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Studies on Macrocyclic Lactone Antibiotics. V.[1] The Structures of Azalomycins F_{3a} and F_{5a}

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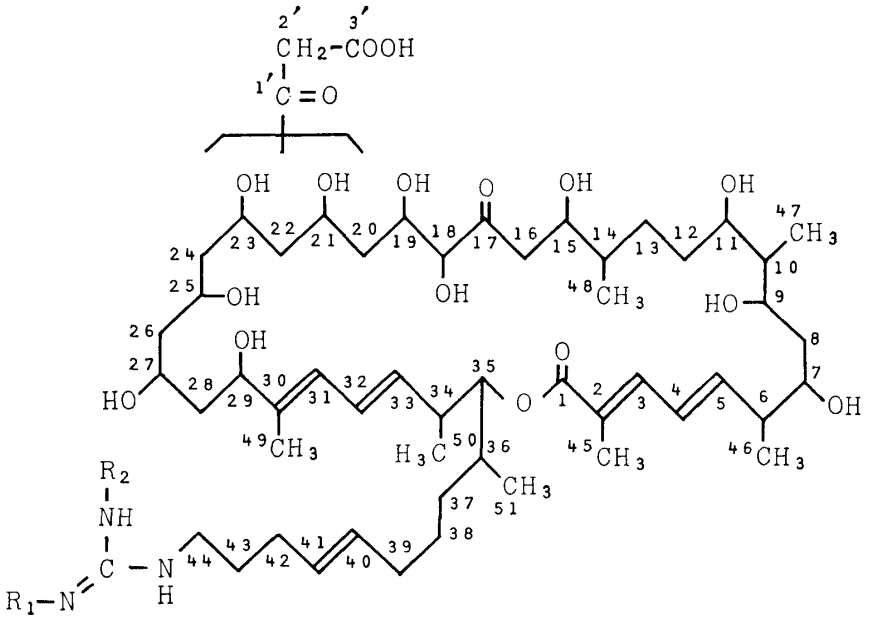
The structures of the antibiotics azalomycin F_{3a} (**1b**) ($C_{55}H_{93}N_3O_{17}$) and azalomycin F_{5a} (**1c**) ($C_{57}H_{97}N_3O_{17}$) were elucidated by comparison of their physicochemical properties with those of F_{4a} (**1a**) ($C_{56}H_{95}N_3O_{17}$). Structural differences among these three compounds lie only in their guanidine moieties, which have no methyl group, one methyl group and two methyl groups in F_{3a}, F_{4a} and F_{5a}, respectively.

Keywords——azalomycin F_{3a}; azalomycin F_{5a}; *Streptomyces hygroscopicus* var. *azalomyceticus*; isolation; purification; macrocyclic lactone antibiotics; skeletal structure; intramolecular hemiketal; malonic acid hemiester

The azalomycin F complex of antibiotics produced by *Streptomyces hygroscopicus* var. *azalomyceticus* was obtained as a mixture of at least five components, from which the three main components, F_3, F_4 and F_5, were isolated.[3-5]

In our previous paper we reported that F_4 was in fact a mixture of two interconvertible compounds, designated as F_{4a} and F_{4b}, and that the latter was the artifact derived from the former.[6a] Similar interconversions were also observed with F_3 and F_5. Therefore the natural compounds were designated as F_{3a} and F_{5a}, and the artifacts were called F_{3b} and F_{5b},



F_{4a} **1a**: $R_1 = CH_3$, $R_2 = H$
F_{3a} **1b**: $R_1 = H$, $R_2 = H$
F_{5a} **1c**: $R_1 = CH_3$, $R_2 = CH_3$

Fig. 1. The Structures of Azalomycins F_{4a} (**1a**), F_{3a} (**1b**) and F_{5a} (**1c**)

