Title

Reconsidering the Structure of Serlyticin-A

Journal

Journal of Natural Products, 82(12)

ISSN

0163-3864

Authors

Tsui, Ka Yi

Tombari, Robert J

Olson, David E

et al.

Publication Date

2019-12-27

DOI

10.1021/acs.jnatprod.9b00859

Peer reviewed

HHS Public Access

Author manuscript

J Nat Prod. Author manuscript; available in PMC 2020 December 27.

Published in final edited form as:

J Nat Prod. 2019 December 27; 82(12): 3464–3468. doi:10.1021/acs.jnatprod.9b00859.

Reconsidering the Structure of Serlyticin-A

Ka Yi Tsui, **Robert J. Tombari**, **David E. Olson***, **Dean J. Tantillo***

Department of chemistry, University of California – Davis, 1 Shied Ave, Davis, CA 95616

Abstract

Serlyticin-A is a secondary metabolite first isolated from a culture of *Serratia ureilytica* grown using squid pen as the sole carbon/nitrogen source. A previous study by Kuo *et al.* demonstrated that it has antioxidative and antiproliferative properties. However, the proposed chemical structure of serlyticin-A is likely incorrect based on the thermodynamic instability of its three contiguous heteroatom-heteroatom bonds. Here, we use quantum chemical calculations to predict ^{1}H and ^{13}C chemical shifts for serlyticin-A, and demonstrate a discrepancy between the calculated and experimental chemical shifts. We then propose several reasonable alternative structures for serlyticin-A. Considering the known antioxidant and antiproliferative activity of hydroxamic acids as well as their stability and prevalence in natural products of bacterial origin, we believe that serlyticin-A is most likely 3-indolylacetohydroxamic acid (**4**). We provide our rationale for this assignment as well as experimental data for pure 3-indolylacetohydroxamic acid obtained via de novo synthesis. This study highlights the power of computational NMR shift prediction to revise chemical structures for natural products like serlyticin-A.

Graphical Abstract

* **Corresponding Author**: djtantillo@ucdavis.edu, deolson@ucdavis.edu.

Author Contributions

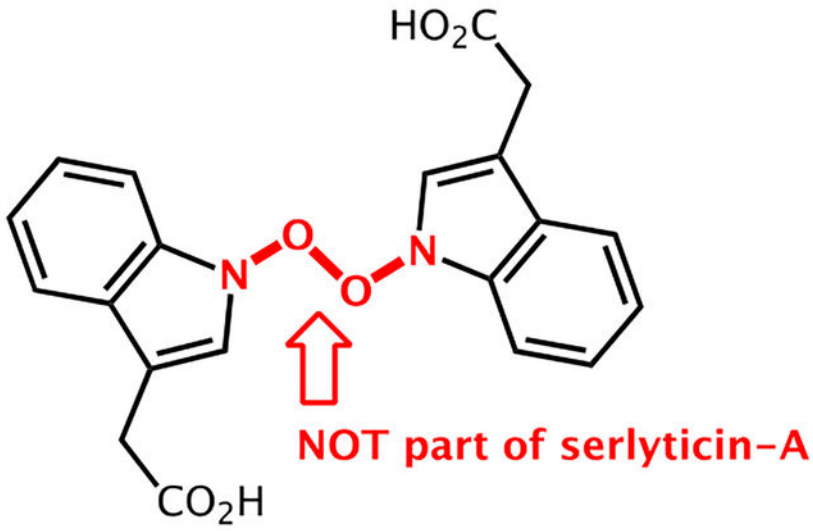
K.Y.T. performed the computational analysis and R.J.T performed the synthesis and experimental characterization, both with guidance from D.E.O. and D.J.T. All authors contributed to writing the paper.

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Details of quantum chemical calculations and characterization data for **4** including ^{1}H and ^{13}C NMR spectra (PDF).

The author declare no conflicts of interest.



The identification of a novel natural product with antioxidant properties is highly beneficial to both the food and pharmaceutical industries. Previous efforts in this area have utilized a variety of organisms with plants being a major source of antioxidant compounds.[1–4] Despite the fact that shellfish waste is a rich source of phenolic antioxidants, there have been relatively few attempts to isolate novel antioxidant natural products from this source.[5,6] In 2012, serlyticin-A (**1**, Figure 1) was identified by Kuo and co-workers as one of the compounds in the *s. ureilytica* culture using squid pen waste as carbon/nitrogen source, and they demonstrated that it has antioxidative and antiproliferative properties.[5] While serlyticin-A certainly has the potential for medicinal and food science applications, we suspect that the reported structure is incorrect for several reasons. First, the structure contains an O–O bond, which, based on the results of our calculations, would be prone to cleavage in solution. Second, the characterization data reported for serlyticin-A, including 1D and 2D NMR, mass spectrometry, UV-vis, and IR, are consistent with multiple potential structures. For example, 1D and 2D NMR data confirm the presence of an indole moiety but do not necessarily support the assignment of the proposed N–O–O–N substructure. Moreover, the reported mass spectrometry data do not match the exact mass of **1**. While select tabulated numerical data were provided in the original report, the actual spectra were not.[7] These factors coupled with the lack of X-ray crystallography data contribute to the ambiguity in the assignment of structure **1** to serlyticin-A. While it is an accepted practice to elucidate chemical structures relying on information obtained from NMR, IR, and UV-Vis, it is not uncommon for structures to be misassigned, leading to the otherwise unnecessary expenditure of additional resources in correcting them.[8–10] Here, we report the use of quantum chemical calculations of NMR chemical shifts[11–13] to demonstrate that the original structure of serlyticin-A may have been misassigned, and we provided several plausible alternative structures.

