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Light-Induced Isomerization of Apoptolidin A leads to Inversion of C2−C3 Double Bond Geometry

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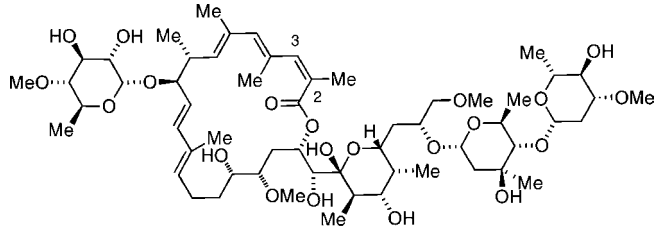
ABSTRACT



The isolation, characterization, and cytotoxicity against H292 cells of apoptolidin G are reported. Apoptolidin G is shown to be derived by a light-induced isomerization of the C2−C3 carbon−carbon double bond of apoptolidin A.

The apoptolidins are a group of macrolides produced by an actinomycete of the genus *Nocardiopsis* (FERM BP-5871).[1] Apoptolidin A (**1**, Figure 1), the first of the class to be identified, was described by Seto and co-workers in 1997 and determined to selectively induce apoptosis in rat glia cells expressing the E1A oncogene.[1a,2] Various shunt metabolites have subsequently been identified from the same actinomycete by the Wender group and assigned the names and structures of apoptolidins B−F (**2**−**6**, Figure 1).[3] Interest in the isolation, synthesis, and study of apoptolidin macrolides is associated with their observed selective cytotoxicity against various cancer cell lines providing an opportunity for the development of a unique class of anticancer therapeutics. Based on a pharmacological analysis, apoptolidin A was demonstrated to be selectively cytotoxic to metabolically active cancer cells that do not exhibit the Warburg effect.[4,5] It was further demonstrated that apoptolidin A inhibits F_0F_1-ATPase; however, subsequent examination of apoptolidin analogues and derivatives suggests the observed in vitro cell cytotoxicity may not be solely associated with this enzymatic inhibition.[6]

The identification and biological evaluation of new apoptolidin congeners provide an opportunity to develop a structure−activity relationship among this class of natural

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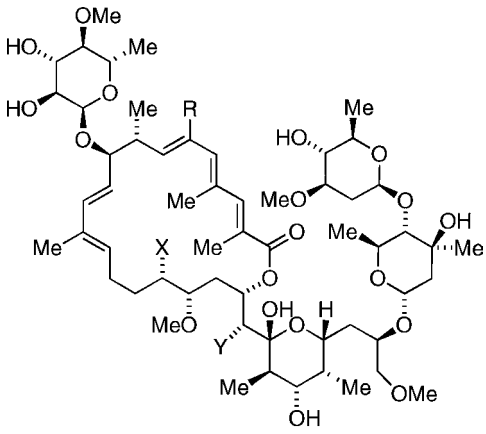
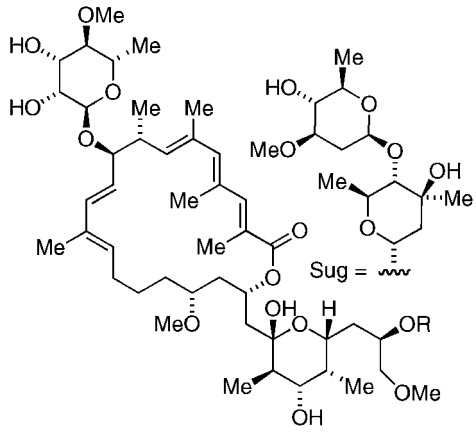


