New Antitumor Substances, FR901463, FR901464 and FR901465

III. Structures of FR901463, FR901464 and FR901465

HIDENORI NAKAJIMA, SHIGEHIRO TAKASE*, HIROSHI TERANO and HIROKAZU TANAKA

Exploratory Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., 5-2-3 Tokodai, Tsukuba, Ibaraki, 300-26, Japan

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During the course of screening fermentation broths for new antitumor agents, we discovered FR901463 (**1**) ($C_{27}H_{42}ClNO_8$), FR901464 (**2**) ($C_{27}H_{41}NO_8$) and FR901465 (**3**) ($C_{27}N_{41}NO_9$) from *Pseudomonas* sp. No. 2663. The taxonomy, fermentation, isolation and physico-chemical properties and biological activities have been described in the preceding papers[1,2]. Herein we report on the structure elucidation of **1**~**3** (see Fig. 1) on the basis of spectroscopic and chemical evidence.

Through the sequential purification of the ethyl acetate extract of fermentation broth was obtained FR901463 (**1**) as colorless needles (mp 102°C). Its IR spectrum and ^1H and ^{13}C NMR spectra appear in the preceding paper[1]. The molecular weight was indicated by FAB-MS (*m/z* 566 $(M+Na)^+$). The M+Na+2 peak with approximately one-third intensity of the *quasi* molecular ion peak was characteristic of the presence of a chlorine atom. The molecular formula of $C_{27}H_{42}ClNO_8$ was determined by interpretation of the ^{13}C NMR (Table 2) and elemental analysis[1]. The ^{13}C spectrum exhibited 27 discrete carbon signals which were attributed to 6 CH_3, 4 CH_2, 12 CH including 5 sp^2, 3 quaternary carbon including one sp^2 and two carbonyl groups, indicating 38 carbon-bound protons. All 4 exchangeable protons (amide NH (δ 6.00 (d, 9), secondary alcohol (δ 2.24 (d, 6.5)) and two tertiary alcohol protons (δ 3.77 (s) and 4.60 (s)) were seen in the ^1H NMR spectrum. The remaining two unsaturations not accounted for by the 8 sp^2 carbons indicated that **1** has two rings. The presence of an acetyl group was inferred from the three-proton singlet at 2.02 ppm and IR absorption band at 1735 cm^{-1} and further substantiated by ^{13}C signals (δ 170.8 (s) and 21.4 (q)). At the early stage of this experiment, FR901463 isolation procedure using methanol resulted in a contamination of its methanol artifact (FAB-MS *m/z* 580 $(M+Na)^+$, δ 3.28 (3H, s)) (**1b** in Fig. 2), as in the case of bafilomycins[3]. This finding reminded us of the presence of a hemiketal ring which was supported by quaternary ^{13}C signal at 96.8 ppm.

A routine analysis of ^1H-^1H COSY and ^{13}C-^1H COSY revealed the following structural fragments (Fig. 3): **I** C-5′~C-2′; **II** C-16~C-9; **III** C-7~C-4; two isolated methylene (C-2 and C-18) and singlet methyl group (C-17). Partial structure **a** (Fig. 4), consisting of **III**, the two isolated methylene and the singlet methyl group, was elucidated by the following key ^1H-^{13}C long-range correlations. The hemiketal carbon C-1 (96.8 ppm) showed ^1H-^{13}C long-range couplings to the exchangeable proton 1-OH (δ 4.60 (s)), non-coupled methylene 2-H_2

Fig. 1. Structures of **1**, **2** and **3**.

