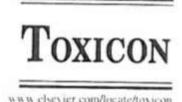


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Short Communication

Isolation of Adda from microcystin-LR by microbial degradation

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Abstract

The intact Adda was isolated from microcystin-LR by a microbial degradation using an isolated Sphingomonas strain. B.9. The reaction of microcystin-LR with cell extract of this strain proceeded smoothly to give the final degradation product by way of two intermediates, linearized microcystin-LR and a tetrapeptide. The purified Adda that was structurally characterized using various spectral data did not show the toxicity to mice or inhibition to protein phosphatase activity in contrast to the native toxin.

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Microcystins, the cyclic heptapeptide toxins produced by freshwater cyanobacteria such as Microcystis, show potent hepatotoxicity and tumor-promoting activity through inhibition of protein phosphatases 1 and 2A (Kuiper-Goodman et al., 1999; Sivonen and Jones, 1990). A toxic incident leading to the deaths of over 50 persons occurred in Brazil in 1996 due to microcystins in the water used for hemodialysis (Jochimsen et al., 1998; Pouria et al., 1998). Now microcystins are threatening human health and life, and many problems associated with these toxins, such as toxic mechanism and biosynthesis, remain unsolved. Microcystins and nodularins (Rinehart et al., 1988), which are also hepatotoxic pentapeptides produced by a brackish cyanobacterium, Nodularia, contain invariably a characteristic β-amino acid, Adda ((2S,3S,8S,9S)-3-amino-9-methoxy-2.6.8-trimethyl-10-phenyldeca-4(E).6(E)-dienoic acid) as one of the constituent amino acids. Adda is essential for the characteristic biological activities of microcystins and

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nodularins because the toxicity disappears completely by ozonolysis of the Adda portion, and the geometrical isomers of Adda do not exhibit biological activity (Harada, 1996). Although the important role of Adda has been widely recognized, intact Adda has not yet been isolated from these hepatotoxins due to its instability under acidic conditions. Actually, acid hydrolysis of microcystin-LR (MCLR) gave an Adda portion with the loss of methanol (Fujii et al., 1997). During the course of an ecological study, we isolated a bacterium that could degrade microcystins and was identified as Sphingomonas sp. In the present study, we isolated the intact Adda from MCLR by microbial degradation using the isolated Sphingomonas strain, B-9.

The B-9 strain could be cultivated in a usual medium composed of peptone, yeast extract and glucose (4:2:1) and the growth reached its maximum in 2 or 3 days. After the disintegration of the cells harvested in Tris-HCl buffer with a French press, a mixture of this extract and MCLR (40 mg) was incubated for 2 days at 27 °C. As shown in Fig. 1, the reaction proceeded smoothly to give the desired product by way of two intermediates, linearized microcystin-LR and tetrapeptide that could be detected by LC/MS. The reaction mixture was adsorbed on an ODS silica gel cartridge

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