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Isolation and Structure Elucidation of Neofusapyrone from a Marine-derived *Fusarium* species, and Structural Revision of Fusapyrone and Deoxyfusapyrone

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Abstract Three polyketides containing a pyrone ring, neofusapyrone, fusapyrone, and deoxyfusapyrone, were isolated from the marine-derived fungus *Fusarium* sp. FH-146. Their structures were determined by extensive 1D- and 2D-NMR and MS spectral analyses. Fusapyrone and deoxyfusapyrone were originally reported as α-pyrone derivatives; however, the revised structures indicate that they are γ-pyrone derivatives. These three compounds exhibited antimicrobial activities.

Keywords fusapyrone, neofusapyrone, *Fusarium* sp. deoxyfusapyrone, antimicrobial activity

Introduction

In our screening program for antimicrobial compounds from endophytic fungi, we have thus far isolated three antimicrobial epoxycyclohexenone derivatives and two eremophilane sesquiterpenes, namely, eremoxylarins A and B, from an xylariaceous endophytic fungus (strain YUA-026) [1, 2]. We also isolated two polyketides, namely, anthracobic acids A and B, from the *Anthracobia* species of fungi [3]. In a continuation of this study, we discovered that an isolate of the marine fungus strain *Fusarium* sp. FH-146, which was obtained from driftwood collected at the Oga peninsula, produced an extract that exhibited antimicrobial activity against *Aspergillus clavatus*. Bioassay-guided fractionation of the extract resulted in the isolation of neofusapyrone (**1**), fusapyrone (**2**), and deoxyfusapyrone (**3**) (Fig. 1). Here, we describe the isolation, structure elucidation, and biological activities of the three related pyrone derivatives along with the revision of the structures of the previously reported antifungal mycotoxins, namely, fusapyrone and deoxyfusapyrone, from *Fusarium semitectum* [4].

Fusarium sp. (section *Liseola*) FH-146 was cultivated on sterilized unpolished rice (total, 580 g) at 25°C for 3 weeks. Purification of the compounds was guided by their antifungal activity and characteristic coloration by TLC. A MeOH extract of the fermented unpolished rice was concentrated. The MeOH extract was then partitioned between ethyl acetate and water. The organic layer was subjected to silica gel column chromatography eluting with an *n*-hexane - EtOAc gradient. Further chromatographic studies using silica and ODS gel yielded three pure compounds, neofusapyrone (**1**, 20 mg), fusapyrone (**2**, 5.3 mg) and deoxyfusapyrone (**3**, 2.5 mg).

Neofusapyrone (**1**) was obtained as an optically active oil. The molecular formula of **1** was deduced as $C_{34}H_{54}O_9$ from HR-FAB-MS. The UV spectrum of **1** exhibited absorption maxima at 239 and 289 nm. This suggested that **1** had a conjugated dienone system in its structure. The IR spectral data showed absorption bands at 3367 and

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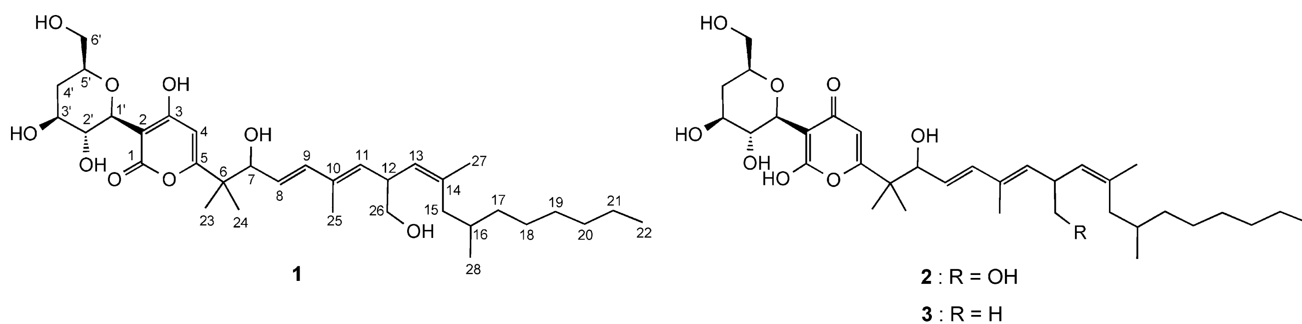

Fig. 1 Structures of neofusapyrone (**1**), fusapyrone (**2**) and deoxyfusapyrone (**3**).

