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SW-163C and E, Novel Antitumor Depsipeptides Produced by *Streptomyces* sp.

II. Structure Elucidation

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SW-163C and E are novel antitumor antibiotics, which belong to quinomycin family, isolated from the culture broth of *Streptomyces* sp. SNA15896. These compounds were determined to be cyclic depsipeptides having 3-hydroxyquinaldic acid as a chromophore and a sulfur-containing intramolecular cross linkage through various spectroscopic analyses.

In the course of our screening program for novel antitumor agents from microbial products, SW-163C and E were isolated from the culture broth of *Streptomyces* sp. SNA15896. These compounds showed potent antitumor activities *in vitro* and *in vivo*. The taxonomy, fermentation, isolation, and biological activities have been reported in the preceding paper[1]. The current report describes the physico-chemical properties and the structural elucidation of SW-163C and E (Fig. 1).

Results

Structural Elucidation of SW-163C (1)

The physico-chemical properties of **1** are summarized in Table 1. The IR spectrum indicated the presence of an ester group at 1735 cm^{-1} and an amide group at 1660 and 1515 cm^{-1}. The molecular formula was established as $C_{52}H_{60}N_{10}O_{14}S_2$ on the basis of HRFAB-MS. The UV absorption maxima were shown at 214, 230, 299, and 359 nm, suggesting the presence of a 3-hydroxyquinaldic acid chromophore[2~4]. The ^{13}C NMR and DEPT spectra of **1** (Table 2) revealed 26 carbons which included four methyl, three methylene, nine methine, five quaternary and five carbonyl carbons. ^1H NMR spectrum of **1** (Table 2) showed 30 proton signals including three exchangeable proton signals at δ 11.69, δ 8.75, and δ 8.31. Because of the molecular formula that had twice the number of the protons and carbons observed in the NMR spectra, SW-163C must be a symmetrical dimer. The direct connectivities of protons and carbons were established by the HMQC spectrum. The COSY and HMBC spectra showed the existence of five partial structures as shown in Fig. 2. For the partial structure A, nine sp^2 carbons, five related aromatic protons with a hydroxyl group and the HMBC correlations (Fig. 2) indicated the presence of a 3-hydroxyquinaldic acid chromophore. The COSY correlations from H-11 to H-12b and NH-20 and the HMBC correlation between H-11 and C-10 showed the partial structure B as a serine residue. The partial structure C was indicated to be an alanine residue by the COSY correlations of H-8 with NH-9 and H-19, and the long-range coupling between H-8 and C-7. The HMBC correlations from H-5 to C-4, C-17, and C-18 and the chemical shifts of H_2-17 (δ 3.54 and δ 2.30) suggested the presence of *N*-methylcysteine residue. Moreover, the chemical shifts of H_2-17, H-5 (δ 6.20), C-17 (δ 43.7), and C-5 (δ 57.1) were similar to those of *N,N'*-dimethylcystine moiety in thiocoraline[3,4] and triostin A[4,5]. These results suggested that the partial structure D comprised a part of a

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Fig. 1. Structures of SW-163C (**1**) and E (**2**).

