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Blazeispirols B, C, E and F, des-*A*-ergostane-type compounds, from the cultured mycelia of the fungus *Agaricus blazei*

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Abstract

Four new des-*A*-ergostane derivatives including blazeispirols B, C, E and F were isolated from the cultured mycelia of fungus *Agaricus blazei* Murill and were established to be (20*S*, 22*R*, 23*R*, 24*S*)-14β,22: 22,25-diepoxy-5-methoxy-des-*A*-ergosta-5,7,9,11-tetraen-23-ol; (20*S*, 22*S*, 23*R*, 24*S*)-14β,22: 22,25-diepoxy-5-methoxy-des-*A*-ergosta-5,7,9-trien-23-ol; (20*S*, 22*S*, 23*R*, 24*S*)-14β, 22: 22, 25-diepoxy-5-methoxy-des-*A*-ergosta-5,7,9,11-tetraene-19,23-diol and (20*S*, 22*S*, 23*R*, 24*S*)-14β,22: 22,25-diepoxy-des-*A*-ergosta-5,7,9-triene-5,23-diol by comparison of extensive 1D and 2D NMR spectral data with that of blazeispirol A.

Keywords: *Agaricus blazei*; Agaricaceae; Basidiomycetes; Higher fungi; des-*A*-Steroid; Blazeispirols A, B, C, D, E and F

1. Introduction

In continuation of our interest in the bioactive secondary metabolites of fungal cultured mycelia, we have examined the cultured mycelia of fungi belonging to Basidiomycetes (Hirotani et al., 1984, 1986, 1987, 1991, 1995; Ino et al., 1984) and Ascomycetes (Furuya et al., 1983). *Agaricus blazei* is an important fungus for producing bioactive compounds. There are many reports of polysaccharides (Kawagishi et al., 1989; Mizuno et al., 1990; Ito et al., 1997; Mizuno et al., 1998), cytotoxic ergosterol derivatives (Kawagishi et al., 1988) and antimutagenic and bactericidal substances (Osaki et al., 1994) from the fruiting bodies of *A. blazei*. However, the chemical examination of the secondary metabolites of the cultured mycelia of this species has not been reported (Ito et al., 1997). In preliminary communications, we have reported the isolation and structural elucidation of unprecedented skeletal compounds, blazeispirols A(**1**), D (**4**), X(**7**), Y(**8**) and Z(**9**) from the cultured mycelia of *A. blazei* (Hirotani et al., 1999, 2000a, 2001) and biosynthesis of blazeispirol A (Hirotani et al., 2000b). Blazeispirols A and D are the first demonstration of the ring A-lost steroid in a living organism. In this paper, we wish to deal with the isolation and structural elucidation of these blazeispirol derivatives and four additional new des-*A*-ergostane-type compounds named blazeispirols B(**2**), C(**3**), E(**5**) and F(**6**) from the cultured mycelia of *A. blazei*.

2. Results and discussion

The cultured mycelia of *A. blazei* Murill were extracted sequentially with methanol at room temperature. The concentrated methanolic extract was diluted with water and partitioned with $CHCl_3$ and EtOAc. Each fraction was dried in vacuo. The chloroform extract (3.86 g) was applied to a silica gel column, and this was separated into fractions A–H. Further purification by reversed phase HPLC afforded compounds **1**–**9** from fractions B, C, D, F and G.

Compound **1**, blazeispirol A, was assigned the molecular formula $C_{25}H_{34}O_4$ (*m*/*z* 398.2484) by HREI–mass spectrometry. The IR spectrum showed the presence of an alcohol absorption (3500 cm^{-1}). Its ^{13}C NMR spectrum showed 25 carbons, and the DEPT spectrum suggested the presence of seven methyls, two methylenes, eight methines, and eight quaternary carbons. Four oxygenated carbon signals were observed at δ 84.0, 84.1,

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85.0 and 107.4, three of which were quaternary carbons. The structure determined from the analyses of 2D NMR spectral data and X-ray crystallography are shown in Fig. 1. Blazeisirol A (**1**) has a des-*A*-ergostane skeleton with an aromatic ring system and with a tetrahydropyran-2-spiro-2′-tetrahydrofuran moiety as a side chain and has been reported to be (20*S*, 22*S*, 23*R*, 24*S*)-14β,22:22,25-diepoxy-5-methoxy-des-*A*-ergosta-5,7,9,11-tetraen-23-ol (Hirotani et al., 1999, 2000a).

