VOL. 52 NO. 8, AUG. 1999 THE JOURNAL OF ANTIBIOTICS pp. 754 — 757

NOTES

Novel Cyclopentanone Derivatives Pentenocins A and B, with Interleukin-1β Converting Enzyme Inhibitory Activity, Produced by *Trichoderma hamatum* FO-6903

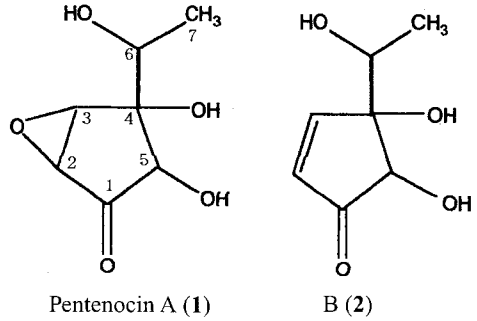
TSUKASA MATSUMOTO, AKI ISHIYAMA, YUUICHI YAMAGUCHI, ROKURO MASUMA, HIDEAKI UI, KAZURO SHIOMI, HARUKI YAMADA and SATOSHI ŌMURA*

Research Center for Biological Function, The Kitasato Institute, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8642, Japan

(Received for Publication June 4, 1999)

Interleukin-1β converting enzyme (ICE, caspase-1) is known as a unique cysteine protease which converts the biologically inactive precursor of interleukin-1β (IL-1β) to its mature biologically active form, an important mediator for inflammation[1~3]. In our screening for novel ICE inhibitor of microbial origin, we found an inhibitory activity in the cultured broth of *Trichoderma hamatum* FO-6903. The active principles were purified from a cultured broth of FO-6903, and their structures were elucidated as novel cyclopentanone derivatives, which we named pentenocins A (**1**) and B (**2**) (structures ware shown in Fig. 1). Herein, we report the fermentation, isolation, structure elucidation and inhibitory activity of pentenocins A and B.

Fig. 1. Structures of pentenocins A (**1**) and B (**2**).



Pentenocin A (**1**) B (**2**)

Materials and Methods

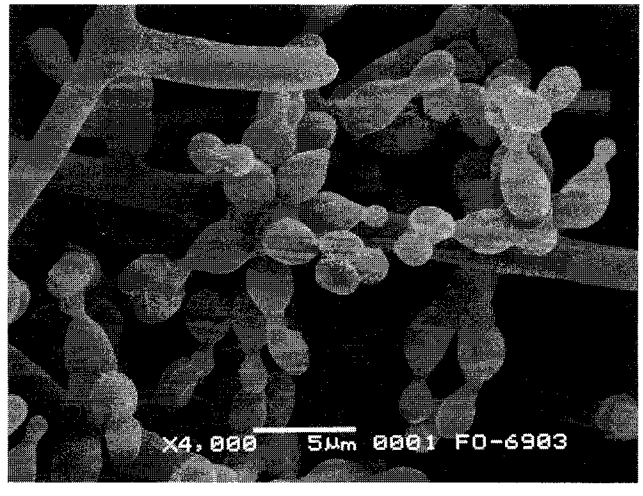
Taxonomy of Producing Organism

The producing fungal strain was isolated from a soil sample collected at Saitama Prefecture, Japan. Morphological properties were examined on potato dextrose agar (Difco), malt extract agar and oat meal agar. Colonies grown on each media were more than 90 mm in diameter after incubation at 25°C for 5 days. This strain grew moderately to form white or whitish green to green colonies which were floccose, generally with compact tufts of conidiophores. The hyphae were branched, smooth-walled and hyaline. Conidiophores were long and thick, often with sterile hyphal elongations. Phialides which grew on short and thick side branches were crowded, short, plump, and 4.0~7.5×3.0~4.0 μm in size. Conidia were short-cylindrical, green, smooth-walled, and 3.0~4.0×2.5~3.0 μm in size (Fig. 2). From the above characteristics, strain FO-6903 was identified as *Trichoderma hamatum*[4] and named *Trichoderma hamatum* FO-6903. The culture was deposited in the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, Japan with an accession number of FERM P -16787.

Fermentation

A loopful of strain FO-6903 from a mature slant culture was inoculated into a 500-ml Erlenmeyer flask containing

Fig. 2. Scanning electron micrograph of *Trichoderma hamatum* FO-6903.