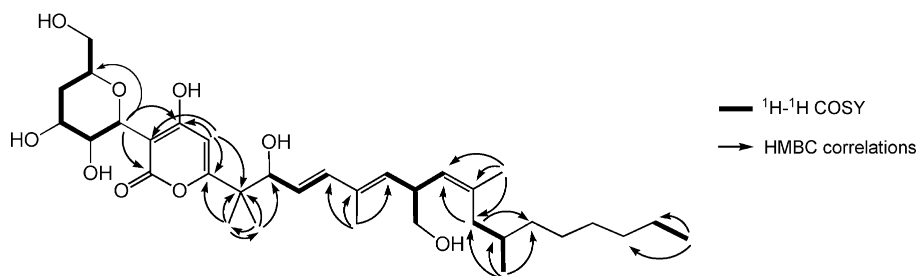

Fig. 2 Selected ¹H-¹H COSY and HMBC correlations for neofusapyrone (**1**).

1675 cm^{-1} indicating the presence of hydroxyl groups and an unsaturated ester group, respectively. The structure of **1** was elucidated based on 1D- and 2D-NMR experiments in CD_3OD. The ^{13}C-NMR spectrum (Table 1) showed the presence of 34 carbon signals, and analysis of the DEPT experiment revealed that the ^{13}C-NMR signals consisted of 23 sp^3, 10 sp^2, and 1 carbonyl carbon atoms. The signals of 10 olefinic carbon atoms indicated the presence of 5 double bonds in **1**. These carbon atoms along with the one carbonyl carbon atom accounted for six degrees of unsaturation; therefore, the remaining two degrees of unsaturation should be due to the presence of two rings in the molecule. In the ^{1}H-NMR spectrum, six methyl groups, many aliphatic methylenes, two oxymethylenes, seven methines including five oxygenated ones, and five olefinic methines were observed. Analysis of the ^{1}H-^{1}H COSY experiment of **1** revealed five spin networks shown as bold lines in Fig. 2. Based on the ^{13}C-NMR data of **1**, the partial structure from C-1′ to C-6′ suggested the existence of a *C*-linked 4-deoxy sugar. The coupling constant of the signals originating from the anomeric proton of **1** indicated a β-configuration of the glycoside linkage. The coupling constant values of H-1′/H-2′ (J=9.7 Hz) and H-2′/H-3′ (J=9.7 Hz) revealed the presence of diaxial couplings. The diaxial orientations of H-1′/H-5′ were confirmed by the NOE correlation from H-1′ to H-5′ in the acetyl derivative **1a** of **1** (Fig. 3a). HMBC correlations between H-1′ and C-1, C-2, and C-3 indicated that a *C*-β-4-deoxy-glucopyranoside moiety was linked to C-2.

The ^{1}H-NMR spectrum (Table 1) showed a singlet olefinic proton at δ_H 6.07; from the ^{13}C-^{1}H COSY experiment, this proton was found to be linked to C-4, and it showed HMBC correlations with C-2, C-3, and C-5. In the ^{13}C-NMR spectrum, three deshielded carbon atoms with chemical shift greater than 160 ppm and two shielded carbons with chemical shift of *ca.* 100 ppm were observed. These NMR data are characteristic of an α-pyrone [5, 6]; this finding was also supported by the IR spectrum [5, 6].

The signals at δ_H 0.89 (3H, t) and those of many aliphatic methylenes suggested the existence of a long-chain fatty acid in **1**. In the HMBC spectrum, the signals H_3-28 correlated with C-15, C-16, and C-17; the signals H_3-27, with C-13, C-14, and C-15; and the signals H_3-22, with C-20 to C-21. This shows that the 2-methyl-octyl moiety (C-15 to C-22 and C-28) is linked to C-14. The linkages of C-10 to C-9, C-11, and C-25 were deduced based on the HMBC correlations of H_3-25 to C-9, C-10, and C-11. HMBC correlations of H_3-23 to C-5, C-6, and C-7 and H_3-24 to C-5, C-6, and C-7 revealed that *geminal* methyls at C-6 were located between C-5 and C-7. Furthermore, HMBC correlations between H-4 and C-6 indicated that a long chain was located at C-5. The