The molecular formula of compound **2**, blazeispirol B, was also determined to be $C_{25}H_{34}O_4$ (*m*/*z* 398.2482) by HREI–mass spectrometry. Its ^{13}C NMR spectrum showed 25 carbons, and the DEPT spectrum suggested the presence of seven methyls, two methylenes, eight methines, and eight quaternary carbons like that of blazeispirol A. Four oxygenated carbon signals were seen at δ 75.1, 82.1, 86.4 and 108.9, three of which were quaternary carbons. The fourth was a hydroxyl methine carbon which was supported by the presence of a proton signal at δ 3.98. One of the quaternary carbons appeared very much deshielded at δ 108.9, reminiscent of an acetal system. As shown in Table 1, ^{13}C and ^{1}H NMR spectroscopic data from C-5 to C-17 and C-19 carbons and protons of **2** were almost identical with that of **1** except for the carbon and proton signal data of the spiroacetal moiety. These data suggested that **2** was a stereoisomer of blazeispirol A (**1**) derived from the spiroacetal structural moiety. To clarify the stereochemistry, the NOESY data for compound **2** were compared in detail to that of blazeispirol A (**1**) of which the orientation of C-22 have been determined to be an *S*-configuration by X-ray analysis (Fig. 2). In the NOE experiment, both **1** and **2** were observed on the same NOEs of the protons connected to the rings B, C, D and E as shown in Fig. 2. On the contrary, the NOEs of the protons connected to the ring F were observed to have quite different cross-peaks. These results indicate that the orientation of C-22 of blazeispirol A and B is different. In the ring F of blazeispirol A, cross-peaks were observed for the following signals: H-23/CH_3-18, CH_3-27/CH_3-21, CH_3-27/H-15 β. Furthermore, in blazeispirol B, cross-peaks were observed only for the following signals: H-23/CH_3-21 and HO-23/H-15β instead of the above NOEs in blazeispirol A. From these results, the C-22 orientation of blazeispirol B has been determined to be an *R*-configuration. Thus, the structure of **2** was determined to be (20*S*, 22*R*, 23*R*, 24*S*)-14β,22:22,25-diepoxy-5-methoxy-des-*A*-ergosta-5,7,9,11-tetraen-23-ol.

Compound **3**, blazeispirol C, had a molecular formula of $C_{25}H_{36}O_4$ (M^+ 400, *m*/*z* 400.2631 HRMS), a two mass unit excess than that of **1**. It showed UV absorptions at 264, 278 and 282 nm, and an hydroxyl (3500 cm^{-1}) absorption in the IR spectrum. The ^{1}H NMR spectrum of **3** was very similar to **1** except for the chemical shift and coupling constants observed for the pair of aromatic protons (δ 6.45 and 5.89) to the two methylene protons (δ 1.91 and 1.53, and 2.70 *m*). The ^{13}C NMR spectrum of **3** was also closely similar to that of **1** except for the appearance of two methylene carbon signals (δ 23.8 and 29.4) instead of the olefinic methine carbon signals (δ 122.4 and 139.1) seen in the spectrum

