VOL. 54 NO. 7, JULY 2001 THE JOURNAL OF ANTIBIOTICS pp. 548 — 553

SNF4435C and D, Novel Immunosuppressants Produced by a Strain of *Streptomyces spectabilis*

II. Structure Elucidation

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(Received for publication March 7, 2001)

SNF4435C and D are new immunosuppressants isolated from the culture broth of a strain of *Streptomyces spectabilis*. Their molecular formulas were determined as $C_{28}H_{31}NO_6$ based on the HRFAB-MS analyses. The structures of SNF4435C and D were elucidated to be novel nitrophenyl pyrones having an intriguing tricyclic ring system and diastereoisomers of each other by spectroscopic analyses including various NMR measurements.

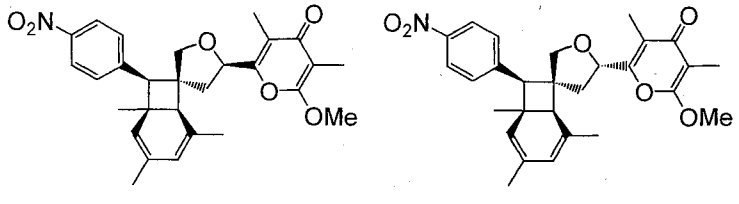
During our screening of microbial products aiming at new immunosuppressants, SNF4435C and D were discovered in the culture broth of a strain *Streptomyces spectabilis* originally isolated from a soil sample collected in the main island of Okinawa, Japan. These compounds showed potent immunosuppressive activities *in vitro* and selectively suppressed B-cell proliferation induced by LPS versus T-cell proliferation induced by Con A. The taxonomy of the producing strain, fermentation, isolation, and biological activities have been described in the preceding paper[1]. In this publication, we describe the physico-chemical properties and the structural elucidation of SNF4435C and D (Fig. 1).

Results

Structural Elucidation of SNF4435C (1)

The physico-chemical properties of **1** are summarized in Table 1. The IR spectrum indicated the presence of a ketone conjugated with a double bond at 1695 cm^{-1} and a nitro group at 1520 and 1350 cm^{-1}. The molecular formula was established as $C_{28}H_{31}NO_6$ on the basis of HRFAB-MS. The ^1H and ^{13}C NMR spectral data for **1** are shown in Table 2.

Fig. 1. Relative structures of SNF4435C (**1**) and D (**2**).



1 **2**

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The ^1H NMR spectrum of **1** showed 31 proton signals which included *p*-substituted benzene at δ 8.18 (2H, d, J= 8.9 Hz) and δ 7.64 (2H, d, J=8.9 Hz), two olefinic protons at δ 5.56 and δ 5.00, and five methyl protons at δ 1.77, 1.70, 1.69, 1.67, and 1.24. The ^{13}C NMR and DEPT spectra of **1** (Table 2) revealed 26 signals corresponding to 28 carbons that were classified into six methyl, two methylene, nine methine, and eleven quaternary carbons. Fourteen degrees of unsaturation were inferred from the molecular formula.

The four partial structures (A~D) were deduced from the analyses of HMQC and HMBC spectra and are shown in Fig. 2. The HMBC correlations for **1** are shown in Table 2.

The proton signals for H-19 and H-18 were indicative of the *p*-substituted phenyl moiety. Moreover, H-19 and H-18 being relatively shifted downfield suggested that a nitro group, having strong inductive effect, attached to C-20. The HMBC correlations from H-16 to C-17 and C-18 showed the unit A. The unit B was determined as a 1,3-cyclohexadiene moiety by the long-range correlations from H-24 to C-10 and C-12, from H-25 to C-12, C-13 and C-14, from H-26 to C-14, C-15 and C-10, and from H-10 to C-11. The unit C was established as a hydrofuran ring by the HMBC correlations from H-7β and H_2-9 to C-6, from H-7α to C-9, and from H_2-7 to C-8. For the unit D, the long-range correlations were observed from H-21 to C-1, from H-22 to C-1, C-2 and C-3, and from H-23 to C-3, C-4 and C-5. Additionally, the degrees of unsaturation of **1** and the chemical shifts at C-1 (δ 161.7) and C-5 (δ 155.5) indicated that C-1 and C-5 were conjugated to the same oxygen atom, resulting in the formation of γ-pyrone system. Connectivities between four partial structures A~D were established using HMBC correlations. The HMBC correlations from H_2-7 and H-6 to C-5 indicated the connection of the unit C and D through C-5 and C-6. Furthermore, the HMBC correlations from H_2-7 to C-16, from H-7β to C-10, and from H-26 to C-16 showed that C-8, C-10, C-15, and C-16 formed a four-membered ring.

