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Apoptolidins E and F, New Glycosylated Macrolactones Isolated from *Nocardiopsis* sp.

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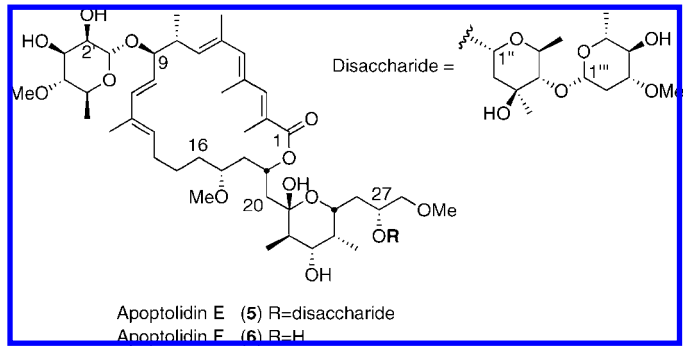
ABSTRACT



Two new glycosylated macrolactones, apoptolidins E (5) and F (6), were isolated from fermentation of the actinomycete *Nocardiopsis* sp. and their structures assigned. Lacking the C16 and C20 oxygens of apoptolidin A (1), these macrolides are also the first members of this family to display a 4-*O*-methyl-L-rhamnose at C9 rather than a 6-deoxy-4-*O*-methyl-L-glucose.

Apoptolidin A (**1**), an aptly named glycosylated macrolactone, was first isolated by Seto and co-workers in 1997 from the fermentation of *Nocardiopsis* sp. using an activity-based screen designed to identify novel compounds that selectively induce apoptosis in cell lines expressing the E1A viral oncogene but have no effect on normal cells. Representative of its striking selectivity, apoptolidin A shows activity in E1A-transformed glial cells at 11 ng/mL but has no effect on normal glial cells even at concentrations >100 000 ng/mL.[1] In the National Cancer Institute's 60 cell line screen, apoptolidin A ranked in the top 0.1% most selective agents of the 37 000 compounds screened up to that time.[2] Due in part to its promising biological activity and its complex structure, apoptolidin has attracted significant interest in its synthesis and modification,[3] the latter greatly assisted by the ready availability of apoptolidin through fermentation (ca. 130 mg/L).[1,4] While efforts to elucidate the mode of action of apoptolidin are ongoing, an impressive early study reported by the Khosla laboratory established the case, at least in part, for its role as an inhibitor of mitochondrial F_1F_0-ATPase.[2,5] A subsequent comparison of the activities of oligomycin, apoptolidin, and various analogues in cell-free (yeast mitochondrial ATPase) and whole cell assays (the original Ad12-3Y1 and 3Y1 cell lines) showed some variation in potency and rank order, suggesting that additional factors could contribute to apoptolidin's activity.[3p] Recently, as part of our synthesis and mode of action studies, we reported the structures and biological activities of three additional naturally occurring apoptolidins: apoptolidins B (**2**), C (**3**), and D (**4**) (Figure 1).[4,6] While isolated in much lower yields than

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

(2) Salomon, A. R.; Voehringer, D. W.; Herzenberg, L. A.; Khosla, C. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 14766–14771.

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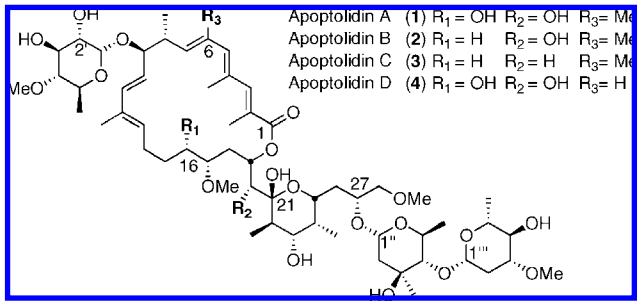

Figure 1. Structures of apoptolidins A−D.

apoptolidin A (**1**), these new analogues showed comparable biological activity in a cell-based assay using H292 (human lung carcinoma) cells and allowed quick access to previously unstudied structures.

