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Structure Revision of Spiroleucettadine, a Sponge Alkaloid with a Bicyclic Core Meager in H-Atoms

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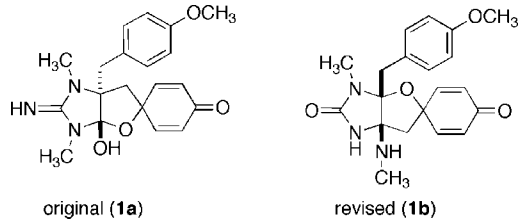
Received May 8, 2008



Our 2004 disclosure of the amino hemiketal-containing spiroleucettadine was met with keen interest by the natural products and synthetic communities. As repeated efforts to synthesize spiroleucettadine failed and questions regarding the original structure elucidation process arose, evidence mounted against the validity of the proposed structure. The low ratio of H/C in the core of spiroleucattadine complicated the original structure elucidation process. Speculation prompted a reisolation of spiroleucettadine from an untouched portion of the original *Luecetta* collection and a thorough analysis of analytical data. In addition, a systematic analysis of candidate structures was performed via density functional theory (DFT) calculations; a favored high scoring structure **1b** was ultimately confirmed to be spiroleucettadine via X-ray analysis of crystalline spiroleucettadine and reinforced the validity of DFT calculations in structure elucidation. We present the revised structure of spiroleucettadine, a bicyclic sponge alkaloid with a scarcity of H-atoms in its core.

Introduction

It has been somewhat surprising to observe the global reaction to our 2004 disclosure of a 2-aminoimidazole containing alkaloid, spiroleucettadine (**1a**).[1] This heterocycle, concluded to possess an uncommon trans-fused imidazolidine−oxalane, must now be revised to structure **1b**, shown in Figure 1, based on the new findings reported herein. The compound was originally isolated from a Fijian *Leucetta* calcareous sponge and we outlined its structural features as being unprecedented. Our report tantalized many laboratories to further explore the properties associated with such a remarkable scaffold. Three nearly simultaneous disclosures by groups in the US, Canada, and Australia each concluded it was not possible to use a ring closure reaction in the synthesis of structure **1a**.[2−4] As an important counter point, the X-ray crystal structure of ergogaline[5] firmly shows that an amino hemiketal embedded in a polycyclic ring system represents a stable moiety.

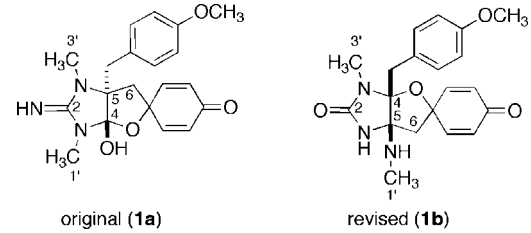


FIGURE 1. Original and revised structure of spiroleucettadine.

Prompted by these synthetic failures, Watson et al. proposed structural alternatives **2** and **3**, shown in Figure 2, and deftly used density functional theory (DFT) calculations to validate their postulate.[4] We were inspired by this accomplishment but were unsettled by the result. While the calculation analysis for

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10.1021/jo800960w CCC: $40.75
Published on Web 10/17/2008

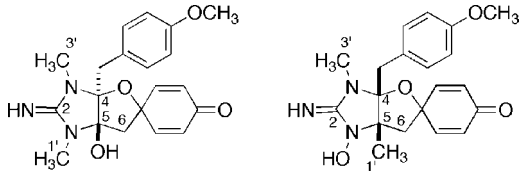

FIGURE 2. Watson's suggested structures **2** and **3**.

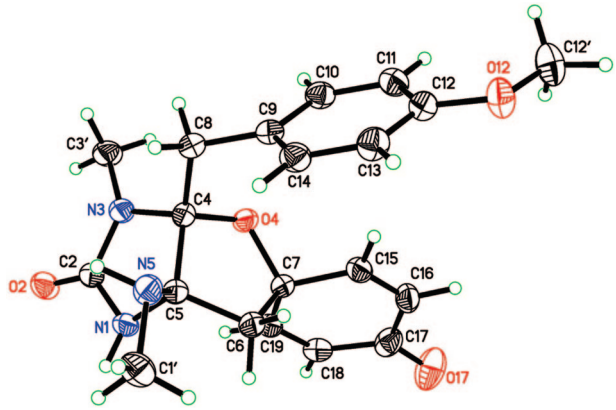

FIGURE 3. X-ray crystal structure of spiroleucettadine.

structure **1a** provided a poor fit to the experimental data, structure **2** was also far from a match. Even though the DFT results for candidate **3** reasonably agreed with the empirical data, in comparison to the large family of *Leucetta* class compounds,[6−8] a C-methyl group did not seem biosynthetically viable. We eventually concluded that our structure elucidation was equivocal and complicated by the circumstance of an unfavorable H/C ratio[9] associated with its bicyclic core, which also contained a plethora of heteroatoms. The results of our further investigation of several alternative structures for spiroleucettadine are outlined below.

