SMTP-1 and -2, Novel Analogs of Staplabin Produced by *Stachybotrys microspora* IFO30018

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Plasminogen is a circulating zymogen of plasmin, a primary fibrinolytic enzyme[1]. Plasminogen binding to fibrin and vascular and blood cells increases the local concentration of the zymogen and accelerates the activation of plasminogen by plasminogen activators[2]. We have previously isolated a novel fungal triprenyl phenol, designed staplabin, which enhances plasminogen binding to fibrin and cultured cells[3]. In the present paper, we report the isolation of two novel analogs of staplabin from cultures of *Stachybotrys microspora* IFO30018.

The analogs, designated SMTP-1 and -2, were isolated as follows. *Sta. microspora* IFO 30018, was grown aerobically at 25°C for 10 days as described previously[3]. In this culture, SMTP-1 and -2 were produced along with staplabin, the major product. The concentrations of SMTP-1, -2 and staplabin were 20.0, 6.3 and 74.2 μg/ml, as determined by HPLC. The cultured broth (6 liters) was centrifuged at 8000 rpm for 10 minutes to obtain supernatant, which was then adjusted to pH 7 with 3 N HCl and applied to an HP-20 column (60 × 330 mm). After washing with 4.9 liters of water, the column was developed successively with 50% MeOH (4.9 liters) and MeOH (4.9 liters). The fraction eluted with MeOH was concentrated *in vacuo*, giving 6.1 g of an oily residue. The residue material was applied to a silica gel column (60 × 255 mm), which was then developed with chloroform and chloroform - MeOH (19 : 1). The chloroform-MeOH fraction was dried *in vacuo* to give 2.5 g of a residue. The residue was dissolved in a small volume of MeOH and subjected in multiple batches to preparative HPLC on an Inertsil PREP-ODS column (30 × 250 mm, GL Sciences). The column was developed at 40°C with acetonitrile - water (49 : 51) at a rate of 25 ml/minute. Purified SMTP-1 (88 mg) was obtained by lyophilizing fractions eluted at retention times of 25.0 ~ 27.0 minutes. To isolate SMTP-2, the fraction eluted from the HP-20 column with 50% MeOH was concentrated *in vacuo* (2.5 g) and applied to a silica gel column (40 × 160 mm). The column was developed successively with chloroform - MeOH (19 : 1) and chloroform - MeOH (9 : 1). The latter fraction was dried *in vacuo*, giving 392 mg of an oily residue. The residue was dissolved in a small volume of MeOH and subjected in multiple batches to preparative HPLC on an Inertsil PREP-ODS column. The column was developed at 40°C with acetonitrile - water (25 : 75) at a rate of 25 ml/minute. Purified SMTP-2 (18.7 mg) was obtained by lyophilizing fractions eluted at retention times of 17.0 ~ 18.0 minutes.

The physico-chemical properties of SMTP-1 and -2 are summarized in Table 1. Both compounds were soluble

Fig. 1. Structures of SMTP-1, -2 and staplabin.

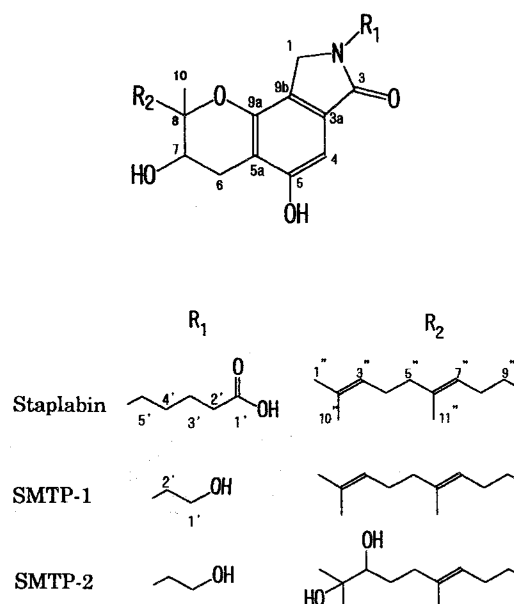


Table 1. Physico-chemical properties of SMTP-1 and -2.

