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Novel Triene-ansamycins, Cytotrienins A and B, Inducing Apoptosis on Human Leukemia HL-60 Cells

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Abstract: Cytotrienins A and B, two novel triene-ansamycins containing a unique 1-aminocyclopropane carboxylic acid moiety, were isolated from the culture broth of *Streptomyces* sp. through a separation procedure guided by the cytotoxic activity *in vitro*. The structures were elucidated on the basis of spectroscopic data. Cytotrienins A and B exhibited a potent apoptosis-inducing activity on human leukemia HL-60 cells.
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In the course of screening for new antitumor secondary microbial metabolites, we have isolated two novel triene-ansamycins, cytotrienins A (**1**) and B (**2**), which possess a unique 1-aminocyclopropane carboxylic acid moiety, from the fermentation broth of *Streptomyces* sp. isolated from a soil sample. Both **1** and **2** exhibited a potent apoptosis-inducing activity on HL-60 cells (a human promyelocytic leukemia cell line).[1] Here we describe mainly the structural elucidation of **1** and **2**.

Cytotrienin A (**1**), a yellowish powder, m.p. 132-135°C from MeOH; $[\alpha]_D^{25}$ +270.6° (*c* 1.00, MeOH); UVλ_{max}^{MeOH} nm (log ε) 262 (4.55), 273 (4.66), 283 (4.54), which suggested the presence of a triene; IRγ_{max}^{KBr}cm^{-1} 3400 (NH, OH), 1720 (ester), 1660 (amide), 1000 (triene). The EIMS spectrum showed a molecular ion $(M)^+$ at *m/z* 648. The molecular formula of **1** was determined as $C_{37}H_{48}N_2O_8$ by HREIMS data [*m/z* 648.3355 $(M)^+$ for $C_{37}H_{48}N_2O_8$, Δ - 5.2 mmu], indicating the index of hydrogen deficiency was fifteen. The ^{13}C NMR spectrum (Table 1) of **1**, assisted by a DEPT experiment, showed 37 carbon signals attributable to onemethoxy, two methyls, ten alkyl methylenes, fourteen methines, and ten quaternary carbons. The ^{13}C-chemical shifts (Table 1) suggested the presence of three oxygenated sp^3 methine carbons ($\delta_{C\text{-}3}$ 79.88 d, $\delta_{C\text{-}11}$ 74.30 d, $\delta_{C\text{-}13}$ 66.69 d) and three carbonyl carbons ($\delta_{C\text{-}1}$ 169.46 s, $\delta_{C\text{-}27}$ 171.64 s, $\delta_{C\text{-}31}$ 169.26 s). The ^1H NMR spectrum (Table 1) showed the presence of five D_2O-exchangeable proton signals due to 20-*NH* and 28-*NH* or 13-*OH*, 19-*OH*, and 22-*OH*. Interpretation of the ^1H and ^{13}C NMR data of **1** (Table 1) facilitated by application of 2D NMR spectra (^1H-^1H DQFCOSY, HMQC,

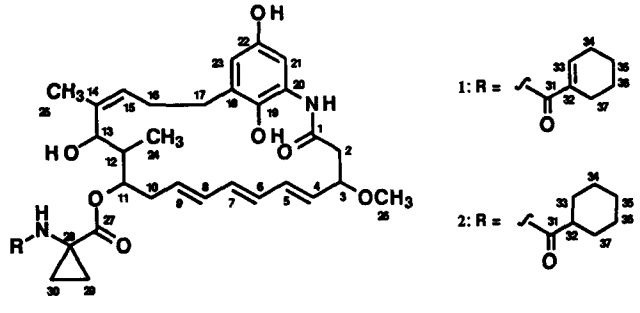

Figure 1. The Structures of Cytotrienins A (**1**) and B (**2**)

Table 1. ^{13}C and ^{1}H NMR Chemical Shifts (δ ppm) for Cytotrienin A (**1**) in DMSO-d_6