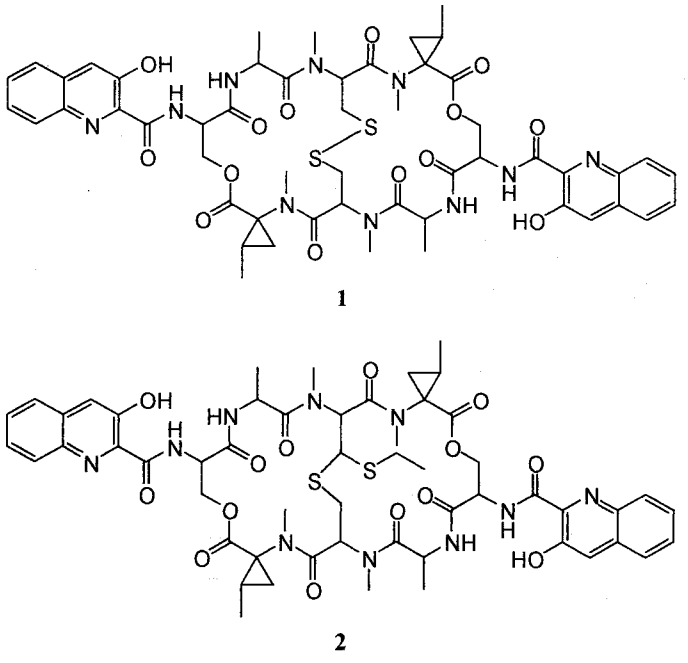
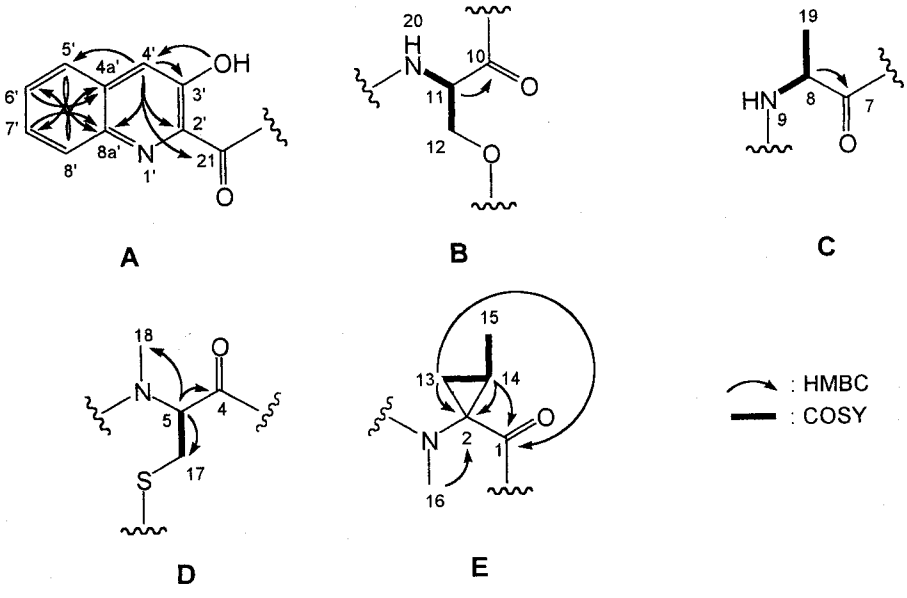


Fig. 2. Partial structures of SW-163C (**1**).



disulfide cross linkage to connect the two equivalent halves. For the partial structure E, the COSY spectrum showed a methyl-methine-methylene spin system. The long-range couplings from H_2-13, H-14, and H-16 to C-2 and from H_2-13 and H-14 to C-1 showed that the partial structure E was a 2-methyl-1-methylaminocyclopropanecarboxylic acid moiety. The connectivities of the partial structures (Fig. 3) were determined by the long-range correlations from H-16 to C-4, from H-18 to C-7, from NH-9 to C-10, and from H_2-12 to C-1. Furthermore, an HMBC correlation

Fig. 3. Connectivities of partial structures of SW-163C (**1**).

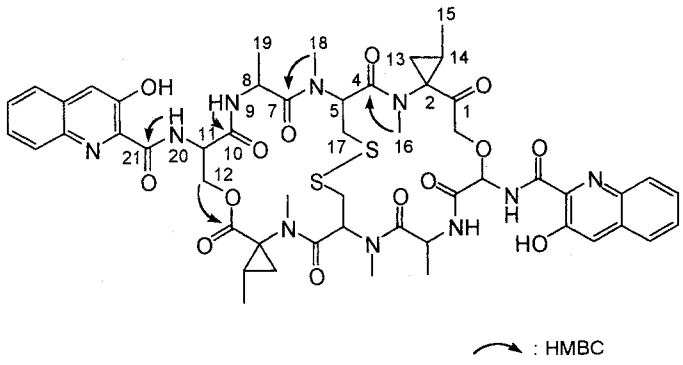


Table 1. Physico-chemical properties of SW-163C (**1**) and E (**2**).

