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**13-BROMOMONOCILLIN I: A NEW WNT-5A EXPRESSION INHIBITOR
PRODUCED BY *POCHONIA CHLAMYDOSPORIA* VAR.
*CHLAMYDOSPORIA***

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Abstract – WNT-5A is a secretory glycoprotein related to the proliferation of dermal papilla cells. While searching for an inhibitor of WNT-5A expression, a new compound, 13-bromomonocillin I (**1**), was isolated from the fermentation broth of the fungus *Pochonia chlamydosporia*, cultured in NaBr-supplemented medium. 13-Bromomonocillin I (**1**) exhibited WNT-5A expression inhibitory activity with an IC₅₀ value of 0.25 μM.

Wingless-type mouse mammary tumor virus integration site family member 5A (WNT-5A) is a secretory glycoprotein that belongs to the WNT family. WNTs are important intercellular signaling molecules that regulate axis formation and organ formation during the fetal stage.^{1,2} We have been studying molecules that regulate dermal papilla cell proliferation to develop a hair-growth stimulant. Consequently, we recently found that WNT-5A was highly expressed in dermal papillae of depilated skin. WNT-5A expression inhibitors promote proliferation of dermal papilla cells.³ Using the inhibitory activity against WNT-5A expression as a bioassay guide, we obtained radicicol (**2**)⁴⁻⁶ as an active compound. In addition, we isolated 10 new radicicol analogues, pochonins G–P,^{7,8} and 10 known radicicol analogues from a culture broth of the fungus TF-0480 that had been identified as *Pochonia chlamydosporia* var. *chlamydosporia* and reported their structures and WNT-5A expression inhibitory activity.

Table 1. Physico-chemical properties of 13-bromomonocillin I (**1**)

Appearance	colorless oil
MW	409.23
Molecular formula	C ₁₈ H ₁₇ BrO ₆
[α] _D ²⁰	+19.6 (c 1.00, acetone)
HR-ESIMS (<i>m/z</i>)	
Found	407.0140
Calcd	407.0130 [M-H] ⁻
UV λ _{max} nm (log ε) (MeOH)	218 (4.24), 264 (4.08), and 317 sh (3.64)
IR ν _{max} cm ⁻¹ (KBr)	3419, 2983, 1709, 1651, 1603, 1581

Pro C₁₈ + YMC-Pack Pro C₁₈ RS, both 5 μm, 20 × 250 mm) eluted with MeCN:H₂O (+0.25% acetic acid) = 35:65 to produce pure compound **1** (40.8 mg) together with known compounds radicicol (**2**) (1608 mg) and monocillin I (**3**) (28.8 mg). Chlorine of **2** must be originated from the cultivation medium containing oatmeal and yeast extract.

The physico-chemical properties of **1** are summarized in Table 1. The appearance of two molecular ion peaks at *m/z* 407 and 409 with equal intensities in the low-resolution ESI-mass spectrum (negative ion-mode) indicated that a bromine atom was incorporated in the molecule. The molecular formula of **1** was established as C₁₈H₁₇BrO₆ by HRESI-MS (found *m/z* 407.0140; calc for C₁₈H₁₆BrO₆ [M-H]⁻, 407.0130, Δ +1.0 mmu). The IR spectrum showed the presence of a hydroxyl (3419 cm⁻¹) and a conjugated carbonyl (1709 cm⁻¹) group. The UV spectrum of **1** showed maximal absorptions (λ_{max}) at 218 and 264 nm, similar to that of radicicol (**2**) [λ_{max} 215, 266, 308 (sh) nm].⁸ The structure of **1** was elucidated using ¹H NMR, ¹³C NMR, DEPT, COSY, HMQC, and HMBC. ¹³C NMR and DEPT spectra of **1** exhibited 18 carbon signals consisting of one methyl, two methylenes, eight methines, and seven quaternary carbons. The ¹H NMR and HMQC spectra of **1** exhibited the signals due to two epoxy protons (δ_H 3.32 and 3.02, δ_C 56.1 and 55.8), four olefinic protons (δ_H 7.42, 6.23, 6.16 and 5.73), and an aromatic proton (δ_H 6.58, δ_C 103.4). The proton network from H₃-1 to H-9 was deduced from the ¹H-¹H COSY spectrum. The small coupling constant (2.3 Hz) between H-4 and H-5 suggested that the epoxide had an *E*-configuration. The coupling constants (*J*_{H-6/H-7} = 10.7 Hz, *J*_{H-8/H-9} = 16.0 Hz) of the olefinic protons indicated that the two olefins (C6–C7 and C8–C9) had *Z*- and *E*-configurations, respectively. The HMBC correlations between H-15 (δ_H 3.32) and C-13, C-14, C-16, and C-17 (δ_C 106.5, 158.8, 161.0, and 112.1, respectively) indicated the presence of a pentasubstituted aromatic ring. Other critical correlations were as follows: from H₂-11 (δ_H 4.38, 3.94) to C-9 (δ_C 131.2), C-10 (δ_C 196.4), C-13 (δ_C 106.5), and C-17 (δ_C 112.1); from H-9 (δ_H 6.16) to C-11 (δ_C 49.0); from H-8 (δ_H 7.42) to C-10; and from

