Journal of Bioscience and Bioengineering
VOL. 127 No. 5, 549–553, 2019

www.elsevier.com/locate/jbiosc

Nanaomycin I and J: New nanaomycins generated by mycothiol-mediated compounds from "*Streptomyces rosa* subsp. *notoensis*" OS-3966

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Received 13 June 2018; accepted 14 October 2018
Available online 29 November 2018

Two new nanaomycin analogs, nanaomycin I and J, were isolated from a cultured broth of an actinomycete strain, "*Streptomyces rosa* subsp. *notoensis*" OS-3966. In our previous study, we have confirmed the occurrence of nanaomycin I (*m/z* = 482 $[M + H]^+$) that lacks a pseudo-disaccharide from the mycothiol of nanaomycin H under same culture condition. In this study, to confirm the structure of nanaomycin I, the strain "*S. rosa* subsp. *notoensis*" OS-3966 was re-cultured and the target compound with *m/z* = 482 $[M + H]^+$ was isolated. Furthermore, we discovered another new analog, designated as nanaomycin J in isolating nanaomycin I. The NMR analyses revealed that the structures of nanaomycin I and J are *N*-acetylcysteine *S*-conjugates without a pseudo-disaccharide and *N*-acetylcysteine *S*-conjugates without a *myo*-inositol of nanaomycin H, respectively. The relative configurations of the tetrahydropyrane moiety of nanaomycin I and J were determined by rotating-frame overhauser effect spectroscopy (ROESY) analysis. Absolute configurations of the *N*-acetylcysteine moiety of nanaomycin I and J were determined by advanced Marfey's analyses for acid hydrolysis of de-sulfurized nanaomycin I and J with Raney nickel. Nanaomycin I and J showed moderate cytotoxicity against several human tumor cell lines.

[**Key words:** Actinomycete; Mycothiol; Nanaomycin I; Nanaomycin J; Metabolite; *Streptomyces*]

Actinomycetes are known to produce natural products with unique chemical structures and many products have been used successfully as drugs in human medicine. These natural products are often isolated along with a variety of analogous compounds. The subtle differences of structure in the analogs, such as the presence or absence of a hydroxyl group, can significantly affect the presence, absence or potency of the biological activity of a compound. For example, nanaomycins A–E were discovered at the Kitasato Institute in 1975 (1–4) from a cultured broth of "*Streptomyces rosa* subsp. *notoensis*" OS-3966 by screening for anti-microbial activity against *Trichophyton* spp. and *Mycoplasma* spp. Nanaomycin A possesses strong anti-microbial activity due to production of the superoxide anion (O_2^-) generated in the process of reduction of the compound (5,6). Nanaomycin analogs are produced in the order of biosynthesis, namely D, A, E and B (7), the anti-microbial activity of nanaomycin A being stronger than other analogs.

The improvement of analytical equipment in recent years has made it possible to discover analogs much more easily. We have performed physicochemical (PC) screening using liquid chromatography mass spectroscopy (LS/MS) and discovered the analogs nanaomycin F and G. Nanaomycins F and G do not exhibit any antimicrobial activity, probably due to the substantial decrease in production of O_2^- and this may be the reason why these two analogs were not identified at the time that other bioactive analogs were discovered (8). Subsequently, another analog, nanaomycin H, was discovered using PC screening (9). Nanaomycin H has a mycothiol moiety which plays a role in protecting against the oxygen toxicity of glutathione in eukaryotes. It is believed that α-glucosamine linked to a *myo*-inositol, pseudo-disaccharide, is retained in the cell and re-enters the mycothiol biosynthesis pathway (10). Actually, we have confirmed the occurrence of another nanaomycin analog (*m/z* = 482 $[M + H]^+$), designated as nanaomycin I, under same culture condition. Nanaomycin I is deduced to be a structure which lacks a pseudo-disaccharide from the mycothiol of nanaomycin H by mass fragments (9). In this study, to confirm the structure of nanaomycin I, the strain "*S. rosa* subsp. *notoensis*" OS-3966 was re-cultured and the target compound with *m/z* = 482 $[M + H]^+$ was isolated. Furthermore, we discovered another new analog, designated as nanaomycin J, that lacks the *myo*-inositol from mycothiol of nanaomycin H in isolating nanaomycin I. Here we report the fermentation, isolation, structure elucidation and biological activity of nanaomycin I and J.

