Structures of Fattiviracin Family, Antiviral Antibiotics

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(Received for publication June 13, 2000)

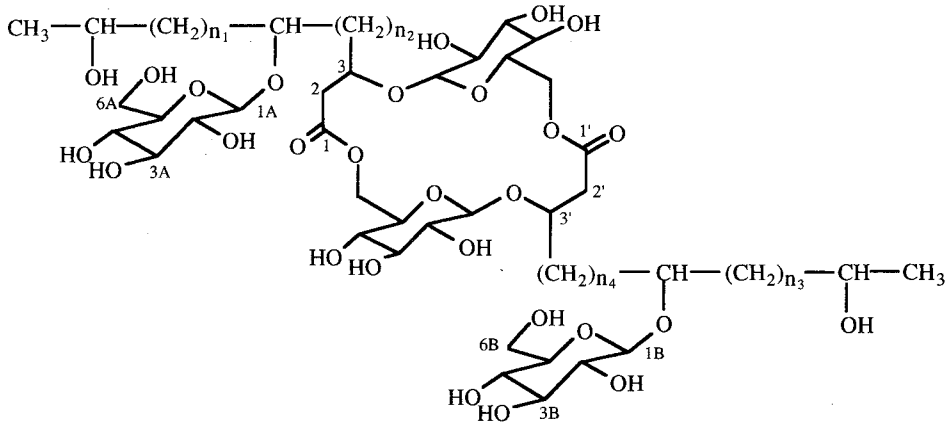
Antiherpetic agents so far clarified in our Labs. are AH-135Y[1], one of glutarimide derivatives, AH-758[2], an antibiotic belonging to bafilomycin, AH-1763 IIa[3], a new antibiotic belonging to pluramycin group and fattiviracin A1[4], a new antibiotic belonging to sugar-fatty acid lactone.

Streptomyces microflavus strain No. 2445 turned out to produce at least 13 derivatives of fattiviracin (fattiviracin FV-1 to FV-13). They were isolated from culture filtrate by using column chromatographies on Diaion HP-10, silica gel, ODS and preparative HPLC with monitoring antiviral activity. Ten derivatives except fattiviracin FV-1 to FV-3 belonged to sugar-fatty acid lactone. Fattiviracin FV-1 to FV-3 remained to be determined because of trace amount of production. As shown in Fig. 1, fattiviracins can be divided into 5 families according to the length of fatty acids moiety. Some of them will be tautomeric isomer each other. In this paper we describe the isolation, structures and biological properties of each representative from 5 families such as FV-4 (**1**), FV-8 (**2**)[5], FV-9 (**3**), FV-10 (**4**) and FV-13 (**5**).

A slant culture of the strain No. 2445 was inoculated into 200 ml Erlenmeyer flask containing 50 ml of seed medium which consisted of glucose 2.0%, starch 3.0%, corn steep liquor 1.0%, soybean flour 1.0%, peptone 0.5%, NaCl 0.3%, $CaCO_3$ 0.3%. The pH was adjusted to 7.0 with NaOH before autoclaving. The seed culture was cultivated for 2 days at 28°C on a rotary shaker set up at 180 rpm. A 200-ml portion of the seed culture was transferred into a 10-liter jar fermentor containing 5 liters of a production medium consisting of glucose 5.0%, corn steep liquor 1.0%, peptone 0.5%, NaCl 0.3%, $CaCO_3$ 0.3% (pH 7.0). The main culture was carried out for 4 days at 28°C under agitation of 350 rpm and aeration of 3.5 liters per minute. The activity reached the maximum in the culture after 4 days.

The purification procedures of fattiviracins were carried out as follows: culture filtrate (80 liters) was adsorbed by

Fig. 1. Structures of fattiviracins.



Fattiviracin family	Carbon number of fatty acid					
	Long chain	n_1	n_2	Short chain	n_3	n_4
Fattiviracin FV-4 (**1**)*, FV-3	22	5	11	22	5	11
Fattiviracin FV-8 (**2**)*, FV-6, FV-7	24	5	13	22	5	11
Fattiviracin FV-9 (**3**)*	24	5	13	24	7	11
Fattiviracin FV-10 (**4**)*, FV-11, FV-12	26	7	13	24	7	11
Fattiviracin FV-13 (**5**)*	28	9	13	24	7	11

* Each derivative with parentheses is a representative of the family.

