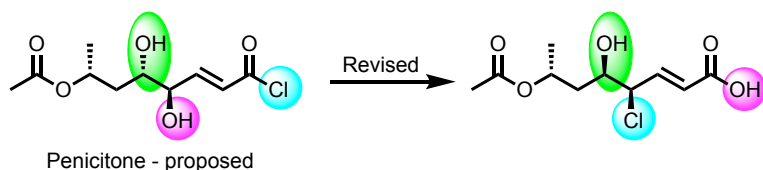


Penicitone: Structural reassignment of a proposed natural product acid chloride

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Supporting Information Placeholder



ABSTRACT: The proposed structure for the natural product penicitone, which contained a chemically improbable acid chloride functional group, was reassigned using a combination of chemical intuition, Computer Assisted Structure Elucidation (CASE) and DFT methods.

Earlier this year Xie *et al.* reported the isolation of penicitone (**1**) from a fungus *Penicillium* sp. YUD18003 obtained from rhizospheric soil associated with the perennial herb *Gastrodia elata* (Scheme 1).¹ Penicitone (**1**) was proposed to contain an acid chloride functional group at position 1 (Figure 1). However, acid chlorides are notoriously unstable, especially when in contact with moisture or the presence of internal nucleophiles (e.g., hydroxy groups). Therefore, given that 1) biosynthesis nominally occurs in an aqueous environment, 2) that part of the purification process involved column chromatograph using a methanol gradient, and 3) two hydroxy groups are proposed which would lead to either a 5- or 6-membered lactone, it seemed very unlikely that the proposed structure was correct. In addition, only one natural product acid chloride (i.e., **2**) (Figure 1) has ever been reported, but it was later proven to be a misassignment.²

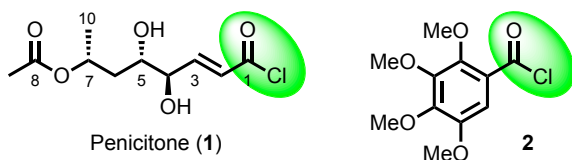


Figure 1. Penicitone **1**, and misassigned natural product **2**.

Misassigned (incorrectly assigned) chemical structures have been well noted and reviewed in the context of natural product elucidation.^{3–8} Elucidation errors are completely understandable in most occurrences, especially given the extensive demands on interpreting spectroscopic information associated with the increasing complexity of natural products and in turn determining precise chemical identity.⁹ That said, with the dawn of computing, computer-assisted structural elucidation (CASE) was soon developed,¹⁰ which greatly assisted in avoiding potential errors arising from subjective interpretation using classical elucidation methods.¹¹ Unsurprisingly, CASE underwent a significant evolution when combined with DFT methods, which improved structural resolving power through conformational searching leading to chemical shift and coupling constant prediction.¹² In fact, this combination of *in silico* approaches has indeed provided considerable benefit in reassigning a range of natural products,^{13,14} especially through the use of ACD/Structure Elucidator (ACD/SE)¹⁵ and a hybrid DFT-parametric computational method, DU8+,^{16,17} and herein a mixture of these methods and chemical principles is applied to address the proposed structure of **1**.

Penicitone (**1**) was reported as a yellow oil with no observations regarding stability and/or degradation. Inspection of the positive ion HRMS-ESI spectrum for **1** revealed two peaks at m/z 273.0504 and m/z 275.0482 respectively. These data were in agreement with the proposed molecular formula $C_{10}H_{15}ClO_5$ (calc. for 273.0506

