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First β-1,6-Glucan Biosynthesis Inhibitor, Bisvertinolone Isolated from Fungus, *Acremonium strictum* and Its Absolute Stereochemistry

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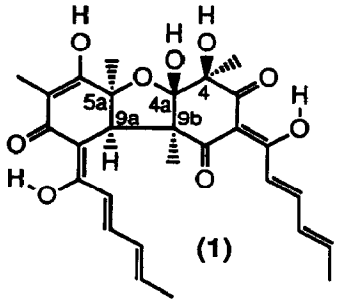
Abstract : Bisvertinolone (**1**) that previously isolated as a fungal yellow metabolite was re-isolated from *Acremonium strictum* as a hyphal malformation inducer in fungi. Bisvertinolone was found to be the first β-1,6-glucan biosynthesis inhibitor, and its absolute stereochemistry determined by spectroscopic analysis.

In investigation of β-glucan biosynthesis inhibitors as hyphal malformation inducers[1], we found a fungus, *Acremonium strictum* exhibited strong activity inducing hyphal malformation in *Phytophthora capsici*. We here describe the isolation of an active substance from the fungus, and the structure (**1**) elucidated as bisvertinolone with the revised relative and absolute stereochemistry, and the biological activity assayed has shown it to be the first β-1,6-glucan biosynthesis inhibitor.

The active compound was transferred from a culture filtrate of *A. strictum* to a weakly acidic EtOAc-soluble fraction, from which an active yellow powder was isolated through four step purification[2]. The molecular formula of **1** was determined to be $C_{28}H_{32}O_9$ by HRFAB-MS. ^1H and ^{13}C NMR spectra and the extensive analysis using DEPT and HSQC revealed that all the protons and carbons, and eight of nine oxygens in the molecule were assigned as 6 methyls, 9 methines, 10 quaternary carbons, 3 carbonyls and 5 hydroxyls, as summarized in Table 1. Two hydroxyl protons that appeared in a very low field (17.4 and 18.4 ppm) must be enolic protons being hydrogen-bonded. Because of many quaternary carbons to be contained in the compound (**1**), only two partial structures, **A** and **B**, could be deduced from the coupling constants and ^1H-^1H COSY (Fig. 1). We, therefore, employed the HMBC technique to clarify H-C relations within three bonds. As the results, **A** and **B** were extended to **A'** and **B'**, respectively, and additional two partial structures, **C** and **D**, were newly deduced (Fig. 1). A connection of **A'** with **C** was attained from 9a-H to be correlated with 8-, 9- and 19-C, as shown in Fig. 2. 9a-H that also correlated with 9b-C in **D** and 25-H3 that correlated with 9a-C in **C** made



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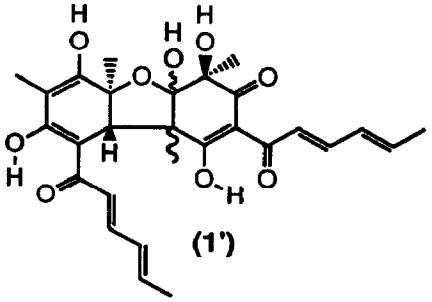

(1')

Table 1 ^{13}C and ^{1}H NMR Spectra Data of Bisvertinolone (1)

Carbon No.	^{13}C-NMR (in C_6D_6) 150 MHz chemical shift (ppm)		^{1}H-NMR (in C_6D_6) 600 MHz chemical shift (ppm)
1	200.5	C=O	
2	107.9	C=	
3	197.1	C=O	
4	79.7	C-O-H	5.00(br.s)
4a	104.7	-(O)-C-O-H	5.20(br.s)
5a	80.4	C-(O)-	
6	164.1	=C-O-H	7.45(brs)
7	111.5	C=	
8	191.7	C=O	
9	100.4	C=	
9a	55.3	CH	4.10(s)
9b	60.5	Q	
10	186.1	=C-O-H	16.40(s)
11	122.4	CH=	7.80(d,J=14Hz)
12	148.6	CH=	7.61(dd,J=10,14Hz)
13	131.5	CH=	5.94(m)
14	143.8	CH=	5.68(m)
15	18.7	CH3	1.38(d,J=5Hz)
16	23.0	CH3	1.27(s)
17	25.7	CH3	1.54(s)
18	7.6	CH3	2.00(s)
19	170.9	=C-O-H	17.40(s)
20	120.7	CH=	6.60(d,J=14Hz)
21	140.0	CH=	7.60(dd,J=10,14Hz)
22	131.4	CH=	5.92(m)
23	137.0	CH=	5.69(m)
24	18.5	CH3	1.53(d,J=5Hz)
25	19.0	CH3	1.63(s)

