Creation of novel caspase-1 inhibitors through biosynthetic studies of berkeleydione

Yudai MATSUDA

Graduate School of Pharmaceutical Sciences, The University of Tokyo

Research aims

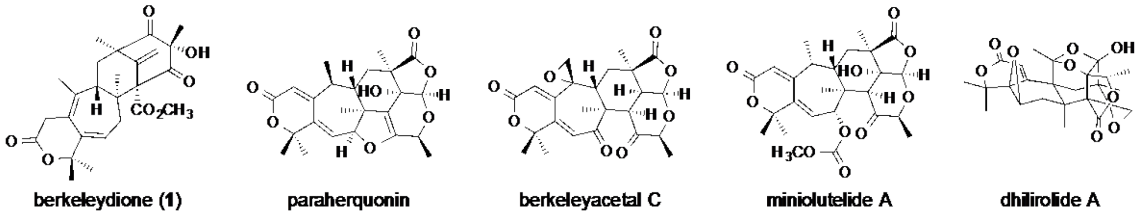

Figure 1. Structures of berkeleydione (**1**) and its related natural products

Berkeleydione (**1**) is a natural product that was isolated as an inhibitor of caspase-1 and as a potential inhibitor of interleukin 1-β production by inflammasomes; it serves as the key intermediate for the biosynthesis of many other meroterpenoids.[1)] Thus, biosynthetic studies of **1** should contribute to clarifying the biosyntheses of many medicinally important natural products. In this study, we aimed to determine the biosynthetic pathway of **1** at the molecular level, and to enzymatically derivatize **1** into a compound with improved biological activity.

Methods

Penicillium brasilianum NBRC 6234 was obtained from the National Institute of Technology and Evaluation. Metabolites derived from each transformant were analyzed using HPLC. Then, *prh* gene-derived compounds were purified via silica-gel chromatography, followed by preparative HPLC, and were characterized using MS and NMR analyses.

Results

To obtain biosynthetic genes for producing berkeleydione (**1**), we initially focused on paraherquonin, which appears to be biosynthesized via **1**. We then performed whole genome sequencing of *P. brasilianum* NBRC 6234, a known producer of paraherquonin, and discovered a putative biosynthetic gene cluster (the *prh* cluster) for paraherquonin. The first

five steps of paraherquonin biosynthesis were predicted to be identical to those of austinol, producing the tetracyclic compound protoaustinoid A (**2**).

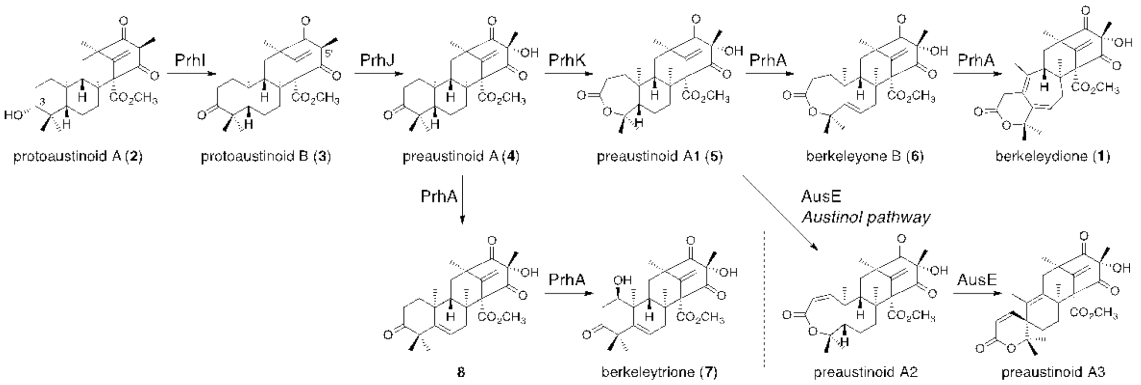

Figure 2. Biosynthetic pathway of berkeleydione (**1**)

The *prh* cluster encodes eight putative tailoring enzymes, including one Fe(II)/α-ketoglutarate(α-KG)-dependent dioxygenase (PrhA), one short-chain dehydrogenase/reductase (SDR; PrhI), and two FAD-dependent monooxygenases (FMOs; PrhJ and PrhK). Since close homologs of PrhI are utilized in the biosyntheses of several other meroterpenoids to oxidize the C-3 alcohol group of the terpene cyclase product, we reasoned that PrhI accepts the cyclized product **2** and performs the C-3 oxidation to yield **3**. The next two conversions would involve two FMOs, PrhJ and PrhK, which are similar to AusB and AusC, respectively. Because AusB and AusC are engaged in C-5′ hydroxylation and Baeyer-Villiger oxidation, respectively, it is most likely that PrhJ and PrhK perform the same functions, and that both generate preaustinoid A (**4**) and preaustinoid A1 (**5**) from **3**. In the austinol pathway, **5** then undergoes two successive oxidations—catalyzed by Fe(II)/α-KG-dependent dioxygenase AusE—to yield preaustinoid A3 with a spirolactone system, via preaustinoid A2. The *prh* cluster does encode a protein homologous to AusE, PrhA; however, given the structural differences between paraherquonin and austinol, PrhA should have an activity distinct from that of AusE. Thus, it seemed that PrhA would accept **5** but generates a different product, berkeleydione (**1**), probably via berkeleyone B (**6**) (Figure 2).

To prove a biosynthetic hypothesis for berkeleydione (**1**), the four genes were introduced into a heterologous host, *A. oryzae* NSAR1, and the resulting transformants were cultivated in the presence of **2**. The transformant with *prhI* alone converted **2** into **3**, which was confirmed to be the analog of **2** with C-3 ketone functionality, and was named protoaustinoid B. Two-gene and three-gene expression systems, [with *prhI* and *prhJ*] and [with *prhI*, prhJ, and *prhK*], respectively, were subsequently analyzed, and their transformants produced **4** and **5**, respectively. Finally, the strain harboring all four genes converted **2** into **1,** as well as into

7. These analyses demonstrated that PrhA had an activity different from that of AusE. We then constructed a three-gene expressing system lacking the FMO gene *prhK*, because the berkeleydione-producing fungus also yielded berkeleytrione (**7**), which seemed to be synthesized without Baeyer-Villiger oxidation. The transformant successfully produced **7**, which should be produced via **8**. Collectively, PrhA is a multifunctional dioxygenase that accepts both **4** and **5** to perform two sequential oxidations.

Conclusion

In this study, we determined the molecular basis for the biosynthesis of berkeleydione (**1**).[2)] In future studies, we will analyze the functions of the enzymes that convert **1** into paraherquonin, and we will construct artificial metabolic pathways using the *prh* genes and their homologs. This could contribute to the production of berkeleydione analogs with improved biological activity.

References

1) Matsuda, Y., Abe, I. (2016) Biosynthesis of fungal meroterpenoids. *Nat. Prod. Rep.* **33:** 26–53.
2) Matsuda, Y. *et al.* (2016) Discovery of key dioxygenases that diverged the paraherquonin and acetoxydehydroaustin pathways in *Penicillium brasilianum*. *J. Am. Chem. Soc.* **138:** 12671–12677.