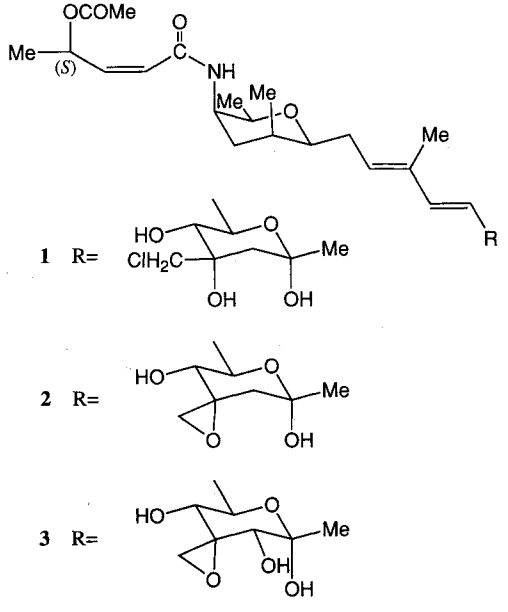


Fig. 2. Plane structures for **1a** and its artifact **1b**.

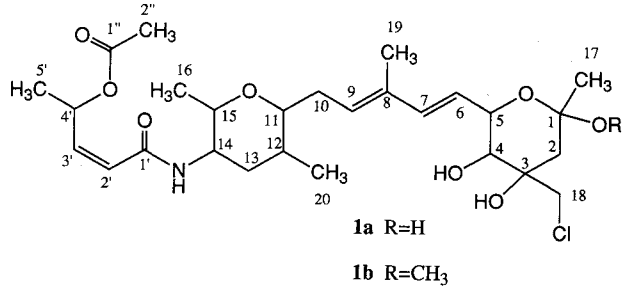
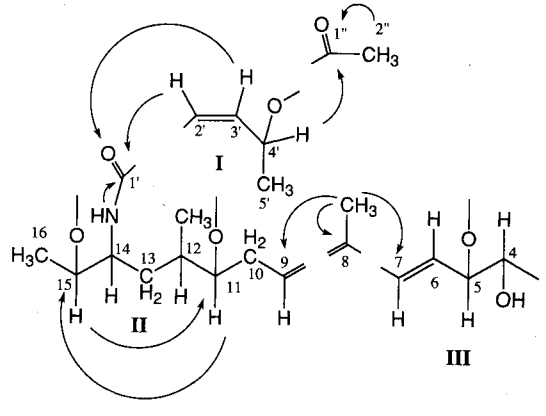
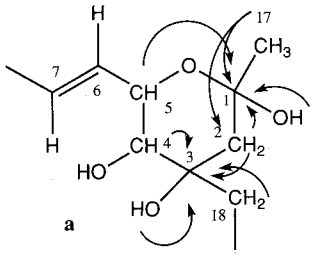


Fig. 3. Substructures **I**~**III** and key ^1H-^{13}C long-range couplings obtained from HMBC and COLOC experiments.



(δ 2.06 (d, 14) and 1.95 (d, 14)) and singlet methyl 17-H_3 (δ 1.40 (3H, s)). The 2-H_2 were in turn coupled to quaternary oxygen-bearing carbon C-3 (73.8 ppm) which exhibited long-range correlations to 3-OH (δ 3.77 (s)), isolated methylene 18-H_2 (δ 3.59 (d, 11) and 3.51 (d, 11)) and 4-H. The knowledge of the correlations extended the fragment **III** to C-7~C-1. The long-range ^{1}H-^{13}C coupling from 5-H to C-1 indicated the presence of a tetrahydropyranose ring and thus partial structure **a** was elucidated. The attachment position of an acetyl group was inferred from the low-field chemical shift of 4'-H (6.25 ppm) in **I** and confirmed by the HMBC correlation between 4'-H and acetyl carbonyl C-1''. The HMBC correlations between 15-H and C-11 and between 11-H and C-15 indicated the presence of a tetrahydropyranose ring in **II** (Fig. 3). The carbonyl signal (165.0 ppm) showed correlations with amide NH, 2'-H (δ 5.72 (dd, 11.5, 1)) and 3'-H (δ 5.91 (dd, 11.5, 8)), indicating linkage of C-1' and C-2'. The broadened methyl singlet at 1.78 ppm (19-H_3) showed HMBC cross peaks to quaternary sp^2 carbon C-8 (134.7 ppm) and two olefinic CH C-9 (129.9 ppm) in **II** and C-7 (138.7 ppm) in **III**, indicating the connections C-9 to C-7. There is only one reasonable connection between C-18 and the chlorine atom and therefore the plane structure of **1** was concluded to be **1a** (Fig. 2). The ^{1}H and ^{13}C NMR spectral assignment are presented in Tables 1 and 2, respectively.

