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Organic structure determination using atomic-resolution scanning probe microcopy

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1. Introduction

1-1. Structure characterization of natural products

- Generally, NMR and MS are used.

  →However, sometimes these are not enough to determine the structure.
- X-ray crystallography is a powerful method.

  →However, it is often difficult to get suitable crystals.

>> In many cases, the resulting structure is confirmed by a complex and lengthy total synthesis.

→Direct observation of molecular structure is required.

1-2. Atomic Force Microscopy (AFM) (Fig 1)

- AFM detects an atomic force between a tip and the sample surface.
- In this paper, the authors used non-contact AFM (NC-AFM) in constant-height mode. → The tip scanned parallel to the surface and recorded frequency shift without contact.

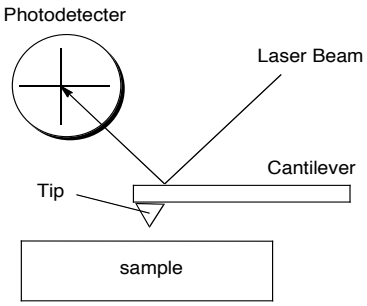


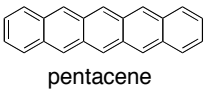
Fig 1. Basic principle of AFM

1-3. Previous work

- The authors could observe one molecule in NC-AFM imaging (Fig 2).[1] Even C–H bond could be observed.
- To detect very small forces,

→AFM was operated in ultrahigh vacuum at 5 K with a very stiff force sensor.

→Stable operation at very small scale could be achieved.



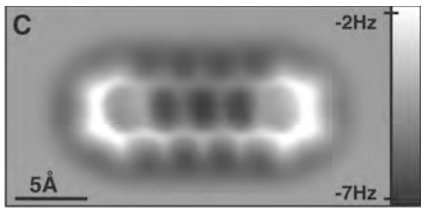


Fig 2. AFM image of pentacene.

- The origin of the dark halo surrounding the molecule is van der Waals force, and the origin of the atomic contrast is the Pauli repulsion (Fig 3).
- The atomic composition and the geometry of the tip have crucial effect for the resolution of the AFM imaging.

→They tried some molecule apex and CO-functionalized tip was the best.

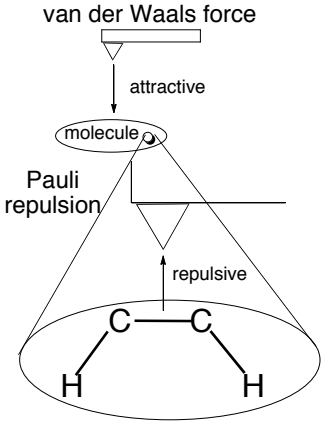


Fig 3. The origin of the contrast.

1-4. This work

1-4-1. Cephalandole A (Fig 4) –Model molecule for this work

• Cephalandole A was isolated from Taiwanese orchid in 2006, but its structure was misassigned (Fig 5).[2]

• The structure was corrected in 2008 by the total synthesis.[3]

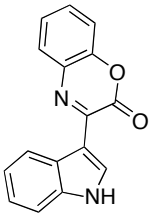


Fig 4. Cephalandole A

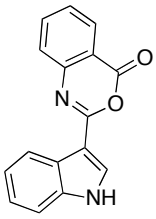


Fig 5. Misassigned structure of Cephalandole A

1-4-2. Purpose of this work

• Structure determination by direct molecule observation with AFM.

2. Results and discussion

2-1. MS and NMR study

• By MS and NMR, there were four possible structures (Fig 6).

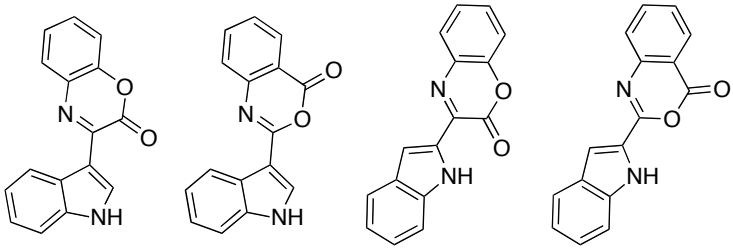


Fig 6. Possible four substructures of Cephalandole A

2-2. AFM measurement

2-2-1 AFM preparation

• How to make CO-functionalized tip (Fig 7)

The tip (50-μm-thick PtIr wire, which apex was coated with Cu) was positioned above a CO molecule on Cu(111) and a voltage pulse of about 2.5 V was applied to pick up the CO.

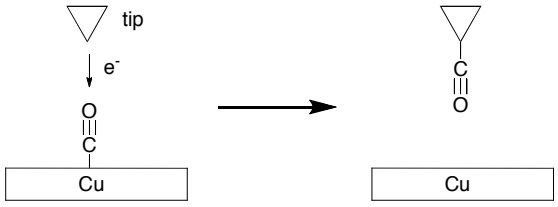


Fig 7. Preparation of CO functionalized tip

• Sample preparation (Fig 8)

A Cu(111) single crystal partly covered with two-monolayer-thick islands of NaCl (thermally evaporated at a sample temperature of 270 K) was used as a substrate. About 10 μg of the molecules was solved in methanol, spread on a silicon wafer and thermally evaporated to the substrate at ~10 K. Two molecules' overlapping was not observed in AFM image.

