4-CHLOROTHREONINE, A HERBICIDAL ANTIMETABOLITE PRODUCED BY *Streptomyces* sp. OH-5093

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(Received for publication May 13, 1994)

In the course of screening for herbicidal substances from antimetabolites produced by microorganisms, we have isolated a metabolite of *Streptomyces* sp. OH-5093 which inhibits the growth of radish and sorghum.The structure was established to be 4-chlorothreonine (**1**). We also found that 4-chlorothreonine is an amino acid antimetabolite with anti-*Candida* activity. 4-Chlorothreonine is a constituent of the naturally occurring syringostatins[1,2] and syringomycin[3,4] and is a product of synthesis[5,6] and enzymatic conversion[7]. However, no papers have reported the occurrence of 4-chlorothreonine as a microbial metabolite nor its herbicidal properties. This note describes the fermentation, isolation, structure identification and biological activities of 4-chlorothreonine.

4-Chlorothreonine was produced by *Streptomyces* sp. OH-5093 in a shake flask fermentation. *Streptomyces* sp. OH-5093 was isolated from a soil sample. A loopful of a slant culture of *Streptomyces* sp. OH-5093 was inoculated in a 50 ml-test tube containing 10 ml of a seed medium consisting of glucose 0.1%, starch 2.4%, peptone 0.3%, meat extract 0.3%, yeast extract 0.5% and $CaCO_3$ 0.4%, pH 7.0, incubated on a reciprocal shaker (300 rpm) at 27°C for 48 hours. The culture broth was transferred into a 500 ml-Erlenmyer flask containing 100 ml of the same seed medium, and incubated at 27°C for 48 hours on a rotary shaker (210 rpm). Vegetative seed culture (2%, v/v) was transferred into a 30-liter jar fermentor containing 20 liters of a production medium consisting of maltose 5.0%, dry yeast 1.5%, Ebios tablets (Asahi Breweries Ltd.) 2.5%, KBr 1.0%, KH_2PO_4 0.05% and $MgSO_4 \cdot 7H_2O$ 0.05%, pH 7.0 and incubated at 27°C for 96 hours with agitation and aeration. The herbicidal activity was assayed by incubating radish (*Rhaphanus sativus* L.) seeds and sorghum (*Sorghum bicolor* Moench) seeds on a defatted cotton piece containing an appropriate amount of a test sample in a test tube (2 × 10 cm) at 27°C for 3 to 4 days under lightening. The antifungal activity was determined by the conventional paper disc (i.d. 8 mm, thick) method using *Candida albicans* KF-1. The reversal of growth inhibition was determined by the addition of 250 μg of an amino acid to the paper disc containing 4-chlorothreonine.

4-Chlorothreonine was purified by the following procedures. Broth filtrate (40 liters) was passed through a column of Dowex 50W × 8 (H^+ type, 3 liters). After washing with water, the active compound was eluted with 1 N and 2 N HCl and neutralized. After deionization by a microacilyzer (Micro acilyzer G3, Asahi Chemical Industry Co., Ltd.), the active material was adsorbed on a column of Dowex 50W × 8 (pyridine type, 400 ml), and then the active compound was eluted by 0.2 M pyridine-HCOOH buffer (pH 3.1 and 4.4). The active fraction was evaporated under reduced pressure to give *ca.* 8 g of a crude active material. This material was dissolved in a small amount of water, put on a column of Sephadex G-10 (200 ml), and developed with water. The active fractions were evaporated under reduced pressure to give 1.7 g of crude material. This material was further purified by a preparative HPLC (Capcell pak C_{18} SG-120, 20 × 250 mm, Shiseido Co., Ltd.) developed with 0.1% trifluoroacetic acid. The active fractions were evaporated under reduced pressure to give 520 mg of crude active material. This material was dissolved in a small amount of methanol, applied on a column of Sephadex LH-20 and developed with 50% methanol. The active fractions were concentrated to give 140 mg of pure compound as white powder. The active compound was identified to be 4-chlorothreonine by physico-chemical properties as follows: appearance, white powder; molecular

Fig. 1. Structure of 4-chlorothreonine.

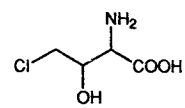

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Table 1. Herbicidal activities of 4-chlorothreonine.

Compound	Rate (μg/tube)	Growth inhibition (%)	
		Radish	Sorghum
4-Chlorothreonine	120	70	80
	30	30	30
Bialaphos	120	90	80
	30	40	50

Radish and sorghum seeds were grown in small test tubes (2 cm × 10 cm) at 27°C for 4 days under lighting. Plant heights were compared.

formula $C_4H_8NO_3Cl$ elucidated by HR FAB-MS ((M+H), *m/z* 154.0287); MP 142~143°C; $[\alpha]_D^{25}$ −81 (*c* 0.43, H_2O); UV $\lambda_{max}^{H_2O}$ nm (ε) 245 (1,600); IR ν_{max} (KBr) cm^{-1} 3400, 3120, 1670, 1640, 1590, 1520, 1400, 1360, 1020, 770, 650, 530; ^1H NMR (400 MHz, D_2O) δ 3.67 (1H, dd, *J*=5.5, 11.5 Hz), 3.71 (1H, dd, *J*=5.5, 11.5 Hz), 3.80 (1H, d, *J*=5.5 Hz), 4.24 (1H, q, *J*=5.5 Hz); ^{13}C NMR (100 MHz, D_2O) δ 172.7 (C=O), 70.0 (CH), 57.4 (CH), 46.6 (CH_2); soluble in water and methanol; hardly soluble in chloroform; color reaction, positive to ninhydrin.

The growth of radish and sorghum was reduced by the treatment of 4-chlorothreonine at more than 30 μg/test tube (Table 1). The compound inhibited the growth of *Candida albicans* on a synthetic medium (inhibition zone: 21 mm at 50 μg/paper disc) and the growth inhibition was reversed by the addition of 250 μg/paper disc of each one of L-alanine, proline, threonine and DL-γ-aminobutyric acid (data not shown). It is suggested that the herbicidal activity was caused by inhibition of amino acid metabolism.

4-Chlorothreonine has been cited as a constituent of syringostatins and syringomycin, metabolites of *Pseudomonas syringae* pv. *syringae*[1~4] and a synthetic compound. This is the first account on the occurrence of 4-chlorothreonine in its free form in nature, together with its herbicidal and anti-*Candida* activities. 4-Chlorothreonine is an important intermediate of β-lactam antibiotics synthesis. Therefore production of 4-chlorothreonine by fermentation is a useful way of providing intermediates of β-lactam antibiotics synthesis.

Acknowledgments

The authors would like to acknowledge Mr. KEN-ICHI SATO for his technical assistance.

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