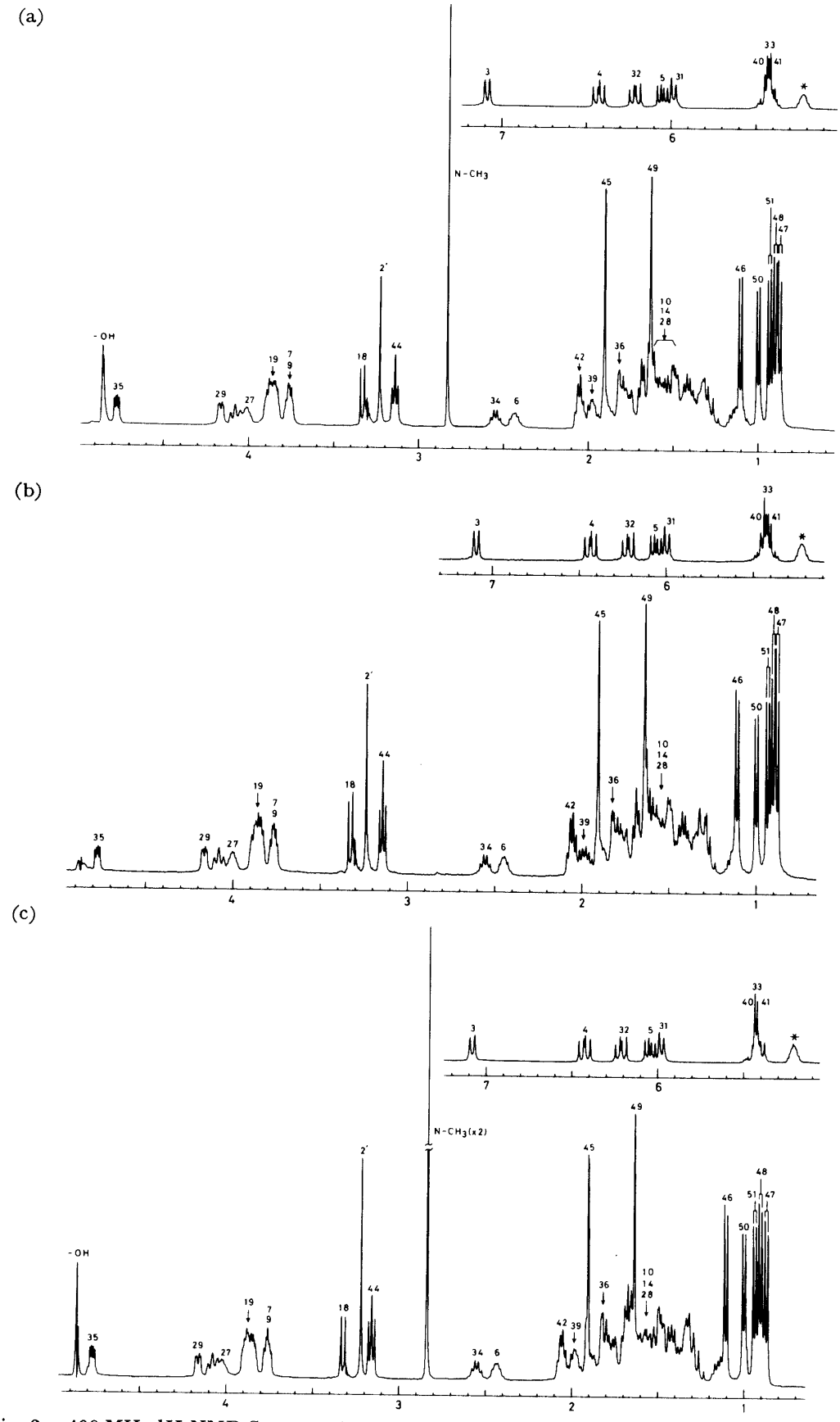


Fig. 2. 400 MHz ^{1}H-NMR Spectra of Azalomycins F_{4a} (a), F_{3a} (b) and F_{5a} (c) in CD_3OD

(a) azalomycin F_{4a}. (b) azalomycin F_{3a}. (c) azalomycin F_{5a}.
*: The methine group bearing the malonylhemiester.

