SAR Study of a Novel Triene-ansamycin Group Compound, Quinotrierixin, and Related Compounds, as Inhibitors of ER Stress-induced XBP1 Activation

II. Structure Elucidation

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Abstract Four novel triene-ansamycin group compounds, quinotrierixin, demethyltrienomycin A, demethyltrienomycin B and demethyltrienomycinol, were isolated from the culture broth of *Streptomyces* sp. PAE37 as inhibitors of ER stress-induced XBP1 activation. The structures of quinotrierixin, demethyltrienomycin A, demethyltrienomycin B and demethyltrienomycinol were determined on the basis of their spectroscopical and chemical properties. All of four possessed 21-membered macrocyclic lactams including triene moieties.

Keywords triene-ansamycin, ER stress, XBP1

We isolated four novel triene-ansamycin group compounds, quinotrierixin (**1**), demethyltrienomycin A (**2**), demethyltrienomycin B (**3**) and demethyltrienomycinol (**4**), from the culture broth of *Streptomyces* sp. PAE37 for SAR study. The taxonomy of the producing strain, fermentation, isolation and biological activities of **1**, **2**, **3** and **4** were reported in the preceding paper [1]. In this paper, we describe the physico-chemical properties and structure elucidation of **1**, **2**, **3** and **4**.

The physico-chemical properties of **1**, **2**, **3** and **4** are summarized in Table 1. The molecular formulae of **1**, **2**, **3**

and **4** were determined to be $C_{37}H_{50}N_2O_8S$ (MW 682), $C_{35}H_{48}N_2O_7$ (MW 608), $C_{34}H_{46}N_2O_7$ (MW 594) and $C_{24}H_{31}NO_5$ (MW 413) from the HRESI-MS measurements in combination with their 1H - and ^{13}C -NMR data, respectively.

The UV absorption maxima at 261, 270 and 281 nm in **1** indicated the presence of a triene moiety [2–9]. The IR spectrum revealed that **1** possesses NH/OH (3450 cm^{-1}), ester (1730 and 1200 cm^{-1}), and amide (1640 and 1500 cm^{-1}) functionalities. These characteristic UV and IR spectra indicated that **1** belongs to a triene-ansamycin group. **2**, **3** and **4** were also confirmed to be triene-ansamycin group compounds from their UV and IR spectra.

In the isolation process of **1**, we also isolated and identified mycotrienin I (**5**, Fig. 1) [2, 3]. Since the UV, 1H -, and ^{13}C -NMR spectra of **1** were quite similar to those of **5**, structural studies on **1** were performed by the comparison with **5**. The 1H -, ^{13}C -, COSY, HMQC and HMBC spectra of **1** were measured, and compared with those of **5** (Table 2-1) [2, 3]. This comparison proved that the partial structures from C-1 to C-17 (a 21-membered macrocyclic lactam except quinone moiety) and from C-28 to C-37 (cyclohexanecarbonylalaninyl moiety) in **5** were completely preserved in **1**. One singlet methyl (δ_H 2.57, H-27) was observed only in **1**, and two aromatic methines in **5** [2, 3] were decreased to one (δ_H 7.41) in **1**. Considering the

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Table 1 Physico-chemical properties of quinotrierixin (**1**), demethyltrienomycin A (**2**), demethyltrienomycin B (**3**) and demethyltrienomycinol (**4**)