Due to the promising biological activities of the previously reported apoptolidins, interest in their biosynthesis and the structural basis for their remarkable selectivities, we have continued to examine the producing organism, *Nocardiopsis* sp., for new apoptolidins. Apoptolidins E (**5**) and F (**6**), both incorporating a 4-*O*-methyl-L-rhamnose in place of the 6-deoxy-4-*O*-methyl-L-glucose typically observed at C9 in the apoptolidins, have been identified and their structures reported herein.

Fermentation of *Nocardiopsis* sp. was performed according to established procedures,[1,4] and subsequent extraction with EtOAc provided a crude mixture consisting of apoptolidins A−F along with isoapoptolidin.[7] Silica gel column chromatography (5% MeOH/$CHCl_3$) separated the apoptolidins lacking hydroxyls (apoptolidins B, C, E, and F) from the more polar apoptolidins (apoptolidins A and D and isoapoptolidin). Final purification using preparative reverse-phase HPLC provided pure apoptolidins E and F, both isolated in <5 mg/L, for characterization and further study.

The molecular formulas for apoptolidins E (**5**) and F (**6**) were determined to be $C_{58}H_{96}O_{19}$ [*m*/*z* 1119.6415 $(M + Na)^+$] and $C_{44}H_{72}O_{13}$ [*m*/*z* 831.4520 $(M + Na)^+$], respectively, by high resolution ESI-TOF MS. The primary structures (Figure 2) were determined using extensive 2D-NMR studies (COSY,

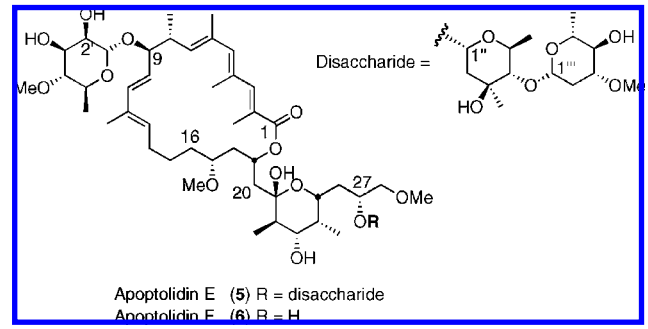

Figure 2. Structures of apoptolidins E (**5**) and F (**6**).

TOCSY, ROESY, HSQC, HMBC) in CD_3OD at room temperature on a 600 MHz instrument (Table 1).

Comparison of the proton NMR spectrum of apoptolidin E (**5**) with that of apoptolidin C (**3**) showed extensive similarities (Table 1), including additional methylenes at C16 and C20 as compared with apoptolidin A (**1**). The notable differences were associated with the sugar appended at C9. After a comparative analysis of ROESY data, apoptolidin E (**5**) was assigned as C2′-*epi*-apoptolidin C. The key difference between the ROESY data of apoptolidin C and that of apoptolidin E is the missing crosspeak for C2′-H and C4′-H in apoptolidin E (Figure 3). This 1,3-diaxial interaction is readily observed in apoptolidin C. Furthermore, C3′-H in apoptolidin E is seen as a doublet of doublets with $J_1 = 9.6$ Hz (trans diaxial coupling with C4′-H) and $J_2 = 3.5$ Hz (axial/equatorial coupling with C2′-H). For comparison, C3′-H in apoptolidin A is a triplet with $J = 8.9$ Hz (trans diaxial couplings with C4′-H and C2′-H).[1b,8] Finally, the C1′-H coupling constant in apoptolidin E is 1.6 Hz as compared to 3.9 Hz observed for apoptolidin C consistent with the change in stereochemistry at C2′.