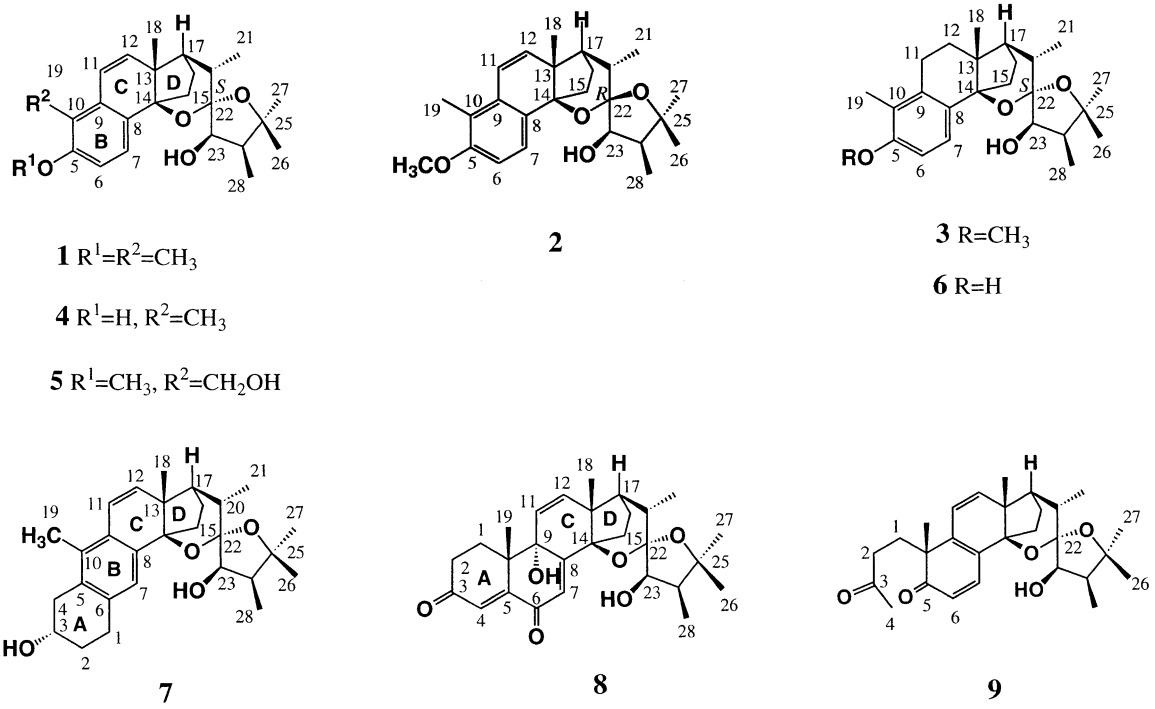

Fig. 1. Structures of blazeispirol A (**1**), B (**2**), C (**3**), D (**4**), E (**5**), F (**6**), X (**7**), Y (**8**) and Z (**9**).

Table 1
^{13}C NMR (100 MHz) and ^{1}H NMR(400 MHz) data (δ in ppm, J in Hz) for blazeispirols A (**1**), B (**2**), C (**3**), D (**4**), E (**5**) and F (**6**)

	1[a]		**2**		**3**		**4**[a]		**5**		**6**	
	$^{\delta}C$	$^{\delta}H$	$^{\delta}C$	$^{\delta}H$	$^{\delta}C$	$^{\delta}H$	$^{\delta}C$	$^{\delta}H$	$^{\delta}C$	$^{\delta}H$	$^{\delta}C$	$^{\delta}H$
5	156.4	–	156.8	–	155.9	–	152.3	–	156.7	–	151.7	–
6	108.6	6.73 *d* (8.5)	108.9	6.57 *d* (8)	108.3	6.76 *d* (7.5)	113.2	6.64 *d* (8)	108.8	6.78 *d* (8.5)	112.7	6.63 *d* (8.5)
7	121.4	7.24 *d* (8.5)	120.9	7.28 *dd* (8, 0.5)	123.5	7.30 *d* (7.5)	121.7	7.14 *d* (8)	124.1	7.36 *d* (8.5)	123.8	7.19 *d* (8.5)
8	132.0	–	130.6	–	132.8	–	132.3	–	132.5	–	133.0	–
9	130.3	–	130.1	–	133.4	–	130.6	–	130.5	–	133.0	–
10	122.5	–	122.8	–	124.2	–	120.0	–	124.5	–	121.5	–
11	122.4	6.54 *d* (10)	122.8	6.57 *d* (10)	23.8	2.70 *m*	122.2	6.51 *dd* (10, 1)	121.5	6.64 *d* (10)	23.8	2.70 *m*
12	139.1	5.89 *d* (10)	138.4	5.88 *d* (10)	29.4	1.91 *ddd* (13.5, 11, 8) 1.53 *ddd* (13.5, 7, 1.5)	139.3	5.90 *d* (10)	140.5	5.96 *d* (10)	29.3	1.91 *ddd* (13.5, 11, 8) 1.53 *ddd* (13.5, 7, 1.5)
13	47.0	–	47.8	–	42.9	–	47.0	–	47.0	–	42.8	–
14	84.0	–	86.4	–	83.5	–	84.0	–	83.8	–	83.4	–
15	37.1	2.44 *ddd* (13.5, 9.5, 5.5) 1.80 *ddd* (13.5, 12.5, 3.5)	36.5	2.25 *ddd* (15, 9, 6) 1.89 *ddd* (15, 4, 1)	38.8	2.52 *dd* (9, 9) 1.74 *m*	37.1	2.44 *ddd* (14, 9.5, 5.5) 1.78 *ddd* (14, 12, 3.5)	37.0	2.44 *ddd* (13.5, 9.5, 5.5) 1.80 *ddd* (13.5, 12.5, 4, 5)	38.8	2.52 *dd* (11, 9) 1.74 *m*
16	25.0	2.05 *ddd* (13, 9.5, 3.5) 1.46 *m*	24.2	1.83 *ddd* (13, 9, 4) 1.52 *ddd* (13, 6, 1)	21.0	2.03 *dd* (9, 9) 1.74 *m*	25.0	2.04 *ddd* (13, 9.5, 3.5) 1.45 *m*	24.9	2.05 *ddd* (13, 9.5, 5.5) 1.45 *m*	20.9	2.03 *dd* (9.5, 8) 1.74 *m*
17	50.7	1.94 *dd* (6, 3.5)	50.1	1.96 *dd* (6, 3)	50.5	1.72 *dd* (6, 3)	50.7	1.94 *dd* (6, 3.5)	50.6	1.95 *dd* (6, 3.5)	50.4	1.70 *dd* (6, 3)
18	15.7	0.90 *s*	15.7	1.12 *s*	14.7	0.92 *s*	15.6	0.89 *s*	15.6	0.89 *s*	14.6	0.92 *s*
19	10.8	2.20	10.9	2.21 *s*	11.1	2.20 *s*	10.8	2.21 *s*	56.3	4.76 *d* (2) 4.81 *d* (12)	11.0	2.10 *s*
20	33.5	2.54 *qdd* (7, 3.5, 1)	39.8	2.56 *qdd* (7, 3, 1)	34.0	2.63 *qd* (7, 3)	33.5	2.53 *qdd* (7, 3.5, 1.5)	33.5	2.57 *qdd* (7, 3, 1.5)	34.0	2.63 *qd* (7, 3)
21	16.4	1.14 *d* (7)	14.1	1.01 *d* (7)	16.7	1.14 *d* (7)	16.4	1.14 *d* (7)	16.4	1.14 *d* (7)	16.7	1.13 *d* (7)
22	107.4	–	108.9	–	107.8	–	107.4	–	107.4	–	107.8	–
23	85.0	3.95 *dd* (4.5, 4.5)	75.1	3.98 *dd* (5, 4)	85.1	3.90 *dd* (5.5, 4)	85.0	3.94 *d* (4.5)	84.9	3.94 *d* (4.5)	85.0	3.90 *dd* (4.5, 4.5)
24	44.1	2.64 *qd* (7, 4.5)	48.6	1.94 *qd* (7, 5)	44.1	2.59 *qd* (7, 4)	44.1	2.62 *qd* (7, 4.5)	44.1	2.62 *qd* (7.5, 4.5)	43.9	2.59 *qd* (7, 4.5)
25	84.1	–	82.1	–	84.0	–	84.1	–	84.2	–	84.0	–
26	25.7	1.16 *s*	24.9	1.32 *s*	25.7	1.18 *s*	25.7	1.16 *s*	25.7	1.16 *s*	25.7	1.17 *s*
27	30.7	1.42 *s*	28.9	1.23 *s*	30.8	1.48 *s*	30.7	1.41 *s*	30.7	1.41 *s*	30.7	1.46 *s*
28	8.7	1.04 *d* (7)	9.6	1.09 *d* (7)	8.6	1.01 *d* (7)	8.7	1.03 *d* (7)	8.7	1.04 *s*	8.6	1.01 *d* (7)
OCH_3	55.6	3.80 *s*	55.6	3.81 *s*	55.6	3.80 *s*	–	–	55.5	3.84 *s*	–	–
OH	–	1.41 *d* (4.5)	–	3.43 *d* (4)	–	1.44 *d* (5.5)	–	1.41 *d* (4.5)	–	1.44 (overlap)	–	–[b]
PhOH	–	–	–	–	–	–	–	4.70 bs	–	–	–	4.52 *s*

