NF00659A$_1$, A$_2$, A$_3$, B$_1$ and B$_2$, Novel Antitumor Antibiotics Produced by *Aspergillus* sp. NF 00659

II. Structural Elucidation

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NF00659A$_1$, A$_2$, A$_3$, B$_1$ and B$_2$, having insecticidal and antitumor activities, were isolated from a culture mycelium of *Aspergillus* sp. NF 00659. The novel structure of NF00659s were determined mainly by spectroscopic studies including various NMR measurements. NF00659s have a common structure which consists of acyl, α-pyrone and 4,5-*seco*-tricyclic diterpene moieties.

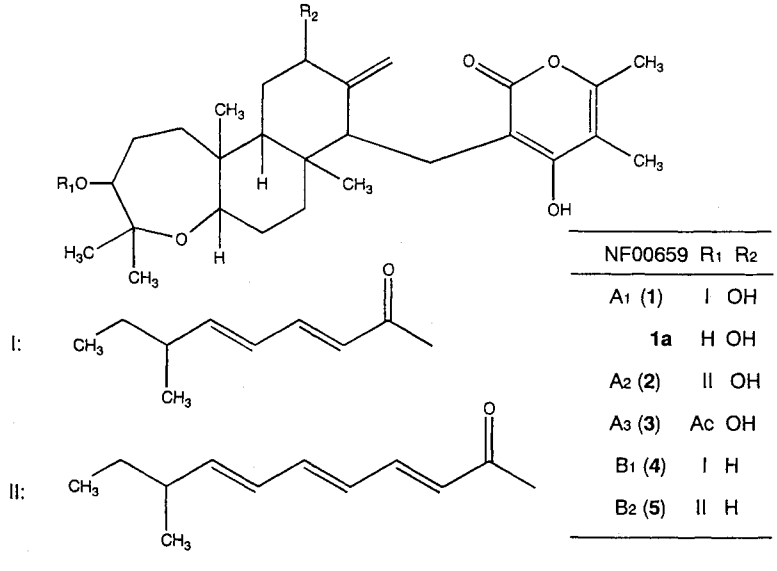
During the course of our screening for insecticides from microbial metabolites, a series of novel bioactive compounds, NF00659A$_1$ (**1**), A$_2$ (**2**), A$_3$ (**3**), B$_1$ (**4**) and B$_2$ (**5**), was isolated from a culture mycelium of *Aspergillus* sp. NF 00659. The structures were determined by spectroscopic analysis as novel 4,5-*seco*-tricyclic diterpene α-pyrone (Fig. 1). These compounds showed potent growth inhibitory activities against human ovarian carcinoma A2780 and human colorectal adenocarcinoma SW480 cells as well as insecticidal activites. The taxonomy, fermentation, isolation and biological activities of NF00659s were reported in the preceding paper[1]. In this paper, we describe the structural elucidation of NF00659s.

Results

Physico-chemical Properties of NF00659A$_1$, A$_2$, A$_3$, B$_1$ and B$_2$

NF00659A$_1$ (**1**), A$_2$ (**2**), B$_1$ (**4**) and B$_2$ (**5**) were isolated as white powders and A$_3$ (**3**) was as colorless crystals. The physico-chemical properties of NF00659s are summarized in Table 1. The molecular weights of NF00659s were obtained with FAB-MS in the positive and negative ion modes. In the positive ion mode, **1**, **2** and **3** gave a dehydrated ion peak $[M-OH]^+$ while **4** and **5** gave no dehydrated ion peak. The strong UV absorption was observed at 265 nm (ε 28,000) for **1** and **4**, and at 300 nm (ε 44,000) for **2** and **5**. The IR spectra of **1**, **2**, **4** and **5** suggested the presence of unsaturated ester (1710~1706

Fig. 1. Structures of NF00659s.



NF00659	R1	R2
A1 (1)	I	OH
1a	H	OH
A2 (2)	II	OH
A3 (3)	Ac	OH
B1 (4)	I	H
B2 (5)	II	H

Table 1. Physico-chemical properties of NF00659A_1 (**1**), A_2 (**2**), A_3 (**3**), B_1 (**4**) and B_2 (**5**).