As with apoptolidin E (**5**), apoptolidin F (**6**) showed similarities to apoptolidin C with additional methylene groups at C16 and C20; however, it was apparent based on mass and NMR data that the disaccharide appended at C27 in other family members is missing in apoptolidin F. To explore whether apoptolidin F is possibly an artifact of isolation, apoptolidin E was stirred in a slurry of silica gel in 10% MeOH/$CHCl_3$ at rt. These conditions emulate the silica gel column chromatography used in the isolation of the apoptolidins, which is considered to be the most likely stage at which the C27-disaccharide could be cleaved. Under these conditions, however, apoptolidin F is not observed by

(3) For a review including early work from the laboratories of Nicolaou, Koert, Sulikowski, Crimmins, Toshima, Fuchs, and Taylor, see: Daniel, P. T.; Koert, U.; Schuppan, J. *Angew. Chem., Int. Ed.* **2006**, *45*, 872–893, and references therein. Additional synthetic studies: (a) Chng, S.-S.; Xu, J.; Loh, T. P. *Tetrahedron Lett.* **2003**, *44*, 4997–5000. (b) Bouchez, L. C.; Vogel, P. *Chem.—Eur. J.* **2005**, *11*, 4609–4620. (c) Wu, B.; Liu, Q.; Jin, B.; Qu, T.; Sulikowski, G. A. *Eur. J. Org. Chem.* **2006**, 277–284. (d) Schuppan, J.; Wehlan, H.; Keiper, S.; Koert, U. *Chem.—Eur. J.* **2006**, *12*, 7364–7377. (e) Wehlan, H.; Dauber, M.; Mujica Fernaud, M. T.; Schuppan, J.; Keiper, S.; Mahrwald, R.; Juarez Garcia, M.-E.; Koert, U. *Chem.—Eur. J.* **2006**, *12*, 7378–7397. (f) Craita, C.; Didier, C.; Vogel, P. *Chem. Commun.* **2007**, *23*, 2411–2413. (g) Kim, Y.; Fuchs, P. L. *Org. Lett.* **2007**, *9*, 2445–2448. (h) Handa, M.; Scheidt, K. A.; Bossart, M.; Zheng, N.; Roush, W. R. *J. Org. Chem.* **2008**, *73*, 1031–1035. (i) Ghidu, V. P.; Wang, J.; Wu, B.; Liu, Q.; Jacobs, A.; Marnett, L. J.; Sulikowski, G. A. *J. Org. Chem.* **2008**, *73*, 4949–4955. (j) Handa, M.; Smith, W. J., III.; Roush, W. R. *J. Org. Chem.* **2008**, *73*, 1036–1039. (k) Crimmins, M. T.; Christie, H. S.; Long, A.; Chadhary, K. *Org. Lett.* **2009**, *11*, 831–834. (l) Ghidu, V. P.; Ntai, I.; Wang, L.; Jacobs, A. T.; Marnett, L. J.; Bachmann, B. O.; Sulikowski, G. A. *Org. Lett.* **2009**, *11*, 3032–3034. (m) Lewis, C. A.; Longcore, K. E.; Miller, S. J.; Wender, P. A. *J. Nat. Prod.* **2009**, *72*, 1864–1869. For additional synthetic studies, including studies connected to mode of action, see: (n) Salomon, A. R.; Zhang, Y.; Seto, H.; Khosla, C. *Org. Lett.* **2001**, *3*, 57–59. (o) Wender, P. A.; Jankowski, O. D.; Tabet, E. A.; Seto, H. *Org. Lett.* **2003**, *5*, 487–490. (p) Wender, P. A.; Jankowski, O. D.; Longcore, K.; Tabet, E. A.; Seto, H.; Tomikawa, T. *Org. Lett.* **2006**, *8*, 589–592.

(4) Wender, P. A.; Sukopp, M.; Longcore, K. *Org. Lett.* **2005**, *7*, 3025–3028.

(5) Salomon, A. R.; Voehringer, D. W.; Herzenberg, L. A.; Khosla, C. *Chem. Biol.* **2001**, *8*, 71–80.

(6) Wender, P. A.; Longcore, K. E. *Org. Lett.* **2007**, *9*, 691–694.

(7) (a) Wender, P. A.; Gulledge, A. V.; Jankowski, O. D.; Seto, H. *Org. Lett.* **2002**, *4*, 3819–3822. (b) Pennington, J. D.; Williams, H. J.; Salomon, A. R.; Sulikowski, G. A. *Org. Lett.* **2002**, *4*, 3823–3825.

