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Assignment of the absolute configuration of blasticidin A and revision of that of aflastatin A

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Abstract—The absolute configuration of blasticidin A, a strong inhibitor of aflatoxin production by *Aspergillus parasiticus*, was assigned by adding the data of relative configurations at its diol and pentaol moieties to previously known stereochemistry. Similarity of the NMR data of blasticidin A to those of aflastatin A allowed us to revise the stereochemistry of the diol and pentaol moieties of aflastatin A.

1. Introduction

Aflatoxins, a group of mycotoxins, show quite potent toxicity and carcinogenicity towards mammals. Their contamination in agricultural products is a serious problem from the viewpoint of not only food safety but also economic loss.[1,2] However, it is difficult to resolve the problem due to lack of an effective method to control aflatoxin production. We have been studying specific inhibitors of aflatoxin production by *Aspergillus parasiticus* since they may be useful to prevent aflatoxin contamination of foods and feeds without incurring a rapid spread of resistant strains. We found aflastatins A and B (AsA and AsB) and blasticidin A (BcA) from *Streptomyces* metabolites as inhibitors of aflatoxin production.[3–6] They strongly inhibited aflatoxin production of *A. parasiticus* by disturbing the primary metabolism of the fungus, which may regulate a pathway leading to expression of aflatoxin biosynthetic enzymes.[7,8]

These compounds have similar unique structures, which are tetramic acid derivatives with a highly oxygenated long alkyl chain. With respect to the stereochemistry of AsA, we have the following information up to now. The absolute configurations at C5′, C4, C6, C33 and C39 of AsA were determined by analysis of small fragment molecules obtained by degradation experiments of AsA.[9] The absolute stereochemistry of the tetrahydropyrane ring moiety of AsA was assigned based on the relative stereochemistry around the ring and the absolute configuration at C33. The polyol fragment **1** was prepared from AsA and its absolute configuration was assigned by applying acetonide and MTPA methods.[10] The stereochemistry of **1** was recently confirmed by its chiral synthesis by Evans et al.[11] The long polyol fragment **3** was also prepared from AsA. The relative configurations from C4 to C8 and from C23 to C31 of **3** were assigned by the *J*-based method,[12] which led to assignment of the whole absolute configuration of AsA (formerly proposed structure in Fig. 1).[9] Recently, however, Kishi and co-workers pointed out that *erythro*/*threo*/*threo*/*threo* is the correct relative stereochemistry at the pentaol moiety (C25–C29) of **3** from their NMR database study.[13] On the other hand, with respect to the stereochemistry of BcA, the absolute configurations at C4, C6, C31–C35, C37 and all chiral centers involved in the polyol fragment **2** were assigned by applying similar methods used for the case of AsA.[6,10] Therefore, determination of the configurations at the diol (C8, C9) and pentaol (C25–C29) moieties of BcA is a remaining problem to complete the assignment of the absolute stereochemistry of BcA. In this Letter, we report the complete assignment of the absolute configuration of BcA, and propose the revised stereochemistry of AsA, which was deduced from similarity of the NMR data of AsA with those of BcA.