	SMTP-1	SMTP-2
Appearance	Light brown oil	Light brown oil
Molecular formula	$C_{25}H_{35}NO_5$	$C_{25}H_{37}NO_7$
HRFAB-MS (m/z)		
Found $(M+H)^+$:	430.2583	464.2694
Calcd:	430.2572 for $C_{25}H_{36}NO_5$	464.2648 for $C_{25}H_{38}NO_7$
UV λ_{max}^{MeOH} nm (ε)	214 (39,700) 254 (7,200) 299 (2,800)	212 (34,000) 252 (8,000) 294 (3,000)
IR ν_{max} (KBr) cm^{-1}	3400, 2920, 1660, 1460, 1340, 1360, 1160, 1080	3400, 2920, 2860, 1720, 1660, 1460, 1380, 1160, 1080
Specific rotation $[\alpha]_D^{20}$	−7.1° (c 2.0, acetone)	−2.7° (c 0.21, acetone)

Table 2. ^{1}H and ^{13}C NMR spectral data for SMTP-1, -2 and staplabin.

Position	Staplabin		SMTP-1		SMTP-2	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	46.6	4.17 (2H, s)	49.0	4.24 (1H, d, J=18.0) 4.31 (1H, d, J=18.0)	47.7	4.27 (2H, s)
3	167.6		170.5		167.6	
3a	131.7		131.4		131.7	
4	99.6	6.63 (1H, s)	101.1	6.72 (1H, s)	99.5	6.65 (1H, s)
5	156.2		156.0		156.0	
5a	111.3		111.3		111.2	
6	26.7	2.47 (1H, dd, J=7.3, 17.5) 2.82 (1H, dd, J=5.3, 17.5)	26.5	2.72 (1H, dd, J=5.0, 16.0) 2.84 (1H, dd, J=4.5, 16.0)	26.6	2.47 (1H, dd, J=5.4, ~18.0) 2.84 (1H, dd, J=5.4, 18.0)
7	66.0	3.73 (1H, dd, J=5.3, 7.3)	67.7	3.82 (1H, m)	66.0	3.74 (1H, dd, J=4.9, 9.2)
8	78.8		79.0		78.6	
9a	148.4		148.1		148.2	
9b	119.3		120.4		119.5	
10	18.2	1.16 (3H, s)	19.3	1.29 (3H, s)	17.9	1.16 (3H, s)
1′	174.4		60.5	3.87 (2H, m)	59.3	3.57 (2H, m)
2′	33.1	2.23 (2H, t, J=7.3)	45.7	3.60 (1H, m) 3.74 (1H, m)	44.5	3.51 (2H, m)
3′	21.8	1.44 (2H, m)				
4′	27.2	~1.6 (2H, m)				
5′	41.1	3.45 (2H, t, J=6.7)				
1″	25.5	1.61 (3H, s)	25.7	1.64 (3H, s)	24.5	0.98 (3H, s)
2″	130.7		131.3		71.5	
3″	124.1	5.04 (1H, t, J=6.6)	123.7	5.04 (1H, t, J=6.4)	77.0	3.04 (1H, d, J=10.2)
4″	26.2	1.99 (2H, m)	26.7	1.98 (2H, m)	29.2	1.16 (2H, m)
5″	39.8	1.94 (2H, m)	39.7	1.92 (2H, m)	36.4	1.90 (2H, m)
6″	134.4		135.6		135.0	
7″	124.2	5.12 (1H, t, J=6.6)	124.2	5.04 (1H, t, J=6.4)	123.5	5.15 (1H, t, J=6.8)
8″	21.1	2.11 (2H, m)	21.6	2.07 (2H, m)	20.9	2.10 (2H, m)
9″	37.2	~1.6 (2H, m)	36.9	~1.6 (2H, m)	37.4	1.64 (2H, m)
10″	17.5	1.53 (3H, s)	17.7	1.55 (3H, s)	26.0	1.02 (3H, s)
11″	15.6	1.54 (3H, s)	15.9	1.54 (3H, s)	15.7	1.59 (3H, s)

The spectra were measured in $CDCl_3$ (for SMTP-1) or DMSO-d_6 (for SMTP-2) at 25°C at 67.9 MHz (for ^{13}C) and 270 MHz (for ^{1}H). The data for staplabin are from ref. 3. The chemical shift was referenced to TMS (δ 0). J=Hz.

