Communications to the Editor

Mer-W8020, A NOVEL DEPSIPEPTIDE WITH ANTI-INFLAMMATORY EFFECT, PRODUCED BY *Streptomyces griseoviridis*

Sir:

In the course of a screening program for new biologically active compounds, we isolated a new depsipeptide, Mer-W8020 from a culture broth of *Streptomyces griseoviridis* Mer-W8020. This compound proved to have an anti-inflammatory effect in some preliminary studies. In this paper, we report the production, isolation, physico-chemical properties and biological activities of Mer-W8020.

The Mer-W8020 producing organism was isolated from a soil sample collected in Fujisawa, Japan, and was classified as *S. griseoviridis* Mer-W8020 (FERM P-13374) according to the method of SHIRLING *et al.*[1].

Fermentation of Mer-W8020 was carried out at 28°C under aeration and agitation in a jar fermentor with the medium consisting of 2.5% glycerin, 0.5% meat extract, 0.5% Polypepton (Nippon Seiyaku), 1% yeast extract (Oriental Koubo), 0.2% NaCl, 0.05% $MgSO_4$, 0.05% KH_2PO_4, 0.32% $CaCO_3$, 0.05% Adekanol LG121 (antifoam, Asahi Denka); pH was adjusted to 7.4 before sterilization.

The production of Mer-W8020 was monitored by TLC analysis (chloroform - methanol - water, 80 : 10 : 1, Ehrlich detection, Rf 0.3). Mer-W8020 was accumulated in mycelium. The cultured broth (970 liters) was filtered and the mycelial cake was extracted with 400 liters of methanol. The methanol extract was concentrated *in vacuo* to an aqueous solution (75 liters), and was diluted with water (25 liters) and then extracted twice with 75 liters of *n*-BuOH. The organic layer was concentrated *in vacuo* to an oily residue. After washing the material with *n*-hexane, methanol was added to the residue. The methanol soluble fraction was concentrated *in vacuo* to dryness. The residue was dissolved in 100 ml of chloroform - methanol (20 : 1) and chromatographed on a column of silica gel (Merck, Art 7734, 1,000 ml). The active substance was eluted with the solvent of chloroform - methanol (20 : 1). The eluate was collected in fractions and examined by TLC (chloroform - methanol - water, 80 : 10 : 1, Ehrlich detection). The fractions containing the organic substances giving Rf 0.3 were combined, concentrated *in vacuo* to dryness, and dissolved in 20 ml of methanol. The methanol solution was purified by chromatography on a column of Sephadex LH-20 (Pharmacia, 160 ml) equilibrated with methanol. The active fractions were collected, concentrated *in vacuo*, and then dissolved in 20 ml of toluene - methanol (20 : 1). The next column chromatography was done with a silica gel (Merck, Art 7734, 500 ml) and active compound was eluted by toluene - methanol (5 : 1). The crude powder (8.2 g) containing Mer-W8020 was obtained from the dried fractions. Finally 5.8 g of Mer-W8020 was obtained as colorless needles by crystallization from toluene - methanol.

The physico-chemical properties of Mer-W8020 are summarized in Table 1. Mer-W8020 was soluble in methanol and acetone, but insoluble in water and *n*-hexane. Mer-W8020 shows positive responses to phosphomolybdic acid and Ehrlich reagents, but is negative to Ninhydrin and Dragendorff reagents. ^1H NMR spectrum of Mer-W8020 is shown in Fig. 1. From NMR spectroscopic studies of the compound derived by chemical degradation, the structure of Mer-W8020 was proposed to be a cyclic depsipeptide. Mer-W8020 turned out to possess only valine residues as a usual amino acid by the analysis of the components with HPLC and TLC. Structurally, Mer-W8020 is composed of a variety of *N*-methylated amino acids, which is also one of the typical characters of this compound. Details on the structure elucidation studies will be reported elsewhere.

The anti-inflammatory effects of Mer-W8020 on adjuvant arthritis were evaluated using the modified method of B. B. NEWBOULD[2]. Mer-W8020 was administered intraperitoneally six times a week from day 0 to 19. The thicknesses of adjuvant-injected

Table 1. Physico-chemical properties of Mer-W8020.

MP	178 ~ 181°C
FAB-MS	*m/z*: 1,472 (M + H)
HRFAB-MS	*m/z*: 1,472.8760
$[\alpha]_D^{23}$	−150.5°C (*c* 0.4, MeOH)
UV λ_{max}^{MeOH} nm (ε)	218 (50,500), 269 (5,700), 281 (4,900), 292 (4,700)
IR ν_{max} (KBr) cm^{-1}	3426, 3354, 2965, 1730, 1640, 1510
Rf value[a]	0.30

[a] TLC: Silica gel TLC plate F_{254} Art. 5715 (Merk). Solvent system: $CHCl_3$ - MeOH - water (80 : 10 : 1).