Table 1. Physico-chemical properties of fattiviracins FV-4 (**1**), FV-8 (**2**), FV-9 (**3**), FV-10 (**4**) and FV-13 (**5**).

	1	**2**	**3**	**4**	**5**
Appearance	colorless oil	colorless oil	colorless oil	colorless oil	colorless oil
Solubility : soluble	H_2O, MeOH	H_2O, MeOH	H_2O, MeOH	H_2O, MeOH	H_2O, MeOH
insoluble	acetone, $CHCl_3$	acetone, $CHCl_3$	acetone, $CHCl_3$	acetone, $CHCl_3$	acetone, $CHCl_3$
Molecular formula	$C_{68}H_{124}O_{28}$	$C_{70}H_{128}O_{28}\cdot H_2O$	$C_{72}H_{128}O_{28}\cdot H_2O$	$C_{74}H_{136}O_{28}\cdot H_2O$	$C_{76}H_{140}O_{28}\cdot H_2O$
FAB-MS	1388	1434	1462	1490	1518
UV λ max nm (MeOH)	end absorption	end absorption	end absorption	end absorption	end absorption
IR ν max cm^{-1} (ATR)	3409, 2925, 2852, 1735, 1461, 1371, 1166, 1081, 1039	3376, 2925, 2852, 1735, 1459, 1369, 1164, 1078, 1041	3403, 2925, 2854, 1735, 1461, 1371, 1166, 1081, 1041	3380, 2923, 2854, 1733, 1459, 1371, 1166, 1079, 1039	3384, 2925, 2854, 1735, 1459, 1376, 1162, 1074, 1039
$[\alpha]_D^{19}$ (c 0.1, MeOH)	-27.5°	-25.8°	-36.8°	-32.3°	-17.6°
HPLC retention time (min)*	9.8	14.7	15.6	19.5	25.4

*μBondapak C_{18} (19 x 300 mm), 90% MeOH, 10 ml/min, 210 nm.

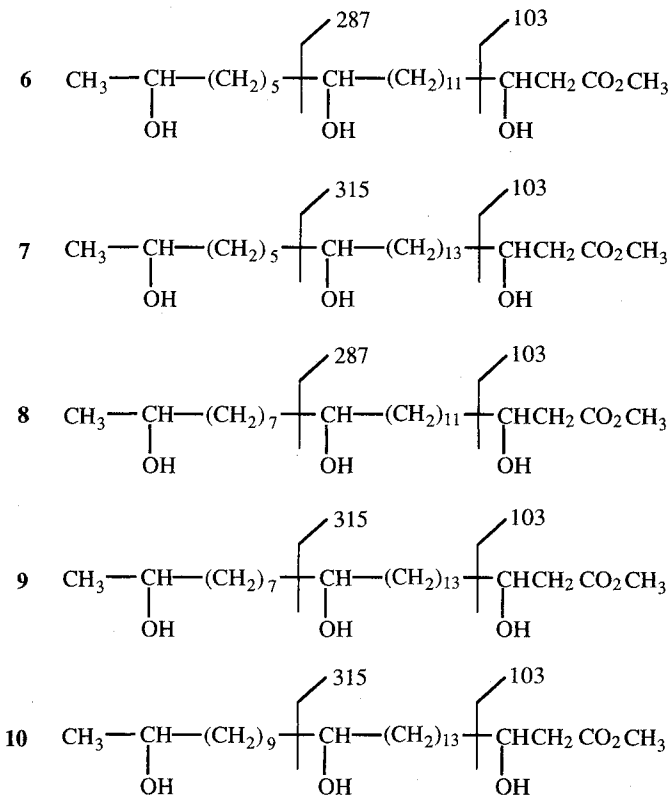
Table 2. ^1H NMR data of fattiviracins FV-4 (**1**), FV-8 (**2**), FV-9 (**3**), FV-10 (**4**) and FV-13 (**5**).