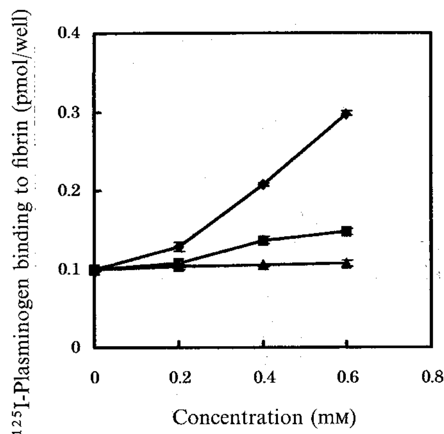
in chloroform, MeOH and DMSO. The UV spectra of SMTP-1 and -2 were similar to that of staplabin, suggesting that both compounds have a chromophore structure related to that of staplabin. Indeed, signals corresponding to those of the ring structure (positions 1~10), which include the chromophore, of staplabin were observed in the ^{1}H and ^{13}C NMR spectra of SMTP-1 and -2 (Table 2). On the other hand, SMTP-1 and -2 lacked the carboxybutyl moiety of staplabin. In these compounds, the side chain was assigned to be hydroxyethyl from the following observations: the nitrogen-bearing methylene signal (2′-H_2) coupled to the C-1 signal in the HMBC spectra; the ^{1}H-^{1}H spin coupling was observed between 2′-H_2 and the oxygen-bearing methylene (1′-H_2, δ_H 3.87 for SMTP-1 and 3.57 for SMTP-2). In the ^{1}H and ^{13}C NMR spectra of SMTP-1, signals quite similar to those for 4,8-dimethyl-3,7-nonadienyl moiety of staplabin were observed (Table 2). The presence of this moiety in SMTP-1 was confirmed by the ^{1}H-^{1}H COSY and HMBC spectra. As compared with SMTP-1, SMTP-2 lacked two olefinic carbon signals but had two oxygen-bearing carbon signals instead. The difference of molecular weight between SMTP-1 and -2 was 34, which corresponded to two hydroxy groups. These observations suggested that two carbons at one of the double bond in the side chain of SMTP-2 are hydroxylated. The positions of the hydroxy groups were assigned to be at 2″ and 3″ from the ^{1}H-^{1}H COSY and HMBC spectra. From these observations, the structures of SMTP-1 and -2 were proposed as shown in Fig. 1.

Staplabin is active in enhancing plasminogen binding to fibrin at concentrations of 0.4~0.6 mM[3]. At the same concentrations, SMTP-1 enhanced ^{125}I-plasminogen binding by 20~30% (Fig. 2). This activity was approximately 5 times lower than that of staplabin. SMTP-2 showed no activity under these conditions.

From these results, it is suggested that carboxybutyl moiety of the staplabin molecule plays a critical role in enhancing plasminogen binding. Further, it may be possible that increasing hydrophilicity or polarity of the dimetylnonyl moiety in the molecule lowers its activity. In addition to characterizing structure-activity relationships of staplabin, these compounds may also be useful for studying the biosynthesis of fungal triprenyl phenols with a hydroxylated prenyl group or a modified benzolactam[4~8].

Fig. 2. Effects of SMTP-1, -2 and staplabin on ^{125}I-plasminogen binding to fibrin.

◆ Staplabin, ■ SMTP-1, ▲ SMTP-2.



Fibrin-coated wells received ^{125}I-plasminogen (50 nM) and the indicated concentration of SMTP-1, -2 and staplabin. After incubation at 37°C for 60 minutes in the absence or presence of 0.2 M ε-aminocaproic acid, the amount of ^{125}I-plasminogen bound was determined as described previously[3]. The values shown represent specific binding, which was calculated by subtracting values obtained in the presence of ε-aminocaproic acid (nonspecific binding) from values obtained in its absence (total binding). Each value represents the mean ± S.D. from triplicate determinations.

Recently, NG-243, a substance effective in potentiating the action of nerve growth factor, was isolated from *Sta. parvispora*[9]. The spectral data for this compound suggest that it may be identical to SMTP-1.

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