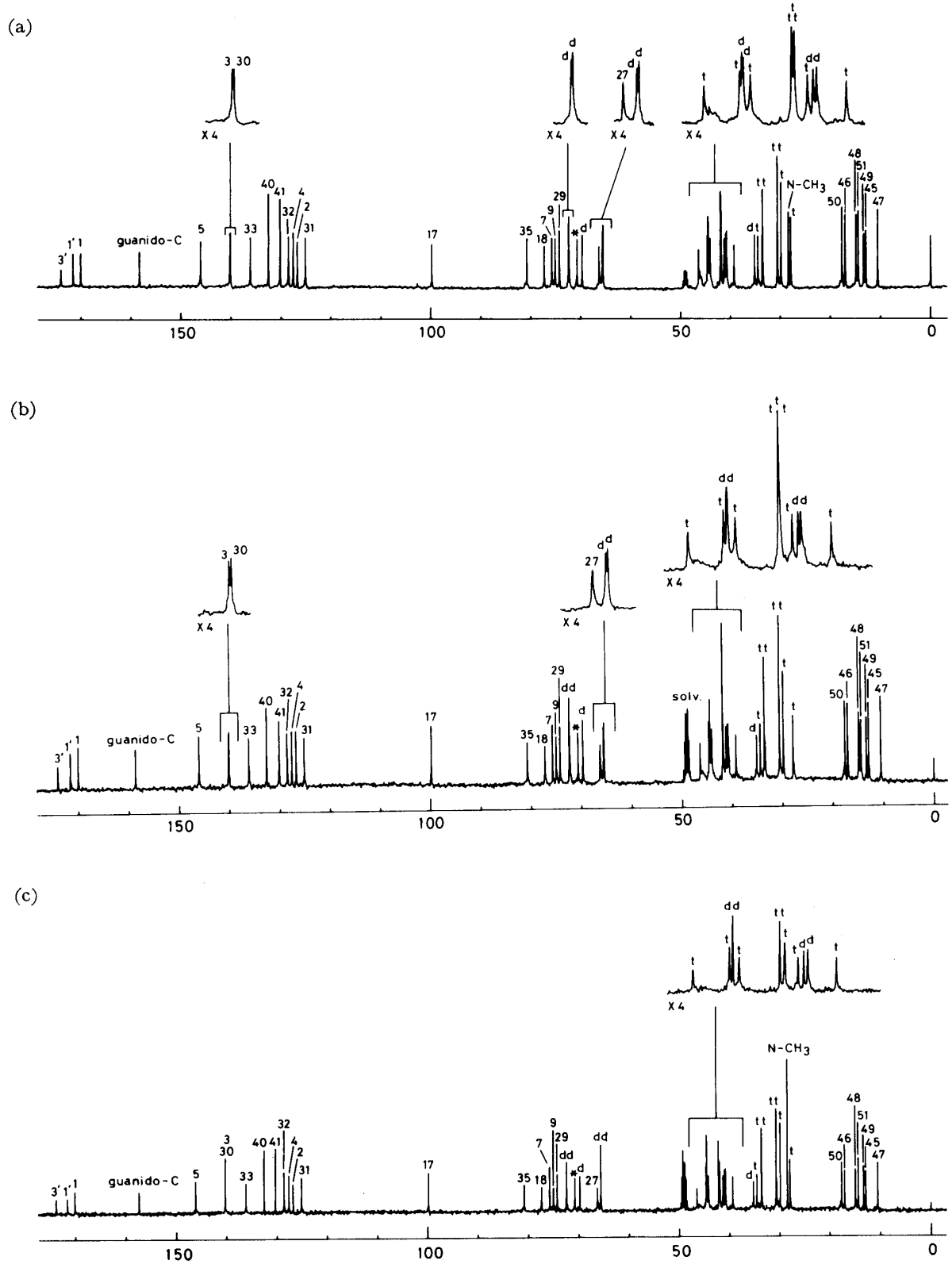


Fig. 3. 100 MHz ^{13}C-NMR Spectra of Azalomycins F_{4a} (a), F_{3a} (b) and F_{5a} (c) in $^{12}CD_3OD$

(a) azalomycin F_{4a}. (b) azalomycin F_{3a}. (c) azalomycin F_{5a}.
*: The carbon bearing the malonyl hemiester.

respectively, as in the case of F_4. This paper deals with the structures of azalomycins F_{3a} (**1b**) and F_{5a} (**1c**).

Isolation of F_{3a} and F_{5a}

The azalomycin F complex provided by Sankyo Company was separated by silica gel column chromatography. Elution of the column with *sec*-butanol–water (9:1) gave F_3 fraction and further elution with *sec*-butanol–water (4:1) yielded the F_4/F_5 fraction. The F_3 and F_5 fractions were each concentrated and the residual crystals were recrystallized from aqueous methanol to afford crude products, which each showed two spots, due to F_{3a} and F_{3b}, and F_{5a} and F_{5b}, respectively, on TLC developed with butanol–acetic acid–water (5:1:1). F_{3a} and F_{5a} were then isolated in pure forms by thin-layer chromatography (TLC) under the same conditions followed by recrystallization from aq. methanol.

These pure compounds were used for the measurements of physicochemical properties, while the degradation experiments of F_{5a} were carried out using the crude preparation, which contained more than 90% F_{5a}.

Structure of F_{3a} (1b)

Azalomycin F_{3a}, fine needles, mp 134—135°C (dec.), $[\alpha]_D^{27}$ +35° (c=1.0, methanol), showed its molecular ion peak in the field desorption (FD) mass spectrum (MS) at m/z 1067 which is smaller by 14 mass units than that of azalomycin F_{4a} (**1a**) (1081), whose skeletal structure has already been elucidated.[6] Its ultraviolet (UV) absorption maxima at 240 nm (ε 41100) and 268 nm (ε 24700) suggested the presence of the same chromophore as in F_{4a} (a conjugated diene and an $\alpha,\beta,\gamma,\delta$-unsaturated carboxylic ester groups).[6a]

Comparison of the ^{1}H-nuclear magnetic resonance (NMR) (400 MHz, Fig. 2 and Table I) and ^{13}C-NMR (100 MHz, Fig. 3 and Table III) spectra with those of F_{4a} revealed that the structural difference between these two compounds lies only in the guanidine moieties, in which a methyl group is present in F_{4a} but not in F_{3a}.

Although no chemical degradation experiment was attempted, the spectroscopic evidence taken together with the analytical data (see "Experimental") indicated the molecular formula of F_{3a} to be $C_{55}H_{93}N_3O_{17}$, and its skeletal structure was elucidated as **1b** (shown in Fig. 1) in which the keto group at C-17 forms a hemiketal ring with a hydroxy group in the molecule.

