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Toward a Stable Apoptolidin Derivative: Identification of Isoapoptolidin and Selective Deglycosylation of Apoptolidin

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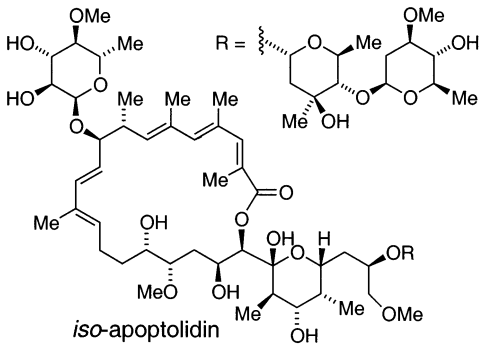
ABSTRACT



Isoapoptolidin was isolated from crude fermentation extracts of the apoptolidin-producing microorganism *Nocardiopsis* sp. Apoptolidin isomerizes to isoapoptolidin upon treatment with methanolic triethylamine to establish a 1.4:1 equilibrium mixture of isoapoptolidin and apoptolidin. Semisynthesis of a peracetylated and deglycosylated derivative of apoptolidin is also described.

In 1998, Hayakawa and co-workers described the structure of apoptolidin in full detail.[1a] An earlier publication provided only the two-dimensional structure of this unique macrolide and indicated apoptolidin selectively induces E1A-transformed cells to undergo apoptosis (programmed cell death).[1b] Using a series of molecular and cell-based pharmacological analyses, Salomon and Khosla later related this cell-specific biological activity to apoptolidin's inhibition of mitochondrial F_0F_1-ATPase.[2] Structurally, the new macrolide features an unsaturated 20-membered macrolactone, a highly substituted cyclic hemiacetal, and several deoxy sugars. During the course of their recently reported total synthesis of apoptolidin, Nicolaou and co-workers encountered difficulties in the final deprotection step.[3,4] For example, they determined that upon standing at room temperature in solution or during chromatographic separation, apoptolidin converted to an isomer they presumed to be its C-21 anomer and produced other unidentified products. Our group is also interested in the synthesis, biology, and chemistry of apoptolidin. As a primary goal we planned to investigate the synthesis of a simplified *stable* derivative of this complex macrolide

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(1) (a) Hayakawa, Y.; Kim, J. W.; Adachi, H.; Shin-ya, K.; Fujita, K.; Seto, H. *J. Am. Chem. Soc.* **1998**, *120*, 3524−3525. (b) Kim, J. W.; Adachi, H.; Shin, Y. K.; Hayakawa, Y.; Seto, H. *J. Antibiot.* **1997**, *50*, 628−630.

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(3) (a) Nicolaou, K. C.; Li, Y.; Fylaktakidou, K. C.; Mitchell, H. J.; Wei, H. X.; Weyershausen, B. *Angew. Chem., Int. Ed.* **2001**, *40*, 3849−3854. (b) Nicolaou, K. C.; Li, Y. W.; Fylaktakidou, K. C.; Mitchell, H. J.; Sugita, K. *Angew. Chem., Int. Ed* **2001**, *40*, 3854−3857.

(4) For other synthetic studies toward apoptolidin: (a) Nicolaou, K. C.; Li, Y.; Weyerhausen, B.; Wei, H.-x. *J. Chem. Soc., Chem. Commun.* **2000**, 307−308. (b) Schuppan, J.; Ziemer, B.; Koert, U. *Tetrahedron Lett.* **2000**, *41*, 621−624. (c) Sulikowski, G. A.; Lee, W. M.; Jin, B.; Wu, B. *Org. Lett.* **2000**, *2*, 1439−1442. (d) Toshima, K.; Arita, T.; Kato, K.; Tanaka, D.; Matsumura, S. *Tetrahedron Lett.* **2001**, *42*, 8873−8876.

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suitable for correlation with material produced by chemical synthesis. An ideal candidate would be the aglycone, apoptolidinone (**2**).[5] Unfortunately, attempts to remove the sugars of apoptolidin by acidic methanolysis have led not to isolation of the aglycone but only to the corresponding sugar methyl glycosides or C-27 deglycosylation accompanied by dehydration within the cyclic hemiacetal.[1a,6] In this communication, we describe the isolation and structure elucidation of isoapoptolidin, produced from fermentation and base-induced isomerization of apoptolidin. We also describe the semisynthesis of a more stable peracetylated/deglycosylated derivative of apoptolidin that serves as our current synthetic target.

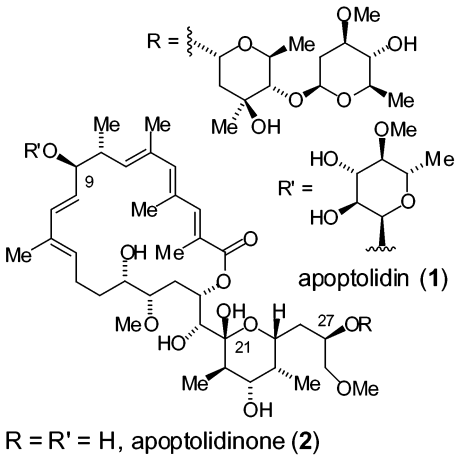

Figure 1. Structure of apoptolidin (**1**) and apoptolidininone (**2**).

