RS-22A, B and C: New Macrolide Antibiotics from *Streptomyces violaceusniger*

II. Physico-chemical Properties and Structure Elucidation

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RS-22A, B and C are novel 36-membered macrolide antibiotics produced by *Streptomyces violaceusniger*. The structures of these antibiotics were unambiguously determined by 1D and 2D NMR and tandem-mass analysis of the oxidative derivative of RS-22C.

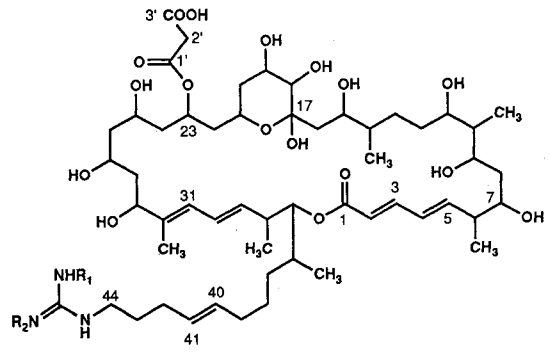
RS-22A (**1**), B (**2**) and C (**3**) are novel 36-membered macrolide antibiotics, produced by *Streptomyces violaceusniger* RS-22, which showed strong antifungal activity. The taxonomic study of the producing strain, fermentation, isolation and biological activity of these antibiotics are reported in the preceding paper.[1] In this paper, we report the physico-chemical properties and structural elucidation of **1**, **2** and **3**. The structures of these antibiotics were determined by 1D and 2D NMR including field gradient (FG)-HMBC and FG-HMQC analyses of **2**. The structural differences among **1**, **2** and **3** were elucidated from B/E linked scan analyses of positive ion fast atom bombardment (FAB) mass spectrometry. The structural sequence was further confirmed by tandem mass spectrometry of the oxidative derivative of **3**.

Results

Physico-chemical Properties

Antibiotics **1**, **2** and **3** were isolated as colorless powders. The physico-chemical properties of these antibiotics are summarized in Table 1. They are soluble in methanol, butanol and dimethylsulfoxide, but hardly soluble in ethyl acetate, chloroform and water. Their UV data show the presence of a diene conjugated to ester group. The molecular formula of **2** was determined to

Fig. 1. Structures of RS-22A (**1**), B (**2**) and C (**3**).



1: $R_1 = H$, $R_2 = H$
2: $R_1 = CH_3$, $R_2 = H$
3: $R_1 = CH_3$, $R_2 = CH_3$

Table 1. Physico-chemical properties of RS-22A (**1**), RS-22B (**2**) and RS-22C (**3**).

	1	2	3
Appearance	Colorless powder	Colorless powder	Colorless powder
Molecular formula	$C_{54}H_{91}N_3O_{17}$	$C_{55}H_{93}N_3O_{17}$	$C_{56}H_{95}N_3O_{17}$
FAB-MS (*m/z*)	1,054.6 $(M+H)^+$	1,068.6 $(M+H)^+$	1,082.7 $(M+H)^+$
HRFAB-MS (*m/z*)			
Calcd:	ND	1,068.6583 $(M+H)^+$	ND
Found:		1,068.6603	
$[\alpha]_D^{28}$	+36.7° (*c* 0.45, MeOH)	+36.7° (*c* 0.45, MeOH)	+36.7° (*c* 0.45, MeOH)
MP (dec)	132~137°	136~139°	129~132°
UV λ_{max}^{MeOH} nm (ε)	233 (22,300, sh), 238 (23,900), 265 (14,900)	234 (26,500, sh), 240 (28,200), 266 (18,400)	233 (22,400, sh), 238 (30,900), 265 (21,200)
IR (KBr) cm^{-1}	3380, 2990, 2960, 1700, 1670, 1640, 1450, 1380, 1255, 1140, 1068, 1000, 970	3380, 2990, 2960, 1700, 1670, 1640, 1450, 1380, 1255, 1140, 1068, 1000, 970	3380, 2990, 2960, 1700, 1670, 1630, 1450, 1380, 1255, 1140, 1067, 1000, 970

ND: Not determined.

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be $C_{55}H_{93}N_3O_{17}$ by HRFAB-MS (*m/z* 1068.6603 $(M+H)^+$, Δ +2.0 mmu). The molecular formulae of **1** and **3** were determined as $C_{54}H_{91}N_3O_{17}$ and $C_{56}H_{95}N_3O_{17}$, respectively by FAB-MS and the total number of carbon detected by 1D ^{13}C NMR, and ^1H-^{13}C COSY.

