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BRIEF COMMUNICATION

Kinanthraquinone, a new anthraquinone carboxamide isolated from *Streptomyces reveromyceticus* SN-593-44

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Abstract

A new anthraquinone derivative, kinanthraquinone (**1**) was isolated from *Streptomyces reveromyceticus* SN-593-44. Its structure was determined by the combination of spectroscopic methods including NMR and MS. Kinanthraquinone had a characteristic carboxamide group and was a rare class of metabolite as an anthraquinone derivative isolated from microbes. It showed moderate cytotoxocity against HL-60 and src^{ts}-NRK cell with IC_{50} value of 7.9 and 10 μM, respectively.

Secondary metabolites produced by microbes such as actinomycetes and fungi have been attractive sources as drugs, agrochemicals, and thier leads or seeds [1]. They are also important as bioprobes, which are useful for investigating biological functions in a chemical biology study [2]. We have isolated several biologically active metabolites, such as cytotrienins [3], epoxyquinols [4], and azaspirene [5] by unique bioassay-guided separations. Reveromycin A [6], which was isolated from *Streptomyces reveromyceticus* SN-593 as an inhibitor of eukaryotic cell growth, belongs to the class of polyketides and is one of the biologically interesting metabolites in both points of activity [7] and biosynthesis [8–10]. In addition to the production of reveromycins, the strain has a capacity to produce polyketides, and we have isolated new furaquinocin derivatives [11]. Over the past decade, we have developed an original method to discover and isolate structurally interesting metabolites using a fraction library combined with spectral database named Natural Product Plot, NPPlot [12]. Based on this structure-oriented method, we have discovered and isolated new metabolites such as verticilactam [13], spirotoamides [14], and pyrrolizilactone [15]. We have recently reported new decaline containing metabolites, wakodecalines A and B, which contained a cyclopentanone-fused decaline skeleton by the application of our method [16]. Here, we applied NPPlot approach to obtain structurally unique metabolites from *S. reveromyceticus* SN-593-44. We discovered an interesting metabolite, which showed a typical UV spectrum for anthraquinones but its *m/z* value was 380 $[M-H]^-$ suggesting it might have contained a nitrogen. Tetracyclines with nitrogen-containing functional groups such as carboxamido moiety are important for their biological activity [17, 18]. Therefore, we focused on this metabolite and isolated a new anthraquinone derivative desinated as kinanthraquinone (Fig. 1).

S. reveromyceticus SN-593-44, whose clone was selected as reveromycin high producing strain after monosporulation process, was cultured in 500 ml cylindrical flask containing 70 ml of a medium (2.0% potato dextrose [Difco, Franklin Lakes, NJ, USA], 1.0% malt extract [Difco, Franklin Lakes, NJ, USA], 1.0% dried yeast [Asahi beer, Tokyo, Japan], 5.0% tomato juice [Table land; Maruzen Foods, Tokyo, Japan], 0.1% K_2HPO_4, 0.1% NaCl, 0.03% $MgSO_4{\cdot}7H_2O$, 0.01% $NaNO_3$, and 0.005% $ZnSO_4{\cdot}\ 7H_2O$) on a rotary shaker at 150 rpm for 5 days at 28 °C. Total 4.9 l of culture broth including mycelia was mixed with the same volume of acetone, and filtered to remove the mycelia. The filterate was evaporated in vacuo to remove acetone, and the remained water extract was adjusted to pH 4 by acetic acid.

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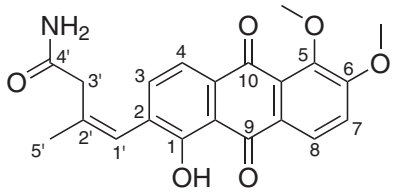

Fig. 1 Structure of compound **1**

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

Position	δ_C	δ_H	Multiplicity (J in Hz)
1	159.6	–	
2	132.4	–	
3	136.6	7.58	d (7.9)
4	119.2	7.75	d (7.9)
4a	133.8	–	
5	150.0	–	
6	159.8	–	
7	115.9	7.25	d (8.6)
8	125.3	8.16	d (8.6)
8a	126.6	–	
9	188.1	–	
9a	115.5	–	
10	181.7	–	
10a	127.1	–	
1′	123.4	6.54	brs
2′	136.4	–	
3′	40.9	3.09	brs, 2H
4′	172.3	–	
5′	24.1	2.04	d (1.2), 3H
1-OH	–	13.23	s
5-OMe	61.3	3.97	s, 3H
6-OMe	56.4	4.00	s, 3H
4′-NH_2	–	5.97	brs
		5.28	brs

^{1}H and ^{13}C NMR spectra were recorded at 500 and 125 MHz, respectively

It was partitioned twice with the half volume of ethyl acetate, and the ethyl acetate layer was evaporated to obtain a 2.4 g of crude extract as dark-red gum. The extract was subjected to SiO_2 column chromatography with chloroform/methanol stepwise elution (chloroform/methanol: 100:0, 100:1, 100:5, 100:10, 100:20, 100:50, 50:50, and 0:100) to obtain 8 fractions. The fraction eluted with 100:20 was separated by C_{18}-high performance liquid chromatography (HPLC) to afford crude **1**. It was further purifed by C_{18}-HPLC to give 2.9 mg of **1** as orange needles; $[\alpha]_D^{29}$ −1.5° (*c* 0.12), UV λ_{max} (MeOH) (log ε): 226 (4.02), 247 (4.00), 269 (4.06), 305sh (3.58), 420 (3.66) nm, infrared (IR)