	1	2
Appearance	Pale yellow crystalline powder	Pale yellow needle
$[\alpha]^{28}_{D}$ (c 0.2, $CHCl_3$)	−78.6°	−157.1°
M.P.	240~243℃	234~237℃
Molecular weight	1112	1140
Molecular formula	$C_{52}H_{60}N_{10}O_{14}S_2$	$C_{54}H_{64}N_{10}O_{14}S_2$
FAB-MS (*m/z*)	1113 $(M+H)^+$	1141 $(M+H)^+$
HRFAB-MS (*m/z*)		
Found:	1113.3815 $(M+H)^+$	1141.4110 $(M+H)^+$
Calcd.:	1113.3810 for $C_{52}H_{61}N_{10}O_{14}S_2$	1141.4123 for $C_{54}H_{65}N_{10}O_{14}S_2$
UV λ_{max}^{MeOH} nm (ε)	214 (78300), 230 (77400), 299 (9800), 359 (9800)	214 (70500), 230 (66200), 299 (9800), 359 (9200)
IR ν_{max} (KBr) cm^{-1}	3380, 2930, 1735, 1660, 1515	3380, 2930, 1735, 1660, 1520
Solubility		
soluble	CH_3CN, DMSO, $CHCl_3$	CH_3CN, DMSO, $CHCl_3$
insoluble	H_2O, *n*-Hexane	H_2O, *n*-Hexane

between NH-20 and C-21 was observed, indicating the chromophore, 3-hydroxyquinaldic acid, was attached to the serine residue. Through a series of analyses, the structure of **1** was determined as shown in Fig. 1.

Structural Elucidation of SW-163E (**2**)

The UV spectrum of **2** (Table 1) showed the absorption maxima at 214, 230, 299, and 359 nm. These characteristic absorptions suggested the presence of a 3-hydroxyquinaldic acid[2~4]. The IR spectrum (Table 1) indicated the presence of an ester group at 1735 cm^{-1} and an amide group at 1660 and 1520 cm^{-1}. These results were characteristic of a depsipeptide with a chromophore, 3-hydroxyquinaldic acid. The molecular formula of **2** was determined to be $C_{54}H_{64}N_{10}O_{14}S_2$ on the basis of HRFAB-MS and NMR spectral analyses. The molecular weight of **2** was 14 mass units higher than that of UK-63,598[2]. Moreover, the similarity of ^{13}C NMR spectra between **2** and UK-63,598 showed that **2** is an UK-63,598 analog[2]. The presence of two alanine residues, two serine residues, two 2-methyl-1-methylaminocyclopropanecarboxylic acid moieties, and

Fig. 4. HMBC correlations of SW-163E (**2**).

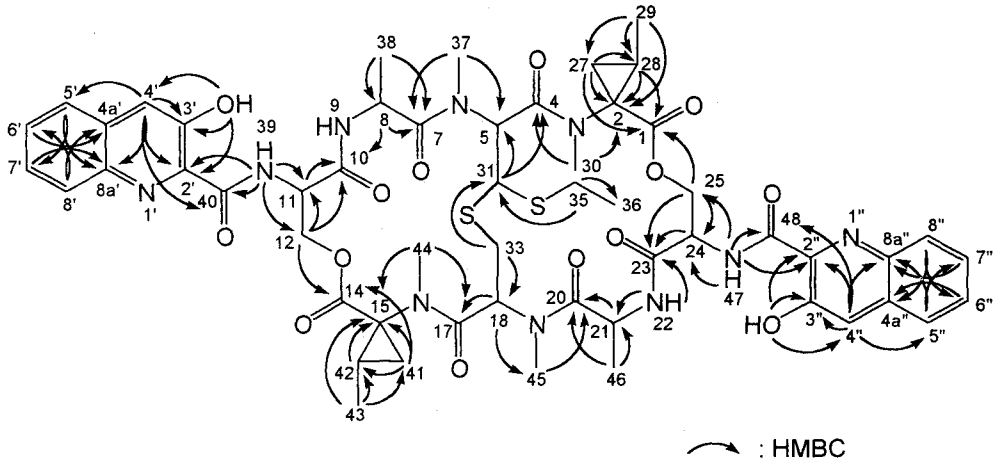


Table 2. ^{1}H and ^{13}C NMR data for SW-163C (**1**) in DMSO-d_6.