Fig. 4. Partial structure **a**.



^{1}H-^{13}C long-range correlations

The (*Z*) geometry of the double bond at C-2' and (*E*) at C-6 were evident from the vicinal coupling constants $J_{2',3'}=11.5$ Hz and $J_{6,7}=16$ Hz, respectively. The high-field ^{13}C chemical shift of C-19 (12.8 ppm) indicated *E* configuration of the double bond[4] at C-8 which was further supported by NOE's between 9-H and 7-H and between 6-H and 19-H_3. The relative configuration on the hemiketal ring was determined as shown in Fig. 5. The large coupling constant of 10 Hz between 4-H (δ 3.46 (dd, 10, 6.5) and 5-H (δ 4.26 (dd, 10, 7)) suggested that the hemiketal ring existed in a chair conformation with 4-H and 5-H in a trans-diaxial orientations. The NOE's between 4-H and one of 2-H_2 (δ 1.95 (d, 14)) and between 5-H and the hemiketal OH (δ 4.60 (s)) would be reasonably accounted for by the 1,3-diaxial relationships. The trans-diaxial disposition of the hemiketal OH

Table 1. ^{1}H NMR signal assignments of **1**, **2** and **3**.

Position	1		2		3	
	δ_H[a] (mult, *J* (Hz))		δ_H (mult, *J* (Hz))		δ_H (mult, *J* (Hz))	
1 - OH	4.60	(s)	3.38	(s)	2.85	(s)
2 axial	1.95	(d, 14)	2.36	(d, 14)	3.66	(d, 12)
equatorial	2.06	(d, 14)	1.66	(d, 14)	2.20[b]	(d, 12)
3 - OH	3.77	(s)				
4	3.46	(dd, 6.5, 10)	3.58	(dd, 10, 10)	3.59	(dd, 10, 10)
4 - OH	2.24	(d, 6.5)	1.66	(d, 10)	1.77	(d, 10)
5	4.26	(dd, 7, 10)	4.25	(dd, 10, 7)	4.22	(dd, 10, 7)
6	5.62	(dd, 7, 16)	5.66	(dd, 7, 16)	5.63	(dd, 7, 16)
7	6.39	(d, 16)	6.37	(d, 16)	6.37	(d, 16)
9	5.56	(br t, 7)	5.53	(br t, 7)	5.54	(br t, 7)
10	2.36	(m)	2.36	(m)	2.37	(m)
	2.24	(m)	2.24	(m)	2.23	(m)
11	3.51	(m)	3.53	(m)	3.52	(m)
12	1.77	(m)	1.77	(m)	1.77	(m)
13	1.94	(m)	1.94	(m)	1.94	(m)
	1.92	(m)	1.91	(m)	1.92	(m)
14	3.89	(m)	3.90	(m)	3.90	(m)
14 - NH	6.00	(d, 9)	5.99	(d, 9)	5.98	(d, 9)
15	3.64	(qd, 7, 2)	3.66	(qd, 7, 2)	3.65	(qd, 7, 2)
16	1.11	(3H, d, 7)	1.11	(3H, d, 7)	1.11	(3H, d, 7)
17	1.40	(3H, s)	1.43	(3H, s)	1.51	(3H, s)
18	3.59	(d, 11)	3.07	(d, 4.5)	2.96	(d, 5)
	3.51	(d, 11)	2.55	(d, 4.5)	2.92	(d, 5)
19	1.78	(3H, s)	1.78	(3H, s)	1.78	(3H, s)
20	1.01	(3H, d, 7)	1.01	(3H, d, 7)	1.01	(3H, d, 7)
2'	5.72	(dd, 11.5, 1)	5.71	(dd, 11.5, 1)	5.72	(dd, 11.5, 1)
3'	5.91	(dd, 11.5, 8)	5.90	(dd, 11.5, 8)	5.90	(dd, 11.5, 8)
4'	6.25	(m)	6.26	(m)	6.25	(m)
5'	1.34	(3H, d, 6.5)	1.33	(3H, d, 6.5)	1.33	(3H, d, 6.5)
2''	2.02	(3H, s)	2.02	(3H, s)	2.02	(3H, s)

[a] 500 MHz in CD_2Cl_2.
[b] In case of **3**, equatorial -OH.

