ANTITUMOR ACTIVITY OF HEPTELIDIC ACID CHLOROHYDRIN

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In the course of our search for new anti-tumor substances, it was found that a strain of *Acremonium* sp. produced a cytotoxic metabolite **1** against tumor cells. It was identified as heptelidic acid chlorohydrin which was formerly reported as a toxic compound to spruce budworm described by Calhoun *et al.*[1].

The producing fungi strain No. 618 was identified as *Acremonium* species, and named *Acremonium* sp. No. 618. Strain No. 618 was isolated from a rotten wood collected at Minoh city, Osaka prefecture, Japan. It has been deposited in the National Institute of Bioscience and Human-Technology Agency of Industrial Science and Technology, Ministry of International Trade and Industry, Tsukuba-shi, Japan, under the accession No. FERM P-14211.

Strain No. 618 was inoculated into 100 ml of potato-dextrose medium in a 500-ml Erlenmeyer flask, and cultured at 28°C for 7 days on a rotary shaker (200 rpm) to obtain the seed culture. One liter of this culture was inoculated into a 10-liter jar fermentor containing 6 liters of potato-dextrose medium. The fermentation was continued for 4 days at 28°C with an air-flow rate of 0.5 v/v/m and an agitation rate of 200 rpm. The pH of the culture filtrate was 5.4.

Active compound **1** (180 mg) was obtained from six liters of culture filtrate. The isolation scheme is summarized in Fig. 1.

The physico-chemical properties of **1** are shown in Table 1. It was soluble in benzene, chloroform, acetonitrile, dimethyl sulfoxide, and methanol, but insoluble in water.

The ^1H and ^{13}C NMR assignments and relative stereochemistry of **1** were confirmed by analyses of 2D H-H COSY, C-H COSY, HMBC and NOESY spectral data as follows; ^1H NMR (300 MHz, $CDCl_3$) δ 7.28 (1H, d, J=4.5 Hz, 12-H), 5.11 (2H, s, 3-H), 4.75 (1H, d, J=12.1 Hz, 7-H), 3.77 (1H, d, J=12.2 Hz, 7-H), 3.52 (1H, d, J=12.1 Hz, 5-H), 2.52 (1H, dt, J=4.5, 11.7 Hz, 11-H), 2.42 (1H, d, J=12.9 Hz, 8-H), 2.07 (1H, m, 13-H), 1.76 (1H, dd, J=3.4, 13.4 Hz, 9-H), 1.50 (1H, bt, 10-H), 1.30 (1H, m, 9-H), 1.25 (1H, m, 8-H), 0.95 (3H, d, J=6.8 Hz, 15-H), 0.88 (3H, d, J=6.8 Hz, 14-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.9 (s, 4-C), 169.9 (s, 1-C), 148.5 (d, 12-C), 129.2 (s, 2-C), 73.7 (s, 6-C), 61.9 (t, 3-C), 53.5 (d, 5-C), 49.2 (d, 10-C), 47.4 (t, 7-C), 40.3 (d, 11-C), 35.2 (t, 8-C), 28.0 (d, 13-C), 21.7 (q, 15-C), 21.6 (t, 9-C), 15.9 (q, 14-C).

Based on the above spectral data, **1** was identified with heptelidic acid chlorohydrin as shown in Fig. 2.

Fig. 1. Isolation and purification of heptelidic acid chlorohydrin.

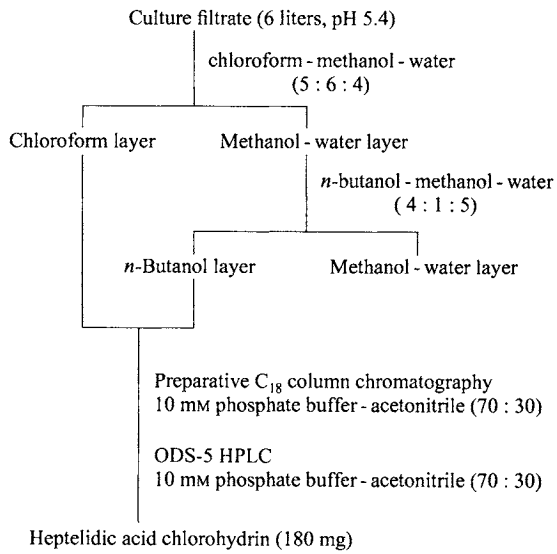
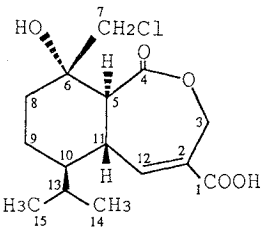


Table 1. Physico-chemical properties of heptelidic acid chlorohydrin.

Appearance	Brown oil
Molecular formula	$C_{15}H_{21}O_5Cl$
FAB-MS	m/z 317 (MH^+)
HRFAB-MS	m/z 317.1152 (Found)
	m/z 317.1156 (Calcd. for $C_{15}H_{22}O_5Cl$)
$[\alpha]_D^{20}$	+3.4° (c 1, MeOH)
UV λ_{max}^{MeOH} nm (ε)	215 (8,000)
IR ν_{max} (KBr) cm^{-1}	3450, 1720

Fig. 2. Structure of heptelidic acid chlorohydrin.



Compound **1** was tested for *in vitro* cytotoxicity of some kinds of human culture cells by the MTT (3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyl tetrazolium bromide)-microculture tetrazolium assay method described by SCUDIERO[2]. The culture cells were exposed to the compound for 72 hours and further 4 hours incubation was carried out for the incorporation of MTT into the cells. IC_{50} values of IMR-90 (normal diploid fibroblast of lung), SW-13 (adrenal cortex adenocarcinoma), G361 (malignant melanoma), IMR-32 (neuroblastoma) were 0.74 μg/ml, 0.13 μg/ml, 0.28 μg/ml, 0.24 μg/ml, respectively.

Heptelidic acid chlorohydrin was reported as a metabolite toxic to spruce budworm[1]. Heptelidic acid, structurally similar compound of heptelidic acid chlorohydrin, was reported to have antimicrobial activity[3] and inhibitory activity of glycelaldehyde-3-phosphate dehydrogenases[4]. This is the first report that heptelidic acid chlorohydrin has cytotoxicity against tumor cells. Thus the compound could be applied for the treatment of tumors such as adrenal cortex adenocarcinoma.

References

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