position	δ_H[a]	COSY	δ_C[b]	HMBC
1			170.0 (s)	12a, 12b, 13a, 13b, 14
2			46.3 (s)	13a, 13b, 14, 16
4			170.6 (s)	5, 16, 17a, 17b
5	6.20 (1H, dd, J = 2.9, 11.6 Hz)	17a, 17b	57.1 (d)	17a, 17b, 18
7			171.0 (s)	8, 18, 19
8	4.50 (1H, m)	9-NH, 19	47.0 (d)	9-NH, 19
9-NH	8.31 (1H, d, J = 5.5 Hz)	8		
10			168.2 (s)	8, 9-NH, 11, 12a, 12b
11	4.86 (1H, dd, J = 4.1, 9.2 Hz)	12b, 20-NH	50.6 (d)	12b
12a	4.35 (1H, d, J = 10.6 Hz)	12b	64.1 (t)	11
b	4.80 (1H, dd, J = 4.1, 10.6Hz)	11, 12a		
13a	1.19 (1H, dd, J = 5.6, 9.5 Hz)	13b, 14	25.0 (t)	14, 15
b	1.52 (1H, dd, J = 5.6, 7.9 Hz)	13a, 14		
14	2.01 (1H, m)	13a, 13b, 15	23.3 (d)	13a, 13b, 15
15	0.99 (3H, d, J = 6.1 Hz)	14	11.7 (q)	13a, 13b
16	3.39 (3H, s)		35.9 (q)	
17a	2.30 (1H, dd, J = 2.9, 13.1 Hz)	5, 17b	43.7 (t)	5
b	3.54 (1H, dd, J = 11.6, 13.1 Hz)	5, 17a		
18	2.74 (3H, s)		29.9 (q)	5
19	1.32 (3H, d, J = 7.0 Hz)	8	15.1 (q)	8, 9-NH
20-NH	8.75 (1H, d, J = 9.2 Hz)	11		
21			167.8 (s)	20-NH, 4'
2'			135.4 (s)	4'
3'			152.9 (s)	4'
4'	7.92 (1H, s)		120.2 (d)	5', 3'-OH
4a'			131.6 (s)	6', 8'
5'	7.92 (1H, d, J = 8.6 Hz)	6'	126.7 (d)	4', 7'
6'	7.64 (1H, m)	5', 7'	128.8 (d)	8'
7'	7.66 (1H, m)	6', 8'	127.7 (d)	5'
8'	7.94 (1H, d, J = 8.6 Hz)	7'	128.5 (d)	6'
8a'			140.5 (s)	4', 5', 7'
3'-OH	11.69 (1H, s)			

[a] ^{1}H NMR at 500 MHz referenced to TMS.

[b] ^{13}C NMR at 125 MHz referenced to DMSO (δ 39.5).

Table 3. ^{1}H and ^{13}C NMR data for SW-163E (**2**) in $CDCl_3$.