	Quinotrierixin	Demethyltrienomycin A	Demethyltrienomycin B	Demethyltrienomycinol
Appearance	Pale yellow powder	Colorless powder	Colorless powder	Colorless powder
Molecular formula	C ₃₇ H ₅₀ N ₂ O ₈ S	C ₃₅ H ₄₈ N ₂ O ₇	C ₃₄ H ₄₆ N ₂ O ₇	C ₂₄ H ₃₁ NO ₅
Molecular weight	682	608	594	413
HRESI-MS (<i>m/z</i> , Pos.)				
Calcd.	705.3186 (as C ₃₇ H ₅₀ N ₂ NaO ₈ S)	631.3359 (as C ₃₅ H ₄₈ N ₂ NaO ₇)	617.3203 (as C ₃₄ H ₄₆ N ₂ NaO ₇)	436.2100 (as C ₂₄ H ₃₁ NNaO ₅)
Found.	705.3210	631.3360	617.3220	436.2110
Optical rotation [α] _D ²²	+210.0° (<i>c</i> 0.10, MeOH)	+142.4° (<i>c</i> 0.13, MeOH)	+75.4° (<i>c</i> 0.10, MeOH)	+38.3° (<i>c</i> 0.12, MeOH)
Melting point (°C)	140~141	125~126	131~132	128~129
UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ)	250 (4.41), 261 (4.47), 270 (4.57), 281 (4.49), 340 (3.52)	213 (4.51), 250 (4.46), 259 (4.48), 270 (4.53), 282 (4.44)	210 (4.48), 250 (4.32), 259 (4.33), 270 (4.39), 281 (4.29)	211 (4.51), 251 (4.27), 260 (4.28), 271 (4.33), 282 (4.23)
IR ν_{max} cm ⁻¹ (KBr)	3450, 2920, 2850, 1730, 1640, 1500, 1450, 1380, 1290, 1200, 1130, 1090, 1000	3420, 2930, 2850, 1740, 1650, 1550, 1450, 1380, 1300, 1210, 1160, 1100, 1000	3420, 2930, 2860, 1730, 1650, 1550, 1450, 1380, 1210, 1160, 1000	3400, 2920, 2860, 1660, 1620, 1560, 1440, 1300, 1230, 1160, 1070, 1000
TLC (R _f)	0.72 ^{a)}	0.31 ^{a)}	0.69 ^{b)}	0.56 ^{b)}
HPLC (R _t , min) ^{c)}	20.8 (80% MeOH)	22.0 (65% MeOH)	20.3 (60% MeOH)	19.7 (45% MeOH)
Solubility				
Soluble:	CHCl ₃ , MeOH	CHCl ₃ , MeOH	MeOH	MeOH
Insoluble:	Hexane, H ₂ O	Hexane, H ₂ O	Hexane, H ₂ O	Hexane, H ₂ O

^{a)} Silica gel TLC (Kieselgel 60F₂₅₄, Merck); mobile phase, CHCl₃ - MeOH (10 : 1).

^{b)} Silica gel TLC (Kieselgel 60F₂₅₄, Merck); mobile phase, CHCl₃ - MeOH (10 : 3).

^{c)} Column, SunFire C₁₈ (Waters, 5 mm, 4.6×250 mm); mobile phase, aqMeOH; flow rate, 0.7 ml/minute.

Fig. 1 Structures of quinotrierixin (**1**), demethyltrienomycin A (**2**), demethyltrienomycin B (**3**), demethyltrienomycinol (**4**), mycotrienin I (**5**) and trienomycin A (**6**).

Table 2-1 ^{13}C - and ^1H -NMR data for quinotrierixin (**1**) and mycotrienin I (**5**) in CDCl_3

1			5		
No.	δ_{C} (ppm)	δ_{H} (ppm)	No.	δ_{C} (ppm)	
1	169.4		1	169.7	
2	44.7	2.51*, 2.72* (2H)	2	44.8	
3	78.8	3.96 (1H, m)	3	79.2	
4	130.8	5.48 (1H, dd, 9.2, 15.4)	4	131.3	
5	133.9	6.06 (1H, dd, 10.5, 15.8)	5	133.7	
6	129.3	5.98 (1H, dd, 10.1, 15.2)	6	129.5	
7	134.1	6.01 (1H, dd, 10.5, 15.2)	7	133.7	
8	133.3	6.03 (1H, dd, 10.5, 15.2)	8	133.2	
9	129.8	5.50 (1H, ddd, 2.8, 10.1, 14.9)	9	129.3	
10	33.0	2.24*, 2.52* (2H)	10	33.0	
11	75.3	4.84 (1H, m)	11	75.2	
12	38.6	1.81* (1H)	12	39.9	
13	68.5	4.51 (1H, brs)	13	68.0	
14	139.4		14	139.9	
15	123.8	5.08 (1H, brd)	15	122.5	
16	26.1	1.85*, 2.13* (2H)	16	25.6	
17	28.8	2.52*, 2.73* (2H)	17	29.4	
18	142.0		18	137.9	
19	178.7		19	188.2	
20	137.7		20	145.4	
21	115.8	7.41 (1H, s)	21	114.5	
22	184.1		22	182.5	
23	147.7		23	133.1	
24	10.0	0.84 (3H, d, 6.8)	24	9.6	
25	20.4	1.74 (3H, brs)	25	20.5	
26	56.7	3.30 (3H, s)	26	56.6	
27	18.0	2.57 (3H, s)			
28	172.9		28	172.9	
29	48.5	4.30 (1H, m)	29	48.5	
30	17.7	1.34 (3H, d, 7.2)	30	17.4	
31	176.5		31	176.6	
32	45.0	2.04 (1H, m)	32	44.9	
33	29.4	1.17*, 1.80* (2H)	33	29.4	
34	25.6	1.16*, 1.79* (2H)	34	25.6	
35	25.6	1.60*, 1.68* (2H)	35	25.5	
36	25.6	1.16*, 1.79* (2H)	36	25.5	
37	29.4	1.17*, 1.80* (2H)	37	29.3	
1-NH		8.18 (1H, s)			
29-NH		5.84 (1H, d, 6.2)			