Keywords: Blasticidin A; Aflastatin A; Aflatoxin; Absolute configuration.
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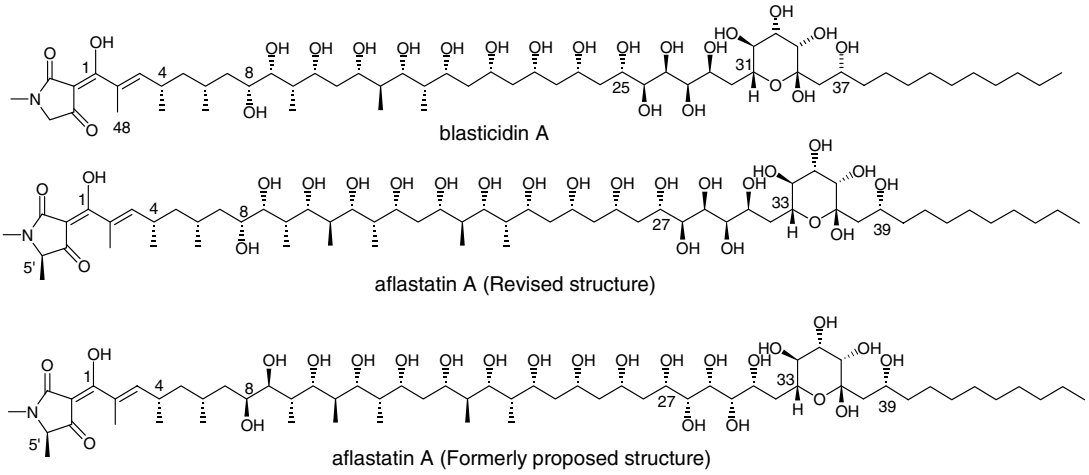
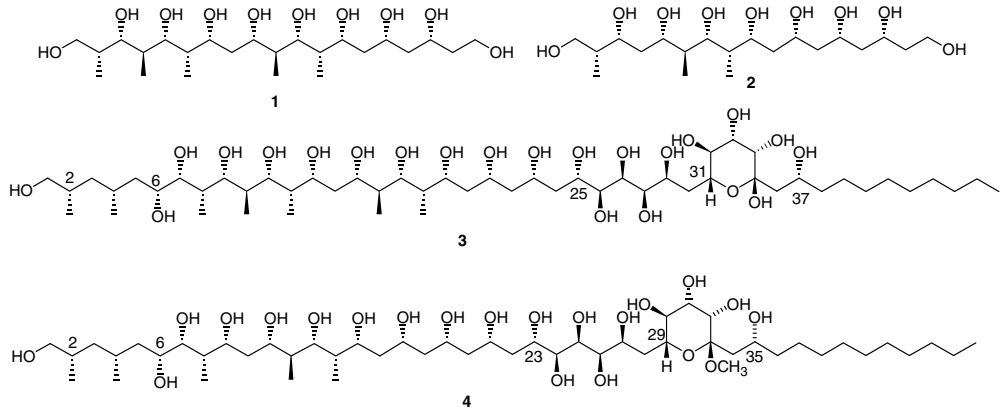


Figure 1. Structures of blasticidin A (BcA) and aflastatin A (AsA).



2. Absolute configuration of blasticidin A

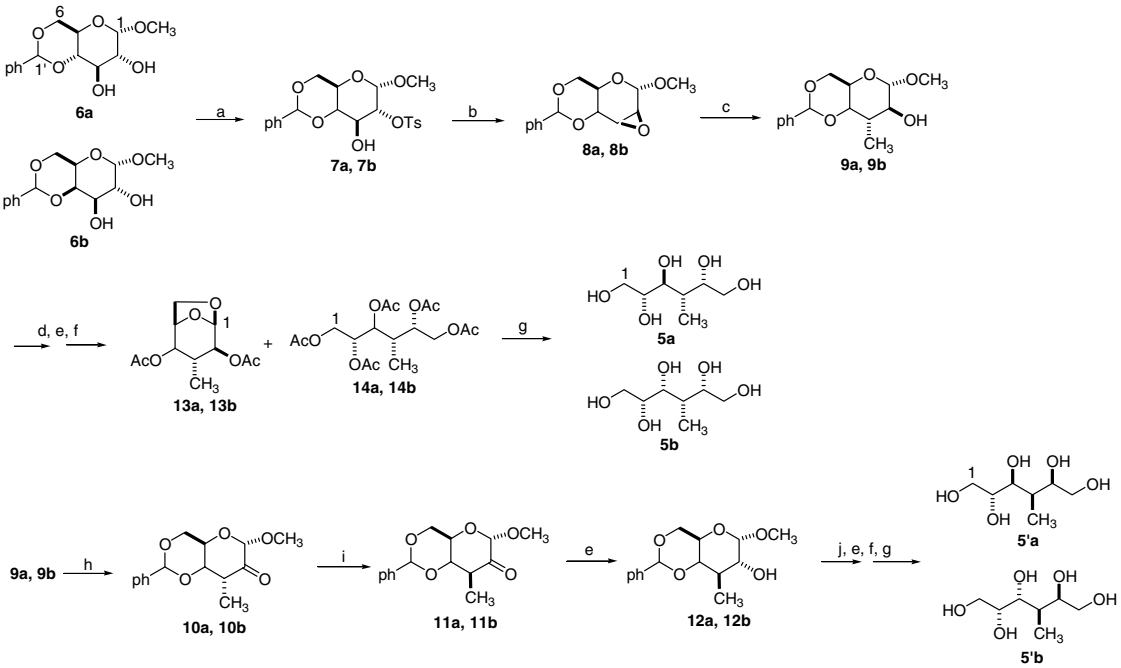
To clarify the configuration at the diol and pentaol moieties of BcA, we analyzed the corresponding parts of its long polyol fragment methyl glycoside **4**, which was prepared from methyl glycoside of BcA as described previously.[14] We assigned the absolute configuration at each moiety as follows.