1	**2**	**3**	**4**	**5**
position δH (ppm, *J* in Hz)	position δH (ppm, *J* in Hz)	position δH (ppm, *J* in Hz)	position δH (ppm, *J* in Hz)	position δH (ppm, *J* in Hz)
2,2' 2.51-2.54 (1H, m)	2,2' 2.50-2.54 (1H, m)	2,2' 2.50-2.53 (1H, m)	2,2' 2.45-2.53 (1H, m)	2,2' 2.50-2.53 (1H, m)
2.59 (1H, dd, 6.10, 15.3)	2.53-2.61 (1H, m)	2.61 (1H, m)	2.59 (1H, dd, 6.10, 15.3)	2.61 (1H, m)
2.76-2.83 (2H, m)	2.77-2.83 (2H, m)	2.76-2.81 (2H, m)	2.79 (2H, dd, 6.71, 15.3)	2.78-2.80 (2H, m)
3,3' 4.07-4.14 (2H, m)	3,3' 4.09-4.14 (2H, m)	3,3' 4.10-4.20 (2H, m)	3,3' 4.07-4.14 (2H, m)	3,3' 4.12-4.14 (2H, m)
15,15' 3.62 (2H, m)	17,15' 3.62-3.64 (2H, m)	17,15' 3.62 (2H, m)	17,15' 3.62-3.64 (2H, m)	17,15' 3.62-3.65 (2H, m)
21,21' 3.69-3.72 (2H, m)	23,21' 3.69-3.72 (2H, m)	23,23' 3.69-3.72 (2H, m)	25,23' 3.70-3.72 (2H, m)	27,23' 3.69-3.70 (2H, m)
22,22' 1.15 (6H, d, 6.10)	24,22' 1.14 (6H, d, 6.10)	24,24' 1.15 (6H, d, 6.10)	26,24' 1.15 (6H, d, 6.10)	28,24' 1.15 (6H, d, 6.10)
23,23' 4.36 (1H, d, 7.33)	25,23' 4.34 (1H, d, 7.93)	25,25' 4.33 (1H, d, 7.32)	27,25' 4.36 (1H, d, 7.94)	29,25' 4.36 (1H, d, 7.94)
4.38 (1H, d, 7.93)	4.36 (1H, d, 7.33)	4.37 (1H, d, 7.32)	4.37 (1H, d, 7.93)	4.37 (1H, d, 7.94)
24,24' 3.13-3.17 (2H, m)	26,24' 3.14-3.20 (2H, m)	26,26' 3.14-3.19 (2H, m)	28,26' 3.13-3.19 (2H, m)	30,26' 3.16-3.19 (2H, m)
25,25' 3.31-3.34 (2H, m)	27,25' 3.31-3.34 (2H, m)	27,27' 3.31-3.35 (2H, m)	29,27' 3.31-3.34 (2H, m)	31,27' 3.31-3.34 (2H, m)
26,26' 3.25-3.30 (2H, m)	28,26' 3.24-3.30 (2H, m)	28,28' 3.25-3.30 (2H, m)	30,28' 3.20-3.30 (2H, m)	32,28' 3.25-3.30 (2H, m)
27,27' 4.18-4.20 (2H, m)	29,27' 4.16-4.20 (2H, m)	29,29' 4.10-4.20 (2H, m)	31,29' 4.16-4.20 (2H, m)	33,29' 4.16-4.21 (2H, m)
28,28' 4.42-4.48 (2H, m)	30,28' 4.09-4.14 (2H, m)	30,30' 4.10-4.20 (2H, m)	32,30' 4.07-4.14 (2H, m)	34,30' 4.12-4.14 (2H, m)
4.10-4.14 (2H, m)	4.42-4.48 (2H, m)	4.42-4.47 (2H, m)	4.42-4.48 (2H, m)	4.42-4.45 (2H, m)
1A,1B 4.29 (1H, d, 7.94)	1A,1B 4.29 (1H, d, 7.94)	1A,1B 4.29 (1H, d, 7.94)	1A,1B 4.29 (1H, d, 7.94)	1A,1B 4.29 (1H, d, 7.94)
4.33 (1H, d, 7.33)	4.32 (1H, d, 7.33)	4.33 (1H, d, 7.33)	4.34 (1H, d, 7.93)	4.34 (1H, d, 7.93)
2A,2B 3.13-3.17 (2H, m)	2A,2B 3.14-3.20 (2H, m)	2A,2B 3.14-3.19 (2H, m)	2A,2B 3.13-3.19 (2H, m)	2A,2B 3.16-3.19 (2H, m)
3A,3B 3.31-3.34 (2H, m)	3A,3B 3.31-3.34 (2H, m)	3A,3B 3.31-3.35 (2H, m)	3A,3B 3.31-3.34 (2H, m)	3A,3B 3.31-3.34 (2H, m)
4A,4B 3.25-3.30 (2H, m)	4A,4B 3.24-3.30 (2H, m)	4A,4B 3.25-3.30 (2H, m)	4A,4B 3.20-3.30 (2H, m)	4A,4B 3.25-3.30 (2H, m)
5A,5B 3.44 (2H, m)	5A,5B 3.44 (2H, m)	5A,5B 3.44 (2H, m)	5A,5B 3.43 (2H, m)	5A,5B 3.44 (2H, m)
6A,6B 3.65-3.68 (2H, m)	6A,6B 3.65-3.68 (2H, m)	6A,6B 3.62-3.68 (2H, m)	6A,6B 3.65-3.68 (2H, m)	6A,6B 3.67-3.68 (2H, m)
3.82-3.86 (2H, m)	3.83-3.85 (2H, m)	3.83-3.85 (2H, m)	3.83-3.85 (2H, m)	3.83-3.84 (2H, m)
methylenes of fatty acid	methylenes of fatty acid	methylenes of fatty acid	methylenes of fatty acid	methylenes of fatty acid
1.30-1.61 (64H, m)	1.30-1.56 (68H, m)	1.30-1.54 (72H, m)	1.30-1.60 (76H, m)	1.30-1.70 (80H, m)