	NF00659A1(**1**)	NF00659A2(**2**)	NF00659A3(**3**)	NF00659B1(**4**)	NF00659B2(**5**)
Appearance	white powder	white powder	white powder	white powder	white powder
MP (℃)	224-226	186-187	272-274	144-145	176-178
Molecular formula	$C_{36}H_{52}O_7$	$C_{38}H_{54}O_7$	$C_{29}H_{42}O_7$	$C_{36}H_{52}O_6$	$C_{38}H_{54}O_6$
Molecular weight	596	622	502	580	606
FAB-MS (*m / z*)	597 $[M+H]^+$	623 $[M+H]^+$	503 $[M+H]^+$	581 $[M+H]^+$	607 $[M+H]^+$
	595 $[M-H]^-$	621 $[M-H]^-$	501 $[M-H]^-$	579 $[M-H]^-$	605 $[M-H]^-$
HRFAB-MS (*m / z*)					
Found:	597.3771	623.3936	503.3009	581.3834	607.3987
Calcd:	597.3791	623.3948	503.3001	581.3842	607.3999
	for $C_{36}H_{53}O_7$	for $C_{38}H_{55}O_7$	for $C_{29}H_{43}O_7$	for $C_{36}H_{53}O_6$	for $C_{38}H_{55}O_6$
UV λ_{max}^{MeOH} nm(ε)	265 (28,000)	300 (44,000)	291 (8,600)	265 (28,000)	300 (44,000)
IR ν max(KBr) cm^{-1}	3400, 3250, 1706, 1676, 1640, 1618, 1571	3400, 3250, 1710, 1675, 1644, 1616, 1568	3400-3200, 1736, 1668, 1636, 1567	3200, 1709, 1664, 1644, 1576, 881	3200, 1707, 1666, 1645, 1617, 1574, 885
$[\alpha]_D^{20}$ ($CHCl_3$)	-4.8° (*c* 1.0)	-11.7° (*c* 0.34)	-9.1° (*c* 1.0)	-2.5° (*c* 0.2)	-16.4° (*c* 0.23)
TLC (Rf value)					
System I[a)]	0.46	0.46	0.13	0.35	0.35
System II[b)]	0.39	0.34	0.48	0.34	0.30

a) Merck, Kieselgel 60F_{254}, *n* -hexane-diethylether (2:5)
b) Merck, RP-18 F_{254s}, CH_3CN-THF-5mM NH_4OAc (7:2:1)

cm^{-1}). On the other hand, IR absorption at 1736 cm^{-1} of **3** suggested the presence of saturated ester instead of unsaturated ester.

Structure Elucidation

Structure of NF00659A_1 (**1**)

The FAB-MS gave pseudomolecular ion peaks at *m/z* 597 $[M+H]^+$, 619 $[M+Na]^+$ and 595 $[M-H]^-$. A dehydrated ion peak was observed at *m/z* 579 $[M-OH]^+$. The molecular formula of **1** was determined to be $C_{36}H_{52}O_7$ by HRFAB-MS (*m/z*: found 597.3771 $[M+H]^+$, calcd 597.3791 for $C_{36}H_{53}O_7$). ^1H and ^{13}C NMR spectra of **1** were measured in DMSO-d_6 and pyridine-d_5 and are summarized in Tables 2 and 3. Thirty six signals in the ^{13}C NMR spectrum were observed, and the result was in accord with that of the HRFAB-MS. They were classified as eight methyl, eight methylene, ten methine and ten quarternary carbons in DEPT spectra. The connectivity of thirty six carbon atoms and fifty protons was assigned by ^1H-^{13}C COSY spectrum. The remaining two protons in the molecule were assigned to two hydroxy protons. The IR spectrum of **1** showed the presence of hydroxyl groups (3400 and 3250 cm^{-1}) and carbonyl groups (1706 and 1676 cm^{-1}). The UV spectrum showed an absorption at 265 nm (ε 28,000) ascribed to a conjugated carbonyl group.

The analysis of ^1H-^1H COSY spectrum revealed six parts of proton spin network, tentatively named partial structures I ~ VI as shown in Fig. 2. The connectivity of these partial structures was determined by COLOC, HMBC and HOHAHA experiments (Fig. 3).

