Stachybotrin C and Parvisporin, Novel Neuritogenic Compounds

II. Structure Determination

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The structures of stachybotrin C and parvisporin have been determined by spectroscopic analyses and chemical derivatization. Stachybotrin C contains a unique pyrano-isoindolinone ring system, while parvisporin has a hydroxyl farnesyl phenol structure.

Stachybotrin C and parvisporin are novel neuritogenic compounds isolated from the culture broth of *Stachybotrys parvispora* F4708. Their taxonomy, fermentation, isolation and physico-chemical properties were reported in the preceding paper[1]. In this publication, we describe the structure determination of stachybotrin C (**1**) and parvisporin (**2**).

Results

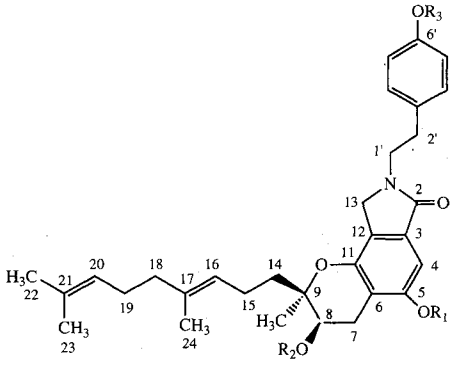
Structural Studies of **1**

Stachybotrin C (**1**) was isolated as a yellowish white powder (**1**. $[\alpha]_D$ $-28.2°$ ($c=0.1$, MeOH), m.p. 89~92°C). The molecular formula was determined to be $C_{31}H_{39}NO_5$ by its molecular ion measurement (m/z, found 505.2840, calcd. 505.2828 for $C_{31}H_{39}NO_5$) in the HREI-MS. Its IR spectrum suggested the presence of hydroxy (3305 cm^{-1}), amide carbonyl (1662 cm^{-1}) and phenyl groups (1615 cm^{-1}), respectively. These physico-chemical properties have been reported in the proceeding paper[1].

The ^{13}C NMR spectrum of **1** demonstrated 31 carbon signals (Table 1). The DEPT spectra indicated the presence of four methyls, eight methylenes, one oxygenated methine, seven olefinic methines and eleven quaternary carbons including nine olefinic carbons and one carbonyl carbon. As shown in Table 1, the ^1H NMR spectrum of **1** showed 36 proton signals which contained *p*-substituted benzene ring protons (δ 6.73, 2H, d, $J=8.4$ Hz and δ 7.01, 2H, d, $J=8.4$ Hz), two olefinic protons (δ 5.08, 2H, m) and an aromatic proton (δ 6.76, 1H, s). The existence of three exchangeable protons and 13 unsaturation equivalents were inferred from the molecular formula. Treatment of **1** with trimethylsilyl diazomethane gave a dimethyl ether **3** (m/z 533; M^+), which was successively converted to a corresponding monoacetate **4** (m/z 575; M^+) by reaction with Ac_2O in pyridine. Thus, these three exchangeable protons were characterized as one alcoholic and two phenolic hydroxyl groups.

The correlation of protons and carbons was determined as shown in Table 1 by the ^1H-^{13}C COSY spectrum. The ^1H-^1H COSY and HMBC spectral analyses allowed us to assign three partial structures, A, B and C (Fig. 2). Units A and B were determined to be a 4,8-dimethyl-3,7-nonadienyl side chain and a *p*-oxyphenethyl group, respectively, by tracing the long range

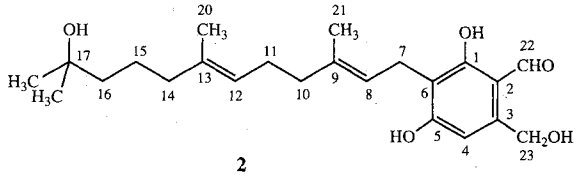
Fig. 1. Structures of stachybotrin C (**1**) and parvisporin (**2**).



1 R_1=H R_2=H R_3=H

3 R_1=CH_3 R_2=H R_3=CH_3

4 R_1=CH_3 R_2=Ac R_3=CH_3



2

Table 1. ^{13}C and ^{1}H NMR data for stachybotrin C (**1**), **3** and **4** (in $CDCl_3$).