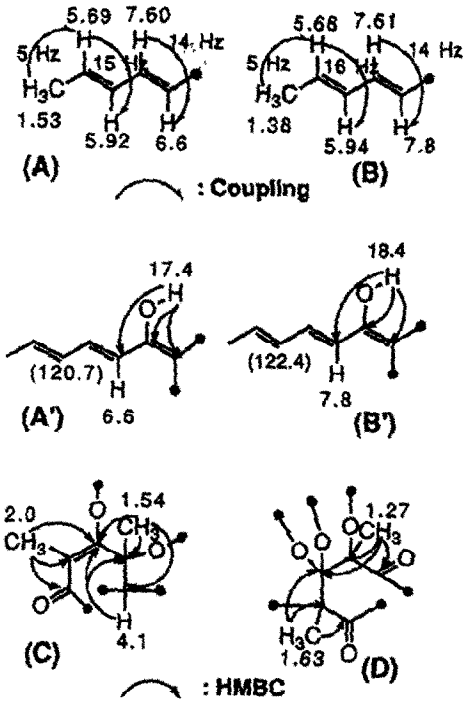

Fig. 1 Partial Structures (A) - (D)

a new bond formation between 9a-C and 9b-C. 2-C in B' could be connected with 1- and 3-carbonyls in D so that the 10-enolic hydroxyl formed hydrogen-bonding with β-positioned 1- or 3-carbonyl. Its hydrogen-bonding with 3-carbonyl rather than 1-one was estimated from the former's higher chemical shift (197.1 ppm) than the latter's (200.5 ppm)[3]. The 1, 3, 10-tricarbonyl system explains well a low chemical shift of H-11 (7.8 ppm) and acidic nature of the compound (1). The carbon skeleton of the compound (1) was thus constructed as in Fig. 2-(a).

The next problem is to identify five oxygens attached on 4-, 4a-, 4a, 5a- and 6-C; either three of which are free hydroxyls and which two making an ethereal bond. ^{13}C NMR spectrum of 1 was measured in a C_6D_6 + CD_3OD solution; the 4-, 4a- and 6-C as well as 10- and 19-C signals appeared as doublets, indicating that the oxygens bonded on these carbons should be free hydroxyls. The ethereal bond was then formed between 4a- and 5a-C to build up a tetrahydrofuran ring. The plane structure of 1 was thus constructed (Fig. 2-(b)). The proposed structure was very similar to the reported one of bisvertinolone (1') that was previously isolated as a yellow metabolite from *Verticillium intertextum* and the structure elucidated using ^{1}H- and ^{13}C-NMR assisted by computer to combine the partial structures [4]. Our physicochemical data of 1, when compared with the published data of 1', revealed that both the compounds were the same. Although the plane structure proposed

by Trifonov *et al.* was different from ours on the direction of enolization of 1,3-dicarbonyl functions, they mentioned that it was not able to be decided by spectral information but arbitrarily chosen as 1'[4]. Our structure with the different enolized carbonyls was unambigously determined by HMBC (Fig. A', B'). Because the relative stereochemistry of bisvertinolone (1') was partly obscure and its absolute stereochemistry being estimated only from a view of biosynthetic hypothesis, we attempted to determine its definitive stereochemistry by spectroscopical evidence. First, NOE experiments upon an irradiation of 9a-H and 16-CH_3 enhanced both 17- and 25-CH_3 signals, indicating that these four groups should be on the same side of the molecule, except for the 4a position, as shown in Fig. 3-(a), in which the previously assigned 5a-9a *trans*-fused stereochemistry was revised. The CPK model consideration showed that 4a-OH should be *trans* to 16- and 25-CH_3, in that stereochemistry the two methyls were near enough (2Å) to each other for the NOE observed, whereas in their *cis*-relations the ca. 4Å distance between the two methyls made NOE unenabled. The relative stereochemistry of **1** thus was postulated as in Fig. 3-(b).