6-Methyl-2,4-octadienoyl moiety (Fig. 2-I) was connected to C-3 position because the ester carbonyl carbon of δ 165.41 (C-1″) was coupled to the three protons of δ 5.89 (2″-H), 7.18 (3″-H) and 4.93 (3-H). The coupling constants of $^2J_{2'',3''}$ and $^2J_{4'',5''}$ of 15.4 and 15.3 Hz for the dienoyl moiety indicated that their relationships are in the *trans* conformation. This partial structure was supported by a good agreement of NMR chemical shifts and UV maximum with those of dendryphiellins reported previously[2].

The structure of diterpene moiety (Fig. 2-II ~ VI) was suggested by the following evidences; the respective three methyl protons of δ 1.40 (18-H_3), 1.11 (20-H_3) and 0.97 (17-H_3) had three coupling carbons of δ 76.17 (C-4), 79.48 (C-3) and 25.51 (C-19), three ones of δ 39.06 (C-10), 34.35 (C-1) and 36.55 (C-9), and four ones of δ 36.63 (C-8), 33.90 (C-7), 36.55 (C-9) and 55.27 (C-14); the oxymethine proton observed at δ 3.81 (5-H) was coupled to three carbons of C-4, C-10 and C-1, suggesting the formation of 4,5-oxy-4,5-*seco*-tricyclic diterpene.

The presence of 5,6-dimethyl-4-oxy-α-pyrone was shown because the carbon signals of C-1′ and C-3′ were observed at δ 165.54 and 166.51 in pyridine-d_5 which

Table 2-1. ^{1}H NMR chemical shifts of NF00659s (400 MHz).

	A_1(**1**)	A_1(**1**)	A-OH(**1a**)	A_2(**2**)
No. of proton	DMSO-*d* 6	pyridine-*d* 5	pyridine-*d* 5	DMSO-*d* 6
1	1.34 (1H, m)	1.35 (1H, m)	1.34 (1H, br.d, J=13.6 Hz)	1.34 (1H, m)
	1.45 (1H, m)	1.64 (1H, m)	1.61 (1H, m)	1.43 (1H,m)
2	1.36 (1H, m)	1.71 (1H, m)	1.90 (1H, m)	1.36 (1H, m)
	1.91 (1H, m)	2.17 (1H, m)	2.28 (1H, m)	1.97 (1H, m)
3	4.93 (1H, br.d, J=10.6Hz)	5.38 (1H, br.d, J=10.8 Hz)	4.17 (1H, br.d, J=11.6 Hz)	4.93 (1H, br.d, J=10.6 Hz)
5	3.56 (1H, br.d, J=10.3Hz)	3.81 (1H, br.d, J=11.0 Hz)	3.80 (1H, dr.d, J=10.6 Hz)	3.56 (1H, br.d, J=10.6 Hz)
6	1.35 (1H, m)	1.61 (1H, m)	1.61 (1H, m)	1.35 (1H, m)
	1.60 (1H, m)	1.92 (1H, m)	1.92 (1H, m)	1.60 (1H, m)
7	1.41 (1H, m)	1.59 (1H, m)	1.58 (1H, m)	1.41 (1H, m)
	2.21 (1H, m)	2.77 (1H, dd, J=6.0, 13.2 Hz)	2.78 (1H, m)	2.22 (1H, m)
9	1.92 (1H, m)	2.35 (1H, m)	2.37 (1H, dd, J=3.3, 11.2 Hz)	1.97 (1H, m)
11	1.68 (2H, m)	1.75 (1H, m), 1.91 (1H, m)	1.85-1.92 (2H, m)	1.68 (2H, m)
12	4.32 (1H, br.s)	4.69 (1H, br.s)	4.69 (1H, br.s)	4.34 (1H, br.s)
14	1.70 (1H, m)	2.27 (1H, br.d, J=11.7 Hz)	2.29 (1H, br.d, J=12.2 Hz)	1.70 (1H, m)
15	2.53 (1H, br.d, J=12.5Hz)	3.16 (1H, br.d, J=13.2 Hz)	3.18 (1H, br.d, J=13.2 Hz)	2.58 (1H, br.d, J=13.2 Hz)
	2.81 (1H, br.t, J=12.5Hz)	3.34 (1H, br.t, J=12.7 Hz)	3.36 (1H, br.t, J=13.2 Hz)	2.82 (1H, br.dd, J=13.1 Hz)
16	4.46 (1H, d, J=2.2Hz)	4.76 (1H, d, J=2.5 Hz)	4.76 (1H, br.d, J=2.2 Hz)	4.47 (1H, d, J=2.2 Hz)
	4.87 (1H, d, J=2.2Hz)	5.05 (1H, d, J=2.5 Hz)	5.07 (1H, br.d, J=2.5 Hz)	4.87 (1H, d, J=2.2 Hz)
17	0.92 (3H, s)	0.97 (3H, s)	1.02 (3H, s)	0.93 (3H, s)
18	1.14 (3H, s)	1.40 (3H, s)	1.49 (3H, s)	1.15 (3H, s)
19	1.14 (3H, s)	1.32 (3H, s)	1.54 (3H, s)	1.15 (3H, s)
20	0.92 (3H, s)	1.11 (3H, s)	1.13 (3H, s)	0.93 (3H, s)
6'	1.78 (3H, s)	1.87 (3H, s)	1.88 (3H, s)	1.80 (3H, s)
7'	2.18 (3H, s)	2.01 (3H, s)	2.01 (3H, s)	2.15 (3H, s)
2"	5.89 (1H, d, J=15.3Hz)	6.06 (1H, d, J=15.6Hz)		5.88 (1H, d, J=15.5 Hz)
3"	7.18 (1H, dd, J=10.5, 15.3Hz)	7.55 (1H, dd, J=11.2, 15.6 Hz)		7.22 (1H, dd, J=11.4, 15.5 Hz)
4"	6.25 (1H, dd, J=10.3, 15.4Hz)	6.25 (1H, dd, J=11.2, 15.6 Hz)		6.33 (1H, dd, J=11.4, 14.7 Hz)
5"	6.16 (1H, dd, J=7.3, 15.4Hz)	6.01 (1H, dd, J=8.3, 15.6 Hz)		6.68 (1H, dd, J=11.0, 14.7 Hz)
6"	2.17 (1H, m)	2.06 (1H, m)		6.15 (1H, dd, J=11.0, 15.4 Hz)
7"	1.33 (2H, m)	1.27 (2H, dq, J=6.8, 6.8 Hz)		5.87 (1H, dd, J=6.6, 15.4 Hz)
8"	0.83 (3H, t, J=7.3Hz)	0.80 (3H, t, J=7.3 Hz)		2.15 (1H, m)
9"	0.99 (3H, d, J=6.6Hz)	0.93 (3H, d, J=6.8 Hz)		1.34 (2H, m)
10"				0.84 (3H, t, J=7.5 Hz)
11"				1.00 (3H, d, J=7.0 Hz)