Carbon No.	**1** δC (100MHz)	**1** δH (*J* in Hz, 400MHz)	**3** δC (100MHz)	**3** δH (*J* in Hz, 400MHz)	**4** δC (100MHz)	**4** δH (*J* in Hz, 400MHz)
2	169.70 (s)		168.74 (s)		168.74 (s)	
3	131.46 (s)		131.49 (s)		131.45 (s)	
4	100.53 (d)	6.76 (s)	96.54 (d)	6.90 (s)	96.49 (d)	6.91 (s)
5	156.34 (s)		158.79 (s)		158.53 (s)	
6	111.12 (s)		111.40 (s)		110.93 (s)	
7	26.59 (t)	2.76 (dd; 5.3, 17.8) 2.96 (dd; 5.3, 17.8)	26.82 (t)	2.74 (dd; 5.7, 17.7) 2.96 (dd; 5.3, 17.7)	24.03 (t)	2.74 (dd; 5.2, 18.3) 3.02 (dd; 5.2, 18.3)
8	67.53 (d)	3.90 (t; 5.3)	67.70 (d)	3.91 (dd; 5.3, 5.7)	69.26 (d)	5.13 (t; 5.2)
9	78.96 (s)		78.97 (s)		77.32 (s)	
11	148.31 (s)		148.04 (s)		147.96 (s)	
12	120.08 (s)		121.72 (s)		121.66 (s)	
13	48.16 (t)	4.13 (d; 17.9) 4.17 (d; 17.9)	47.93 (t)	4.13 (d; 17.1) 4.19 (d;17.1)	47.90 (t)	4.14 (d; 17.0) 4.22 (d; 17.0)
14	36.90 (t)	1.62 (m)	36.87 (t)	1.62 (m)	36.78 (t)	1.60 (m)
15	21.57 (t)	2.11 (m)	21.60 (t)	2.11 (m)	21.51 (t)	2.09 (m)
16	123.65 (d)	5.08 (m)	123.57 (d)	5.08 (m)	123.21 (d)	5.06 (m)
17	135.67 (s)		135.80 (s)		135.91 (s)	
18	39.61 (t)	1.95 (m)	39.63 (t)	1.95 (m)	39.61 (t)	1.95 (m)
19	26.59 (t)	2.03 (m)	26.62 (t)	2.03 (m)	26.61 (t)	2.03 (m)
20	124.13 (d)	5.08 (m)	124.13 (d)	5.08 (m)	124.12 (d)	5.06 (m)
21	131.66 (s)		132.66 (s)		132.69 (s)	
22	25.60 (q)	1.66 (s)	25.65 (q)	1.66 (s)	25.65 (q)	1.66 (s)
23	17.60 (q)	1.57 (s)	17.65 (q)	1.57 (s)	17.64 (q)	1.57 (s)
24	15.86 (q)	1.56 (s)	15.90 (q)	1.56 (s)	15.86 (q)	1.54 (s)
25	19.15 (q)	1.33 (s)	19.20 (q)	1.34 (s)	19.90 (q)	1.31 (s)
5-OCH_3			55.86 (q)	3.86 (s)	55.84 (q)	3.86 (s)
8-OAc					170.40 (s)	
					21.11 (q)	2.07 (s)
1'	44.37 (t)	3.75 (m)	44.34 (t)	3.80 (t; 7.6)	44.35 (t)	3.80 (t; 7.4)
2'	33.76 (t)	2.85 (t; 7.3)	33.97 (t)	2.92 (t; 7.6)	33.98 (t)	2.92 (t; 7.4)
3'	129.60 (s)		130.75 (s)		130.73 (s)	
4'	129.60 (d)	7.01 (d; 8.4)	129.63 (d)	7.16 (d; 8.7)	129.67 (d)	7.17 (d; 8.6)
5'	115.45 (d)	6.73 (d; 8.4)	113.99 (d)	6.83 (d; 8.7)	113.99 (d)	6.83 (d; 8.6)
6'	154.99 (s)		158.21 (s)		158.22 (s)	
7'	115.45 (d)	6.73 (d; 8.4)	113.99 (d)	6.83 (d; 8.7)	113.99 (d)	6.83 (d; 8.6)
8'	129.60 (d)	7.01 (d; 8.4)	129.63 (d)	7.16 (d; 8.7)	129.67 (d)	7.17 (d; 8.6)
6'-OCH_3			55.22 (q)	3.77 (s)	55.21 (q)	3.77 (s)

