[Chem. Pharm. Bull. 30(5)1658—1668(1982)]

Studies on Macrocyclic Lactone Antibiotics. II.[1] Partial Structures of Azalomycin F_{4a}

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(Received October 14, 1981)

Degradations of azalomycin F_{4a} were carried out, and the structures of the products were determined.

Keywords——azalomycin F_{4a}; macrocyclic lactone antibiotics; degradation; ozonolysis; periodate oxidation; partial structures

In the preceding paper[1] we showed that azalomycin F_{4a} possesses partial structures composed of a conjugated dienoic acid (or ester) moiety, a conjugated diene system, and an isolated olefinic bond, as well as multiple oxygen functions. Since attempts to obtain crystals of F_{4a} or of its derivatives suitable for X-ray crystallographic analysis have so far been unsuccessful, this compound and its derivatives were subjected to ozonolyses and to periodate oxidations to obtain degradation products which might facilitate the structural study of F_{4a}. This paper deals with the structures of such degradation products.

Results of Degradation Experiments

1. Ozonolysis of Azalomycin F_{4a}——Azalomycin F_{4a}, on ozonization in methanol at −78°C followed by decomposition of the ozonide by sodium borohydride ($NaBH_4$) treatnent for 24 h, afforded degradation products **2a** ($C_6H_{15}N_3O$), **3a** ($C_{10}H_{22}O_3$), ethylene glycol (**4a**, $C_2H_6O_2$) and **5a** ($C_{30}H_{62}O_{14}$). All of these products were transformed to their acetates **6a**, **8a**, **9a** and **10a**, respectively. Compound **3a** could not be obtained in pure form, and it was purified as its acetate **8a**.

Compound **5a** was obtained as a mixture of 4 stereoisomers epimeric at C-17[3] and at C-30. Stereoisomerism at C-17 allowed separation of the epimers at this position by thin-layer chromatography (TLC) to give a pair of mixtures of C-30 epimers. One of these mixtures which showed a higher *Rf* value on TLC was purified and designated as **5a**.

An alkaline hydrolysis of **2a** afforded methylamine (trapped as its hydrochloride) and 4-aminobutanol which was identified as its *N*,*O*-dibenzoate (**7**) by comparison with an authentic specimen.

A short (1 h) $NaBH_4$ treatment of the ozonide yielded, in addition to the products mentioned above, an ester **11a** ($C_{13}H_{26}O_5$) and an acidic compound **12** ($C_{33}H_{64}O_{17}$). Compound **11a** was obtained as a mixture of epimers at C-2, and they were separated as their acetates **13a** and **14a**. An alkaline hydrolysis of **11a** gave **3a** and lactic acid. Compound **12** was unstable and decomposed during the purification process. An alkaline hydrolysis of the crude **12** gave rise to **5a** and malonic acid.

Decomposition of the ozonide with sodium borodeuteride ($NaBD_4$, 24 h treatment) followed by work-up and separations as in the case of $NaBH_4$ treatment afforded **2b**, **3b**, **4b** and **5b** (1*d*, 2*d*, 2*d* and 3*d* incorporations, respectively). A short treatment (1 h) of the ozonide with $NaBD_4$ gave **11b**, and acetylation of **11b** yielded **13b** and **14b**.

2. **Periodate Oxidation of 5a and 5b**——Compound **5a** was oxidized with sodium periodate ($NaIO_4$) in water at room temperature. The crude mixture of oxidation products, on $NaBH_4$ reduction followed by acetylation, afforded **15a** and **16a** with loss of a $C_3H_7O_2$ unit involved in **5a**, and $NaBD_4$ reduction followed by acetylation yielded **15b** and **16b**.

The same degradation reaction of **5b** using $NaBH_4$ as the reducing agent gave **15a** and **16c**.

3. **Ozonolysis of Acetylation Product of F_{4a}**——F_{4a} suspended in anhydrous dioxane was acetylated with acetic anhydride in pyridine using 4-dimethylaminopyridine as the catalyst. ^{13}C-Nuclear magnetic resonance (NMR) spectrum of the crude acetylation product showed no peak due to hemiketal carbon but instead a signal due to a carbonyl carbon conjugated with a double bond (δ 192.2). Ozonization of the crude acetate followed by $NaBH_4$ treatment and reacetylation afforded, in addition to **6a**, **8a** and **9a**, compound **17** which could presumably be derived *via* a $\Delta^{15(16)}$-17-keto compound formed by 15(16)-deacetoxylation.

