Cycloelatanene A and B: absolute configuration determination and structural revision by the crystalline sponge method†

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Cycloelatanene A and B are marine natural products first reported a few years ago. Their relative structures had been elucidated by an extensive NMR study and found to be epimers. However, their absolute configurations had not been established because they were isolated in only minute quantities as oily compounds. In this study, the complete structures of cycloelatanene A and B, including absolute configurations, were determined by the crystalline sponge method. The structure analysis confirmed the unique tricyclic structure involving a spiro[5.5]undecene skeleton. One stereogenic centre at C4 was revised as a result of this analysis. Since it only took 1–2 weeks to complete the experiments using the crystalline sponge method (guest-soaking followed by crystallographic analysis), this method is now highly recommended as a first port of call to achieve complete natural product structure elucidation.

This great advantage has recently been applied to the absolute structure determination of natural products, where no other means except for the crystalline sponge method could directly address the absolute configuration of the compounds.† The present study reports on the absolute configuration determination of cycloelatanene A and B via the crystalline sponge method. This methodology has not only been able to confirm the unique triyclic framework of the cycloelatanenes, which possess a spirocarbon center, but also led to the revision of one of the chiral centers in these structures (Fig. 1).

Results and discussion

[(ZnI2)3(tpt)2(cyclohexane)] (3, tpt = 2,4,6-tris-(4-pyridyl)-1,3,5-triazine)† was used as the crystalline sponge in this study. A single crystal of 3 (typically approx. 200 × 150 × 100 μm3) was

Fig. 1. Reported relative structures of cycloelatanene A (1) and B (2) as elucidated by a detailed NMR study. The compounds are epimers with opposite configuration at C4. Note that these structures have been revised as shown in Fig. 2.
placed in a vial and immersed in a small amount of cyclohexane (45 μL). For cycloelatanene B (2), five replicate vials were prepared and a 1,2-dichloroethane solution (5 μL) of 2 (1 μg μL⁻¹, 5 μg) was added to each of them. The vials were equipped with a syringe needle for slow solvent evaporation. Guest soaking was carried out at 50 °C for 1 d. After confirming solvent evaporation, all the guest-absorbed crystals were subjected to a short exposure time sample screening with an in-house X-ray diffractometer. One crystal was selected that produced the best diffraction pattern giving sharp and non-split spots and the highest resolution. The single-crystal X-ray diffraction experiment was performed on an in-house X-ray diffractometer with Mo Kα radiation.

Due to the inclusion of the chiral guest, the net host–guest structure becomes chiral and the inherent centrosymmetric C2/e space group of host 3 turns into the non-centrosymmetric C2 space group, as confirmed based on the extinction rule systematic absence of reflections. The crystallographic analysis revealed two independent guest molecules per asymmetric unit with estimated populations of 71.0% and 14.5% (Fig. 2). The Flack parameter calculated by the Parsons’ method with restraint on atomic displacement parameters (SIMU and RIGU). The five chiral centers in the skeleton were determined to be 4R, 5S, 6R, 8R, 9S. As a result of this analysis, the stereochemical assignment at the C4 stereogenic center was revised. The revised structure of cycloelatanene B (newly denoted as 5) has the same relative stereochemistry as structure 1 on the methoxy group at C4 (Fig. 2b). The sample was recovered from the vials and the 1H NMR of the compound was acquired and compared to the natural product before the soaking procedure. No changes in the chemical shifts for the H4 or 13- OCH₃ signals were evident (Fig. S3†), thus eliminating the possibility of epimerization having occurred at the C4 stereogenic center during the soaking experiments.

According to the previous NMR studies, cycloelatanene A and B are epimers with opposite configurations at C4. Thus the structure of cycloelatanene A should also be revised as 4 (as denoted in Fig. 2b). To conclusively determine the structure of cycloelatanene A, the compound was also subjected to structure analysis by the crystalline sponge method. After some trial-and-error experiments, the soaking conditions were optimized and the diffraction study was performed with the best-diffracted single-crystal on an in-house X-ray diffractometer; for this compound, Cu Kα radiation gave better quality data than Mo Kα radiation.