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1389-1723/$ – see front matter
https://doi.org/10.1016/j.jbiosc.2018.10.013

MATERIALS AND METHODS

General experimental procedures Silica gel and octadecylsilyl (ODS) silica gel were purchased from Fuji Silysia Chemical (Aichi, Japan). All solvents were purchased from Kanto Chemical (Tokyo, Japan).

High-resolution electrospray ionization mass spectrometry (HRESIMS) spectra were measured using an AB Sciex QSTAR Hybrid LC/MS/MS Systems (AB Sciex, Framingham, MA, USA). Nuclear magnetic resonance (NMR) spectra were measured using a Bruker AVANCE III HD600 (Bruker, Kanagawa, Japan), with ^{1}H NMR at 600 MHz and ^{13}C NMR at 150 MHz in CD_3OD or DMSO-d_6. The chemical shifts are expressed in parts per million (ppm) and are referenced to residual and CHD_2OD (3.31 ppm) and CHD_2SOCD_3 (2.50 ppm) in the ^{1}H NMR spectra and CD_3OD (49.0 ppm) and DMSO-d_6 (39.5 ppm) in the ^{13}C NMR spectra. The ultra violet (UV) spectra were measured with a Hitachi U-2810 spectrophotometer (Hitachi, Tokyo, Japan). Optical rotation was measured on a JASCO model DIP-1000 polarimeter (Jasco, Tokyo, Japan). The infrared radiation (IR) spectra (KBr) were taken on a Horiba FT-710 Fourier transform IR spectrometer (Horiba, Kyoto, Japan).

Fermentation of "*S. rosa* subsp. *notoensis*" OS-3966 "*S. rosa* subsp. *notoensis*" OS-3966 strain has been preserved as freeze-dried status with 10% skim-milk. The produce culture was performed using same condition that of nanaomycin H described following. This strain was cultured on agar slants consisting of 1.0% starch, 0.3% NZ amine, 0.1% yeast extract, 0.1% meat extract, 1.2% agar and 0.3% $CaCO_3$. A loop of spores of the strain OS-3966 was inoculated into 100 mL of the seed medium, consisting of 2.4% starch, 0.1% glucose, 0.3% peptone, 0.3% meat extract, 0.5% yeast extract and 0.4% $CaCO_3$ (adjusted to pH 7.0 before sterilization) in a 500-mL Erlenmeyer flask. The flask was incubated on a rotary shaker (210 rpm) at 27°C for 3 days. A 1-mL portion of the seed culture was transferred to Erlenmeyer flasks (total 60), each containing 100 mL of starch medium consisting of 2% soluble starch, 0.5% glycerol, 1.0% defatted wheat germ, 0.3% meat extract, 0.3% dry yeast and 0.3% $CaCO_3$ (adjusted to pH 7.0 before sterilization) and fermentation was carried out on a rotary shaker (210 rpm) at 27°C for 6 days.

Isolation of nanaomycin I and J The 6-days old cultured broth (6 L) of strain OS-3966 was extracted with an equal amount of methanol (MeOH) and evaporated. The MeOH extract (3.2 g) was separated to six fractions by silica gel column chromatography with chloroform ($CHCl_3$) and MeOH solvent system ($CHCl_3$:MeOH = 50:1, 25:1, 10:1, 6:4, 4:6, 0:1, stepwise separation). The $CHCl_3$:MeOH = 4:6 (1.02 g) fraction was separated by middle pressure liquid chromatography (MPLC) with an ODS column (10%–100% gradient system with MeOHaq, ϕ25 × 100 mm). The collective fraction (416.5 mg), including nanaomycin I, was separated by MPLC using an ODS column (10%–100% gradient system with MeOHaq, ϕ25 × 100 mm). The final purification was carried out by preparative HPLC (Inertsil ODS-4, ϕ14 × 250 mm) using a 20% MeOHaq (0.1% formic acid) isocratic solvent system to obtain nanaomycin I (9.4 mg). The other collective fraction (163.8 mg), including nanaomycin J, was separated by MPLC using ODS column chromatography (10%–100% gradient system with MeOHaq, ϕ25 × 100 mm). The final purification was carried out by semi-preparative HPLC (YMC-Triart PFP, ϕ10 × 250 mm) using a 30% MeOHaq (0.1% formic acid) isocratic solvent system to obtain nanaomycin J (5.5 mg).