[a] The data for compounds **1** and **4** are cited from Hirotani et al., 2000a, 2001.
[b] –No observed OH signal.

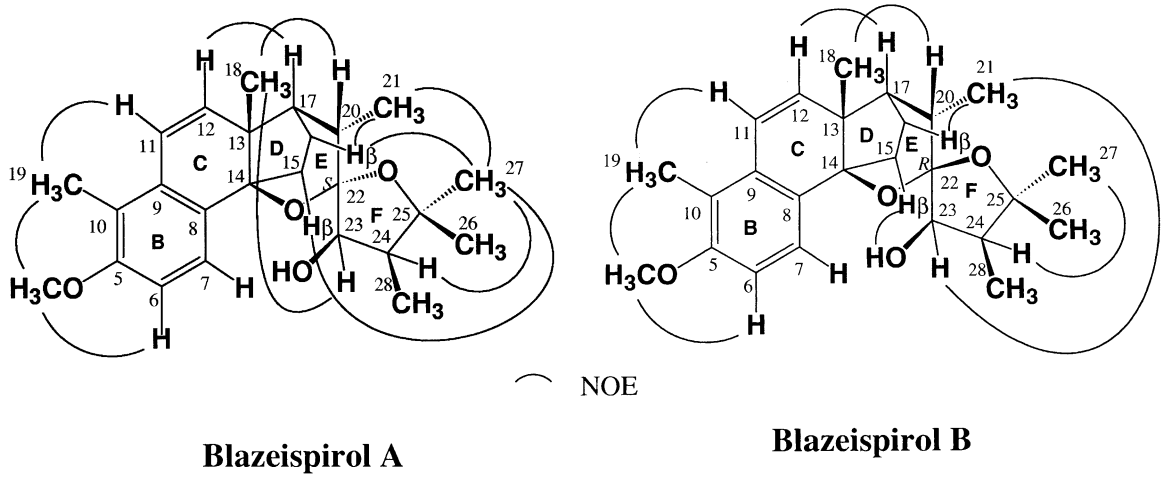

Fig. 2. NOESY interaction in blazeispirols A and B.