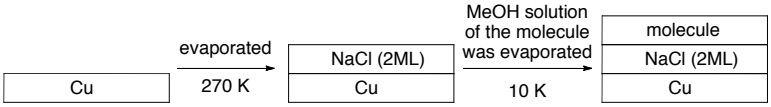


Fig 8. Preparation of the sample

2-2-3 NC-AFM measurement of Cephalandole A

• In the AFM image, most of the intermolecular bonds and cyclic systems can be observed (Fig 9)

→Structures **3** and **4** could be ruled out because of the connection position of indole core.

→Structure **1** and **2** are possible.

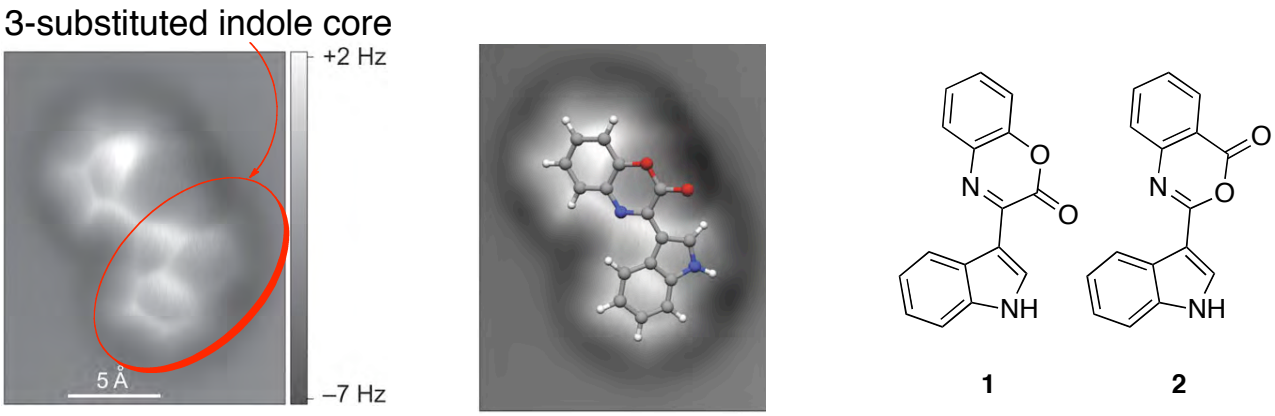


Fig 9. AFM image of Cephalandole A

• However, ester group could not be observed.

The possible reason is non-planar adsorption of the molecule due to the different atomic species and non-planar structure of the molecule itself (Fig 10).

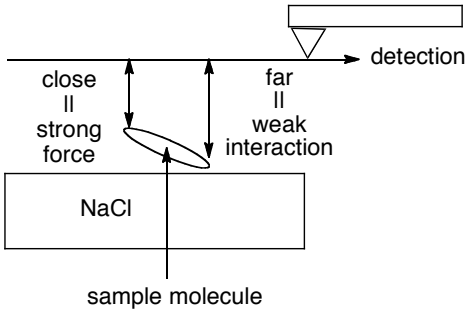


Fig 10. The reason why ester group cannot be observed.

2-2-4 Adsorption position

• Then, they determined the adsorption position of the molecule on NaCl.

• They used STM constant-current feedback to control the tip height, while recording AFM signal at the same time (Fig 11a-c).

→For this method, the substrate and the molecule were observed at the same time.

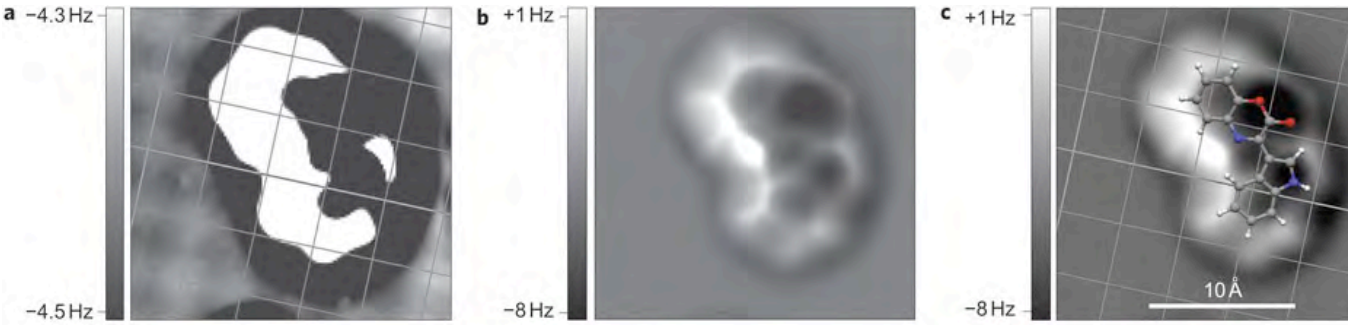


Fig 11. (a)STM and (b) AFM image of Cephalandole A on NaCl.

