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NOTE

AN483, a new anti-MRSA compound from *Streptomyces* sp.

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Gram-positive eubacteria are representative of pathogenic microorganisms. Especially, *Staphylococcus aureus* is the most clinically important of these pathogens because of its exceptional virulence, stress tolerance and capacity to accumulate antimicrobial resistances.[1,2] Antibiotic-resistant strains of pathogenic bacteria are increasingly prevalent in hospitals and the community. Of antibiotic-resistant pathogens, the one that has gained the most attention is methicillin-resistant *S. aureus* (MRSA) because of its high frequency of infection as well as its resistance to many other antibiotics.[3,4] Overall, in the United States and the United Kingdom, 40–60% of nosocomial *S. aureus* strains are methicillin resistant. The increased use of vancomycin, the last anti-MRSA antibiotic in the last century, resulted in the prevalence of vancomycin-resistant *Enterococcus*. Although some anti-MRSA drugs such as linezolid and daptomycin have been introduced in recent years,[5] new classes of anti-MRSA agents are still needed.

In the course of our continued screening for new anti-MRSA agents from microbial resources,[6] a new anthracenone-type antibiotic (**1**) was isolated from liquid fermentation cultures of *Streptomyces* sp. AN100483 (Figure 1). The anthracenone-type compounds are rare metabolites. To the best of our knowledge, only three anthracenone-type compounds have been reported that include WS9761,[7] Q6916Z[8] and dimeric oxanthromicin.[9] Especially, compound **1** is chlorinated unlike other compounds. In this paper, we report the fermentation, isolation, structure determination and antibacterial activity of **1**.

The actinomycetal strain AN100483 was isolated from a soil sample that was collected at *Colocasia esculenta* field, Baekam Mountain, Gyeongsangbuk-do, Korea, and identified as *Streptomyces* sp. based on 16S DNA sequence. Fermentation was carried out in a liquid culture medium containing soluble starch 1%, glucose 2%, soybean meal 2.5%, beef extract 0.1%, yeast extract 0.4%, NaCl 0.2%, K_2HPO_4 0.025% and $CaCO_3$ 0.2% (adjusted to pH 7.2 before sterilization). A portion of the strain AN100483 from a mature plate culture was inoculated into a 500-ml Erlenmeyer flask containing 80 ml of the above sterile seed liquid medium and cultured on a rotary shaker (150 r.p.m.) at 28 °C for 3 days. For the production of an active compound, 5 ml of the seed culture were transferred into 500 ml Erlenmeyer flasks containing 100 ml of the above medium, and cultivated on a rotary shaker (150 r.p.m.) for 10 days at 28 °C. After incubation, the fermented liquid cultures (3 l) were extracted with 80% acetone. The acetone extracts were concentrated *in vacuo* to an aqueous solution, which was then extracted with an equal volume of EtOAc three times. The EtOAc extract (1.2 g) was subjected to silica gel (56 × 200 mm, Merck Art No. 7734.9025) column chromatography, followed by stepwise elution with $CHCl_3$–MeOH (100:1, 50:1, 25:1). Each fraction was tested for anti-MRSA activity. The active fractions eluted with $CHCl_3$–MeOH (50:1) were pooled and concentrated *in vacuo*. The resultant residue (285 mg) was purified by preparative HPLC equipped with Waters 510 HPLC pump and Waters 996 Photodiode Array Detector. The reverse phase HPLC column (20 × 150 mm, YMC C18) was eluted with CH_3CN–H_2O (65:35) containing 0.035% trifluoroacetic acid at a flow rate of 6 ml per min to afford 3.3 mg of **1** with retention time of 28 min.

The molecular formula of **1** was determined as $C_{17}H_{15}ClO_4$ on the basis of the HRESI-MS [$(M-H)^-$, 317.0564 *m/z* (−1.6 m.m.u. error)] in combination with the ^1H and ^{13}C NMR data (Table 1). Together with HRESI-MS data, the M+2 peak having approximately one-third intensity of the molecular ion peak indicated the presence of a chlorine atom. Compound **1** gave characteristic UV maxima at 231, 279, 310 and 348 nm. IR absorptions of **1** at 1687 and 3448 cm^{-1} suggested the presence of carbonyl and hydroxyl moieties, respectively.