Structure of F_{5a} (1c)

Azalomycin F_{5a}, fine needles, mp 126—127°C (dec.), $[\alpha]_D^{27}$+44° (c=1.0, methanol), showed its molecular ion peak in the FD–MS at m/z 1095, which is larger by 14 mass units than that of F_{4a}. Its UV absorption maxima again appear at 240 nm (ε 38300) and 268 nm (ε 22700), indicating the presence of the same chromophores as in F_{4a} and F_{3a}.

Comparison of the ^{1}H-NMR (Fig. 2 and Table II) and ^{13}C-NMR (Fig. 3 and Table III) spectra of F_{5a} with those of F_{4a} suggested that their structures differ only in the numbers of *N*-methyl groups. The ^{1}H and ^{13}C signals due to *N*-methyl groups of F_{5a} appeared at almost the same chemical shifts as the corresponding signals of F_{4a}, but these signals of F_{5a} were twice as intense as those of F_{4a}. These data indicated that F_{5a} has two magnetically equivalent *N*-methyl groups in its guanidine moiety, whereas F_{4a} has one methyl group in this moiety; this conclusion is consistent with the mass spectroscopic data described above. From these spectroscopic and analytical data (see "Experimental") the molecular formula of F_{5a} was determined to be $C_{57}H_{97}N_3O_{17}$.

In order to determine the positions of the *N*-methyl groups and also to verify the identity of other parts of the structures of F_{4a} and F_{5a}, F_{5a} was subjected to ozonolysis under the same reaction condition as in the case of F_{4a}.[6b] Sodium borohydride ($NaBH_4$) treatment of the ozonide gave the products **2**, **3** (as a mixture of the epimers at C-2), **4** (ethylene glycol), **5** ($C_{33}H_{64}O_{17}$; a malonyl hemiester of **6**) and **6** ($C_{30}H_{62}O_{14}$) (both **5** and **6** were obtained as mixtures of the epimers at both C-17 and C-30). Acetylation of **3** yielded a pair of epimers, **7** and **8**,