4. **Ozonolysis of Partial Hydrogenation Products of F_{4a}**——A mixture of partially hydrogenated products of F_{4a} (3 molar equivalents of H_2 taken up) was subjected to an ozonolysis followed by $NaBH_4$ treatment for the decomposition of the ozonides. A mixture of basic products separated on an Amberlite IR-120B column was hydrolyzed with 3 N KOH (ethanol–water, 1 : 1) to give a mixture of aminoalcohols. Acetylation of the mixture followed by gas chromatographic (GC) separation afforded **18**, **19** and **20**. The carbon chains of these products should be derived from Δ^{30}, Δ^{31} and Δ^{32}-compounds formed *via* non-selective partial hydrogenation of the diene system, C-30—C-33.

5. **Periodate Oxidation of F_{4a}**——$NaIO_4$ oxidation of F_{4a} in methanol–water (2 : 1) at room temperature followed by $NaBH_4$ treatment of the oxidation products afforded a mixture. Alkaline hydrolysis at room temperature to cleave the ester linkage, followed by acetylation and diazomethane treatment, yielded a products mixture which was separated by silica gel column chromatography affording a guanidine derivative **21** and a methyl ester **22**, together with other products not discussed in this paper. Compound **21** was unstable and was subjected to ozonolysis without further purification using $NaBH_4$ for decomposition of the ozonide. Reacetylation of the products mixture followed by chromatographic separation yielded **6a**, **8a**, **9a** and **23**.

Structures of Degradation Products

Structural assignments of the degradation products were generally based on their spectral[4] and analytical data.

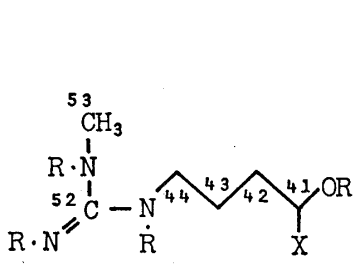
The structure of **2a** was indicated by its molecular ion peak (M^+, m/z 145) in its field desorption mass spectrum (FD–MS), and ultraviolet (UV) and ^1H-NMR spectra, and its structure was confirmed by analytical and spectral data for its acetate **6a**. Elemental analysis of **6a** proved the presence of 3 nitrogen atoms, and the ^1H- and ^{13}C-NMR spectra showed the presence of an *N*-methyl group, and of a 4 methylene carbon chain between an acetoxyl group and a nitrogen function, whose sequence was established by simple decoupling experiments (for the data see "Experimental"). The structure of **2a** was also supported by the fact that **2a** gave, on alkaline hydrolysis, 4-aminobutanol (purified as its *N*,*O*-dibenzoate (**7**)) and methylamine (trapped as its hydrochloride).

The deuterated position in **2b** was determined from its ^1H-NMR spectrum in which the signal due to H-41 appearing at δ 3.72 gave one-proton integration.

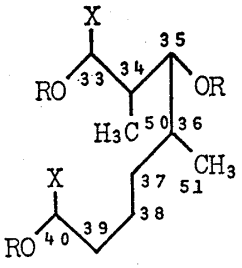
In the ^1H-NMR spectrum of **8a**, all the signals due to non-equivalent protons appeared separately and their assignments were established by decoupling experiments (see Table II and "Experimental"). Hence, the structure of **3a** was determined.

According to these assignments, one deuterium incorporation at C-33 and another at C-40 were found for **8b** (consequently for **3b**), from the integrations of the signals at δ 4.01 and 3.95, and at δ 4.04.

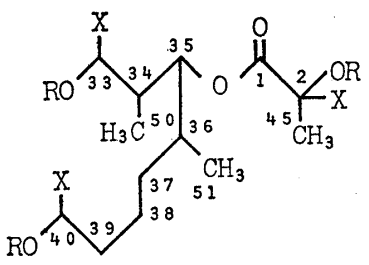
The structure of **11a** (and consequently those of **13a** and **14a**) was easily determined from



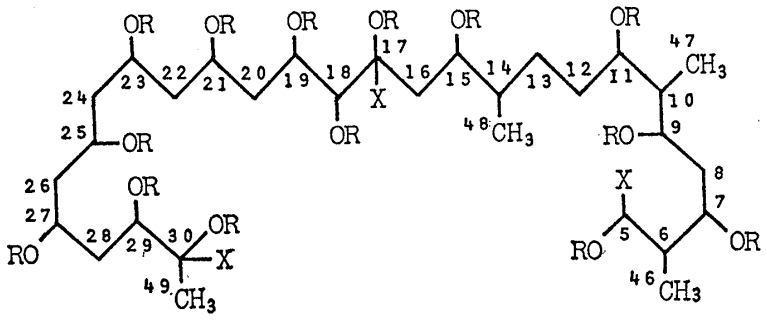
2a: R=H, X=H
2b: R=H, X=D
6a: R=Ac, X=H
6b: R=Ac, X=D