Table 2. ^{13}C NMR signal assignments of **1**, **2** and **3**.

Position	1		2		3	
	δ_C	(mult)	δ_C	(mult)	δ_C	(mult)
1	96.8	(s)	96.7	(s)	97.8	(s)
2	40.5	(t)	41.8	(t)	69.2	(d)
3	73.8	(s)	58.1	(s)	59.7	(s)
4	70.4	(d)	68.1	(d)	68.2	(d)
5	71.6	(d)	73.8	(d)	73.1	(d)
6	124.8	(d)	124.7	(d)	124.3	(d)
7	138.7	(d)	138.3	(d)	138.5	(d)
8	134.7	(s)	134.8	(s)	134.8	(s)
9	129.9	(d)	129.8	(d)	130.1	(d)
10	32.4	(t)	32.4	(t)	32.4	(t)
11	81.1	(d)	81.2	(d)	81.2	(d)
12	29.6	(d)	29.6	(d)	29.6	(d)
13	36.2	(t)	36.3	(t)	36.2	(t)
14	47.4	(d)	47.4	(d)	47.4	(d)
15	76.3	(d)	76.3	(d)	76.3	(d)
16	18.0	(q)	17.9	(q)	17.9	(q)
17	29.1	(q)	29.1	(q)	26.5	(q)
18	49.2	(t)	48.0	(t)	43.5	(t)
19	12.8	(q)	12.7	(q)	12.7	(q)
20	15.3	(q)	15.2	(q)	15.3	(q)
1'	165.0	(s)	165.0	(s)	165.0	(s)
2'	122.7	(d)	122.8	(d)	122.8	(d)
3'	144.0	(d)	143.9	(d)	143.9	(d)
4'	68.9	(d)	68.9	(d)	68.9	(d)
5'	20.2	(q)	20.2	(q)	20.2	(q)
1"	170.8	(s)	170.6	(s)	170.6	(s)
2"	21.4	(q)	21.4	(q)	21.4	(q)

125 MHz in CD_2Cl_2.

and the one of 2-H_2 was established by observation of W coupling between C-1 hydroxyl proton and the 2-H_{axial}. The NOE correlation observed between 4-H and 18-H_2 denied axial-disposition of 18-H_2. The equatorial-orientation of 18-H_2 was further supported by observation of W coupling between C-3 hydroxyl proton and 2-H_{axial}. The relative stereostructure of the tetrahydropyranose ring C-11 ~ C-15 was suggested to be as shown in Fig. 6. Two NOE's 15-H/11-H and C-14 amideNH/C-12 methyl protons pointed out the 1,3-diaxial relationships in a chair conformation. The small ^1H-^1H coupling constant (2 Hz) between 14-H and 15-H suggested that 14-H is equatorial. It was assumed that the tetrahydropyranose ring would not adopt a typical chair form because of unfavorable 1,3-diaxial interaction between C-14 amideNH and C-12 methyl group. The gross structure of **1** including relative stereochemistry was determined as depicted in Fig. 1. The (*S*) assignment of C-4' stereochemistry was established by the determination of the degradation product corresponding to C-1' ~ C-5' part. Mild acid treatment of **1** (conc HCl - dioxane (2:5), room temperature, 24 hours) afforded (*S*)-5-methyl-2(5H)-furanone[5] ($[\alpha]_D^{23}$ +107° (*c* 0.5, $CHCl_3$); lit. $[\alpha]_D^{20}$ +103.5° ($CHCl_3$)).

