Pergamon

Tetrahedron Letters, Vol. 38, No. 42, pp. 7399-7402, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)01734-6

Structure of Blasticidin A

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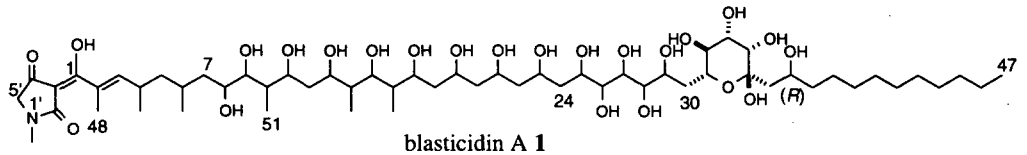
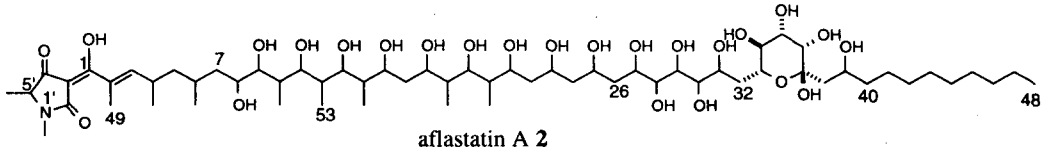
Abstract: The structure of blasticidin A was characterized as **1**, which is a tetramic acid derivative with a highly oxygenated long alkyl chain similar to aflastatin A **2**. © 1997 Elsevier Science Ltd.

Blasticidin A, an antibiotic, was found in 1955 in the culture broth of *Streptomyces griseochromogenes*.[1] In 1968, Kono *et al.* reported its isolation and detailed physicochemical properties,[2] but its structure has not been elucidated. Recently, during the course of our search for inhibitors of aflatoxin production by *Aspergillus parasiticus*, aflastatin A was isolated as a specific inhibitor from *Streptomyces* sp. MRI142 and its structure was characterized as **2**.[3,4] Since close homology was noticed between the physicochemical properties of aflastatin A and blasticidin A, the biological activity of blasticidin A was reexamined, and it became clear that blasticidin A inhibits aflatoxin production as strongly as aflastatin A.[5] This finding prompted us to investigate the structure of blasticidin A. In this paper, we describe the preliminary structural elucidation of blasticidin A.



blasticidin A **1**



aflastatin A **2**

Blasticidin A was isolated from a mycelial MeOH extract of *S. griseochromogenes* IFO 13413 as a white powder by *n*-BuOH extraction, precipitation from $CHCl_3$/MeOH(3:1) and from THF, and finally by reverse-phase HPLC under basic conditions.[6] The molecular formula of **1** was determined as $C_{58}H_{107}NO_{23}$, which is smaller than that of **2** by C_4H_8O, from analysis of the HR-FABMS spectrum and NMR spectra. The UV spectrum of **1**[2] was the same as that of **2**,[3] indicating that **1** and **2** have a similar chromophore. ^1H and ^{13}C NMR spectra of **1** closely resembled those of **2**, and the presence of a common partial structure (A) was clarified by analyzing the DQF-COSY, DQF-relayed COSY, HMQC, and HMBC spectra of **1** (Fig. 1). From the UV and NMR spectra, the presence of a tetramic acid moiety in **1** was also suggested. Other small partial structures common to **1** and **2** were identified from the spectra, but it was difficult to determine the remainder of

the structure of **1** by further NMR analysis with the intact molecule. Therefore, oxidation of **1** with $NaIO_4$ was performed to obtain fragment molecules according to the method used for the preparation of fragments of **2**[4] with slight modification.

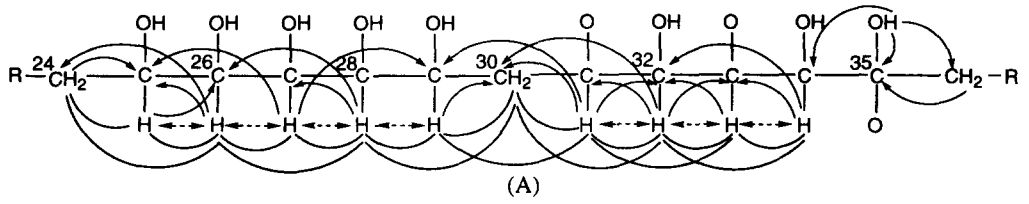


Figure 1. COSY, Relayed COSY and HMBC correlations observed in partial structure A.
COSY ◄----►, Relayed COSY ⌣, HMBC (H→C) ⌣

