Structure of Apoptolidin, a Specific Apoptosis Inducer in Transformed Cells

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Several oncogenes have been demonstrated to sensitize cells to apoptosis. Thus, specific apoptosis inducers in cells expressing such oncogenes may be useful as anticancer agents for treating certain types of tumors. In the course of our screening for specific apoptosis inducers in transformed cells, we isolated a novel 20-membered macrolide, apoptolidin (**1**), from *Nocardiopsis* sp. **1** induced apoptotic cell death in rat glia cells transformed with the adenovirus E1A oncogene[1] (IC_{50} 11 ng/mL) but not in normal glia cells or normal fibroblasts (IC_{50} >100 μg/mL). In the previous paper,[2] we described the production, isolation, physicochemical properties, and biological activity of **1**. We report here the structure elucidation of **1** based on NMR spectral data in CD_3OD.

The molecular formula of **1** was determined to be $C_{58}H_{96}O_{21}$ from high-resolution FABMS [*m*/*z* 1151.6357 $(M + Na)^+$, Δ+1.5 mmu]. The ^{13}C NMR spectrum confirmed the presence of 58 carbons and the HMQC spectrum established all one-bond $^1H-^{13}C$ connectivities (Table 1). COSY and HMBC experiments generated a polyketide chain (**2**) and three hexoses (**3**−**5**) as the partial structures (Figure 1). Methanolysis (1% HCl-MeOH, 50 °C, 30 min) of **1** followed by hydrolysis yielded D-oleandrose[3] (**5**), L-olivomycose[4] (**4**), and a novel sugar, 6-deoxy-4-*O*-methyl-L-glucose[5] (**3**). Their anomeric configurations in **1** were identified as β for **5** and α for **3** and **4** by the proton coupling constants (Table 1).

Long-range correlations from 19-H to C-1 and from 25-H to C-21 constructed a 20-membered macrolide ring and a six-membered hemiketal ring, respectively. The three glycosidic linkages were formed on the basis of long-range couplings between 1′-H and C-9, 1″-H and C-27, 1‴-H and C-4″, and 4″-H and C-1‴. High-field carbon shifts for the allylic methyls (Table 1) and a large vicinal coupling constant (J_{10-11} = 15.0 Hz) indicated all (*E*) configurations for the five olefinic bonds, which were confirmed by a NOESY experiment as shown in Figure 2.

A clue to the absolute stereochemistry of the macrolide ring was found between the 6-deoxy-4-*O*-methyl-L-glucose moiety and the vicinal ring methines (J_{8-9} = 9.0 Hz). Their configurations were identified as (8*R*,9*R*) based on NOEs from 5′-H to 8-Me and from 1′-H to 9-H and 10-H. Significant NOEs between 9-H and 11-H, 11-H and 13-H, and 13-H and 16-H established stereochemical relationships from C-9 to C-16 and a loop-type conformation for C-13 to C-16. A carbon chain representing C-15 to C-20 was required to be in a zigzag-type conformation by

Table 1. ^{13}C (125 MHz) and ^1H (500 MHz) NMR Data for Apoptolidin in CD_3OD

no.	δ_C	δ_H	no.	δ_C	δ_H (J = Hz)
1	172.7		1′	96.0	4.85 (3.5)
2	123.7		2′	73.6	3.44 (9.0, 3.5)
3	149.2	7.41	3′	74.9	3.76 (9.0, 9.0)
4	133.1		4′	87.4	2.76 (9.0, 9.0)
5	147.0	6.23	5′	68.2	3.78 (9.0, 6.5)
6	133.4		6′	18.4	1.29 (6.5)
7	142.9	5.27	4′-OMe	61.1	3.61
8	38.9	2.79			
9	84.2	3.87	1″	99.5	4.97 (4.0)
10	126.4	5.26	2″	45.5	1.96 (13.0)
11	141.2	6.21			1.84 (13.0, 4.0)
12	134.8		3″	73.0	
13	133.3	5.71	4″	85.8	3.37 (9.5)
14	24.7	2.50, 2.09	5″	67.4	3.70 (9.5, 6.0)
15	36.4	1.52, 1.44	6″	19.0	1.25 (6.0)
16	74.6	3.47	3″-Me	22.9	1.36
17	83.8	2.75			
18	38.4	2.20, 1.78	1‴	101.9	4.86 (11.5, 1.0)
19	72.4	5.32	2‴	37.2	2.47 (12.0, 5.0, 1.0)
20	75.4	3.57			1.32 (12.0, 11.5, 11.5)
21	101.3		3‴	82.0	3.21 (11.5, 9.0, 5.0)
22	36.4	2.08	4‴	77.1	3.01 (9.0, 9.0)
23	73.8	3.76	5‴	73.2	3.24 (9.0, 6.0)
24	40.6	1.76	6″	18.4	1.31 (6.0)
25	69.4	3.99	3‴-OMe	57.4	3.46
26	37.2	1.62, 1.49			
27	76.8	3.48			
28	76.8	3.36			
2-Me	14.2	2.14			
4-Me	18.0	2.21			
6-Me	16.6	1.97			
8-Me	18.4	1.17			
12-Me	12.2	1.71			
22-Me	12.4	1.06			
24-Me	5.3	0.92			
17-OMe	61.4	3.40			
28-OMe	59.5	3.30			

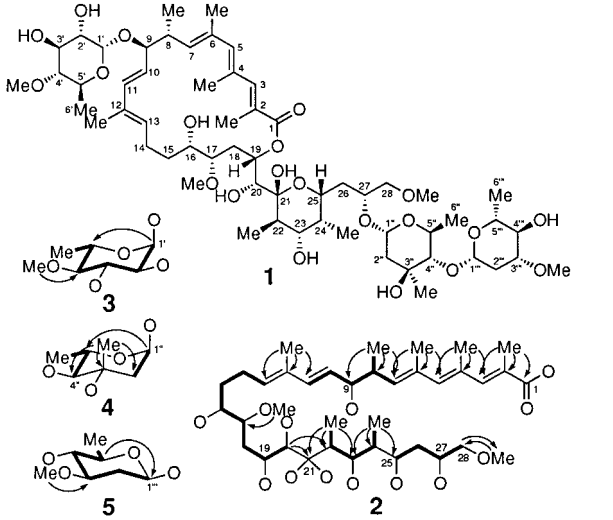

Figure 1. Structure and partial structures of apoptolidin. Bold lines show proton spin networks and arrows show $^1H-^{13}C$ long-range correlations.