Table 1. ^{1}H NMR Data for Apoptolidins C (**3**), E (**5**), and F (**6**)[a]

	3[b]	**5**	**6**		**3**[b]	**5**	**6**
carbon	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)	carbon	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)
3	7.16	7.16	7.16	4-Me	2.12	2.14	2.14
5	6.08	6.08	6.08	6-Me	1.89	1.89 (1.1)	1.89
7	5.18 (9.6)	5.17	5.16	8-Me	1.13 (6.6)	1.12 (6.6)	1.12 (6.6)
8	2.73	2.61 (15.6, 9.0, 6.6)	2.60	12-Me	1.67	1.66	1.66
9	3.84 (9.6, 9.0)	3.85 (9.0)	3.84	22-Me	1.05 (6.6)	1.04 (6.6)	1.04 (6.6)
10	5.21 (16.0, 9.6)	5.13 (15.6, 9.0)	5.13 (15.6, 9.0)	24-Me	0.90 (6.6)	0.90 (6.6)	0.90 (7.2)
11	6.17 (16.0)	6.19 (15.6)	6.19 (15.6)	17-OMe	3.26	3.27	3.26
13	5.58 (9.6, 5.8)	5.59 (9.6, 5.9)	5.58 (9.6, 5.9)	28-OMe	3.34	3.33	3.36
14	1.96	1.96	1.96	1′	4.82 (3.9)	4.76 (1.6)	4.76 (1.5)
	2.31	2.30	2.30	2′	3.40	3.74	3.74 (3.4, 1.7)
15	1.20	1.22	1.21	3′	3.72	3.77 (9.6, 3.5)	3.78
	1.34	1.35	1.34	4′	2.72 (9.0, 9.0)	3.10 (9.6)	3.10 (9.6)
16	1.33	1.33	1.32	5′	3.74	3.67 (9.6, 6.0)	3.67 (9.6, 6.6)
	1.60	1.60	1.60	6′	1.26 (6.6)	1.28 (6.0)	1.29 (6.0)
17	2.92	2.92	2.91	4′-OMe	3.58	3.57	3.57
18	1.74	1.75	1.73	1″	4.98 (4.2)	4.98 (4.1)	--
	1.96	1.96	1.95	2″	1.80	1.80	--
19	5.18	5.18	5.18		1.95	1.95	--
20	1.80	1.80	1.77	4″	3.33	3.33	--
	2.10 (14.4, 5.5)	2.10 (14.4, 5.6)	2.11 (14.4, 5.4)	5″	3.74	3.74	--
22	1.81	1.81	1.81	6″	1.24 (6.6)	1.23 (6.0)	--
23	3.75	3.76	3.78	3″-Me	1.34	1.35	--
24	1.74	1.74	1.76	1‴	4.83 (10.2, 2.0)	4.83 (9.6, 1.9)	--
25	4.07 (9.6, 2.5, 2.1)	4.07 (9.6, 2.5)	4.18 (10.2, 2.6)	2‴	1.29	1.29	--
26	1.43	1.42 (14.4, 9.6, 2.9)	1.31		2.44 (12.0, 5.0, 2.0)	2.44 (12.0, 5.0, 1.7)	--
	1.43	1.67	1.66	3‴	3.17	3.17 (10.0, 9.0, 5.0)	--
27	3.74	3.75	3.83	4‴	2.97 (9.6, 9.6)	2.97 (9.0, 9.0)	--
28	3.41	3.41	3.36	5‴	3.21	3.21 (9.6, 6.0)	--
	--	--	3.28 (10.8, 4.0)	6‴	1.28 (6.6)	1.28 (6.0)	--
2-Me	2.06	2.06	2.06	3‴-OMe	3.43	3.43	--

[a] Data for apoptolidins E (**5**) and F (**6**) recorded in CD_3OD, 600 MHz. See Supporting Information for complete characterization data. [b] Apoptolidin C (**3**) data adapted from ref 4.

analytical HPLC even after 3 h. Analogous NMR data that is described for stereochemical assignment of apoptolidin E was also observed for apoptolidin F.