Table 1 ^{13}C and ^{1}H NMR data for neofusapyrone (**1**) in CD_3OD[a]

No.	δ_C	δ_H	HMBC
1	167.1 s		
2	100.2 s		
3	171.1 s		
4	101.2 d	6.07 (1H, s)	2, 3, 5, 6
5	171.9 s		
6	45.5 s		
7	78.1 d	4.33 (1H, d, 7.4)	6, 8, 9, 23, 24
8	126.3 d	5.58 (1H, dd, 15.4, 7.4)	10
9	139.0 d	6.27 (1H, d, 15.4)	25
10	134.3 s		
11	135.1 d	5.32 (1H, d, 9.3)	9, 13, 25
12	42.1 d	3.47∼3.53 (1H, m)	11, 13, 26
13	126.9 d	5.05 (1H, d, 8.9)	15, 26, 27
14	137.1 s		
15	41.2 t	2.06 (1H, dd, 13.2, 6.7)	13, 14, 17, 27, 28
		1.92 (1H, dd, 13.2, 8.2)	
16	32.3 d	1.51∼1.60[b]	
17	38.2 t	1.03∼1.08 (1H, m)	
		1.23∼1.30[b]	
18	28.4 t	1.23∼1.30[b]	
19	30.7 t	1.23∼1.30[b]	
20	33.0 t	1.23∼1.30[b]	
21	23.7 t	1.23∼1.30[b]	
22	14.5 q	0.89 (3H, t, 6.6)	20, 21
23	22.7 q	1.15 (3H, s)	5, 6, 7, 24
24	20.2 q	1.21 (3H, s)	5, 6, 7, 23
25	13.4 q	1.79 (3H, s)	9, 10, 11
26	66.8 t	3.36 (1H, dd, 10.4, 7.3)	11, 13
		3.42 (1H, dd, 10.4, 5.9)	11, 13
27	24.0 q	1.67 (3H, s)	13, 14, 15
28	20.6 q	0.87 (3H, d, 6.4)	15, 16, 17
1′	75.7 d	4.47 (1H, d, 9.7)	1, 2, 3, 2′, 5′
2′	73.5 d	4.00 (1H, t, 9.7)	2, 1′
3′	74.1 d	3.55∼3.64[b]	
4′	36.5 t	1.93∼1.97 (1H, m)	
		1.51∼1.60[b]	
5′	78.4 d	3.55∼3.64[b]	
6′	65.7 t	3.55∼3.64[b]	

[a] ^{1}H and ^{13}C NMR were measured at 400 MHz and 100 MHz, respectively.

[b] Multiplicity patterns were unclear due to signals overlapping.

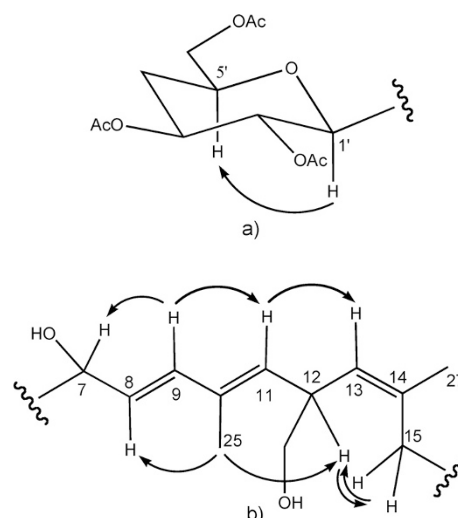

Fig. 3 Selected NOE correlations for acetyl derivative **1a** and neofusapyrone (**1**).

geometrical isomerism of the double bond (C-8 and C-9) was determined as 8*E* based on the coupling constants ($J_{8,9}$=15.4 Hz). Based on the NOEs observed from H-9 to H-11, H-11 to H-13, H_3-25 to H-8 and H-12, and H-15 to H-12, the geometry was established as 10*E*, 13*Z* (Fig. 3b). Thus, the gross structure of **1** was assigned as neofusapyrone.