[M+ Na⁺]), and the corresponding isotopic peak (calc. for 275.0476 [M+ Na⁺]), which was also in the appropriate ~3:1 isotopic ratio expected for incorporation of a chlorine atom. Evaluation of the reported ¹³C NMR spectrum showed the presence of two carbonyl peaks at δ_C 172.0 and 170.1 ppm. A HMBC cross peak between the carbonyl at δ_C 172.0, and Me-9 (δ_H 2.08 ppm), confirmed this resonance as the proposed acetoxy group. The remaining peak at δ_C 170.1 ppm, which was originally proposed to be a carbonyl chloride, also falls within the chemical shift range of carboxylic acids and their derivatives (e.g., esters). However, there was no indication from the 2D NMR spectra that an ester was present, and the molecular formula ruled out the possibility of a primary amide, and therefore it was tentatively assigned as a carboxylic acid. Presuming the mass spectrometry results were reflective of the isolate, and that the acid chloride was indeed a carboxylic acid, suggested that one of the hydroxy groups (i.e., C4 or C5) could instead be a chloro substituent. This notion afforded two possible constitutional isomers, **3** and **4** (Figure 2); however, this assumed that the assignment of the remaining molecular framework was correct.

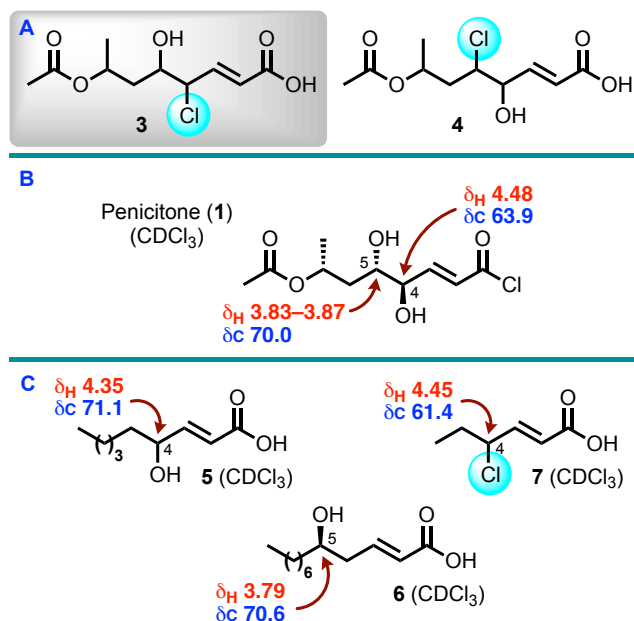


Figure 2. A) New candidate constitutional isomers; B) ¹H and ¹³C NMR chemical shifts for penicitone (**1**) at C4 and C5; C) ¹H and ¹³C NMR chemical shifts at C4 and C5 for **5-7**.

¹H and ¹³C NMR chemical shift comparisons to related, albeit less complex known systems (i.e., **5-7**) (Figure 2), then enabled **3** to be identified as a lead candidate. The key chemical shift comparisons justifying this decision resided mostly on the ¹³C NMR data. For example, position C4 of **5** (δ_C 71.1)¹⁸ and C5 of **6** (δ_C 70.6)¹⁹ matched more closely the chemical shift of C5 of **1** (δ_C 70.0), whereas position C4 of **7** (δ_C 61.4)²⁰ aligned well with C4 of **1** (δ_C 63.9) (Figure 2).

Subsequently, the proposed structure for **1** with the reported ¹³C NMR chemical shift assignments, were entered into ACDLabs Structure Elucidator (ACD/SE), and chemical shift prediction performed using empirical methods. Specifically, the HOSE code-based method, the neural

networks, and the incremental approach of chemical shift prediction was utilised, whereby the average deviations of the ¹³C chemical shifts determined by these methods are denoted as *d_A*, *d_N* and *d_I*, correspondingly.^{10,15} From this analysis the average and maximum deviations were deemed large enough to raise suspicion that the proposed structure for **1** was questionable (Figure 3A). Of note was that the lefthand portion of the proposed structure **1**, as highlighted in green, was predicted to be correctly deduced by Xie *et al.*, but multiple erroneous assignments existed in the righthand fragment (highlighted in yellow). A Molecular Connectivity Diagram (MCD) was then generated (Figure 3B) using data from the lefthand fragment (i.e., in green), and the reported HMBC and COSY correlations for the remaining free atoms (i.e., in yellow), to obtain a predicted structure. Reassuringly, the predicted structure matched the reassignment lead candidate for **3** (Figure 3C).