Position	^{13}C	^{1}H	(*J*/Hz)	Position	^{13}C	^{1}H	(*J*/Hz)
1	169.46 s	—		19-*OH*		7.62 s[a]	
2	41.69 t	2.80 m		20	125.90 s	—	
		2.54 m		20-*NH*		10.06 s[a]	
3	79.88 d	4.01 m		21	107.08 d	6.40 s[b]	
4	130.39 d	5.69 dd[c]	(15.6, 8.8)	22	149.27 s	—	
5	134.49 d	6.59 dd[c]	(15.6, 9.3)	22-*OH*		8.82 s[a]	
6	128.95 d	6.33 dd[c]	(14.7, 9.3)	23	115.29 d	6.40 s[b]	
7	134.09 d	6.31 dd[c]	(14.7, 9.8)	24	9.22 q	0.64 d	(6.8)
8	132.59 d	6.15 dd[c]	(15.0, 9.8)	25	20.99 q	1.60 s	
9	130.39 d	5.88 td[c]	(15.0, 10.3, 3.4)	26	55.69 q	3.20 s	
10	32.44 t	2.44 dt	(10.7, 3.4)	27	171.64 s	—	
		2.16 m		28	33.31 s	—	
11	74.30 d	4.61 dq	(10.3, 4.8, 2.0)	28-*NH*		8.49 s[a]	
12	37.48 d	1.66 m		29	16.68 t	1.35 m	
13	66.69 d	4.41 br s		30	16.45 t	1.04 m	
13-*OH*		4.11 d[a]	(5.4)	31	169.26 s	—	
14	139.38 s	—		32	133.01 s	—	
15	122.12 d	4.99 br d	(7.8)	33	132.68 d	6.59 m	
16	25.02 t	2.16 m		34	24.81 t	2.16 m	
17	31.24 t	2.82 m				1.94 m	
		2.04 m		35	21.24 t	1.60 m	
18	131.39s	—		36	21.79 t	1.60 m	
19	140.39 s	—		37	23.75 t	2.16 m	

[a]The signals disappeared after D_2O exchange. [b]The signals were separated in MeOH-d_4 at $\delta_{H\text{-}21}$ 6.52 br d (J = 2.9 Hz) and $\delta_{H\text{-}23}$ 6.48 br d (J = 2.9 Hz). [c]The signals showed better coupling pattern in pyridine-d_5.

HMBC and NOESY)[2] suggested that **1** consisted of a triene portion (C-1 ~ C-17), a hydroquinone moiety (C-18 ~ C-23), and an aminocyclopropane carboxylic acid side chain (C-27 ~ C-37) as shown in Fig.2.

The ^{1}H-^{1}H DQFCOSY spectrum of **1**, which showed cross-peaks for H_2-2 to H-13, H-15 to H-17, and H-33 to H_2-37, suggested the sequence of C-2 ~ C-13, C-15 ~ C-17, and C-33 ~ C-37. The HMBC cross-peaks (Fig. 2) indicated the junction of C-2 to C-20 via an amide group at C-1, C-13 to C-15, and also demonstrated the connections for C-17 to the hydroquinone unit and C-11 to the side chain. The assignment of the 13-*OH* group (δ_H 4.11 d) was supported by the ^{1}H-^{1}H DQFCOSY cross-peak for 13-*OH*/H-13, and the HMBC cross-peaks for 13-*OH*/C-12, C-13, and C-14. The position of 24-methyl was assigned by the ^{1}H-^{1}H DQFCOSY cross-peak for H-12/H_3-24. The assignments of 25-methyl and 26-methoxy were confirmed by the HMBC cross-peaks for H_3-25/C-13, C-14, C-15 and H_3-26/C-3, respectively.

On the other hand, the presence of meta-coupled aromatic protons was supported by a ^{1}H-^{1}H long-range coupling for H-21/H-23 (J = 2.9 Hz, observed in MeOH-d_4). The ^{13}C NMR chemical shifts for quaternary aromatic carbons of C-19 (δ_C 140.39 s) and C-22 (δ_C 149.27 s) implied that two *OH* groups were substituted at C-19 and C-22. The assignments of 19-*OH* (δ_H 7.62 s) and 22-*OH* (δ_H 8.82 s) were verified further by the HMBC cross-peaks for 19-*OH*/C-18, C-19, C-20, and 22-*OH*/C-21, C-22, and C-23. The presence of these

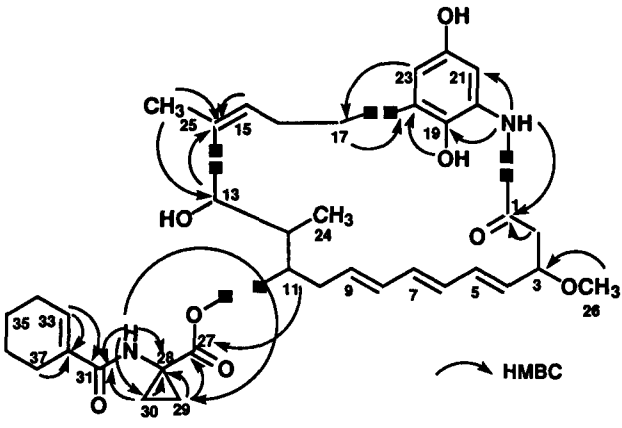

Figure 2. The partial Structure of Cytotrienin A (**1**)

phenolic hydroxyl groups was also supported by oxidation of **1** in 1% methanolic $FeCl_3$ solution (30 min., r.t.). The connection between 20-*NH* (δ_H 10.06 s) and C-20 (δ_C 125.90 s) was proved by the HMBC cross-peaks for 20-*NH*/C-19, C-20, and C-21.