2.1. Stereochemistry at the diol moiety of 4

Since the absolute configurations at C4 and C8 of **4** were confirmed, determination of the relative stereochemistry either from C4 to C7 or from C6 to C8 was enough for assignment of the absolute configurations at C6 and C7 of **4**. We first tried to elucidate the relative configurations by the *J*-based method.[12] The values of $^{3}J_{H,H}$ and $^{2,3}J_{C,H}$ were obtained from DQFCOSY, HETLOC and PFGHMBC spectra. However, any relative configurations involved in the C4–C8 moiety of **4** could not be assigned due to difficulty encountered in determination of the conformations around the bonds C4–C5, C6–C7 and C7–C8 by the obtained *J* values.

Therefore, we next synthesized four model stereoisomers, **5a**, **5b**, **5′a** and **5′b**, which could cover all possible stereochemistry for C6–C8 of **4**, with expectation that one of them shows similar $^{3}J_{H,H}$ values to those observed in **4**. The synthetic procedure of these model compounds is summarized in Scheme 1. We chose **9a**, **9b**, **12a** and **12b** as precursors of **5a**, **5b**, **5′a** and **5′b**, respectively. Compounds **9a** and **12a** were prepared from methyl 4,6-*O*-benzylidene-α-D-glucopyranoside **6a** according to the known procedure.[15] By using the same procedure as that used in preparation of **9a** and **12a**, **9b** and **12b** could be synthesized from methyl 4,6-*O*-benzylidene-α-D-galactopyranoside **6b** as follows. The reaction of **6b** with 1-tosylimidazole and NaH afforded known monotosylate **7b**,[16,17] which was easily converted to epoxide **8b** by NaH. Compound **9b** was obtained by the reaction of **8b** with methylmagnesium chloride. After conversion of **9b** to **11b**, $NaBH_4$ reduction of **11b** afforded **12b** together with its diastereomer at C2.

Acid hydrolysis of **12a**, followed by $NaBH_4$ reduction, afforded crude **5′a**, which was once acetylated for purification and deacetylation of acetylated **5′a** afforded pure **5′a**. When **9a** was similarly treated with acid and $NaBH_4$, **14a** was obtained as a minor product after acetylation. By the reaction, **13a** was obtained as a major product due to the much higher population of a 1,6-anhydro form than that of a hemiacetal one in the acidic solution during hydrolysis.[18] Removal of the acetyl groups of **14a** afforded **5a**. Similarly to the case of **9a**,



Scheme 1. Reagents and conditions: (a) 1-tosylimidazole, NaH, DMF; (b) NaH; (c) CH_3MgCl; (d) 1 M HCl:1,4-dioxane (1:1), 100 °C, 5 h; (e) $NaBH_4$; (f) $(CH_3CO)_2O$, pyridine; (g) MeONa; (h) $(CF_3CO)_2O$, DMSO; (i) Et_3N; (j) 1 N H_2SO_4, 110 °C, 1.5 h.

Table 1. $^3J_{H,H}$ Values in **5a**, **5′a**, **5b**, **5′b** and **4**[a]

Compound	$^3J_{H\text{-}2,H\text{-}3}$	$^3J_{H\text{-}3,H\text{-}4}$	$^3J_{H\text{-}4,H\text{-}5}$
5a	7.5	3.7	2.3
5′a	8.0	2.4	4.0
5b	5.0	4.3	4.3
5′b	[b]	8.0	1.8
4	4.2[c]	4.2[d]	[e]

[a] Spectra were obtained in pyridine-d_5. *J* Values were confirmed by conventional decoupling experiments.
[b] Cannot be determined due to signal overlappings.
[c] $^3J_{H\text{-}6,H\text{-}7}$.
[d] $^3J_{H\text{-}7,H\text{-}8}$.
[e] Not determined.

acid hydrolysis of **9b** followed by $NaBH_4$ reduction and acetylation afforded **13b** and **14b** as major and minor products, respectively. The stereochemistry of **14b** was unambiguously confirmed by that of **13b**. Compound **5′b** was obtained from **12b** as a major product.