correlations in the HMBC spectrum as shown in Fig. 2. Unit C was established through long range correlations from H-4, H_2-7 and H_2-13. The signal for H-4 displayed contours with three aromatic ring carbons at C-2, C-5 and C-6 and a carbonyl carbon at C-11, whereas H_2-13 correlated with C-2, C-3, C-11 and C-12. Two aromatic carbons at C-5 and C-11 were oxygenated on the basis of ^{13}C NMR shift trends of δ 156.34 and 148.31 as shown in Table 1 and located at the meta position which was confirmed by the HMBC correlations of H_2-7 with C-5, C-6 and C-11. Its IR absorption at 1662 cm^{-1} due to an amide group and the chemical shifts of C-2 (δ 169.70) and C-13 (δ 48.16) indicated that these carbons were conjugated to a nitrogen atom, resulting in the formation of an unique trisubstituted isoindolinone ring. The H_2-7 protons also showed correlations with two oxygenated carbons at δ 67.53 (C-8) and δ 78.96 (C-9), which was estimated to form a ring system with C-5 or C-11.

The connectivities of three partial structures were also established by the HMBC experiments as shown in Fig. 3. The observation of clear correlations from H-1′ to C-2 and C-3, and the chemical shift of C-1′ at δ 44.37 indicated the methylene for C-1′ bonded to a nitrogen atom. The linkage of units A and C was determined by the HMBC correlations of H_3-25 with C-8, C-9 and C-14, and H-8 with C-9 and C-14, respectively. In the HMBC spectrum of **3**, two methoxy methyl protons showed contours with each of C-5 and C-6′, indicating that these two phenolic hydroxyl groups were attached at C-5 and C-6′ in **1**. In the ^{1}H NMR spectrum of **3** and **4** (Table 2), the down field shift from δ 3.91 to 5.13 of the proton at H-8 by acetylation suggested the location of a secondary hydroxyl group at C-8. As a result, the remaining C-9 carbon is linked to an oxygen atom at C-11 to establish a pyrane ring. Thus, the total structure of **1** was elucidated as shown in Fig. 1.

Fig. 2. Partial structures of stachybotrin C (1) deduced by ^1H-^1H COSY and HMBC.

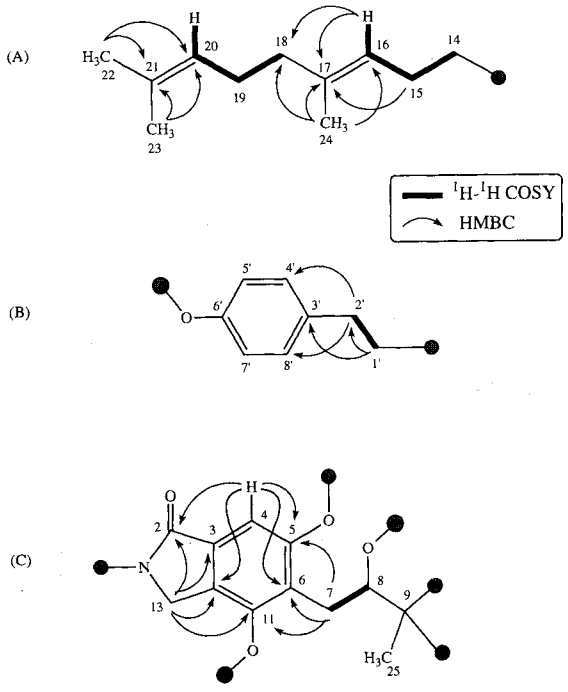
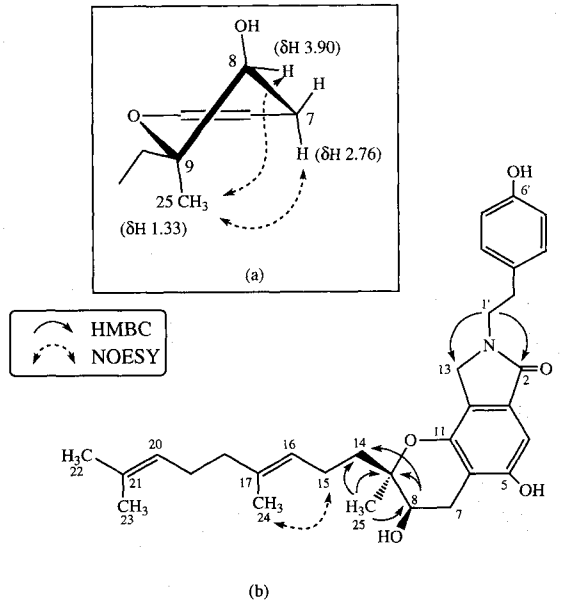


Fig. 3. Relative configuration of stachybotrin C (1).