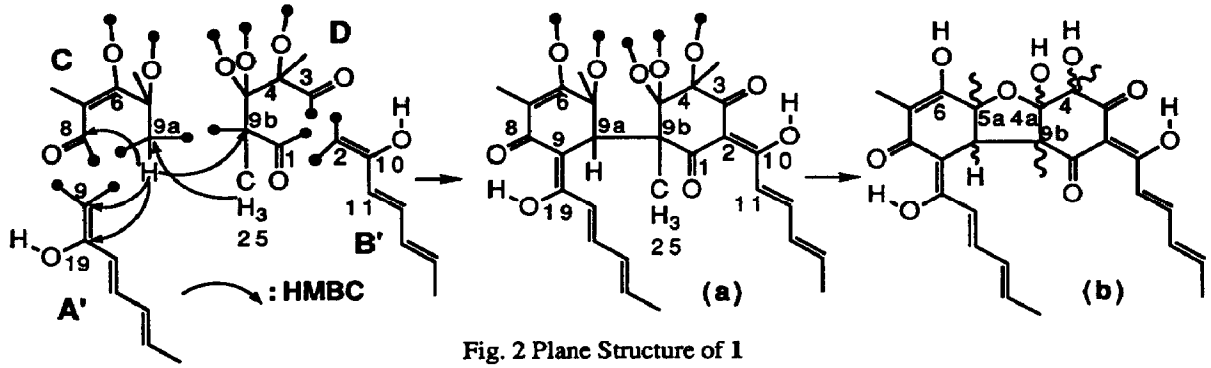

Fig. 2 Plane Structure of 1

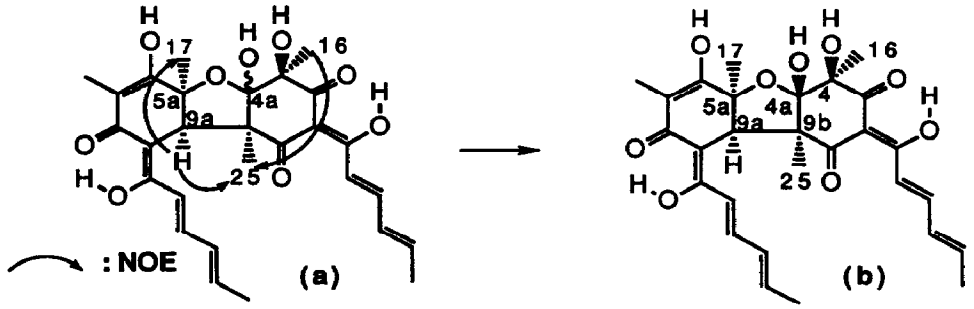

Fig.3 Stereochemistry of Bisvertinolone (1)

The CD spectrum of **1** was very similar to the reported one of bisvertinolone[4] showing a strong splitting Cotton effect at 390nm ($\Delta\varepsilon$ -27) and 330nm ($\Delta\varepsilon$ +14). This should be originated from exciton chirality caused by interaction between two conjugated chromophores. A set of enantiomers were constructed by use of the CPK model; among them, the anticlockwise orienting enantiomer (**1**) should have the minus first Cotton, thus the correct absolute stereochemistry. The active compound (**1**), hence bisvertinolone was shown to have 4*R*, 4a*S*, 5a *S*, 9a*R* and 9b*R*.