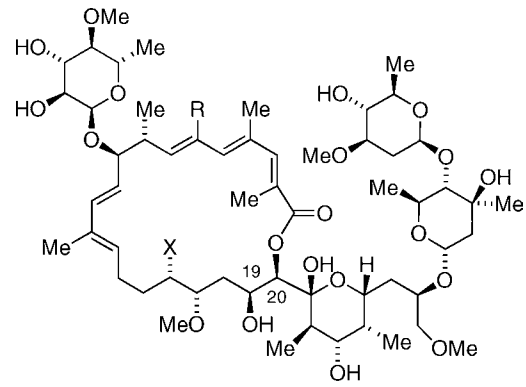
apoptolidin A (R = Me; X = Y = OH, **1**)
apoptolidin B (R = Me; X = H; Y = OH, **2**)
apoptolidin C (R = Me; X = Y = H, **3**)
apoptolidin D (R = H; X = Y = OH, **4**)



apoptolidin E (R = Sug, **5**)
apoptolidin F (R = H, **6**)

Figure 1. Structures of apoptolidins A−F (**1**−**6**).

products. Several structural modifications have been introduced to the apoptolidin A skeleton by degradation, semisynthesis, and total synthesis.[7,8] Another source of new structural types, as mentioned above, has been fermentation that to date has yielded five new metabolites (apoptolidins B−F).[3] Finally, as is frequently the case in natural product isolation, unique apoptolidins have been inadvertently produced during the isolation and purification process. This appears to be the case among the isoapoptolidins (**7**−**9**, Figure 2) that result from an acyl migration from the C19 to



isoapoptolidin A (R = Me; X = Y = OH, **7**)
isoapoptolidin B (R = Me; X = H; Y = OH, **8**)
isoapoptolidin D (R = H; X = Y = OH, **9**)

Figure 2. Structures of isoapoptolidins A, B, and D (**7**−**9**).

C20 hydroxyl group resulting in a one-atom ring expansion of the macrolactone.[9] During the course of producing apoptoldin by fermentation we recently identified a new apoptolidin A isomerization. Herein we describe the isolation, structure elucidation, and biological evaluation of this new congener (apoptolidin G).

Following apoptolidin production by *Nocardiopsis* (FERM BP-5871), we isolated a minor (less than 5 mg/L) apoptolidin from an aged crude extract. Isolation was achieved by normal-phase HPLC (10% methanol in dichloromethane) following purification by flash chromatography (silica gel, 5% methanol in dichloromethane then 15% methanol in dichloromethane). The new apoptolidin had a slightly shorter retention time then apoptolidin A (normal phase, 10% methanol/dichloromethane).

The newly identified apoptolidin closely resembled apoptolidin A by NMR analysis and displayed a blue-shifted UV absorption maximum (320−278 nM) relative to apoptolidin A. Inspection of the ^{1}H NMR suggested the core of the new isolate was a 20-membered macrolactone and not the corresponding ring expanded isomer (isoapoptolidin).[7e,9] Preliminary assignments for the new congener were based on comparison to the extant chemical shifts for apoptolidin A, which revealed that primary structural differences were apparently localized in the trienoate region from C-1 to C-8 (Tables 1 and 2).

Taken together, these data implied that the new congener was likely a geometrical isomer located within the trieonate chromophore.

The remaining structural features were established by a series of two-dimensional NMR experiments. HSQC experi-

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Table 1. Comparison of ^{1}H NMR Data for Apoptolidin G and Apoptolidin A (ppm, CD_3OD, 600 MHz)