Table 2. NMR data (500 MHz, acetone-*d*₆) for 13-bromomonocillin I (**1**) and radicicol (**2**)

position	13-bromomonocillin I (1)			radicicol (2) ⁸		
	δ_C	mult.	δ_H (<i>J</i> in Hz)	δ_C	mult.	δ_H (<i>J</i> in Hz)
1	18.5	q	1.53 d (6.9)	18.5	q	1.55 d (6.6)
2	71.7	d	5.40 ddq (3.8, 3.3, 6.9)	71.9	d	5.43 ddq (3.8, 3.3, 6.6)
3	37.1	t	2.41 dt (15.3, 3.8)	37.0	t	2.43 ddd (15.1, 3.3, 2.7)
			1.77 ddd (15.3, 8.4, 3.3)			1.78 ddd (15.1, 8.5, 3.8)
4	56.1	d	3.02 ddd (8.4, 3.8, 2.3)	56.1	d	3.04 dt (8.5, 2.7)
5	55.8	d	3.32 br s	55.8	d	3.19 br s
6	135.6	d	5.73 dd (10.7, 3.8)	135.9	d	5.75 dd (10.7, 3.6)
7	130.3	d	6.23 dd (10.7, 9.2)	130.4	d	6.24 dd (10.7, 9.6)
8	138.7	d	7.42 dd (16.0, 9.2)	139.0	d	7.50 dd (15.9, 9.6)
9	131.2	d	6.16 d (16.0)	131.2	d	6.12 d (15.9)
10	196.4	s		199.1	s	
11	49.0	t	4.38 d (16.0)	46.5	t	4.44 d (16.2)
			3.94 d (16.0)			3.90 d (16.2)
12	137.8	s		136.4	s	
13	106.5	s		115.6	s	
14	158.8	s		157.9	s	
15	103.4	d	6.58 s	103.8	d	6.57 s
16	161.0	s		160.6	s	
17	112.1	s		111.0	s	
18	168.7	s		168.8	s	

H-2 (δ_H 5.40) to C-18 (δ_C 168.7). These data suggested that compound **1** has a 14-membered macrolactone the same as **2**. The ¹³C NMR spectrum of **1** was similar to that of **2**, with the exception of the signal of the quaternary carbon at C-13 (δ_C 106.5) in the former (Table 2). The higher shift of C-13 of **1** than that of the C-13 (δ_C 115.6) of **2** indicated that the bromine atom was attached to C-13 instead of the chlorine atom of **2**.¹³ These data demonstrated that compound **1**, named 13-bromomonocillin I, was a new analogue of **2** with the replacement of the chlorine atom in **2** by a bromine atom (Figure 1).

The relative stereochemistry of **1** was assigned according to the results of the NOESY experiment (Figure 2). The NOE correlations between H₃-1/H-11a and H-11b, H₃-1/H-4, H-3a and H-3b/H-5, and H-5/H-8 indicated that the relative configurations of **1** at C-2, C-4, and C-5 were the same as those of **2**.

The WNT-5A expression inhibitory activity and cytotoxicity against dermal papilla cells of compounds **1–3** are shown in Table 3. The WNT-5A expression inhibitory activity of 13-bromomonocillin I (**1**) is stronger than that of monocillin I (**3**) and as potent as that of radicicol (**2**). The cytotoxicity of **1** against dermal papilla cells is more than five times weaker than that of **3**. These data indicate that a halogen atom at C-13 in the radicicol-type 14-membered macrolactone contributes to enhancement of WNT-5A expression inhibitory activity and the reduction of cytotoxicity, and that chlorine and bromine atoms at this position had similar effects. We have already found that active compounds with WNT-5A

