and triethylamine provided oxazoline **18** as the product, and no elimination occurred. A stereoselective elimination method to generate dehydroamino acid derivatives was reported by Sai et al.[33] Under these conditions (EDC, copper chloride, 80 °C in toluene), elimination provided only the *Z*-dehydroamino product from substrate **17**. Assuming a *syn* elimination transition state, the *Z*-isomer **19** should be the major product.[33] Global deprotection of Boc with TMSI and treatment with ammonia in methanol provided isoroquefortine C.[28]

To make the thermodynamically unstable *E*-isomer using the same dehydration conditions, we needed the *threo* isomer. To this end, protected ester **12** was treated with NBS in an acetonitrile/water mixture to afford the *anti* β-hydroxy-α-bromo derivative **20** (Scheme 4). Azide displacement of the bromine (**21**) and reduction of the azide provided amine **22**. Coupling between **5** and **22** by 2-(1*H*-9-azobenzotriazole-1-yl)-1,1,3,3-tetramethylammonium hexafluorophosphate (HATU) afforded **23**. Using EDC dehydration conditions provided the *Z*-isomer **19** and *E*-isomer **24** as a 1:1 mixture of isomers. Significant amounts of the thermodynamically unstable *E*-isomer were produced by *syn* elimination under these conditions. The *E*/*Z* isomers were easily separable by silica gel chromatography. Treatment of **24** with TMSI and then ammonia in methanol completed the first total synthesis of roquefortine C.

(23) Horenstein, B. A.; Nakanishi, K. *J. Am. Chem. Soc.* **1989**, *111*, 6242.
(24) Kim, D.; Li, Y.; Horenstein, B. A. *Tetrahedron Lett.* **1990**, *31*, 7119.
(25) Poisel, H.; Schmidt, U. *Chem. Ber.* **1975**, *108*, 2547.
(26) Schmidt, U. G., H.; Leitenberger, V.; Lieberknecht, A.; Mangold, R.; Meyer, R.; Riedl, B. *Synthesis* **1992**, 487.
(27) Wild, J. Diplomarbeit. University of Stuttgart, Stuttgart, Germany, 1982.
(28) Schiavi, B.; Richard, D. J.; Joullié, M. M . *J. Org. Chem.* **2002**, *67*, 620.
(29) Richard, D. J.; Schiavi, B.; Joullié, M. M. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 11971.
(30) Nicolaou, K. C.; Claremon, D. A.; Barnette, W. E.; Seitz, S. P. *Tetrahedron* **1985**, *41*, 4835.
(31) Crich, D.; Huang, X. *J. Org. Chem.* **1999**, *64*, 7218.
(32) Nicolaou, K. C.; Jain, N. F.; Natarajan, S.; Hughes, R.; Solomon, M. E.; Li, H.; Ramanjulu, J. M.; Takayanagi, M.; Koumbis, A. E.; Bando, T. *Angew. Chem., Int. Ed.* **1998**, *37*, 2714.
(33) Sai, H.; Ogiku, T.; Ohmizu, H. *Synthesis* **2003**, 201.
(34) (a) Roothaan, C. C. J. *Rev. Mod. Phys.* **1951**, *23*, 69. (b) Hall, G. G. *Proc. R. Soc.* **1951**, *A205*, 541. c) Hehre, W. J.; Radom, L.; Schleyer, P. R.; Pople, J. A. *Ab Initio Molecular Orbital Theory*; Wiley: New York, 1985.