RESULT AND DISCUSSION

Our investigations began with the structural optimization of **1**. We observed a long O–O bond (~2.10 Å) in all relevant conformers of **1** optimized in the gas phase and solution

(Figure 2). Computed Wiberg bond orders for the O–O bond were less than 0.32 for all conformers within 3 kcal/mol of the lowest energy conformer, suggesting that **1** is prone to fragment in both gas phase and solution. Optimization of these conformers while constraining the O–O bond to 1.48 Å (a reasonable distance for a covalent O–O bond) led to structures that are at least 15 kcal/mol higher in free energy than their unconstrained versions. Additionally, the calculated ^{13}C and ^{1}H NMR shifts for **1** deviate greatly from the reported NMR shifts (Table 1, Figure 2, and Table S1). For the constrained structure of **1**, although the ^{13}C shifts are close to the experimental shifts, several ^{1}H shifts deviate greatly from the experimental shifts (Figure 2 and Table S1). Given the accuracy of such calculations,[11–13] serlyticin-A is likely misassigned.

Kuo *et al.* reported ^{1}H-^{1}H COSY (correlation spectroscopy) and HMQC (heteronuclear multiple bond coherence) correlations, that are consistent with serlyticin-A containing an indole-3-acetic acid moiety. This led us to consider structural alternative structures possessing this group. Our predicted ^{13}C chemical shifts for the indole-3-acetic acid dimer (Figure 2 and Table S1) matched the experimental shifts very well with only one calculated ^{13}C chemical shift (C1) falling outside the acceptable range. Therefore, derivatives of this structure were examined further. Such structures included hydrazine, **2**, hydroxylamine **3**, and hydroxamic acid **4** (Figure 3). All these structures have computed chemical shifts that are consistent with the reported shifts for serlyticin-A, though **4** is the closest match (Table 1, Figures 3–4 and Table S3). Additional structures considered that were not good matches are described in the SI (Figure S1, Tables S5 and S6). Serlyticin-A has demonstrated antioxidant properties in a DPPH radical scavenging assay.[5] Compounds **2–4** all have the potential to serve as effective radical scavengers due to the presence of N–O or N–N bonds. However, we deemed **4** to be the most likely structure of serlyticin-A for several reasons. First, to the best of our knowledge, *N*-amino indole natural products have not previously been reported. Second, while *N*-hydroxy indole natural products are known, these are rare and often difficult to isolate.[14] Examples of notable *N*-hydroxyindole-containing natural products include stephacidin B, versicoamide F, nocathiacin-I, thiazomycin, coproverdine, notoamide G, and *N*-hydroxy-β-carbolines.[15–23] The overwhelming majority of *N*-hydroxyindole-containing natural products possess substitution at the 2-position of the indole, likely a consequence of the tendency of indoles to be oxidized at C2. Finally, **4** contains a hydroxamic acid group—a structural motif that is found in numerous natural products of bacterial origin.[24–27] Hydroxamic acids are excellent siderophores and bacteria often produce them to sequester iron from their environment. In fact, **4** itself has been shown to coordinate metals.[28] Furthermore, many hydroxamic acids possess anti-proliferative properties, like that observed for serlyticin-A, due to their ability to potently inhibit histone deacetylases (HDACs).[29,30] Next, we synthesized **4** from the methyl ester of indole acetic acid by converting the ester into the hydroxamic acid.[31] We have characterized the product and the data agree with that available from previous studies (see SI for details).[32,33] The ^{1}H and ^{13}C NMR chemical shifts for synthesized **4** agree with the calculated shifts (Table 1 and Figure 4; see SI for additional detail). While Kuo *et al.* described serlyticin-A as a yellow powder with IR absorption bands of 3366 cm^{-1} and 1713 cm^{-1} corresponding to a hydroxy group and carbonyl moiety, respectively, we found compound **4** to be a tan powder with IR absorption bands at 3422, 3218, 1636, and 740 cm^{-1}. Unfortunately, given the lack of raw