	δ_H			δ_H			δ_H			δ_H	
carbon	**G**	**A**	carbon	**G**	**A**	carbon	**G**	**A**	carbon	**G**	**A**
1			16	3.2	3.47	6-Me	*1.59*	*1.97*	1″	5.02	4.97
2			17	3.33	2.75	8-Me	1.08	1.17	2″	1.78, 1.97	1.84, 1.96
3	*6.08*	*7.41*	18	1.69, 2.41	1.78, 2.2	12-Me	1.73	1.71	3″		
4			19	5.2	5.32	22-Me	1.04	1.06	4″	3.3	3.37
5	*5.87*	*6.23*	20	3.79	3.57	24-Me	0.88	0.92	5″	3.72	3.7
6			21			17-OMe	3.56	3.4	6″	1.2	1.25
7	*4.93*	*5.27*	22	2.1	2.08	28-OMe	3.33	3.3	3″-Me	1.33	1.36
8	2.8	2.79	23	3.77	3.76	1′	4.79	4.85	1‴	4.82	4.86
9	3.77	3.87	24	1.7	1.76	2′	3.37	3.44	2‴	1.28, 2.43	1.32, 2.47
10	5.33	5.26	25	4.06	3.99	3′	3.7	3.76	3‴	3.17	3.21
11	6.21	6.21	26	1.44, 1.69	1.49, 1.62	4′	2.7	2.76	4‴	2.96	3.01
12			27	3.9	3.48	5′	3.72	3.78	5‴	3.17	3.24
13	5.68	5.71	28	3.38	3.36	6′	1.24	1.29	6‴	1.26	1.31
14	2.18, 2.41	2.09, 2.5	2-Me	*2.01*	*2.14*	4′-OMe	3.56	3.61	3‴-OMe	3.42	3.46
15	1.42, 1.73	1.44, 1.52	4-Me	*1.7*	*2.21*						

ments were used to assign all direct $^{1}H-^{13}C$ connectivities. COSY and HMBC experiments confirmed the presence of the apoptolidin A polyketide chain and all functional groups were preserved in the new congener. Long-range correlations from H19 to C1 and throughout the hemiketal region confirmed the 20-membered macrolactone linkage (Figure 3). Similarly, the positions of pendant *O*-methyl groups and sugars were verified by long-range correlations.

The geometric configurations of the trienoate double bonds were determined by analysis of a ROESY experiment as shown in Figure 3, which demonstrated that the configuration of the C2−C3 olefinic bond had inverted to the *Z* configuration and that the remaining four olefins retained the *E* configuration. This structural change resulted in a conformational change of the macrolide relative to apoptolidin A resulting in rotation about bonds C3−C4 and C5−C6 as indicated by diagnostic ROESY cross peaks, including from H7 to 4-Me with concurrent anisotropic sheilding of trieoate olefinic protons (H3, H5 and H7, Table 1) and shielding of corresponding vinyl methyls (2-Me, 4-Me and 6-Me, Table 1) relative to the corresponding chemical shifts observed in apoptolidin A.

The isolation of a small amount of a presumed light-induced isomer of apoptolidin A prompted us to procure more of this material for further studies. Accordingly, a sample of apoptolidin A was dissolved in acetone-d_6 in a borosilicate glass NMR tube. The sample was irradiated using a 275 W sunlamp for 16 h. A similar experiment was performed using methanol-d_4 solution of apoptolidin A. The samples were removed from irradiation and analyzed by ^{1}H NMR for the appearance of a new isomer. In the acetone sample, the isomerization was clean but not complete,

Table 2. Comparison of ^{13}C NMR Data for Apoptolidin G and Apoptolidin A (ppm, CD_3OD, 600 MHz)