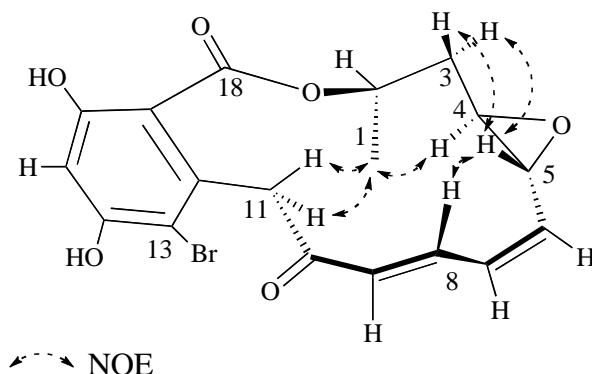


Figure 2. Key NOESY correlations for 13-bromomonocillin I (**1**)

Table 3. WNT-5A expression inhibitory activities of 13-bromomonocillin I (**1**), radicicol (**2**), and monocillin I (**3**)

compound	IC_{50} (μM) WNT-5A	TC_{50} (μM) ^a
13-bromomonocillin I (1)	0.25	>15.3
radicicol (2)	0.19	15.3
monocillin I (3)	1.93	2.90

^a Half maximal toxic concentration against dermal papilla cells

expression inhibitory activity also exert dermal papilla cell proliferation (will be reported in another article), and the new WNT-5A expression inhibitor, 13-bromomonocillin I (**1**), is expected as a potent hair-growth stimulant.

EXPERIMENTAL

General Experimental Procedures

NMR spectra were recorded at 500 MHz using a JEOL ECA 500 spectrometer (JEOL Ltd.). Chemical shifts were reported in parts per million (ppm), and the solvent peaks were utilized as an internal standard. The coupling constants (J) were given in hertz (Hz). Electrospray ionization (ESI) mass spectra were obtained using a Micromass Platform LC mass spectrometer (Micromass Ltd.). Optical rotations were measured using an AUTOPOL V digital polarimeter (Rudolph Research Analytical). UV spectra were recorded using a V-520 UV/Vis spectrophotometer (JASCO). IR spectra were measured using a Spectrum One FT-IR spectrometer (Perkin Elmer).

Microorganism

Strain TF-0480 was isolated from a soil sample collected in Fujioka City, Tochigi Prefecture, Japan. The strain was deposited at the National Institute of Advanced Industrial Science and Technology in Japan as FERM BP-8332.

Biological assay

Inhibition of WNT-5A expression: quantification of mRNA using the QuantiGene method

Human dermal papilla cells were cultured in MEM (Invitrogen) containing 12% fetal bovine serum (FBS). The dermal papilla cells in the fifth subculture generation were sowed in a 96-well plate at a density of 1×10^4 cells/well and cultured overnight. The medium was then replaced with one that did or did not contain the test compounds and the culture was continued for an additional 24 h. After 24 h, the amount of WNT-5A mRNA was measured using a QuantiGene High Volume Kit (Bayer Medical) by the branched DNA (bDNA) signal amplification method.¹⁴ In accordance with the manufacturer's protocol, the cells were lysed with a lysis mixture and the lysis solution was added to the capture plate. Next, a set of probes specific to WNT-5A³ was added, and the reaction proceeded at 53 °C for 20 h. After the plate was washed using $0.1 \times$ SSC (3.0 M NaCl and 0.3 M sodium citrate) containing 0.03% lauryl sulfate, an amplification probe containing bDNA was added and reacted at 46 °C for 1 h. After the plate was washed again, a probe labeled with alkaline phosphatase was added and reacted at 46 °C for 1 h. After the plate was washed for last time, the Lumi-Phos Plus substrate was added, and the reaction proceeded at 46 °C for 30 min. Luminescence was measured using a WALLAC 1420 ARVOSx (Perkin Elmer).

Dermal papilla cell cytotoxicity

Dermal papilla cells were sowed in a 96-well plate to yield a density of 5×10^3 cells/well and cultured for 16 h in MEM containing 12% FBS. The medium was then replaced with either a medium to which no compounds had been added or a medium that contained the test compounds, and the culture continued for an additional 24 h. The medium was then replaced with a medium containing 10% Alamar BlueTM (Wako Pure Chemical Industries), and the culture was continued for an additional 4 h. Finally, the fluorescence intensity (Ex 544 nm, Em 590 nm) was measured using a WALLAC 1420 ARVOSx.

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