

Ab Initio Structure Determination of Ambuic Acid with Synchrotron Powder Diffraction and Solid-state NMR

Y. Zhang,¹ J.K. Harper,² P.L. Lee,¹ R. Von Dreele,¹ D.M. Grant²
¹Advanced Photon Source (APS), Argonne National Laboratory, Argonne, IL, U.S.A.
²Department of Chemistry, University of Utah, Salt Lake City, UT, U.S.A.

Introduction

The *ab initio* structure determination of complicated crystal structures from x-ray powder diffraction data is becoming routine as a result of the development of synchrotron instruments [1] and computational algorithms [2]. However, the structures of large size organic molecular structures with only H, C, and O elements remain difficult to determine because of problems such as overlapping of the Bragg peaks, lack of high-resolution data, and radiation damage to the samples. Organic samples also show severe radiation damage under the high brilliance of a synchrotron x-ray beam. The measurement of solid-state nuclear magnetic resonance (NMR) chemical shift tensors can give valuable independent information, such as information on chemical purity, phase purity, the number of molecules in an asymmetric unit (Z'), models with accurate bond distances, bond angles, most flexible torsion angles, and especially reliable coordinates for hydrogen atoms [3]. By using direct spacing methods with starting models from NMR experiments, the number of parameters required for structure determination can be greatly reduced and the structural ambiguity can be avoided.

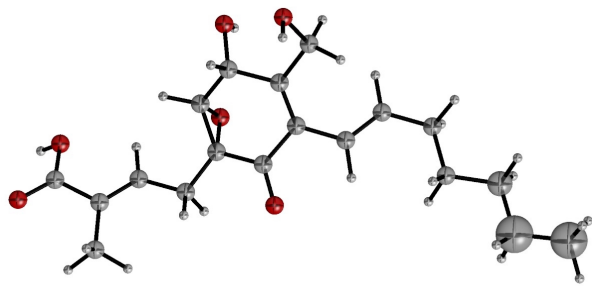


FIG. 1. Oak Ridge Thermal Ellipsoid Plot Program (ORTEP) plot of the ambuic acid structure.

Ambuic acid is an antibiotic obtained from *Pestalotiopsis microspora*, an endophytic fungus commonly found in tropical plants. The sample used here was obtained from *P. microspora* cultured from the lesions of a pandanus leaf. The formula of this compound is $C_{19}H_{26}O_6$ (51 total atoms with 25 nonhydrogen atoms) (Fig. 1). It is difficult to obtain diffraction-quality single crystals of this compound. Solid-state NMR studies for this compound have been carried out to provide the stereochemistry and rough conformational information [4]. However, because of degeneracy in the best fit of the NMR data, the values of certain flexible torsion angles cannot be obtained. The packing of molecules in the unit

cell is also inaccessible, preventing the global calculation of NMR tensors. The combination of both x-ray and NMR results will give accurate structural information that cannot be obtained by simply x-ray powder diffraction or NMR alone.

Methods and Materials

Synchrotron x-ray powder diffraction data for ambuic acid were collected at the 1-BM beamline at the APS by using a Mar345 image plate detector. The x-ray beam was focused onto the detector with a wavelength of 0.619 Å. The sample-to-detector distance was calibrated with a National Institute of Standards and Technology (NIST) LaB_6 standard as 502 mm. The ambuic acid sample was loaded into a 0.8-mm capillary, and diffraction data were collected at room temperature.

The data were averaged with five 20-second exposure images, and the powder pattern was integrated with a 30° azimuthal angle range by using the Fit2d program [5]. Indexing with the Dicvol program [6] gave a monoclinic cell with a $P2_1$ space group giving lattice parameters of $a = 15.514 \pm 0.001$ Å, $b = 4.3937 \pm 0.0002$ Å, $c = 14.2012 \pm 0.0005$ Å, $\beta = 110.316 \pm 0.004^\circ$, $Z = 2$, Cell volume = 907.79 ± 0.08 Å³. The structure was solved with DASH [7] and PSSP [8] programs by using the direct spacing methods with the starting model given by the solid-state NMR experiments.

The Rietveld refinement was carried out by the GSAS program [9], with all the bond distances and angles constrained at the values given by the solid-state NMR results. The model was fine-tuned back and forth between the NMR model and x-ray model until the fit converged with both NMR and x-ray data. The final x-ray refinement gave $wRp = 6.46\%$, $Rp = 8.26\%$, and $R(F^2) = 5.21\%$.

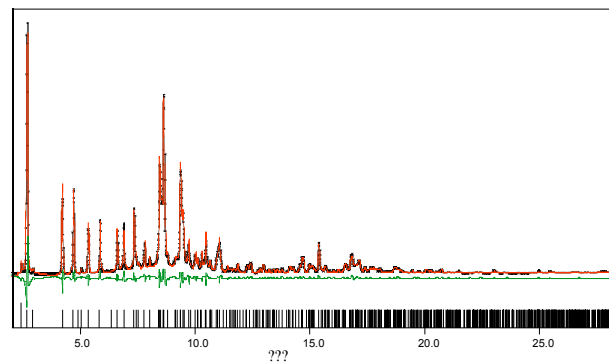


FIG. 2. The Rietveld fit of the ambuic acid powder diffraction data.

Results and Discussion

Results on the conformation of the tail atoms of the ambuic acid molecule given here are significantly different from the result given by NMR alone, which indicates that NMR alone may give structural information with ambiguity.

Since the use of an area detector can shorten the data collection time from hours to minutes, no radiation damage was observed. This research clearly shows that data collected within minutes can have high quality and be sufficient for structure solution and Rietveld refinement.

Our results demonstrate that solid-state NMR and x-ray powder diffraction are complementary to each other, with the limitations of one method being the strength of the other. The combination of these two independent techniques will expand structural determination capabilities by allowing the use of powder samples and will increase the accuracy of the structural information.

Acknowledgments

The work at the APS was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract No. W-31-109-ENG-38. Support for D.M. Grant's research was provided by the National Institutes of Health under Grant No. GM 08521-40. Computer support for all tensor computations was provided by the Center for High Performance Computing at the University of Utah.

References

- [1] J.C. Lang, G. Srajer, J. Wang, and P.L. Lee, *Rev. Sci. Instrum.* **70**, 4457-4462 (1999).
- [2] *Crystal Structure Determinations from Powder Diffraction Data*, *Trans. Am. Cryst. Soc.* **37**, edited by A. Clearfield (Pittsburgh, PA, 2002).
- [3] J.K. Harper, A.E. Mulgrew, J.Y. Li, D.H. Barich, G.A. Strobel, and D.M. Grant, *J. Am. Chem. Soc.* **123**, 9837-9842 (2001).
- [4] J.K. Harper, D.H. Barich, J.Z. Hu, G.A. Strobel, and D.M. Grant, *J. Org. Chem.* **68**, 4609-4614 (2003).
- [5] A.P. Hammersley, *FIT2D V5.18 Reference Manual VI.6*, ESRF Internal Report, EXP/AH/95-01 (1995).
- [6] A. Boulouf and D. Louër, *J. Appl. Cryst.* **24**, 987-993 (1991).
- [7] W.I.F. David, K. Shankland, and N. Shankland, *Chem. Commun.* **8**, 931-932 (1998).
- [8] S. Pagola and P.W. Stephens, *Mater. Sci. Forum* **321-324**, 40-45 (2000).
- [9] A.C. Larson and R.B. Von Dreele, *GSAS - General Structure Analysis System*, Report LA-UR-86-748 (Los Alamos National Laboratory, Los Alamos, NM, 1987).