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Blazeispirol D and Z, as the actual intermediates of blazeispirol A biosynthesis from the cultured mycelia of the fungus *Agaricus blazei*

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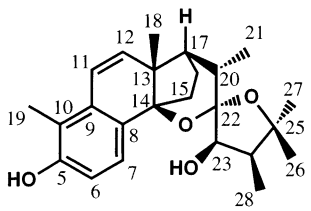
Abstract—Two novel blazeispirol derivatives, blazeispirol D and Z, as the actual intermediates of blazeispirol A biosynthesis have been isolated from the cultured mycelia of *Agaricus blazei* (Agaricaceae). The structures were elucidated to be (20*S*,22*S*,23*R*,24*S*)-14β, 22: 22, 25-diepoxy-des-*A*-ergosta-5,7,9,11-tetraene-5,23-diol and (20*S*,22*S*,23*R*,24*S*)-14β,22:22,25-diepoxy-23-hydroxy-4,5-seco-ergosta-6,8,11-triene-3,5-dione by comparison of extensive 1D and 2D NMR spectral data with that of blazeispirol A. It is discussed that the compounds are the actual intermediates of blazeispirol A biosynthesis.

In a preceding paper, we reported the structure elucidation of blazeispirols A, X and Y, an unprecedented skeleton from the cultured mycelia of the fungus *Agaricus blazei* Murill,[1,2] and the biosynthetic pathway of blazeispirol A.[3] As ring A-lost steroidal compounds such as blazeispirol A have not been reported in living organisms, it seems likely that they are formed by diagenetic degradation of steroid.[4,5] This degradation could proceed only by the pathway proposed for 3-oxygenated triterpenoids such as β-amyrin, lupane, oleanane and ursane.[6–9] To find the actual intermediate of blazeispirol A as the first demonstrated des-*A*-steroid in a living organism, we examined in detail the methanol extract of the *A. blazei* cultured mycelia and isolated two new blazeispirol derivatives, which seemed to be the actual intermediates of blazeispirol A biosynthesis, named blazeispirol D (**1**) and Z (**2**) (Fig. 1).

According to essentially the same procedure as described in the previous paper,[1,2] the methanol extract of the mycelia of *A. blazei* cultured in a medium of 25.8 L was partitioned between chloroform and water. The $CHCl_3$ extract (3.86 g) was chromatographed on silica gel using a gradient solvent of toluene–AcOEt (0–50%). In the previous paper,[2] we reported the isolation of blazeispirol A (620 mg), X (4.1 mg) and Y (2.1 mg). This paper describes the structure elucidation of two additional blazeispirol derivatives, **1** and **2**.

Blazeispirol D (**1**, 9.2 mg: 0.24% yield from the $CHCl_3$ extract)[11] had the molecular formula $C_{24}H_{32}O_4$ as shown by the high-resolution EI mass spectrum, which had a molecular ion at m/z 384.2307 $[M]^+$($C_{24}H_{32}O_4$ requires 384.2300). The presence of hydroxy groups was indicated from the IR absorption at 3430 cm^{-1}. The ^{13}C



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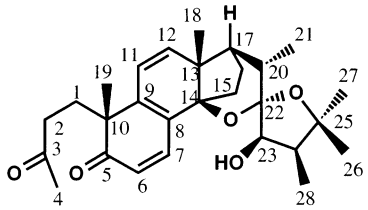

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Figure 1. Structures of blazeispirol D(**1**) and Z(**2**).

Keywords: fungi; natural products; des-*A*-steroid; NMR.

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NMR spectrum showed 24 carbons and the DEPT spectrum suggested the presence of six methyls, two methylenes, eight methines and eight quaternary carbons.[10] The ^{13}C NMR data of **1** was almost identical to that of blazeispirol A, except for the disappearance of the signals of the methoxymethyl carbon (δ 55.6 ppm) and its related carbons. This was supported by the disappearance of the signal at δ 3.80 ppm in the ^{1}H NMR spectrum of **1** relative to that of blazeispirol A. All these data led to the conclusion that the structure of **1** was 5-demethylblazeispirol A, (20*S*,22*S*,23*R*,24*S*)-14β,22:22,25-diepoxy-des-*A*-ergosta-5,7,9,11-tetraene-5,23-diol.