The molecular formula of fusapyrone (**2**) was deduced as $C_{34}H_{54}O_9$ from HR-FAB-MS, indicating that the molecular formulae of **2** and **1** were identical. The UV spectrum of **2** exhibited absorptions at λ_{max} 239 and 283 nm. The ^{1}H- and ^{13}C-NMR spectra of **2** (Table 2) also displayed a close similarity to those of **1**. In the NMR data of **2**, the main significant difference was the shift in the signals of a pyrone group, suggesting that **2** was a γ-pyrone derivative of **1**. The signals due to an olefinic methine at δ_H 5.81 and δ_C 108.1 and due to quaternary carbon atoms at δ_C 182.2, 170.0, 168.9, and 97.6 indicated the presence of a γ-pyrone system (C-1 to C-5). The presence of the γ-pyrone moiety was also confirmed by the comparison of NMR and IR data of reported γ-pyrone-type compounds [5, 6]. Unambiguous signals in the ^{1}H- and ^{13}C-NMR spectra of **2** were assigned based on HMBC experiments (Table 2). Thus, the structure of compound **2** was determined as shown in Fig. 1.

Deoxyfusapyrone (**3**) had a molecular formula of $C_{34}H_{54}O_8$ as determined based on the HR-FAB-MS and NMR data (Table 3); this suggested that **3** was a deoxy compound of **2**. Most of the signals in the ^{1}H- and ^{13}C-NMR spectra of **3** were similar to those of **2** (Table 3). However, a feature of these spectra is the disappearance of the hydroxymethyl group present in **2** and the appearance of characteristic signals due to a methyl group [δ_H 0.97 (3H, d, J=6.6 Hz, H_3-26), δ_C 22.2 (C-26)]. In the HMBC spectrum of **3** (Table 3), correlations between H_3-26 and C-11, C-12, and C-13 indicated that the methyl group was linked to C-12. Consequently, **3** was elucidated as a 26-deoxy derivative of compound **2**.

Based on these structures, we conducted a literature

Table 2 ^{13}C and ^{1}H NMR data for fusapyrone (**2**) in CD_3OD^a

No.	δ_C	δ_H	HMBC
1	170.0 s		
2	97.6 s		
3	182.2 s		
4	108.1 d	5.81 (1H, s)	2, 3, 5, 6
5	168.9 s		
6	44.9 s		
7	78.0 d	4.37 (1H, d, 7.2)	5, 6, 8, 9, 23, 24
8	127.0 d	5.58 (1H, dd, 15.6, 7.2)	10
9	138.4 d	6.26 (1H, d, 15.6)	7, 10, 25
10	134.4 s		
11	134.6 d	5.29 (1H, d, 9.2)	9, 25
12	42.1 d	3.47~3.53 (1H, m)	11, 26
13	127.0 d	5.04 (1H, d, 9.0)	15, 27
14	137.0 s		
15	41.2 t	2.05 (1H, dd, 13.3, 6.5) 1.92 (1H, dd, 13.3, 8,0)	13, 14, 16, 17, 27, 28
16	32.3 d	1.59~1.61[b]	
17	38.2 t	1.10~1.19 (1H, m) 1.27~1.33[b]	
18	28.4 t	1.27~1.33[b]	
19	30.7 t	1.27~1.33[b]	
20	33.1 t	1.27~1.33[b]	
21	23.7 t	1.27~1.33[b]	
22	14.5 q	0.89 (3H, t, 6.5)	20, 21
23	22.3 q	1.11 (3H, s)	5, 6, 7, 24
24	20.2 q	1.19 (3H, s)	5, 6, 7, 23
25	13.4 q	1.67 (3H, s)	9, 10, 11
26	66.9 t	3.33~3.43 (2H, m)	11, 13
27	24.0 q	1.77 (3H, s)	13, 14, 15
28	21.3 q	0.86 (3H, d, 6.5)	15, 16, 17
1′	76.7 d	4.48 (1H, d, 9.7)	1, 2, 3, 2′, 5′
2′	73.3 d	4.12 (1H, t, 9.7)	3′
3′	74.7 d	3.59~3.68[b]	
4′	36.2 t	1.85~1.94 (1H, m) 1.59~1.61[b]	
5′	77.7 d	3.59~3.68[b]	
6′	65.6 t	3.59~3.68[b]	

[a] ^{1}H and ^{13}C NMR were measured at 400 MHz and 100 MHz, respectively.
[b] Multiplicity patterns were unclear due to signals overlapping.