3a: R=H, X=H
3b: R=H, X=D
8a: R=Ac, X=H
8b: R=Ac, X=D



11a: R=H, X=H
11b: R=H, X=D
13a, 14a: R=Ac, X=H
13b, 14b: R=Ac, X=D



5a: R=H, X=H
5b: R=H, X=D
10a: R=Ac, X=H
10b: R=Ac, X=D

Fig. 1

the fact that its alkaline hydrolysis afforded **3a** and lactic acid. The deuterated positions (at C-2, C-33 and C-40) in **13b** and **14b** demonstrated that these carbons were originally present in double bonds in F_{4a}, and this indicated that the hydroxy function at C-35 should have formed the ester bond in F_{4a}.

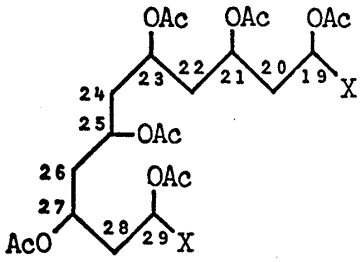
Compound **5a**, a mixture of epimers at C-30, showed the molecular ion peak at m/z 646 in its FD–MS, and its elemental analysis was in accord with the molecular formula $C_{30}H_{62}O_{14}$ (see "Experimental"). This was supported by analytical and spectral data for its acetylated derivative **10a** whose FD–MS showed the molecular ion peak at m/z 1234, indicating incorporation of 14 acetyl groups, and whose ^{13}C-NMR spectrum exhibited, in addition to the signals of acetyl groups, signals of 4 methyl groups, 10 methylene groups and 16 methine groups. Of these, the signals assigned as C-49 appeared as two quartets (δ 16.1 and 15.0), and the signals due to a methylene group appeared as two triplets (δ 35.1 and 34.0). Such a spectrum was seen for **5a** because it was still a mixture of epimers at C-30 as mentioned before. The structure of compound **5a** was still too complex for complete spectroscopic analysis, and it was, therefore, elucidated from the data for its degradation products **15a** and **16a**, and their deuterated derivatives **15b**, **16b** and **16c**, as well as of compound **23** obtained by ozonolysis of the periodate oxidation product of F_{4a}. The structure of **5a** will, therefore, be discussed in the following paper.

FD–MS of **5b** and of **10b** showed molecular ion peaks at m/z 649 and 1237, respectively, demonstrating incoroporation of 3 deuterium atoms into **5b** by $NaBD_4$ reduction of the ozonide of F_{4a}. Deuteration at the C-5 position of **5b** was determined by analysis of the ^{1}H-NMR spectrum of **10b** in which the integration of the signal at δ 3.95 (assigned as H_2-5) corresponded to one proton.

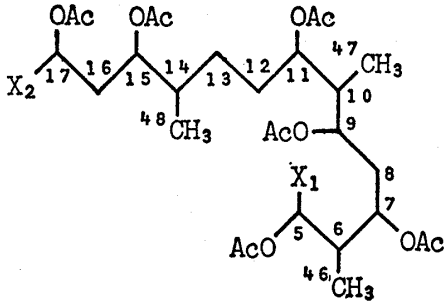
The ^{1}H-NMR spectrum of **15a** apparently showed 5 signals due to CH-OAc, CH_2-OAc, acetyl methyls and two sets of CH_2 groups with 2: 2: 9: 2: 3 relative intensity ratio, respecti-

vely. Its ^{13}C-NMR spectrum exhibited, in addition to the signals of acetyl carbons, only 6 signals of which three are due to carbons bearing acetoxyl groups and the others are due to methylene carbons without acetoxyl substitution. One of these methylene signals appearing at δ 39.6 was shown to have only half the intensity of the others. These NMR data (see Table III, and "Experimental") and a fragment ion peak in the electron impact mass spectrum (EI-MS) at m/z 417 ($M^+-CH_2CH_2OAc$) indicated that the compound possesses $4\times$CH-OAc, $2\times CH_2$-OAc and $5\times CH_2$ including a symmetric center carbon (a triplet at δ 39.6 in ^{13}C-NMR). Since it is the product of a periodate oxidation it should have no 1,2-glycol moiety, and, hence, its skeletal structure was determined unambiguously. The fact that **15a** has optical rotation (see "Experimental") indicates the presence of a symmetric plane at C-24.

One deuterium incorporation at each terminal carbon (C-19 and C-29) in **15b** was apparent from its ^{1}H-NMR spectrum and EI-MS data (m/z 418).