100 ml of seed medium consisting of glucose 2.0%, yeast extract (Oriental Yeast Co.) 0.2 %, $MgSO_4 \cdot 7H_2O$ 0.05%, Polypepton (Nihon Pharmaceutical Co.) 0.5%, KH_2PO_4 0.1%, agar 0.1% (adjusted to pH 6.0 before sterilization) and cultured on a rotary shaker (200 rpm) at 28°C for 2 days. One milliliter of the seed culture was inoculated into each 500-ml Erlenmeyer flasks containing 100 ml of production medium consisting of dextrin 3.0%, glycerol 1.0 %, wheat germ (Sigma) 0.5%, Phermamedia (Traders Protein) 1.0%, soybean peptide (Fuji Oil Co.) 0.5%, $NaNO_3$ 0.2%, $CaCO_3$ 0.2%, allophane (Shinagawa Chemicals Co., LTD.) 0.5%, and 1% (v/v) of trace salts solution which contained 1g/liter of $FeSO_4 \cdot 7H_2O$, $CoCl_2 \cdot 6H_2O$, $MgCl_2 \cdot 4H_2O$, and $ZnSO_4 \cdot 5H_2O$ (adjusted to pH 6.5 before sterilization). The fermentation was carried out at 27°C for 4 days on a rotary shaker (200 rpm).

Isolation of Active Substances

Active substances were purified by the following procedures: Ethanol (2 liters) was added to the cultured broth (2 liters) and the mixture was stirred for 30 minutes. The mixture was centrifuged at 3,500 rpm for 30 minutes and then supernatant was evaporated *in vacuo* to remove ethanol. The aqueous solution was partitioned between H_2O and EtOAc, and the aqueous layer was applied to a column of Diaion HP-20 (500 milliliters, Mitsubishi Chemical Co, Japan). Unabsorbed fraction was concentrated to small volume and chromatographed on a Sephadex LH-20 column (2.7×91 cm) using 25% methanol as the eluent. The active fractions were collected and evaporated *in vacuo*. The resulting active substance was dissolved in a small amount of H_2O, applied to ODS column (Develosil C_{18}, i.d. 25×250 mm, flow rate 2.0 ml/minute) and eluted with water. At retention times from 40 to 50 minutes, active substances were eluted. They were concentrated and purified by reversed phase HPLC column (Daiso Pak C_{18} BP, i.d. 20×250 mm) with 0.05% TFA, and active fractions were re-chromatogrphed on the same column with water to gave **1** and pure **2** (5 mg). Compound **1** was further purified by HPLC (Aquasil silica, i.d. 20×250 mm; EtOAc saturated with water) to afford a pure light brown resin (13.9 mg).

Spectroscopic Studies

UV spectra were recorded on a Hitachi U-2000 spectrophotometer. IR spectra were recorded on a Horiba FT-210 FT-IR spectrometer. FAB-MS spectra were recorded on JMS-DX300 and JMS-AX505 HA mass spectrometers. The various NMR spectra were obtained on a Varian Unity 400 spectrometer.

Assay of ICE Activity

ICE activity was monitored essentially as described by THORNBERRY *et al.*[1]. Briefly, recombinant human ICE was used as a source of enzyme, and fluorogenic substrate, Ac-Tyr-Val-Ala-Asp-Amino methyl coumarin (AMC), was used. The release of AMC as an enzyme activity was monitored over time using a Fluoroskan II fluorometer (Labosystems) at a excitation and emission of 355 and 460 nm, respectively.

Results and Discussion

Physico-chemical properties of **1** and **2** are summarized

Table 1. Physico-chemical properties of pentenocin A (**1**) and B (**2**).

	1	**2**
Appearance	Light brown resin	Colorless resin
Molecular formula	$C_7H_{10}O_5$	$C_7H_{10}O_4$
FAB-MS (positive) HR FAB-MS (m/z)	175(M+H)$^+$	159(M+H)$^+$
Found(M+H)$^+$	175.0610	159.0674
Calcd.	175.0606	159.0657
UV λ_{max}^{H2O} nm	220	210
IR ν_{max} cm^{-1}	3400, 1765, 858	3400, 1712, 1681, 1385
Color reaction		
20% H_2SO_4	positive	positive
Ninhydrin	negative	negative
Dragendorff 's reagent	negative	negative
Orcinol-H_2SO_4	positive	positive

Table 2. ^{13}C and ^{1}H NMR data of **1** and **2**.

Carbon	**1**		**2**	
	δ_C	δ_H	δ_C	δ_H
1	205.4	-	208.4	-
2	55.0	3.57 (1H, d, J=3.0)	131.9	6.15 (1H, d, J=6.0)
3	58.3	3.86 (1H, d, J=3.0)	165.5	7.47 (1H, d, J=6.0)
4	76.5	-	78.7	-
5	75.9	4.06 (1H, s,)	70.7	3.91 (1H, s,)
6	66.0	3.80 (1H, q, J=6.5)	69.2	3.68 (1H, q, J=6.5)
7	17.0	1.15 (3H, d, J=6.5)	17.9	1.11 (3H, d, J=6.5)
OH		4.65 (1H, s,)		4.79 (1H, s,)
		4.95 (1H, s,)		4.85 (1H, s,)
		5.60 (1H, s,)		5.30 (1H, s,)