Bisvertinolone (**1**) induced morphological malformation at 10 μg/disk in growing hyphae of *Phytophthora*

capsici, whose hyphal cell wall has been reported to compose in major of β-1,3-glucan with highly branched β-1,6-glucan and of cellulose in minor[6]. In order to clarify which glucosidic linkage of these three different glucans to be inhibited by the action of bisvertinolone, we first examined an *in vitro* enzyme assay for β-1,3-glucan synthetase[7]. Although our previously isolated two hyphal malformation inducers, (+)-isoepoxidon and ophiobolin A, showed inhibitory activity in this bioassay, **1** showed only weak activity, the IC_{50} at 400 μg/ml [1), 5)]. We then examined inhibitory activity of bisvertinolone to cellulose biosynthesis in the bacterium, *Acetobacter xylinum*[8], however, it showed no activity[9]. It induced morphological changes in cells of *Saccharomyces cerevisiae*, whose cell wall composed of β-1,3-glucan branched with β-1,6-glucan and mannan. This suggests that **1** might inhibit the biosynthesis of β-1,6-glucan. So, we cultured *P. capsici* with and without bisvertinolone, and the cell walls were collected from both the cultured mycelia[10]. The hot water extracts obtained from the cell wall cultured with **1** was twelve fold heavier than that of the extracts cultured without **1**[11]. The ^{13}C NMR spectrum of the hot water extracts showed the carbon signals only of β-1,3-glucan but not of β-1,6-glucan[12]. These experimental results indicate that bisvertinolone (**1**) is the β-1,6-glucan biosynthesis inhibitor first found in nature. Because an *in vitro* assay of β-1,6-glucan synthetase is not available yet, we are now making effort to establish it. After that, we will be able to show the direct evidence that bisvertinolone is a β-1,6-glucan biosynthesis inhibitor.

References and Notes

1. Fukushima, Y.; Sakagami, Y.; Marumo, S., *Bioorganic & Medicinal Chem. Lett.*, **1993**, *3*, 1219.
2. Purification procedure: silica gel column chromatography, counter current distribution (EtOAc-0.2M Tris-HCl buffer pH 8.0), Sephadex LH-20 column chromatography and silica gel TLC. Physicochemical properties of bisvertinolone (**1**) were mp 157-158℃, $[\alpha]_D^{25}$ -495° (MeOH, c=0.1),
   FAB MS m/z=535($M+Na)^+$, 513($M+H)^+$, HRFAB MS m/z=513.2181(found) $C_{28}H_{33}O_9$, calcd. 513.2153
3. This also indicated that the C-3 signal was broadened in the ^{13}C NMR spectrum in C_6D_6 + CD_3OD, whereas the C-1 signal was not.
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5. In reference 1), we reported that (+)-isoepoxidon induced hyphal malformation activity at 100 μg/disk and inhibited β-1,3-glucan synthetase at 50 μg/ml (IC_{50}) and that ophiobolin A did at 10 μg/disk and 25 μg/ml.
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8. Omura, S.; Tanaka, Y.; Hisatome, K.; Miura, S.; Takahashi, Y.; Nakagawa, A.; Iwai, H.; Woodruff, H., *J. Antibiot.*, **1988**, *41*, 1910.
9. Bisvertinolone did not affect cellulose biosynthesis nor growth of *A. xylinum* up to 1 mg/disk.
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11. The cell wall fraction prepared by ref. 9) was successively treated with 1) hot water, 2) cold alkaline water and 3) hot alkaline water. The weights of the extracts per dry cell wall gram were as follows; the culture with bisvertinolone: 1) 448 mg, 2) 21 mg, 3) 44.2 mg and 4) residue 655 mg; the culture without bisvertinolone: 1) 32 mg, 2) 26 mg, 3) 69 mg and 4) 829 mg.
12. The chemical shifts of hot water extracts [102.7(C1), 73.6(C2), 84.1(C3), 68.2(C4), 75.6(C5) and 60.9(C6) ppm] was identical to those of laminaritetraose (β-1,3-glucan) measured and different from those of β-1,6-glucan in literature; Block, K.; Pederson, C.; Pederson H., *Adv. Carbohydr. Chem. Biochem.*, **1984**, 42, 193.

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