15a: X=H
15b: X=D



16a: $X_1=X_2=H$
16b: $X_1=H$, $X_2=D$
16c: $X_1=X_2=D$

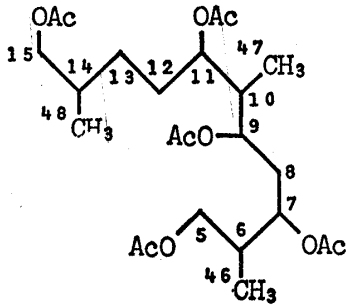


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Fig. 2

TABLE I. ^{1}H- and ^{13}C-NMR Data for **16a** and **17**

Carbon No.	**16a** ^{1}H Signal			**16a** ^{13}C Signal		**17** ^{1}H Signal			**17** ^{13}C Signal	
5	4.02 3.98	dd, dd,	$J_{5-5}=11.2$, $J_{5-6}=5.9$ $J_{5-5}=11.2$, $J_{5-6}=6.3$	65.3	t	4.02 3.98	dd, dd,	$J_{5-5}=11.2$, $J_{5-6}=5.9$ $J_{5-5}=11.2$, $J_{5-6}=6.3$	65.2	t
6	2.11	m,	$J_{6-5}=5.9$ and 6.3, $J_{6-7}=4.9$, $J_{6-46}=7.0$	36.1	d	2.11	m,	$J_{6-5}=5.9$ and 6.3, $J_{6-7}=4.9$, $J_{6-46}=7.2$	35.9	d
7	4.94	ddd,	$J_{7-6}=4.9$, $J_{7-8}=5.1$ and 6.9	72.4	d	4.94	ddd,	$J_{7-6}=4.9$, $J_{7-8}=5.1$ and 6.9	72.2	d
8	2.00 1.83	m, m,	$J_{8-7}=5.1$ and 6.9	33.5	t	2.00 1.86	m, m,	$J_{8-7}=5.1$ and 6.9	33.3	t
9	4.80	ddd,	$J=7.8$, 7.1 and 3.9	72.0	d	4.80	ddd,	$J=7.8$, 7.1 and 3.9	71.8	d
10	1.83	m,	$J_{10-47}=7.0$	39.3	d	1.81	m,	$J_{10-47}=7.2$	39.1	d
11	5.00	ddd,	$J=8.1$, 5.5 and2.8	72.1	d	5.02	ddd,	$J=8.1$, 5.5 and 2.8	71.8	d
12	1.72 1.40	m, m,		29.9	t	1.67 1.46	m, m,		29.5	t
13	1.32 1.10	m, m,		28.0	t	1.34 1.14	m, m,		29.1	t
14	1.72	m,	$J_{14-48}=6.8$	36.3	d	1.78	m,	$J_{14-15}=6.1$ and 6.6, $J_{14-48}=7.0$	32.4	d
15	4.89	ddd,	$J=5.0$, 5.0 and 8.0	74.1	d	3.91 3.88	dd, dd,	$J_{15-15}=11.0$, $J_{15-14}=6.1$ $J_{15-15}=11.0$, $J_{15-14}=6.6$	68.8	t
16	1.83	m,		29.4	t					
17	4.07	m,		61.1	t					
46	0.97	d,	$J_{46-6}=7.0$	13.6	q	0.96	d,	$J_{46-6}=7.2$	13.5	q
47	0.97	d,	$J_{47-10}=7.0$	9.8	q	0.97	d,	$J_{47-10}=7.2$	9.7	q
48	0.89	d,	$J_{48-14}=6.8$	14.8	q	0.93	d,	$J_{48-14}=7.0$	16.8	q

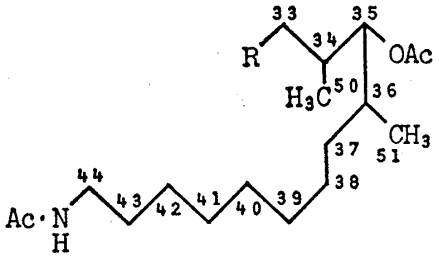
In the ^{1}H-NMR spectrum of **17** all the signals of non-equivalent protons appeared separately, which allowed complete assignment of these signals by simple decoupling experiments. Selective ^{1}H-^{13}C decoupling experiments by irradiating the resonance positions of all the proton signals permitted complete assignment of the ^{13}C signals (see Table I). These data demonstrate that this compound consists of the following functional groups; $2\times CH_2$-OAc, $3\times$CH-OAc, $3\times$CH, $3\times CH_2$ and $3\times CH_3$, and these data completely define the structure of **17**.