Blazeispirol Z (**2**, 4.2 mg: 0.11% yield) had the molecular formula $C_{28}H_{38}O_5$ as shown by the high-resolution FAB mass spectrum, which had a molecular ion at m/z: 477.2617 $[M+Na]^+$ ($C_{28}H_{38}O_5Na$ requires 477.2592).[11] In the UV spectrum **2** showed an absorption at 382 (log ε 3.77) characteristic of a steroid trienone.[12] The IR spectrum of **2** showed the presence of an acetyl carbonyl group (1715 cm^{-1}) and an α,β-unsaturated carbonyl group (1650 cm^{-1}). The ^{13}C and ^{1}H NMR spectrum of **2** showed 28 carbon atoms and seven methyl signals, which suggested an ergostane skeleton with a spiroacetal structure moiety as a side chain similarly to blazeispirol A. Futhermore, one of the seven methyl signals was observed at δ 2.03 ppm in the ^{1}H NMR spectrum indicating the presence of an acetyl methyl group. The acetyl methyl signal (δ 2.03) showed correlation to the quaternary carbon C-3 (δ 207.7: ^{2}J) and the methylene carbon C-2 (δ 38.9: ^{3}J), both proton signals of which showed correlation to the quaternary carbon C-10 (δ 50.6: ^{3}J) (Table 1). Another methyl group at δ 1.20, also correlated to the carbonyl carbon C-5 (δ 204.9: ^{3}J), methylene carbon C-1 (δ 32.6: ^{3}J) and olefin carbon C-9 (δ 142.7: ^{3}J). Blazeispirol Z (**2**) is, therefore, (20*S*,22*S*,23*R*,24*S*)-14β,22:22,25-diepoxy-23-hydroxy-4,5-seco-ergosta-6,8,11-triene-3,5-dione.

The presence of blazeispirol derivatives (Fig. 2) in a cultured mycelia of *A. blazei* was the first example of the occurrence of natural steroids in which the A-ring had been lost. In a recent paper, we proposed hypothetical intermediates A, B and C including the ring A-lost compound in blazeispirol A biosynthesis (Fig. 2).[3] Until now, des-*A*-steroids and triterpenoids have been found only in the field of geochemistry such as the studies on

Table 1. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) data (δ in ppm, J in Hz) for blazeispirol Z(**2**)

C/H	δ_H[a]	δ_C[a]	^{1}H–^{13}C long range correlations[b]	
			^{2}J	^{3}J
1	2.20 dd (12, 4.5)	32.6		C-9
	1.97 m			C-5
2	1.85 ddd (9.5, 9.5, 4.5)	38.9	C-1	C-4, C-10
	2.16 m		C-1, C-3	C-10
3	–	207.7		
4	2.03 s	29.9	C-3	C-2
5	–	204.9		
6	6.07 d (10)	123.9		C-8, C-10
7	7.41 dd (10, 0.5)	140.9		C-5, C-9, C-14
8	–	130.7		
9	–	142.7		
10	–	50.6		
11	5.96 bd (9.5)	121.4	C-9	C-8, C-10, C-13
12	6.05 d (9.5)	143.7		C-9, C-14, C-17
13	–	48.2		
14	–	82.8		
15	2.59 ddd (13, 9.5, 5.5)	36.8	C-14, C-16	C-13
	1.66 ddd (13, 12.5, 3.5)			
16	2.06 m	25.6		C-13, C-14
	1.39 m		C-17	C-20
17	1.98 dd (6, 3.5)	50.6	C-20	C-12, C-14, C-15, C-22
18	0.93 s	15.3	C-13	C-12, C-14, C-17
19	1.20 s	25.5	C-10	C-1, C-5, C-9
20	2.49 qd (7, 4.5)	33.6	C-17, C-21	C-23
21	1.12 d (7.0)	16.3	C-20	C-17, C-22
22	–	107.7		
23	3.90 d (4.5)	84.7		C-25
24	2.48 qd (7, 4.5)	44.2	C-28	C-22, C-26, C-27
25	–	84.6		
26	1.16 s	25.8	C-25	C-24, C-27
27	1.39 s	30.7	C-25	C-24, C-26
28	1.02 d (7.0)	8.6	C-24	C-23, C-25