Structure Elucidation of **2**

The ^{13}C NMR spectrum of **2** showed 55 carbon signals, in which one carbon at 49.4 ppm was detected in the ^{13}C NMR in MeOH-d_3. One guanidino carbon at 158.2 ppm, three carbonyl carbons at 168.9, 171.6 and 174.2 ppm and ten olefinic carbons at 120.7, 125.1, 128.6, 130.3, 130.4, 132.5, 136.2, 139.9, 146.7 and 147.4 ppm indicate that **2** possesses two rings in the molecule because eleven degrees of unsaturation are required from its molecular formula. Analyses of ^1H-^{13}C COSY and FG-HMQC spectra clarified the presence of 7 methyl carbons, 16 methylene carbons, and 26 methine carbons including 9 olefinic and 12 oxygenated methine carbons and one quaternary hemiacetal carbon at 99.7 ppm. Since three overlapped peaks at 33.6, 30.6 and 65.5 ppm were observed, the total number of carbon signals is consistent with the required carbon number (Table 2).

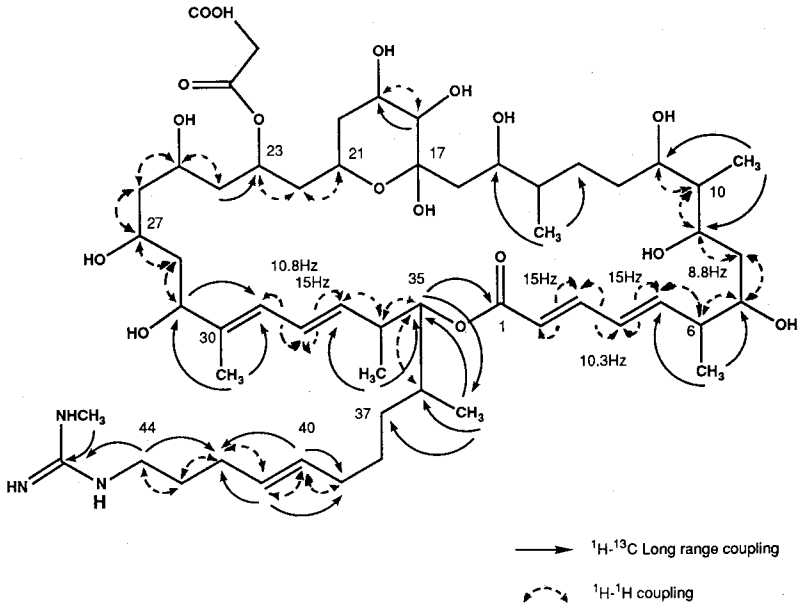
The structure of **2** was deduced by FG-DQF-COSY, ^1H-^{13}C COSY, FG-HMQC and FG-HMBC. Fig. 2 shows ^1H-^1H couplings detected by FG-DQF-COSY and long-range couplings observed in the FG-HMBC spectrum. The sequential assignments of C-2 to C-11 was deduced from FG-DQF-COSY. The long-range couplings between doublet methyl protons at 1.09 ppm (6-Me) and two methines (H-5 and H-7), and between doublet methyl protons at 0.86 ppm (10-Me) and two oxygenated methines (H-9 and H-11) detected in FG-HMBC spectrum supported the partial structure. The sequential assignments for C-13 to C-15, C-21 to C-37 and C-39 to guanidinomethyl were also determined by the analyses of FG-DQF-COSY and FG-HMBC data as shown in Fig. 2. The assignments of the six membered hemiacetal was executed by comparison with the NMR data for azalomycins,[2] copiamycin,[3] malolactomycins[4] and shurimycins.[5] The position of lactone was revealed from the long-range coupling between H-35 and C-1, detected in the FG-HMBC spectrum. To confirm the presence of nine free secondary hydroxy groups, deuterium-induced isotope shifts in ^{13}C NMR were applied.[6] The large upfield shifts due to deuterium substitutions of hydroxyl groups were observed for nine oxymethines at C-7, C-9, C-11, C-15, C-18, C-19, C-25, C-27 and C-29 (data not shown). The upfield shift was not observed for the oxymethines at C-21, C-23 and C-35. This results shows

Table 2. ^{13}C NMR data of RS-22A (**1**), RS-22B (**2**) and RS-22C (**3**) in MeOH-d_4 (100 MHz).