characterization data in the original report,[5] we cannot be sure if these differences are meaningful. Kuo and co-workers reported UV absorption bands (solvent not indicated) for serlyticin-A at 244, 261, and 299 nm, implying that the molecule could contain an indole moiety. We analyzed **4** by UV-Vis absorption in MeOH and found absorption bands at 218, 230, and 280 nm, a reasonable match to the reported values assuming different solvents were used. Structure **1**, with the molecular formula $C_{20}H_{16}N_2O_6$, has a calculated exact mass of 380.1008. The reported ESI-MS $[m + H]^+$ *m/z* was 381.2629, a difference of > 0.15 from the calculated m/z of **1** $[m + H]^+$. We suspect that the mass reported by Kuo and co-workers reflected a noncovalent dimer of the natural product. This type of dimerization is commonly detected in ESI-MS,[34,35] and would be anticipated for a hydroxamic acid such as **4**. The dimer of **4** has a calculated *m/z* $[2m + H]^+$ of 381.1563, which is closer to the reported mass for serlyticin-A than the calculated *m/z* for **1**. While our liquid chromatography mass spectrometry (LC-MS) analysis of **4** did not identify a dimer, this is not unexpected, as different instruments are known to yield different ionization patterns for the same molecule.[36]

Overall, the chemical and biological data reported for serlyticin-A[5] are consistent with a compound such as **4**, but comparison with an authentic sample will be necessary to confirm this hypothesis.[7] Regardless, the variety of factors described above make it highly unlikely that **1** is the correct structure of serlyticin-A. In the field of organic chemistry, structural misassignments are obviously problematic.[8] Many such misassignments can be avoided through judicious application of NMR chemical shifts calculations.[9–10,11–13] Here, we have demonstrated that the originally proposed structure for serlyticin-A is likely incorrect, a conclusion based on (1) computations that point to the likelihood that the central O–O bond in the proposed structure is weak, (2) deviations between the reported and calculated chemical shifts for **1**, and (3) the unprecedented nature of such an *N*-oxidized indole natural product lacking substitution at C2. Furthermore, the high prevalence and known antioxidant/antiproliferative properties of naturally occurring hydroxamic acids is consistent with our proposal that serlyticin-A is likely 3-indolylacetohydroxamic acid (**4**) or a closely related compound.

EXPERIMENTAL SECTION

Computational Methods.

Quantum chemical calculations were performed using *Gaussian09*.[37] Structural optimizations and frequency calculations were performed with both B3LYP/6–31+G(d,p)[38] in the gas phase and PCM(MeOH)-B3LYP/6–31+G(d,p).[39,40] Both sets of optimized structures were subjected to NMR calculations using the gauge-including atomic orbital (GIAO) method.[12] Results from both approaches are consistent with each other. Shown in the text are the results from NMR calculations on optimizations performed with PCM(MeOH); for gas phase results, please see Supporting Information (SI). Since the NMR experiments reported by Kuo and co-workers were performed on a sample dissolved in deuterated methanol, our ^{13}C and ^{1}H shifts were calculated with PCM(MeOH)-mPW1PW91/6–311+G(2d,p).[41] Chemical shifts were linearly scaled using scaling factors obtained from cheshirenmr.info (slope = −1.0754 for ^{1}H and −1.0399 for ^{13}C; intercept =

31.8463 for ^{1}H and 186.5993 for ^{13}C).[11,42] In order to sufficiently sample conformational space, multiple systematic conformational search runs were performed on each compound using *Spartan10*.[43] These conformational search runs used molecular mechanics, explicitly the Merck Molecular Force Field (MMFF). However, all resulting conformers were subjected to single point calculations at the B3LYP/6–31+G(d,p) level. Then, geometric optimizations were performed on conformers within 5 kcal/mol of the lowest energy conformer, with and without implicit solvent (i.e., PCM(MeOH)). NMR calculations were only performed on the optimized conformers within 3 kcal/mol of the lowest energy conformer. The geometries of all conformers for each structure were confirmed to be minima (no imaginary frequencies). The chemical shifts of these contributing conformers were averaged using Boltzmann-weighting. This procedure is well precedented for computational NMR studies.[11–13] In addition, an optimization constraining the fragmentable O–O bond of the originally proposed structure for serlyticin-A, **1** (Figure 1), was performed to assess its thermodynamic stability. Natural Bond Orbital (NBO)[44] analysis calculations were performed on all contributing conformers of **1** to obtain Wiberg bond orders.[45]

General Experimental Procedures.