The $^3J_{H\text{-}2,H\text{-}3}$ and $^3J_{H\text{-}3,H\text{-}4}$ values observed in the ^1H NMR spectra of **5a**, **5b**, **5′a** and **5′b** are listed in Table 1. Among them, only the values observed in **5b** ($^3J_{H\text{-}2,H\text{-}3} = 5.0$ Hz, $^3J_{H\text{-}3,H\text{-}4} = 4.3$ Hz) were comparable to those in the C6–C8 moiety of **4** ($^3J_{H\text{-}6,H\text{-}7} = {}^3J_{H\text{-}7,H\text{-}8} = 4.2$ Hz), indicating that the relative configurations for both C6–C7 and C7–C8 were assigned as *threo*. Based on the absolute configuration at C8, the absolute stereochemistry at C6 and C7 of **4** was determined.

2.2. Stereochemistry at the pentaol moiety of 4

To assign the absolute stereochemistry at the pentaol moiety based on the absolute configurations at C21 and C29 of **4**, the relative configurations from C21 to C29 of **4** were elucidated by the *J*-based method. Each configuration for C21–C23, C25–C26 and C26–C27 was assigned as *syn*, *threo* and *threo*, respectively, from each dominant conformation of C21–C22, C22–C23, C25–C26 and C26–C27 deduced from the typical *J* values (Fig. 2), but the configurations for C23–C24, C24–

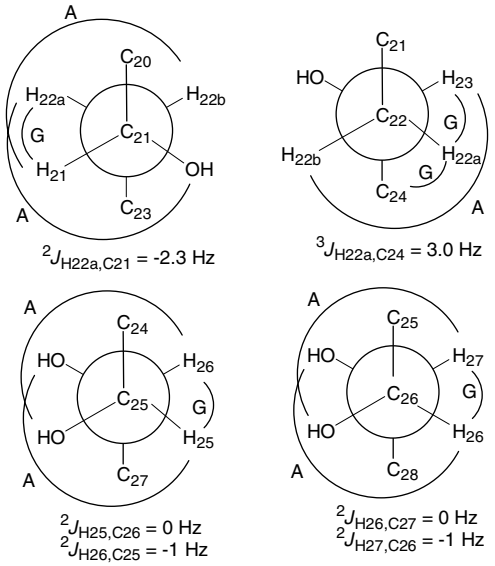


Figure 2. Dominant conformations for C21–C22, C22–C23, C25–C26 and C26–C27 of **4** deduced from the *J*-based method. $^3J_{H,H}$ Values are listed in Table 2. A, *anti*. G, *gauche*. The typical *J* values used for the method in general are 9–12 Hz ($^3J_{H,H}$), 6–8 Hz ($^3J_{C,H}$) and 0 to −2 Hz ($^2J_{C,H}$) for *anti* relationship, 7–10 Hz ($^3J_{H,H}$), 5–7 Hz ($^3J_{C,H}$) and 0–+2 Hz ($^2J_{C,H}$) for *anti* relationship in the case of diol system, 2–4 Hz ($^3J_{H,H}$), 1–3 Hz ($^3J_{C,H}$) and −5 to −7 Hz ($^2J_{C,H}$) for *gauche* relationship, and 0–3 Hz ($^3J_{H,H}$), 1–3 Hz ($^3J_{C,H}$) and −4 to −6 Hz ($^2J_{C,H}$) for *gauche* relationship in the case of diol system.[12]

Table 2. NMR data for the diol and pentaol moieties of **3** and **4**[a]