Table 3 ^{13}C and ^{1}H NMR data for deoxyfusapyrone (**3**) in CD_3OD^a

No.	δ_C	δ_H	HMBC
1	169.9 s		
2	97.7 s		
3	181.7 s		
4	107.9 d	5.83 (1H, s)	2, 3, 5, 6
5	169.0 s		
6	44.9 s		
7	77.8 d	4.36 (1H, d, 7.0)	5, 6, 8, 9, 23, 24
8	126.5 d	5.55 (1H, dd, 15.5, 7.0)	7, 10
9	138.6 d	6.20 (1H, d, 15.5)	7, 25
10	131.6 s		
11	139.0 d	5.29 (1H, d, 9.7)	9, 25
12	33.0 d	3.59~3.86[b]	
13	132.0 d	5.05 (1H, d, 8.8)	15, 27
14	133.9 s		
15	41.0 t	1.95 (1H, dd, 13.9, 6.4) 1.90 (1H, m)	13, 14, 16, 17, 27, 28
16	32.3 d	1.59~1.62[b]	
17	38.3 t	1.01~1.10 (1H, m) 1.20~1.40[b]	19
18	28.4 t	1.20~1.40[b]	
19	30.7 t	1.20~1.40[b]	
20	33.1 t	1.20~1.40[b]	
21	23.7 t	1.20~1.40[b]	
22	14.4 q	0.87 (3H, t, 7.1)	20, 21
23	22.6 q	1.11 (3H, s)	5, 6, 7, 24
24	20.1 q	1.19 (3H, s)	5, 6, 7, 23
25	13.1 q	1.73 (3H, s)	9, 10, 11
26	22.2 q	0.97 (3H, d, 6.6)	11, 12, 13
27	23.9 q	1.63 (3H, s)	13, 14, 15
28	21.2 q	0.84 (3H, d, 6.5)	15, 16, 17
1′	76.7 d	4.49 (1H, d, 9.7)	1, 2, 3, 2′, 5′
2′	73.3 d	4.12 (1H, t, 9.7)	2, 1′
3′	74.7 d	3.59~3.68[b]	
4′	36.2 t	1.86~1.90 (1H, m) 1.59~1.62[b]	
5′	77.8 d	3.49~3.53 (1H, m)	
6′	65.6 t	3.59~3.68[b]	

[a] ^{1}H and ^{13}C NMR were measured at 400 MHz and 100 MHz, respectively.
[b] Multiplicity patterns were unclear due to signals overlapping.

search and found that compound **1** matched with a mycotoxin, namely, fusapyrone from *F. semitectum*, which was reported by Evidente *et al.* [4]. However, our ^{1}H- and ^{13}C-NMR data of **1** were inconsistent with those of the reported fusapyrone. Furthermore, based on the detailed analysis of the NMR data of these compounds, the proton and carbon resonances of the reported fusapyrone [4] were indistinguishable from those of **2**. This provided evidence that the reported fusapyrone and **2** were identical. Thus, our study is the first to report the spectral data of **1**; therefore, **1** has been named neofusapyrone, and the structures assigned for fusapyrone and deoxyfusapyrone in ref. 4 should be reassigned to **2** and **3**, respectively. In addition, complete assignment of the NMR spectroscopic data for the acetyl

Table 4 MIC values (μg/ml) of neofusapyrone (**1**), fusapyrone (**2**) and deoxyfusapyrone (**3**)

Microorganisms	**1**	**2**	**3**	Amphotericin B	Chloramphenicol
Aspergillus clavatus F318a	6.25	25	3.12	0.15	—[a]
Candida albicans ATCC 2019	>100	>100	>100	0.62	—[a]
Pseudomonas aeruginosa ATCC 15442	>100	50	12.5	—[a]	1.25
Staphylococcus aureus NBRC 13276	>100	>100	>100	—[a]	5

[a] Not tested.

derivative **1a** of **1** was also accomplished.

Over the past 20 years, some structurally similar fungal metabolites containing a pyrone moiety, long chain, and polyalcohol moiety have been reported. Dactylfungins A and B and YM-202204 were isolated from *Dactylaria parvispora* and the marine-derived fungus *Phoma* sp., respectively [6, 7]. It is interesting to note that these pyrone derivatives exhibited antifungal activity. Fusapyrone reported in previous studies by Evidente *et al.* [8, 9] also possessed antifungal activity. The minimum inhibitory concentrations (MIC) of **1**, **2**, and **3** against bacteria and fungi were determined by the agar dilution method, and the results are shown in Table 4. Comparison of the MIC values of these compounds with those of some known antimicrobial compounds (Table 4) showed that **1**~**3** possessed antimicrobial activities. Fusapyrone (**2**) was less potent than **1** against *Aspergillus clavatus*. The behavior of **1** and **2** appears to indicate that their activities are related to the change in the pyrone ring moiety. The fact that the antimicrobial activity of **2** was lower than that of **3** suggests that the presence of an OH group at position 26 might be important for the antimicrobial activity of **3**. Further detailed biological studies on **1**~**3** are in progress.