Table 3. ^{13}C NMR data of fattiviracins FV-4 (**1**), FV-8 (**2**), FV-9 (**3**), FV-10 (**4**) and FV-13 (**5**).

1		2		3		4		5	
position	δc (ppm)	position	δc (ppm)	position	δc (ppm)	position	δc (ppm)	position	δc (ppm)
1, 1'	173.5, 172.5	1, 1'	172.5, 173.4	1, 1'	172.4, 173.4	1, 1'	172.5, 173.4	1, 1'	172.4, 173.4
2, 2'	42.2, 42.4	2, 2'	42.2, 42.3	2, 2'	42.2, 42.3	2, 2'	42.3, 42.4	2, 2'	42.2, 42.3
3, 3'	78.2, 78.3	3, 3'	78.2, 78.3	3, 3'	78.1, 78.1	3, 3'	78.2, 78.2	3, 3'	78.2, 78.2
15, 15'	80.5, 81.0	17, 15'	80.5, 80.9	17, 15'	80.5, 80.9	17, 15'	80.5, 81.0	17, 15'	80.5, 81.0
21, 21'	68.6, 68.6	23, 21'	68.6, 68.6	23, 23'	68.5, 68.5	25, 23'	68.6, 68.6	27, 23'	68.6, 68.6
22, 22'	23.6, 23.6	24, 22'	23.6, 23.6	24, 24'	23.6, 23.6	26, 24'	23.6, 23.6	28, 24'	23.6, 23.6
23, 23'	104.0, 104.0	25, 23'	104.0, 105.0	25, 25'	103.9, 104.3	27, 25'	103.3, 104.0	29, 25'	103.9, 104.3
24, 24'	75.2, 75.3	26, 24'	75.2, 75.3	26, 26'	75.1, 75.2	28, 26'	75.2, 75.3	30, 26'	75.3, 75.3
25, 25'	77.7, 77.9	27, 25'	77.7, 78.0	27, 27'	77.6, 78.0	29, 27'	77.7, 77.9	31, 27'	77.7, 77.9
26, 26'	71.9, 72.0	28, 26'	71.8, 71.9	28, 28'	71.7, 71.9	30, 28'	71.8, 72.0	32, 28'	71.8, 71.9
27, 27'	78.2, 78.3	29, 27'	78.2, 78.3	29, 29'	78.1, 78.1	31, 29'	78.2, 78.2	33, 29'	78.2, 78.2
28, 28'	65.3, 65.3	30, 28'	66.0, 65.2	30, 30'	65.2, 65.2	32, 30'	65.3, 65.3	34, 30'	65.2, 65.2
1A, 1B	103.4, 103.7	1A, 1B	103.4, 103.7	1A, 1B	103.4, 103.6	1A, 1B	103.4, 103.7	1A, 1B	103.4, 103.7
2A, 2B	75.2, 75.2	2A, 2B	75.1, 75.2	2A, 2B	75.1, 75.2	2A, 2B	75.2, 75.2	2A, 2B	75.2, 75.3
3A, 3B	77.7, 77.9	3A, 3B	77.7, 77.9	3A, 3B	77.6, 78.0	3A, 3B	77.7, 77.9	3A, 3B	77.7, 77.9
4A, 4B	71.6, 71.7	4A, 4B	71.6, 71.6	4A, 4B	71.5, 71.5	4A, 4B	71.6, 71.6	4A, 4B	71.6, 71.6
5A, 5B	78.1, 78.1	5A, 5B	78.0, 78.0	5A, 5B	78.1, 78.1	5A, 5B	78.1, 78.1	5A, 5B	78.0, 78.0
6A, 6B	62.8, 62.9	6A, 6B	62.8, 62.9	6A, 6B	62.8, 62.9	6A, 6B	62.9, 62.9	6A, 6B	62.9, 63.0