Carbon	δ_C	δ_H	Coupled protons	$^3J_{H,H}$ (Hz)
4	27.9(27.8)[b]	2.15(2.14)	H-4,H-5a	7.3(7.5)
			H-4,H-5b	6.0(5.0)
5	42.2(42.3)	1.79(1.84)(Ha)	H-5a,H-6	4.4(4.5)
		1.67(1.75)(Hb)	H-5b,H-6	9.2(9.0)
6	69.9(69.5)	4.26(4.29)	H-6,H-7	4.2(4.0)
7	77.4(77.9)	4.02(4.00)	H-7,H-8	4.2(4.0[c])
21(23)	71.2(71.1)	4.63(4.67)	H-21,H-22a(H-23,H-24a)	3.0(3.0)
			H-21,H-22b(H-23,H-24b)	8.8(8.0)
22(24)	42.1(42.1)	2.58(2.59)(Ha)	H-22a,H-23(H-24a,H-25)	3.0(3.0)
		2.16(2.14)(Hb)	H-22b,H-23(H-24b,H-25)	8.8(8.0)
23(25)	72.2(72.2)	4.68(4.67)	H-23,H-24(H-25,H-26)	7.6(7.5)
24(26)	76.2(76.2)	4.33(4.34)	H-24,H-25(H-26,H-27)	<2(3.0)
25(27)	71.5(71.5)	4.97(4.96)	H-25,H-26(H-27,H-28)	4.4(5.0)
26(28)	75.4(75.4)	4.50(4.49)	H-26,H-27(H-28,H-29)	3.4(3.0)
27(29)	70.8(70.9)	4.93(4.92)	H-27,H-28a(H-29,H-30a)	6.5(8.0)
			H-27,H-28b(H-29,H-30b)	6.5(6.0)
28(30)	37.6(37.5)	3.18(3.17)(Ha)	H-28a,H-29(H-30a,H-31)	3.5(3.5)
		2.49(2.47)(Hb)	H-28b,H-29(H-30a,H-31)	9.2(10.0)
29(31)	72.8(72.8)	4.21(4.24)		

[a] Spectra were obtained in pyridine-d_5.
[b] The values in parentheses indicate the chemical shifts in **3** cited from Ref. 9.
[c] This value is corrected from the one (8.0 Hz) reported previously.

C25 and C27–C29 could not be assigned because ambiguous medium J values were obtained around the bonds of C23–C24, C24–C25 and C27–C28. On the other hand, the NMR database method clearly showed that the relative configuration from C23 to C27 was *erythro*/*threo*/*threo*/*threo* from the profile of the observed $^3J_{H,H}$ values ($^3J_{H\text{-}23,H\text{-}24} = 7.6$ Hz, $^3J_{H\text{-}24,H\text{-}25} = <2$ Hz, $^3J_{H\text{-}25,H\text{-}26} = 4.4$ Hz, $^3J_{H\text{-}26,H\text{-}27} = 3.4$ Hz),[13] which confirmed the configurations for C25–C26 and C26–C27 assigned by the J-based method and determined the stereochemistry for C23–C24 and C24–C25. This relative stereochemistry from C21 to C27 afforded the absolute configuration at the pentaol moiety of **4** based on the configuration at C21. From the results obtained, the absolute configuration of BcA was completely assigned as shown in Figure 1.

3. Revision of the absolute configuration at diol and pentaol moieties of aflastatin A

Table 2 summaries δ and $^3J_{H,H}$ values around the diol and pentaol moieties of **3** and **4**. The values observed in the moiety from C23 to C31 of **3** coincided well with those in the corresponding C21–C29 moiety in **4**, indicating that both moieties should have the same relative stereochemistry. The NMR data at C4–C7 of **3** were also very similar to those at C4–C7 of **4**, which showed that **3** and **4** have the same relative configuration from C4 to C7. From these observations, we propose the revised absolute stereochemistry of AsA which is shown in Figure 1.[†]

[†] Revised structure of AsA (Fig. 1) indicates that our previous assignment of the configurations for C4–C5, C7–C8, C25–C26 and C29–C30 of **3** by the J-based method[9] should be reconsidered.

In conclusion, we have assigned the absolute configuration of blasticidin A and revised that of aflastatin A. They have very similar stereochemistry through the overall structures.

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Supplementary data

Experimental section including spectral data of this article. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.02.024.

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