Experimental

General

Optical rotations were measured with a Horiba model SEPA-300 polarimeter, whereas IR and UV spectra were recorded with JASCO J-20A and Shimadzu UV mini-1240 spectrophotometer, respectively. Mass spectra were obtained using a JEOL JMS-700 instrument, and ^{1}H- and ^{13}C-NMR spectra were acquired with a JEOL EX-400 spectrometer. Chemical shifts are given on a δ (ppm) scale with TMS as an internal standard. Column chromatography was conducted on silica gel 60 (Kanto Chemical Co., Inc., Japan) and ODS (Fuji Silysia, Japan). TLC was carried out using precoated silica gel plates (Merck), and spots were detected by spraying with 10% vanillin in H_2SO_4 followed by heating, or by UV irradiation.

The Producing Strain

A Fungal Strain *Fusarium* sp. (section *Liseola*) FH-146 was isolated from driftwood collected in May, 2004 from the Oga peninsula, Akita, Japan. The isolation procedure was as follows. The driftwood samples were surface-sterilized successively with 70% EtOH for 1 minute, 5% sodium hypochlorite for 5 minutes and 70% EtOH for 1 minute, then rinsed in sterile water for two times. The sterilized samples were dried on sterilized paper and cut into 1 cm pieces. The peaces were placed on plates of Potato-Dextrose-Agar (PDA) containing chloramphenicol (100 mg/liter). After incubation at 25°C for 7 days, the hyphal tips of the fungi on the plates were removed from the agar plates and transferred to PDA plates (slant). The strain FH-146 was isolated and grew on slants of PDA as white colored culture. Identification of this strain was carried out at Centraalbureau voor Schimmelcultures (The Netherlands). The strain FH-146 has been deposited at our laboratory of the Faculty of Agriculture, Yamagata University, Yamagata, Japan.

Fermentation, Extraction and Isolation

The fungal strain FH-146 was cultivated on sterilized unpolished rice (total 580 g, 20 g/petri dish×29) at 25°C for 3 weeks. The moldy unpolished rice was extracted MeOH, and MeOH extract was concentrated. The resulting aqueous concentrated was partitioned into *n*-hexane layer, EtOAc layer and aqueous layer. The EtOAc layer was purified by bioassay-directed fractionation employing a combination of several column chromatographies. The EtOAc layer (2.3 g) was chromatographed on a silica gel column with mixture of *n*-hexane and EtOAc, and EtOAc and MeOH to obtain an active fraction (EtOAc : MeOH, 50/50). The fraction (430 mg) was subjected to ODS column chromatography by eluting with H_2O and an increasing ratio of MeOH. The 50% MeOH eluate (300

mg) was chromatographed on a silica gel column (EtOAc-MeOH) to yield neofusapyrone (**1**, 20 mg), fusapyrone (**2**, 5.3 mg) and deoxyfusapyrone (**3**, 2.5 mg).

The Physico-chemical Properties of 1, 2, and 3

Neofusapyrone (**1**): oil; $[\alpha]_D^{20}$ −23.6 (*c* 1.0, MeOH); UV (MeOH) λ_{max} (ε) 239 (31,000), 289 (10,000); IR (KBr) ν_{max} 3367, 2958, 2924, 1675, 1587, 1455, 1375, 1050 cm^{-1}; FAB-MS: 605 $[M-H]^-$. HR-FAB-MS: 605.3685 ($[M-H]^-$, $C_{34}H_{53}O_9$, calcd. 605.3690).

Fusapyrone (**2**): oil; $[\alpha]_D^{20}$ −22.9 (*c* 0.31, MeOH); UV (MeOH) λ_{max} (ε) 239 (30,000), 283 (8,000) nm; IR (KBr) ν_{max} 3403, 2960, 2925, 1652, 1506, 1452, 1031 cm^{-1}; FABMS: 605 $[M-H]^-$. HR-FAB-MS: 605.3698 ($[M-H]^-$, $C_{34}H_{53}O_9$, calcd. 605.3690).

