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Blazeispirol A, an Unprecedented Skeleton from the Cultured Mycelia of the Fungus *Agaricus blazei*

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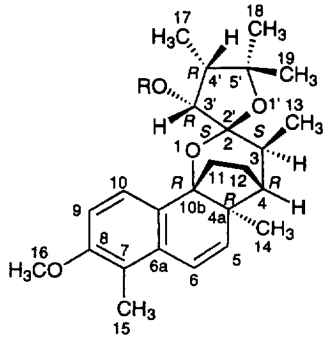
Abstract

Blazeispirol A, an unprecedented skeleton has been isolated from the cultured mycelia of *Agaricus blazei*(Agaricaceae). Its structure was elucidated to be the previously unknown spiro [4,10b-ethanonaphtho[1,2-b]pyran-2,2'-furan] skeleton which was established by extensive 1D and 2D NMR spectral data and X-ray analysis.

Keywords: fungi; natural products; NMR; X-ray crystal structure

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[1-6] and Ascomycetes[7]. *Agaricus blazei* is an important fungus for producing bioactive compounds. There are some reports of polysaccharides and steroid derivatives from the fruiting bodies of *A.blazei*.[8-10]. However, the chemical examination of the secondary metabolites of the cultured mycelia of this species has not been reported [11]. We now report the isolation of an unprecedented skeleton compound, named blazeispirol A(**1a**), whose structure was elucidated by spectral data including X-ray analysis.



1a : R = H
1b : R = *p*-Br-Bz

The chloroform solubles(1.475 g) of the methanol extract of the mycelia of *A. blazei* cultured in a medium of 18.8 l were subjected to column chromatography over silica gel followed by HPLC to furnish blazeispirol A (**1a**)(304.2 mg)(20.6% yield from the $CHCl_3$ solubles)[12]. $C_{25}H_{34}O_4$, colorless powders, $[\alpha]_D^{29}$ -28.6°(c, 0.21, $CHCl_3$).

The presence of a hydroxyl group was indicated from its IR absorption at 3510 cm^{-1}. Its ^{13}C NMR spectrum showed 25 carbons and the DEPT spectrum suggested the presence of seven methyls, two methylenes, eight methines, eight quaternary carbons. Four oxygenated carbon signals were seen at δ 84.0, 84.1, 85.0 and 107.4, three of which were quaternary

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carbons. The fourth was a hydroxyl methine carbon which was supported by the presence of a proton signal at δ 3.95. One of the quaternary carbons appeared very much deshielded at δ 107.4, reminiscent of an acetal system.

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

C/H	δ_H*	δ_C*	^{1}H-^{13}C long-range correlations **	
			^{2}J	^{3}J
2	-	107.4		
3	2.54 qdd(7, 3, 1)	33.5	C-2, C-4, C-13	C-12
4	1.94 dd(6, 3.5)	50.7	C-3, C-4a	C-2, C-5, C-10b, C-11, C-13
4a	-	47.0		
5	5.89 d (10)	139.1	C-4a	C-4, C-10b
6	6.54 d(10)	122.4		C-4a, C-7
6a	-	130.3		
7	-	122.5		
8	-	156.4		
9	6.73 d(8.5)	108.6	C-8	C-7, C-10a
10	7.24 d(8.5)	121.4		C-6a, C-8, C-10b
10a	-	132.0		
10b	-	84.0		
11	1.80 ddd(13.5, 12.5, 3.5)	37.1	C-12	C-4, C-10a
	2.44 ddd(13.5, 9.5, 5.5)		C-12	C-4a
12	1.46 m	25.0	C-4, C-11	C-3
	2.05 ddd(13, 9.5, 3.5)			C-4a
13	1.14 d(7)	16.4	C-3	C-2, C-4
14	0.90 s	15.7	C-4a	C-4, C-5, C-10b
15	2.20 s	10.8	C-7	C-6a, C-8
16	3.80 s	55.6		C-8
17	1.04 d(7)	8.7	C-4'	C-3', C-5'
18	1.16 s	25.7	C-5'	C-19, C-4'
19	1.42 s	30.7	C-5'	C-18, C-4'
3'	3.95 dd(4.5, 4.5)	85.0		C-5'
4'	2.64 qd(7, 4.5)	44.1	C-17, C-5'	C-18, C-19
5'	-	84.1		
OH	1.41 d(4.5)	-		C-2, C-4'