Fig. 2. Structures and mass fragmentations of **6**, **7**, **8**, **9** and **10**.



twentieth volume of Diaion HP-10 (Mitsubishi Chemical Industries, Ltd.). After washing with 75% MeOH, fattiviracins were eluted with 90% MeOH. The 90% MeOH fraction was applied to a silica gel column and eluted with $CHCl_3$ - MeOH - H_2O (7 : 3 : 0.5). The fractions containing fattiviracins were pooled and applied to ODS column and eluted with 80% MeOH and 90% MeOH. Further purification was carried out by preparative HPLC on a C_{18} column (μBondapak C_{18}, 19 mm×300 mm, Waters Associates). Elution was carried out with 90% MeOH and a flow rate of 10 ml/minute. Detection was achieved by UV 210 nm. The retention times of FV-1, FV-2, FV-3, FV-4, FV-5, FV-6, FV-7, FV-8, FV-9, FV-10, FV-11, FV-12 and FV-13 were 5.6, 6.6, 7.4, 9.8, 10.8, 11.4, 12.7, 14.7, 15.6, 19.5, 20.8, 21.6 and 25.4 minutes, respectively. This HPLC was repeated to give 4 mg of FV-1, 1 mg of FV-2, 4 mg of FV-3, 170 mg of FV-4 (**1**), 20 mg of FV-5, 30 mg of FV-6, 30 mg of FV-7, 120 mg of FV-8 (**2**), 160 mg of FV-9 (**3**), 90 mg of FV-10 (**4**), 30 mg of FV-11, 10 mg of FV-12 and 30 mg of FV-13 (**5**) from 80 liters of culture filtrate.

Physico-chemical properties of **1**, **2**, **3**, **4** and **5** are summarized in Table 1. The molecular formula of each derivative were determined by using FAB-MS and NMR techniques. The ^1H and ^{13}C NMR spectra of **1**, **2**, **3**, **4** and **5** were very similar to those of fattiviracin A1 except for the number of methylene protons derived from fatty acid (Tables 2 and 3). Degradations of **1**, **2**, **3**, **4** and **5** were carried out to determine the sugar molecules and the length of two alkyl chains. Each one of **1**, **2**, **3**, **4** and **5** was reacted in 2 N HCl-MeOH at 100°C for 2 hours, then extracted with $CHCl_3$. The sugar molecules in each aqueous layer (sugar fraction) were identified as methyl-β-D-glucopyranoside with the spectral data and by comparison with authentic samples. From $CHCl_3$ layer (lipid fraction), fatty acid methyl esters were purified by HPLC. Upon acid methanolysis, fatty acid methyl esters were obtained as follows: compound **6** from **1**, compounds **6** and **7** from **2**, compounds **7** and **8** from **3**, compounds **8** and **9** from **4**, and compounds **9** and **10** from **5**. The length of fatty acid chain and the position of hydroxy groups were determined by FAB-MS and EI-MS (Fig. 2). Thus, the structures of **1**, **2**, **3**, **4** and **5** were proposed as described in Fig. 1.

Antiviral activities of these compounds against HSV-1 were examined with plaque reduction assay[6]. Compounds **1**, **2**, **3**, **4** and **5** showed the antiviral activities of 3.1, 2.7, 2.9, 2.5 and 2.9 μg/ml against HSV-1, respectively. Antiviral activities of these compounds were almost the same regardless of their length of fatty acid chains.

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