Nanaomycin I: pale yellow amorphous solid; $[\alpha]_D^{26}$ −142.7° (c = 0.1, MeOH), IR (KBr) ν_{max} cm^{-1}: 3451, 1646, 1527; UV λ_{max} nm (ε): 204 (15,392), 231 (15,969), 267 (sh), 353 (5050); ^{1}H and ^{13}C NMR data are shown in Table 1; HRESI-MS m/z 482.1129 $[M + H]^+$ (calculated value for $C_{21}H_{24}NO_{10}S$: m/z 482.1115).

Nanaomycin J: pale yellow amorphous solid; $[\alpha]_D^{26}$ −125.5° (c = 0.1, MeOH); IR (KBr) ν_{max} cm^{-1}: 3463, 1643, 1527; UV λ_{max} nm (ε): 204 (24,075), 231 (18,746), 267 (sh), 353 (5778); ^{1}H and ^{13}C NMR data are shown in Table 1; HRESI-MS m/z 643.1833 $[M + H]^+$ (calculated value for $C_{21}H_{24}NO_{10}S$: m/z 643.1803).

Absolute configurations of the *N*-acetylcysteine moiety of nanaomycin I and J Advanced Marfey's analyses for acid hydrolysis of a de-sulfurized nanaomycin I and J with Raney nickel were used to determine the absolute configurations of the *N*-acetylcysteine moiety of both nanaomycin I and J.

Raney nickel (10 mg) was added to a solution of either nanaomycin I (0.9 mg, 1.87 μmol) or nanaomycin J (1.1 mg, 1.71 μmol) in MeOH (500 μL) and stirred at room temperature. The suspensions were then purged with H_2 atmosphere under 1 atm. After stirring for 18 h (nanaomycin I) and 6 days (nanaomycin J) at room temperature, the suspensions were filtered through a filter pad and washed with MeOH (1.0 mL × 3) and H_2O (1.0 mL × 3), respectively. MeOH filtrates and H_2O filtrates were concentrated *in vacuo*.

To hydrolyze the acetyl moiety of *N*-acetylcysteine, the H_2O fractions derived from nanaomycin I and J processed as above were dissolved in 500 μL of 6 M hydrochloric acid (HCl), followed by heat treatment at 100°C for 12 h. The filtrates were concentrated to dryness *in vacuo* and the residue was dissolved in 1 mL of H_2O. Twenty microliters of 1 M $NaHCO_3$ and 50 μL of N^{α}-(5-fluoro-2,4-dinitrophenyl)-L-leucinamide (D-FDLA) were added to the 50 μL of hydrolysates, followed by incubation at 37°C for 1 h. The mixtures were neutralized by addition of 20 μL of 1 M HCl and then concentrated to dryness *in vacuo*. The resultant dried residues were dissolved in 1 mL acetonitrile, followed by passage through a filter. Similarly, the standard L- and D-alanine were derivatized according to the method mentioned

TABLE 1. ^{1}H and ^{13}C NMR chemical shifts of nanaomycin I and nanaomycin J.

