Actinoplanic Acid A: A Macrocyclic Polycarboxylic Acid Which Is a Potent Inhibitor of Ras Farnesyl-Protein Transferase

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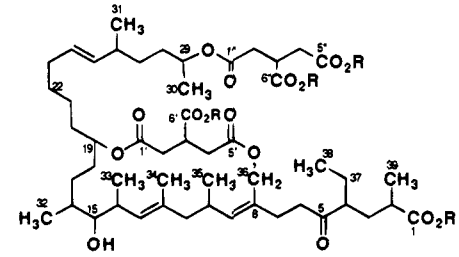
The *ras* genes are found mutated in 50% of colon and 90% of pancreatic carcinomas.[1] These oncogenes can lead to an unregulated cell division in these and many other prominent carcinomas.[2] Farnesyl-protein transferase[3] (FPTase) is an enzyme that catalyzes the farnesylation[4] of protein encoded by *ras* (Ras or p21) at cysteine 186, which is an essential step for association of Ras with the cell membrane and promotion of cell transforming activity.[4] Therefore, selective inhibitors of FPTase have the potential to be used as anticancer agents, particularly in colon and pancreatic cancers. Indeed, FPTase inhibitors reduce the number of the tumorigenic phenotypes of cells transformed by *ras* in both cell culture and animal models.[5]

We recently reported chaetomellic acids[6] and fusidienol[7] as specific inhibitors of FPTase. Continued screening for potent and novel inhibitors of FPTase led to the isolation of actinoplanic acid A (**1**) from an *Actinoplanes* species.[8] Actinoplanic acid A is a novel and complex 20-membered macrocyclic bislactone tetracarboxylic acid that inhibits recombinant human FPTase[9] with an IC_{50} of 230 nM. Inhibition of FPTase by actinoplanic acid A is selective and competitive with respect to FPP[10a] (K_i = 98 nM) and uncompetitive with respect to acceptor peptide ras-CVIM[10b] (K_i = 4 μM). Furthermore, actinoplanic acid A appears to be selective for FPTase as it did not inhibit human squalene synthase ($IC_{50} \gg 1$ μM) or bovine brain geranyl-geranyl protein transferase I ($IC_{50} \gg 1$ μM). Herein, we describe the isolation and structure determination of actinoplanic acid A (**1**).



1: R = H (Actinoplanic Acid A)

2: R = CH_3

Actinoplanic acid A was produced by fermentation of *Actinoplanes* sp. (MA 7066; ATCC 55532) in liquid nutrient medium. The filtered broth was acidified to pH 1.5 and extracted with ethyl acetate. Gel filtration (Sephadex LH-20) of the ethyl acetate extract followed by purification by preparative reverse phase HPLC[11] yielded actinoplanic acid A (10 mg/L) as a gum.[12]

Analysis of HRFABMS and ^{13}C NMR spectra of actinoplanic acid A led to a molecular formula $C_{51}H_{80}O_{16}$, which indicated 12 degrees of unsaturation. The ^{13}C NMR spectrum (Table 1) of actinoplanic acid A in CD_3OD exhibited 51 carbons, which in conjunction with the DEPT spectrum revealed carbon signals for eight methyls, 18 methylenes (one being oxygenated), 11 methine (three bearing oxygen), four olefinic methine and two nonprotonated olefinic, an acyclic ketone, four acid carbonyls [actinoplanic acid A formed a tetramethyl ester[13] (**2**) upon reaction with diazomethane], and three ester/lactone carbonyls. The 500 MHz ^1H NMR spectrum (Table 1) in CD_3OD of **1** showed six methyl doublets, a methyl triplet, a vinylic methyl, an *E*-olefin (J = 15.5 Hz), two trisubstituted olefins, and several allylic or α-to-carbonyl CH_2 groups. The ^1H NMR spectrum was very complex, and many overlapping signals in the upfield region of the spectrum could be interpreted only with the help of multiple 2D NMR spectra. As the resonances in the ^{13}C NMR spectrum were well resolved, the HMQC (J = 140 Hz) experiment served not only in identifying the protons attached to a specific carbon but also in deciphering the ^1H-domain chemical shifts of some of the protons. Detailed analysis of phase-sensitive $^1H-^1H$ COSY, one-step relayed $^1H-^1H$ COSY, TOCSY, and $^1H-^{13}C$ COSY allowed construction of six partial

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(10) (a) FPP, Farnesyl pyrophosphate; (b) CVIM, cysteine-valine-isoleucine-methionine.

