

Madindolines, Novel Inhibitors of IL-6 Activity from *Streptomyces* sp. K93-0711

II. Physico-chemical Properties and Structural Elucidation

SATOSHI TAKAMATSU, YONG-PIL KIM, AKIKO ENOMOTO,
MASAHICO HAYASHI, HARUO TANAKA,
KANKI KOMIYAMA and SATOSHI ŌMURA*

The Kitasato Institute and School of Pharmaceutical Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108, Japan

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Interleukin 6 (IL-6) is a multifunctional cytokine that acts on immune response, hematopoiesis, acute phase reactions and the nervous system¹⁾. Thus, IL-6 plays one of the central regulatory roles in host defense mechanisms. However, it has been demonstrated that IL-6 produced by tumor cells causes cancer cachexia, and also stimulates the proliferation of tumor cells in an autocrine/paracrine manner^{2~5)}. Therefore, it may be possible that the inhibition of IL-6 activity relieves cancer cachexia and suppress the growth of IL-6 dependent tumor cells.

In the course of a search for inhibitor of IL-6 activity of microbial origin, we have reported a new IL-6 inhibitor, chlovalicin, together with ovalicin produced by *Sporothrix* sp. FO-4649^{6,7)}. Continuation of the screening, led to the discovery of new selective inhibitors of IL-6 activity, named madindolines A (1) and B (2) in the cultures of *Streptomyces* sp. K93-0711 isolated from a soil sample (Fig. 1). Taxonomic studies of the producing strain, the isolation procedure and the biological

characteristics of 1 and 2 were reported in a previous paper⁸⁾. This paper describes physico-chemical properties and structural determination of 1 and 2.

Madindolines A (1) and B (2) were finally purified by HPLC (Senshu Pak Pegasil ODS, 5 μm, i.d. 20 × 250 mm; solv. sys., CHCl₃ - CH₃OH = 1:1; detection, UV at 210 or 254 nm) of an active fraction eluted with CHCl₃ - CH₃OH (100:1) on silica gel column chromatography as described previously⁸⁾. Physico-chemical properties of 1 and 2 are summarized in Table 1. The molecular formula of 1 was determined as C₂₂H₂₇NO₄ by HR-FAB-MS. In the UV spectrum, 1 showed characteristic UV maximal absorptions of a 3a-hydroxyindoline at 245 nm and 299 nm⁹⁾. The IR absorptions at 3390 cm⁻¹ and 1695 cm⁻¹ showed the presence of a hydroxy group and an α,β-unsaturated carbonyl moiety in the molecule. The ¹H NMR spectrum of 1 (Table 2) showed the

Fig. 1. Structures of madindolines A (1) and B (2).

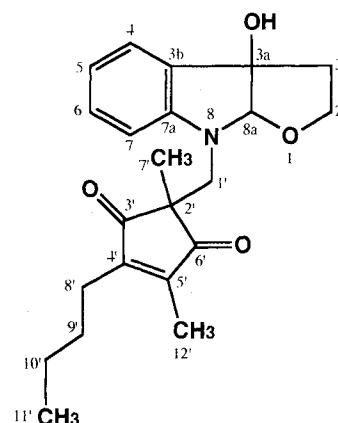


Table 1. Physico-chemical data of 1 and 2.

	Madindoline A (1)	Madindoline B (2)
Appearance	Light yellow needles	Light yellow needles
MP	80~84°C	105~107°C
[α] _D ²⁴	+44.4° (c 0.3, MeOH)	+25.6° (c 0.3, MeOH)
Molecular formula	C ₂₂ H ₂₇ NO ₄	C ₂₂ H ₂₇ NO ₄
Pos. FAB-MS (m/z)	370 (M + H) ⁺	370 (M + H) ⁺
HR Pos. FAB-MS (m/z)	Found. 370.2020 (M + H) ⁺ Calcd. 370.2018 (as C ₂₂ H ₂₈ NO ₄) 207 (4.38), 245 (4.26), 2.99 (3.28) 3390, 2927, 1741, 1695, 1603, 1487, 1381, 1281, 756	Found. 370.2036 (M + H) ⁺ Calcd. 370.2019 (as C ₂₂ H ₂₈ NO ₄) 208 (4.40), 246 (4.29), 304 (3.28) 3410, 2927, 1738, 1693, 1597, 1489, 1380, 1282, 756
UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ε)		
IR $\nu_{\text{max}}^{\text{KBr}}$ cm ⁻¹		
Color reaction		
50% H ₂ SO ₄ + Δ	Positive	Positive
Iodine	Positive	Positive
EHRLICH's reagent + Δ	Positive	Positive
DRAGENDORFF's reagent	Negative	Negative
Ninhydrin reagent	Negative	Negative

Table 2. ^{13}C and ^1H NMR chemical shifts of **1** and **2** in CDCl_3 .