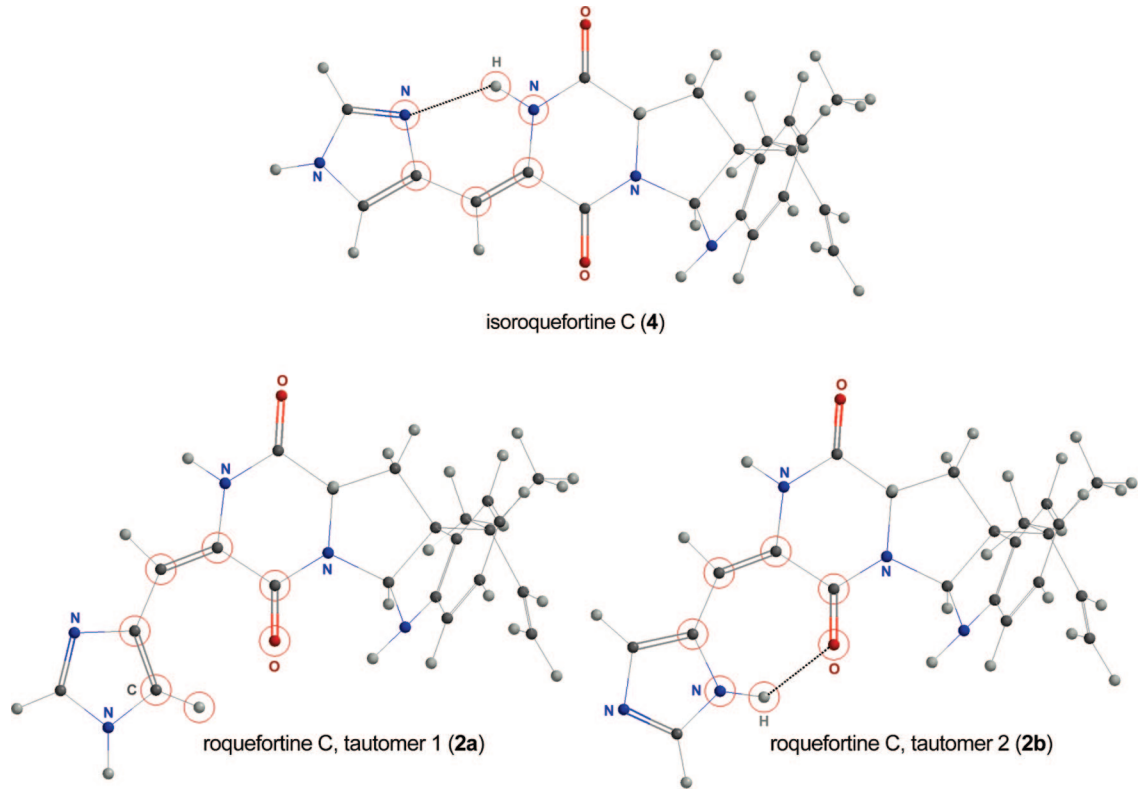


Figure 5. 3-Dimensional structural representations of isoroquefortine C, tautomer 1 of roquefortine C, and tautomer 2 of roquefortine C based on HF/6-31G* geometry optimizations. Both isoroquefortine C (**4**) and tautomer (**2**) of roquefortine C (**2b**) have intramolecular H-bonds between the imidazole and diketopiperazine rings. The atoms circled in red denote the centers (sp^2, nitrogen, carbon, and hydrogen atoms) involved in the "rings" representing the coplanar double bond networks.

Figure 6. Vinyl imidazole. The barrier to rotation of the exocyclic double bond out of coplanarity was modeled using HF/6-31G*. If the double bond rotates out of coplanarity with the conjugated imidazole ring, the penalty is in the range of 17–23 kJ/mol, depending on the starting tautomer and conformer.

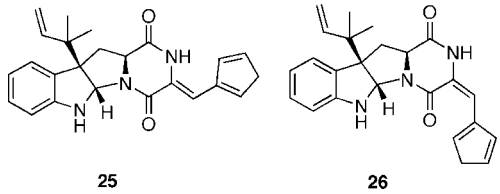


Figure 7. Cyclopentadiene analogues of isoroquefortine C and roquefortine C (**25** and **26**, respectively). The isoroquefortine C analogue (**25**) is 14 kJ/mol more stable than the roquefortine C analogue (**26**) from HF/6-31G* and 9 kJ/mol from T1.

Computational studies on roquefortine C (**2**) and isoroquefortine C (**4**) were undertaken to understand why the synthesis of roquefortine C was at first elusive, and why roquefortine C so readily isomerized to isoroquefortine C photochemically. Lowest energy conformers were established for the most stable tautomers of isoroquefortine C and roquefortine C (Figure 4) using molecular force field (MMFF) molecular mechanics. Equilibrium geometries were then calculated using the Hartree–Fock method with the 6-31G* basis set (HF/6-31G*).[34] Additionally, calculations were performed with the T1 model that was developed to provide accurate heats of formation.[35] The Spartan 06 program was employed.[36]

When comparing the lowest energy tautomer of roquefortine C (**2b**) to isoroquefortine C (**4**), we find that roquefortine C is nearly 17 kJ/mol higher in energy than isoroquefortine C, according to the HF/6-31G* model and 15 kJ/mol higher according to the T1 model (Tables 1 and 3). These energy differences clearly demonstrate the stability of isoroquefortine C over roquefortine C.