Chemical shifts are given in ppm with reference to TMS as 0.00ppm.

was in accord with 4-hydroxy-α-pyrone ring system of aszonapyrone A[3]. The methyl proton of δ 1.87 (6'-H_3) was coupled to four carbons of δ 107.73 (C-4'), 166.51 (C-3'), 155.24 (C-5') and 17.02 (C-7'). To prove the constitution of the aromatic ring moiety, alcohol (**1a**) was prepared from **1** by a methanolysis with sodium methoxide as described in experimental section. The UV maximum of **1a** at 290 nm supported the presence of the α-pyrone ring moiety[3,4].

Furthermore, the methylene protons observed at δ 3.16 and 3.34 (15-H_2) was coupled to three carbons of C-1', C-2' and C-3', suggesting the connection of α-pyrone and diterpene moieties as shown in Fig. 3. All carbons of **1** were assigned and then two hydroxyl groups in the molecule were assigned at the C-12 and C-3' positions from their chemical shifts. Therefore the plane structure of NF00659A_1 (**1**) was deduced to be tricyclic diterpene α-pyrone as shown in Fig. 1.

Structure of NF00659A_2 (**2**)

The molecular formula of NF00659A_2 (**2**) was elucidated as $C_{38}H_{54}O_7$ from the HRFAB-MS, which was two more carbon atoms, two more protons than that of **1**. ^{1}H and ^{13}C NMR spectra of **2** closely similar to those of **1** except for the C-2" ~ 5" region of the side-chain, where dienoyl moiety was extended to trienoyl. In agreement, the olefinic protons of the side-chain clearly showed correlations to the neighbouring olefinic and a methine proton (8"-H) signals in ^{1}H-^{1}H COSY experiments. The coupling constants $^2J_{2'',3''}$, $^2J_{4'',5''}$ and $^2J_{6'',7''}$ of 15.5, 14.7 and 15.4 Hz for **2** indicated that their relationships are in the *trans* conformation. Signals for remainder of the structure of NF00659A_2 equivalent to that in **1** could be assigned by a series of ^{1}H-^{1}H, ^{1}H-^{13}C

Table 2-2. ^{1}H NMR chemical shifts of NF00659s (400 MHz).