(11) Column, Zorbax RX C-8 (22 × 250 mm); eluent, 55% aqueous acetonitrile containing 0.3% TFA; flow rate, 7 mL/min for 30 min followed by 8 mL/min. Actinoplanic acid A was eluted between 41 and 47 min.
(12) $[\alpha]^{25}_D$ 23° (*c* 0.13, CH_3OH); IR (ZnSe) ν_{max} 3600-2600 (br), 2932, 1713, 1450, 1379, 1250, 1177, and 967 cm^{-1}; positive ion FABMS (*m/z*) 949 (M + H), 971 (M + Na), 987 (M + K); negative ion FABMS (*m/z*) 947 (M - H); HRFABMS (*m/z*) 971.5389 (calcd for $C_{51}H_{80}O_{16}$ + Na, 971.5344).
(13) CIMS (*m/z*) 1005 (M + H); ^1H NMR ($CDCl_3$, assigned using $^1H-^1H$ COSY, relayed COSY, and TOCSY experiments) H-2 (δ 2.34, m), H-3 (1.68, 1.72), H-4 (2.46), H-6 (2.42, 2.58), H-7 (2.21, m; 2.35, m), H-9 (5.16, d, J = 8.5 Hz), H-10 (2.66, m), H-11 (1.84, m; 2.18, dd, J = 12.5, 3 Hz), H-13 (4.83, d, J = 11 Hz), H-14 (2.48, m), H-15 (3.13, dd, J = 10, 1.5 Hz), H-16 (1.58), H-17 (1.00; 1.32), H-18 (1.26), H-19 (4.70, m), H-20 (1.30; 1.50), H-21 (1.30), H-22 (1.34), H-23 (1.95, m), H-24 (5.33, dt, J = 15, 7 Hz), H-25 (5.21, dd, J = 15.5, 8 Hz), H-26 (2.0, octet, J = 6.5 Hz), H-27 (1.20, 1.60), H-28 (1.50), H-29 (4.87, quintet, J = 6.5 Hz), H-30 (1.19, d, J = 6.5 Hz), H-31 (0.95, d, J = 7 Hz), H-32 (0.99, d, J = 6.5 Hz), H-33 (1.02, d, J = 6.5 Hz), H-34 (1.57, br s), H-35 (0.87, d, J = 6.0 Hz), H-36 (4.44, d, J = 13 Hz; 4.94 , d, J = 13 Hz), H-37 (1.40, 1.58), H-38 (0.85, t, J = 7.5 Hz), H-39 (1.13, d, J = 7 Hz), H-1′ (2.50, dd, 17, 6.5 Hz; 2.96, dd, J = 17, 8 Hz), H-3′ and H-3″ (3.26, quintet, J = 6.5 Hz), H-4′ (2.45 and 2.67, each dd, J = 15.5, 6 Hz), H-2″ and H-4″ (2.60, m; 2.77 and 2.72, dd, J = 17, 7.5 Hz), CO_2CH_3 (3.75, 3.691, 3.689, 3.67, all s).

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Table 1. ^1H and ^{13}C NMR Assignments and HMBC Correlations of Actinoplanic Acid A in CD_3OD Solution