	Nanaomycin I[a]		Nanaomycin J[b]	
Position	δ_H	δ_C	δ_H	δ_C
1	4.13 (q, J = 7.2 Hz, 1H)	72.6	4.21 (q, J = 7.2 Hz, 1H)	75.5
3	4.50 (m,1H)	62.8	4.69 (m, 1H)	64.6
4	1.82 (d, J = 13.5 Hz, 1H)	29.9	2.06 (d, J = 14.1 Hz, 1H)	31.3
	2.13 (dd, J = 13.5, 12.0 Hz, 1H)		2.27 (dd, J = 14.1, 4.8 Hz, 1H)	
4a		58.4		60.1
5		189.2		190.7
5a		132.9		134.6
6	7.45 (d, J = 7.6 Hz, 1H)	118.6	7.56 (dd, J = 7.5, 0.9 Hz, 1H)	119.9
7	7.72 (dd, J = 8.1, 7.6 Hz, 1H)	137.0	7.69 (dd, J = 8.4, 7.5 Hz, 1H)	137.9
8	7.28 (d, J = 8.1 Hz, 1H)	123.6	7.24 (dd, J = 8.4, 0.9 Hz, 1H)	124.6
9		160.3		162.6
9a		114.4		116.0
10		198.7		200.5
10a		75.9		77.5
11	1.61 (d, J = 7.2 Hz, 3H)	15.5	1.76 (d, J = 7.2 Hz, 3H)	16.1
12	2.39 (dd, J = 15.3, 12.0 Hz, 1H)	40.3	2.61 (d, J = 6.0 Hz, 2H)	41.6
	2.54 (dd, J = 15.3, 3.6 Hz, 1H)			
13		172.1		175.6
2′	4.19 (m, 1H)	51.3	4.50 (m, 1H)	53.7
3′	2.33 (dd, J = 12.6, 8.4 Hz, 1H)	31.9	2.40 (dd, J = 12.6, 9.0 Hz, 1H)	33.8
	2.64 (dd, J = 12.6, 4.8 Hz, 1H)		2.77 (dd, J = 12.6, 5.1 Hz, 1H)	
5′		169.2		173.2
6′	1.72 (s, 3H)	22.2	1.87 (s, 3H)	22.4
1″			5.03 (d, J = 3.0 Hz, 1H)	92.4
2″			3.76 (dd, J = 10.2, 3.0 Hz, 1H)	56.1
3″			3.68 (dd, J = 10.2, 9.0 Hz, 1H)	72.6
4″			3.37 (dd, J = 9.0, 9.0 Hz, 1H)	72.4
5″			3.79 (ddd, J = 9.0, 6.0, 3.0 Hz, 1H)	73.2
6″			3.69 (dd, J = 12.0, 6.0 Hz, 1H)	62.8
			3.79 (dd, J = 12.0, 3.0 Hz, 1H)	
10a-OH	7.14 (s, 1H)			
4′-NH	8.15 (d, J = 7.8 Hz, 1H)			

[a] Recorded for ^{1}H-NMR at 600 MHz, for ^{13}C-NMR at 150 MHz in DMSO-d_6.
[b] Recorded for ^{1}H-NMR at 600 MHz, for ^{13}C-NMR at 150 MHz in methanol-d_4.

above. The D-FDLA derivatives of the hydrolysate and the standard amino acids were subjected to LC/MS analysis at 40°C using the following gradient program: solvent A, H_2O with 0.1% formic acid; solvent B, MeOH with 0.1% formic acid; linear gradient 50–100% of B from 2 to 10 min.

Cytotoxic activity Cytotoxic activity of nanaomycin I and J was evaluated using WST-8 (Kishida Chemical, Osaka, Japan) against eight cell lines, namely HL-60 (human promyelocytic leukemia cells), Jurkat (human acute lymphocytic leukemia cell line), THP-1 (human acute monocytic leukemia cell line), HeLa S3 (human cervical cancer cell line), A549 (Human lung carcinoma cell line), Panc1 (human pancreas carcinoma cell line), HT29 (human colon adenocarcinoma cell line) and H1299 (human non-small lung carcinoma cell line) as described previously (10). Briefly, HL-60, Jurkat and THP-1 cell lines were seeded in 96-well plates (3×10^5 cells per well) and cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (Nacalai Tesque, Kyoto, Japan) supplemented with 10% fetal bovine serum (FBS), 100 IU/mL penicillin and 100 μg/mL streptomycin (Life Technologies Japan, Tokyo, Japan) and 1 mM sodium pyruvate (Life Technologies Japan). HeLa S3, A549, Panc1, HT29 and H1299 cell lines were seeded in 96-well plates (5×10^3 cells per well) and cultured in Dulbecco's modified Eagle's medium (Wako Pure Chemical Industries, Osaka, Japan) supplemented with 10% FBS and 100 IU/mL penicillin and 100 μg/mL streptomycin (Life Technologies Japan) at 37°C under 5% CO_2. After overnight culture, nanaomycin I and nanaomycin J, dissolved in MeOH at appropriate final concentrations (1.0, 3.0, 10, 30 and 100 μM), were added into each well. Staurosporine, as used positive control, was added to wells at final concentration 2.0 μM. After incubating for 48 h at 37°C, the WST-8 solution was added to each well and incubated at 37°C for 4 h. The absorbance at 450 nm of each well was measured using a Corona Grating Microplate Reader SH-9000 (Corona Electric, Ibaraki, Japan). Cytotoxicity was determined by comparing the resulting absorbances with the mean absorbance of the control wells (without nanaomycins, considered as 100% viability).