Fig. 2. Partial structures of SNF4435C (**1**) and D (**2**).

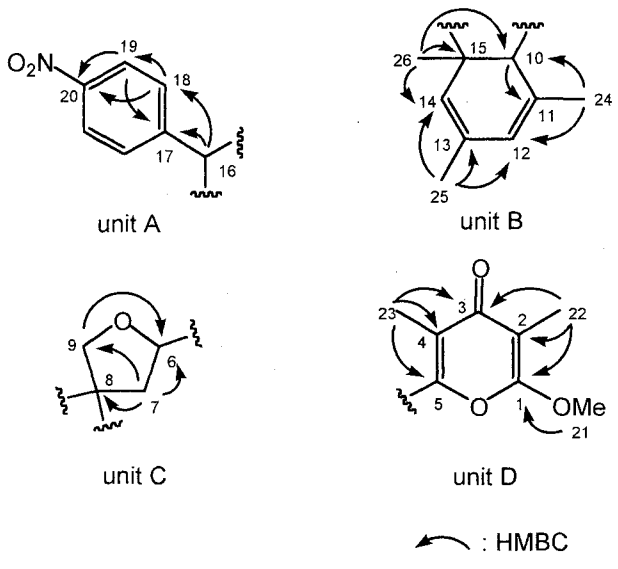


Table 1. Physico-chemical properties of SNF4435C (**1**) and D (**2**).

	SNF4435C	SNF4435D
Appearance	Pale-yellow powder	Pale-yellow powder
$[\alpha]^{26}_D$ (c 0.1, $CHCl_3$)	−105.6°	+84.8°
Molecular weight	477	477
Molecular formula	$C_{28}H_{31}NO_6$	$C_{28}H_{31}NO_6$
FAB-MS (m/z)	478 $(M+H)^+$	478 $(M+H)^+$
HRFAB-MS (m/z)		
Found:	478.2248 $(M+H)^+$	478.2217 $(M+H)^+$
Calcd.:	478.2230 for $C_{28}H_{32}NO_6$	478.2230 for $C_{28}H_{32}NO_6$
UV λ_{max}^{MeOH} nm (ε)	271 (19300)	271 (21500)
IR ν_{max} (KBr) cm^{-1}	2950, 2850, 1695, 1600, 1520, 1350	2950, 2850, 1695, 1600, 1520, 1350
Solubility		
soluble	MeOH, DMSO, $CHCl_3$	MeOH, DMSO, $CHCl_3$
insoluble	H_2O, *n*-hexane	H_2O, *n*-hexane

Table 2. ^{1}H and ^{13}C NMR data for SNF4435C (**1**) in DMSO-d_6.

position	δ_H[a]		NOE	δ_C[b]	HMBC
1				161.7 (s)	22, 21
2				98.2 (s)	22
3				179.1 (s)	22, 23
4				118.3 (s)	23
5				155.5 (s)	6, 7α, 7β, 23
6	4.78	(1H, dd, J = 7.3, 9.1 Hz)	7α, 23	72.9 (d)	7β, 9α, 9β
7α	2.61	(1H, dd, J = 7.3, 13.2 Hz)	6, 7β, 16	44.5 (t)	6, 9α, 10, 16
7β	2.43	(1H, dd, J = 9.1, 13.2 Hz)	7α		
8				51.5 (s)	7α, 7β, 10, 16
9α	4.10	(1H, d, J = 9.8 Hz)	9β, 18	69.7 (t)	7α, 10, 16
9β	3.82	(1H, d, J = 9.8 Hz)	9α		
10	2.90	(1H, s)	16, 24, 26	50.1 (d)	7β, 9β, 12, 14, 24, 26
11				131.1 (s)	10, 24
12	5.56	(1H, s)	25, 24	122.9 (d)	10, 14, 24, 25
13				130.0 (s)	12, 25
14	5.00	(1H, s)	18, 25, 26	122.1 (d)	10, 12, 16, 25, 26
15				42.0 (s)	10, 14, 16, 26
16	3.86	(1H, s)	7α, 10, 18, 26	61.9 (d)	7α, 7β, 9α, 9β, 14, 18, 26
17				145.8 (s)	16, 18, 19
18	7.64	(2H, d, J = 8.9 Hz)	9α, 14, 16, 19	129.5 (d)	16
19	8.18	(2H, d, J = 8.9 Hz)	18	123.1 (d)	18
20				146.1 (s)	18, 19
21	3.95	(3H, s)	24	55.8 (q)	
22	1.69	(3H, s)		6.8 (q)	
23	1.77	(3H, s)	6	8.9 (q)	
24	1.70	(3H, s)	21, 10, 12	21.8 (q)	10, 12
25	1.67	(3H, d, J = 1.2 Hz)	12, 14	22.4 (q)	12, 14
26	1.24	(3H, s)	10, 14, 16	29.9 (q)	10, 14, 16

[a] ^{1}H NMR at 500 MHz referenced to TMS.