Results and Discussion

The biogenetic-based assumption that the three nitrogen atoms of spiroleucettadine ($C_{20}H_{24}N_3O_4$) were associated with a 2-aminoimidazole[10] was an attractive and logical assignment but ultimately proved to be incorrect. We embarked on two parallel approaches to obtain further clarifying data. The first involved a successful reisolation of spiroleucettadine to provide additional authentic compound. Especially fruitful was obtaining a crystalline sample for X-ray analysis, providing the structure **1b**, shown in Figure 3. The second involved the drafting, prior to obtaining the crystal, of 15 additional structural and stereoisomers as candidates (see Figure S7 in the Supporting Inforamtion). These were all evaluated by DFT calculations and the results favored structure **1b**.

In view of the previous errors, more convincing data were sought to further affirm the revised structure **1b** by obtaining a new sample. Our repository contained two distinct morphological types of *Leucetta* sponges that were considered as starting

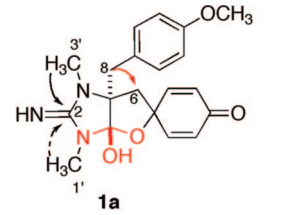

FIGURE 4. Original structure (**1a**) with significant HMBC correlations (CD_3OD).[1]

points for reisolation efforts. One set possessed a lemon yellow color, oval shape, and regular surface (Supporting Information, Figure S1a) versus the other with an atypical highly corrugated surface (Supporting Information, Figure S1b). No collections of the smooth surface type have ever yielded spiroleucettadine, so we focused on an untouched portion (170 g dry weight) of the original sample (coll no. 00111 obtained from Fiji). Extraction with MeOH followed by partitioning of the concentrated extract between dichloromethane and water afforded the crude organic phase (692 mg) used in the next step. Repetitive reversed-phase chromatography afforded 3.6 mg of spiroleucettadine that exhibited ^{1}H and ^{13}C NMR properties in accord with the published values.[1]

The reisolated compound was the starting point for the next step that involved obtaining crystalline material. This began by dissolving 0.9 mg of spiroleucettadine in 300 μL of $CDCl_3$ for a vapor diffusion experiment employing hexanes as the cosolvent. After 2 weeks at −7 °C several very small colorless platelike crystals of spiroleucettadine formed that were suitable for X-ray analysis, but this required using an advanced light source facility. The result of this examination (shown in Figure 3) was that the diffraction pattern obtained was consistent with structure **1b**. Highlights of the crystallographic study are as follows. The compound crystallizes as a network of four hydrogen-bonded molecules, in the centric monoclinic space group $P2_1/c$ (Supporting Inforamtion, Figure S6). Surprisingly, the centrosymmetric nature of the space group indicates the presence of a racemic mixture of molecules. The optical rotation data (in CH_3OH) are relevant and include $[\alpha]_D$ −27.1 of our original sample[1] and $[\alpha]_D$ −5.1 of the new sample. It appears that scalemic mixtures of spiroleucettadine are being isolated. This phenomenon is not unique and bears remarkable similarity to the racemic crystal structure observed for pericosine E.[11] The biosynthetic basis for this observation is unclear yet a number of racemic and scalemic mixtures of natural products exist in the literature.[12−14]

The new 2D NMR data obtained on spiroleucettadine verified a critical inconsistency noted by Watson.[4] The correlation we reported[1] from H-8 to C-6 in Figure 4 for **1a** in methanol-d_4 was actually due to a solvent J_{CH} peak. This embarrassing error cast serious doubt on the validity of the amino hemiketal functional group shown in structure **1a**. Another unsettling element of the original 2D NMR data set was the lack of parity