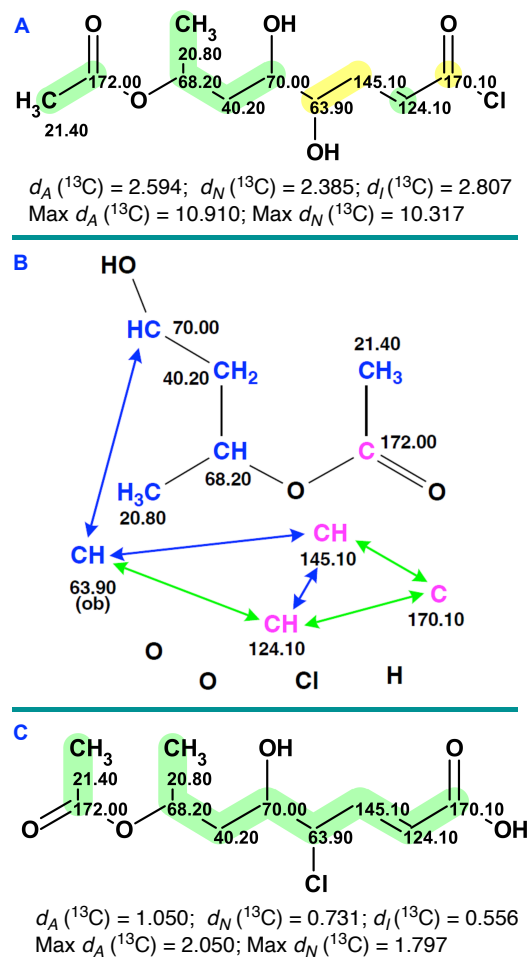


Figure 3. A) ACD/SE analysis of penicitone (**1**): each atom is color coded to denote a difference between the experimental and calculated ¹³C NMR chemical shifts – green represents a difference of 0 to 3 ppm, whereas yellow indicates a >3 to 15 ppm difference; B) Molecular connectivity diagram (MCD) created for **1** using ACD/SE. Carbon atom hybridizations color coded as: *sp*² – violet and *sp*³ – blue. Label “ob” is set by the program to the carbon atom for which neighboring with a heteroatom is obligatory. HMBC connectivities are marked by green arrows, while COSY connectivities by blue arrows. C) Lead candidate for reassignment (i.e., **3**).

With the carbon skeleton and atom connectivities in place (i.e., **3**), assignment of the relative stereochemistry was addressed, and to achieve this goal DU8ML,¹⁷ the DFT empowered NMR chemical shift and coupling constant prediction program was employed. Therefore, the four possible diastereomers (i.e., **8-11**) allowed for **3**, were subjected to both ¹³C NMR chemical shift and ¹H-¹H coupling constant prediction, and then compared to the reported experimental observations. Of the four potential diastereomers, candidate **11**-(4*R*, 5*R*, 7*R*) [**11**-(4*S*, 5*S*, 7*S*)] provided the lowest rmsd (root mean square deviation) values in both categories [i.e., rmsd (*J*_{HH}) = 1.3 Hz; rmsd (δ_C) = 1.26 ppm] (Figure 4A). Further support for **11** came from consideration of the reported NOESY spectrum, which showed distinctive correlations between 4-H and 5-H, in addition to 5-H and 7-H (Figure 4B). Critical to the DFT analysis was the observation that hydrogen bonding between the C5-OH hydrogen atom and the C7-OCOMe oxygen atom gives rise to an 8-membered ring that locks the conformation (Figure 4C). That is, all low energy conformers in the 5*S*,7*S* (5*R*,7*R*) diastereomeric series (i.e., **9** and **11**) display intramolecular cyclic hydrogen bonding, because it allows for the methyl at C8 (i.e., the terminus of the carbon chain) to assume a pseudo-equatorial position. For diastereomers in the 5*S*,7*R* (5*R*,7*S*) series (i.e., **8** and **10**), the same type of hydrogen bonding exists, but the conformers are higher in energy.