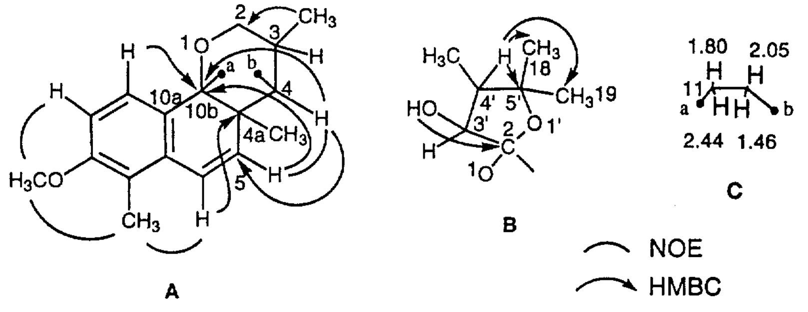
* Spectra obtained in $CDCl_3$ referenced to $CHCl_3$ at δ 7.26(^{1}H) and 77.0(^{13}C). ** Direct ^{1}H-^{13}C correlations from HMQC and ^{1}H-^{13}C long-range correlations from HMBC.

The ^{1}H and ^{13}C NMR spectra suggested the presence of two -CH=CH- groups and four aromatic quaternary carbons. Furthermore, H-6 (δ 6.54) showed correlation to the quaternary carbon C-4a (δ 47.0) and H-10 (δ 7.24) to C-10b (δ 84.0: ^3J) which showed a correlation from H-5 (δ 5.89), thus confirming the presence of 1,2-dihydronaphthalene(Table 1). Additionally, the methine proton at δ 1.94 showed HMBC correlation with C-4a (δ 47.0), C-3 (δ 33.5), C-2 (δ 107.4), and C-10b (δ 84.0), thus establishing the presence of a tetrahydropyran ring. Next, in the NOE experiment this molecule showed the connectivity H-5(δ 5.89) with H-4(δ 1.94) which was correlated to C-5 (δ 139.1: ^3J) and C-10b (δ 84.0: ^3J) in the HMBC spectrum. This confirmed that the tetrahydropyran ring and the 1, 2-dihydronaphthalene ring, sharing the same carbons(C-10b and C-4a), fused each other to form a naphtho[1,2-b]pyran structure **A**.

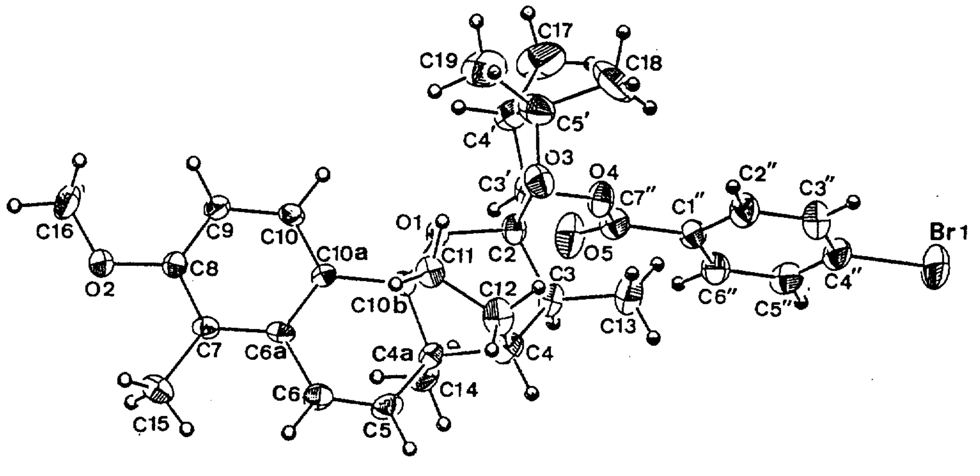
A carbinol proton at δ 3.95 showed COSY correlation with protons at δ 1.41(3'-OH) and δ 2.64 (H-4').