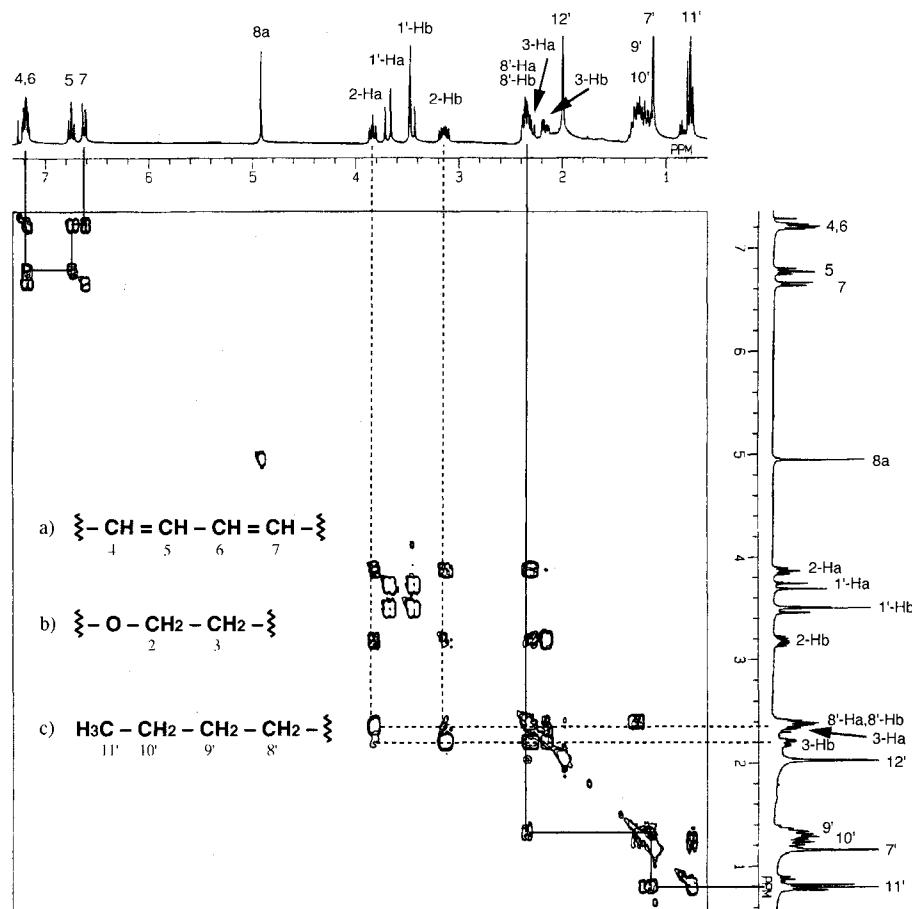
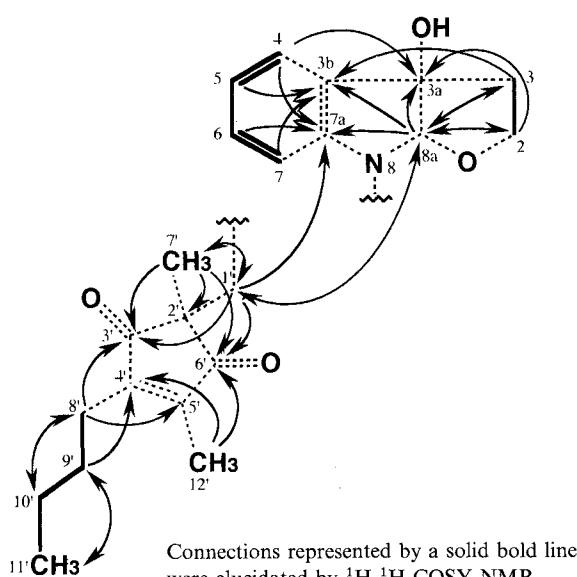
C	Madindoline A (1)			Madindoline B (2)		
	^{13}C	M	^1H (M, J value in Hz)	^{13}C	M	^1H (M, J value in Hz)
2	66.6	t	3.84 (1H, m) 3.14 (1H, m)	66.8	t	3.85 (1H, ddd, 12.0, 7.9, 1.5) 3.17 (1H, ddd, 12.0, 9.2, 5.0)
3	41.1	t	2.33 (1H, dd, 12.0, 9.0) 2.17 (1H, ddd, 12.0, 5.0, 1.5)	41.2	t	2.33 (1H, ddd, 12.0, 7.9, 1.5) 2.19 (1H, ddd, 12.0, 9.2, 1.5)
3a	88.0	s		87.9	s	
3b	129.5	s		129.4	s	
4	123.5	d	7.19 (1H, m)	123.5	d	7.19 (1H, m)
5	119.1	d	6.75 (1H, ddd, 7.3, 7.3, 0.5)	118.9	d	6.74 (1H, ddd, 7.2, 7.2, 1.0)
6	130.5	d	7.19 (1H, m)	130.5	d	7.19 (1H, m)
7	108.0	d	6.63 (1H, d, 8.0)	107.8	d	6.62 (1H, d, 8.0)
7a	150.5	s		150.4	s	
8a	106.2	d	4.93 (1H, s)	105.0	d	4.92 (1H, s)
1'	53.7	t	3.69 (1H, d, 14.2) 3.45 (1H, d, 14.2)	52.1	t	3.69 (1H, d, 14.9) 3.49 (1H, d, 14.9)
2'	50.6	s		50.5	s	
3'	206.4	s		205.9	s	
4'	157.7	s		160.3	s	
5'	156.6	s		153.7	s	
6'	206.3	s		206.6	s	
7'	17.3	q	1.12 (3H, s)	17.5	q	1.12 (3H, s)
8'	23.5	t	2.38 (1H, m) 2.36 (1H, m)	24.0	t	2.46 (1H, m) 2.37 (1H, m)
9'	29.9	t	1.29 (2H, m)	29.5	t	1.39 (2H, m)
10'	22.7	t	1.19 (2H, m)	22.9	t	1.25 (2H, m)
11'	13.8	q	0.76 (3H, t, 7.0)	13.8	q	0.86 (3H, t, 7.1)
12'	9.4	q	2.00 (3H, s)	9.1	q	1.94 (3H, s)

