Chemical stability of petrichorins

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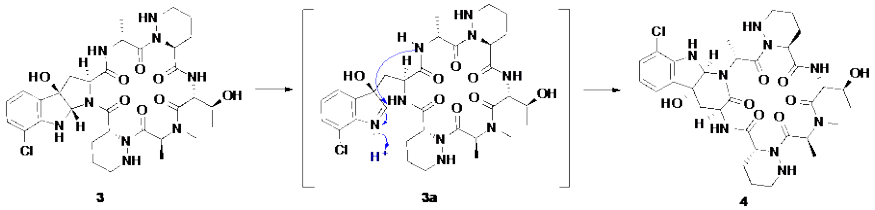

ABSTRACT: The structure of petrichorin C1 (**4**) converted from petrichorin C (**3**) was determined by using NMR and x-ray. The chemical stability of petrichorins A and C (**1** and **3**) was investigated by NMR, X-ray and calculations.

In our recent efforts to discover unique biologically active compounds from underexploited rare actinomycetes, we identified three piperazyl cyclopeptides petrichorins A-C (**1**-**3**) (Fig. 1) from *Lentzea flaviverrucosa* DSM 44664.[1] These compounds, especially petrichorin A (**1**), showed potent anti-proliferative activity with IC_{50} values at the nanomolar level. Petrichorin A (**1**) is an unsymmetrical heterodimer, and petrichorin B (**2**) is a symmetrical homodimer, while petrichorin C (**3**) is a hexaminoacid monomer comprising one half of **1**.