All reagents were obtained commercially unless otherwise noted. Reactions were performed using glassware that was oven dried (120oC) unless otherwise stated. Air- and moisture-sensitive liquids and solutions were transferred via syringe. Organic solutions were concentrated under reduced pressure (~5 Torr) by rotary evaporation. Solvents were purified by passage under 12 psi N2 through activated alumina columns. Chromatography was performed using Fisher Chemical™ Silica Gel Sorbent (230–400 Mesh, Grade 60). Compounds purified by chromatography were typically applied to the adsorbent bed using the indicated solvent conditions with a minimum amount of added dichloromethane as needed for solubility. Thin layer chromatography (TLC) was performed on Merck silica gel 60 F254 plates (250 μm). Visualization of the developed chromatogram was accomplished by fluorescence quenching or by staining with butanolic ninhydrin, or aqueous ceric ammonium molybdate (CAM).

Nuclear magnetic resonance (NMR) spectra were acquired on either a Bruker 400 operating at 400 and 100 MHz for 1H and 13C, respectively, and are referenced internally according to residual solvent signals. Data for 1H NMR are recorded as follows: chemical shift (δ, ppm), multiplicity (s, singlet; br s, broad singlet; d, doublet; t, triplet; q, quartet; quint, quintet; sext, sextet; m, multiplet), integration, and coupling constant (Hz). Data for 13C NMR are reported in terms of chemical shift (δ, ppm). Infrared spectra were recorded using a Thermo Scientific Nicolet iS10 spectrometer with Smart iTX Accessory (diamond ATR) and are reported in frequency of absorption. Low-resolution mass spectra were obtained using a Waters Acuity Arc LC-MS.

Synthesis of Methyl 2-(1H-indol-3-yl)acetate.

To a solution of indole acetic acid (0.400 g, 2.28 mmol, 1.0 equiv) in MeOH (22.8 mL, 0.1 M) at room temperature was added concentrated hydrochloric acid (0.76 mL, 9.14 mmol, 4.0 equiv). The mixture was stirred overnight at 65 °C, cooled to room temperature, and then concentrated under reduced pressure. 100 mL of saturated NaHCO3 was added and then

extracted with Et2O (3 × 100 mL). The combined organic extracts were washed with brine (1 × 150 mL), dried over Na2SO4, and concentrated under reduced pressure to yield a brown oil (0.410 g, 95%). 1H NMR (CD3OD, 400 MHz) δ 7.51 (d, 1H, J = 8.0 Hz), 7.33 (d, 1H, J = 8.0 Hz), 7.14 (s, 1H), 7.10 (t, 1H, J = 7.5 Hz), 7.01 (t, 1H, J = 7.5 Hz), 3.75 (s, 2H), 3.66 (s, 3H) ppm; 13C NMR (CD3OD, 100 MHz) δ 174.81, 139.98, 128.54, 124.64, 122.47, 119.88, 119.34, 112.25, 108.50, 52.32, 31.83 ppm; IR (Smart iTX Diamond) 3404, 2952, 2683, 1719 cm-1; LC-MS (ES+) calcd for C11H11NO2 [M + H] 190.08 found 190.23.

Synthesis of *N*-hydroxy-2-(1H-indol-3-yl)acetamide.

To a solution of methyl 2-(1H-indol-3-yl)acetate (0.062 g, 0.33 mmol, 1.0 equiv) and NaOH (0.066 g, 1.64 mmol, 5.0 equiv) in (1:1) MeOH/THF (1.3 mL, 0.25 M) at room temperature was added 50% aqueous hydroxylamine (0.65 mL, 10.49 mmol, 32.0 equiv). The mixture was stirred for 4.5 h at room temperature, and then concentrated under reduced pressure. 10 mL of 2 M HCl(aq) was added and the solution was extracted with (3 × 20 mL) EtOAc. The combined organic extracts were washed with brine (1 × 20 mL), dried over Na2SO4, and concentrated under reduced pressure to yield a tan solid. (0.061 g, 98%). 1H NMR (CD3OD, 400 MHz) δ 7.57 (d, 1H, J = 8.0 Hz), 7.33 (d, 1H, J = 8.0 Hz), 7.17 (s, 1H), 7.09 (t, 1H, J = 7.4 Hz), 7.01 (t, 1H, J = 7.4 Hz), 3.57 (s, 2H) ppm; 13C NMR (CD3OD, 100 MHz) δ 171.66, 138.07, 128.49, 124.77, 122.50, 119.87, 119.35, 112.26, 108.88, 30.92 ppm; IR (Smart iTX Diamond) 3422, 3218, 3057, 1636, 1544, 1081, 740 cm-1; LC-MS (ES+) calcd for C10H10N2O2 [M + H] 191.07 found 191.29.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

We gratefully acknowledge computational support from NSF XSEDE program. R.J.T. is supported by NIH Training Grant T32GM113770.

REFERENCES AND NOTES

(1). (a)Chang SS; Ostric-Matijasevic B; Hsieh OL; Huang CL J. Food Sci 1977, 42, 1102–1106. (b)Inatani R; Nakatani N; Fuwa H; Seto H Agric. Biol. Chem 1982, 46, 1661–1666.(c)Cuvelier ME; Berset C; Richard H J. Agric. Food Chem 1994, 42, 665–669.