Position	1	2	3	Position	1	2	3
1	169.0 s	168.9 s	169.1 s	26	44.1 t	44.1 t	44.1 t
2	120.8 d	120.7 d	120.8 d	27	66.3 d	66.1 d	66.1 d
3	146.7 d	146.7 d	146.8 d	28	40.8 t	40.8 t	40.8 t
4	130.4 d	130.4 d	130.4 d	29	74.3 d	74.3 d	74.3 d
5	147.4 d	147.4 d	147.5 d	30	139.9 s	139.9 s	140.0 s
6	46.5 d	46.5 d	46.6 d	30-Me	13.4 q	13.3 q	13.3 q
6-Me	16.9 q	16.9 q	16.9 q	31	125.1 d	125.1 d	125.1 d
7	75.5 d	75.5 d	75.6 d	32	128.6 d	128.6 d	128.7 d
8	39.4 t	39.3 t	39.4 t	33	136.2 d	136.2 d	136.2 d
9	74.8 d	74.7 d	74.7 d	34	41.0 d	41.0 d	41.0 d
10	44.3 d	44.4 d	44.4 d	34-Me	17.5 q	17.5 q	17.6 q
10-Me	10.4 q	10.4 q	10.4 q	35	80.4 d	80.3 d	80.4 d
11	72.4 d	72.2 d	72.3 d	36	35.0 d	34.9 d	35.0 d
12	33.6 t	33.6 t	33.6 t	36-Me	14.2 q	14.0 q	14.1 q
13	30.6 t	30.6 t	30.67 t	37	34.4 t	34.5 t	34.5 t
14	41.7 d	41.6 d	41.6 d	38	27.9 t	27.9 t	27.9 t
14-Me	14.9 q	14.8 q	14.91 q	39	33.7 t	33.6 t	33.7 t
15	72.3 d	72.3 d	72.4 d	40	132.7 d	132.5 d	132.6 d
16	41.8 t	41.7 t	41.8 t	41	130.2 d	130.3 d	130.3 d
17	99.8 s	99.7 s	99.8 s	42	30.6't	30.6 t	30.7 t
18	77.3 d	77.1 d	77.3 d	43	29.8 t	29.8 t	29.8 t
19	69.7 d	69.7 d	69.8 d	44	41.9 t	41.9 t	42.1 t
20	41.2 t	41.2 t	41.3 t	1′	171.7 s	171.6 s	171.7 s
21	65.5 d	65.5 d	65.5 d	2′	49.7 t[a]	49.6 t[a]	49.6 t[a]
22	44.6 t	44.6 t	44.6 t	3′	174.1 s	174.2 s	174.1 s
23	70.6 d	70.6 d	70.7 d	N′-Me		28.3 s	28.5 s
24	43.8 t	43.9 t	44.0 t	N″-Me			28.5 s
25	65.5 d	65.5 d	65.5 d	N-CN_2	158.7 s	158.2 s	157.3 s

[a] Detected in MeOH-d_3.

Fig. 2. ^{1}H-^{1}H coupling of RS-22 B (**2**) detected by FG-DQF COSY and ^{1}H-^{13}C long-range coupling of RS-22 B (**2**) determined by FG-HMBC.



that the hydroxy group at C-21 formed the six membered hemiacetal ring with C-17 carbonyl group and the hydroxy groups at C-23 and C-35 were acylated. Three methylene carbons at C-8, C-26, and C-28 in the 1, 3-polyol system shifted upfield due to γ-effects of the deuterium isotope. Deuterium induced isotope shifts were also observed in the nitrogen bearing carbons at C-44 and *N*-methyl carbons. Since the methylene protons at C-2′ were rapidly exchanged by deuterium in MeOH-d_4 solution,[7] the carbon signal at C-2′ could not be detected in ^{13}C NMR of **2** in MeOH-d_4. However the signal was detected in ^{13}C NMR in MeOH-d_3. All carbon and proton signals of **2** were completely assigned and are listed in Tables 2 and 3.

The sequential information for the side chain was obtained from the collision induced dissociation (CID) mass spectrum using the B/E linked scan technique as shown in Fig. 3. Intense fragment ions at *m/z* 210, 182, 168, 154, 155, 101, 87 and 73 show the side-chain functionality of **2**. The fragment ion at *m/z* 965 was assigned to a demalonylated ion. The diagnostic ions from **2**, for the assignment of the double-bond position in the side chain moiety, are the allylic bond cleavage products having *m/z* 155 and 101.

The configuration of the diene system at C-2 and C-4 was determined as 2*E*, 4*E* from the large *trans* coupling constants, $J=15$ Hz. Though the signals of H-40 and H-41 are poorly resolved in the normal spectrum because of the overlapping of those signals with H-33, the large *trans* coupling constants ($J=15$ Hz) could be observed in FG-HMQC spectrum of **3**. Thus the *E* geometry of the double bond at C-40 was established. The *E* Configuration of the double bond at C-30 was established, because the ^{13}C chemical shift (δ_C 13.3 ppm) of the olefinic methyl (30-Me) resembles the chemical shift data[8] for the olefinic methyl carbon possessing the *E*-configuration and negative nuclear Overhauser effects (NOEs) between the olefinic methyl proton at 30-Me (δ 1.64 ppm) and the olefinic proton at H-32 (δ 6.2 ppm) were observed.

Thus, the structure of **2** was determined based on the result of the NMR studies and CID mapping of positive FAB mass spectrometry, as shown in Fig. 1.