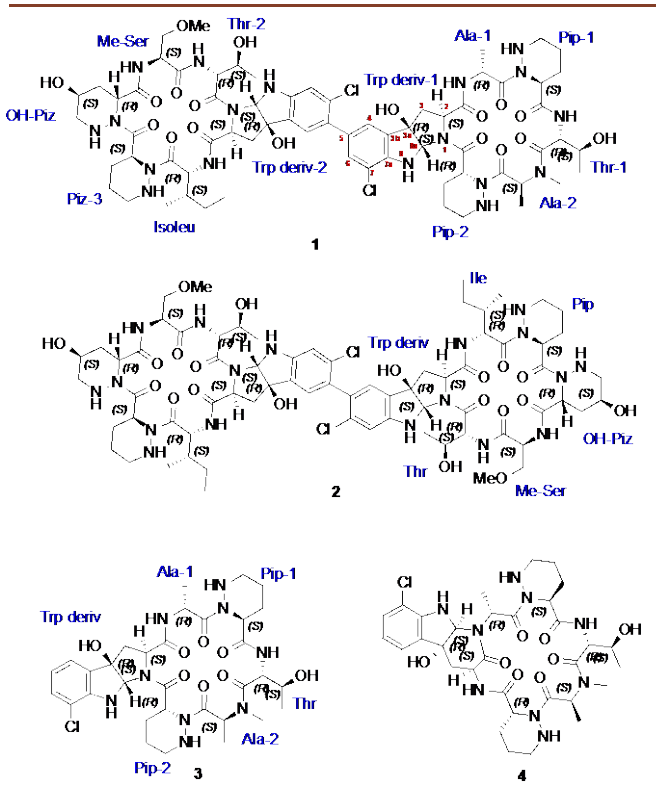

Fig. 1. Structures of compounds **1-4**

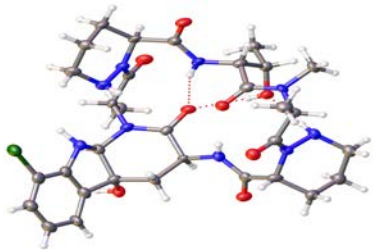

Fig. 2. X-ray structure of compound **4.**

During our analysis of compound **3**[2] (about 5 milligram in 0.5 mL of acetone-d_6 with 2 drops of DMSO-d_6), we observed an obvious change of the ^{1}H NMR spectrum after overweekend NMR experiments. We found that compound **3** was almost completely converted to a new compound (**4**) when NMR tubes were stored at 4°C. Both 1D and 2D (COSY, TOCSY, HSQC, HMBC, and NOESY) NMR data (Table S1, Fig. S1-6) of the sample were collected, and the structure of compound **4** was readily determined. Suitable crystals (0.15 × 0.06 × 0.04 m) for an X-ray crystallographic study were also obtained from aqueous methanol at room temperature after repeated recrystallization. The final structure and the absolute configuration of **4** were then unambiguously confirmed by single-crystal X-ray diffraction with Flack(x)= -0.01(2), and Hooft(y) = 0.071(16)[3] (Fig. 2, Fig. S7, Fig. S8, and Tables S2-S4).

Because the mechanism behind the apparent conversion of **3** to **4** was not immediately clear to us, we decided to explore this phenomenon further. By comparing these two compounds, we hypothesized (Fig. 3) that an acid-catalyzed elimination (C8a-C1 bond cleavage) of **3** is followed by the nucleophilic attack of the amide nitrogen in the Ala moiety to C8a to yield **4**. To shed light into this proposed mechanism, we carried out DFT calculations of **3**, **3a**, and **4** at the ωB97XD/6-31G* level, including the effect of the solvent (acetone) using SMD,[4] as implemented in Gaussian 09.[5] The ωB97XD functional was developed by Head-Gordon and co-workers, using a version of Grimme's D2 dispersion model, and was successfully employed in recent theoretical studies.[6] After exhaustive exploration of the conformational landscape with MMFF we found hundreds of conformations of each target structure. Given the size of the system under study, we narrowed down the conformational size following a workflow including geometry optimizations at increasingly larger levels to filter out duplicates and high-energy shapes (see SI). As shown in

Fig. 4, the calculations supported the transformation of **3** into **4**. The opening of **3** to give the corresponding imine **3a** is endergonic, with a ΔG=9.1 kcal/mol. On the other hand, the transformation of **3a** into **4** by nucleophillic attack of the amide nitrogen present in the Ala moiety is exergonic (ΔG=10.5 kcal/mol). Overall, compound **4** is 1.4 kcal/mol more stable than the precursor **3**, and the energy gap significantly increases considering only the enthalpy (ΔH = -3.8 kcal/mol), consistent with the experimental findings.

Unfortunately, the reaction intermediate **3a** couldn't be chemically trapped nor isolated in our experimental setting, suggesting that the intermediates are solvolytically very unstable. **4** was not evaluated for its bioactivity due to lack of material after analysis.

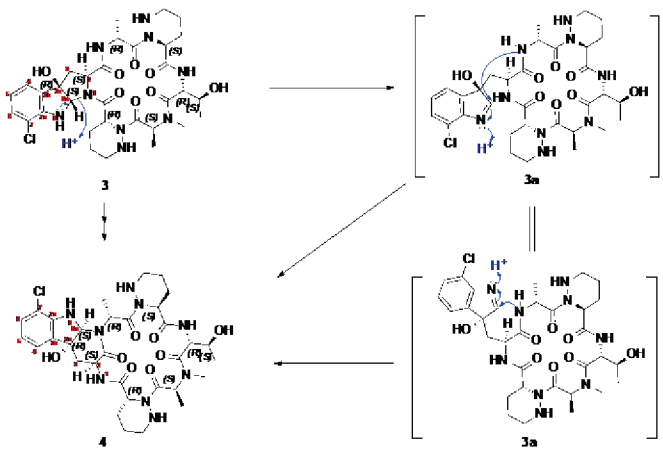

Fig. 3. Proposed conversion of petrichorin C (**3**) to petrichorin C1 (**4**). Rearrangement and ring expansion illustrated stepwise for clarity.