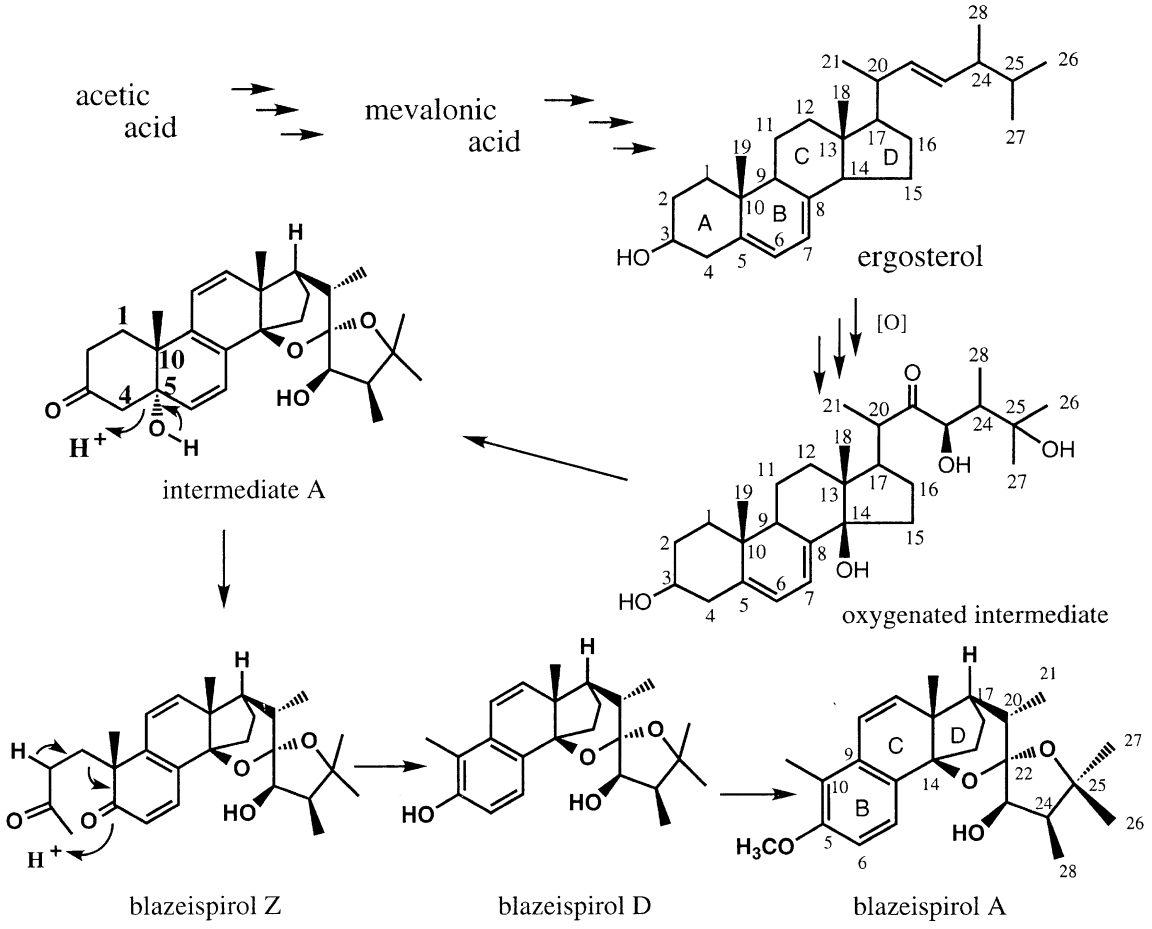

Fig. 3. Some actual intermediates of the blazeispirol A biosynthesis.

of **1**. On the basis of these observations, compound **3** was identified to be (20*S*, 22*S*, 23*R*, 24*S*)-14β, 22:22,25-diepoxy-5-methoxy-des-*A*-ergosta-5,7,9-trien-23-ol.

Compound**4**, blazeispirol D had a molecular formula of $C_{24}H_{32}O_4$ by HREI mass spectrum, with a molecular ion at *m*/*z* 384.2307 $[M]^+$ ($C_{24}H_{32}O_4$ requires 384.2300). The presence of hydroxyl groups was indicated from the IR absorption at 3430 cm^{-1}. The ^{13}C NMR spectrum showed 24 carbons, and the DEPT spectrum suggested the presence of six methyls, two methylenes, eight methines, and eight quaternary carbons. The ^{13}C NMR spectroscopic data for **4** was almost identical to that of **1**

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 **4** relative to that of **1**. All these data led to the conclusion that the structure of **4** 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.