position	δC^a	δH^b	HMBC[c] C → H	position	δC^a	δH^b	HMBC[c] C → H
1	180.0		H-2, H-39	27	33.8	1.20, m, 1.62, m	H-25, H-29, H-31
2	39.1	2.26, m	H-4, H-39	28	34.9	1.51, m	H-26, H-29, H-30
3	35.9	1.67, m	H-2, H-4, H-39	29	73.0	4.86, m	H-30
4	52.5	2.57, m	H-2, H-38	30	20.2	1.18, d, 6.0	H-29
5	215.5		H-4, H-7	31	21.5	0.94, d, 6.5	H-25, H-26
6	41.7	2.54, m, 2.68, m	H-7	32	18.9	0.97, d, 7.5	H-15
7	29.5	2.14, dd, 13.5, 1.2 2.37, dd, 14.5, 1.2	H-9, H-36	33	18.8	0.99, d, 7.5	H-13, H-15
8	133.3		H-7, H-9, H-36	34	15.9	1.56, brs	H-11
9	136.9	5.18, brd, 8	H-7, H-11, H-35, H-36	35	23.2	0.88, d, 7	H-9
10	30.7	2.70, m	H-9, H-11, H-35	36	62.8	4.44, d, 13 4.93, d, 13	H-7, H-9
11	50.3	1.86, m, 2.18, m	H-9, H-13, H-34, H-35	37	26.4	1.41, m, 1.59, m	H-4, H-38
12	134.5		H-11, H-13, H-34	38	11.8	0.85, t, 7.5	H-4
13	131.3	4.84, m	H-11, H-15, H-33, H-34	39	18.6	1.11, d, 7.0	H-2
14	37.7	2.53, m	H-13, H-15, H-33	1′	174.9		H-19, H-2′, H-3′
15	82.1	3.05, dd, 10, 1.0	H-13, H-32, H-33	2′	36.6	2.53, m, 2.82, m	H-3′
16	37.0	1.62, m	H-15, H-32	3′	39.3	3.20, appt, 7.5	H-2′, H-4′
17	26.7	1.00, m, 1.31, m	H-15, H-19, H-32	4′	37.9	2.42, dd, 15.5, 10 2.70, m	H-2′, H-3′
18	32.7	1.26, m	H-19	5′	175.2		H-36, H-3′, H-4′
19	76.8	4.72, m		6′	172.3		H-2′, H-3′, H-4′
20	34.0	1.33, m, 1.50, m	H-19, H-22	1″	173.0		H-29, H-3″
21	25.7	1.27, m, 1.36, m	H-22, H-23	2″	36.7	2.57, m, 2.68, m	H-3″
22	30.5	1.34, m	H-23, H-24	3″	38.7	3.16, quint, 6.5	
23	33.4	1.96, m	H-22, H-24, H-25	4″	36.1	2.54, m, 2.72, m	H-3″
24	130.1	5.37, dt, 15.5, 6.5	H-22, H-23, H-25	5″	175.0*		H-3″
25	137.3	5.25, dd, 15.5, 8.0	H-23, H-24, H-26, H-31	6″	176.8*		H-3″
26	38.1	2.04, quint, 6.5	H-24, H-25, H-31				

[a] 125 MHz. [b] 500 MHz. [c] $^nJ_{CH} = 7$ Hz. Correlations observed with severly overlapped protons are not shown and were not used for structural elucidation purposes; m, either true multiplet or overlapped signals; appt, apparent triplet; quint, quintet; *, interchangable assignments.

structures (C38−C39, C6−C7, C9−C11, C13−C30, C2′−C4′, and C1″−C4″). The largest of these, C13−C30, contained a number of overlapping proton signals, and final verification of this fragment as well as others came from HMBC ($^nJ_{CH} = 7$ Hz) experiments. The methyl groups gave strong two- and three-bond HMBC correlations to their respective neighboring carbons. This was of paramount importance in verification of the partial structures and determination of key connectivities. For example, both H-2 and H_3-39, besides giving the expected correlations within substructure C38−C39, were correlated to C-1 (δ 180.0); H-4 gave a correlation to C-5 (δ 215.5), which was also correlated to H_2-6 and H_2-7, thus linking the first two substructures. Similarly, correlations from H_2-7 to C-8 and C-9 connected the third piece to the newly created fragment C1−C7. Correlations from H-19, one of the well-dispersed ^1H signals, were critical to verification of the connectivities of C-17, C-18, C-20, and C-21 (all of the ^1H shifts in this segment were between δ 1.00 and 1.50 ppm). Based on the HMBC correlations of H-2′, H-3′, and H-4′ and H-2″, H-3″, and H-4″ to the respective carbonyl groups, the C2′−C4′ and C2″−C4″ partial structures were assembled to give two 1,2,3-tricarboxypropane moieties (tricarballylic acid). The ester and bis-lactone linkages were established from the HMBC correlations as follows: H-19 to C-1′ (δ 174.9); H_2-36 to C-5′ (δ 175.2); and H-29 to C1″ (δ 173.0).

The geometries of the olefinic bonds in actinoplanic acid A were determined to be all *E* on the basis of NOESY correlations of methyl ester **2**, which showed correlations of H-9 to H-7 (δ 2.35), H-11 (δ 1.84), and a weak correlation to H-35; H-13 to H-11, H-15, and H-33. Relative stereochemistry of the chiral centers could not be determined, except for that of C-14 and C-15. H-15 shows a large coupling with H-14 ($J = 10$ Hz) and a small coupling with H-16 ($J = 1$ Hz), thus indicating its anti and syn relationship to the respective protons. Based on the spectroscopic data, structure **1** is proposed for actinoplanic acid A.

The substitution pattern of actinoplanic acid A is uniquely arranged on a tridecanoic acid backbone and is probably derived from a mixed acetate/propionate biosynthesis. It is uniquely esterified with two units of carballyllic acid residues, one in the form of bis-lactone, to give the 20 membered macrocycle. Carballyllic acid has been reported to be a part of fumonisins, a class of mycotoxins derived from *Fusarium moniliforme*.[14]

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