	δ_C			δ_C			δ_C			δ_C	
carbon	**G**	**A**	carbon	**G**	**A**	carbon	**G**	**A**	carbon	**G**	**A**
1	172.1	172.7	16	73.2	74.6	6-Me	16.1	16.6	1″	99.5	99.5
2	*128.7*	*123.7*	17	86.0	83.8	8-Me	18.4	18.4	2″	45.2	45.5
3	*138.4*	*149.2*	18	37.8	38.4	12-Me	12.0	12.2	3″	73.1	73.0
4	133.5[a]	133.1	19	75.8	72.4	22-Me	12.4	12.4	4″	86.0	85.8
5	*139.2*	*147.0*	20	76.2	75.4	24-Me	5.4	5.3	5″	67.5	67.4
6	132.9[a]	133.4	21	101.4	101.3	17-OMe	61.0	61.4	6″	18.0	19.0
7	*133.0*	*142.9*	22	36.8	36.4	28-OMe	59.5	59.5	3″-Me	22.7	22.9
8	39.6	38.9	23	77.3	73.8	1′	95.5	96.0	1‴	101.9	101.9
9	82.7	84.2	24	40.7	40.6	2′	73.9	73.6	2‴	37.3	37.2
10	126.4	126.4	25	68.6	69.4	3′	75.0	74.9	3‴	82.0	82.0
11	141.4	141.2	26	37.8	37.2	4′	87.6	87.4	4‴	77.0	77.1
12	135.1	134.8	27	75.8	76.8	5′	68.2	68.2	5‴	73.0	73.2
13	133.6	133.3	28	77.3	76.8	6′	18.4	18.4	6‴	18.3	18.4
14	24.1	24.7	2-Me	23.0	14.2	4′-OMe	61.0	61.1	3‴-OMe	57.3	57.4
15	36.8	36.4	4-Me	17.8	18.0						

[a] Denotes interchangeable assignments.

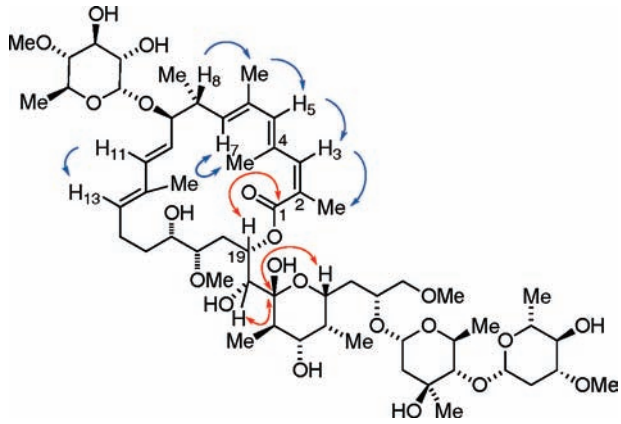

Figure 3. Selected ROESY (blue arrows) and HMBC (red arrows) correlations establishing the structure of apoptolidin G (**10**).

resulting in ~5:1 mixture of an isomerized product and apoptolidin A. In the methanol sample, isomerization was observed resulting in a similar ratio of products; however, the NMR showed other minor degradation products. The sample in acetone was concentrated and the resulting photoisomerized product was purified from the mixture using flash chromatography (silica gel, 2.5% methanol in dichloromethane then 10−15% methanol in dichloromethane). The material isolated from the direct photolysis of apoptolidin A was identical to the minor metabolite we isolated from the fermentation extract. We now suggest this macrolide be named apoptolidin G, in accord with earlier minor metabolites isolated from the apoptolidin producer FERM BP-5871.

Over 25 apoptolidins, derived by semisynthesis, total synthesis, and isolation, have been evaluated for cytotoxicity against apoptolidin sensitive cancer cell lines. The H292 human lung cancer cell shows good sensitivity to apoptolidins and various congeners. We compared the cytotoxic effect of apoptolidin A to apoptolidin G against H292 cells. As observed previously, apoptolidin A displayed an EC_{50} below 50 nM when exposed to H292 cells for 4 days. In the same cell viability assay, apoptolidin G showed a modest decrease in cytotoxicity (EC_{50} 150 nM) relative to apoptolidin A. This observation is in accord with earlier SAR studies which show that considerable structural modification is tolerated within the aglycone of the apoptolidins. Notably, the observed minimal loss of of cell cytotoxicty by extended irradiation of apoptolidin A with UV light has implications for future photaffinity experiments using apoptolidin A derived chemical probes.

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Supporting Information Available: Procedure for the photoisomerization of apoptolidin A to **10**. ^{1}H and ^{13}C and COSY, TOCSY, ROESY, HSQC, and HMBC spectra, and HRMS for apoptolidin G (**10**). This material is available free of charge via the Internet at http://pubs.acs.org.

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