Compound **5**, blazeispirol E, $C_{25}H_{34}O_5$ showed UV absorptions at 230, 255, 263, 272, 308 nm and a hydroxyl (3440 cm^{-1}) absorption in the IR spectrum. The ^{13}C NMR spectrum of **5** was very similar to that of **1**, except for the downfield shift of the C-7, C-10 and C-19 carbon signals (δ 124.1, 124.5 and 56.3) relative to that of **1** (δ 121.4, 122.5 and 10.8), respectively (Table 1). The downfield shift (from δ 10.8 to 56.3) indicated that the C-19 methyl carbon has been changed into hydroxymethyl carbon. This was supported by the fact that the downfield shifts seen in C-7 and C-10 carbons are due to substitution effects. These data led us to the conclusion that the structure of **5** was 19-hydroxymethylblazeispirol A, (20*S*, 22*S*, 23*R*, 24*S*)-14β,22:22, 25-diepoxy-des-*A*-ergosta-5,7,9,11-tetraene-19,23-diol.

Compound **6**, blazeispirol F, where analyzed for $C_{24}H_{34}O_4$ showed absorptions in the UV spectrum at 225, 278 nm and a hydroxyl (3420 cm^{-1}) absorption in the IR spectrum. In the mass spectrum the molecular ion peak was observed at *m*/*z* 387 $[M+H]^+$ suggesting that **6** was demethylblzeispirol C. The ^{13}C spectral data of **6** were very similar to those of **3** except for the carbon signals of C-5, C-6 and the disappearance of a carbon signal at δ 55.6 (as seen in **3**). On the basis of this spectroscopic evidence, compound **6** was identified as (20*S*, 22*S*, 23*R*, 24*S*)-14β, 22:22, 25-diepoxy-des-*A*-ergosta-5,7,9-triene-5,23-diol.

The presence of blazeispirols A–F (Fig. 1) 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 (Hirotani et al., 2000b). Until now, des-*A*-steroids and triterpenoids have been found only in the field of geochemistry such as in the studies on black shales and sediments (Peakman et al., 1986; Trendel et al., 1989). Although the loss of ring A may be initiated by a microbially induced oxidative opening of ring A, the mechanism is not yet known (Peakman and Maxwell, 1988). Therefore, the finding of intermediates proposed previously is very important to reveal the nature of the loss of ring A. In a quite recent paper, we reported the isolation of two blazeispirol derivatives D (**4**) and Z (**9**) as the actual intermediates of Blazeispirol A (Hirotani et al., 2001). The mechanism of the loss of ring A has been proposed to be the loss of ring A of intermediate A via its corresponding ring A-opened product **9** (Fig. 3). The reaction

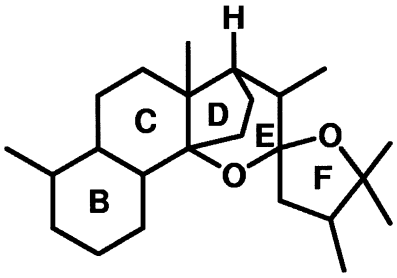

Fig. 4. Blaziespirane skeleton.

mechanism of blazeispirol D (**4**) biosynthesis through **9** can be assumed to occur from intermediate A by the cleavage of C-4, C-5 and C-1, C-10 bonds on a retro-aldol condensation and a Michael reaction as shown in Fig. 3 (Hirotani et al., 2001).

In this paper, we have described six blazeispirol derivatives, in which the A-ring had been lost, including four new blazeispirol derivatives. These compounds are a group of naturally occurring steroids built on a des-*A*-ergostane-type skeleton in which C-14, C-22 and C-25 are appropriately oxidized to form a 14, 22:22, 25-diepoxy structure. For convenience, this basic structure was designated the "blazeispirane" skeleton as shown in Fig. 4. Surprisingly, these blazeispirol derivatives have not been detected in the fruiting body of *A. blazei*. We recently examined the distribution of the blazeispirol derivatives in the cultured mycelia of eight other *Agaricus* species. Blazeispirol A (**1**) was detected in the extract of the cultured mycelia of four of the eight species.

3. Experimental

3.1. General

Optical rotation: Jasco DIP-370 ($CHCl_3$), UV: Hitachi 340 (alcohol), IR: Jasco FT/IR-200 (KBr discs and NaCl film), EIMS: Jeol JMS-AX505 H, FABMS: Jeol JMS-AX505 HA, ^{1}H and ^{13}C NMR: Varian UNITY 400 (400 and 100 MHz respectively in $CDCl_3$). HPLC was performed by Senshu Pak ODS (10×300 mm and 20×300 mm) coupled with a UV detector and a differential refractometer.

3.2. Isolation and culture of Agaricus blazei mycelium

After sterilizing the cultivated fruiting body of *A. blazei,* a small piece of the stipe was inoculated onto a malt agar medium. Developing colonies of the new hyphae from the stipe were transferred to malt agar in Petri dishes. The pure mycelia were subcultured for 2 weeks and grown in a 500-ml Erlenmeyer flask in 125 ml of medium containing 10 g sucrose, 30 g malt extract, 5

g yeast extract in 1l of dist. H_2O. Usually, each flask was seeded with 5 of the 10 mm plugs cut from the potato dextrose agar culture.