The ^1H and ^{13}C NMR data (Table 2) supported by HMQC data suggested the presence of three aromatic methines (δ_H 6.37, s, δ_C 102.9; δ_H 6.78, brs, δ_C 118.6; and δ_H 6.92, brs, δ_C 111.8), an aromatic methyl (δ_H 2.74, s, δ_C 24.5), a $CHCH_3$ (δ_H 1.45, q, $J = 6.6$, δ_C 28.4 and δ_H 4.50, d, $J = 6.6$, δ_C 39.3), a methoxy, nine sp^2 quaternary carbons (δ_C 164.8, 164.5, 160.8, 152.5, 146.9, 146.0, 122.5, 111.7 and 110.9) and a ketone carbonyl (δ_C 190.7). In the HMBC spectrum (Figure 2), the two aromatic methine protons at δ_H 6.78 (H-7) and δ_H 6.92 (H-5) have the long-range correlations with carbons at δ_C 111.8 (C-5) and δ_C 118.6 (C-7), respectively, and also with the sp^2 quaternary carbons at δ_C 122.5 (C-8a) and δ_C 164.6 (C-6). In addition, the aromatic

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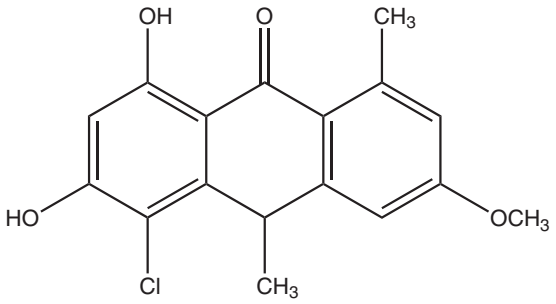

Figure 1 Chemical structure of AN483 (**1**).

Table 1 Physico-chemical properties of 1

Appearance	*Reddish-yellow powder*
$[\alpha]_D$	+82.2(c 0.1 MeOH)
ESI-MS (*m/z*)	317 $(M-H)^-$, 319 $(M+H)^+$
HRESI-MS (m/z)	
Found.	317.0564
Calcd.	317.0580 $(M-H)^-$
Molecular formula	$C_{17}H_{15}ClO_4$
UV λ_{max} nm (log ε)	202 (4.54), 212 (sh 4.42), 231 (4.07), 243 (3.90),
(MeOH)	279 (3.76), 310 (3.87), 348 (3.88)
IR (KBr) γcm^{-1}	3448, 2918, 2850, 1687, 1603, 1358, 1258, 1212

Table 2 ^1H and ^{13}C NMR spectral data for 1

Position	*δ_H(mult., J_{HH})*	*δ_C*
1		160.8 C
2	6.37 (1H, s)	102.9 CH
3		164.8 C
4		110.9 C
4a		146.9 C
5	6.92 (1H, brs)	111.8 CH
6		164.5 C
6-OCH_3	3.90 (3H, s)	56.1 CH_3
7	6.78 (1H, brs)	118.6 CH
8		146.0 C
8a		122.5 C
9		190.7 C
9a		111.7 C
10	4.50 (1H, q, 6.6)	39.3 CH
10a		152.5 C
1′	1.45 (3H, d, 6.6)	28.4 CH_3
1′′	2.74 (3H, s)	24.5 CH_3

^1H and ^{13}C NMR spectral data were measured at 500 and 125 MHz, respectively, in CD_3OD. The assignments were aided by HMQC, HMBC and NOESY.