Although the methylene proton signals from C-34 to C-37 were overlapped inthe ^1H NMR spectrumn of **1**, the assignment of the cyclohexene ring was assisted by the respective HMQC cross-peaks (Table 1), and also supported by analysis of the ^1H-^1H DQFCOSY cross-peaks from H-33 to H-37 and the HMBC cross-peaks for H-33/C-34, C-35, C-37. The presence of the cyclohexene carbonyl group was clarified by the HMBC cross-peaks for H-33/C-31, C-32; H_2-37/C-31, C-32. This result was also suggested by EIMS fragments *m/z* 109 for C_7H_9O and *m/z* 81 for C_6H_9.

Moreover, the detailed analyses of the remaining ^1H and ^{13}C NMR signals ($\delta_{C\text{-}27}$ 171.64 s; $\delta_{28\text{-}NH}$ 8.49 s, $\delta_{C\text{-}28}$ 33.31 s; $\delta_{H\text{-}29}$ 1.35 m, $\delta_{C\text{-}29}$ 16.68 t; $\delta_{H\text{-}30}$ 1.04 m, $\delta_{C\text{-}30}$ 16.45 t), in combination with 2D NMR spectra, confirmed unambiguously the presence of the 1-aminocyclopropane carboxylic group as described as follows. First, the presence of two vicinal cyclopropane methylenes (C-29 and C-30) was inferred by the ^1H-^1H DQFCOSY cross-peak for H_2-29/H_2-30, and demonstrated further from the respective ^1H-^{13}C coupling constant (both were J_{CH} 165 Hz) by INEPT (non-decoupling) data. Subsequently, the adjacency of the cyclopropane ring was suggested by the HMBC correlation H_2-29/C-28 and H_2-30/C-28. Finally, the connections from C-27 to C-31 via 28-*NH* were proved by the HMBC cross-peaks for the 28-*NH*/C-28, C-29, C-30, and C-31, and suggested further by the HMBC cross-peaks for H_2-29/C-27. Therefore, the above results provided evidence of the presence of a cyclohexene carbonyl 1-aminocyclopropane carboxylic group. The side chain was connected obviously at the position 11 by the HMBC cross-peaks for H-11/C-27 and H_2-29/C-27.

From all the spectroscopic experimental data described above, the structure of **1** was elucidated to be a novel member of the ansamycin group possessing a 21-membered macrocyclic lactam ring and a unique 1-aminocyclopropane carboxylic side-chain, as shown in Fig. 1. The structure of **1** was similar to mycotrienin II[3] except for the 11-side chain. Cytotrienin A (**1**) possessed a unique *N*-cyclohexene carbonyl-1-aminocyclopropane carboxylic group at C-11, while mycotrienin II had a *D*-alanine unit at C-11.

The geometry of the triene was determined as all *E* in view of the coupling constants of $J_{4,5}$, $J_{6,7}$ and $J_{8,9}$, which were 15.6 Hz, 14.7 Hz and 15.0 Hz, respectively. NOE's were observed between 15-H at δ_H 4.99 and H_3-25 at δ_H 1.60 s. This result indicated that the geometry of the double bond was 14 *Z*. The NOE data suggested the stereochemistry at carbons 3, 11, 12, 13 was similar to that of (+)- mycotrienin II.[4] The detailed investigation of the stereochemistry is in progress.

Cytotrienin B (**2**)[5] was isolated from a more non-polar portion than compound **1**. The EIMS spectrum of **2**, which displayed a molecular ion $(M)^+$ at *m/z* 650, showed two more mass units than **1**. The molecular

formula of **2** was determined as $C_{37}H_{50}N_2O_8$ by HRFABMS data [*m/z* 651.3704 $(M+H)^+$ for $C_{37}H_{51}N_2O_8$, Δ + 5.8 mmu], indicating the index of hydrogen deficiency was fourteen. The spectral data of **2**[5] were similar to those of **1**. The ^1H NMR spectrum of **2** showed the presence of five D_2O-exchangeable protons, the same as in the case of **1**. The ^{13}C NMR signals were especially different from **1** at δ_{C-31} 176.43 s, δ_{C-32} 43.69 d, and δ_{C-33} 29.23 t. The analyses of 2D NMR spectral data indicated that the structure of **2** had a cyclohexane ring at C-31, and was only different from the structure of **1** at the position 32 and 33. Namely, this result was suggested by the ^1H-^1H COSY cross-peaks H-32 to H_2-37 and also supported by the respective HMQC cross-peaks (Table 1). Furthermore, the assignment of the cyclohexane carbonyl group at 28-*NH* was established by the HMBC cross-peaks for H-32/C-31 and 28-*NH*/C-31. Thus, the structure of cytotrienin B (**2**) was determined to be the structure **2**. By the same means of **1**, the geometry of the triene in **2** was also deduced to be all *E* and the double bond at C-14 was *Z* configuration.