3.2.1. Extraction procedure and separation of MeOH extract of the mycelia

After 5 weeks culture (206 flasks), the mycelia (2.25 kg, fr. wt) were harvested with nylon cloth, homogenized with MeOH (8 l) in a Waring blender and allowed to stand for 1 week at room temp. The homogenate was filtered and the residue was re-extracted with the same solvent (5.7 l). The filtrates were combined and the organic solvent was removed under red. pres. The residue was extracted with $CHCl_3$ (6 times in a total of 4.6 l), dried and evapd. to dryness. The $CHCl_3$ extract (3.86 g) was subjected to chromatography over silica gel (Wakogel C-200, 220 g). Elution with 800 ml toluene (fraction A), 650 ml (fraction B), 2750 ml (fraction C), 1000 ml toluene–EtOAc (19:1) (fraction D); 800 ml toluene–EtOAc (9:1) (fraction E); 700 ml toluene–EtOAc (8:2) (fraction F); 480 ml toluene–EtOAc (8:2) and 300 ml toluene–EtOAc (1:1; 300 ml) (fraction G); 650 ml and EtOAc (500 ml) (fraction H) yielded the crude mixture of compound **1** (fraction B 1.1 g), compounds **2**, **3** (fraction C 0.19 g), compounds **4**,**6** (fraction D 0.23 g), compounds **5**, **7**, **9** (fraction F 0.23 g) and compound **8** (fraction G 0.19 g). Further purification was achieved by HPLC. Compound **1** (620 mg) was isolated from fraction B by HPLC (Senshu Pak ODS, ϕ 20×300 mm, MeOH, 6 ml min^{-1} flow rate, Rt, 18.6 min). Compounds **2**, **3** (2.9 and 53.2 mg) were isolated from fraction C by HPLC (Senshu Pak ODS, ϕ 10×300 mm, 92% MeOH, 3 ml min^{-1} flow rate, Rt, 12.2 min and 13.9 min, respectively). Compounds **4**, **6** (9.2 and 2.4 mg) were isolated from fraction D by HPLC (Senshu Pak ODS, ϕ 10×300 mm, 85% MeOH, 3 ml min^{-1} flow rate, Rt, 12.0 min and 15.8 min, respectively). Fraction F was further separated into four subfractions (F-1, F-2, F-3 and F-4) by HPLC (Senshu Pak ODS, ϕ 10×300 mm, 90% MeOH, 2.5 ml min^{-1} flow rate). Compounds **5**, **9** (22.0 and 4.2 mg) were isolated from fraction F-2 by HPLC (Senshu Pak ODS, ϕ 20×300 mm, 90% MeOH, 3 ml min^{-1} flow rate, Rt, 38.6 and 37.4 min, respectively). Compound **7** (4.0 mg) was also isolated from fraction F-3 by HPLC (Senshu Pak ODS, ϕ 10×300 mm, 90% MeOH, 3 ml min^{-1} flow rate, Rt, 56.2 min). After repeated HPLC under different conditions (Senshu Pak ODS, ϕ 10×300 mm, 90% MeOH, 3 ml min^{-1} flow rate, 6.9 min and 75% MeOH, 3 ml min^{-1} flow rate, 12.0 min), compound **8** (2.4 mg) was isolated from fraction G.

3.2.2. Blazeispirol A (*1*)

Colorless powder, $[\alpha]_D^{29}$ −28.6 (*c*, 0.21, $CHCl_3$); UV λ_{max} (EtOH) nm (log ϵ): 226 (4.45), 258 (3.76), 266 (3.86), 274 (3.78), 305 (3.18). IR ν_{max} (KBr) cm^{-1}: 3500, 2960, 2930, 1580, 1475, 1260, 1100, 970. HREIMS *m*/*z*: 398.2482 $[M]^+$ ($C_{25}H_{34}O_4$ requires 398.2457). EI–MS *m*/*z* (ret. int.): 398 $[M]^+$ (55), 380 $[M–H_2O]^+$ (33), 299 (33), 225 (38), 216 (86), 208 (34), 199 (22), 97 (33), 28 (100). CD λ_{max} (MeOH) nm 308.7 (−3.22), 277.6 (+2.80), 250.8 (−1.60). For ^{13}C and ^1H NMR spectral analyses see Table 1.

3.2.3. Blazeispirol B (*2*)

Colorless powder, $[\alpha]_D^{22}$ −17.0 (*c*, 0.33, $CHCl_3$); IR ν_{max} (NaCl) cm^{-1}: 3480, 2965, 2930, 1580, 1470, 1260, 1100, 960. HREIMS *m*/*z*: 398.2484 $[M]^+$ ($C_{25}H_{34}O_4$ requires 398.2457). EI–MS *m*/*z* (ret. int.): 398 $[M]^+$(39), 380 $[M–H_2O]^+$ (35), 299 (19), 225 (43), 216 (95), 204 (53), 199 (29), 97 (40), 32 (100). For ^{13}C and ^1H NMR spectral analyses see Table 1.