First, to obtain a chromophoric fragment, **1** was oxidized with $NaIO_4$, and followed by $NaBH_4$ reduction. In this case, the oxidation reaction was stopped when the amount of a product **3**[7] reached maximum, since a tetramic acid moiety of the chromophore was labile to excess $NaIO_4$. The molecular formula of **3**, whose UV spectrum is the same as that of **1**, was determined as $C_{16}H_{25}NO_4$ from its HR-FABMS spectrum. The structure **3** was determined by analysis of the NMR spectra. Next, a fragment **4** was obtained by $NaIO_4$ oxidation of **1** followed by $NaBH_4$ reduction and acetylation.[8] The HR-FABMS spectrum of **4** indicated that the molecular formula was $C_{38}H_{60}O_{18}$. By detailed analysis of COSY, HMQC and HMBC spectra, its structure was determined as **4**. Finally, **1** was oxidized with $NaIO_4$, and the reaction mixture was extracted with CH_2Cl_2. From the CH_2Cl_2 extracts, fragment **5** was obtained.[9] It was characterized as **5** by analysis of the MS and NMR spectra. The optical rotation value revealed that it has 3-(*R*) configuration.[10]

Because all carbon atoms of **1** were involved in structure A or fragment **3**,**4** or **5**, its total carbon skeleton could be deduced from them. Based on the fact that **1** had no carboxylic acid, acetoxymethyl or hydroxymethyl groups in its molecule, the carbon skeleton of **1** was easily reconstructed to afford a large partial structure (B) as shown in Fig. 2.

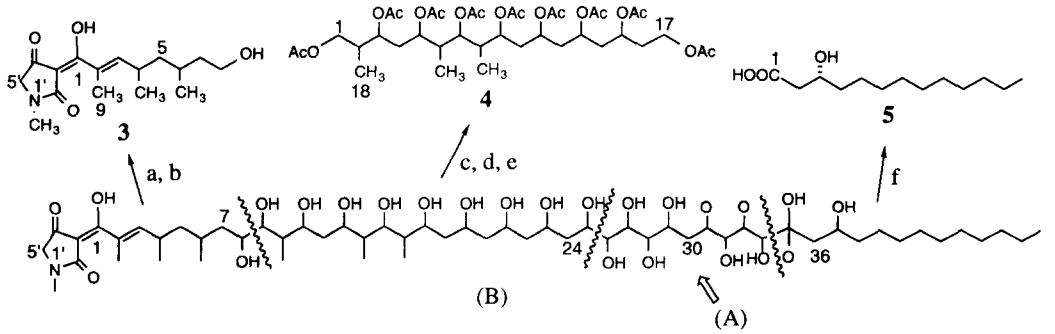


Figure 2. Degradation experiments of **1**.
a)$NaIO_4$, MeOH, rt, 5h, b)$NaBH_4$, MeOH, rt, 1h, 36% (2 steps), c)$NaIO_4$, MeOH, rt, 14h, d)$NaBH_4$, MeOH, 0℃, 1h, e)Ac_2O, DMAP, pyridine, rt, 24h, 6.3% (3 steps), f)$NaIO_4$, MeOH, rt, 20h, 27%

From the structure (B) and molecular formula of **1**, determination of the position of an ether linkage was the only remaining problem. The formation of a tetrahydropyran ring by the ether linkage between C-31 and C-35 was revealed by the *J* values and NOEs around the ring protons as shown in Fig. 3. Thus, the total structure of blasticidin A was determined as **1**. The assignments of protons and carbons in the NMR spectra of **1** are summarized in Table 1.

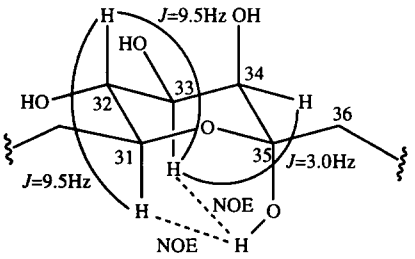


Figure 3. Relative stereochemistry of the tetrahydropyran ring.