This is the first example in a series of triene-ansamycin antibiotics, demonstrating that the compounds involve a 1-aminocyclopropane carboxylic acid unit, such as cytotrienins A (**1**) and B (**2**). Both **1** and **2** are clearly different from the triene-ansamycin antibiotics possessing *D*-alanine such as mycotrienins,[3] or *L*-alanine such as ansatrienins[6] at C-11. These compounds were considered to be a series of related biogenetic analogs of mycotrienins.[3] It is interest in attempting to study the biosynthesis of **1** and **2**, especially for the 1-aminocyclopropane carboxylic acid moiety. Both **1** and **2** induced apoptosis to HL-60 cells with ED_{50} values of 7.7 nM, which were the same dose range for their growth inhibitory activities. Detailed discussion of the biological activities of **1** and **2** will be reported elsewhere.

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5. Cytotrienin B (**2**): a yellowish powder, m.p. 163 ~ 165°C from MeOH; $[\alpha]_D^{25}$ +211.2° (*c* 1.00, MeOH); UVλ_{max}^{MeOH} nm (log ε) 260 (4.49), 271 (4.59), 282 (4.48); IRγ_{max}^{KBr}cm^{-1} 3400 (NH, OH), 1720 (ester), 1650 (amide), 1000 (triene). ^1H NMR (400MHz, DMSO-d_6) δ 2.76 (1H, dd, *J* = 13.2, 4.9 Hz, H-2), 2.51 (1H, m, H-2), 4.02 (1H, td, *J* = 10.7, 4.9 Hz, H-3), 5.35 (1H, dd, *J* = 15.1, .8.8 Hz, H-4), 6.09 (1H, dd, *J* = 15.1, 8.3 Hz, H-5), 6.04 (1H, dd, *J* = 16.1, 8.3 Hz, H-6), 5.95 (1H, dd, 16.1, 7.8 Hz, H-7), 6.04 (1H, dd, *J* = 15.0, 7.8 Hz, H-8), 5.67 (1H, td, *J* = 15.1, 10.3, 3.9 Hz, H-9), 2.35 (1H, dt, *J* = 9.3, 4.9 Hz, H-10), 2.16 (1H, m, H-10), 4.63 (1H, br d, *J* = 10.3 Hz, H-11), 1.62 (1H, m, H-12), 4.44 (1H, br s, H-13), 4.19 (1H, d, *J* = 5.4 Hz, 13-*OH*), 4.92 (1H, br d, *J* = 7.3 Hz, H-15), 2.16 (2H, m, H-16), 2.84 (1H, m, H-17), 1.99 (1H, m, H-17), 7.50 (1H, s, 19-*OH*), 10.07 (1H, s, 20-*NH*), 6.32 (1H, br d, *J* = 2.4 Hz, H-21), 8.26 (1H, s, 22-*OH*), 6.37 (1H, br d, *J* = 2.4 Hz, H-23), 0.59 (3H, d, *J* = 6.8 Hz, H-24), 1.55 (3H, s, H-25), 3.17 (3H, s, H-26), 8.49 (1H, s, 28-*NH*), 1.20 (1H, m, H-29), 0.99 (1H, m, H-29), 1.38 (1H, m, H-30), 1.28 (1H, m, H-30), 2.06 (1H, td, *J* = 8.3, 3.4 Hz, H-32), 1.68 (1H, m, H-33), 1.57 (1H, m, H-33), 1.62 (2H, m, H-34), 1.16 (2H, m, H-35), 1.16 (2H, m, H-36), 1.82 (1H, m, H-37), 1.19 (1H, m, H-37). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.52 (s, C-1), 41.59 (t, C-2), 79.88 (d, C-3), 130.38 (d, C-4), 134.61 (d, C-5), 128.96 (d, C-6), 134.05 (d, C-7), 132.53 (d, C-8), 130.51 (d, C-9), 32.56 (t, C-10), 74.17 (d, C-11), 37.38 (d, C-12), 66.85 (d, C-13), 139.41 (s, C-14), 122.08 (d, C-15), 25.02 (t, C-16), 31.27 (t, C-17), 131.49 (s, C-18), 140.63 (s, C-19), 125.87 (s, C-20), 107.09 (d, C-21), 149.23 (s, C-22), 115.50 (d, C-23), 9.16 (q, C-24), 21.06 (q, C-25), 55.71 (q, C-26), 171.66 (s, C-27), 32.84 (s, C-28), 16.68 (t, C-29), 17.17 (t, C-30), 176.43 (s, C-31), 43.69 (d, C-32), 29.23 (t, C-33), 25.39 (t, C-34), 25.49 (t, C-35), 25.50 (t, C-36), 28.90 (t, C-37).
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