A crude sample of apoptolidin was produced by fermentation of *Nocardiopsis* sp. following the procedure of Hayakawa and co-workers.[1b,7] In addition to apoptolidin, a second major fermentation product was isolated following preparative HPLC. This compound shared a common molecular weight with apoptolidin according to high-resolution mass spectrometry and closely resembled apoptolidin by ^{1}H and ^{13}C NMR. Initially, we considered the possibility that this material was the C-21 anomer of apoptolidin; however, this assumption was proven to be incorrect following two-dimensional NMR analysis. The key $^{1}H-^{1}H$ COSY and HMBC correlations that led us to conclude that isoapoptolidin is the result of an acyl migration from the C-19 to C-20 hydroxyl group are highlighted in Figure 2. This rearrangement expands the 20-membered lactone of apoptolidin to a 21-membered lactone. We determined that the isomerization of apoptolidin to isoapoptolidin is catalyzed by base. For example, a sample of apoptolidin in methanol, when treated with a trace of triethylamine, eventually equilibrates to a 1.4:1 mixture of isoapoptolidin and apoptolidin as determined by ^{1}H NMR.

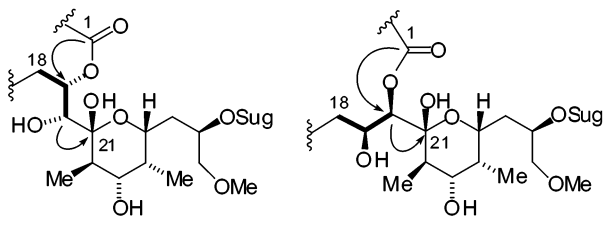

Figure 2. Selected $^{1}H-^{1}H$ COSY (—) and HMBC (→) correlations for apoptolidin and isoapoptolidin.

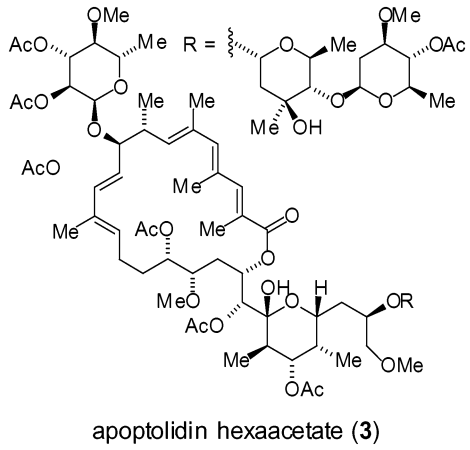

Figure 3. Structure of apoptolidin hexaacetate (**3**).

A sample of crude apoptolidin, obtained by fermentation, was peracetylated under standard acylation conditions to afford apoptolidin hexaacetate (**3**) in 38% yield. The assigned structure of **3** was supported by extensive NMR analysis in order to confirm that no undesired C-19 to C-20 acyl migration accompanied peracetylation. Not surprisingly, apoptolidin hexaacetate is considerably more stable than apoptolidin and shows a lesser tendency to rearrange or decompose even upon extended treatment with triethylamine, being 70% unchanged after 56 h. In contrast to apoptolidin, treatment of apoptolidin hexaacetate (**3**) with anhydrous methanolic hydrochloric acid results in a rapid and high-yielding deglycosylation of C-28 to give **4** in 83% yield (Figure 4).[8] Acetylation of **4** gives hexaacetate **5**. The latter product is currently the primary target of our apoptolidin synthetic program.

(5) Synthesis of apoptolidinone: Schuppan, J.; Wehlan, H.; Keiper, S.; Koert, U. *Angew. Chem., Int. Ed* **2001**, *40*, 2063−2066.

(6) Salomon, A. R.; Zhang, Y. B.; Seto, H.; Khosla, C. *Org. Lett.* **2001**, *3*, 57−59.

(7) Fermentation and isolation was conducted at Stanford University by A. Gulledge and O. Jankowski (Wender group) and A. Salomon (Khosla group).

(8) Under similar conditions, apoptolidin decomposed in less than 12 h. The peracetylated psuedoaglycone **4** underwent successive deacetylation via transesterifications leading to decomposition after 56 h.

(9) Assay was performed as previously described; see refs 2b and 6.

(10) We cannot distinguish whether isoapoptolidin or apoptolidin is first produced biosynthetically and subsequently isomerize by an acyl shift to the other under the fermentation conditions. In other words, isoapoptolidin may very well be the first-formed natural product that isomerizes to apoptolidin.

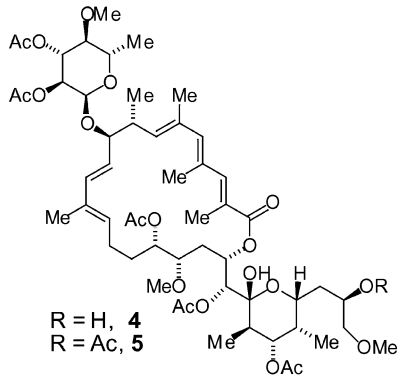

Figure 4. Semisynthetic derivatives of apoptolidin.

The inhibition of mitochondrial ATPase of apoptolidin derivatives **3**−**5** were compared to apoptolidin by an in vitro yeast ATPase assay.[9] Apoptolidin has a K_i of 0.38 μM, while apoptolidin derivatives **3**−**5** have a K_i >100 μM. These findings suggest that free hydroxyl groups are important for binding of apoptolidin to its mitochondrial target. Indeed of the three derivatives (**3**−**5**), the most active inhibitor was **4**, which has a free hydroxyl group at C-27.

In conclusion, we have identified isoapoptolidin a 21-membered macrolactone presumably the result of a 1,2-acyl migration of apoptolidin.[10] We have also prepared by semisynthesis apoptolidin derivatives **3**−**5**. These derivatives are poor inhibitors of yeast ATPase.

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Supporting Information Available: Full characterization data for apoptolidin, isoapoptolidin, and compounds **3** and **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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