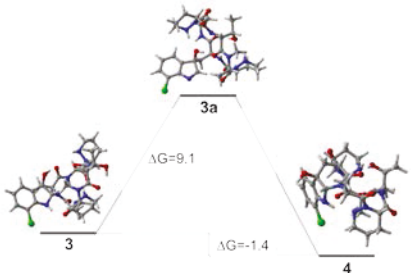

Fig. 4. Free energy reaction profile computed for the transformation of **3** into **4** at the SMD/ωB97X-D/6-31G* level. The global minima structures found for each compound are shown and the energies are given in kcal/mol.

Compared with **3**, the symmetric homodimer, petrichorin B (**2**), was relatively stable. We believe that the left hemisphere (**5**, not detected[1]) of compound **1**, which is exactly the same as the right and left hemispheres of **2**, is stable.

Petrichorin C (**3**) makes up the right hemisphere of petrichorin A (**1**), and because of this we surmised that **1** might suffer innate instability stemming from **3**. However, we failed observe an obvious change of **1** when routinely stored at -20 ºC for chemical analysis and bioassay evaluation. Therefore, we surmised that while compound **1** could undergo similar conversion as **3** (Fig. 5), the dimeric **1** seemed more kinetically stable.

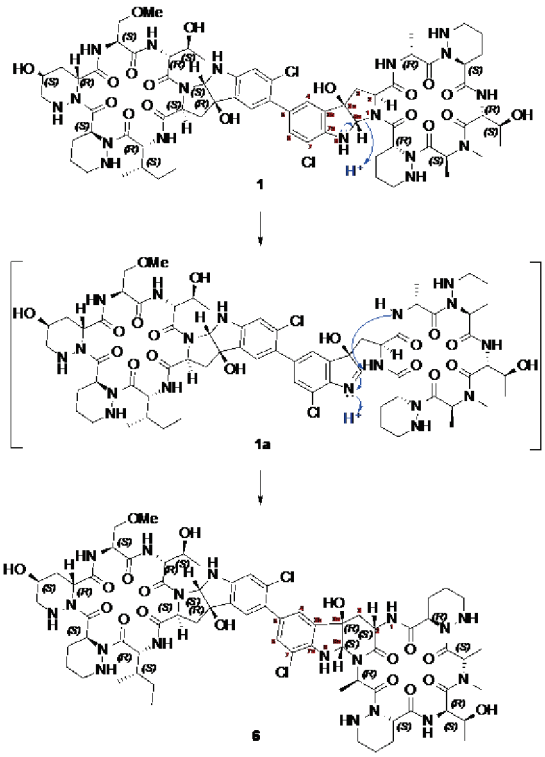

Fig. 5. Proposed conversion of **1** into **6**.

To model the stability of compound **1**, we performed DFT calculations at the SMD/ ω B97X-D/6-31G* level following the same computational procedure discussed above. We started by computing the Gibbs free energy difference between **1** (a heterodimer of **5** and **3**) and **6** (a heterodimer of **5** and **4**). The results showed a ΔG value of 5.4 kcal/mol, indicating that **6** is thermodynamically more stable than **1** (Fig. 6). This gap is larger than that found for **3** and **4** (1.4 kcal/mol), suggesting that the **1→6** conversion should be more favored. However, since **6** has not been isolated nor identified, we suspected that the isomerization might kinetically slowed. To validate our hypothesis, we computed the energy of **1a**, the right hemispheres of which are the equivalent heterodimers of intermediates **3a** (Fig. 6). Our calculations demonstrated that the **1**/**1a** gap (Δ G=11.6 kcal/mol) is indeed considerably larger than that of **3**/**3a** (Δ G=9.1 kcal/mol), possibly accounting for the kinetic stability of **1**, consistent with our observations while previously working with the compound. Although **6** is thermodynamically more stable than **1**, the higher energy barrier between **1** and **1a** than that between **3** and **3a** makes the conversion from **1** to **6** kinetically harder and slower than that from **3** to **4**.