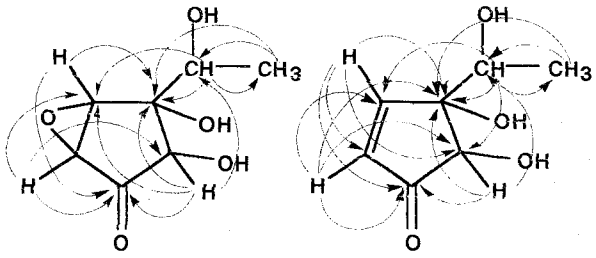
^{1}H (400 MHz) and ^{13}C (100 MHz) spectra were measured in DMSO-d_6.
Coupling constants are given in Hz.

in Table 1, and ^{1}H and ^{13}C NMR spectral data are in Table 2. The HMQC experiments revealed the connectivity of each proton and carbon. The molecular formula of **1** and **2** were determined by HR FAB-MS and ^{13}C NMR spectrum analyses as $C_7H_{10}O_5$, and $C_7H_{10}O_4$, respectively.

The IR spectrum of **1** suggested the presence of hydroxyl (3400 cm^{-1}) and carbonyl (1765 cm^{-1}) groups. In ^{13}C NMR spectrum, a signal at 205.4 ppm showed existence of carbonyl group. In ^{1}H NMR, **1** gave a signal at $\delta_{7\text{-}H3}$ 1.15 ppm based on methyl coupled to the oxymethine at $\delta_{6\text{-}H}$ 3.80 ppm with J=6.5 Hz. Two methine proton signals, $\delta_{2\text{-}H}$ 3.57 and $\delta_{3\text{-}H}$ 3.86, were coupled with J=3.0 Hz, and signals of 2-CH (δ_C 55.0, J_{CH}=195.28 Hz) and 3-CH (δ_C 58.3, J_{CH}=191.40 Hz) proved the presence of an epoxy moiety between C-2 and C-3. The 4-(1-hydroxyethyl)-cyclopentanone ring was revealed by HMBC as shown in Fig. 3. Consequently, the structure of **1** was elucidated.

The structure of **2** was determined by comparison of its spectra with those of **1**. The ^{1}H and ^{13}C NMR spectra were similar to those of **1** (see Table 2). However, two epoxymethine signals were absent, and two orefinic signals of 2-CH (δ_C 131.9, δ_H 6.15, J=6.0 Hz) and 3-CH (δ_C 165.5, δ_H 7.47, J=6.0 Hz) were observed in **2**. The IR absorption at 1681 cm^{-1} also suggested the α,β-unsaturated ketone. In addition to above spectral evidences, all the assignments and structure were ascertained by the HMBC experiment (Fig. 3), and the structure of **2** was elucidated. The new compounds, **1** and **2** were named pentenocins A and B, respectively. Details of stereochemistry are now underway.

Fig. 3. HMBC correlations of **1** and **2**.



Both pentenocins A (**1**) and B (**2**) inhibited ICE weakly, and IC_{50} were 575 μM and 250 μM, respectively. Detailed investigations on other biological activities are also now underway.

Acknowledgments

The authors wish to thank Dr. T. SUNAZUKA, School of Pharmaceutical Sciences, Kitasato University, for useful discussion. A part of work was supported by funds from the Japan Keirin Association.

References

1) THORNBERRY, N. A.; H. G. BULL, J. R. CALAYCAY, K. T.

CHAPMAN, A. D. HOWARD, M. J. KOSTURA, D. K. MILLER, S. M. MOLINEAUX, J. R. WEIDNER, J. AUNINS, K. O. ELLISTON, J. M. AYALA, F. J. CASANO, J. CHIN, G. J.-F. DING, L. A. EGGER, E. P. GAFFNEY, G. LIMJUCO, O. C. PALYHA, S. M. RAJU, A. M. ROLANDO, J. P. SALLEY, T.-T. YAMIN, T. D. LEE, J. E. SHIVELY, M. MACCROSS, R. A. MUMFORD, J. A. SCHMIDT & M. J. TOCCI: A Novel heterodimeric cysteine protease is required for interleukin-1β processing in monocytes. Nature 356: 768~774, 1992

2) CERRETTI, D. P.; C.J. KOZLOSKY, B. MOSLEY, N. NELSON, K. V. NESS, T. A. GREENSTREET, C. J. MARCH, S. R. KRONHEIM, T. DRUCK, L. A. CANNIZZARO, K. HUEBNER & R. A. BLACK: Molecular cloning of the interleukin-1β converting enzyme. Science 256: 97~100, 1992

3) TOCCI, M. J.: Structure and function of interleukin-1 beta converting enzime. Vitam Horm 53: 27~63, 1997

4) DOMSCH, K. H.; W. GAMS & T.-H. ANDERSON: Compendium of Soil Fungi, Volume I., pp.794~809, IHW-Verlag, 1993