Fig. 1. 400 MHz ^{1}H NMR spectrum of Mer-W8020 in CD_3OD.

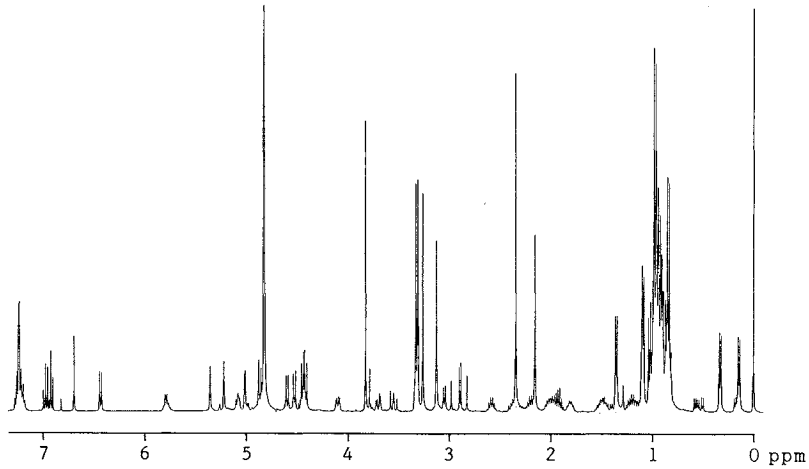

Table 2. Effect of Mer-W8020 on adjuvant arthritis in rats.

Day	Drug	Dose (mg/kg)	n	Paw thickness (mm)	
				Injected	Non-injected
Day 7	Control	—	9	10.0±0.4	5.8±0.1
	Mer-W8020	1	4	9.3±0.7	5.6±0.1
		10	6	8.3±0.1**	5.4±0.1*
Day 14	Control	—	9	13.1±0.1	9.8±0.4
	Mer-W8020	1	4	12.4±0.2*	8.1±0.8
		10	6	12.2±0.2**	7.7±0.5**
Day 19	Control	—	9	13.4±0.2	11.6±0.2
	Mer-W8020	1	4	12.5±0.4	9.7±0.8*
		10	6	11.7±0.2**	9.0±0.6**

Each value represents the mean±S.E. Significant difference from the control (vehicle): * $P<0.05$. ** $P<0.01$.

and non-injected paws were measured with a caliper on day 7, 14 and 19. As shown in Table 2, Mer-W8020, at doses of 1 and 10 mg/kg, significantly suppressed the swelling in both paws. Thus, Mer-W8020 turned out to have a potential activity with an anti-inflammatory effect on adjuvant arthritis, a model of chronic inflammation. Mer-W8020 also showed anti-inflammatory activity on carrageenin-induced paw edema, a model of acute inflammation, after intraperitoneal injection to SD rats at doses of 1 ~ 10 mg/kg (data not shown). It is well known that non-steroidal anti-inflammatory drugs such as indomethacin show anti-inflammatory and analgesic effects. Therefore, the analgesic effects of Mer-W8020 were examined using a model of acetic acid-induced writhing. Mer-W8020 showed marked analgesic effects in this model after intraperitoneal injection to ICR mice at doses of 1 ~ 10 mg/kg (data not shown). These results indicated that Mer-W8020 shows not only anti-inflammatory but also analgesic actions.

Components of cascades to yield arachidonate and its metabolites are known to be important mediators of inflammation. For example, phospholipase A_2[3], cyclooxygenase[4] and 5-lipoxygenase[5] have been shown to cause inflammation with increased activities frequently being associated with inflammation diseases. Mer-W8020 did not have direct inhibitory effects on these enzymes at the maximum dose of 100 μg/ml. Therefore, the precise mechanism by which Mer-W8020 suppressed the inflammation on adjuvant arthritis and carrageenin induced paw edema may be different from direct inhibitory actions on these enzymes. The mechanisms of action of Mer-W8020 are now under study.

Mer-W8020 showed no antimicrobial activities against *Bacillus subtilis* ATCC6633, *Staphylococcus*

aureus 209P and *Candida albicans* 3147 at a concentration of 50 μg/ml in disk diffusion assay. Mer-W8020 also has no growth inhibitory activity against mice leukemia cell L1210 when Mer-W8020 was added at 10 μg/ml to the culture medium. No acute toxicity was observed when Mer-W8020 was intraperitoneally injected into ICR mice at a dose of 100 mg/kg. Thus, Mer-W8020 was a low toxic anti-inflammatory agent with analgesic activity, and proved to be a potential compound for the clinical treatment of inflammation diseases such as rheumatoid arthritis or systemic lupus erythematosus (SLE).

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K. SAKAI
K. ISSIKI
S. HIRANO
K. OKAMURA
H. TONE

Central Research Laboratories,
Mercian Corporation,
9-1, Johnan 4-chome, Fujisawa
251, Japan

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