M: multiplicity. ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a Varian XL-400.

following signals; aromatic signals at δ 7.19 (2H, m); δ 6.75 (1H, ddd, J =7.3, 7.3 and 0.5 Hz) and δ 6.63 (1H, d, J =8.0 Hz); oxymethine signal at δ 4.93 (1H, s); oxymethylene signals at δ 3.84 (1H, m) and δ 3.14 (1H, m); methylene signals at δ 2.33 (1H, dd, J =12.0 and 9.0 Hz) and δ 2.17 (1H, ddd, J =12.0, 5.0 and 1.5 Hz). These chemical shifts were in good agreement with those of 3a-hydroxyindoline⁹⁾. The partial structures of C4~7 and C2~3 of **1** were confirmed by the ^1H - ^1H COSY spectrum (Figs. 2a and 2b), which suggested the presence of 3a-hydroxyindoline in the molecule of **1**. Further, an another partial structure of butyl moiety (C-8'~C-11') was also assigned (Fig. 2c). Based on HMBC experiment (J =4 Hz), the presence of 4-butyl-2,5-dimethylcyclopentene-1,3-dione was demonstrated besides 3a-hydroxyindoline (Fig. 3). In addition, HMBC spectrum of **1** clarified the connection of two partial skeletons. In the HMBC spectrum of **1**, the correlation from H-1' (δ 3.69 and δ 3.45) to C-7a (δ 150.5), C-8a (δ 106.2), C-2' (δ 50.6), C-3' (δ 206.4) and C-6' (δ 206.3), and from H-8a (δ 4.93) to C-1' (δ 53.7) were observed (Fig. 3). Consequently, the structure of madindoline A (**1**) was determined to be as shown in Fig. 1.

Compound **2** gave the same molecular formula ($\text{C}_{22}\text{H}_{27}\text{NO}_4$) as that of **1**. The UV and IR spectra of **2** were also similar to those of **1**. In the ^1H - ^1H COSY and the HMBC spectra of **2**, compound **2** was assumed to possess the same planar structure as **1**. In the ^1H and ^{13}C NMR spectra (Table 2), slight differences in chemical shift between **1** and **2** were observed on cyclopentene-dione skeleton. Taking into consideration the spectroscopic data, **2** appears to be a stereoisomer of **1** at the C-3a, C-8a or C-2' position. Further work is under way to determine the stereochemistry of **1** and **2**.

The synthesis of a 3a-hydroxyindoline, by photo-sensitized oxygenation of tryptophol has been reported by SAITO *et al.*⁹⁾. Further, KAWASHIMA *et al.* reported 3a-hydroxy-6-fluoro-indoline as biomodification product from 6-fluorotryptophan in the cultured broth of *Streptomyces* sp. H-63¹⁰⁾. The dioxocyclopentene ring is necessary for inhibition of IL-6 activity based on preliminary experiments (unpublished data). It will be of interest to determine the relationships between the unique structure and the activity including stereochemistry, mechanism of action and the biosynthetic origins of the madindolines.

Fig. 2. ^1H - ^1H COSY of **1** in CDCl_3 .Fig. 3. Key ^1H - ^{13}C long range couplings detected by HMBC experiment on **1** and **2**.

Connections represented by a solid bold line were elucidated by ^1H - ^1H COSY NMR. Arrows show $^1\text{H} \rightarrow ^{13}\text{C}$.

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