Statistical analysis The experiment was performed in triplicate and values are expressed as mean±standard deviation ($n = 3 \pm$ S.D.). In all analyses, $P < 0.05$ indicates statistical significance versus control (Student's *t*-test).

RESULTS AND DISCUSSION

Structures of nanaomycin I and J Nanaomycin I was isolated as a pale yellow amorphous solid. The HRESIMS of nanaomycin I produced the $[M + H]^+$ at *m/z* 482.1110, indicating the molecular formula was $C_{21}H_{24}NO_{10}S$ (calculated value for *m/z* 482.1115). The absorption maxima of UV spectrum were observed at 232, 267 (sh) and 353 nm, which closely resembles those of nanaomycin H (9). When the ^1H -NMR in CD_3OD (Table S1) of nanaomycin I was compared with that of nanaomycin H, the proton signals derived from a pseudo-dissacharide observed in nanaomycin H disappeared. These results suggest that nanaomycin I is an *N*-acetylcysteine *S*-conjugate without the pseudo-dissacharide of nanaomycin H. The gross structure of nanaomycin I was confirmed by analyses of two-dimensional (2D) NMR data, including ^1H–^1H correlation spectroscopy (COSY), hetero-nuclear multiple quantum connectivity (HMQC) and hetero-nuclear multiple-bond connectivity (HMBC) spectra in DMSO-d_6 (Figs. S1–S5). The ^1H–^1H COSY and HMQC analysis revealed the presence of four partial structures a (C-6–C-8), b (C-1–C-11), c (C-4–C-12) and d (C-2′–C-3′), as shown Fig. 1A. Analysis of HMBC data confirmed the presence of a 2,3-disubstituted phenol, based on correlations from H-6 to C-9a, from H-7 to C-5a and C-9, and from H-8 to C-9 and C-9a. The HMBC correlations from H-6 to C-10, from H-7 to C-5 and from H-8 to C-10 indicated by the dotted arrows in Fig. 1 were observed as weak $^4J_{CH}$ couplings (Fig. S5). The HMBC correlations from H-1 to C-3, C-4a and C-10a, from H-3 to C-4a, from H-4 to C-10a, from 11-Me to C-10a and from C-10a-OH to C-4a and C-10a established the connection between the partial structures b and c in conjunction with forming a 5-hydroxy-tetrahydropyran. The conjugation of 2,3-disubstituted phenol and 5-hydroxy-tetrahydropyran via 1,4-benzoquinone was confirmed by the HMBC correlations from H-1 to C-4a and C-10a, from H-3 to C-4a, from H-4 to C-5 and C-10a, and from H-6 to C-5. The presence and position of the carboxylic acid were confirmed by HMBC correlations from H-3 to C-13 and from H-12 to C-13. The partial structure d and HMBC correlations from H-2′ to C-1′ and C-5′, from H-3′ to C-1′, from 6′-Me to C-5′ and from C-2′-NH to C-2′ and C-5′revealed the presence of *N*-acetylcysteine. The position of *N*-acetylcysteine was confirmed by HMBC correlations of H-3′ to C-4a through a sulfur atom. Thus, the gross structure of nanaomycin I was assigned as shown in Fig. 1. The partial relative configuration of nanaomycin I was determined by ROESY spectra (Fig. S6) that gave cross peaks for H-3/11-Me in tetrahydropyran moiety (Fig. 2A). To determine the absolute configuration of the *N*-acetylcysteine moiety, nanaomycin I was defined by advanced Marfey's analysis (11) of the desulfurized analog of nanaomycin I derivatized with Raney nickel (12) as a catalyst. Treatment of nanaomycin I by Raney nickel for desulfurization and the advanced Marfey's procedure led to identification of the absolute configuration of the alanine as the *S* form (Figs. S7 and S8). Thus, the structure of nanaomycin I was determined, as shown in Fig. 3.