Deoxyfusapyrone (**3**): oil; $[\alpha]_D^{20}$ −23.3 (*c* 0.23, MeOH); UV (MeOH) λ_{max} (ε) 240 (30,000), 284 (8,000) nm; IR (KBr) ν_{max} 3413, 2964, 2927, 1648, 1508, 1457,1263, 1024 cm^{-1}; FAB-MS: 589 $[M-H]^-$. HR-FAB-MS: 589.3736 ($[M-H]^-$, $C_{34}H_{53}O_8$, calcd. 589.3740).

Acetylation of Neofusapyrone (1)

Neofusapyrone (**1**, 42 mg) in pyridine (2 ml) was acetylated with acetic anhydride (2 ml) at room temperature overnight. The reaction mixture was poured into water and extracted with EtOAc (5 ml×3). The organic layer was washed with saturated NaCl and dried over Na_2SO_4 and concentrated *in vacuo* to give a residue, which was purified by silica gel column chromatography to yield the pentaacetate (**1a**, 36 mg) as an amorphous powder.

NMR and MS data of 2′,3′,6′,7,26-*O*-pentaacetyl-neofusapyrone (**1a**) are as follows: ^{13}C-NMR (100 MHz, $CDCl_3$): δ 12.8 (C-25), 14.0 (C-22), 19.4 (C-24), 19.5 (C-28), 20.4, 20.6, 20.7, 20.8, 20.9 (5×$C\underline{H}_3$C(O)–), 22.6 (C-21), 22.8 (C-23), 23.6 (C-27), 27.2 (C-18), 29.5 (C-19), 30.9 (C-16), 31.8 (C-20), 32.5 (C-4′), 37.0 (C-12), 37.1 (C-17), 40.0 (C-15), 43.4 (C-6), 64.8 (C-6′), 67.1 (C-26), 70.8 (C-2′), 71.3 (C-3′), 74.4 (C-5′), 74.9 (C-1′), 77.0 (C-7), 97.5 (C-2), 100.0 (C-4), 120.4 (C-8), 124.2 (C-13), 133.1 (C-10), 133.6 (C-11), 137.1 (C-14), 140.0 (C-9), 162.8 (C-1), 168.2 (C-3), 169.3, 169.5, 170.0, 170.3, 170.6 (5×$CH_3\underline{C}$(O)–), 171.1 (C-5) ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.82 (3H, t, 6.5, H_3-28), 0.86 (3H, t, 6.5, H_3-22), 1.03∼1.14 (1H, m, H_2-17), 1.19 (3H, s, H_3-23 or 24), 1.21 (3H, s, H_3-23 or 24), 1.23∼1.26 (8H, m, H_2-18, 19, 20, 21), 1.55∼1.63 (1H, m, H-16), 1.65 (3H, s, H_3-27), 1.74 (3H, s, H_3-25), 1.84∼1.90 (2H, m, H_2-15), 1.94∼1.97 (1H, m, H-4′), 1.97, 1.98, 1.99, 2.01, 2.09 (15H, each s, $CH_3\underline{C}$(O)–), 2.26∼2.28 (1H, m, H-4′), 3.64∼3.70 (1H, m, H-12), 3.86∼3.96 (3H, m, H-5′, H_2-26), 4.17 (1H, dd, 12.2, 2.9, H-6′), 4.23 (1H, dd, 12.2, 7.4, H-6′), 4.85 (1H, d, 9.3, H-1′), 4.99 (1H, d, 9.2, H-13), 5.06∼5.15 (2H, m, H-2′ and 3′), 5.28 (1H, d, 9.2, H-11), 5.42 (1H, dd, 15.5, 7.7, H-8), 5.55 (1H, d, 7.7, H-7), 5.88 (1H, s, H-4), 6.27 (1H, d, 15.5, H-9), 8.87 (1H, br. s, OH-4). FAB-MS: 839 $[M+Na]^+$.

Assay for Antimicrobial Activity

MIC was determined by the agar dilution method, using nutrient agar for bacteria, Sabouraud agar for *Candida albicans* and PD agar for *Aspergillus clavatus*. Bioassay procedure was the same as article [1].

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