Structures of **1** and **3**

Compounds **1**, **2** and **3** share common structural features, but **1** and **3** differ from **2** with respect to the absence of an *N*-methyl group and the presence of two *N*-methyl groups respectively, as described below (Fig. 1). Molecular weights of **1**, **2** and **3** were determined to be 1053, 1067 and 1081, respectively, by positive ion FAB mass spectrometry. ^{1}H NMR and ^{13}C NMR assignments for **1** and **3**, which were executed by comparison with the data of **2** are listed in Tables 2 and 3, respectively. In the ^{1}H NMR and ^{13}C NMR of **1**, the *N*-methyl proton signal at around δ_H 2.8 ppm and the *N*-methyl carbon signal at around δ_C 28 ppm could not be observed. However, two-equivalent signals assigned as the methyl groups of an *N*-alkyl-*N*′,*N*″-dimethyl trisubstituted guanidino group were observed at δ_H 2.83 ppm (6H, s)

Table 3. ^{1}H NMR data of RS-22A (**1**), RS-22B (**2**) and RS-22C (**3**) in MeOH-d_4 (400 MHz).

Position	1	2	3
2	5.82 (1H, d, J=15 Hz)	5.82 (1H, d, J=15 Hz)	5.82 (1H, d, J=15 Hz)
3	7.17 (1H, dd, J=10, 15 Hz)	7.18 (1H, dd, J=10, 15 Hz)	7.18 (1H, dd, J=10, 15 Hz)
4	6.26 (1H, dd, J=10, 15 Hz)	6.26 (1H, dd, J=10, 15 Hz)	6.26 (1H, dd, J=10, 15 Hz)
5	6.2 (1H, dd, J=8.8, 15 Hz)	6.1 (1H, dd, J=8.8, 15 Hz)	6.1 (1H, dd, J=8.8, 15 Hz)
6	2.42 (1H, m)	2.42 (1H, m)	2.42 (1H, m)
6-Me	1.1 (1H, d, J=6.8 Hz)	1.09 (1H, d, J=6.8 Hz)	1.09 (1H, d, J=6.8 Hz)
7	3.75 (1H, m)	3.75 (1H, m)	3.75 (1H, m)
8	1.42, 1.72 (each 1H, m)	1.46, 1.72 (each 1H, m)	1.46, 1.72 (each 1H, m)
9	3.78 (1H, m)	3.8 (1H, m)	3.8 (1H, m)
10	1.44 (1H, m)	1.44 (1H, m)	1.44 (1H, m)
10-Me	0.87 (1H, d, J=6.8 Hz)	0.86 (1H, d, J=6.8 Hz)	0.86 (1H, d, J=6.8 Hz)
11	3.88 (1H, m)	3.88 (1H, m)	3.88 (1H, m)
12	1.32, 1.59 (each 1H, m)	1.32, 1.59 (each 1H, m)	1.32, 1.59 (each 1H, m)
13	1.28 (2H, m)	1.28 (2H, m)	1.28 (2H, m)