[a] Spectra obtained in $CDCl_3$ referenced to $CHCl_3$ at δ 7.26 (^{1}H) and 77.0 (^{13}C).
[b] Direct ^{1}H–^{13}C correlations from HMQC and ^{1}H–^{13}C long range correlations from HMBC.

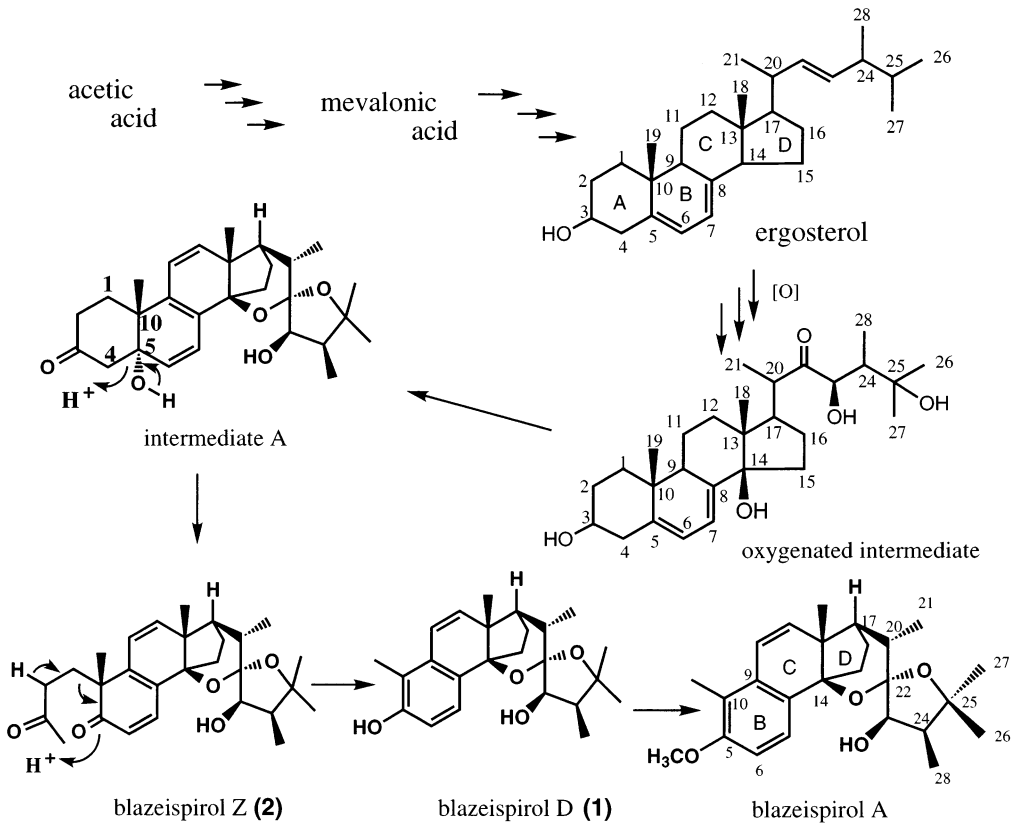

Figure 2. Some actual intermediates of the blazeispirol A biosynthesis.

black shales and sediments.[5,9] Although the loss of ring A may be initiated by a microbially induced oxidative opening of ring A, the mechanism is not yet known.[13] Therefore, the finding of an intermediate such as blazeispirol Z(**2**) is very important to reveal the nature of the loss of ring A. Blazeispirol A seems to be formed by loss of ring A of intermediate A via its corresponding ring A-opened product **2** (Fig. 2). The reaction mechanism of blazeispirol D (**1**) biosynthesis through **2** can be assumed from intermediate A by the cleavage of C-4, C-5 and C-1, C-10 bonds on retro aldol condensation and Michael reaction as shown in Fig. 2.