position	δ_H[a]	δ_C[b]	position	δ_H	δ_C
1		169.35 (s)	38	1.34 (3H, d, J = 7.3 Hz)	16.76 (q)
2		47.21 (s)	39-NH	8.89 (1H, d, J = 3.1 Hz)	
4		170.56 (s)	40		168.55[e] (s)
5	6.42 (1H, d, J = 8.2 Hz)	60.51 (d)	41a	1.31 (1H, m)	26.24 (t)
7		173.08 (s)	b	1.84 (1H, dd, J = 5.8, 7.9 Hz)	
8	4.85 (1H, m)	46.17 (d)	42	1.68 (1H, m)	24.61 (d)
9-NH	6.42 (1H, d, J = 8.2 Hz)		43	1.05 (3H, d, J = 6.1 Hz)	11.78 (q)
10		167.65 (s)	44	3.51 (3H, s)	36.56 (q)
11	4.95 (1H, m)	50.78 (d)	45	2.88 (3H, s)	29.75 (q)
12a	4.66 (1H, d, J = 11.0Hz)	63.81 (t)	46	1.39 (3H, d, J = 7.0 Hz)	17.92 (q)
b	4.84 (1H, m)		47-NH	8.91 (1H, d, J = 3.1 Hz)	
14		169.59 (s)	48		168.63[e] (s)
15		47.02 (s)	2'		133.73[f] (s)
17		171.61 (s)	3'		153.76 (s)
18	6.15 (1H, br.d)	54.39 (d)	4'	7.67[c] (1H, s)	121.20[g] (d)
20		173.21 (s)	4a'		132.42[h] (s)
21	4.97 (1H, m)	45.66 (d)	5'	7.72 (1H, m)	126.78 (d)
22-NH	6.77 (1H, d, J = 8.2 Hz)		6'	7.50 (1H, m)	128.91[i] (d)
23		167.36 (s)	7'	7.50 (1H, m)	127.64[j] (d)
24	4.86 (1H, m)	51.69 (d)	8'	7.76 (1H, m)	128.66 (d)
25a	4.68 (1H, d, J = 11.3 Hz)	64.30 (t)	8a'		141.23[k] (s)
b	4.75 (1H, dd, J = 4.9, 11.3 Hz)		3'-OH	11.34[d] (1H, s)	
27a	1.20 (1H, m)	26.00 (t)	2"		133.79[f] (s)
b	1.88 (1H, dd, J = 5.8, 8.2 Hz)		3"		153.76 (s)
28	1.74 (1H, m)	25.03 (d)	4"	7.68[c] (1H, s)	121.29[g] (d)
29	1.19 (3H, d, J = 6.4 Hz)	11.92 (q)	4a"		132.43[h] (s)
30	3.40 (3H, s)	35.90 (q)	5"	7.72 (1H, m)	126.78 (d)
31	5.03 (1H, d, J = 8.2 Hz)	47.86 (d)	6"	7.50 (1H, m)	129.01[i] (d)
33a	2.72 (1H, dd, J = 5.5, 15.9 Hz)	26.57 (t)	7"	7.50 (1H, m)	127.69[j] (d)
b	3.63 (1H, dd, J = 2.4, 15.9 Hz)		8"	7.76 (1H, m)	128.66 (d)
35	2.53 (2H, m)	25.00 (t)	8a"		141.30[k] (s)
36	1.30 (3H, t, J = 7.3 Hz)	13.68 (q)	3"-OH	11.36[d] (1H, s)	
37	2.84 (3H, s)	31.82 (q)			

[a] ^{1}H NMR at 500 MHz referenced to TMS.

[b] ^{13}C NMR at 125 MHz referenced to $CDCl_3$ (δ 77.0).

[c, d, e, f, g, h, i, j, and k] These signals were interchangeable, respectively.

two 3-hydroxyquinaldic acid chromophores was revealed by the COSY, HMQC, and HMBC spectra. Furthermore, the connectivities of these partial structures were deduced from the HMBC correlations (Fig. 4). The ester linkages between the serine residues and the 2-methyl-1-methylaminocyclopropanecarboxylic acid moieties were confirmed by the HMBC correlations from H-12 to C-14 and from H-25 to C-1, respectively. The alanyl-serinyl connections *via* the amide bonds were shown by the long-range couplings from H-8 to C-10 and from NH-22 to C-23. The long-range correlations from NH-39 and NH-47 to C-40 and C-48 of the carbonyl carbons for the chromophore units were observed, respectively. Discriminative assignments of C-40 and C-48 signals were difficult because the chemical shift values of these carbons at δ 168.63 and δ 168.55 were quite close. However,

attachment of each 3-hydroxyquinaldic acid moiety to the corresponding serine residue was clear. The long-range couplings between H-30 and C-4, H-37 and C-7, H-44 and C-17, and H-45 and C-20 established four *N*-methylamide linkages, respectively. For the thioacetal cross linkage, the HMBC correlations from H-18 to C-45 and C-17, from H-31 to C-5 and C-4, and from H-37 to C-5 indicated that **2** had a macrocyclic structure. Furthermore, the thioacetal moiety was established by the HMBC correlations from H-35 and H-33 to C-31 and from H-33 to C-18. Therefore, the structure of **2** was determined to be that shown in Fig. 1. All of the assigned proton and carbon signals are listed in Table 3.