Nanaomycin J was isolated as a pale yellow amorphous solid. The HRESIMS of nanaomycin J produced the $[M + H]^+$ at *m/z* 643.1788, indicating the molecular formula was $C_{27}H_{35}N_2O_{14}S$ (calculated value for *m/z* 643.1803). The absorption maxima of UV spectrum were observed at 231, 267 (sh) and 353 nm, similar to those of nanaomycin H. When the ^1H-NMR in CD_3OD of nanaomycin J was compared with that of nanaomycin H, the proton signals derived from the inositol observed in nanaomycin H had

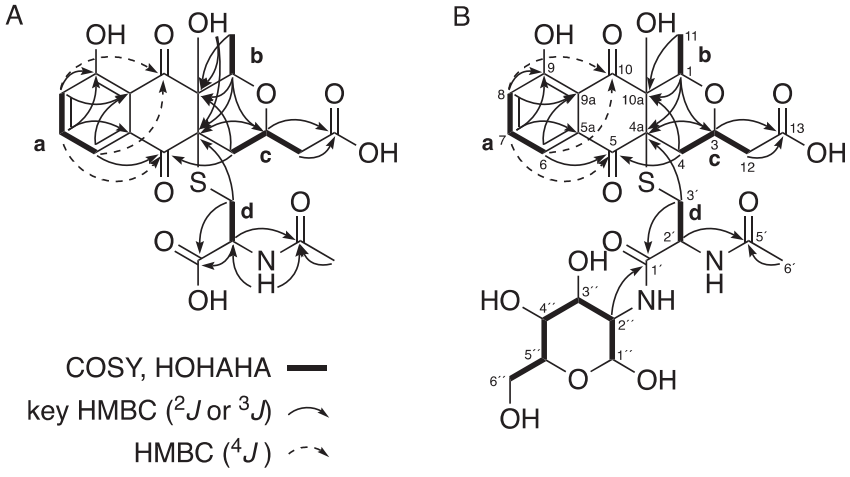

FIG. 1. Selected 2D-NMR correlations of (A) nanaomycin I and (B) nanaomycin J.

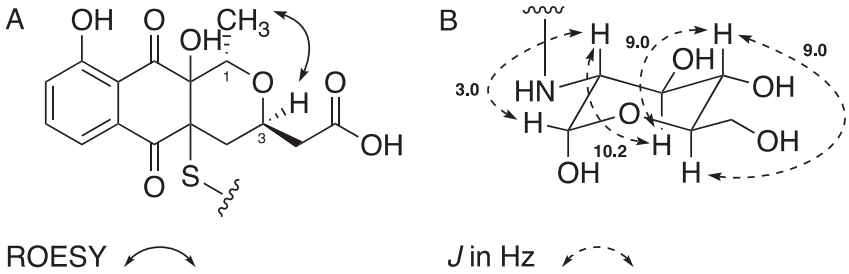

FIG. 2. Analyses of relative configurations: (A) ROESY correlation in the tetrahydropyran moiety of nanaomycin I and nanaomycin J, (B) Coupling constants of the sugar moiety of nanaomycin J.

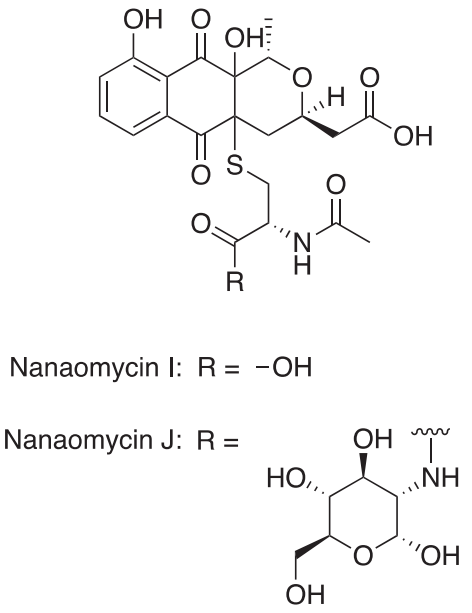

FIG. 3. The gross structures of nanaomycin I and nanaomycin J.