(35) T1 is an efficient procedure for calculating heats of formation of uncharged, closed-shell molecules comprising H, C, N, O, F, S, Cl, and Br. It follows the G3(MP2) recipe [Curtiss, L. A.; Redfern, P. C.; Raghavachari, K.; Rassolov, V.; Pople, J. A. *J. Chem. Phys.* **1999**, *110*, 4703.] but substitutes a HF/6-31G* for the MP2/6-31G* geometry, eliminates both the HF/6-31G* frequency and the QCISD(T)/6-31G* energies and approximates the MP2/G3MP2Large energy using dual basis set RI-MP2 techniques. Taken together, these changes reduce computation time by 2–3 orders of magnitude. Atom counts, Mulliken bond orders, and HF/6-31G* and RI-MP2 energies are introduced as variables in a linear regression fit to a set of ~1100 G3(MP2) heats of formation. The T1 procedure reproduces these values with a mean absolute error of 1.8 kJ/mol. It reproduces experimental heats of formation for a set of ~1800 organic molecules from the NIST thermochemical database with a mean absolute error of 8.5 kJ/mol. Heats of formation of flexible molecules have been approximated by the heats of formation of their lowest-energy conformer. This has been identified by examining all conformers for molecules with fewer than 100 conformers and by examining a random sample of 100 conformers for molecules with more than 100 conformers. In terms of both overall error and errors for individual systems, T1 provides a better account of the experimental thermochemistry than any practical quantum chemical method that we have previously examined. The T1 model will be included with the release of the Spartan electronic structure program. A database of ~40000 T1 calculations for both rigid and flexible organic molecules will also be available (scheduled for late Summer 2008).

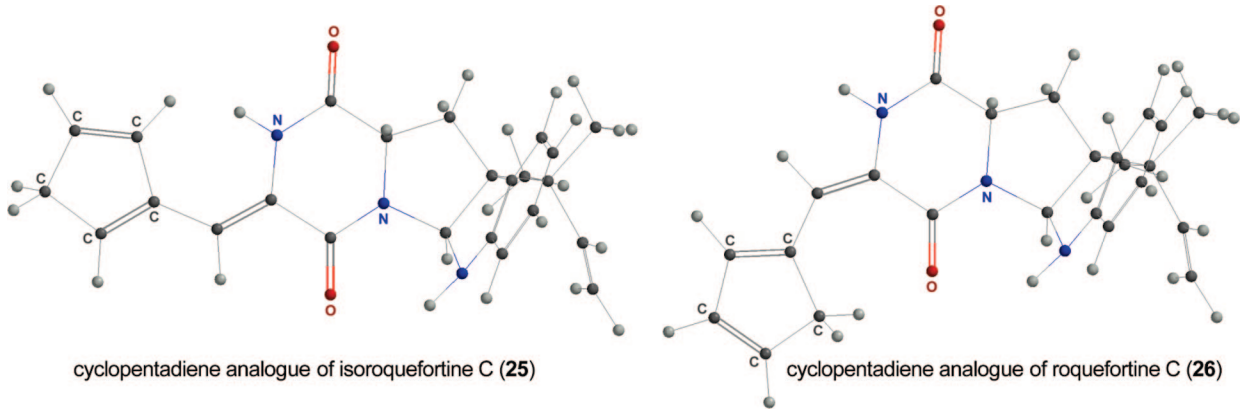


Figure 8. 3-Dimensional structural representations of cyclopentadienyl analogues of isoroquefortine C and roquefortine C (**25** and **26**) based on HF/6-31G* geometry optimizations. The conjugated double bonds in **25** are nearly coplanar while those in **26** have bond angles that deviate from ideal sp^2 geometries. These double bond networks (in both **25** and **26**) involve the exocyclic double bond and the double bond of the Cp ring that it is conjugated to.

Table 1. Relative Energies for Isoroquefortine C (**4**) and for the two Tautomers of Roquefortine C (**2a** and **2b**) Calculated by the Hartree/Fock Method with the 6-31G* Basis Set

isomer	relative energies (kJ/mol)	Boltzmann distribution
4	0.0	>99.5%
2a	32	<0.5%
2b	17	<0.5%

Table 2. Relative Energies for the Cyclopentadiene Analogues (Cp Replaces Imidazole) of Isoroquefortine C (**25**) and Roquefortine C (**26**) Calculated by the Hartree/Fock Method with the 6-31G* Basis Set

isomer	relative energies (kJ/mol)	Boltzmann distribution
25	0.0	>99.5%
26	14	<0.5%

Table 3. Relative Energies for Isoroquefortine C (**4**) and for Tautomer (**2**) of Roquefortine C (**2b**) Calculated by the T1 Method[35]

isomer	relative energies (kJ/mol)	Boltzmann distribution
4	0.0	>99.5%
2b	15	<0.5%