Discussion

SW-163C (**1**) and E (**2**) are novel antitumor antibiotics, which belong to quinomycin family, produced by *Streptomyces* sp. SNA15896. Compounds **1** and **2** showed potent antitumor effects against P388 leukemia without toxicity and change in body weight at a dose of 1.0 mg/kg and 0.01 mg/kg, respectively[1]. The structures of **1** and **2** were determined to be cyclic octadepsipeptides with 3-hydroxyquinaldic acid as a chromophore. They have high structural similarity to the UK-65,032 complex[2]. Compound **1**, which possesses a 2-fold axis of symmetry, is related to triostin A[5], thiocoraline[3,4], and BE-22179[4,6]. Compounds **1** and **2** differ only in the sulfur-containing intramolecular cross linkage in the peptide portion, and the other structures such as 3-hydroxyquinaldic acid chromophore and the sequence of amino acids in the peptide are identical (Fig. 1). However, the antitumor activity of **2** is approximately 100 fold more potent than that of **1**[1]. This result suggests that the difference in intramolecular cross linkage between **1** and **2** influences antitumor activity. The elucidation of this structure-activity relationship might lead to more effective antitumor agents[7~11].

Experimental

UV spectra were recorded on a Hitachi U-3210 spectrophotometer. IR spectra were measured on a JASCO IR-810 infrared spectrophotometer. FAB-MS were obtained with a JEOL JMS-HX110 mass spectrometer. Optical rotations were measured on a JASCO DIP-1000 digital polarimeter. NMR spectra were recorded on a JEOL JNM-A500 NMR spectrometer. Melting points were measured on a Yanaco micro melting point apparatus.

References

1) KUROSAWA, K.; K. TAKAHASHI & E. TSUDA: SW-163C and E, novel antitumor depsipeptides produced by *Streptomyces* sp. I. Taxonomy, fermentation, isolation and biological activities. J. Antibiotics 54: 615~621, 2001
2) RANCE, M. J.; J. C. RUDDOCK, M. S. PACEY, W. P. CULLEN, L. H. HUANG, M. T. JEFFERSON, E. B. WHIPPLE, H. MAEDA & J. TONE: UK-63,052 complex, new quinomycin antibiotics from *Streptomyces braegensis* subsp. *Japonicus*; Taxonomy, fermentation, isolation, characterization and antimicrobial activity. J. Antibiotics 42: 206~217, 1989
3) BAZ, J. P.; L. M. CAÑEDO, J. L. FERNÁNDEZ PUENTES & M. V. SILVA ELIPE: Thiocoraline, a novel depsipeptide with antitumor activity produced by a marine *Micromonospora*. II. Physico-chemical propeties and structure determination. J. Antibiotics 50: 738~741, 1997
4) BOGER, D. L. & S. ICHIKAWA: Total syntheses of thiocoraline and BE-22179: Establishment of relative and absolute stereochemistry. J. Am. Chem. Soc. 122: 2956~2957, 2000
5) OTSUKA, H.; J. SHOJI, K. KAWANO & Y. KYOGOKU: Structure confirmation of triostin A by ^{1}H and ^{13}C magnetic resonance. J. Antibiotics 29: 107~110, 1976
6) OKADA, H.; H. SUZUKI, T. YOSHINARI, H. ARAKAWA, A. OKURA & H. SUDA: A new topoisomerase II inhibitor, BE-22179, produced by a streptomycete. I. Producing strain, fermentation, isolation and biological activity. J. Antibiotics 47: 129~135, 1994
7) FOX, K. R.: Footprinting studies of the interaction of quinomycin antibiotic UK63052 with DNA: Comparison with echinomycin. J. Antibiotics 43: 1307~1315, 1990
8) SEARLE, M. S. & G. WICKHAM: Hoogsteen versus Watson-Crick A-T basepairing in DNA complexes of a new group of 'quinomycin-like' antibiotics. FEBS Lett. 272: 171~174, 1990
9) SEARLE, M. S.: Binding of quinomycin antibiotic UK-65,662 to DNA: ^{1}H-n.m.r. studies of drug-induced changes in DNA conformation in complexes with $d(ACGT)_2$ and $d(GACGTC)_2$. Biochem. J. 304: 967~979, 1994
10) CHEN, H. & D. J. PATEL: Solution structure of a quinomycin bisintercalator-DNA complex. J. Mol. Biol. 246: 164~179, 1995
11) BOGER, D. L.; S. ICHIKAWA, W. C. TSE, M. P. HEDRICK & Q. JIN: Total syntheses of thiocoraline and BE-22179 and assessment of their DNA binding and biological properties. J. Am. Chem. Soc. 123: 561~568, 2001