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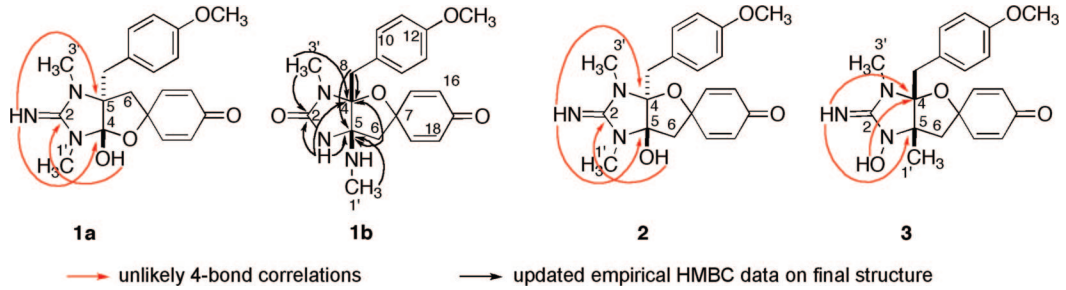

FIGURE 5. Correlation data ($CDCl_3$) from spiroleucettadine on original structure **1a**, final structure of spiroleucettadine **1b**, and alternate structures **2**[4] and **3**.[4]

displayed between the two *N*-methyl groups; the expected correlation from the protons of 3′-*N*-CH_3 to C-2 was observed but none were present from the protons of 1′-*N*-CH_3 to C-2. Finally, the 5,5-trans geometry was suspect as it was based on a very weak $^1H-^1H$ ROESY correlation. At this point it is important to summarize several important insights of Figure 5, based on new NMR data in $CDCl_3$, making it possible to favor structure **1b**. Only one *N*-CH_3 group exhibited coupling to C-2, so the inequality of the two *N*-methyl groups persisted. An important new strong correlation (Supporting Information, Figure S5) from the same heteroproton (either an NH or an OH) to C-2, C-4, and C-5 unambiguously meant that structure **1a** could be ruled out. This new correlation also made Watson's alternate structures **2** and **3** unlikely because it would require 4-bond couplings. In summary, only **1b** was in harmony with the attached heteroproton HMBC data.

During the course of this reinvestigation it became clear that structure elucidation can be backbreaking or inaccurate when the ratio of H/C is less than 1 for a core substructure or an entire molecule. Under such a circumstance the process of assembling partial or total structures becomes tenuous as the NMR data sets will be less useful and other approaches must be used. Faced with this dilemma, we turned to DFT calculations to evaluate original structure **1a** alongside Watson's two alternatives (**2** and **3**), and an additional 13 possibilities (Figure S7 in the Supporting Information). We found it useful to evaluate the degree of fit using two parameters designated as the "mean absolute error (MAE)" and "% Score." The MAE is calculated in a standard fashion[15] while the % Score is defined by the following equation: % Score = [$\sum$ of carbons − $\sum$ error points/$\sum$ of carbons]100. On the basis of extensive DFT evaluations of model compounds, we define acceptable agreement as follows: MAE < 2.2 and % Score > 85. The results for the top three new alternate structures, **1b**, **4**, and **5**, plus that of **1a**, **2**, and **3** are summarized in Figure 6. The very poor match of the rating parameters for **1a** [% Score = 65 and MAE = 4.3] and **2** [% Score = 75 and MAE = 3.6] are in accord with the observations of Watson.[4] The calculation results for **4** are not within our defined acceptable range. While structure **3** provides an acceptable fit, it can be ruled out based on the discussion presented above. The two remaining structures **1b** and **5** provide good matches, but only the former is in harmony with the HMBC data discussed above. Thus, the final list could be pruned to a single candidate structure, **1b**.

The revised structure of spiroleucettadine as **1b** is now secure. The new structural core, comprised of a methylamino-furoimidazolone array, contains an enticing functional group moiety for total synthesis. It is reminiscent of the hydroxy-furoimidazolone array present in the *Agelas*-derived slagenins.[16] The overall pathway taken to amend the original structure of **1a** to **1b** provides some important lessons learned. Caution should be exercised during the structure elucidation or dereplication phase of metabolites having three or more nitrogens isolated from a calcareous sponge. Its compounds may not necessarily possess an amino imidazole residue. More importantly, the outcome of this study underscores the value of total synthesis as a powerful tool to interrogate the possibility that a densely heteroatom-functionalized structure, assigned based solely on NMR and mass spec data, could be equivocal. The value of conducting an X-ray analysis is obvious but such results are not completely error free. Finally, as shown by the analysis of the structures in Figure 6, a powerful approach involves overlaying the DFT results with those obtained by HMBC experiment.