Table 4. Relative Energies for the Cyclopentadiene Analogues (Cp Replaces Imidazole) of Isoroquefortine C (**25**) and Roquefortine C (**26**) Calculated by the T1 Method[35]

isomer	relative energies (kJ/mol)	Boltzmann distribution
25	0.0	98%
26	10	2%

Examination of the equilibrium geometry structures for roquefortine C (**2b**) and isoroquefortine C (**4**) reveals that both compounds adopt conformations with an internal H-bond (Figure 5). In roquefortine C (**2b**), the hydrogen bonding is between a carbonyl oxygen on the diketopiperazine ring and the NH on the imidazole ring. In isoroquefortine C (**4**), the hydrogen bond forms between an NH bond on the diketopiperazine ring and the N on the imidazole ring. This observation suggests that the large calculated energy difference between the two isomers (17 kJ/mol by the HF/6-31G* method and 15 kJ/mol by the T1 method) cannot be explained on the basis of hydrogen-bonding stabilization alone.

Note that both roquefortine C and isoroquefortine C are planar (or nearly so) with respect to the orientation of the diketopiperazine and imidazole rings (Figure 5). Coplanarity is necessary for the exocyclic (*E*/*Z*) double bond and the ring (imidazole and diketopiperazine) double bonds to conjugate. The preference for planar arrangements in roquefortine C and isoroquefortine C was modeled using vinyl imidazole[37] (Figure 6), where, according to the HF/6-31G* model, the barrier to rotation is in the range of 17–23 kJ/mol, depending on the starting tautomer and conformer. This is the same order of magnitude as the difference in energy between roquefortine C (**2b**) and isoroquefortine C (**4**).

The "ring" involving the H-bond in roquefortine C (Figure 5, **2b**) is made up of seven atoms (five sp^2 centers, a nitrogen and the hydrogen atom), while the ring in isoroquefortine C (**4**, Figure 5) is made up of only six atoms (four sp^2 centers, a nitrogen, and a hydrogen). Achieving ideal sp^2 bond angles (120°) will be more difficult for roquefortine C than for isoroquefortine C and the calculated structures are in agreement: roquefortine C has bond angles up to 134° while the largest bond angle calculated for isoroquefortine C is 127°.

We contend that the difference in stability of roquefortine C and isoroquefortine C is primarily due to the difference in the energy penalty required to achieve coplanarity and not to the difference in hydrogen-bond strengths. This view is supported by additional calculations on analogues in which a cyclopentadiene replaces imidazole (Figure 7). While there is no possibility for intramolecular hydrogen bonding in these analogues, the HF/6-31G* energy difference (14 kJ/mol favoring the isoroquefortine C analogue) is nearly the same as in the imidazole systems. The T1 energy difference is 10 kJ/mol. As expected, double bonds in the diketopiperazine and cyclopentadiene rings for **25** are nearly coplanar, and bond angles in the roquefortine analogue **26** are further removed from ideal values (Figure 8).

(36) Spartan 06 is available from Wavefunction, Inc., 18401 Von Karman Avenue, Suite 370, Irvine, CA 92612; www.wavefun.com.

(37) Although tautomer **1** of roquefortine C **2a** does not have the potential to hydrogen bond, its coplanar double bond network (circled atoms in **2a**, Figure 5) also resembles a seven-membered "ring" involving six sp^2 centers and one hydrogen atom. Here too, the bond angles deviate from ideal sp^2 geometries. Since tautomer **2a** does not gain any stabilization from H-bonding as tautomer **2b** does, it has a higher relative energy (34 kJ/mol) than tautomer **2b** (17 kJ/mol) by the HF/6-31G* method.

The computational calculations are summarized in Tables 1−4. The computational studies support the notion that achieving coplanarity of the diketopiperazine and imidazole rings requires greater distortion of the sp^2 centers in roquefortine than in isoroquefortine C. We believe that this is the major cause of the thermodynamic differences in stability between the two isomeric compounds.

Acknowledgment. We thank the NSF (CHEM-0130958) for support of this work.

Supporting Information Available: Experimental procedures, characterization, and spectra for compounds **12–24**, **2**, **4**. This material is available free of charge via the Internet at http://pubs.acs.org. (The coordinates for the 3D structures of roquefortine C, isoroquefortine C, and cyclopentadiene derivatives **25** and **26**, resulting from HF/6-31G* geometry optimizations can be found as pdb files).

JA800067Q