Table 1. ^{1}H and ^{13}C Assignments of **1**[a]

C-No.	δ_C	δ_H	C-No	δ_C	δ_H
1	191.2		28-OH		4.14
2	135.2		29	68.6	3.82
3	139.2	5.46 d (9)	29-OH		4.11
4	29.9	2.52	30	35.8	2.05, 1.48
5	44.6	1.33, 0.94	31	70.2	3.62
6	26.2	1.86	32	71.2	3.18[g]
7	42.4	1.23	32-OH		4.57
8	68.0	3.56	33	70.7	3.56[g]
9	74.6	3.27	34	73.0	3.41[g]
10	39.6	1.61	34-OH		4.45
11	71.6	3.89	35	98.4	
12	34.8[b]	1.48, 1.33	35-OH		6.11
13	71.0	3.93	36	41.6	1.82, 1.42
13-OH[f]		4.73	37	68.5	3.88
14	41.7	1.63	38	38.2	1.30
15	76.0	3.44	39	24.9[d]	1.25
15-OH		4.57	40	28.7[d]	1.23
16	38.2	1.51	41	29.0[d]	1.23
17	73.4	3.78	42	29.1[d]	1.23
17-OH		4.70[e]	43	29.1[d]	1.23
18	41.3[b]	1.55	44	29.2[d]	1.23
19	67.5[c]	3.87	45	31.3	1.23
20	41.9[b]	1.55	46	22.1	1.23
21	67.8[c]	3.78	47	14.0	0.84 t (6.5)
21-OH		4.73[e]	48	13.2	1.69
22	44.5[b]	1.53	49	21.4	0.88 d (6.5)
23	67.8[c]	3.80	50	20.8	0.86 d (6.5)
23-OH		4.70	51	8.7	0.83 d (7)
24	40.9	1.85, 1.35	52	10.5	0.68 d (6.5)
25	69.7	3.62	53	5.9	0.79 d (6.5)
25-OH		4.66	2'	174.4	
26	74.3	3.25	3'	99.7	
26-OH		4.57	4'	189.4	
27	69.4	3.82	5'	55.9	3.31
27-OH		4.11	6'	28.4	2.71
28	72.5	3.44			

[a] Spectra were obtained in DMSO-d_6 on a JEOL GX-500. [b,c,d,e] May be interchanged. [f] Each hydroxy proton was assigned from the COSY spectrum except for 35-OH, which was assigned from the HMBC spectrum. [g] $J_{31,32}$ = 9.5Hz, $J_{32,33}$ = 9.5Hz and $J_{33,34}$ = 3.0Hz were observed in the spectrum obtained in DMSO-d_6 + 3%D_2O. Coupling constants in Hertz are given in parentheses

The structure of blasticidin A **1** is similar to that of aflastatin A **2**. It is a tetramic acid derivative with a highly oxygenated long alkyl chain. A saturated hydrocarbon skeleton forms the end part of the alkyl chain. There are a few differences between the structures of **1** and **2** as follows. A methyl group in the tetramic acid moiety of **2** is not present in the corresponding part of **1**. The length of the carbon chain and the number or position of methyl or hydroxyl groups in the part corresponding to fragment **4** of **1** are different from those in the counterpart of **2**. The length of the hydrocarbon end skeleton of **1** is longer than that of **2** by one carbon unit. Studies on the stereochemistry and biosynthesis of **1** are now in progress.

Acknowledgement: This work was supported by a grant from JSPS Program for Research for the Future.