The relative stereochemistry of **1** were determined by its NOESY data and ^1H NMR coupling constants (Fig. 3). The NOE between a methyl of H_3-25 at δ 1.33 and H-7 (δ 2.76) indicated that these groups are spatially close to each other and that the H_3-25 methyl group has also an axial orientation. Additionally, a NOE correlation between H_3-25 and H-8 (δ 3.90) revealed that H-8 was equatorialy oriented and the hydroxyl group at C-8 was assigned as trans to the H_3-25 methyl group. The geometry at C-16 and C-17 was determined to be 16E by the observation of a NOE between H-15 (δ 2.11) and H_3-24 (δ 1.56).

Table 2. ^{13}C and ^1H NMR data for parvisporin (2) (in $CDCl_3$).

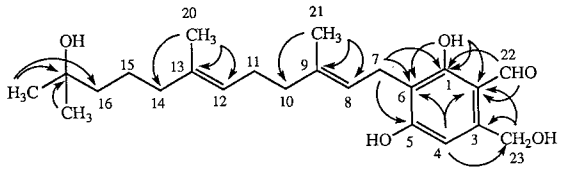
Carbon No.	2	
	δc (100MHz)	δH (*J* in Hz, 400MHz)
1	163.85 (s)	
2	111.79 (s)	
3	143.32 (s)	
4	108.91 (d)	6.42 (s)
5	162.46 (s)	
6	114.22 (s)	
7	21.22 (t)	3.38 (d; 6.9)
8	121.70 (d)	5.18 (dd; 1.1, 6.9)
9	137.60 (s)	
10	39.24 (t)	2.10 (m)
11	25.38 (t)	2.13 (m)
12	123.63 (d)	5.03 (dt; 1.1, 5.4)
13	135.12 (s)	
14	39.64 (t)	1.92 (m)
15	22.23 (t)	1.41 (m)
16	42.97 (t)	1.41 (m)
17	72.19 (s)	
18	29.20 (q)	1.23 (s)
19	29.20 (q)	1.23 (s)
20	16.03 (q)	1.55 (s)
21	16.03 (q)	1.78 (s)
22	193.17 (d)	10.15 (s)
23	62.11 (t)	4.85 (s)

Structure of 2

Parvisporin (**2**) has the molecular formula $C_{23}H_{34}O_5$ determined by its pseudo-molecular ion at *m/z* 389.2322 $(M-H)^-$ (calcd. 389.2328 for $C_{23}H_{33}O_5$) in its HRFAB-MS spectrum. Its UV spectrum (λ_{max} nm (MeOH) 206 (log ε1.67), 295 (0.58)) and IR spectrum as described in the preceding paper[1] were clearly different from those of **1**.

The ^1H NMR spectrum of **2** (Table 2) exhibited 4 methyl groups (δ 1.23 × 2, δ 1.55 and δ 1.78), two olefinic protons (δ 5.03 and δ 5.18), an aromatic proton (δ 6.42), an aldehyde proton (δ 10.15) and a phenolic hydroxy proton (δ 12.70). The ^{13}C NMR spectrum (Table 2) demonstrated 23 carbon signals, which were assigned to four methyl, seven methylene, one quaternary (δ 72.19), ten sp^2 carbons including three sp^2 methine carbons and one aldehyde carbon by its DEPT spectrum. The presence of an aldehyde group was also ascertained by the characteristic IR absorption at 1725 cm^{-1}, and the extence of three exchangeable protons (hydroxy and/or

Fig. 4. HMBC correlations observed in parvisporin (**2**).



phenolic) were supposed by the molecular formula and the degree of saturation.

