Sterins A and B, New Antioxidative Compounds from *Stereum hirsutum*

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Free radicals are involved in the pathogenesis of various human diseases. There is growing interest in new free radical scavengers having the potential as protective agents against active oxygen-related human diseases[1,2].

In search for new antioxidants[3~5], we have discovered a basidiomycete strain of *Stereum hirsutum* as a producer of antioxidative compounds. The genus *Stereum* is known to produce various sesquiterpenes, benzaldehydes and benzofurans[6~8]. We herein describe the fermentation of the producing strain, isolation, structure determination and antioxidative activity of sterins A and B.

The sterins-producing strain was generated from tissue culture of the fruiting body of *Stereum hirsutum* collected at Dukyu-National Park, Korea and was confirmed as a basidiomycete strain by formation of clamp-connection. The strain grown on potato sucrose agar medium was used to inoculate two 500 ml Erlenmeyer flasks containing 100 ml of the seed medium consisting of yeast extract 0.4%, malt extract 1.0% and glucose 0.4% (pH 6.0 before sterilization). The flasks were shaken on a rotary shaker for three days at 27°C. The seed culture was transferred into a 5-liter jar fermenter containing 3 liters of the above medium for production of sterins, and cultivation was carried out at 28°C for 12 days with aeration of 2 liters/minute and agitation of 250 rpm. Sterins were produced at 7 days of cultivation and reached to maximum at 11 days.

Sterins were isolated from the fermentation broth by monitoring the antioxidative activity. The fermentation broth was separated into supernatant and mycelia by centrifugation. The supernatant was applied to a column of Diaion HP-20, and the column was washed with 30% aqueous MeOH, and then eluted with 70% aqueous MeOH.

After concentration of the eluate *in vacuo*, the resultant residue was partitioned between ethyl acetate and H_2O. The ethyl acetate-soluble portion was concentrated and then subjected to a silica gel column eluting with $CHCl_3$: MeOH (50 : 1~1 : 1, stepwise) to give two active fractions. An active fraction was chromatographed on a Sephadex LH-20 column with 70% aqueous MeOH, followed by a reversed phase preparative HPLC (column: Metasil 5u ODS, 21.2×250 mm) eluting with 50% aqueous MeOH at a flow rate of 5.5 ml/minute to give sterin A (3 mg, retention time: 34 minutes). Another fraction from silica gel column was purified by Sephadex LH-20 column chromatography eluting with 70% aqueous MeOH to afford small amount of sterin B (<1 mg).

The chemical structures of sterins were determined by various spectroscopic analyses. The ^{1}H NMR spectrum of sterin A in CD_3OD exhibited the signals due to an AMX spin system [δ 6.87 (1H, dd, J=8.6, 2.8 Hz), 6.81 (1H, d, J=2.8 Hz), 6.63 (1H, d, J=8.6 Hz)] for 1,2,4-trisubstituted benzene, two olefinic protons [δ 6.31 and 5.69 (1H each, d, J=9.8 Hz)], a spin system of furanose including a characteristic signal of anomeric proton at δ 5.47 and two methyls at δ 1.41 (Table 1). The ^{13}C NMR spectrum revealed the signals for five methines and three quaternary carbons in aromatic or olefinic region, an anomeric carbon at δ 102.1, three methines and a methylene for furanose and an oxygenated quaternary carbon at δ 76.1 and two methyls at δ 27.5.

The above NMR spectral analyses indicated that the aglycone of sterin A was closely related to 6-methoxy-2,2-dimethylchromene, a fungal metabolite from the mushrooms *Lactarius fuliginosus* and *L. picinus*[9]. The furanose was assigned by the ^{1}H-^{1}H COSY experiment, and its chemical shifts and ^{1}H coupling constants suggested to be ribose[10]. The structure of sterin A was assigned by the HMBC experiment, which showed the long-range correlations from an olefinic proton at δ 6.31 to C-1, C-5 and C-6, and from an anomeric proton at δ 5.47 to C-4 at δ 150.3 (Table 1). Therefore the structure of sterin A was determined as a chromene glycoside. The deduced structure was supported by ESI-mass measurement in positive ion mode showing a molecular ion peak at m/z 331 $(M+Na)^+$.