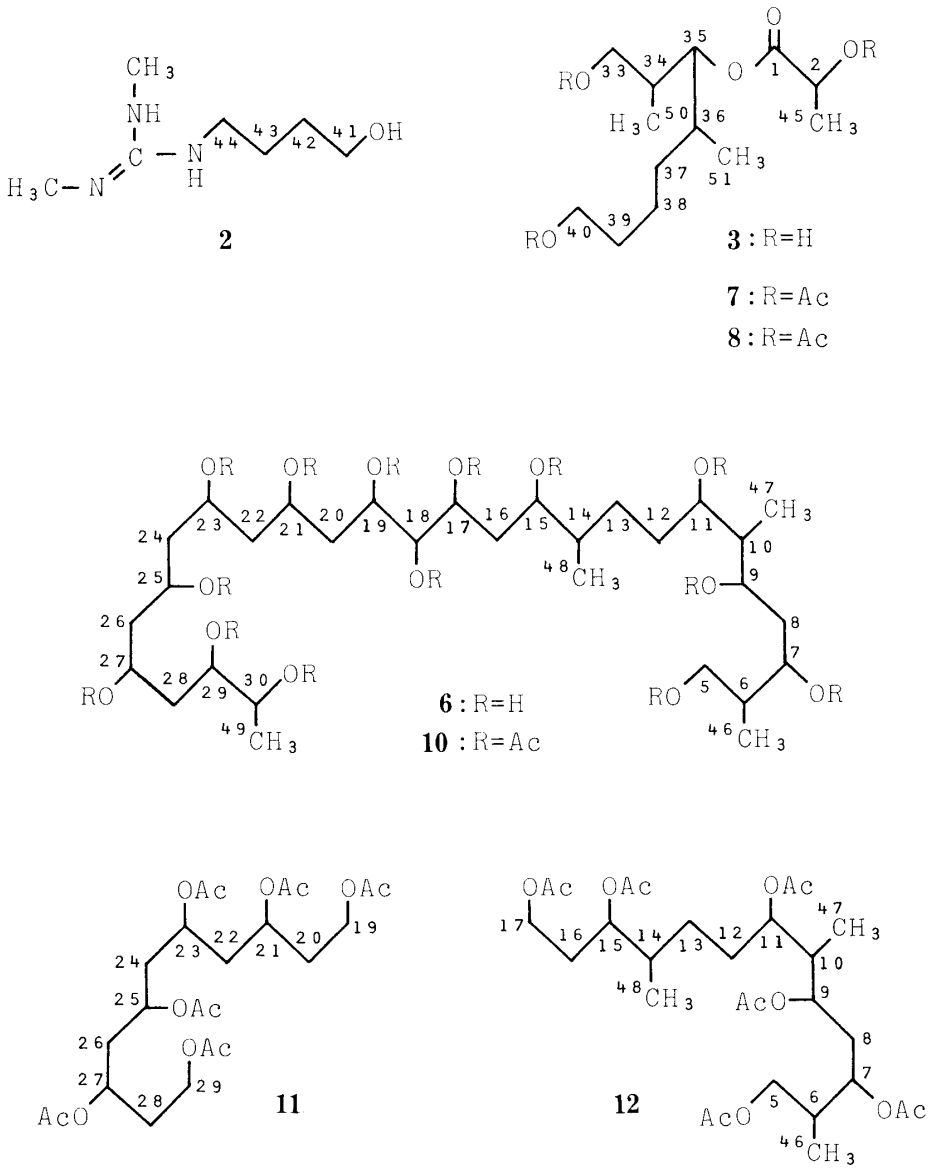


Fig. 4. The Structures of Degradation Products

which were separated by gas chromatography. Compounds **4** and **6** were converted to their acetates **9** and **10**, respectively. Periodate oxidation of **6** followed by $NaBH_4$ treatment and by subsequent acetylation afforded the compounds **11** and **12**.

The structures of the products **5**, **6**, **7**, **8**, **9**, **10**, **11** and **12** were identified spectroscopically as well as chromatographically by comparison with the same products obtained from the degradation of F_{4a} (compounds **12**, **5a**, **13a**, **14a**, **10a**, **15a** and **16a** in ref. 6*b*, respectively).

The ^{1}H-NMR spectrum of compound **2** exhibited a signal due to *N*-methyl groups at δ 2.86 (6 protons). Alkaline hydrolysis of this compound resulted in the formation of 4-aminobutanol (trapped as its *N*,*O*-dibenzoate) and methylamine (trapped as its benzoate). This result indicated that compound **2** has an *N*,*N*′,*N*″-trisubstituted guanidine moiety, and, consequently, the structure of azalomycin F_{5a} was determined as **1c** (shown in Fig. 1) in which the keto group at C-17 forms a hemiketal ring with a hydroxy group in the molecule. If the keto groups in F_{3a}, F_{4a} and F_{5a} form 6-membered hemiketal rings with their hydroxy group at C-21, the position of malonyl hemister formation should be restricted to C-23 in these compounds.

Experimental

General——Melting points were taken with a Yamato MP-1 apparatus and are uncorrected. UV spectra were measured on a Shimadzu apparatus (model UV-300), and the maxima are given in nm (extinction ε). IR spectra were measured on a Japan Spectroscopic Co. apparatus (model IR-S) and are recorded in cm^{-1}. ^1H-NMR spectra and ^{13}C-NMR spectra were measured on a JEOL JNM FX-400 (^1H: 400.5 MHz, ^{13}C: 100.7 MHz) machine; chemical shifts are given in ppm (in δ) relative to tetramethyl silane (TMS) (=0 ppm) as an internal standard and coupling constants are recorded in Hz (J). Mass spectra were measured on a Shimadzu LKB-9000 apparatus (EI-MS) or on a JEOL JMS-01SG-2 apparatus (FD-MS). Optical rotation was measured on a Japan Spectroscopic Co. apparatus (model DIP-181).

TLC was carried out on Merck DC-Fertigplatten (Kieselgel 60 F-254), and gas chromatography was performed on a Shimadzu GC-4A, GC-4APF or GC-4BPFT machine.

Azalomycin F complex was provided by Sankyo Co.

Column Chromatography of Azalomycin F Complex——A methanol solution of the F complex (10 g) was mixed with celite (25 g) and the solvent was evaporated off. The mixture was suspended in *sec*-butanol and applied to a column prepared with 500 g of silica gel in *sec*-butanol. The mixture was eluted with *sec*-butanol–water (9: 1). The first 5 l of eluate gave foreruns containing F_1 and F_2, and the next 3 l afforded a crude F_3 fraction containing *ca.* 1.5 g of a mixture of F_1, F_2 and F_3 (more than 60% of F_3). After further elution with this solvent mixture, yielding a 3 l eluate containing F_3 and F_4, a 6 l eluate containing *ca.* 5 g of F_4 and a 3 l eluate containing F_4 and F_5, the solvent system was changed to *sec*-butanol–water (4: 1). Elution with 5 l of the second solvent mixture yielded *ca.* 0.5 g of almost pure F_5.

Recrystallization of the crude F_3 fraction from aq. methanol gave a crude preparation of F_{3a} (more than 60% of F_{3a}), and recrystallization of F_5 fraction from aq. methanol afforded a crude preparation of F_{5a} (more than 90% of F_{5a}).