14	1.58 (1H, m)	1.58 (1H, m)	1.58 (1H, m)
14-Me	0.91 (1H, d, J=6.8 Hz)	0.91 (1H, d, J=6.8 Hz)	0.91 (1H, d, J=6.8 Hz)
15	3.86 (1H, m)	3.86 (1H, m)	3.86 (1H, m)
16	1.83 (2H, m)	1.83 (2H, m)	1.83 (2H, m)
18	3.34 (1H, d, J=9.3 Hz)	3.35 (1H, d, J=9.3 Hz)	3.35 (1H, d, J=9.3 Hz)
19	3.86 (1H, m)	3.86 (1H, m)	3.86 (1H, m)
20	1.3, 1.9 (each 1H, m)	1.3, 1.9 (each 1H, m)	1.3, 1.9 (each 1H, m)
21	4.08 (1H, m)	4.08 (1H, m)	4.08 (1H, m)
22	1.5, 1.67 (each 1H, m)	1.5, 1.67 (each 1H, m)	1.5, 1.67 (each 1H, m)
23	5.23 (1H, m)	5.21 (1H, m)	5.21 (1H, m)
24	1.65~1.7 (2H, m)	1.65~1.7 (2H, m)	1.65~1.7 (2H, m)
25	3.86 (1H, m)	3.85 (1H, m)	3.85 (1H, m)
26	1.58 (2H, m)	1.58 (2H, m)	1.58 (2H, m)
27	3.86 (1H, m)	3.85 (1H, m)	3.85 (1H, m)
28	1.58 (2H, m)	1.58 (2H, m)	1.58 (2H, m)
29	4.16 (1H, m)	4.16 (1H, m)	4.16 (1H, m)
30-Me	1.64 (3H, s)	1.64 (3H, s)	1.65 (3H, s)
31	5.97 (1H, d, J=10.7 Hz)	5.97 (1H, d, J=10.7 Hz)	5.97 (1H, d, J=10.7 Hz)
32	6.2 (1H, dd, J=10.7, 15.1 Hz)	6.2 (1H, dd, J=10.7, 15.1 Hz)	6.21 (1H, dd, J=10.7, 15.1 Hz)
33	5.4 (1H, dd, J=8.8, 15.1 Hz)	5.4 (1H, dd, J=8.8, 15.1 Hz)	5.4 (1H, dd, J=8.8, 15.1 Hz)
34	2.52 (1H, m)	2.51 (1H, m)	2.52 (1H, m)
34-Me	0.99 (1H, d, J=6.9 Hz)	0.99 (1H, d, J=6.9 Hz)	0.99 (1H, d, J=6.9 Hz)
35	4.76 (1H, dd, J=3.8, 8.8 Hz)	4.75 (1H, dd, J=3.8, 8.8 Hz)	4.76 (1H, dd, J=3.9, 8.3 Hz)
36	1.77 (1H, m)	1.77 (1H, m)	1.77 (1H, m)
36-Me	0.92 (3H, d, J=6.8 Hz)	0.92 (3H, d, J=6.8 Hz)	0.92 (3H, d, J=6.8 Hz)
37	1.1 (2H, m)	1.1 (2H, m)	1.1 (2H, m)
38	1.34 (2H, m)	1.38 (2H, m)	1.38 (2H, m)
39	1.85~2.0 (2H, m)	1.85~2.0 (2H, m)	1.85~2.0 (2H, m)
40	5.44 (1H, m)	5.44 (1H, m)	5.44 (1H, m)
41	5.42 (1H, m)	5.42 (1H, m)	5.42 (1H, m)
42	2.06 (2H, m)	2.06 (2H, m)	2.06 (2H, m)
43	1.62 (2H, m)	1.62 (2H, m)	1.62 (2H, m)
44	3.15 (2H, t, J=6.9 Hz)	3.14 (2H, t, J=6.9 Hz)	3.16 (2H, t, J=6.4 Hz)
2′	3.25 (2H, m)	3.25 (2H, m)	3.25 (2H, m)
N′-Me	—	2.83 (3H, s)	2.83 (3H, s)
N″-Me	—	—	2.83 (3H, s)