The structure of **2** was also determined by the 2D NMR experiments. The proton spin network in the ^1H-^1H COSY spectrum provided the fragment structure C-7 ~ C-14. Additionally, four methyl protons showed HMBC correlations with their relevant carbons as follows; 18-H_3 to C-16 and C-17, 19-H_3 to C-17, 20-H_3 to C-12, C-13 and C-14 and 21-H_3 to C-8, C-9 and C-10. These results clarified the structure of an alkyl side chain (C-7 ~ C-21). The geometry of the two double bonds were determined to be 8E and 12E by the observation of NOEs between H-7 (δ 3.38) and H_3-21 (δ 1.78), H-11 (δ 2.13) and H_3-20 (δ 1.55), respectively. Two of the aromatic carbons for C-1 at δ 163.85 and for C-5 at δ 162.46 and the methylene carbon for C-23 at δ 62.11 were considered due to attached hydroxy groups on the basis of their chemical shift trends. The positioning of the substituent groups on the benzene ring was established by the observation of HMBC correlations as illustrated in Fig. 4. Thus we assigned the total structure of **2** as shown in Fig. 1.

Discussion

In the course of our screening program for neuritogenic compounds, we have isolated two novel compounds designated stachybotrin C and parvisporin from the culture broth of *Stachybotrys parvispora* F4708. Stachybotrin C induces neurite outgrowth of PC12 cells and enhances the survival of cultured neurons from rat brain. Parvisporin shows marginal neuritogenic activity and no cell survival activity[1].

Structural studies revealed that stachybotrin C had a unique pyranoisoindolinone ring system, and that it is related to stachybotrins A and B isolated as antibacterial and antifungal active compounds from *Stachybotrys* sp.[2]. The significant difference between stachybotrin C and stachybotrins A and B is that an additional phenol ring combines with a piranoisoindolinone ring in stachybotrin C. Recently, NAKAMURA *et al.* reported novel endothelin receptor antagonists stachybocins[3,4] and spirodihydrobenzofuranlactams[5] produced by *Stachybotrys* sp.. Their structures are also related to those of parvisporin A_1.

As stachybotrin C exhibits neurotrophic effects on cultured neurons from the central nervous system, we expect that it might be a good candidate for the pharmaceutical agent which prevents the neuronal cell death caused by cerebral ischemia.

Since various congeners of stachybotrin C are detected in the fermentation broth of the same organism, isolation of other parvisporins is in progress.

Experimental

General

Melting point was determined with a Yanagimoto micro-melting point apparatus and uncorrected. Optical rotation was measured on a Jasco DIP-360 polarimeter. IR spectrum was recorded on a Perkin-Elmer 1760 FT-IR spectrophotometer. UV spectrum was obtained with a Hitachi 220A spectrophotometer. MS spectra were determined with a Joel JMX-SX 102 mass spectrometer. NMR spectra were measured on a Joel JMN-GX 400 spectrometer at ambient temperature using the solvent peaks as internal references. Preparative HPLC was performed with a Waters Model 600E system.

Methylation of Stachybotrin C (**1**)

A solution of **1** (50 mg) in ethanol (5 ml) was stirred with trimethylsilyl diazomethane (5 ml) for 40 hours at room temperature. After evaporation of the solvent *in vacuo*, the residue was subjected to preparative HPLC (column: Senshu-Pak PEGASIL ODS, 10 i.d. × 150 mm; 75% CH_3CN; flow rate 4.5 ml/minute; detection UV, 215 nm). The appropriate fractions were pooled and concentrated to give a dimethyl ether (**3**) (43.4 mg).

3: Colorless oil, EI-MS *m/z* 533(M^+), IR ν cm^{-1} (Neat) 3367, 2930, 1670, 1611, 1514, 1475, UV λ_{max} nm (MeOH) 212 (log ε 15.52), 257 (4.43), 302 (1.05), ^1H and ^{13}C NMR in Table 1.

Acetylation of **3**

3 (10 mg) was stirred with acetic anhydride (1 ml) in dry pyridine (1 ml) for 16 hours at room temperature. The reaction mixture was poured into water (5 ml) and extracted by ethyl acetate (5 ml). The organic layer was washed successively with dil HCl (5 ml × 4) and water (5 ml × 4), and concentrated *in vacuo* to give monoacetate (**4**) (6.7 mg).

4: Colorless oil, EI-MS *m/z* 575, IR ν cm^{-1} (Neat) 2928, 2855, 1742, 1688, 1611, 1514, 1475, UV λ_{max} nm (MeOH) 215 (log ε 7.89), 258 (1.69), 300 (0.52), ^{1}H and ^{13}C NMR in Table 1.

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