(2). Farombi EO; Britton G; Emerole GO Food Res. Int 2000, 33, 493–499.

(3). Canadanovic-Brunet JM; Djilas SM; Cetkovic GS; Tumbas VT; Mandic AI; Canadanovic VM Int. J. Food Sci.Technol 2006, 41, 667–673

(4). Diouf PN; Stevanovic T; Cloutier A Food Chem 2009, 113, 897–902.

(5). Kuo Y-H; Hsu H-C; Chen Y-C; Liang T-W; Wang S-L J. Agric. Food Chem 2012, 60, 9043–9047. [PubMed: 22897632]

(6). Seymour TA; Li SJ; Morrissey MT J. Agric. Food Chem 1996, 44, 682–685.

(7). Attempts to contact the authors of ref. 5 requesting an authentic sample and/or original spectra went unanswered.

(8). Nicolaou KC; Snyder SA Angew. Chem. Int. Ed 2005, 44, 1012–1044.

(9). Elyashberg M; Blinov K; Molodtsov S; Smurnyy Y; Williams AJ; Churanova T J. Cheminformatics. 2009, 1:3.

(10). Elyashberg M; Williams AJ; Blinov K Nat. Prod. Rep 2010, 27, 1296–1328 [PubMed: 20480119]

(11). Lodewyk MW; Siebert MR; Tantillo DJ Chem. Rev 2012, 112, 1839–1862. [PubMed: 22091891]

(12). Grimblat N; Sarotti AM Chem. Eur. J 2016, 22, 12246–12261. [PubMed: 27405775]

(13). Willoughby PH; Jansma MJ; Hoye TR Nat. Protoc 2014, 9, 643–660. [PubMed: 24556787]

(14). Somei M Adv. Heterocycl. Chem 2002 82, 101–155.

(15). Qian-Cutrone J; Huang S; Shu YZ.; Vyas D; Fairchild C; Menendez A; Krampitz K; Dalterio R; Klohr SE; Gao Q J. Am. Chem. Soc 2002, 124, 14556–14557. [PubMed: 12465964]

(16). Liu L; Wang L; Bao L; Ren J; Bahadur Basnet B; Liu R; He L; Han J; Yin WB; Liu H Versicoamides F-H Org Lett 2017, 19, 942–945. [PubMed: 28181808]

(17). Singh SB; Herath K; Yu NX; Walker AA; Connors N Tetrahedron. Let 2008, 49, 6265–6268.

(18). Zhang C; Herath K; Jayasuriya H; Ondeyka JG; Zink DL; Occi J; Birdsall G; Venugopal J; Ushio M; Burgess B; Masurekar P; Barrett JF; Singh SB J. Nat. Prod 2009, 72, 841–847. [PubMed: 19334707]

(19). Urban S; Blunt JW; Munro MH J. Nat. Prod 2002, 65, 1371–1373. [PubMed: 12350170]

(20). Tsukamoto S; Kato H; Samizo M; Nojiri Y; Onuki H; Hirota H; Ohta T J. Nat. Prod 2008, 71, 2064–2067. [PubMed: 19053517]

(21). Susanna TS; Chan A; Pearce N; Page MJ; Kaiser M; Copp BR J. Nat. Prod 2011, 74, 1972–1979. [PubMed: 21846091]

(22). Santos AK; Machado LL; Bizerra AM; Monte FJ; Santiago GM; Braz-Filho R; Lemos TL Nat. Prod. Commun 2012, 7, 729–730. [PubMed: 22816293]

(23). Costa EV; Pinheiro ML; Xavier CM; Silva JR; Amaral AC; Souza AD; Barison A; Campos FR; Ferreira AG; Machado GM; Leon LL J. Nat. Prod 2006, 69, 292–294. [PubMed: 16499336]

(24). Neilands JB Science. 1967, 156, 1443–1447. [PubMed: 4304945]

(25). Challis GL Microbiology. 2008, 154, 1555–1569. [PubMed: 18524911]

(26). Miller MJ Chem. Rev 1989, 89, 1563–1579.

(27). Tsuji N; Kobayashi M; Nagashima K; Wakisaka Y; Koizumi K J. Antibiot 1974, 49, 14691.

(28). Arrabal MJ; González PV; Gámez CC; Misiego AS; de la Peña AM Analyst, 1994, 119, 1537–1540.

(29). Marks PA; Richon VM; Rifkind RA J. Natl. Cancer. Inst 2000, 92, 1210–1216. [PubMed: 10922406]

(30). Marks PA Expert Opin. Investig. Drugs 2010, 19, 1049–1066.

(31). Holson E and Olson D U.S. Patent 9745613B2, 2017.