[b] ^{13}C NMR at 125 MHz referenced to DMSO (δ 39.5).

Thus, the total structure of **1** was determined to be as shown in Fig. 1.

Structural Elucidation of SNF4435D (**2**)

The molecular formula of **2** was determined as $C_{28}H_{31}NO_6$ by HRFAB-MS. Most physico-chemical properties of **2** were the same as those of **1** and its data are summarized in Table 1. However, there was significant difference of the optical rotations between **1** and **2**. Furthermore, as shown in Table 3, the ^{1}H and ^{13}C NMR spectra of **2** were similar to those of **1**. The analyses of 2D NMR spectra showed that the total structure of **2** was the same as that of **1** (Fig. 1). All of the protons and the carbons to be assigned are listed in Table 3.

Relative Stereochemistries of SNF4435C (**1**) and D (**2**)

Information on the relative structures of **1** and **2** was obtained by NOESY spectra and NOE difference spectroscopic experiments (Fig. 3). In the case of **1**, NOE correlations from H-26 to H-10 and H-16 and from H-7α to H-16 indicated that all of these protons resided on the same face of the molecule. Moreover, the observation of correlation between H-21 and H-24 showed the γ-pyrone was spatially close to the unit B, the 1,3-cyclohexadiene

Table 3. ^{1}H and ^{13}C NMR data for SNF4435D (**2**) in DMSO-d_6.

position	δ_H[a]		NOE	δ_C[b]		HMBC
1				161.6	(s)	22, 21
2				97.8	(s)	22
3				178.9	(s)	22, 23
4				118.2	(s)	23
5				155.7	(s)	6, 7α, 7β, 23
6	4.96	(1H, dd, J = 7.3, 8.9 Hz)	7β, 23	71.9	(d)	7α, 9α, 9β
7α	2.32	(1H, dd, J = 8.9, 13.4 Hz)	7β	43.9	(t)	6, 9α, 9β, 10, 16
7β	2.62	(1H, dd, J = 7.3, 13.4 Hz)	6, 7α, 10			
8				50.9	(s)	7α, 7β, 9α, 9β, 10, 16
9α	3.72	(1H, d, J = 9.2 Hz)	9β, 18	69.8	(t)	6, 7β, 10, 16
9β	4.04	(1H, d, J = 9.2 Hz)	9α			
10	2.83	(1H, s)	7β, 16, 24, 26	53.8	(d)	7α, 7β, 9α, 9β, 12, 14, 16, 24, 26
11				131.9	(s)	10, 24
12	5.65	(1H, s)	25, 24	122.9	(d)	10, 14, 24, 25
13				130.13	(s)	12, 25
14	4.93	(1H, s)	18, 25, 26	122.1	(d)	10, 12, 16, 25, 26
15				42.1	(s)	10, 14, 16, 26
16	3.93	(1H, s)	10, 18, 26	59.6	(d)	7α, 7β, 9α, 9β, 10, 14, 18, 26
17				144.3	(s)	16, 18, 19
18	7.56	(2H, d, J = 8.7 Hz)	9α, 14, 16, 19	130.10	(d)	16, 19
19	8.15	(2H, d, J = 8.7 Hz)	18	123.4	(d)	18
20				146.2	(s)	18, 19
21	3.47	(3H, s)		55.0	(q)	
22	1.64	(3H, s)		6.7	(q)	
23	1.85	(3H, s)	6	8.9	(q)	
24	1.80	(3H, s)	10, 12	22.0	(q)	10, 12
25	1.69	(3H, d, J = 1.2 Hz)	12, 14	21.8	(q)	12, 14
26	1.25	(3H, s)	10, 14, 16	30.1	(q)	10, 14, 16

[a] ^{1}H NMR at 500 MHz referenced to TMS.

[b] ^{13}C NMR at 125 MHz referenced to DMSO (δ 39.5).