Another possible conversion mechanism is the epoxide formation at C3a-C8a in the tryptophan unit with the amide nitrogen (position-1) as a leaving group to form the intermiediate **3a'** or **1a'**, followed by the attack of the other amide nitrogen in the Ala moiety to C8a to produce **4** or **6**. Our calculations showed that this path has much higher barriers (ΔG=17.4 kcal/mol for **3a'** and 13.5 kcal/mol for **1a'**) than those calculated for the iminie intermdiates **3a** or **1a**. In addition, compound **1a'** derived from the dimer (**1**) is 3.9 kcal/mol more stable than the corresponding epoxide (compound **3a'**) derived from monomer **3** (Fig. S9-S12), which is inconsistent with our experimental observation.

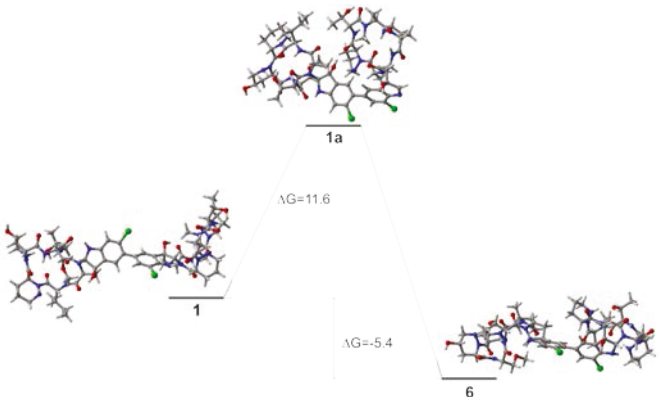

Fig. 6. Free energy reaction profile computed for the transformation of **1** into **6** at the SMD/ωB97X-D/6-31G* level. The global minima structures found for each compound are shown and the energies are given in kcal/mol.

A third possible conversion mechanism could be that **3** or **1** opens at the amide bond between the hydroxyhexahydropyrrolo[2,3-*b*]indole-2-carboxamide (HPIC) and Ala moieties to yield **3a"** or **1a"**, which might be catalyzed by the aqueous formic acid residue from the HPLC separation. The Ala amine subsequently nucleophilically attacked the electron-poor atom C-8a of HPIC, which is flanked by two nitrogen atoms with higher electronegativity than C-8a, to generate **3b"** or **1b"**. The same amino group in **3b"** or **1b"** would react with the carboxylic acid group at the 2-position of HPIC to produce the unexpected compound **4**. Although the energy barrier between **3** (or **1**) and **3a"/3b"** (or **1a"/1b"**) is lower than that between **3** (or **1**) and **3a** (or **1a**) (Fig. S13-S16), the opening and closing of the macrocyclic peptide appeared to be unlikely.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/xxxxxx.

Characterization data, NMR spectra of products, X-ray experimental details, and general computational information (PDF).

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. [a]Li and Sarotti made equal contribution to this manuscript.

Notes

The authors declare no competing financial interest

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REFERENCES

(1) Li, C.; Hu, Y.; Wu, X.; Stumpf, S. D.; Qi, Y.; D'Alessandro, J. M.; Nepal, K. K.; Sarotti, A. M.; Cao, S.; Blodgett, J. A. V. Discovery of unusual dimeric piperazyl cyclopeptides encoded by a Lentzea flaviverrucosa DSM 44664 biosynthetic supercluster. *Proc. Natl. Acad. Sci. U.S.A.* **2022** Apr 26;119(17):e2117941119. doi: 10.1073/pnas.2117941119. Epub 2022 Apr 19. PMID: 35439047