Table I. Assignment of ^1H-NMR Signals of Aazlomycin F_{3a} (in CD_3OD)

Signal No.	Chem. shift (ppm)	Multiplicity and coupling constant (J in Hz)	Assignment[a]
1.	0.88	d, $J_{47-10}=7.0$	H_3-47
2.	0.90	d, $J_{48-14}=7.0$	H_3-48
3.	0.94	d, $J_{51-36}=6.8$	H_3-51
4.	1.00	d, $J_{50-34}=6.8$	H_3-50
5.	1.11	d, $J_{46-6}=6.8$	H_3-46
6.	1.64	d, $J_{40-31}=1.6$	H_3-49
7.	1.82	m, $J_{36-35}=4.0$, $J_{36-51}=6.8$	H-36
8.	1.90	d, $J_{45-3}=1.6$	H_3-45
9.	1.99	m,	H_2-39
10.	2.06	m,	H_2-42
11.	2.45	m, $J_{6-5}=8.8$, $J_{6-46}=6.8$, $J_{6-7}=4.8$	H-6
12.	2.55	m, $J_{34-33}=8.8$, $J_{34-35}=8.0$, $J_{34-50}=6.8$	H-34
13.	3.14	t, $J_{44-43}=6.8$	H_2-44
14.	3.23	s,	H_2-2′
15.	3.33	d, $J_{18-19}=10.0$	H-18
16.	3.77	m, $J_{7-6}=4.8$	H-7
17.	4.00	m,	H-27
18.	4.17	dd, $J_{29-28}=8.9$ and 3.6	H-29
19.	4.78	dd, $J_{35-34}=8.0$, $J_{35-36}=4.0$	H-35
20.	5.22	m,	[b]
21.	5.42	m, $J_{41-40}=15.0$	H-41
22.	5.43	dd, $J_{33-32}=14.9$, $J_{33-34}=8.8$	H-33
23.	5.44	m, $J_{40-41}=15.0$	H-40
24.	5.99	d, $J_{31-32}=11.2$	H-31
25.	6.06	dd, $J_{5-4}=14.7$, $J_{5-6}=8.8$	H-5
26.	6.21	dd, $J_{32-31}=11.2$, $J_{32-33}=14.9$	H-32
27.	6.43	dd, $J_{4-3}=11.2$, $J_{4-5}=14.7$	H-4
28.	7.09	d, $J_{3-4}=11.2$	H-3

a) Assignments were made on the basis of decoupling experiments.
b) The methine group bearing the malonyl hemiester.

Isolation of F_{3a} and F_{5a}——The crude preparation of F_{3a} (10—15 mg) obtained from column chromatography followed by recrystallization was applied to a TLC plate (20 cm × 20 cm, 0.25 mm thick) which was developed with a solvent system composed of butanol–acetic acid–water (5: 1: 1) for 18—36 h. During development, the top of the plate was exposed to air to allow the solvents to evaporate. F_{3a} fraction was obtained by extraction of the appropriate region of plate silica gel and the extract was filtered to remove insoluble materials. The crude F_{3a} thus obtained was recrystallized from aq. methanol to give fine needles; *Anal.* Calcd for $C_{55}H_{93}N_3O_{17}$: C, 61.83; H, 8.77; N, 3.93; O, 25.46. Found: C, 61.45; H, 8.98; N, 3.73; O, 25.40., mp 134—135°C (dec.), $[\alpha]_D^{27}$ +35° (c=1.0, methanol), FD-MS m/z: 1067 (M^+), UV λ_{max}^{MeOH} nm (ε): 240 (41100), 268 (24700), IR ν_{max}^{KBr} cm^{-1}: 3400 (br s), 2940 (s), 1680 (br s), 1635 (s), 1600 (s), 1435 (m), 1380 (s), 1290 (m), 1245 (s), 1090 (s), 1065 (s), 970 (m), ^{1}H-NMR: see Fig. 2 and Table I, ^{13}C-NMR: see Fig. 3 and Table III.

F_{5a} was purified in the same way as in the case of F_{3a}, yielding fine needles; *Anal.* Calcd for $C_{57}H_{97}N_3O_{17}$: C, 62.44; H, 8.92; N, 3.83; O, 24.81. Found: C, 62.12; H, 9.24; N, 3.77; O, 25.09., mp 126—127°C (dec.), $[\alpha]_D^{27}$ +44° (c=1.0, methanol), FD-MS m/z: 1095 (M^+), UV λ_{max}^{MeOH} nm (ε): 240 (38300), 268 (22700), IR ν_{max}^{KBr} cm^{-1}: 3400 (br s), 2930 (s), 1700 (m), 1635 (br s), 1600 (s), 1445 (m), 1370 (s), 1250 (s), 1075 (br s), 965 (m), ^{1}H-NMR: see Fig. 2 and Table II, ^{13}C-NMR: see Fig. 3 and Table III.

Interconversion of F_{3a} and F_{3b}, and F_{5a} and F_{5b}——Methanol solutions of F_{3a} and of F_{3b} were allowed to stand at room temperature. Conversion of one to the other was observed in both solutions by TLC analysis after 7 d. Decomposition of these compounds was also observed. Similarly, the interconversion of F_{5a} and F_{5b}, and their decomposition were observed on standing of their methanol solutions at room temperature.