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5. Blasticidin A inhibited aflatoxin production in *A. parasiticus* at the concentration of 0.5 μg/mL completely without inhibiting its growth, which was the same activity as aflastatin A showed. The biological activity of blasticidin A as an inhibitor of aflatoxin production will be published in detail elsewhere.
6. **1** : HR-FABMS (positive, glycerol matrix) *m/z* 1208.7153 $(M+Na)^+$ (Calcd for $C_{58}H_{107}NO_{23}Na$, 1208.7132); $[\alpha]^{22}_D$ +10.8° (*c* 1.0, DMSO).
7. **3** : HR-FABMS (positive, NBA matrix) *m/z* 318.1687 $(M+Na)^+$ (Calcd for $C_{16}H_{25}O_4NNa$, 318.1681); UV λ_{max} (nm) (ε) (MeOH-H_2O, 1:1): 299(7,300), 246(14,400); (MeOH-0.01N NaOH, 1:1): 298(6,300), 245(12,300); (MeOH-0.01N HCl, 1:1): 314(6,800), 236(8,600);$[\alpha]^{18}_D$ +37.6° (*c* 0.1, MeOH); δ_H (CD_3OD, 500MHz) 5.67(dq, *J*=9.5, 1.5Hz, H-3), 3.58(H-8), 3.55(H-5'), 2.87(H-6'), 2.64(H-4), 1.82(d, *J*=1.5Hz, H-9), 1.69(H-6), 1.49(H-7a), 1.34(H-7b), 1.34(H-5a), 1.11(H-5b), 0.98(d, *J*=6.5Hz, H-10), 0.90(d, *J*=6.5Hz, H-11); δ_C (CD_3OD, 500MHz) 195.8(C-1), 192.7(C-4'), 176.8(C-2'), 143.1(C-3), 137.3(C-2), 102.5(C-3'), 60.9(C-8), 57.1(C-5'), 46.2(C-5), 41.7(C-7), 31.7(C-4), 28.9(C-6'), 28.2(C-6), 21.1(C-10), 20.4(C-11), 13.5(C-9); HMBC correlations ($^nJ_{CH}$=8Hz): H-3 to C-1, 4, 5, 9 and 10, H-5 to C-3, 4, 6 and 10, H-7 to C-5, 6, 8 and 11, H-8 to C-6 and 7, H-9 to C-1, 2 and 3, H-10 to C-3, 4 and 5, H-11 to C-5, 6 and 7, H-5' to C-2' and 4', H-6' to C-2' and 5'.
8. **4** : HR-FABMS (positive, glycerol matrix) *m/z* 827.3677 $(M+Na)^+$ (Calcd for $C_{38}H_{60}O_{18}Na$, 827.3677); $[\alpha]^{18}_D$ +7.1° (*c* 0.1, MeOH); δ_H (CD_3OD, 500MHz) 4.99(H-3), 4.99(H-15), 4.93(H-13), 4.90(H-5), 4.88(H-11), 4.84(H-9), 4.82(H-7), 4.07(H-17), 3.91(H-1), 2.11(H-6), 2.05(H-8), 2.02(H-2), 1.96(H-16a), 1.93(H-10a), 1.92(H-4a), 1.92(H-12), 1.82(H-14), 1.82(H-10b), 1.81(H-16b), 1.81(H-4b), 0.95(d, *J*=7Hz, H-18), 0.92(d, *J*=7Hz, H-19), 0.91(d, *J*=7Hz, H-20), 2.10, 2.04, 2.03, 2.03, 2.03, 2.02, 2.02, 2.01 and 2.01(Ac); δ_C (CD_3OD, 500MHz) 76.0(C-7), 73.7(C-9), 73.0(C-5), 72.8(C-3), 70.2(C-11), 69.9(C-13), 69.6(C-15), 66.8(C-1), 61.7(C-17), 40.0(C-6), 39.9(C-14), 39.7(C-12), 39.1(C-8), 38.1(C-10), 36.9(C-2), 34.2(C-16), 32.2(C-4), 11.4(C-19), 11.0(C-18), 8.9(C-20), ~21 and ~172(Ac).; HMBC correlations ($^nJ_{CH}$=8Hz): H-1 to C-2, 3 and 18, H-2 to C-18, H-3 to C-1, 2, 4 and 5, H-5 to C-6, H-6 to C-19, H-7 to C-5, 6, 8, 9 and 20, H-8 to C-20, H-9 to C-7, 8, 10, 11 and 20, H-11 to C-9, 10, 12 and 13, H-13 to C-11, 12, 14 and 15, H-15 to C-14, 16 and 17, H-17 to C-15 and 16, H-18 to C-1, 2 and 3, H-19 to C-5, 6 and 7, H-20 to C-7, 8 and 9.
9. **5** : HR-FABMS (positive, NBA matrix) *m/z* 253.1764 $(M+Na)^+$ (Calcd for $C_{13}H_{26}O_3Na$, 253.1780); $[\alpha]^{22}_D$ -13.8° (*c* 0.05, $CHCl_3$) [Lit.[10] $[\alpha]_D$ -15° (*c* 2.0, $CHCl_3$)]; δ_H (CD_3OD, 500MHz) 3.86(H-3), 2.31(dd, *J*=15, 4.5Hz, H-2), 2.22(dd, *J*=15, 8.5Hz, H-2), 1.43(H-4 and 5a), 1.28(H-5b, H-6 ~ H-12), 0.89(t, *J*=6.8Hz, H-13); δ_C (CD_3OD, 500MHz) 180.8(C-1), 70.4(C-3), 45.4(C-2), 38.0(C-4), 33.1(C-11), 30.8, 30.5 and 26.7(C-5,6,7,8,9,10), 23.7(C-12), 14.4(C-13).
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(*Received in Japan* 17 *July* 1997; *revised* 20 *August* 1997; *accepted* 22 *August* 1997)