(2) Petrichorin C1 (**4**) White solid; $[\alpha]_D^{25}$ +12.0 (c = 0.5 mg/mL, MeOH). UV (MeOH) λ_{max} (log ε) 212 (3.08), 241 (2.70), 297 (2.21) nm; IR (MeOH) ν_{max} 3614, 3162, 3001, 2941, 2295, 2253, 1634, 1446, 1374, 1036, 916, 749 cm-1; ^{1}H (400 MHz, acetone-d_6 with 2 drops of DMSO-d_6) δ_H 7.48 (d, 10), 7.24 (d, 7.6), 7.14 (d, 7.6), 6.77 (d, 7.6), 6.44 (m), 5.43 (dd, 12.7, 2.8), 5.16 (br s), 5.15 (d, 4.2), 5.04 (ol), 5.02 (ol), 5.02 (ol), 4.87 (m), 4.71 (m), 4.26 (br s), 3.85 (d, 12), 3.76 (m), 3.27 (s), 2.99 (m), 2.78 (m), 2.71 (m), 2.45 (m), 2.34 (m), 2.28 (br d, 12.9), 1.93 (t, 12.9), 1.70 (m), 1.62 (m), 1.44 (m), 1.43 (m), 1.40 (d, 6.8), 1.29 (m), 1.28 (m), 1.27 (d, 6.8), 1.10 (d, 5.9) (see Table S1), and ^{13}C NMR (100 MHz, acetone-d_6 with 2 drops of DMSO-d_6) δ_C 171.9, 171.8, 171.7 (×2), 169.8, 169.3, 145.6, 136.6, 129.3, 122.3, 121.2, 116.0, 81.8, 79.0, 67.1, 59.3, 55.9, 55.5, 55.0, 53.8, 48.7, 47.1, 47.0, 40.3, 36.9, 23.8, 23.7, 22.5, 22.4, 20.2, 14.5, 14.3 (see Table S1); HRMS (ESI-TOF) m/z: 718.3073, $[M + H]^+$ (calcd for $C_{32}H_{45}ClN_9O_8^+$, 718.3074).

(3) X-ray Crystallography of compound **4** (CCDC number: 2005096, deposited on May 20, 2020; Fig. S4). Suitable crystals (0.15 × 0.06 × 0.04 mm) of compound 4 for an X-ray crystallographic study were obtained from aqueous methanol at room temperature after repeated re-crystallization. Crystals mounted on a diffractometer were collected data at 100 K. The intensities of the reflections were collected by means of a Bruker APEX DUO CCD diffractometer

(Cu$K\alpha$ radiation, λ=1.54178 Å), and equipped with an Oxford Cryosystems nitrogen flow apparatus. The collection method involved 1.0° scans in ω at -30°, -55°, -80°, 30°, 55°, 80°, and 115° in 2θ. Data integration down to 0.84 Å resolution was carried out using SAINT V8.37 Å with reflection spot size optimization. Absorption corrections were made with the program SADABS. The structure was solved by the Intrinsic Phasing methods and refined by least-squares methods again F^2 using SHELXT-2014 and SHELXL-2014 with OLEX 2 interface. Crystallographic refinement details have been delineated within crystallographic information file (*.cif). Deposition Number 2005096 (compound 4) contains the supplementary crystallographic data for this paper. These data are provided free of charge by the joint Cambridge Crystallographic Data Centre and Fachinformationszentrum Karlsruhe Access Structures service www.ccdc.cam.ac.uk/structures. .

(4) Marenich, A. V.; Cramer, C. J.; Truhlar, D. G. Universal solvation model based on solute electron density and on a continuum model of the solvent defined by the bulk dielectric constant and atomic surface tensions. *J. Phys. Chem. B*, **2009**, *113*, 6378–6396.

(5) Frisch, M. J. et al., Gaussian 09, Revision B.01, Gaussian, Inc., Wallingford, CT, 2009. For the full reference, see the Supporting Information.

(6) Chai, J.-D.; Head-Gordon, M. Long-range corrected hybrid density functionals with damped atom-atom dispersion corrections. *Phys. Chem. Chem. Phys.*, **2008**, *10*, 6615–6620.

(7) Johansson, D. G. A.; Wallin, G.; Sandberg, A.; Macao, B.; Åqvist, J.; Härd, T. Protein autoproteolysis: conformational strain linked to the rate of peptide cleavage by the pH dependence of the N --> O acyl shift reaction. *J. Am. Chem. Soc.* **2009**, *131*, 9475–9477.

ASSOCIATED CONTENT