Chemical shifts in ppm from TMS as internal standard. * Obscured by overlapping signals.

Table 2-2 ^{13}C - and ^1H -NMR data for demethyltrienomycin A (**2**) and trienomycin A (**6**) in CDCl_3

2			6		
No.	δ_{C} (ppm)	δ_{H} (ppm)	No.	δ_{C} (ppm)	
1	168.6		1	168.5	
2	43.3	2.50, 2.68 (2H, m)	2	43.5	
3	78.6	4.04 (1H, brs)	3	78.5	
4	129.5	5.54 (1H, dd, 8.5, 14.9)	4	130.6	
5	133.4	6.20 (1H, dd, 9.5, 16.1)	5	133.5	
6	129.4	6.05 (1H, dd, 10.0, 15.5)	6	129.3	
7	134.1	6.06 (1H, dd, 10.0, 15.5)	7	134.1	
8	133.4	5.98 (1H, dd, 11.0, 15.1)	8	133.4	
9	129.4	5.51 (1H, m)	9	129.4	
10	33.3	2.25*, 2.43* (2H)	10	33.1	
11	75.6	4.87 (1H, m)	11	75.5	
12	40.4	1.72* (1H)	12	39.6	
13	66.5	4.46 (1H, brs)	13	68.4	
14	133.4	5.46*, (1H)	14	138.6	
15	129.3	5.36 (1H, m)	15	124.7	
16	34.9	2.16*, 2.28* (2H)	16	29.3	
17	35.7	2.43* (2H)	17	36.2	
18	143.7		18	144.1	
19	111.0	6.11 (1H, s)	19	110.8	
20	138.1		20	138.4	
21	106.2	7.36 (1H, s)	21	105.7	
22	157.3		22	157.2	
23	111.9	6.41 (1H, s)	23	111.9	
24	9.6	0.87 (3H, d, 6.6)	24	9.8	
25	56.8	3.29 (3H, s)	25	56.8	
26	173.0		26	172.9	
27	48.5	4.35 (1H, m)	27	48.5	
28	17.8	1.31 (3H, d, 7.0)	28	17.8	
29	176.5		29	176.6	
30	45.0	2.02 (1H, m)	30	45.1	
31	29.4	1.18*, 1.73* (2H)	31	29.5	
32	25.6	1.15*, 1.75* (2H)	32	25.6	
33	25.7	1.59*, 1.68* (2H)	33	25.5	
34	25.6	1.15*, 1.71* (2H)	34	25.7	
35	29.4	1.18*, 1.75* (2H)	35	29.7	
			14-CH ₃	20.3	
13-OH		5.10 (1H, brs)			
1-NH		7.62 (1H, s)			
27-NH		5.98* (1H)			

Chemical shifts in ppm from TMS as internal standard. * Obscured by overlapping signals.

Fig. 2-1 Structures of **1** and **2** elucidated by ^1H - ^1H COSY, NOE and HMBC experiments.

