NK154183A and B, Antitumor Substances Produced by *Streptomyces* sp.

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In the course of antitumor activity screening program, dunaimycin analogs, NK154183A and B (Fig. 1), were isolated from the culture broth of *Streptomyces* sp. NK154183. In this paper, we report their production, isolation, structure elucidation and biological properties.

The strain NK154183, which was isolated from a soil sample and identified to be *Streptomyces* sp., was cultured in the seed medium composed of soluble starch 2%, glucose 0.5%, peptone 0.5%, meat extract 0.5%, soybean meal 0.5%, KH_2PO_4 0.05%, $MgSO_4$ 0.05% and $CaCO_3$ 0.2% (adjusted at pH 7.2 before sterilization). The seed culture was carried out on a rotary shaker (220 rpm) at 27°C for 48 hours in 500-ml Erlenmeyer flasks containing 100 ml of the seed medium. Then, the seed culture (300 ml) was inoculated to a 30-liter jar fermenter containing 20 liters of the production medium (dextrin 2.0%, galactose 2.0%, corn steep liquor 0.5%, Bacto-Soytone (DIFCO) 1.0%, $(NH_4)_2SO_4$ 0.2%, $CaCO_3$ 0.2% and antifoam 0.08%). The fermentation was carried out at 27°C for 4 days with aeration (10 liters/minute) under constant agitation (270 rpm).

NK154183A and B were isolated from the mycelia by monitoring the antitumor activity. The culture broth (60 liters) of *Streptomyces* sp. NK154183 was filtered. And the resulting mycelial cake was treated with Me_2CO (3 liters) and filtered. The extract was evaporated under the reduced pressure to give aqueous solution. The resulting aqueous solution was extracted with EtOAc and then the EtOAc extract was dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give a brown oil (5.8 g). The oily residue was applied on a silica gel column and eluted with a step wise gradient of EtOAc-MeOH. The active fractions were separated into A and B. The fraction A was eluated with EtOAc - MeOH (15 : 1) eluent and concentrated *in vacuo* to give A (236 mg), the other fractions were eluated with EtOAc - MeOH (10 : 1) eluent and to give B (460 mg). The fraction A was chromatographed on a silica gel column with $CHCl_3$ - MeOH (25 : 1) and the active fractions were evaporated to dryness giving a powder material (34 mg). From this powder material, white powder (10.8 mg) was given by using preparative silica gel TLC (EtOAc - MeOH, 7 : 1). Finally, NK154183A (6.2 mg) was crystallized from

Fig. 1. Structures of NK154183A and B.

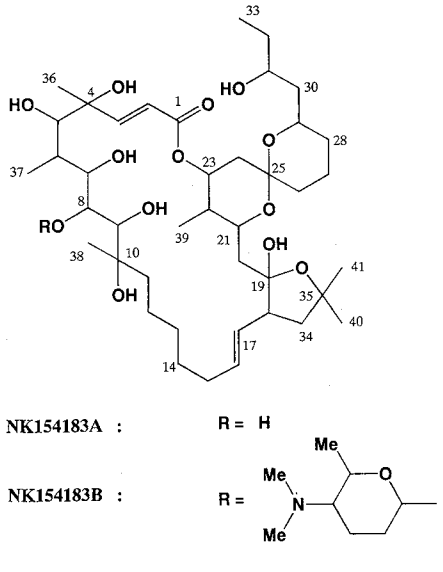

Table 1. Physico-chemical properties of NK154183A and B.

	NK154183A	NK154183B
Appearance	Colorless prism	Colorless prism
FAB-MS *m/z*	753 $[M-OH]^-$, 769 $[M-H]^-$	912 $[M+H]^+$, 934 $[M+Na]^+$
HR-MS Found.	753.4764	912.6131
Calcd.	753.4789 ($C_{41}H_{69}O_{12}$)	912.6048 ($C_{49}H_{86}NO_{14}$)
Molecular formula	$C_{41}H_{70}O_{13}$ (MW: 770)	$C_{49}H_{85}NO_{14}$ (MW: 911)
UV λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$)	217 (125)	219 (165)
IR ν_{max} (KBr) cm^{-1}	3460, 2940, 1720, 1280, 1170, 1090, 1060, 980	3470, 2950, 1725, 1280, 1180, 990, 975
Rf[a] value 1)	0.55	0.57
2)	0.80	0.30
Color reaction positive	H_2SO_4, $KMnO_4$	H_2SO_4, $KMnO_4$

[a] Slica gel TLC (Kieselgel 60F 0.25 mm, Merck) was used with developing solvent 1) $CHCl_3$ - MeOH (8 : 1), 2) EtOAc - MeOH (4 : 1).

Me_2CO as colorless crystals. The fraction B was chromatographed on a silica gel column with $CHCl_3$-MeOH (100:3) and the active fractions were evaporated to a powder material (274 mg). This material was applied on a TOYOPEARL HW-40 gel column eluting with MeOH to yield a crude powder (195 mg) and on a silica gel column with $CHCl_3$-MeOH-NH_4OH (100:1:1) to give a powder material (105 mg), successively. From this powder material, white powder (58 mg) was given by using preparative silica gel TLC ($CHCl_3$-MeOH-NH_4OH, 300:20:3). Finally, NK154183B (18.0 mg) was crystallized from Et_2O-hexane as colorless crystals.