Ozonolysis of Azalomycin F_{5a}——1) F_{5a} (900 mg) was ozonized in methanol (50 ml) at −78°C, and the ozonide was decomposed by treatment with $NaBH_4$ (1 g in 10 ml methanol) for 1 h. The reaction solution was neutralized with conc. HCl and concentrated. A mixture of the ozonolysis products, containing a large amount of salt, was extracted with methanol to separate the products from salt, and the extract was again concentrated. This process was repeated several times. The concentrate was dissolved in 50 ml of water

Table II. Assignment of ^{1}H-NMR Signals of Azalomycin F_{5a} (in CD_3OD)

Signal No.	Chem. shift (ppm)	Multiplicity and coupling constant (J in Hz)	Assignment[a]
1.	0.87	d, J_{47-10} = 7.0	H_3-47
2.	0.91	d, J_{48-14} = 7.0	H_3-48
3.	0.94	d, J_{51-36} = 6.9	H_3-51
4.	1.00	d, J_{50-34} = 6.8	H_3-50
5.	1.10	d, J_{46-6} = 6.8	H_3-46
6.	1.64	d, J_{49-31} = 1.6	H_3-49
7.	1.82	m, J_{36-35} = 4.0, J_{36-51} = 6.9	H-36
8.	1.91	d, J_{45-3} = 1.6	H_3-45
9.	1.98	m,	H_2-39
10.	2.06	m,	H_2-42
11.	2.43	m, J_{6-5} = 8.8, J_{6-46} = 6.8, J_{6-7} = 4.8	H-6
12.	2.55	m, J_{34-33} = 8.8, J_{34-35} = 8.0, J_{34-50} = 6.8	H-34
13.	2.84	s,	2(N-CH_3)
14.	3.15	t, J_{44-43} = 6.8	H_2-44
15.	3.22	s,	H_2-2′
16.	3.33	d, J_{18-19} = 10.2	H-18
17.	3.76	m, J_{7-6} = 4.8	H-7
18.	4.02	m,	H-27
19.	4.17	dd, J_{29-28} = 8.8 and 3.6	H-29
20.	4.78	dd, J_{35-34} = 8.0, J_{35-36} = 4.0	H-35
21.	5.21	m,	[b]
22.	5.41	m, J_{41-40} = 14.9	H-41
23.	5.42	dd, J_{33-32} = 14.9, J_{33-34} = 8.8	H-33
24.	5.43	m, J_{40-41} = 14.9	H-40
25.	5.98	d, J_{31-32} = 11.2	H-31
26.	6.05	dd, J_{5-4} = 14.7, J_{5-6} = 8.8	H-5
27.	6.21	dd, J_{32-31} = 11.2, J_{32-33} = 14.9	H-32
28.	6.42	dd, J_{4-3} = 11.2, J_{4-5} = 14.7	H-4
29.	7.08	d, J_{3-4} = 11.2	H-3

a) Assignments were made on the basis of decoupling experiments.
b) The methine group bearing the malonyl hemiester.

TABLE III. ^{13}C-NMR Data for Azalomycins F_{3a}, F_{4a} and F_{5a}

Signal No.	F_{3a}	F_{4a}	F_{5a}	Multiplicity	Assignment[a]
1.	10.49	10.52	10.52	q	47
2.	12.89	12.88	12.87	q	45
3.	13.35	13.33	13.33	q	49
4.	14.37	14.37	14.35	q	51
5.	14.86	14.87	14.93	q	48
6.	17.04	17.00	17.05	q	46
7.	17.60	17.63	17.64	q	50
8.	27.87	27.89	27.90	t	
9.		28.36	28.40	q	N-CH_3
10.			28.40	q	N-CH_3
11.	29.79	29.84	29.84	t	
12.	30.56	30.57	30.62	t	
13.	30.56	30.57	30.62	t	13[b]
14.	33.59	33.57	33.59	t	
15.	33.59	33.57	33.59	t	
16.	34.46	34.47	34.49	t	
17.	35.15	35.16	35.17	d	
18.	39.26	39.24	39.31	t	
19.	40.77	40.73	40.77	d	
20.	40.91	40.90	40.95	d	
21.	41.20	41.19	41.25	t	
22.	41.88	41.85	41.90	t	
23.	41.88	41.98	42.12	t	
24.	41.88	41.98	42.12	t	
25.	44.04	44.04	44.16	t	
26.	44.45	44.40	44.46	d	
27.	44.45	44.49	44.46	d	
28.	44.62	44.58	44.64	t	
29.	46.10	46.10	46.10	t	2′
30.	46.39	46.37	46.44	t	
31.	65.52	65.53	65.59	d	
32.	65.61	65.62	65.59	d	
33.	66.27	66.32	66.29	d	27
34.	69.71	69.70	69.73	d	
35.	70.68	70.69	70.75	d	c)
36.	72.35	72.34	72.37	d	
37.	72.35	72.40	72.42	d	
38.	74.24	74.24	74.26	d	29
39.	75.02	75.10	75.03	d	9
40.	75.76	75.78	75.77	d	7
41.	77.25	77.27	77.33	d	18
42.	80.77	80.77	80.81	d	35
43.	99.79	99.78	99.79	s	17[d]
44.	125.14	125.12	125.10	d	31
45.	126.76	126.74	126.75	s	2
46.	127.62	127.57	127.58	d	4
47.	128.54	128.52	128·56	d	32
48.	130.13	130.19	130.27	d	41
49.	132.63	132.51	132.54	d	40
50.	136.17	136.14	136.15	d	33
51.	140.08	140.09	140.18	s	30
52.	140.20	140.17	140.18	d	3
53.	146.06	146.07	146.10	d	5
54.	158.69	158.27	157.37	s	e)
55.	170.06	170.05	170.10	s	1
56.	171.63	171.60	171.63	s	1′
57.	174.10	174.06	173.87	s	3′