and δ_C 28.5 ppm in the ^{1}H and ^{13}C NMR of **3**, respectively. The structural differences in the side chains were supported by the CID spectra obtained from positive FAB mass spectrometries of **1** and **3** using the B/E linked scan technique. Fourteen mass unit differences between the sequential daughter ions of **2** and those of **1** or **3** clearly show the structural differences in the terminal guanidino group as shown in Fig. 3.

Corroboration of Structure of 3 by Tandem Mass Analysis of the Derivative (3a)

Further structural information of **3** was obtained from the CID spectrum of a derivative (**3a**) prepared from **3** by oxidative cleavage of the hemiacetal ring and demalonylation by alkaline hydrolysis, using tandem mass spectrometry. In the NMR in MeOH-d_4, **3a** was detected as the hemiacetal form (**3b**) which is a 1:1 equilibrium mixture of α and β-anomers as shown in Fig. 4. The hemiacetal signals at C-19 were observed at

Fig. 3. Cleavage assignments for protonated RS-22A (**1**), B (**2**) and C (**3**) detected by collision-induced dissociation mass spectra.

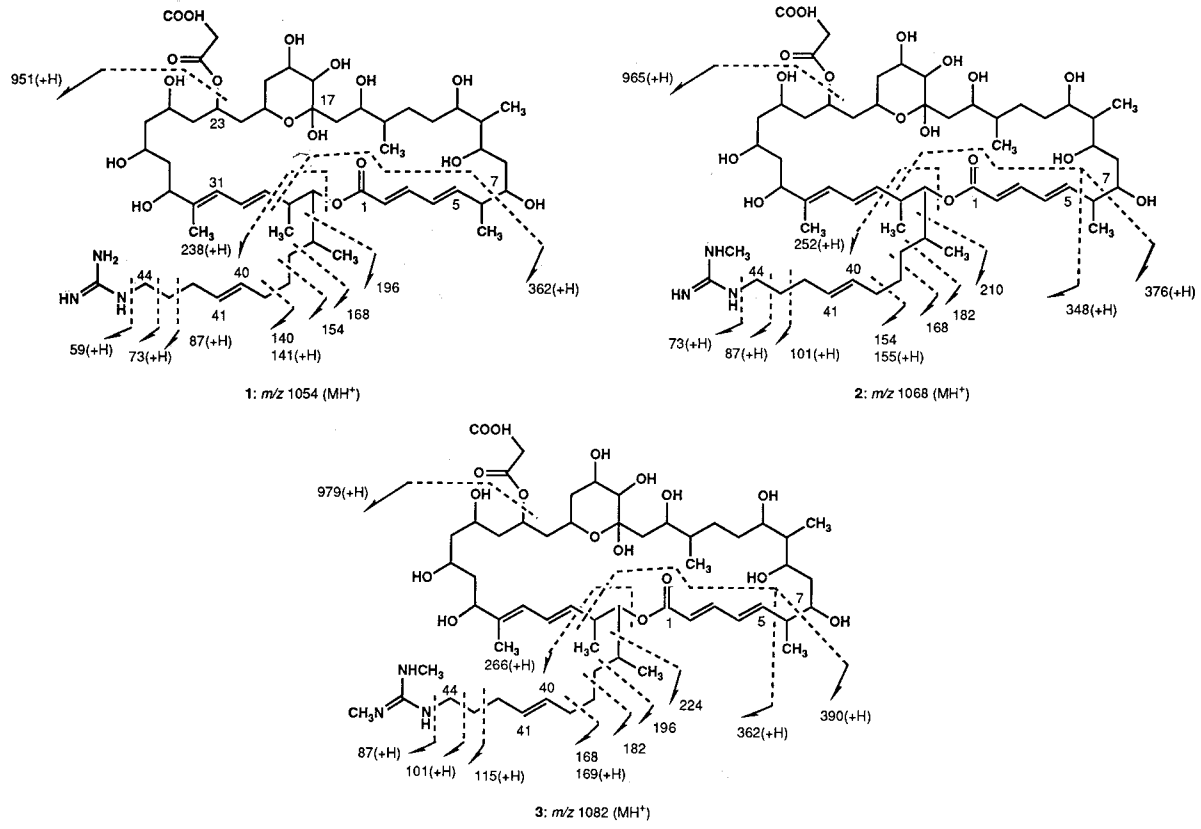
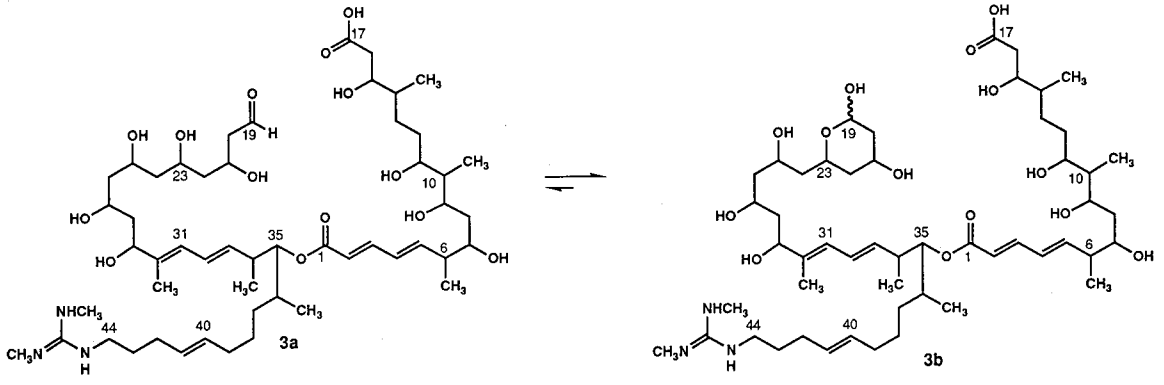


Fig. 4. Structure of oxidative derivative (**3a**) prepared from RS-22C (**3**) and its hemiacetal form (**3b**) in MeOH.