In addition, H-4' (δ 2.64) showed HMBC correlation with C-5' (δ 84.1), C-18 (δ 25.7) and C-19 (δ 30.7), while 3'-OH (δ1.41) showed cross peak with C-2 (δ107.4), thus leading to a tetrahydrofuran structure **B**.

One of the methylene protons at δ 1.80 (H-11) showed COSY cross peaks with three protons at δ 2.44, δ 2.05 and δ 1.46 which were only mutually coupled giving rise to partial structure **C**. Furthermore, H-11 (δ 1.80) showed HMBC correlation with C-10a(δ 132.0) and C-4 (δ 50.7), suggesting that partial structure **C** is connected with partial structure **A**. We were able to piece together the partial structures to give the total structure (**1a**). The absolute configuration of blazeispirol A was finally established by X-ray analysis[13] of its *p* -bromobenzoate as depicted in the structure (**1b**)[14]. Thus, the structure of **1a** was determined as (2*S*, 3*S*, 3'*R*, 4*R*, 4a*R*, 4'*R*, 10b*R*)(3, 4, 4', 5')-tetrahydro-3'-hydroxy-8-methoxy-(3,4',4a,5',5',7)-hexamethyl-spiro[2*H*-4,10b-ethanonaphtho[1,2-b]pyran-2,2'(3'*H*)-furan].



A **B** **C**



1b

Spiroketals occur widely as substructures of naturally occurring substances from many sources, including microbes, plants, fungi and marine organisms, and have many kinds of biological activities[15]. To our knowledge, no other spiroketal comprising a dihydronaphthalene unit has been reported from nature. The investigation of the biosynthesis as well as the biological activity of **1a** is currently under way.

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[12] **1a**: 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, 2915, 1580, 1475, 1260, 1100. 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-H2O]$^+$(33), 299(33), 225(38), 216(86), 208(34),199(22), 97(33), 28(100).

[13] Single crystal X-ray analysis of (**1b**). Data were collected on an AFC5R Rigaku automated four-circle diffractometer, Cu Ka radiation (l = 1.54178 Å), graphite monochromator. $C_{32}H_{37}O_5Br$ (581.55), crystal dimensions 0.20 x 0.40 x 0.20 mm, orthorhombic, space group $p2_12_12_1$, 296 K, a = 13.321(3), b = 31.360(6), c = 7.073(3),Å, V = 2955.(1) Å^3, Dc = 1.307g/cm^3, Z = 4, F(000) = 1216, μ = 21.88 cm^{-1}. A total of 5618 reflections were collected in the 6°< 2θ <126.4° range using ω–2θ scan. 2809 reflections were unique, and from these, 2065 were assumed as observed (Fo > 3σ (Fo). Lorentz, polarization effects and absorption correlations were applied. Three standard reflections monitored every 97 reflections indicated no significant intensity variation. The structure was solved by direct methods (SHELX-86[1]). Hydrogen atoms were set in calculated positions and fixed at their positions. The structure was refined by full-matrix least-squares using anisotropic thermal parameters for all non-hydrogen atoms. The refinement converged to R = 0.037, Rw = 0.033, GOF = 2.37 and a final difference map revealed no peaks greater than 0.16 e$^-$ /Å^3. The absolute configuration was determined through refinement of the inverted configuration (R=0.054, Rw=0.050). Complete details of the structure investigation are available at request from the Cambridge crystal Data Centre, 12 Union Road, Cambridge CB2 1EZ, England.

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[14] Synthesis of blazeispirol A *p*-bromobenzoate(**1b**). A mixture of blazeispirol A(**1a** 58.2mg), *p*-bromobenzoylchloride (250.4 mg) and pyridine(5.0 ml) was stirred at 90°C for 24 hr. After being worked up, **1b** was recrystallized from MeOH to afford 17.4 mg as colorless plates. **1b**: m.p. 176-177°C (found; C, 66.17; H, 6.33. $C_{32}H_{37}O_5Br$ requires C, 66.09; H, 6.41). $[\alpha]_D^{29}$ - 68.0 (*c*, 0.10, $CHCl_3$).

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