Structure determination of **16a** could be achieved by comparisons of its NMR data with those of **17** (see Table I). The ^{13}C-NMR spectrum of **16a** showed signals due to $2\times CH_2$-OAc, $4\times$CH-OAc, $3\times$CH, $4\times CH_2$ and $3\times CH_3$, *i.e.*, two carbon units less ($1\times$CH-OAc and $1\times CH_2$) than in **17**. Chemical shifts and coupling modes of the signals of the system, C-5—C-14, in the ^{1}H- and ^{13}C-NMR of **16a** indicated close similarity of its partial structure to the structure of **17**, suggesting the overlapping of this system (C-5—C-14) in these two compounds. The structure of **16a** was, thus, elucidated.

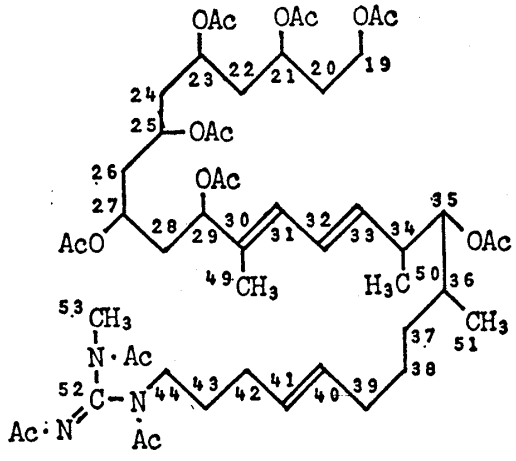
The ^{1}H-NMR spectra of **16b** and **16c** showed deuterium incorporation at C-17, and at C-5 and C-17, respectively.

Three nitrogen-containing compounds **18**, **19** and **20** obtained from ozonolysis of the partial hydrogenation products of F_{4a} should be derived from the same partial segment in the F_4 molecule, and, therefore, should have a common carbon skeleton. Their ^{13}C-NMR spectra evidently show that the differences in their structures lie in the numbers of methylene groups (see "Experimental").

On comparisons of the ^{1}H- and ^{13}C-NMR spectra of **18** with those of **8a**, the signals due to the partial structure, the system C-33—C-36, of **18** showed close similarity to the spectra of



18: R=OAc
19: R=CH_2OAc
20: R=CH_2CH_2OAc



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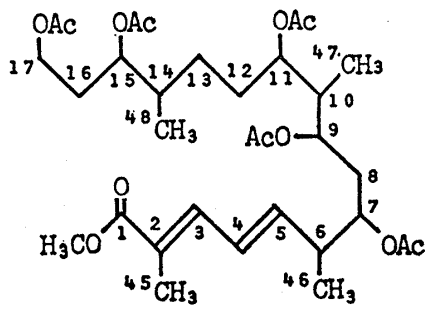

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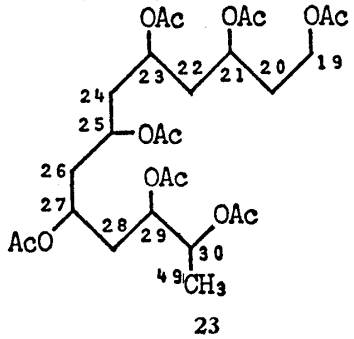

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Fig. 3

TABLE II. ^{1}H- and ^{13}C-NMR Data for **8a** and **18**

Carbon No.	8a ^{1}H Signal			8a ^{13}C Signal		18 ^{1}H Signal			18 ^{13}C Signal	
33	4.01	dd,	$J_{33-33}=11.5$, $J_{33-34}=5.0$	66.0	t	4.01	dd,	$J_{33-33}=11.0$, $J_{33-34}=4.5$	66.1	t
	3.95	dd,	$J_{33-33}=11.5$, $J_{33-34}=6.0$			3.92	dd,	$J_{33-33}=11.0$, $J_{33-34}=6.0$		
34	2.09	m,	$J_{33-34}=5.0$ and 6.0, $J_{34-50}=7.0$, $J_{34-35}=8.1$	34.4	d	2.11	m,	$J_{34-33}=4.5$ and 6.0, $J_{34-35}=8.0$, $J_{34-50}=6.8$	34.5	d
35	4.82	dd,	$J_{35-34}=8.1$, $J_{35-36}=4.0$	77.2	d	4.83	dd,	$J_{35-34}=8.0$, $J_{35-36}=4.0$	77.6	d
36	1.73	m,	$J_{36-35}=4.0$, $J_{36-51}=6.8$	34.2	d	1.72	m,	$J_{36-35}=4.0$, $J_{36-51}=7.0$	34.3	d
37	1.22	m,		33.4	t				7 triplets	
	1.12	m,							33.8	
38	1.39	m,		23.5	t				29.6 x 2	
39	1.61	m,	$J_{39-40}=6.8$	28.8	t				29.4	
40	4.04	t,	$J_{40-39}=6.8$	64.3	t			1.20—1.35	29.2	
41									27.0	
42									26.9	
43						1.48	m,	$J_{43-44}=6.0$		
44						3.22	dt,	$J_{44-43}=6.0$	39.7	t
50	0.97	d,	$J_{50-34}=7.0$	14.4	q	0.97	d,	$J_{50-34}=6.8$	14.5	q
51	0.88	d,	$J_{51-36}=6.8$	13.4	q	0.86	d,	$J_{51-36}=7.0$	13.6	q