HRFAB-MS measurement of FR901464 (**2**) yielded a molecular formula of $C_{27}H_{41}NO_8$ (HCl less than **1**) which was consistent with the ^{13}C NMR data (Table 2). ^1H NMR spectrum of **2** was quite similar to that of **1** except that a new isolated methylene protons (δ 3.07 (d, 4.5) and 2.55 (d, 4.5)) appear at the expense of the

Fig. 5. Relative stereochemistry of the hemiketal ring.

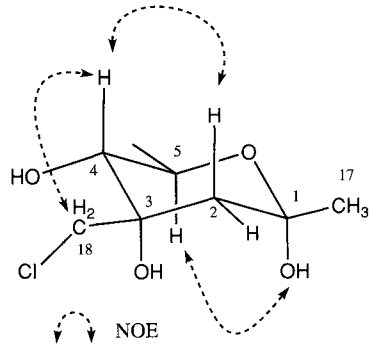
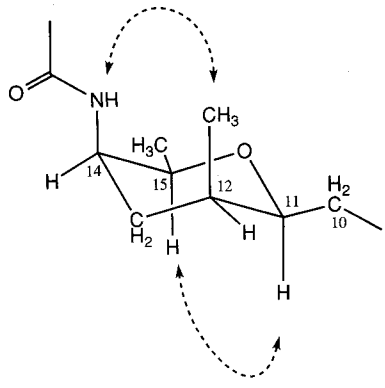


Fig. 6. Proposed relative stereostructure of C-1 ~ C15.



non-coupled methylene group 18-H_2 in **1**. The small geminal coupling constant (4.5 Hz) was quite characteristic of the presence of an epoxide methylene group which was confirmed by J_{CH} = 178 Hz and by the high field shift of quaternary ^{13}C signal at C-3. The molecular formulae difference between **2** and **1** was reasonably explained by elimination of HCl followed by formation of epoxide. The stereochemical identity between **1** and **2** was accomplished as follows. Base treatment (*t*-BuOK in dry THF, 0°C, 10 minutes) of **1b** yielded its epoxide derivative which is identical in all respects (MS, ^1H NMR and $[\alpha]_D$) with the methyl glycoside derivative of **2**, derived from methanol treatment of **2**.

HRFAB-MS and ^{13}C NMR data (Table 2) established the molecular formula of **3** as $C_{27}H_{41}NO_9$. The molecular formula of **3** differs from that of **2** by one extra oxygen atom. The difference of ^{13}C spectra between **3** and **2** lies in the hemiketal moieties. The C-2 methylene in **2** was replaced by a secondary alcohol group in **3**. The axial-orientation of 2-H was established by observation of W coupling between 2-H and 1-OH and further supported by NOE between 2-H and 4-H.

From the above information, the structures of **1**, **2** and **3** are determined to be (2*Z*)-3-[6-[(2*E*,4*E*)-5-(4-Chloromethyl-3,4,6-trihydroxy-6-methyltetrahydropyran-2-yl)-3-methylpenta-2,4-dienyl]-2,5-dimetyltetrahydropyran-3-ylcarbamoyl]-1-methylallyl acetate, (2*Z*)-

3-[6-[(2*E*,4*E*)-5-(4,7-Dihydroxy-7-methyl-1,6-dioxaspiro[2.5]oct-5-yl)-3-methylpenta-2,4-dienyl]-2,5-dimethyltetrahydropyran-3-ylcarbamoyl]-1-methylallyl acetate and (2*Z*)-3-[2,5-Dimethyl-6-[(2*E*,4*E*)-3-methyl-5-(4,7,8-trihydroxy-7-methyl-1,6-dioxaspiro[2.5]oct-5-yl)penta-2,4-dienyl]tetrahydropyran-3-ylcarbamoyl]-1-methylallyl acetate, respectively, as depicted in Fig. 1. With the structure of **1** and **2** in hand, the question was raised whether **1** is an HCl adduct artifact of **2** during the isolation process. Direct LC-MS examination on the cultured broth detected the presence of **1**, **2** and **3**. We therefore assume that **1** is not an artifact but a metabolite.

References

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