methyl protons at δ_H 2.74 (H_3-1'') were long-ranged coupled to the sp^2quaternary carbon at δ_C 146.0 (C-8), C-7 and C-8a. These spectral data suggested the presence of an 1,2,3,5-tetrasubstituted benzene with the methyl and the methoxy substituents at C-1 and C-5, respectively. The methyl protons (H_3-1') of the $CHCH_3$ group have the HMBC correlations with the sp^2 quaternary carbons at δ_C 146.9 (C-4a) and δ_C 152.5 (C-10a), and the methine proton (H-10) of the $CHCH_3$ group was long-ranged coupled to the sp^2 quaternary carbons at δ_C 111.7 (C-9a) and δ_C 110.9 (C-4), C-4a, C-5, C-8a and C-10a. In addition, the HMBC correlations from the remaining aromatic proton at δ_H 6.37 (H-2) to the oxygenated sp^2 quaternary carbons at δ_C 160.8 (C-1) and δ_C 164.8 (C-3), C-4 and C-9a. This spectral data suggested the presence of a 1,2,3,4,5-pentasubstituted benzene that was linked to the 1,2,3,5-tetrasubstituted benzene by the $CHCH_3$ group between C-4a and C-10a. This was confirmed by the NOEs between H-10/H_3-1' and H-5 (Figure 2). Also the presence of the methyl and methoxy at C-8 and C-6, respectively, was confirmed by NOEs from 6-OCH_3 to H-5/H-7, and from H_3-1'' to H-7. Considering the low-field-shifted chemical shifts of C-4a and C-10a, the ketone carbon (C-9) should be located between C-8a and C-9a. Also considering the chemical shifts of C-4 and the molecular formula, C-4 should be chlorinated. Thus, the structure of **1** was determined as shown in Figure 1.

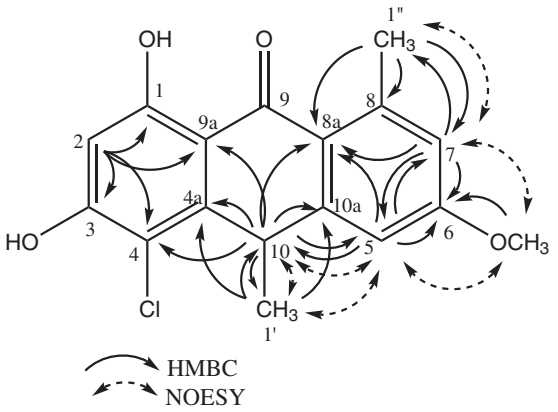

Figure 2 HMBC and NOE correlations of **1**.

The antibacterial activity of **1** was evaluated with serial two-fold dilutions starting with 128 μg ml^{-1} according to our previously reported method.[10] Compound **1** exhibited antibacterial activity against *S. aureus* RN4220, methicillin-resistant *S. aureus* (MRSA; *S. aureus* CCARM 3167 and *S. aureus* CCARM 3506), and quinolone-resistant *S. aureus* (*S. aureus* CCARM 3505 and *S. aureus* CCARM 3519) with MIC of 32 μg ml^{-1}. As a positive control, ciprofloxacin showed antibacterial activity against *S. aureus* RN4220, MRSA (*S. aureus* CCARM 3167 and *S. aureus* CCARM 3506), and quinolone-resistant *S. aureus* (*S. aureus* CCARM 3505 and *S. aureus* CCARM 3519) with MIC of 0.125, 4 and 128 μg ml^{-1}, respectively. Compound **1** also showed antibacterial activity against other pathogenic bacteria including *Enterococcus faecalis* KCTC 5191 and *Bacillus cereus* KCTC 1661 with MIC of 32 μg ml^{-1}. However, **1** did not have antibacterial activity against some gram-negative bacteria including *Pseudomonas aeruginosa* and *Acetobacter baumannii* at 128 μg ml^{-1}.

Compound **1** is a new chlorinated compound containing the anthracenone skeleton. The anthracenone antibiotics are rare metabolites since, to the best of our knowledge, only three compounds have been reported that include WS9761, Q6916Z and dimeric oxanthromicin which have been isolated from *Streptomyces* sp. and *Actinomadura* sp. WS9761 has been reported as an androgen-receptor antagonist.[7] Q6916Z has been known to inhibit phospholipase A2.[8] Oxanthromicin, a dimeric compound, has been reported to show antimicrobial activity against dermatophytic fungi, *Candida albicans* and *S. aureus*.[9] Oxathromicin glycosides such as adxanthromycin A and B have been reported to inhibit cell division.[11–13]

In summary, AN483 is a new chlorinated anthracenone, a rare metabolite, isolated from a strain of *Streptomyces* sp. AN100483. AN483 showed antibacterial activity against gram-positive pathogenic bacteria including MRSA and QRSA.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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