	A_3(**3**)	B_1(**4**)	B_2(**5**)
No. of proton	pyridine-*d* 5	DMSO-*d* 6	DMSO-*d* 6
1	1.31 (1H, m)	1.34 (1H, m)	1.35 (1H, m)
	1.60(1H, m)	1.45 (1H, m)	1.46 (1H, m)
2	1.89 (1H, m)	1.42 (1H, m)	1.42 (1H, m)
	2.10 (1H, br.dd, J=12.1,13.2 Hz	1.93 (1H, m)	1.93 (1H, m)
3	5.24 (1H, br.d, J=10.6 Hz)	4.90 (1H, br.d, J=10.7 Hz)	4.90 (1H, d, J=10.6 Hz)
5	3.76 (1H, br.d, J=11.0 Hz)	3.59 (1H, br.d, J=10.8 Hz)	3.58 (1H, br.d, J=11.4 Hz)
6	1.83-1.98 (2H, m)	1.32 (1H, m)	1.32 (1H, m)
		1.57 (1H, m)	1.57 (1H, m)
7	1.58 (1H, m)	1.35 (1H, m)	1.35 (1H, m)
	2.76 (1H, dd, J=5.9, 13.2 Hz)	2.20 (1H, m)	2.20 (1H, m)
9	2.35 (1H, dd, J=3.3, 12.8 Hz)	2.00 (1H, m)	2.00 (1H, m)
11	1.78-1.84 (2H, m)	1.50 (1H, m), 1.37 (1H, m)	1.50 (1H, m), 1.37 (1H, m)
12	4.68 (1H, br.s)	2.00 (1H, m), 2.38 (1H, m)	2.00 (1H, m), 2.39 (1H, m)
14	2.27 (1H, br.d, J=10.7 Hz)	1.98 (1H, m)	1.98 (1H, m)
15	3.15 (1H, br.d, J=13.2 Hz)	2.37 (1H, br.d, J=12.8 Hz)	2.38 (1H, br.d, J=12.6 Hz)
	3.33 (1H, br.t, J=12.8 Hz)	2.70 (1H,br.t, J=12.1 Hz)	2.70 (1H, br.t, J=12.3 Hz)
16	4.74 (1H, br.d, J=2.6 Hz)	4.15 (1H, br.s)	4.15 (1H, br.s)
	5.05 (1H, br.d, J=2.2 Hz)	4.43 (1H, br.s)	4.43 (1H, br.s)
17	0.95 (3H, s)	0.96 (3H, s)	0.98 (3H, s)
18	1.27 (3H, s)	1.13 (3H,s)	1.13 (3H, s)
19	1.31 (3H, s)	1.13 (3H,s)	1.13 (3H, s)
20	1.02 (3H, s)	0.92 (3H, s)	0.92 (3H, s)
6'	1.88 (3H, s)	1.83 (3H, s)	1.82 (3H, s)
7'	2.01 (3H, s)	2.11 (3H, s)	2.11 (3H, s)
2"	2.05 (3H, s)	5.92 (1H, d, J=15.3 Hz)	5.89 (1H, d, J=15.0 Hz)
3"		7.18 (1H, dd, J=9.9, 15.1 Hz)	7.22 (1H, dd, J=11.4, 16.4 Hz)
4"		6.25 (1H, dd, J=9.9, 15.5 Hz)	6.34 (1H, dd, J=11.4, 14.7 Hz)
5"		6.16 (1H, dd, J=7.0, 15.3 Hz)	6.69 (1H, dd, J=10.6,14.7 Hz)
6"		2.18 (1H, m)	6.15 (1H, dd, J=11.0, 15.0 Hz)
7"		1.32 (2H, m)	5.88 (1H, dd, J=7.0, 15.0 Hz)
8"		0.83 (3H, t, J=7.4 Hz)	2.16 (1H, m)
9"		0.99 (3H, d, J=6.8 Hz)	1.33 (2H, m)
10"			0.83 (3H, t, J=7.3 Hz)
11"			0.99 (3H, d, J=6.7 Hz)

Chemical shifts are given in ppm with reference to TMS as 0.00ppm.

