



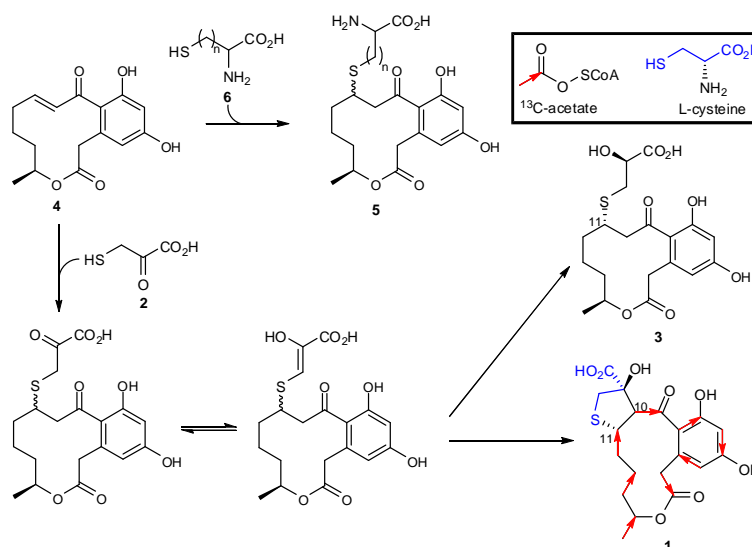
## Involvement of L-cysteine in the detoxification process of curvularins

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Curvularins, first described in the 1960's, constitute a group of metabolites of particular interest in polyketide biosynthesis investigations. Cyclothiocurvularins A (**1**) and B, have been recently isolated by us from the culture medium of a marine-derived *Penicillium* sp. DRF2. Compound **1** is a curvularin condensed with a 3-mercaptolactate (**2**) residue. In order to establish the origin of the sulfur-bearing moiety, a series of experiments have been performed. Feeding experiments using [1-<sup>13</sup>C]acetate, [1,2-<sup>13</sup>C<sub>2</sub>]acetate and [U-<sup>13</sup>C<sub>3</sub><sup>15</sup>N]-L-cysteine showed, after (+)-HRESIMS and <sup>13</sup>C NMR analyses, that the benzene and the macrocyclic moieties of **1** were formed by the condensation of eight intact acetate units, as observed in previous biosynthetic studies [1]. Feeding experiments with [U-<sup>13</sup>C<sup>15</sup>N]-L-cysteine exhibited 100% of incorporation at the of 3-mercaptolactate moiety and unambiguously established L-cysteine as the precursor of **2**. Sumalarin C (**3**) [2] and its isomer 11R have also been isolated from the culture of *Penicillium* sp. DRF2, as well as a pair of isomers (**5**, n=1) corresponding to  $\alpha,\beta$ -dehydrocurvularin (**4**) condensed with L-cysteine (**6**, n=1). We suspected that **5** was a product of spontaneous Michael-addition of L-cysteine into  $\alpha,\beta$ -dehydrocurvularin, as a non-natural product. In order to test this hypothesis, homocysteine (**6**, n=2) was added to the growth medium of *Penicillium* sp. DRF2 as it was made with L-cysteine. Extensive chromatographic purification led to the isolation of **5** (n=2), confirming our hypothesis. Considering that cyclothiocurvularins A and B did not present cytotoxic activity, that a very high incorporation rate of cysteine into cyclothiocurvularin A was observed, that diastereoisomers of **1**, **3**, and **5** were also isolated from the growth medium, cyclothiocurvularins appears to be a detoxification product of  $\alpha,\beta$ -dehydrocurvularin, by incorporation of L-cysteine after its transformation into **2**. 3-mercaptolactate (**2**) is

probably the reactive species to be condensed to the polyketide chain after the aromatic ring construction and cyclization. Compound **2** probably reacts with **4** via a Michael type addition to the conjugated double bond, as a post-functionalization of the curvularin backbone. The incorporation of 3-mercaptolactate into fungal natural products has been rarely observed, but appears to be a detoxification process of highly cytotoxic secondary metabolites, such as  $\alpha,\beta$ -dehydrocurvularin.



### References:

- [1] Arai, K., Rawlings, B.J., Yoshizawa, Y. and Vederas, J. C. 1989. J. Am. Chem. Soc. 111: 3391.  
 [2] Meng, L., Li, X., Lv, C., Li, C., Xu, G., Huang, C., Wang, B. 2013. J. Nat. Prod. 76:2145.

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