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(5) Methyl 6-deoxy-4-*O*-methyl-α-L-glucopyranoside: mp 69−71 °C (colorless needles); ^1H NMR (500 MHz, D_2O) δ 4.61 (d, J = 4.0 Hz, 1-H), 3.61 (dq, J = 9.5, 6.5 Hz, 5-H), 3.56 (dd, J = 10.0, 9.0 Hz, 3-H), 3.46 (dd, J = 10.0, 4.0 Hz, 2-H), 3.44 (3H, s, OMe), 3.27 (3H, s, OMe), 2.82 (dd, J = 9.5, 9.0 Hz, 4-H), 1.18 (3H, d, J = 6.5 Hz, 6-H_3); ^{13}C NMR (125 MHz, D_2O) δ 99.8, 85.6, 73.1, 72.2, 67.2, 60.6, 55.7, 17.4; $[\alpha]^{25}_D$ −162° (*c* 0.55, H_2O). Treatment of the methyl glycoside with benzoyl chloride in pyridine at room temperature for 22 h gave its dibenzoate. The absolute configuration was established by the negative CD curve of the dibenzoate. See: Harada, N.; Nakanishi, K. *J. Am. Chem. Soc.* **1969**, *91*, 3989.

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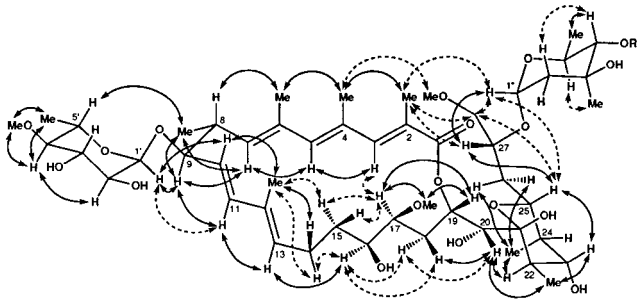

Figure 2. NOESY data summary for apoptolidin. Arrows indicate significant (solid) and less significant (dashed) NOEs. NOEs between vicinal protons are not shown.

NOEs between 15-H_2 and 17-H, 16-H and 18-H_2, 17-H and 19-H, and 18-H_2 and 20-H. In this conformation, the two large coupling constants (J_{17-18b} = 10.0 Hz and J_{18a-19} = 11.5 Hz) revealed an anti relationship for 17- and 19-oxygens. The most important NOE was observed between 3-H and 17-H to fix the macrolide ring conformation including a conjugated-carbonyl rotation. The endo arrangement of 17-H required exo orientations for 16-OH and 18-Hb (δ 1.78), indicating the remaining macrolide-ring configurations to be (16*S*,17*S*,19*S*).

NOEs from 2-Me to 27-H and 28-H_2 suggested intramolecular folding so as to bring C-27 and C-28 close to 2-Me. The proton on C-20 displayed a significant NOE with 18-Hb and a small coupling with 19-H (J_{19-20} < 2 Hz), thereby showing its stereochemistry to be (20*R*). NOEs from 23-H to 25-H and from 22-H to 20-H and 24-Me established the relative stereochemistry of a chair-form tetrahydropyran ring composed of C-21 to C-25. Their absolute configurations were determined to be (21*R*,22*R*,23*S*,24*R*,25*R*) based on NOEs from 25-H to 27-H, 28-H_2, and 1″-H. The remaining asymmetrical center was identified as (27*R*) by NOEs between 2-Me and 1″-H and between 4-Me and 28-OMe.

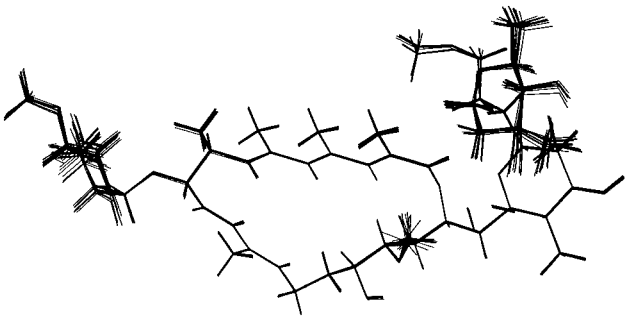

Figure 3. Superposition of 10 structures derived from DADAS of apoptolidin without the oleandrose moiety.

The stereochemistry of **1** thus obtained was confirmed by distance analysis in dihedral angle space (DADAS)[6] using a JEOL MolSkop system, which gave 10 final structures (Figure 3) starting with 100 randomly generated structures. Among the 10 structures, all possible pairs showed root-mean-square deviation (RMSD) values in the range of 0.13−0.54 Å, and none had NOE or van der Waals distance violations larger than 0.28 Å.

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Supporting Information Available: ^{1}H NMR, ^{13}C NMR, HMQC, COSY, HMBC, NOESY, and 1-D COSY spectra in CD_3OD for **1**; distance limits for DADAS of **1**; CD spectrum of methyl 2,3-*O*-dibenzoyl-6-deoxy-4-*O*-methyl-α-L-glucoside (27 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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