COSY and HMBC experiments. Therefore the structure of NF00659A_2 (**2**) was determined as shown in Fig. 1.

Structure of NF00659A_3 (**3**)

The molecular formula of NF00659A_3 (**3**) was elucidated as $C_{29}H_{42}O_7$ from the HRFAB-MS, which was two more carbon atoms, two more protons and one more oxygen atom than that of **1a**. UV maximum and IR spectrum were closely similar to those of **1a**. However, an ester absorption band in the IR spectrum was observed at 1736 cm^{-1}. ^{1}H and ^{13}C NMR spectra of **3** were also closely similar to those of **1a**, except for additional acetyl signals at δ_H 2.08 (3H, s), and δ_C 170.24 (C-1″) and 21.18 (C-2″). The ester linkage of the acetyl group between the carbonyl carbon and the oxymethine (C-3) was deduced from a coupling between δ_C 170.24 (C-1″) and δ_H 5.24 (3-H) in HMBC. Therefore the structure of NF00659A_3 (**3**) was determined as 3-acetyl-**1a** shown in Fig. 1.

Structure of NF00659B_1 (**4**)

The molecular formula of NF00659B_1 (**4**) was elucidated as $C_{36}H_{52}O_6$ from the HRFAB-MS, which was one less oxygen atom than that of **1**. UV maximum and IR spectra were closely similar to those of **1**. However, **4** differed from **1**, **2** and **3** in lacking dehydrated ion peak in the positive FAB-MS spectrum. Furthermore, the NMR signal of the oxymethine group (C-12) of **1** was replaced by the ^{1}H and ^{13}C signals of a methylene group at δ_H 2.00 and 2.38, and δ_C 29.95, respectively. The chemical shifts of the *exo*-methylene protons of **4** showed high field shift from δ 4.46 and 4.87 to δ 4.15 and 4.43, supporting that the hydroxyl group was replaced to a proton at the position (C-12). Therefore the structure of NF00659B_1 (**4**) was determined as 12-

Table 3. ^{13}C NMR chemical shifts of NF00659s (100 MHz).

	A_1(**1**)	A_1(**1**)	A-OH(**1a**)	A_2(**2**)	A_3(**3**)	B_1(**4**)	B_2(**5**)
No. of Carbon	DMSO-*d* 6	pyridine-*d* 5	pyridine-*d* 5	DMSO-*d* 6	pyridine-*d* 5	DMSO-*d* 6	DMSO-*d* 6
1	34.35	35.42	35.81	34.40	35.04	34.33	34.27
2	26.90	27.90	31.73	26.95	27.80	27.01	27.10
3	78.31	79.48	77.93	78.36	79.62	78.47	78.46
4	75.20	76.17	77.89	75.25	75.99	75.20	75.15
5	74.10	75.15	74.31	74.14	75.08	73.55	73.51
6	26.78	27.81	27.95	26.81	27.80	26.81	26.76
7	33.90	34.98	35.13	33.98	34.98	33.33	33.27
8	36.63	37.54	37.60	36.66	37.55	36.71	36.66
9	36.55	37.82	37.72	36.57	37.82	42.19	42.12
10	39.06	40.00	40.02	39.09	39.96	36.71	36.66
11	31.45	32.95	32.99	31.50	32.96	23.44	23.20
12	71.11	72.48	72.54	71.24	72.49	29.95	29.89
13	146.31	147.55	147.67	146.09	147.55	148.50	148.47
14	55.27	56.22	56.26	55.18	56.20	54.97	54.92
15	24.10	25.45	25.44	24.15	25.45	22.04	22.20
16	116.21	116.72	116.59	116.34	116.73	108.96	108.91
17	27.72	28.21	28.31	27.75	28.18	28.31	28.27
18	22.71	23.31	22.70	22.70	23.16	22.78	22.73
19	25.05	25.51	26.10	25.08	25.45	25.07	25.03
20	20.05	20.69	20.75	20.04	20.66	20.34	20.29
1'	164.22	165.54	165.52	164.30	165.52	164.13	164.10
2'	99.42	101.16	101.19	99.72	101.16	101.81	101.67
3'	165.88	166.51	166.46	165.50	166.48	165.47	165.42
4'	106.86	107.73	107.71	106.77	107.72	106.40	106.39
5'	154.72	155.24	155.18	154.66	155.24	154.62	154.55
6'	9.84	10.31	10.28	9.87	10.31	10.26	10.22
7'	16.82	17.02	16.99	16.85	17.02	16.99	16.95
1"	165.41	166.51		165.36	170.24	164.85	165.10
2"	119.23	120.11		119.71	21.18	119.29	119.74
3"	145.22	145.92		144.87		145.26	144.97
4"	150.40	150.72		127.69		150.42	127.80
5"	126.53	127.26		141.67		126.55	141.80
6"	38.04	38.96		128.15		38.04	128.20
7"	28.60	29.41		145.83		28.63	145.93
8"	11.43	11.81		38.08		11.45	37.90
9"	19.22	19.57		28.89		19.24	28.60
10"				11.50			11.44
11"				19.43			19.36