(32). Cohen W and Erlanger BF J. Am. Chem. Soc 1960, 82, 3928–3934.

(33). Boularot A; Giglione C; Petit S; Duroc C; Alves de Sousa R; Larue C; Cresteil T; Daredel F; Artaud I; Meinnel T. J. Med. Chem 2007, 50, 10–20. [PubMed: 17201406] In this report, NMR were taken in DMSO rather than chloroform, only a carbonyl peak was reported in IR (1638 cm^{-1}), no UV data was reported, and elemental analysis was reported instead of MS data.

(34). Pan H Rapid Commun. Mass Spectrom 2008, 22, 3555–3560. [PubMed: 18853406]

(35). Ding J; Anderegg RJ J. Am. Soc. Mass Spectrom 1995, 6, 159. [PubMed: 24214113]

(36). Jiang H; Somogyi A; Timmermann BN; Gang David. R. Rapid Commun. Mass Spectrom 2006, 20, 3089–3100. [PubMed: 16991102]

(37). Frisch MJ; Trucks GW; Schlegel HB; Scuseria GE; Robb MA; Cheeseman JR; Scalmani G; Barone V; Mennucci B; Petersson GA; et al. Gaussian 09, Revision D. 01; Gaussian, Inc.: Wallingford, CT, USA, 2010.

(38). (a)Becke AD J. Chem. Phys 1993, 98, 5648–5652.(b)Lee C; Yang W; Parr RG Phys. Rev. B: Condens. Matter Mater. Phys 1988, 37, 785–789.(c)Miehlich B; Savin A; Stoll H; Preuss H Chem. Phys. Lett 1989, 157, 200–206.

(39). (a)Cammi R; Mennucci B; Tomasi J Chem. Rev 2005, 105, 2999–3093 [PubMed: 16092826] (b)Leszczynski J Ed.; World Scientific Publishing Co. Pte. Ltd.: Singapore, 2003; Vol. 8.

(40). Cheeseman JR; Trucks GW; Keith TA; Frisch MJ J. Chem. Phys 1996, 104, 5497–5509.

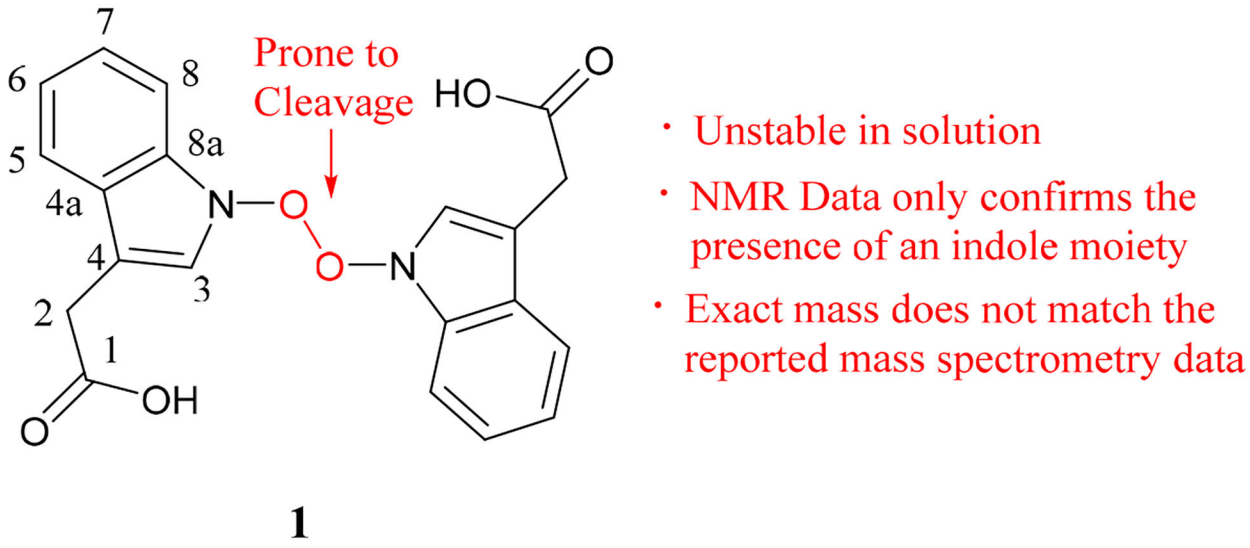
(41). Adamo C; Barone V J. Chem. Phys 1998, 108, 664–675.

(42). Scaling factors were obtained from chesirenmr.info.

(43). Spartan, Version10 (Wavefunction, Ind.: Irvine, CA, USA, 2010).

(44). NBO 7.0. Glendening ED, Badenhoop J,K, Reed AE, Carpenter JE, Bohmann JA, Morales CM, Karafiloglou P, Landis CR, and Weinhold F, Theoretical Chemistry Institute, University of Wisconsin, Madison (2018).