The molecular formula of sterin B was established as $C_{12}H_{14}O_3$ by high-resolution EI-mass measurement (m/z 206.0949 M^+ +0.6 mmu). Sterin B was readily decomposed in room temperature. The ^{1}H NMR spectrum of sterin B showed the signals attributable to an aldehyde

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Table 1. ^{1}H and ^{13}C NMR spectral data of sterins A and B in CD_3OD.

No.	Sterin A			Sterin B		
	δ_H	δ_C	HMBC (H→C)	δ_H	δ_C	HMBC (H→C)
1		148.8			161.7	
2	6.63 (1H, d, J = 8.6)^a	117.0	C-1,4,6	6.93 (1H, d, J = 8.4)^a	116.5	C-4,6
3	6.87 (1H, dd, J = 8.6, 2.8)	118.3	C-1,5	7.63 (1H, dd, J = 8.4, 1.8)	131.2	C-1,5,CHO
4		150.3			129.5	
5	6.81 (1H, d, J = 2.8)	115.6	C-1,3,7	7.95 (1H, d, J = 1.8)	130.1	C-1,3,7,CHO
6		122.1			126.1	
7	6.31 (1H, d, J = 9.8)	122.1	C-1,5,6,9	6.89 (1H, d, J = 16.2)	120.8	C-1,5,8,9
8	5.69 (1H, d, J = 9.8)	131.9	C-6,9	6.48 (1H, d, J = 16.2)	139.9	C-6,9,10,11
9		76.1	C-7		71.3	
10,11	1.41 (6H, s)	27.5	C-8,9,10,11	1.42 (6H, s)	29.7	C-8,9,10,11
CHO				9.79 (1H, s)	192.8	C-3,4
1'	5.47 (1H, d, J = 4.4)	102.1	C-4,2',3',4'			
2'	4.24 (1H, m)	72.0				
3'	4.11 (1H, dd, J = 6.3, 3.4)	70.8	C-1'			
4'	4.24 (1H, m)	85.6				
5'	3.87 (1H, dd, J = 12.0, 3.0)	62.6	C-3'			
	3.75 (1H, dd, J = 12.0, 3.7)					

^{a} Proton resonance integral, multiplicity, and coupling constant (J = Hz) are in parentheses.

Fig. 1. Structures of sterins A and B.
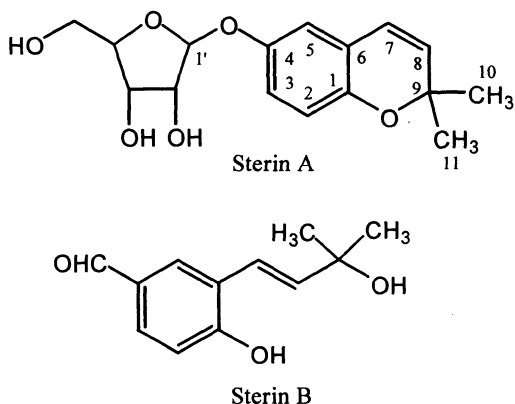
sterin A, the difference being that an olefinic set with *cis*-configuration and furanose in sterin A were displaced with *trans*-configuration and an aldehyde, respectively, in sterin B. The structure of sterin B was finally assigned as shown in Fig. 1 by HMBC experiment, which showed the long-range correlations from an olefinic proton at δ 6.89 to C-1 and C-5, and from an aldehyde proton at δ 9.79 to C-3 and C-4 (Table 1).

The antioxidative activity of sterin A was investigated by means of a lipid peroxidation inhibition test using rat liver microsomes[5]. Sterin A inhibited lipid peroxidation with an IC_{50} value of 8 μg/ml. This activity was about 10 times less active than vitamin E, which was used as a control. The antioxidative activity of sterin B could not be estimated because of its instability.

proton at δ 9.79, an AMX spin system [δ 7.95 (1H, d, J = 1.8 Hz), 7.63 (1H, dd, J = 8.4, 1.8 Hz), 6.93 (1H, d, J = 8.4 Hz)] for 1,2,4-trisubstituted benzene, two olefinic protons [δ 6.89 and 6.48 (1H each, d, J = 16.2 Hz)] with *trans*-geometry and two overlapped singlet methyls at δ 1.42 (Table 1). The structure of sterin B was similar to

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