In this paper, we have described that we have been able to find the intermediates B and C from the MeOH extract of the cultured mycelia of the fungus *A. blazei*. This is the first demonstration of the intermediate of the ring A-lost steroid in a living organism. Investigation of a much more direct intermediate is currently under way.

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10. **1**: colorless solid, $[\alpha]_D^{22}$−33.6 (*c*, 0.22, $CHCl_3$); UV λ_{max} (MeOH)nm (log ε): 230 (4.25), 260 (3.77), 266 (3.88), 276 (3.81), 309 (3.26). IR ν_{max} (KBr) cm^{-1}: 3430, 2970, 2930, 1630, 1580, 1460, 1270, 970. HREIMS *m*/*z*: 384.2307 $[M]^+(C_{24}H_{32}O_4$ requires 384.2300). EI-MS *m*/*z* (ret. int.): 384 $[M]^+$(12), 366 $[M-H_2O]^+$(9), 298(13), 246(17), 202(19), 149(71), 133(19), 119(84), 105(49), 91(56), 83(100). ^1H NMR (400 MHz, $CDCl_3$): δ 0.89 (3H, s, H-18), 1.03 (3H, d, *J*=7, H-28), 1.14 (3H, d, *J*=7, H-21), 1.16 (3H, s, H-26), 1.41 (3H, s, H-27), 1.45 (1H, m, H-16), 1.78 (1H, ddd, *J*=14, 12, 3.5, H-15), 1.94 (1H, dd, *J*=6, 3.5, H-17), 2.04 (1H, ddd, *J*=13, 9.5, 3.5, H-16), 2.21 (3H, s, H-19), 2.44 (1H, ddd, *J*=14.5, 9.5, 5.5, H-15), 2.53 (1H, qdd, *J*=7, 3.5, 1.5, H-20), 2.62 (1H, qd, *J*=7, 4.5, H-24), 3.94 (1H, d, *J*=4.5, H-23), 5.90 (H, d, *J*=10, H-12), 6.51 (1H, dd, *J*= 10, 1, H-11), 6.64 (1H, d,

$J=8$, H-6), 7.14 (1H, d, $J=8$, H-7). ^{13}C NMR ($CDCl_3$): δ 8.7 (C-28), 10.8 (C-19), 15.6 (C-18), 16.4 (C-21), 25.0 (C-16), 25.7 (C-26), 30.7 (C-27), 33.5 (C-20), 37.1 (C-15), 44.1 (C-24), 47.0 (C-13), 50.6 (C-17), 84.0 (C-14), 84.1 (C-25), 85.0 (C-23), 107.4 (C-22), 113.2 (C-6), 120.0 (C-10), 121.7 (C-7), 122.2 (C-11), 130.6 (C-9), 132.3 (C-8), 139.3 (C-12), 152.3 (C-5).

11. **2**: slightly yellow solid, $[\alpha]_D^{22}$ −87.5 (*c*, 0.08, $CHCl_3$); UV λ_{max} (MeOH)nm (log ε): 226 (3.91), 382 (3.77). IR ν_{max} (KBr) cm^{-1}: 3440, 2970, 2930, 1715, 1650, 1460, 1360, 1160, 970. HRFABMS *m/z*: 477.2617 $[M+Na]^+$ ($C_{28}H_{38}O_5Na$ requires 477.2592). EI-MS *m/z* (ret. int.): 454$[M]^+$(19), 436 $[M-H_2O]^+$(41), 384(21), 297(22), 285(22), 255(19), 227(22), 211(78), 202(53), 185(41), 169(33), 141(24), 97(100).
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