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Cytotoxic 6-Alkylsalicylic Acids from the Endophytic *Streptomyces laceyi*

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Abstract Two new 6-alkylsalicylic acids, salaceyins A and B were isolated by bioassay-guided fractionation from the culture of the endophytic *Streptomyces laceyi* MS53 and their structures were determined on the basis of spectroscopic data. Salaceyins A and B exhibited modest cytotoxicity against a human breast cancer cell line (SKBR3) with IC_{50} values of 3.0 and 5.5 μg/ml, respectively.

Keywords alkysalicylic acid, *Streptomyces*, cytotoxic

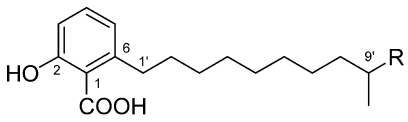
Introduction

In our search for biologically active agents of natural origin, the culture broth of an endophytic actinomycete strain exhibited cytotoxic activity against a human breast cancer (SKBR3) cell line. Bioassay-guided fractionation of an EtOAc extract of a culture broth of *Streptomyces laceyi* MS53 resulted in the isolation of two new 6-alkylsalicylic acids (**1** and **2**, Fig. 1). Several 6-alkylsalicylic acid derivatives, biogenetically formed by the condensation of a fatty acid and three molecules of malonyl-CoA, have been isolated from plant sources including Anacardiaceae plants and *Ginkgo biloba* L., and exhibited antibacterial, antioxidant, insecticidal, and molluscidal activities [1～9]. We report herein the isolation and structural determination of two new 6-alkylsalicylic acids, together with cytotoxic activities of the isolates against a human breast cancer cell line.

Results and Discussion

An endophytic *Streptomyces* strain (MS53) was isolated from a surface-sterilized stem of *Ricinus communis* L. Chemotaxonomic tests showed that the cell wall of the strain contained LL-diaminopimelic acid, indicating that it has cell-wall type I; no diagnostic sugars were detected in the hydrolysates of whole cells. Using partial 16S ribosomal DNA sequence analysis, the strain was identified to be closely related to *Streptomyces laceyi*. The 16S ribosomal DNA sequence identity was 99.38% (1460/1469 bp) [10～12].

Compound **1** was obtained as a colorless amorphous powder and was shown to possess a molecular formula of



1 R = CH_3
2 R = CH_2CH_3

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

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Table 1 NMR data for salaceyins A (**1**) and B (**2**) in $CDCl_3$[a]

	δ_C		δ_H	
	1	**2**	**1**	**2**
1	110.36	110.25		
2	163.63	163.67		
3	115.85	115.84	6.85 d (8.0)	6.87 d (7.8)
4	135.40	135.35	7.34 t (7.6, 8.0)	7.37 t (7.8)
5	122.75	122.69	6.75 d (7.6)	6.77 d (7.2)
6	147.77	147.61		
COOH	175.64	175.74		
1′	36.48	36.51	2.96 t (7.6)	2.98 t (7.8)
2′	32.02	32.07	1.58 m	1.61 m
3′	29.92[b]	29.49[b]	1.23∼1.35[b]	1.23∼1.39[b]
4′	29.81[b]	29.49[b]	1.23∼1.35[b]	1.23∼1.39[b]
5′	29.65[b]	29.67[b]	1.23∼1.35[b]	1.23∼1.39[b]
6′	29.49[b]	29.83[b]	1.23∼1.35[b]	1.23∼1.39[b]
7′	27.41	27.10	1.23∼1.35[b]	1.23∼1.39[b]
8′	39.03	36.63	1.14 m	1.23∼1.39[b]
9′	27.95	34.40	1.49 octet	1.12 m
10′	22.64	30.01[b]	0.83 d (6.8)	1.23∼1.39
11′	22.64	11.40	0.83 d (6.8)	0.86 overlap
12′	—	19.22	—	0.84 overlap
OH	—	—	10.98 br s	11.01 br s

[a] TMS was used as the internal standard. Chemical shifts are shown in the δ scale with J values (Hz) in paraentheses.
[b] Interchangeable within the same column.

$C_{18}H_{28}O_3$ by positive HRFABMS. The ^{1}H NMR spectrum of compound **1** displayed characteristic signals for an AMX spin system (1,2,3-trisubstituted benzene ring) at δ_H 6.85 (1H, doublet, J=8.0 Hz, H-3), 7.34 (1H, triplet, J=7.6 and 8.0 Hz, H-4), and 6.75 (1H, doublet, J=7.6 Hz, H-5). In the aliphatic region, a benzylic methylene signal at δ_H 2.96 (2H, triplet, J=7.6 Hz, H-1′), a homobenzylic methylene signal at δ_H 1.58 (2H, broad quintet like multiplet, H-2′), the signals coupled to each other at δ_H 1.49 (1H, octet like multiplet, H-9′), and 0.83 (6H, doublet, H-10′ and H-11′) as well as the overlapped signals [δ_H 1.23∼1.35 (10H, multiplet, H-3′, 4′, 5′, 6′, and 7′) and 1.14 (2H, multiplet, H-8′)], suggested the presence of a 9-methyldecyl unit, which was substantiated by the ^{13}C NMR and mass spectra. The results obtained from the 1D and 2D NMR spectra indicated that compound **1** is a salicylic acid derivative with a 9-methyldecyl substitution in the molecule. The position of this alkyl group was determined unambiguously as C-6 by salient HMBC correlations (H-5/C-1, C-1′, H-1′/C-1, C-5, C-6). Therefore, the structure of the new compound, **1** was elucidated as 6-(9-methyldecyl)salicylic acid.