3.2.4. Blazeispirol C (*3*)

Colorless powder, $[\alpha]_D^{22}$ −19.0 (*c*, 0.21, $CHCl_3$); λ_{max} (MeOH) nm (log ϵ): 220 (4.11), 264(3.31), 278 (3.31), 282 (3.30). IR ν_{max} (KBr) cm^{-1}: 3500, 2970, 2930, 1600, 1480, 1260, 1100, 970. HREIMS *m*/*z*: 400.2631 $[M]^+$ ($C_{25}H_{36}O_4$ requires 400.2614). EI–MS *m*/*z* (ret. int.): 400 $[M]^+$ (4), 382 $[M–H_2O]^+$ (1), 301 (11), 227 (5), 204 (49), 149 (13), 118 (36), 87 (100). For ^{13}C and ^1H NMR spectral analyses see Table 1.

3.2.5. Blazeispirol D (*4*)

Colorless solid, $[\alpha]_D^{22}$ −33.6 (*c*, 0.22, $CHCl_3$); 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 $[M\text{-}H_2O]^+$ (9), 298 (13), 246 (17), 202 (19), 149 (71), 133 (19), 119 (84), 105 (49), 91 (56), 83 (100). For ^{13}C and ^1H NMR spectral analyses see Table 1.

3.2.6. Blazeispirol E (*5*)

Slight yellow solid, $[\alpha]_D^{22}$ −14.3 (*c*, 0.28, $CHCl_3$); UV λ_{max} (MeOH) nm (log ϵ): 230 (4.25), 255 (3.69), 263 (3.75), 272 (3.67), 308 (3.25). IR ν_{max} (KBr) cm^{-1}: 3440, 2970, 2930, 1630, 1580, 1470, 1260, 1090, 970. HRFABMS *m*/*z*: 414.2405 $[M]^+$ ($C_{25}H_{34}O_5$ requires 414.2406). EI–MS *m*/*z* (ret. int.): 414 $[M]^+$ (1), 380 (5), 299 (33), 270 (46), 212 (21), 199 (51), 197 (31), 153 (10), 141 (15), 97 (21), 83 (100). For ^{13}C and ^1H NMR spectral analyses see Table 1.

3.2.7. Blazeispirol F (*6*)

Colorless powder, $[\alpha]_D^{30}$ −51.2 (*c*, 0.1, $CHCl_3$); UV λ_{max} (MeOH)nm (log ε): 225 (3.16), 278 (2.71). IR ν_{max} (NaCl) cm^{-1}: 3420, 2970, 2930, 1470, 1280, 1160, 970. HRFABMS *m*/*z*: 387.2530 $[M+H]^+$ ($C_{24}H_{35}O_4$ requires 387.2535). For ^{13}C and ^1H NMR spectral analyses see Table 1.

3.2.8. Blazeispirol X (7)

Colorless solid, $[\alpha]_D^{22}$ +85.2 (*c*, 0.27, $CHCl_3$); UV λ_{max} (MeOH)nm (log ε): 222 (4.32), 228 (4.36), 234 (4.23), 272 (3.90). IR ν_{max} (KBr) cm^{-1}: 3440, 2960, 2920, 1715, 1455, 1380, 970. HREIMS *m/z*: 438.2753 $[M]^+$ ($C_{28}H_{38}O_4$ requires 438.2770). EI–MS *m/z* (ret. int.): 438 $[M]^+$ (43), 420 $[M–H_2O]^+$ (34), 339 (24), 321 (16), 265 (25), 256 (100), 242 (29), 221 (23), 209 (22), 165 (28), 97 (53).

*3.2.9. Blazeispirol Y (**8**)*

Colorless solid, $[\alpha]_D^{22}$ −68.5 (*c*, 0.09, $CHCl_3$); UV λ_{max} (MeOH) nm (log ϵ): 279 (4.23). IR ν_{max} (KBr) cm^{-1}: 3440, 2970, 2930, 1690, 1650, 1630, 1600, 1270, 970. HRFABMS *m/z*: 491.2435 $[M+Na]^+$ ($C_{28}H_{36}O_6Na$ requires 491.2410). EI–MS*m/z* (ret. int.): 468 $[M]^+$ (32), 450 $[M\text{-}H_2O]+$ (23), 433 (24), 370 (69), 351 (25), 295 (32), 256 (32), 197 (100), 179 (51), 137 (72), 109 (53), 97 (99), 85 (78).

*3.2.10. Blazeispirol Z (**9**)*

Slight 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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