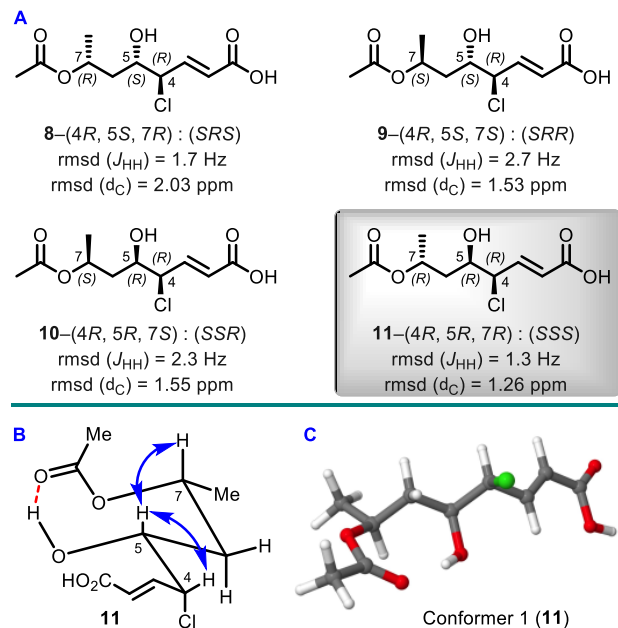


Figure 4. A) Diastereomer candidates for **3** include **8** to **11**; B) NOESY correlation diagram match to diastereomer **11**; and C) lowest energy conformation showing an 8-membered ring formed through hydrogen bonding to the acetyl oxygen.

Electron circular dichroism (ECD) calculations were then undertaken, using Time-Dependent DFT (TD-DFT),²¹ to determine the absolute stereochemistry. Predicted ECD curves were generated for **11**-(4*R*, 5*R*, 7*R*) and **11**-(4*S*, 5*S*, 7*S*) using the TD- ω B97xD/6-311+G(d,p)/b3lyp/6-311+G(d,p) level of theory, with a polarizable continuum model (PCM) to account for solvent (methanol). The curves

were compared to the reported experimental ECD (Figure 5), which indicated that the correct enantiomer was the 4*R*, 5*R*, 7*R* isomer.

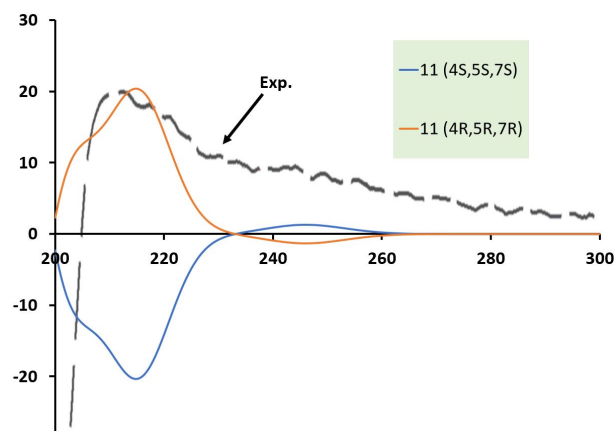


Figure 5. Computed electron circular dichroism (ECD) spectra with the overlaid reported experimental spectra. Note: The placement of the experimental curve is approximate as the raw numerical data was not reported.

In conclusion, reassignment of penicitone (**1**) as a chlorinated polyketide, containing a carboxylic acid residue and not an acid chloride, was achieved through a combination of chemical principle analysis supported by CASE and DFT methods. This is a prime example whereby chemical intuition can be particularly useful in identifying erroneously assigned natural products.

ASSOCIATED CONTENT

Data Availability Statement

The data underlying this study are available in the published article and its Supporting Information.

Supporting Information

Supplementary data containing ACD Elucidator structure generations, DU8+ Cartesian co-ordinates and ECD calculations are available in the supplementary material of this article. The Supporting Information is available free of charge on the ACS Publications website.

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Notes

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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