disappeared. These results suggest that nanaomycin J is *N*-acetylcysteine *S*-conjugate without an inositol of nanaomycin H. The gross structure of nanaomycin J was confirmed by analyses of 2D NMR data, including ^{1}H−^{1}H COSY, HMQC and HMBC spectra in DMSO-d_6 (Figs. 1B, S9−S13). The ^{1}H−^{1}H COSY and HMQC analysis revealed the presence of four partial structures **a**−d, as shown Fig. 1B. The HMBC correlations from H-1 to C-3, C-4a and C-10a, from H-3 to C-4a and C-13, from H-4 to C-5 and C-10a, from H-6 to C-5 and C-9a, from H-7 to C-5a and C-9, from H-8 to C-9 and C-9a and from 11-Me to C-10a revealed connectivity of three partial structures **a**−c via 1,4-benzoquinone (Fig. 1B). The partial structure d and HMBC correlations from H-2′ to C-5′, from H-3′ to C-1′ and from 6′-Me to C-5′ revealed the presence of *N*-acetylcysteine. The position of *N*-acetylcysteine was confirmed by HMBC correlations from H-3′ to C-4a through a sulfur atom (Fig. 1B). The presence and connectivity of an α-glucosamine was determined by the HMBC correlations from H-2′ to C-1′, detailed HOHAHA analysis (Figs. S14−S16) and analysis of coupling constants (Fig. 2B). The partial relative configuration of nanaomycin J was determined by ROESY spectra (Fig. S17) that gave cross peaks for H-3/11-Me in the tetrahydropyran moiety (Fig. 2A). To determine the configuration of the *N*-acetylcysteine moiety, nanaomycin J was also defined by advanced Marfey's analysis of the acid hydrolysate derived from desulfurization of nanaomycin J with Raney nickel as a catalyst (Fig. S7). The procedure led to identification of the absolute configuration of the alanine as the *S* form (Fig. S8). Thus, the structure of nanaomycin J was determined, as shown Fig. 3.

Biological activity of nanaomycin I and J Nanaomycin I and J were evaluated for possible cytotoxicity against a range of cell lines, HL-60, Jurkat, THP-1, HeLa S3, A549, Panc1, HT29 and H1299 cells, using previously reported methods (9). When these cells were treated with staurosporine, a positive control, at 2 μM for 48 h, the viability of HL-60, Jurkat, THP-1, HeLa S3, A549, Panc1, HT29 and H1299 cells were 2.4 ± 1.3%, 5.1 ± 3.5%, 1.4 ± 1.7%, 5.8 ± 1.0%, 13.6 ± 1.3%, 12.1 ± 2.1%, 35.9 ± 3.4% and 12.1 ± 2.7%, respectively. When these cells were treated with nanaomycin I at 100 μM for 48 h, the viability of HL-60, Panc1 and HT-29 cells were 65 ± 17%, 67 ± 4.5% ($P < 0.05$) and 68 ± 12% ($P < 0.05$), respectively. Nanaomycin J showed cytotoxicity against HL-60 cells, viability being 72 ± 16% at 100 μM for 48 h.

Two new nanaomycins were isolated from cultured broth of same condition of nanaomycin H and these compounds were considered to mycothiol-mediated compounds. The structure of nanaomycin I was determined to be a lack of pseudo-disaccharide from the mycothiol of nanaomycin H as expected. The planar structure of nanaomycin I was identical with that of 5R-(*N*-acetyl-L-cysteinyl)-14*S*-hydroxy-dihydrokalafungin (5R-AcCys-14*S*-OH-DHK) isolated from a mutant of the *act*VA-ORF4 gene for anti-norhodin biosynthesis in *Streptomyces coelicolor* A3(2) (13). However, the ^{1}H-NMR spectrum of nanaomycin I in DMSO-d_6 was different from that of 5R-AcCys-14*S*-OH-DHK. Despite the same absolute configuration of *N*-acetylcysteine, the optical rotation of nanaomycin I ($[\alpha]_D^{26}$ −142.7°, $c = 0.1$, MeOH) was the opposite when compared to 5R-AcCys-14*S*-OH-DHK ($[\alpha]_D^{23}$ + 116°, $c = 0.1$, MeOH). Furthermore, nanaomycin A and dihydrokalafungin, one of the biosynthetic precursors of nanaomycin I and 5R-AcCys-14*S*-OH-DHK, respectively, are enantiomers (1,14). These results suggest that nanaomycin I is a diastereomer of 5R-AcCys-14*S*-OH-DHK.

Nanaomycin J possessed *N*-acetylcysteine *S*-conjugate without a *myo*-inositol. In general, α-glucosamine linked to a *myo*-inositol, pseudo-disaccharide, is retained in the cell and re-enters the mycothiol biosynthesis pathway (10). To our knowledge, there is no previous report regarding secondary metabolites containing a mycothiol without inositol.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jbiosc.2018.10.013.

ACKNOWLEDGMENTS

The authors declare no conflict of interest. This study was supported by funds from the Institute for Fermentation, Osaka (IFO), Japan. We thank Ms. Noriko Sato, School of Pharmacy, Kitasato University, for measurements of NMR.

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