Structure Revision of Aspergillides A and B, Cytotoxic 14-Membered Macrolides from *Aspergillus ostianus*, by X-ray Crystallography

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m-Bromobenzoates of aspergillides A and B gave crystals suitable for X-ray crystallography, resulting in the revision of the structures of both compounds as **1** and **3**, respectively.

We have reported the structures of aspergillides A, B, and C, which were obtained from marine-derived fungus *Aspergillus ostianus* and which each had a unique 14-membered macrolactone ring.[1] The structures of aspergillides A and B were proposed to be **3** and **5**, respectively, based on NMR analyses including NOEs and ^{1}H,^{1}H-coupling constants.[1] Recently, Hande and Uenishi succeeded in the total synthesis of a compound possessing the proposed structure **3**, and found that its NMR data were different from that of aspergillide A but identical to that of aspergillide B.[2] Therefore, the structure of aspergillide B ought to be revised to **3**. This change throws the real structure of aspergillide A into question.

In our previous study, the absolute configurations at the 4- and 13-positions of aspergillide A had been firmly established by the modified Mosher's method[3] carried out for the compound itself (C-4) and its methanolysis product (C-13). Thus, our most likely error was incorrect assignment of the stereochemistry at C-3 and C-7.

In order to reexamine the structures of aspergillides A and B, we performed a large-scale (45 L) cultivation of the fungus again, and obtained two compounds, labeled I (9.2 mg) and II (48.5 mg), whose physical properties (NMR, TLC, and HPLC) were identical to those of aspergillides A and B, respectively.

The *p*-bromobenzoate of II failed to crystallize, although the *m*-bromobenzoates[4] of I and II afforded nice crystals suitable for X-ray crystallography. The results are shown in Figure 1. The X-ray structures, **2**-X-ray (I-*m*-bromobenzoate) and **4**-X-ray (II-*m*-bromobenzoate), unambiguously established the absolute stereochemistry of aspergillides A (**1**) and B (**3**). The latter structure naturally accords with the one revised by Hande and Uenishi,[2] and the former one has an unexpected cis bridge at C-3 and C-7.

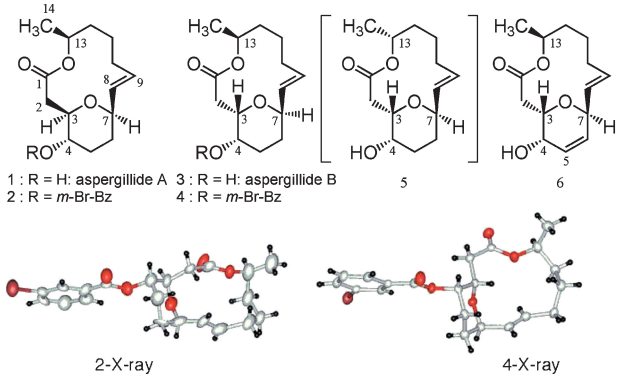

Figure 1. (above) Correct structures of aspergillides A (**1**) and B (**3**) and incorrect structure **5** reported as aspergillide B. Structure **6** of aspergillide C was reconfirmed in this study. (below) X-ray structures of **2** and **4**.

Since the correct structures **1** and **3** were obtained, we rechecked the previous experiments for aspergillides A and B to clarify the causes of the mistakes. First, we had assigned a small peak appearing at the cross section of H-3 and H-8 as a real cross peaks between the protons and overlooked the cross peaks between H-2a and H-8, which were relevant to the cis juncture at C-3 and C-7 in the NOESY spectrum (400 MHz, $CDCl_3$)[1] of aspergillide A, leading to the trans bridge structure **3**. However, since the chemical shift of H-3 (δ 4.23) was very close to that of H-7 (δ 4.21), it could be a cross peak between H-8 and H-7. Change of the solvent to DMSO-d_6 resulted in satisfactory separation of the signals [H-7 (δ 4.03) and H-3 (δ 3.96)], and distinct NOE cross peaks were observed between the protons.[5] The cross peaks between H-8 (δ 5.88) and H-2a (δ 2.55) were also observed. Secondly, although the absolute stereochemistry at C-4 of aspergillide B (previously proposed as **5**) had been determined by the modified Mosher's method, that at C-13 was assumed, without an experiment, to be opposite to that at C-13 of aspergillide A (previously proposed as **3**), because the stereochemistry at C-3, -4, and -7 of both compounds had been thought to be the same.

With respect to structure **6** of aspergillide C, the absolute configurations as C-4 and -13 had been established by the modified Mosher's method.[1] Moreover, because of the presence of C_5=C_6 in **6**, separation of the ^{1}H NMR signals was much better than that observed for aspergillides A and B, we were able to reconfirm the trans stereochemistry at C-3 and C-7 of **6**.

Our comparison of the structures of aspergillides A (**1**) and B (**3**) led us to wonder whether or not the two compounds could be equilibrated via a *retro*-Michael reaction (cleavage of C_3–O forming C_2=C_3). Allowing **3** on silica gel to stand for 24 h did not yield a trace of **1**. Reaction of **1** with sodium methoxide afforded a methanolysis product as a single isomer.[1] Therefore, it seems most likely that both compounds are genuine natural products.

We would like to express our sincere gratitude to Professor Jun'ichi Uenishi of Kyoto Pharmaceutical University for his kind discussions about the structures and chemistry of aspergillides.

References and Notes

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2 S. M. Hande, J. Uenishi, *Tetrahedron Lett.* **2009**, *50*, 189.
3 I. Ohtani, T. Kusumi, Y. Kashman, H. Kakisawa, *J. Am. Chem. Soc.* **1991**, *113*, 4092.
4 Crystallographic data reported in this manuscript have been deposited with Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC-710343 for **2** and -710344 for **4**. Copies of the data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge, CB2 1EZ, UK; fax: +44 1223 336033; or deposit@ccdc.cam.ac.uk).
5 Supporting Information is also available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

Published on the web (Advance View) April 5, 2009; doi:10.1246/cl.2009.384