In the crystallographic analysis, one guest molecule per asymmetric unit was clearly observed. As expected, cycloelatanene A (4) was shown to have an inverted methoxy group at the C4 center (Fig. 2b). The Flack parameter calculated by the Parsons’ method was 0.264(9). The high Flack value is presumably due to insufficient guest occupancy (~47%) at the binding site of the crystalline sponge. Despite the Flack parameter value, the absolute configuration of 4 is suggested to be 4S, 5S, 6R, 8R, 9S by analogy with the absolute configuration deduced for its C4-epimer, 5, based on the similar optical rotations between 4 and 5.†

As a result of the revision of the C4 stereogenic center, as deduced by the crystalline sponge method, the 1D NOE NMR spectra acquired for cycloelatanenes A (4) and B (5) were reanalyzed. The revised data are given in Fig. 3 (see also Fig. S2†) and in Tables S1 and S2, respectively. A number of additional weak NOE correlations in both cycloelatanenes had also been observed and due to the close nature of some of the chemical shift assignments in both compounds, it is highly likely that both sets of protons were inadvertently irradiated (e.g. δ 1.68/1.62 and 3.63/3.67 in 4 and 1.68/1.70 in 5). This meant that
some NOEs reported in the original study required revision and reassignment. In addition, based on the NOE data, the proton signals assigned to H10a and H10b in the original report of the structures of the cycloelatanenes should be reversed.

After close re-examination of the data, key NOE enhancements placed 12-CH$_3$, 14-CH$_3$, 15-CH$_3$, H7ab, H8, H10a and H11ab at the convex of the rigid and bent tricyclic framework in both 4 and 5. The two epimers 4 and 5 could be discriminated by an additional key NOE enhancement observed between the two substituents placed in close proximity in the concave region of the framework: i.e., H10b with 13-OCH$_3$ in 4 and H10b with H4 in 5 in the concave region of the molecules (see Fig. S2†). On this basis the C4 stereogenic center could be deduced for both cycloelatanenes A and B and was consistent with the findings obtained from the crystalline sponge method.

Further support for the revised assignment of the C4 stereogenic center in the cycloelatanenes was based on analysis of the coupling constants between the olefinic H3 and the methine H4. The crystal structures reveal that the dihedral angles $\psi_{H3-C3-C4-H4}$ are $63^\circ$ and $83^\circ$ for 4 and 5, respectively (Fig. 4).

The observed $J_{H3-H4}$ values of 6.0 and 1.6 Hz for 4 and 5, respectively, are thus consistent with the crystal structures as the $J$ value decreases with increasing $\psi$ value in the range of $0^\circ < \psi < 90^\circ$ (Karplus equation). The $J_{H3-H4}$ value for 4 (6.0 Hz) is also consistent with the values observed for structurally related compounds 6–10, which showed $J$ values of 4–7 Hz [ref. 10–12] with the proton facing up at the stereogenic centre of interest (C4 in 4). Laureacetal A (6) has had its absolute configuration confirmed.$^{15}$

**Conclusions**

In conclusion, the crystalline sponge method has been used to unequivocally deduce the absolute configuration of cycloelatanenes A and B, which possess five chiral centers. While initial NMR spectroscopy was successful in elucidating the gross structures and the relative configuration for four of the five chiral centers, the sponge method has provided confirmation of the absolute configuration, resulting in one chiral center being revised. Reanalysis of the 1D NOE NMR experiments in combination with a comparison of the coupling constants for structurally related natural products also supports the final stereogenic center reassignment.

The crystalline sponge method is a revolution in complete structure determination, particularly in the area of natural products chemistry. Its ability to establish the absolute configuration of highly complex bioactive natural products is extraordinary and is certain to pave the way for the re-analysis of many synthetic or natural compounds, especially in the field of drug discovery. Based on these findings, it is recommended that structure determination of new chiral entities be based on combining NMR spectroscopy and mass spectrometry with the crystalline sponge method where possible. Whilst limitations of this approach include the necessity for expertise in sponge methodology and crystallography and the fact that not all molecules will be absorbed by the sponge method, this complete structure elucidation package approach, where achievable, is remarkable.

**Acknowledgements**

The authors thank Daniel Anthony Dias for extracting Cycloelatanene A and B and assigning their preliminary relative structures. This research was promoted as a part of JST-ACCEL and by KAKENHI, MEXT (24000009).

**References**

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