8a in terms of the chemical shifts and the coupling modes, indicating the overlapping of the carbon skeleton of **8a** with the partial structure of **18** (see Table II). Since **18** possesses 4 additional methylene carbons as compared to the carbon chain of **8a** (consequently of **3a**), fragments **2a** and **3a** should be connected directly through the C-40—C-41 linkage which originally formed a C–C double bond in F_{4a}.

The structures of **19** and **20**, which have one and two additional methylene groups, respectively, as compared to the carbon skeleton of **18** were thus easily determined.

Compound **23** was obtained as a mixture of epimers at the carbon bearing a methyl group (C-30), as indicated by its ^{1}H-NMR spectrum exhibiting two doublets at δ 1.20 and 1.22 (3 proton integration with these two), and in its ^{13}C-NMR spectrum exhibiting two quartets at δ 16.1 and 15.0. The ^{13}C-NMR spectrum revealed the presence of $1\times CH_2$-OAc, $6\times$CH-OAc,

TABLE III. ^{13}C-NMR Data for **15a** and **23**

Carbon No.	15a ^{1}H Signal		15a ^{13}C Signal		23 ^{1}H Signal			23 ^{13}C Signal	
19	4.06	m	60.5	t	4.18	t	$J_{19-20}=7.5$	60.6	t
20	1.88	m	33.6	t	1.92	m		33.6	t
21	4.99	m	66.7	d	5.00	m		66.8	d
22	1.82	m	39.1	t	1.84	m		39.2	t
23	4.99	m	67.5	d	5.00	m		66.8, 67.0 or 67.5	d
24	1.82	m	39.6	t	1.84	m		39.5	t
25	4.99	m	67.5	d	5.00	m		66.8, 67.0 or 67.5	d
26	1.82	m	39.1	t	1.84	m		39.5	t
27	4.99	m	66.7	d	5.00	m		66.8, 67.0 or 67.5	d
28	1.88	m	33.6	t	1.84	m		35.2	t[a]
29	4.06	m	60.5	t				34.2	
30					5.00	m		70.6	d
49					5.00	m	$J_{30-49}=7.0$	70.5 70.4	d[a]
					1.20 1.22	d[a],	$J_{49-30}=7.0$	16.1 15.0	q[a]

a) Signal splitting due to the stereoisomerism at C-30.

$5\times CH_2$ and $1\times CH_3$, suggesting the overlapping of its partial structure, the system C-19—C-29, with the carbon skeleton of **15a** (see Table III). The partial structure, C-30—C-49, was determined by decoupling between methyl protons and a proton on a carbon bearing an acetoxyl group.

The UV absorption maximum (262 nm, ε 19000), and ^1H- and ^{13}C-NMR signals of **22** indicate the presence of an $\alpha,\beta,\gamma,\delta$-unsaturated methyl ester group bearing a methyl substitution at the α carbon, and in addition, of $1\times CH_2$-OAc, $4\times$CH-OAc, $3\times$CH, $4\times CH_2$ and $3\times CH_3$ groups, suggesting the overlapping of the partial structure of the compound, the system C-5—C-17, with the carbon skeleton of **16a** (see Table I for **16a** and Table IV for **22**).

From the UV and NMR spectral data mentioned above taken together with the partial structure, C-3—C-7, determined by decoupling experiments, the system C-1—C-17 was established.

(*E*)-Orientation of the C(4)=C(5) bond was determined from the large coupling constant between H-4 and H-5 (J=15.1 Hz), and (*E*)-orientation of the C(2)=C(3) bond was based on the fact that irradiation of the CH_3-45 signal (on a 100 MHz apparatus) gave 8% nuclear Overhauser effect (NOE) enhancement of the H-4 signal, while on irradiations of H-3 and H-5, *ca.* 12% NOE enhancements were observed for the H-5 and H-3 signals, respectively.