Chemical shifts are given in ppm with reference to TMS as 0.0ppm.

deoxy-**1** shown in Fig. 1.

Structure of NF00659B_2 (**5**)

The molecular formula of NF00659B_2 (**5**) was elucidated as $C_{38}H_{54}O_6$ from the HRFAB-MS, which was one less oxygen atom than that of **2**. The positive FAB-MS gave no dehydrated ion peak as was the case of **4**. UV maximum of **5** suggested the presence of trienoyl moiety, which were coincident with that of **2**. The NMR signals of the trienoyl moiety of **5** were coincident with those of **2**. Signals for remainder of the structure of **5** equivalent to that in **4** could be assigned by a series of ^1H-^1H, HMQC and HMBC experiments. Therefore the structure of NF00659B_2 (**5**) was determined as 12-deoxy-**2** shown in Fig. 1.

Discussion

NF00659s were isolated as novel antitumor antibiotics from the extract of *Aspergillus* sp. NF 00659. The structures of NF00659s established by spectroscopic analysis, mainly NMR techniques, consist of common three parts, acyl side-chain, α-pyrone and 4,5-*seco*-tricyclic diterpene moieties. The skeleton of NF00659s has a novel 4,5-oxy-4,5-*seco* diterpene structure derived

Fig. 2. Partial structures and chemical shifts δ_H (δ_C) from ^1H-^1H and ^1H-^{13}C COSY experiments for NF00659A$_1$.

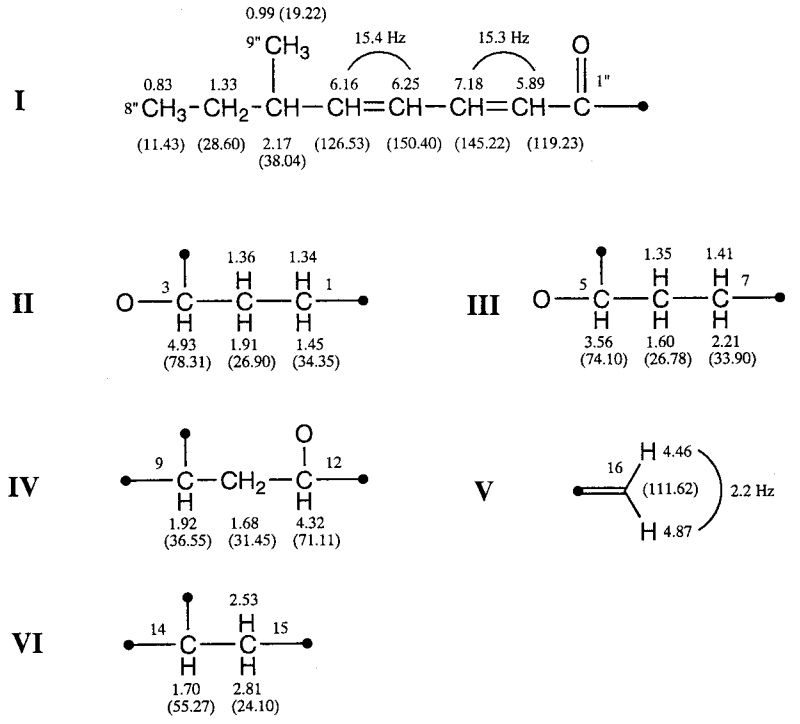
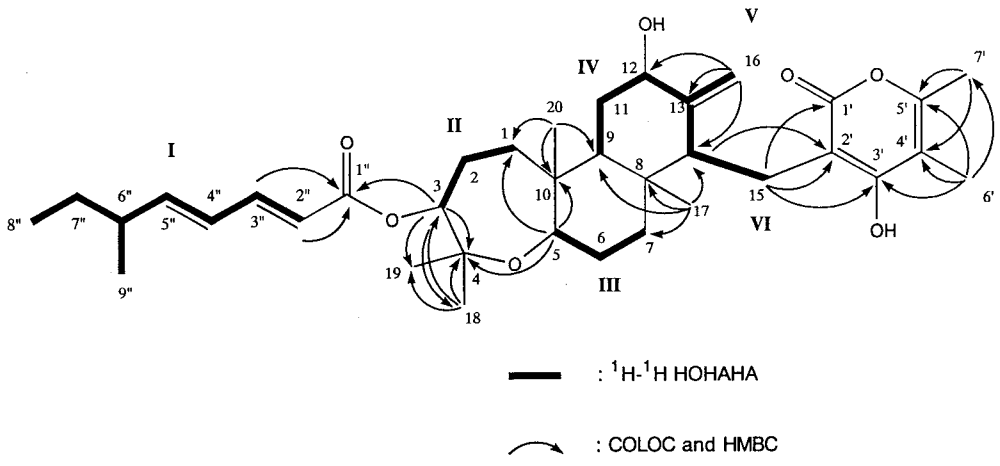