Fig. 2-2 Structures of **3** and **4** elucidated by ^1H - ^1H COSY, NOE and HMBC experiments.

difference in the molecular formula between **1** and **5** (CH_2S), the attachment of SCH_3 group at C-21 or C-23 in **1** was thus speculated. The attachment of SCH_3 group at C-23 was determined by the observation of ^1H - ^{13}C long-range couplings from H-17 (δ_{H} 2.52, 2.73) and SCH_3 (δ_{H} 2.57) to C-23 (δ_{C} 147.7), and from 1-NH (δ_{H} 8.18) to C-21 (δ_{C} 115.8) in the HMBC experiment (Fig. 2-1). The linkage of cyclohexanecarbonylalanine at C-11 was confirmed by the observation of H-11 at δ_{H} 4.84 [8]. The geometry of C-4, C-6 and C-8 were determined to be all *E* by the coupling constants of $J_{4,5}=15.4$ Hz, $J_{6,7}=15.2$ Hz and $J_{8,9}=15.2$ Hz, respectively. The geometry of C-14 was determined to be *Z* by the ^{13}C chemical shift of C-25 (δ_{C} 20.4) and NOE observation between H-15 (δ_{H} 5.08) and H-25 (δ_{H} 1.74) (Fig. 2-1). From the above findings, the structure of **1** was determined as shown in Fig. 1. The ^1H - and ^{13}C -NMR spectral data for **1** are summarized in Table 2-1.

The UV, ^1H -, and ^{13}C -NMR spectra of **2** were quite similar to those of trienomycin A (**6**, Fig. 1) [8], which was also isolated from the broth. The 2D NMR spectra (COSY, HMQC and HMBC) analyses of **2** proved that the most of the partial structures in **6** were preserved in **2**. One singlet methyl (δ_{H} 1.80) in **6** [8] had disappeared in **2** and one sp^2 methine (H-14, δ_{H} 5.46) was observed only in **2**. From the difference in the molecular formula between **2** and **6** (CH_2), the detachment of CH_3 group at C-14 in **2** was thus speculated. The vicinal spin network from H-2 to H-17 (Fig. 2-1) observed in the COSY spectrum of **2** proved this structure. The geometry of C-4, C-6, C-8, and C-14 were determined to *E*, *E*, *E*, and *Z*, respectively, by the coupling constants ($J_{4,5}=14.9$ Hz, $J_{6,7}=15.5$ Hz, $J_{8,9}=15.1$ Hz) and NOE between H-14 and H-15 (Fig. 2-1). The structure of **2** was thus determined as shown in Fig. 2-1. The ^1H - and ^{13}C -NMR data for **2** and **6** were summarized in Table 2-2.

Table 2-3 ^{13}C - and ^1H -NMR data for demethyltrienomycin B (**3**) and demethyltrienomycinol (**4**) in CD_3OD

3			4		
No.	δ_{C} (ppm)	δ_{H} (ppm)	No.	δ_{C} (ppm)	δ_{H} (ppm)
1	171.0		1	171.3	
2	46.6	2.40*, 2.58* (2H)	2	46.6	2.50* (1H), 2.65 (1H, dd, 4.3, 12.2)
3	72.0	4.37 (1H, m)	3	71.7	4.49 (1H, m)
4	135.3	5.60 (1H, dd, 8.1, 15.0)	4	134.3	5.71 (1H, dd, 7.9, 15.0)
5	132.9	6.03 (1H, dd, 9.4, 15.2)	5	132.8	6.12 (1H, dd, 9.4, 15.0)
6	131.3	6.06 (1H, dd, 9.4, 15.2)	6	131.0	6.14 (1H, dd, 9.5, 15.1)
7	134.4	6.07 (1H, dd, 9.7, 14.9)	7	134.9	6.14 (1H, dd, 9.5, 15.1)
8	134.6	6.07 (1H, dd, 9.7, 14.9)	8	134.2	6.12 (1H, dd, 9.4, 15.0)
9	130.0	5.55 (1H, ddd, 5.1, 9.7, 15.0)	9	131.6	5.72 (1H, m)
10	37.1	2.02*, 2.55* (2H)	10	41.5	2.19*, 2.37* (2H)
11	72.3	4.96* (1H)	11	69.0	3.82 (1H, m)
12	39.2	1.50*, 1.63* (2H)	12	41.6	1.56*, 1.66* (2H)
13	66.9	4.47 (1H, dd)	13	68.2	4.66 (1H, m)
14	139.7		14	139.4	
15	125.7	5.06 (1H, dd)	15	126.6	5.19 (1H, dd, 7.0)
16	29.8	2.06*, 2.19* (2H)	16	30.2	2.12*, 2.33* (2H)
17	37.0	2.40* (2H)	17	37.3	2.46* (2H)
18	144.8		18	145.1	
19	114.0	6.25 (1H, s)	19	113.9	6.35 (1H, s)
20	139.8		20	139.8	
21	107.8	6.81 (1H, s)	21	107.8	6.91 (1H, s)
22	158.6		22	158.6	
23	112.6	6.33 (1H, s)	23	112.7	6.40 (1H, s)
24	18.6	1.57 (3H, s)	24	19.1	1.71 (3H, s)
25	173.9				
26	49.7	4.22 (1H, q, 7.2)			
27	17.2	1.27 (3H, d, 7.2)			
28	179.1				
29	45.8	2.13* (1H)			
30	30.4	1.19*, 1.67* (2H)			
31	26.6	1.15*, 1.68* (2H)			
32	26.8	1.60*, 1.66* (2H)			
33	26.6	1.15*, 1.68* (2H)			
34	30.8	1.19*, 1.67* (2H)			