Table IV. ^1H- and ^{13}C-NMR Data for **22**

Carbon No.	^1H Signal	^{13}C Signal
1		168.8 s
2		126.3 s
3	7.16 dq, J_{3-4}=10.8, J_{3-45}=1.4	138.0 d
4	6.38 dd, J_{4-3}=10.8, J_{4-5}=15.1	127.3 d
5	5.96 dd, J_{5-4}=15.1, J_{5-6}=8.5	142.0 d
6	2.61 m, J_{6-5}=8.5, J_{6-46}=7.2	40.9 d
7	4.89 m	73.6 d
8	1.82 m	34.3 t
9	4.76 m	71.7 d
10	1.72 m, J_{10-47}=7.2	39.3 d
11	4.99 m	72.0 d
12	1.34 m	29.8 t
13	1.34 m	28.1 t
14	1.70 m, J_{14-48}=7.2	36.3 d
15	4.89 m	74.1 d
16	1.82 m	29.3 t
17	4.07 m	61.1 t
45	1.95 d, J_{45-3}=1.4	12.6 q
46	1.04 d, J_{46-6}=7.2	16.5 q
47	0.92 d, J_{47-10}=7.2	9.9 q
48	0.89 d, $J_{48\ 14}$=7.2	14.8 q
$-OCH_3$	3.77 s	51.7 q

Experimental

General——See ref. 1).

Ozonolysis of Azalomycin F_{4a}——1) F_{4a} (1.03 g) was ozonized in methanol (40 ml) at −78°C, and the ozonide was decomposed with sodium borohydride ($NaBH_4$) by addition of 1.2 g (in water, 10 ml) initially and 1.1 g (in water, 10 ml) after stirring for 10 h; the solution was further stirred for 14 h. The reaction solution was passed through a column of Amberlite IR-120B (60 ml) to adsorb a basic product, and the column was washed with water (200 ml). The washing water was combined with the reaction solution after passage through the column (fr. 1). The column was then eluted with aq. 1 N HCl (300 ml) and the eluate was neutralized and concentrated (fr. 2). Fr. 2, containing a large amount of salt, was extracted with methanol to separate an ozonolysis product from salt, and the extract was again concentrated. This process was repeated several times, and finally the concentrate was extracted with butanol to obtain almost salt free

basic product **2a** (80 mg), which was acetylated with acetic anhydride in pyridine to give **6a**. Fr. 1 was concentrated to *ca.* 20 ml, and this was extracted with ethyl acetate. The extract was concentrated (220 mg of oil, mainly **3a**) and the residue was acetylated and chromatographed on a silica gel column to afford **8a** (150 mg). The water layer after extraction of fr. 1 was passed through a column of Amberlite IRA-410 to remove boric acid, and the eluate was concentrated to dryness to afford 510 mg of a neutral fraction. This was separated by high performance liquid chromatography (HPLC) using a Shimadzu gel SCR-100 column to give ethylene glycol (**4a**) and **5a**. Acetylation yielded ethylene glycol diacetate (**9a**) and **10a**, respectively.

2) F_{4a} (1.05 g) was ozonized in methanol (40 ml) at −78°C, and the ozonide was decomposed by 1 h's treatment with $NaBH_4$ (1.1 g in methanol, 10 ml). The reaction was quenched with Amberlite IR-120B. The filtered solution was diluted with water (40 ml), and the solution was concentrated to *ca.* 20 ml. This was extracted with ethyl acetate, and the extract after removal of the solvent by evaporation (210 mg, a mixture containing **3a** and **11a**) was acetylated to give an acetate mixture (290 mg). Silica gel column chromatography with benzene–acetone (92 : 8) afforded crude **13a** (95 mg) and **14a** (120 mg), and they were purified by GC (10% OV-1, 1.5 m, 220°C). The aqueous fraction was concentrated to give *ca.* 850 mg of mixture containing **4a** and **12**, and a small amount of **6a**. TLC separation (butanol–acetic acid–water, 5 : 1 : 1) of **12** followed by its HPLC purification afforded almost pure **12**.

3) F_{4a} (530 mg) was ozonized in methanol (20 ml) at −78°C, and the ozonide was decomposed with sodium borodeuteride ($NaBD_4$) by addition of 580 mg (in water, 5 ml) initially and 560 mg (in water, 5 ml) after stirring for 10 h, and the solution was further stirred for 14 h. Work up and separation as in the case of 1) gave rise to **2b** (35 mg), **3b**, d_2-ethylene glycol (**4b**) and **5b**. Of these **3b**, **4b** and **5b** were isolated as their acetates **8b** (90 mg), d_2-ethylene glycol diacetate (**9b**) and **10b**, respectively.