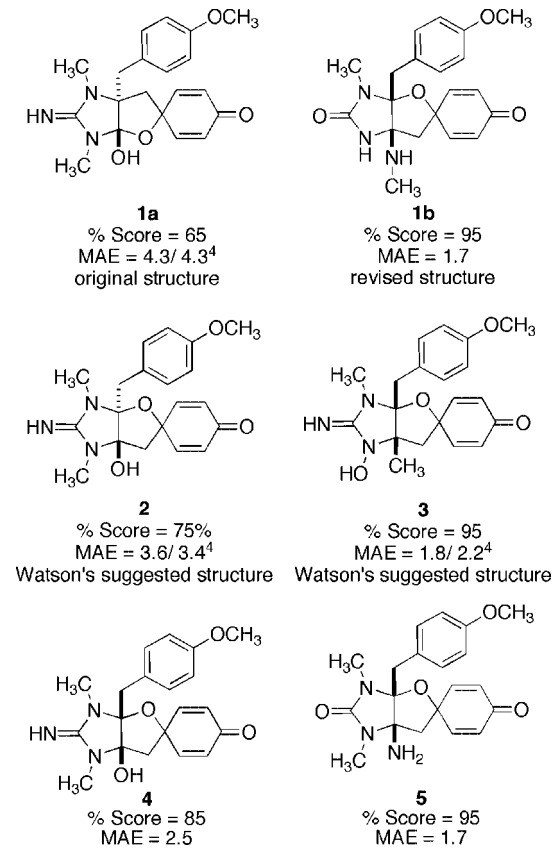

FIGURE 6. DFT calculation results for spiroleucettadine (**1b**) and other candidate structures.

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Experimental Section

Biological Material, Collection, and Identification. Specimens of *Leucetta* (coll. no. 00111) (6.2 kg, wet weight) were collected with use of scuba in 2000 from Caesar's Rock, Fiji. Taxonomic identification was based on comparison of the biological features to other samples in our repository. Voucher specimens and underwater photos are available.

Extraction and Reisolation of Spiroleucettadine (1b). The sponge (170 g, dry weight) was extracted with MeOH followed by partitioning of the concentrated extract between dichloromethane and water. The crude organic phase (692 mg) was purified via reversed-phase HPLC to afford **1b** (3.6 mg).

Spiroleucettadine (1b). ^{1}H and ^{13}C NMR properties (see the Supporting Information, Figures S2−S5 and Table S1) were in accord with the published values.[1] The NMR data recorded in $CDCl_3$ are reported in Table S1 in the Supporting Information. $[\alpha]^{23}_{D}$ −5.1 (*c* 0.56 in MeOH). HRESIMS *m*/*z* $[M + H]^+$ 370.1758 (calcd for $C_{20}H_{24}N_3O_4$ 370.1761). Crystals were too small to allow for a melting point to be determined.

Density Functional Theory Calculations. Carbon chemical shifts predictions were performed by ChemNMR (version 8.0), ACD (version 8.0), Spartan 06, and Gaussian 03. The PC computer used for Spartan 06 possessed an Athlon 64 × 2 5600+ (2.80 GHz, 1MB × 2) processor and 4 GB DDR2 SDRAM at 667 MHz with 222GB disk space. Gaussian 03 was run on a Unix machine with 8 GB RAM with 72 GB disk space. The Spartan calculations were performed by the basis set, B3LYP/6-31G*//B3LYP/6-31G*. Other basis sets described in Table S1 in the Supporting Information were performed by Gaussian 03. Tables S2−S17 in the Supporting Information provide detailed results for calculated ^{13}C vs experimental shifts. The calculation results for 16 spiroleucettadine candidate structures (Figure S7 and Tables S2−S17 in the Supporting Information) and the seven diastereomers of **4** (Figure S9) are given in the Supporting Information.

Crystallization Method. Spiroleucettadine (0.9 mg) was dissolved in 300 μL of $CDCl_3$ for a vapor diffusion experiment employing hexanes as the cosolvent. After two weeks at −7 °C several colorless plate-like crystals of spiroleucettadine formed that were suitable for synchrotron crystallographic analysis.

Acknowledgment. This work is supported by grants from the National Institutes of Health (RO1 CA 47135) and National Science Foundation (NSF-CHE-0342912). Samples for synchrotron crystallographic analysis were submitted through the SCrALS (Service Crystallography at Advanced Light Source) program. Crystallographic data were collected at Beamline 11.3.1 at the Advanced Light Source (ALS), Lawrence Berkeley National Laboratory. The ALS is supported by the U.S. Department of Energy, Office of Energy Sciences, under contract DE-AC03-76SF00098. Additional support for crystallographic data comes from the NSF MRI grant CHE-05-21569.

Supporting Information Available: Underwater and above water photos, 1D and 2D NMR data, DFT calculation data, and crystallographic information. This material is available free of charge via the Internet at http://pubs.acs.org.

JO800960W