(45). (a)Reed AE; Weinstock RB; Weinhold F J. Chem. Phys 1985, 83, 735–746.(b)Glendening DE; Weinhold F, F. J. Comp. Chem 1998, 19, 610–627.(c)Weinhold F J. Comp. Chem 2012 33, 2363–2379 [PubMed: 22837029]



1

Figure 1.
The previously proposed structure of serlyticin-A.

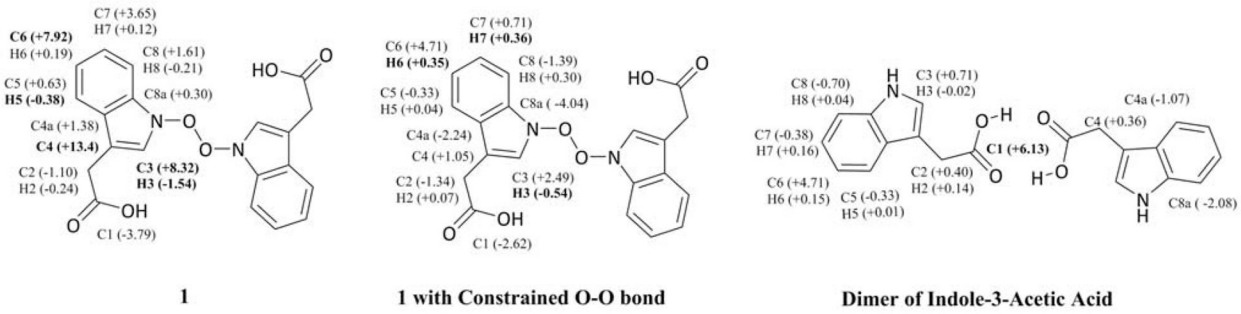


Figure 2.

Deviations between calculated ^{13}C and ^{1}H chemical shifts and the reported experimental shifts for **1**, constrained **1**, and a dimer of indole-3-acetic acid. Deviations of less than 6 ppm for ^{13}C and less than 0.3 ppm for ^{1}H shifts are considered acceptable.[13–15] Deviations exceeding these limits are bolded.

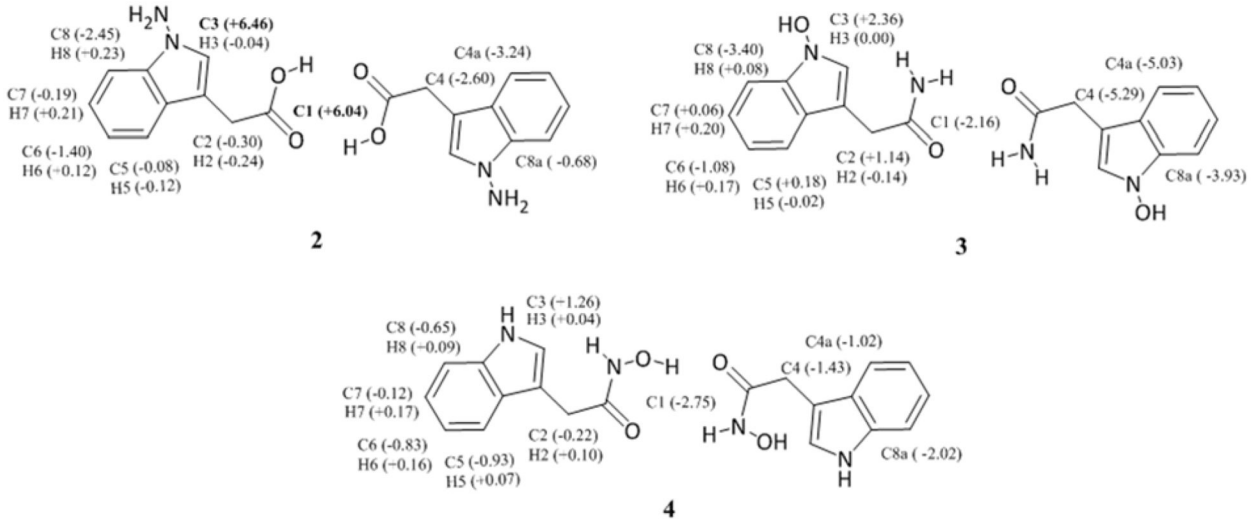


Figure 3.

Alternative structures examined. Deviations of less than 6 ppm and less than 0.3 ppm between experimental and computed ^{13}C and ^{1}H shifts, respectively, are considered acceptable.[13–15] Deviations exceeding these limits are bolded. For unsymmetrical dimers, shifts for the two monomers were averaged.