a) Assignments were made on the basis of ^{1}H-^{13}C selective decoupling experiments. *b*) One of these two triplets.
c) The carbon bearing the malonyl hemiester. *d*) The carbon forming the hemiketal. *e*) Guanido carbon.

and the aqueous solution was passed successively through columns of IRA-410 and IRC-50. The latter column was eluted with 1 N HCl and the eluate was neutralized with 1 N NaOH. The basic product **2** was extracted with methanol from the concentrate, which contained a large amount of salt, by repeating this process several times. The product **2** was finally purified by HPLC (ODS-3, Whatman).

2 ($C_7H_{17}N_3O$)——IR ν_{max}^{KBr} cm^{-1}: 3600—2700 (br, s), 2940, 1630 (br, s), 1450, 1370, 1180, 1050. EI-MS *m/z*: 159 (M^+). ^{1}H-NMR (in CD_3OD) δ: 3.60 (2H, t, J=6.3 Hz, H_2-41), 3.24 (2H, t, J=7.1 Hz, H_2-44), 2.86 (6H, s, *N*–CH_3), 1.68 (2H, m, H_2-43), 1.60 (2H, m, H_2-42). ^{13}C-NMR (in CD_3OD) δ: 157.3 (s, guanido carbon), 62.4 (t, C-41), 42.5 (t, C-44), 30.5 (t, C-42 or C-43), 28.4 (q, *N*–CH_3), 26.7 (t, C-42 or C-43).

2) A solution obtained from ozonolysis of F_{5a} followed by $NaBH_4$ treatment in the same way as in 1) was passed through a column of Amberlite IR-120 B (40 ml) to remove the basic product **2**, and the column was washed successively with methanol (100 ml) and water (50 ml). The washing solvents was combined with the reaction solution after passage through the column. The combined solution was concentrated to *ca.* 20 ml, and this was extracted with ethyl acetate. The extract was concentrated (130 mg of oil, mainly **3** as a mixture of epimers at C-2) and the residue was acetylated. Separation of the acetate mixture by silica gel column chromatography followed by gas chromatographic purification gave **7** (40 mg) and **8** (55 mg). These compounds were identical with the same products obtained from the degradation of F_{4a} (**13a** and **14a**, respectively, in Ref. 6*b*). The water layer after extraction of **3** was concentrated to give a mixture of compounds **4** (ethylene glycol), **5** (as a mixture of epimers at both C-17 and C-30) and a small amount of **6** (as a mixture of epimers at both C-17 and C-30). The compounds **5** and **6** were indistinguishable on TLC from the same degradation products of F_{4a} (**12** and **5a**, respectively in Ref. 6*b*). This product mixture was further treated with $NaBH_4$ (360 mg) in water for 18 h. The reaction solution after successive passage through columns of Amberlite IR-120B and of IRA-410 was concentrated (210 mg of oily mixture of **4** and **6**). The mixture was acetylated and the acetates **9** and **10** were separated by silica gel column chromatography. Ethylene glycol diacetate **9** was identified by comparison with an authentic specimen, and **10** was identified by chromatographic and spectroscopic comparisons with the same product from F_{4a} (**10a** in Ref. 6*b*).

Alkaline Hydrolysis of Compound 2——Compound **2** (40 mg) in aq. 3 N NaOH (10 ml) was heated at reflux (6 h) under gentle bubbling of nitrogen, and the evolved gas was passed through aq. 1 N HCl to trap the generated amine. The HCl was evaporated off to give the amine hydrochloride, which was treated with benzoyl chloride in aq. alkali to give *N*-methylbenzamide. The remaining reaction solution was, after cooling to room temperature, treated with benzoyl chloride to afford 4-aminobutanol *N*,*O*-dibenzoate.

Periodate Oxidation of 6——A solution of **6** (110 mg) and $NaIO_4$ (120 mg) in water was stirred for 4 h at room temperature. Excess periodate was decomposed with ethylene glycol. Water was evaporated off *in vacuo*, and the residue was extracted with methanol. The extract was concentrated, and the residue was treated with $NaBH_4$ (50 mg) in water (15 ml) under ice cooling. The reaction solution was then stirred with Amberlite IR-120B and with IRA-410, successively. Water was evaporated off to give an oily product mixture. Acetylation of the products followed by gas chromatographic separation afforded **11** and **12**. They were identical with the same products obtained from the degradation of F_{4a} (**15a** and **16a**, respectively, in Ref. 6*b*).

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