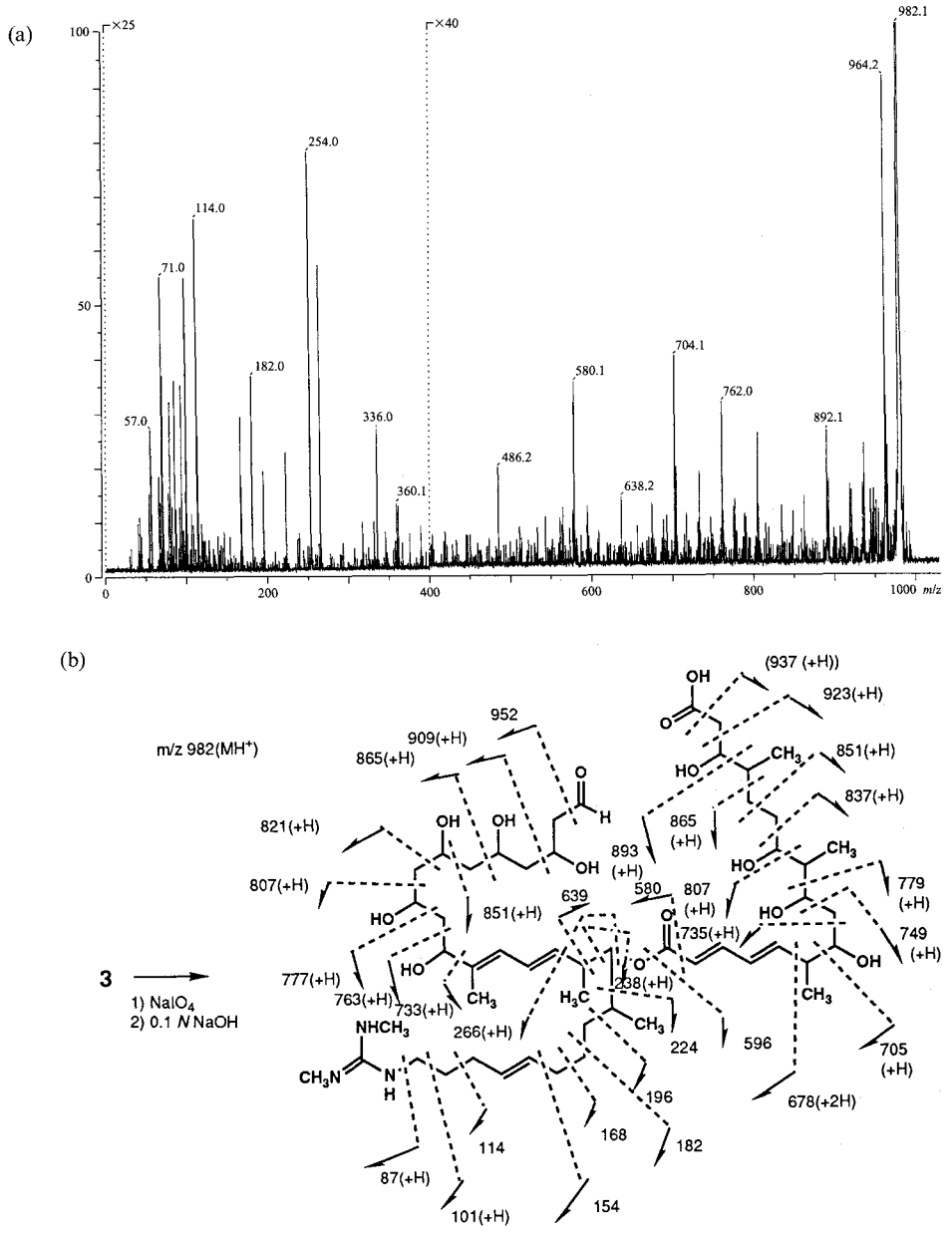
97.5 ppm and 95.2 ppm in the ^{13}C NMR, and at 4.69 ppm (dd, $J=3.5$, 7.5 Hz) and 4.16 ppm (dd, $J=2.2$, 9.7 Hz) in the ^{1}H NMR. Mass selection and collision-induced dissociation of the protonated derivative **3a** using tandem mass spectrometry (Fig. 5a), exhibits a series of ions derived from C-1 ~ C-17 together with C-19 ~ C-44 guanidino group (Fig. 5b). The CID spectrum strongly supported the proposed structure.

Discussion

The structures of new 36-membered macrolide antibiotics, RS-22A, B and C isolated from a culture broth of *Streptomyces violaceusniger* have been determined by 1D and 2D NMR and FAB mass analyses including tandem mass spectrometry. RS-22A, B, and C are related to polyol macrolide antibiotics such as azalomycins,[2] copiamycin,[3] malolactomycin,[4] shurimycins,[5] scopafungin,[9] guanidylfungins,[10] amycins[11] and RP-63834.[12] The proposed structures of RS-22A, B and C closely resemble those of azalomycins F_{3a}, F_{4a} and F_{5a},[2] respectively. The structure difference between RS-22 and azalomycins is the absence of a 2-CH_3 in the α, β, γ, δ-unsaturated lactone moiety. The structures of RS-22B and C also resemble those of shurimycins A and

Fig. 5. Determination of structural functionality of **3a** by tandem mass spectrometry.

(a) Mass spectrum resulting from CID of the *m/z* 982 ion produced by FAB ionization of **3a**, (b) Cleavage assignments for protonated **3a** detected in panel (a).



3a

B, respectively. RS-22B and C differ from shurimycins A and B by the absence of a 28-CH_3, respectively.

Experimental

General

IR spectra were recorded on a Shimadzu FTIR-8100 M. UV spectra were obtained by a Hitachi 220A spectrophotometer. NMR spectra were recorded on Jeol JMN-α-400 and JMN-α-600 spectrometers. Chemical shifts are reported relative to TMS in MeOH-d_4 or MeOH-d_3. FG-DQF-COSY, ^1H-^{13}C COSY, FG-HMQC and FG-HMBC spectra were obtained by conventional methods. Mass spectra including B/E linked scan experiments were taken by a Jeol JMN-HX-110. CID Mass spectrum of **3a** was acquired using a Jeol JMS-HX-110/HX-110 tandem mass spectrometer. Samples were dissolved in glycerol and ionized by FAB using 10 kV Xe atoms. CID Mass spectrum was acquired at unit mass produced using 3 kV collision cell voltage at He collision gas pressure corresponding to approximately 30% of the precursor ion beam.