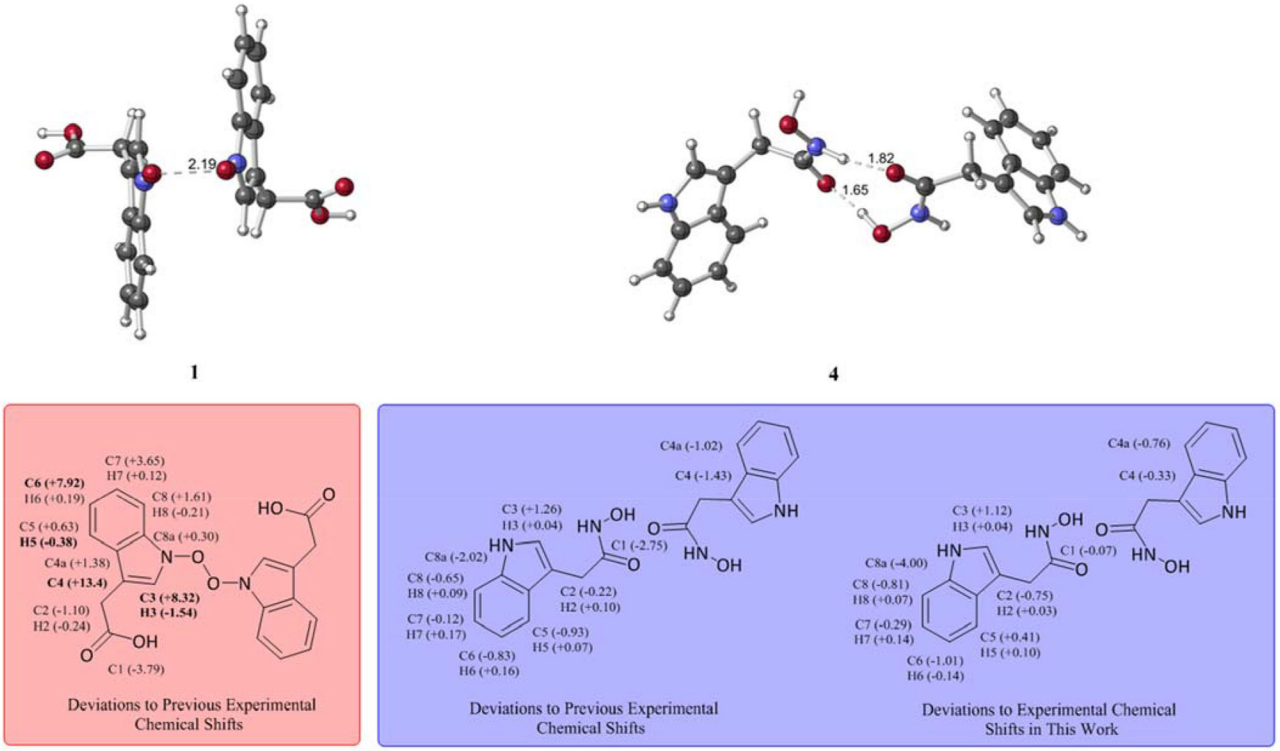


Figure 4.

Summary of NMR results. Deviations between calculated chemical shifts of **1** and its reported experimental shifts (left). Deviations between calculated chemical shifts of **4** and the previously reported experimental shifts for serlyticin-A (center) and newly determined experimental shifts for **4** (right). Deviations of less than 6 ppm for ^{13}C and less than 0.3 ppm for ^{1}H shifts are considered acceptable.[13–15] Deviations exceeding these limits are bolded. For unsymmetrical dimers, shifts for the two monomers were averaged.

Table 1.

Summary of Computed NMR Results for **1**–**4**

Atom Label	Exp. δ (p.p.m)	1	2	3	4
C1	177.5	173.71	**183.54**	175.33	174.74
C2	32.8	31.70	32.50	33.94	32.58
C3	124.5	132.82	**130.96**	126.86	125.76
C4	109.6	**123.00**	107.00	104.31	108.17
C4a	128.8	**130.18**	125.56	123.76	127.78
C5	119.5	120.13	119.4	119.68	119.75
C6	119.7	**127.62**	118.30	118.62	118.87
C7	122.3	125.95	122.11	122.36	122.18
C8	112.1	113.71	109.64	108.70	111.45
C8a	138.0	138.30	137.32	134.07	135.98
MAD		4.21	2.34	2.46	1.07
H2	3.68	3.44	3.44	3.54	3.78
H3	7.14	**5.60**	7.10	7.14	7.18
H5	7.54	**7.15**	7.42	7.52	7.61
H6	6.99	7.18	7.11	7.16	7.15
H7	7.07	7.19	7.28	7.27	7.24
H8	7.33	7.12	7.56	7.41	7.40
MAD		0.45	0.16	0.10	0.10

[a]Abbreviation: MAD, mean absolute deviation. Deviations of less than 6 ppm for 13C and less than 0.3 ppm for H shifts are considered acceptable.9−11 Chemical shifts exceeding the accepted deviations are bolded.