Chemical shifts in ppm from TMS as internal standard. * Obscured by overlapping signals.

As in the case of **2**, the ^{13}C -NMR spectrum of **3** was also quite similar to that of **6**. The comparison of ^{13}C -NMR spectrum between the **3** and **6** (Table 2-2 and Table 2-3) proved that the most partial structures in **6** were preserved in **3**, whereas two methyl signals [δ_{C} 9.8 (C-24) and δ_{C} 56.8 (C-25)] in **6** [8] were disappeared in **3**. Considering the difference in the molecular formula between **3** and **6** (C_2H_4), the detachment of two methyl groups (C-24 and C-25 in **3**) was speculated. The vicinal spin network from H-2 to H-13 (Fig. 2-2) observed in the COSY spectrum of **3**,

and the difference of ^{13}C chemical shift in C-3 (δ_{C} 72.0 in **3** and δ_{C} 78.5 in **6**) proved this structure. The geometry of C-4, C-6, C-8, and C-14 were determined to *E*, *E*, *E*, and *Z*, respectively, by the coupling constants ($J_{4,5}=15.0$ Hz, $J_{6,7}=15.2$ Hz, $J_{8,9}=14.9$ Hz) and NOE between H-15 and H-25. The structure of **3** was determined as shown in Fig. 1. The ^1H - and ^{13}C -NMR data for **3** were listed in Table 2-3.

The ^1H - and ^{13}C -NMR spectra of **4** were related to those of **3**. The signals due to cyclohexanecarbonylalanine

in **3** was disappeared in **4**. Considering the difference in the molecular formula between **3** and **4** ($C_{10}H_{15}NO_2$), the detachment of this acyl chain in **4** was speculated. The chemical shift of H-11 (δ_H 4.96 in **3** and δ_H 3.82 in **4**) also supported this structure. The geometry of C-4, C-6, C-8, and C-14 were determined to *E*, *E*, *E*, and *Z*, respectively, by the coupling constants ($J_{4,5}=15.0$ Hz, $J_{6,7}=15.1$ Hz, $J_{8,9}=15.0$ Hz) and NOE between H-15 and H-25 (Fig. 2-2). Thus, the structure of **4** was determined as shown in Fig. 1. The 1H - and ^{13}C -NMR data for **4** were listed in Table 2-3.

Studies on the relative stereochemistry of **1**~**4** are now under way.

Experimental

Melting points were determined with Yanagimoto micro melting point apparatus and are uncorrected. Mass spectra were measured with a JEOL JMS-T100LC mass spectrometer. Optical rotations were made with a JASCO P-1030 polarimeter using a micro-cell (light path 100 mm). UV spectra and IR spectra were recorded on a Hitachi U-1800 spectrophotometer and a Horiba FT-210 spectrometer in KBr disc, respectively. 1H - and ^{13}C -NMR spectra were recorded on a JEOL JNM-AL operating at 300 and 75 MHz, respectively. LC/MS system (Waters Corp., USA) with the photo diode array detector (2996) and mass analyzer (micromass ZQ) was used for analysis.

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