(c) Cl grid and molecular structure are overlaid.

2-3. DFT calculations

Working Hypothesis: Comparing with calculated absorption position of two molecules, most probable structure can be determined.

Method: Highly optimized plane-wave code CPMD was used and the Perdew-Burke-Ernzerhof exchange correlation functional was applied. *Ab initio* norm-conserving pseudopotentials with a plane-wave cut-off energy of 100 Ry was used.

2-3-1 Calculation for adsorption position

- In calculation, the structure **1** and **2** were first put on the position of the experiment result. Then, all atoms except the two bottom NaCl layers were relaxed.

→The calculated adsorption positions of each structure were shown (Fig 12b and c).

- Comparing with the experimental position (Fig 12a), a good agreement of adsorption position and orientation for **1**, but significant mismatch for **2**. →Therefore, structure **2** was conclusively ruled out.

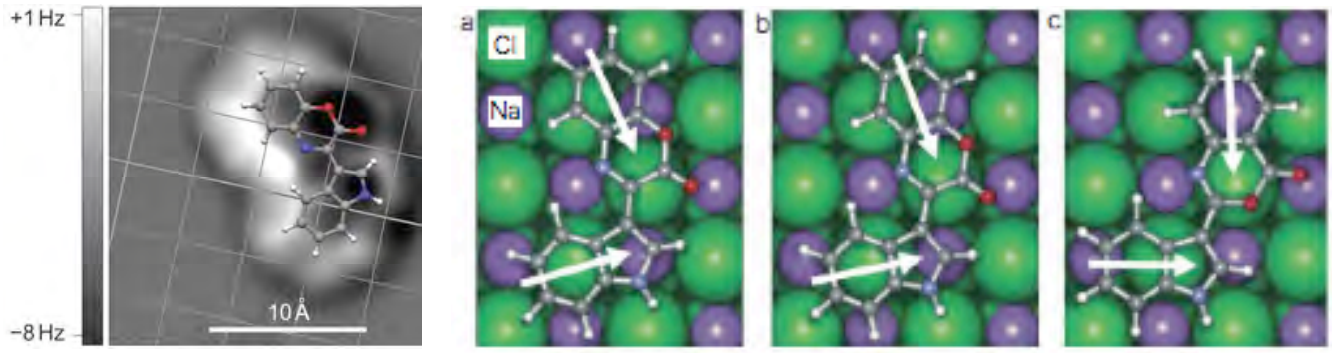


Fig 12. The molecular on NaCl (a) experiment (b) calculation about **1** (c) calculation about **2**

2-3-2 AFM image simulations - Support the structure **1**

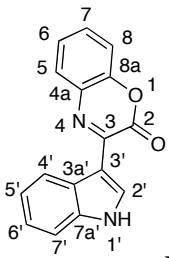
- AFM image of **1** was simulated using DFT (Fig 13a).
- DFT for **1**: *h*(O1) = 2.68 A, *h*(O2) = 2.52 A and *h*(N1') = 2.69 A

→O and N atom is close to NaCl.

- DFT for **2**: *h*(O2) = 2.92 A

→Oxygen atom is far from NaCl.

>> Comparing with experiment (Fig 13b), the agreement with the calculations is excellent.



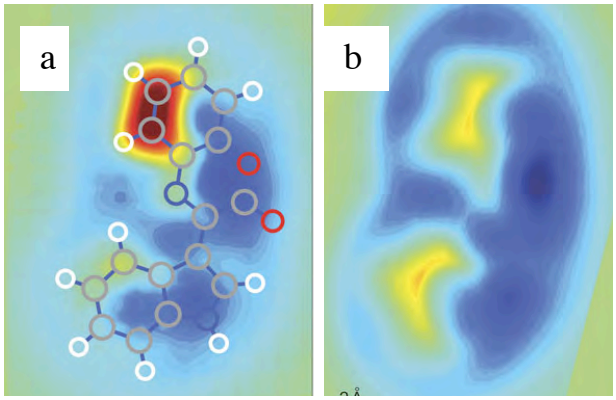


Fig 13. (a) Simulated and (b) experimental AFM images.

3. Conclusion

They could achieve the structure determination of Cephalandole A with combination of AFM and DFT calculation without synthesis.

4. Reference

[1] Gross, L.; Mohn, F.; Moll, N.; Liljeroth, P.; Meyer, G. *Science*, **2009**, *325*, 1110-1114.

[2] Wu, P-L.; Hsu, Y-L.; Jao, C-W. *J.Nat. Prod*. **2006**, *69*, 1467-1470.

[3] Mason, J.; Bergman, J.; Janosik, T. *J.Nat. Prod*. **2008**, *71*, 1447-1450.