A recent report by Sulikowski et al.[3l] combines traditional synthesis with biosynthesis and suggests that incorporation of the C9-sugar in apoptolidin A might precede macrolactonization and attachment of the C27-disaccharide follows. The isolation of apoptolidin F is consistent with this insightful hypothesis; however, alternative biosynthetic sequencing of pathways cannot be excluded at this time. Furthermore, the incorporation of 6-deoxy-4-*O*-methyl-L-glucose (apoptolidins A−D) and 4-*O*-methyl-L-rhamnose (apoptolidins E−F) could occur using the same glycosyltransferase (GT) given the substrate flexibilty that has been reported within this family of enzymes.[9]

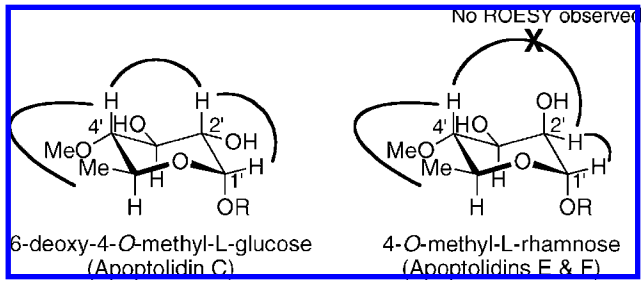

Figure 3. Key ROESY correlations for the C9-sugar in apoptolidins C, E, and F.

As expected from earlier work,[3e,n,p,7b,10,11] apoptolidin F, which lacks the disaccharide subunit, exhibits significantly reduced activity (greater than an order of magnitude loss in potency) as compared to apoptolidin A when tested for growth inhibition against the H292 cell line. In this same assay, apoptolidin E gave somewhat variable results; how-

(8) Coupling constants for the sugar protons of apoptolidin A were originally determined following methanolysis and hydroylsis of apoptolidin A to provide the individual sugars (D-oleandrose, L-oliomycose, and 6-deoxy-4-*O*-methyl-L-glucose) for NMR analysis.

(9) For a review regarding post-polyketide synthase modifications, see: Rix, U.; Fischer, C.; Remsing, L. L.; Rohr, J. *Nat. Prod. Rep.* **2002**, *19*, 542–580.

(10) Wender, P. A.; Jankowski, O. D.; Tabet, E. A.; Seto, H. *Org. Lett.* **2003**, *5*, 2299–2302.

(11) Nicolaou, K. C.; Li, Y.; Sugita, K.; Monenschein, H.; Guntupalli, P.; Mitchell, H. J.; Fylaktakidou, K. C.; Vourloumis, D.; Giannakakou, P.; O'Brate, A. *J. Am. Chem. Soc.* **2003**, *125*, 15443–15454.

ever, its activity was on par with that of apoptolidin A (EC_{50} < 100 nM). This result, taken together with the recent observation that omission of the C9 sugar in apoptolidin D leads to less than an order of magnitude loss in activity in H292 cells,[3l] suggests that the C9 sugar is not directly involved in the interaction between the apoptolidins and their cellular target(s). Further studies on the activities of these compounds and their mode of action are in progress.

In conclusion, two new apoptolidins, apoptolidins E and F, isolated from the fermentation of *Nocardiopsis* sp., were characterized on the basis of extensive NMR spectroscopy and by comparison with other known congeners. This family of natural products is thus expanded to now include structures with varied sugars appended at C9 as well as those lacking the disaccharide typically observed at C27. Importantly, the natural production and subsequent isolation of these analogues provides facile access to structures of interest in ongoing synthesis, biosynthesis, and mode of action studies.

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Supporting Information Available: Complete spectroscopic data including ^{1}H NMR, COSY, TOCSY, HSQC, HMBC, ROESY, and IR. This material is available free of charge via the Internet at http://pubs.acs.org.

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