Fig. 3. ^1H-^1H HOHAHA, COLOC and HMBC experiments for NF00659A$_1$.



from fungi. The biogenesis of NF00659s is surmised to be the result of a combination of the mevalonate-geranyl-geranyl pyrophosphate route with acetate-polyketide route reported in the biogenesis of colletotrichins[5]. Formation of the ether linkage of NF00659s is probably derived by concendation of 10,11-15,16-diepoxy-geranyl-geranyl pyrophosphate proposed by CARMELY *et al.*[6]. The stereochemistry of NF00659s is under investigation.

Experimental

General

Melting points were determined with a Yanako micro-melting point apparatus and were uncorrected. Optical rotations were measured on a JASCO model DIP-370 digital polarimeter. UV-VIS spectra were measured on a Shimadzu UV-265 spectrometer. IR spectra were measured on a Perkin-Elmer 1640 spectrometer. FAB- and HRFAB-MS spectra were obtained with a JEOL JMS-AX505HA mass spectrometer. The ^1H and ^{13}C NMR spectra were recorded on JEOL GX400 and α400 spectrometers.

Methanolysis of **1**

NF00659A$_1$ (**1**, 10.0 mg) was dissolved in methanol (2 ml). Sodium methoxide-methanol (1 mmol/liter, 0.2

ml) was added and allowed to stand for 4 hours at room temperature. The resulting solution was extracted with *n*-hexane after addition of water (10 ml), and then extracted with ethyl acetate. The ethyl acetate phase was washed with brine, dried over anhydrous sodium sulfate and concentrated *in vacuo*. The residue (6.1 mg) was purified by a preparative TLC (Kiesel gel 60 F_{254}, Merck) developed with *n*-hexane - acetone (1 : 1). The main quenching band (Rf value 0.5) under UV light at 254 nm was scratched, extracted with *n*-hexane - acetone (1 : 1) and the extract was concentrated *in vacuo*. The resulting product was crystallized from *n*-hexane - ethyl acetate to yield **1a** (5 mg).

Alcohol (**1a**); colorless crystal, molecular weight 460 (FAB-MS *m/z*: 483 $[M+Na]^+$, 461 $[M+H]^+$, 443 $[M-OH]^+$, 459 $[M-H]^-$), molecular formula $C_{27}H_{40}O_6$ (HRFAB-MS *m/z*: found 461.2868 $[M+H]^+$, calcd 461.2903 for $C_{27}H_{41}O_6$); mp 276~278°C; UV λ_{max}^{MeOH} nm: 290 (ε 7,600); IR ν_{max} (KBr) cm^{-1}: 3404, 3300, 1674, 1640, 1568, 1261, 1068 and 1028.

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