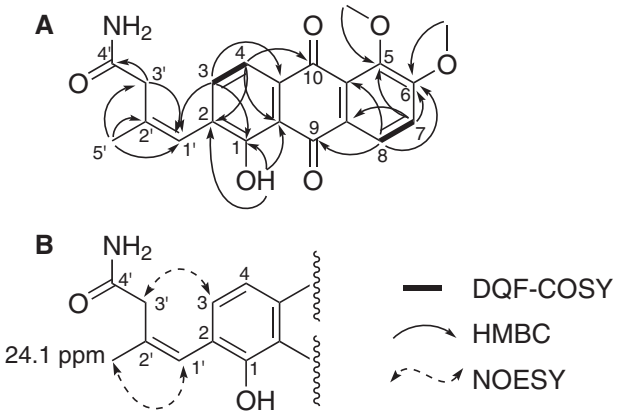

Fig. 2 Key 2D NMR correlations for determination of the planar structure (**a**) and NOESY correlations for confirmation of geometry at $\Delta^{1'}$ (**b**)

(ATR) ν_{max} (cm^{-1}): 3419, 2915, 2850, 1733, 1660, 1623, 1560, 1457, 1413, 1365, 1276, 1253, 1195, 1072, 781.

The molucular formula of **1** was determined to be $C_{21}H_{19}NO_6$ by HRESITOFMS (found: *m/z* 380.1111 $[M-H]^-$, calculated for $C_{21}H_{18}NO_6$ 380.1134). An anthraquinone skeleton was implied by the typical UV spectrum showing maximum absorption at 226, 247, 269, and 420 nm. The IR spectrum suggested the presence of amide group (1733 and 1560 cm^{-1}) and ketone (1733 cm^{-1}) (Figure S1). The ^{1}H nuclear magnetic resonance (NMR) spectrum (Figure S2) showed three methyl signals including two methoxy signals at δ_H 2.04 (d, $J = 1.2$ Hz), 3.97, and 4.00 and an olefine signal at δ_H 6.54 suggesting the presence of a double bond (Table 1). Two pair of aromatic signals at δ_H 7.25 and 8.15 (both: d, $J = 8.6$ Hz) and δ_H 7.58 and 7.75 (both: d $J = 7.9$ Hz) were observed implying the presence of two tetra substituted benzene rings. It also contained three exchangeable signals at δ_H 5.28 (brs), 5.97 (brs), and 13.23 (s), which did not show any correlation with a carbon signal in heteronuclear single quantum correlation (HSQC) spectrum, implying the presence of an amine and hydroxyl group. The ^{13}C NMR spectrum showed 21 signals including a methyl signal at δ_C 24.1 attached to an olefine carbon, two methoxy signals at δ_C 56.4 and 61.3, a methylene signal at δ_C 40.9, and three carbonyl signals at δ_C 172.3, 181.7, and 188.1, which supported the presence of an amide group and quinone moiety (Figure S3). The planar structure was established by the interpretation of 2D NMR spectra including HSQC, DQF-COSY, and heteronuclear multiple bond correlation (HMBC) (Fig. 2). The connections between protons and carbons were confirmed by the correlations in HSQC spectrum (Figure S4). The DQF-COSY revealed the proton spin networks between H-3/H-4 and H-7/H-8 (Figure S5), which were supported by the coupling patterns in ^{1}H NMR spectrum. Overall strucutre was

established by long range correlations in HMBC spectrum (Figure S6). Anthroquinone skeleton was confirmed by HMBC correlations from H-4 to a ketone at C-10 and from H-8 to a ketone at C-9. Two methoxy groups were attached to C-5 and C-6 by HMBC correlations from methoxy signals to C-5 and C-6, respectively. The exchangeable signal at δ_H 13.23 (s) showed HMBC correlations to C-1, C-2, and C-9a suggesting the attachment of the hydroxyl group at C-1. The carboxyamide chain was constructed by HMBC correlations from Me-5′ to C-1′, C-2′, and C-3′ and from H-3′ to C-1′, and C-4′ with the consideration of ^{13}C NMR chemical shift value of C-4′ at δ_C 172.3 and the molecular formula. It was attached to C-2 by HMBC correlation from H-3 to C-1′. The geometry of $\Delta^{1'}$ was assigned as *Z* by ^{13}C NMR chemical shift value of Me-5′ at δ_C 24.1, which was supported by NOESY correlations between H-3/H-3′ and H-1′/Me-5′ (Figure S7). Therefore, the sctructure of **1** was determined to be an anthraquinone carboxamide desinated as kinanthraquinone.

We evaluated the cytotoxicities of **1** against the human cervical epidermoid carcinoma cell line, HeLa, human promyelocytic leukemia cell line, HL-60, and rat kidney cells infected with ts25, a T-class mutant of *Rous sarcoma* virus Prague strain, *src*^{ts}-NRK. Also, their antimicrobial activities against *Staphylococcus aureus* 209, *Escherichia coli* HO141, *Aspergillus fumigatus* Af293, *Pyricularia oryzae* kita-1, and *Candida albicans* JCM1542 were tested. Compound **1** showed moderate cytotoxicity against HL-60 and *src*^{ts}-NRK cells with IC_{50} value of 7.9 and 10 μM, respectively. Antimicrobial effects was not observed by the concentration up to 100 μM.

Kinanthraquinone was isolated from *S. reveromyceticus* SN-593-44. It had an anthraquinone skeleton with a carboxamide group and was a rare class of metabolite as a natural product isolated from microbes. Recetly, similar anthraquinone carboxamides were reported as minor metabolites of lugdunomycins from *Streptomyces* sp. QL37 [19]. The carboxamide group might be biosynthesized from a carboxylic acid by a amidotransferase [20], which has not been identifed in the strain yet. We are going to proceed a study to reveal a biosynthetic mechanism of the metabolite and also investigate biological activities.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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