The physico-chemical properties of NK154183A and B are summarized in Table 1. NK154183A and B are soluble in MeOH, Me_2CO, EtOAc, $CHCl_3$ and Et_2O, while they are insoluble in *n*-hexane, toluene and H_2O. The molecular formula of NK154183A was determined to be $C_{41}H_{70}O_{13}$ from the result of HRFAB-MS spectrometry (Found: *m/z* 753.4764, Calcd: *m/z* 753.4789), which was supported by ^{13}C NMR data (Table 2). The molecular formula of NK154183B was determined to be $C_{49}H_{85}NO_{14}$ from the result of HRFAB-MS spectrometry (Found: *m/z* 912.6131, Calcd: *m/z* 912.6048), which was also supported by ^{13}C NMR data (Table 2). Their UV and IR spectra showed almost the same absorption as shown in Table 1.

In the ^{13}C NMR spectra, similar signal patterns were observed between NK154183B and dunaimycin[1]. Structure elucidation of NK154183A and B was achieved by the detailed NMR spectral analyses and the comparison with dunaimycin. The ^{13}C NMR spectral data of NK154183A and B and their assignments are shown in Table 2. The 1D and 2D NMR data of NK154183A and B indicated that they were 24-membered macrolides like as dunaimycin D2S. Whereas dunaimycin D2S had nine *C*-methyl groups, the 1D and 2D NMR data of NK154183B accounted for eight *C*-methyls. Thus, C-28 methyl, which appeared at δ_C 11.4 in dunaimycin D2S, was absent in NK154183B. Recently, ossamycin which is 24-membered macrolide and absent C-28 methyl has been reported from Lilly Laboratories[2]. So, we tried the comparison of NK154183B and ossamycin by the single crystal X-ray analysis. The X-ray data (unpublished) confirmed the structure of NK154183B to be ossamycin. A comparison of 1D and 2D NMR spectra between NK154183A and B indicated the existence of amino sugar attached at C-8 in NK154183B. From this result, it was shown that NK154183A was an aglycone of NK154183B. The aglycone, NK154183A, is novel macrolide according to our research for new compounds.

NK154183A and B exhibited antitumor activity *in vitro* against the human colon adenocarcinoma (SW1116) and NIH swiss mouse embryo cell (NIH 3T3). The IC_{50} values of NK154183A were found to be 0.89 μg/ml and 1.21 μg/ml, while those of NK154183B were 5.22 μg/ml and 8.19 μg/ml, respectively. The acute toxicities intravenously for mice of NK154183A and B were found to be 1.5 mg/kg (LD_{50}) and 1.25 mg/kg (LD_{50}), respectively. They showed antifungal activities against some species of phytopathogens, for example, *Pyricularia oryzae* (MIC A: 0.78 μg/ml, B: 0.39 μg/ml), *Collerichum frgariae* (MIC A: 0.78 μg/ml, B: 1.56 μg/ml), but inactive against Gram-positive and negative bacterium.

Table 2. ^{13}C NMR assignments for NK154183A, B and Dunaimycin D2S.

Carbon	NK154183A[a]	NK154183B[a]	Dunaimycin D2S[b]
1	165.0	164.7	164.7
2	119.8	119.2	119.3
3	149.4	148.7	148.7
4	75.4	74.6	74.9
5	79.9	79.4	79.5
6	35.3	35.5	35.6
7	78.2	77.2	77.3
8	72.7	86.1	86.0
9	73.8	74.3	74.4
10	76.2	74.6	74.6
11	35.5	30.2	39.9
12	23.3	23.5	29.7
13	30.1	30.8	23.6
14	29.1	29.1	29.1
15	32.6	35.4	32.5
16	134.2	133.7	133.7
17	129.5	129.0	129.2
18	54.3	54.1	54.2
19	106.4	106.1	106.1
20	38.5	38.2	38.2
21	68.0	67.7	67.8
22	34.6	34.5	34.5
23	69.9	69.5	69.6
24	39.8	39.9	35.5
25	98.5	98.1	98.2
26	34.2	34.0	29.0
27	19.3	19.2	26.8
28	30.9	32.4	30.6
29	65.8	65.5	67.1
30	44.0	43.9	41.4
31	68.2	67.9	68.2
32	29.8	29.6	30.2
33	10.1	10.0	10.1
34	43.4	43.0	43.2
35	81.8	81.6	81.7
36	28.3	28.5	28.6
37	5.4	5.5	6.0
38	22.1	22.0	22.2
39	6.0	5.9	5.6
40	28.9	28.8	28.8
41	30.4	30.2	30.3
1′		98.3	98.4
2′		29.9	29.2
3′		20.0	20.0
4′		61.8	61.9
5′		72.3	72.3
6′		13.3	13.4
7′		43.3	43.3
28-CH_3			11.4

[a] Measured at 100 MHz in $CDCl_3$; chemical shifts in ppm from TMS.
[b] From ref 1. (125 MHz in $CDCl_3$).

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