Preparation of **3a**

To a solution of **3** (15.8 mg) in MeOH-H_2O (1.5 ml, 1 : 1) was added $NaIO_4$ (9 mg). After stirring for 11 hours,

9 mg of $NaIO_4$ was added to the reaction mixture, which was continuously stirred for 4 hours. The resulting solution was diluted with brine (8 mg) and extracted with *n*-BuOH (8 ml, 3 times). The combined organic extracts were washed with water and evaporated *in vacuo*. The residue was dissolved in MeOH (1.5 ml) and to the resulting solution was added 0.1 N NaOH solution (1.5 ml). The reaction mixture was stirred for 12 hours at room temperature. The solution was neutralized with diluted HCl, washed with EtOAc (7 ml, 3 times), and concentrated. The residue was purified by preparative HPLC (Capcell Pak C-18 column, Shiseido, Japan, MeOH - H_2O, 8 : 2) to give pure **3a** (4.3 mg).

FAB-MS *m/z* 982 $(M+H)^+$; ^1H NMR (600 MHz, MeOH-d_4) δ 5.81 (1H, d, $J=15$ Hz, 2-H), 7.23 (1H, dd, $J=10$, 15 Hz, 3-H), 6.28 (1H, dd, $J=10$, 15 Hz, 4-H), 6.19 (1H, dd, $J=8$, 15 Hz, 5-H), 2.43 (1H, m, H-6), 1.12 (1H, d, $J=7$ Hz, 6-Me), 3.71 (1H, m, 7-H), 1.53, 1.7 (each 1H, m, 8-H), 3.78 (1H, m, 9-H), 1.57 (1H, m, 10-H), 0.9 (1H, d, $J=6.2$ Hz, 10-Me), 3.86 (1H, m, 11-H), 1.33, 1.57 (each 1H, m, 12-H), 1.23, 1.32 (each 1H, m, 13-H), 1.4 (1H, m, 14-H), 0.9 (1H, d, $J=6.2$ Hz, 14-Me), 3.75 (1H, m, 15-H), 2.2 (1H, dd, $J=10.2$, 15 Hz, 16_a-H), 2.34 (1H, dd, $J=4$, 15 Hz, 16_b), 4.66 (1/2H, dd, $J=2.2$, 9.7 Hz, 19_a), 4.69 (1/2H, dd, $J=3.5$, 7.5 Hz, 19_b), 1.25, 1.35 (each 1H, m, 20-H), 3.5 ~ 4.1 (4H, m, 21-H, 23-H, 25-H, 27-H), 1.1 ~ 2.1 (6H, m, 22-H, 24-H, 26-H), 1.6 (2H, m, 28-H), 4.21 (1H, dd, $J=3.5$, 8.8 Hz, 29-H), 1.69 (3H, s, 30-Me), 5.97 (1H, d, $J=10$ Hz, 31-H), 6.27 (1H, dd, $J=10$, 15 Hz, 32-H), 5.47 (1H, m, 33-H), 0.99 (3H, d, $J=6.6$ Hz, 34-H), 4.79 (1H, dd, $J=5$, 7 Hz, 35-H), 1.78 (1H, m, 36-H), 0.92 (3H, d, $J=6.2$ Hz, 36-Me), 1.1 (2H, m, 37-H), 1.4 (2H, m, 38-H), 1.97 (2H, m, 39-H), 5.41 (1H, m, 40-H), 5.42 (1H, m, 41-H), 2.06 (2H, m, 42-H), 1.65 (2H, m, 43-H), 3.16 (1H, t, $J=7.4$ Hz, 44-H), 2.8 (3H × 2, s, N-Me × 2).

^{13}C NMR (600 MHz, MeOH-d_4, detected by FG-HMQC and FG-HMBC) δ 169 (s, C-1), 120.8 (d, C-2), 146.5 (d, C-3), 130.2 (d, C-4), 147.1 (d, C-5), 44.1 (d, C-6), 16.8 (q, C-6Me), 75.4 (d, C-7), 39.6 (t, C-8), 75 (d, C-9), 44.4 (d, C-10), 10.5 (q, C-10Me), 72.4 (d, C-11), 33.2 (t, C-12), 29.9 (t, C-13), 39.7 (d, C-14), 15.6 (q, C-14Me), 74.1 (d, C-15), 41.4 (t, C-16), 180.5 (s, C-17), 95.2 (d, C-19a), 97.1 (d, C-19b), 35.5, 40.5, 42.2, 42.7, 43 (each t, C-20, C-22, C-24, C-26, C-28), 67, 75, 75.1 (each d, C-21, C-23, C-27), 66.5 (d, C-25), 74.4 (d, C-29), 140.1 (s, C-30), 12.6 (q, C-30Me) 125.4 (d, C-31), 128.2 (d, C-32), 136.4.(d, C-33), 40.8 (d, C-34), 17.9 (q, C-34Me), 80.6 (d, C-35), 35.6 (d, C-36), 14.3 (q, C-36Me), 34.2 (t, C-37), 27.9 (t, C-38), 33.6 (t, C-39), 132.6 (d, C-40), 130.2 (d, C-41), 30.6 (t, C-42), 29.8 (t, C-43), 42.0 (t, C-44), 28.2 (s, N-Me × 2), 157.2 (s, N-CN2).

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