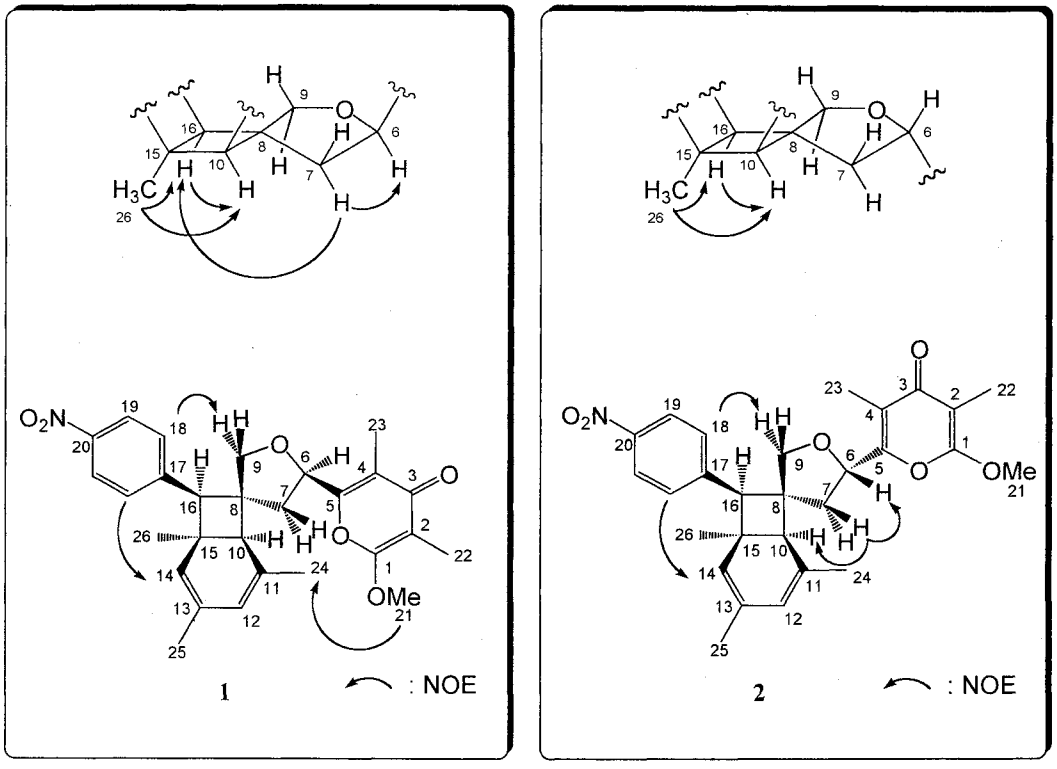
moiety. The relative stereochemistry of **1** was determined to be as shown in Fig. 3.

In the case of **2**, the NOE correlations from H-26 to H-10 and H-16 and from H-10 to H-7β and H-16 showed that all of these protons resided on the same face of the molecule. The relative stereochemisty of **2** around the four-membered ring was very similar to that of **1**. As compared with H-21 of **1** (δ 3.95), H-21 of **2** (δ 3.47) was considerably shifted upfield, suggesting that H-21 was placed in the shielding area of the *p*-nitrobenzene. This result was indirectly supported by the NOE correlation between H-6 and H-7β. Consequently, the relative stereochemistry of **2** was determined to be that shown in Fig. 3. SNF4435C (**1**) and D (**2**) were indicated to be diastereoisomers of each other. The absolute stereochemistries of **1** and **2** remain unassigned.

Discussion

In this study, SNF4435C (**1**) and D (**2**), isolated from the culture broth of a strain *Streptomyces spectabilis* were determined as novel nitrophenyl pyrones. Although aureothin[2] and spectinabilin[3] have been known as antibiotics of this class, our structural study revealed that **1**

Fig. 3. Relative stereochemistries of SNF4435C (**1**) and D (**2**).



and **2** are new members of structurally unique nitrophenyl pyrones. These compounds, having an intriguing tricyclic ring system, are diastereoisomers of each other. Their structures are different from those of known immunosuppressants, such as cyclosporin A (CsA)[4] and tacrolimus (FK-506)[5]. Interestingly, **1** and **2** selectively suppressed B-cell proliferation *versus* T-cell proliferation[1]. This finding shows that the mechanism involved in the immunosuppressive actions of **1** and **2** is clearly different from those of CsA and FK-506 which suppress the immune system by blocking T-cell activation[6,7]. In addition, the inhibitory activity of **2** is four fold as potent as that of **1** on the mitogen induced lymphocyte blastogenesis. This fact suggests that the difference in stereochemistries between **1** and **2** affects their biological activities. The analysis on the structure-activity relationships between these compounds may contribute to the development of new immunosuppressants.

Experimental

UV spectrum was recorded on a Hitachi U-3210 spectrophotometer. IR spectrum was measured on a JASCO IR-810 infrared spectrophotometer. FAB-MS was obtained with a JEOL JMS-AX505WA mass spectrometer. Optical rotation was measured on a JASCO DIP-1000 digital polarimeter. NMR spectrum was recorded on a JEOL JNM-A500 NMR spectrometer.

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