The ^{1}H and ^{13}C NMR spectra of compound **2** were almost superimposable with those of **1**, except for the branch moiety of the 9-methyldecyl group of the latter compound. The absence of the *iso*-type methyl branch signals (9-methyldecyl unit) and the presence of the upfield shift signal for the terminal methyl group at δ_C 11.40 (C-11′) due to the γ-effect of the methyl substitution at C-9′ indicated that compound **2** has an *anteiso*-type methyl branched chain rather than an *iso*-type 9-methyldecyl unit as in **1** [13]. The stereochemistry of C-9′ position was inferred to be *S* based on the positive optical rotation of *anteiso*-type compounds [14]. Accordingly, the structure of the new compound, **2** was assigned as 6-(9 methylundecyl)salicylic acid and confirmed using HRFABMS and 2D NMR techniques.

Salaceyins A (**1**) and B (**2**) were evaluated against human breast cancer cell line (SKBR3) and both compounds exhibited modest cytotoxicity with IC_{50} values of 3.0 and 5.5 μg/ml, respectively. Salicylic acids with alkyl side chains were previously reported to exhibit antibacterial and molluscidal activities [2∼4]. More specifically, anacardic acids isolated from Anacardiaceae plants showed

synergistic antibacterial effects against methicillin-resistant *Staphylococcus aureus*, which has troubled hospitals worldwide with persistent infections [4, 5, 15, 16]. Therefore, it is obvious that more research is needed to investigate the antibacterial activity of **1** and **2**.

Experimental

All melting points were determined on an Electrothermal 9100 instrument without correction. Optical rotation was measured on a JASCO P-1020 polarimeter. UV spectra were measured on a Shimadzu UV-1601 UV-visible spectrophotometer. NMR spectra were recorded on a Varian UNITY 400 spectrometer with $CDCl_3$. ESI-MS and HRFABMS were obtained on Platform quadrupole and JMS-HX110A/HX110A Tandem Mass spectrometers, respectively. Preparative HPLC was carried out on a Varian system.

Determinations of the isomer of diaminopimelic acid and the whole-cell sugar pattern were carried out as described by Hasegawa *et al.* using colonies from agar plates (ISP2 medium) [11]. A 16S rRNA gene sequence of strain MS53 was amplified by PCR using universal bacterial 16S rDNA primers (forward primer adapted from primer fD1 of Weisburg *et al.* [12]; reverse primer adapted from primer p1525r of Chun and Goodfellow [10]). The 16S rDNA was sequenced using a DNA automated sequencer (Applied Biosystems) and an Amersham Pharmacia Biotech Cy5 Thermo Sequenase dye terminator kit.

MS53 was cultured in a seed agar medium, ISP4. The fully grown mycelia or spore suspension was used to inoculate a 1000-ml baffled flask containing 300-ml of a seed medium consisting of yeast extract 0.3%, Bacto-peptone 0.5%, malt extract 0.3%, glucose 1.0%, sucrose 10.3%, and $MgCl_2{\cdot}6H_2O$ 0.1%, which is a modified YEME broth. The flasks were cultivated on a rotary shaker (170 rpm) for 3 days at 28°C. For the production cultures, 300 ml of the modified YEME medium in 1000-ml baffled flasks was inoculated with the seed culture, and the flasks were incubated at 28°C and 170 rpm for 7 days. The cultured broth (8 liters) was extracted with EtOAc twice and these extracts were filtered through a fritted funnel *in vacuo* to remove insolubles. The volume of the filtrate was reduced *in vacuo* and then partitioned between EtOAc and H_2O to give the organic extracts (1.3 g). Fractionation of the EtOAc extract was initiated by Silica gel chromatography using a $CHCl_3$ - MeOH gradient as mobile phase, and fractions obtained were pooled based on TLC analysis. Fraction 4, eluted with $CHCl_3$ - MeOH (15 : 1), was passed through a Sephadex LH-20 column using $CHCl_3$ - MeOH (1 : 1) and further purified by HPLC [YMC-Pack Pro C18, 250×20 mm i.d., MeCN - H_2O (0.05% TFA) gradient, 10 ml/minute] to afford compound **1** (10.0 mg, 0.77% w/w) and compound **2** (2.0 mg, 0.15% w/w).

Salaceyin A (**1**). Colorless amorphous powder; UV (MeOH): λ_{max} (log ε) 244 (3.72), 310 nm (3.51); ^1H and ^{13}C NMR data, see Table 1; ESI-MS *m/z* 291.4 $[M-H]^-$, 583.7 $[2M-H]^-$; HRFABMS *m/z* 293.2119, calculated for $C_{18}H_{29}O_3$, 293.2117.

Salaceyin B (**2**). Colorless amorphous powder; $[\alpha]_D^{24}$ +5.0 (*c* 0.1, $CHCl_3$); UV (MeOH): λ_{max} (log ε) 244 (3.56), 310 nm (3.35); ^1H and ^{13}C NMR data, see Table 1; ESI-MS *m/z* 305.4 $[M-H]^-$, 611.6 $[2M-H]^-$; HRFABMS *m/z* 307.2274, calculated for $C_{19}H_{31}O_3$, 307.2273.

Compounds **1** and **2** were evaluated for cytotoxicity against human breast cancer (SKBR3) cell line according to established protocols (positive control, geldanamycin: IC_{50} value of 40 nM) [17].

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