4) Ozonolysis of F_{4a} (510 mg) followed by treatment with $NaBD_4$ as in the case of 2) yielded **13b** (35 mg) and **14b** (50 mg) after the same work-up and separation procedures as in 2).

2a (as Hydrochloride)——UV λ_{max}^{MeOH} nm (ε): 207 (2070). IR ν_{max}^{NaCl} cm^{-1}: 3600—2700 (br s), 1680—1630 (br s), 1025. FD-MS *m/z*: 145 (M^+). ^{1}H-NMR (in D_2O) δ: 3.72 (2H, m, H_2-41), 3.33 (2H, m, H_2-44), 2.93 (3H, s, N–CH_3), 1.8—1.2 (4H, m, H_2-42 and H_2-43).

2b (as Hydrochloride)——FD-MS *m/z*: 146 (M^+). ^{1}H-NMR (in D_2O): Integration of the signal at δ 3.72 gave 1H.

6a——*Anal.* Calcd for $C_{14}H_{23}N_3O_5$: C, 53.66; H, 7.40; N, 13.41. Found: C, 54.13; H, 7.33; N, 13.35. ^{1}H-NMR (in $CDCl_3$) δ: 4.08 (2H, m, H_2-41), 3.58 (2H, m, H_2-44), 3.12 (3H, s, CH_3-53), 2.28, 2.24 and 2.19 (9H, 3s, three *N*-acetyls), 2.06 (3H, s, *O*-acetyl), 1.80—1.55 (4H, m, H_2-42 and H_2-43). ^{13}C-NMR (in $CDCl_3$) δ: 181.7, 171.1, 171.0 and 170.9 (4s, acetyl carbonyls), 145.9 (s, C-52), 63.5 (t, C-41), 46.5 (t, C-44), 35.1 (q, C-53), 26.0 and 25.1 (2t, C-42 and C-43), 25.4, 23.7, 23.0 and 20.9 (4q, acetyl methyls). UV λ_{max}^{MeOH} nm (ε): 206 (3570), 234 (8660). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2940 (m), 1735—1690 (br s), 1630 (s), 1370 (s), 1210 (s), 1170 (m).

8a——*Anal.* Calcd for $C_{16}H_{28}O_6$: C, 60.74; H, 8.92. Found: C, 60.98; H, 8.96. $[\alpha]_D^{25}$ −0.3° (*c*=3.5, methanol). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2885 (m), 1735 (s), 1370 (m), 1240 (s), 1100 (m), 1050 (m). EI-MS *m/z* (%), 257 (23), 256 (6), 215 (73), 196 (32), 174 (92), 121 (100). ^{1}H-NMR (in $CDCl_3$) δ: 4.82 (1H, dd, *J*=4.0, 8.1 Hz, H-35), 4.04 (2H, t, *J*=6.8 Hz, H_2-40), 4.01 (1H, dd, *J*=5.0, 11.5 Hz, H-33), 3.95 (1H, dd, *J*=6.0, 11.5 Hz, H-33), 2.25, 2.23 (9H, 3s, acetyl methyls), 2.09 (1H, m, H-34), 1.73 (1H, m, H-36), 1.61 (2H, m, H_2-39), 1.39 (2H, m, H_2-38), 1.22 (1H, m, H-37), 1.12 (1H, m, H-37), 0.97 (3H, d, *J*=7.0 Hz, CH_3-50), 0.88 (3H, d, *J*=6.8 Hz, CH_3-51). ^{13}C-NMR (in $CDCl_3$) δ: 171.1, 171.0 and 170.7 (3s, acetyl carbonyls), 77.2 (d, C-35), 66.0 (t, C-33), 64.3 (t, C-40), 34.4 (d, C-34), 34.2 (d, C-36), 33.4 (t, C-37), 28.8 (t, C-39), 23.5 (t, C-38), 20.9, 20.8, 20.7 (acetyl methyls), 14.4 (q, C-50), 13.4 (q, C-51).

8b——EI-MS *m/z* (%): 259 (16), 258 (6), 216 (42), 198 (14), 174 (90), 56 (100). ^{1}H-NMR (in $CDCl_3$): Integration of the signal at δ 4.04 gave 1H, and integration of the signals at δ 4.01 and 3.95 gave 0.5H each.

5a——*Anal.* Calcd for $C_{30}H_{62}O_{14}$: C, 55.57; H, 9.66; O, 34.63. Found: C, 55.26; H, 9.55; O, 33.68. FD-MS *m/z*: 646 (M^+).

5b——FD-MS *m/z*: 649 (M^+), as a mixture of epimers at C-30.

10a——IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2960 (w), 1735 (s), 1370 (s), 1260—1200 (br s), 1045 (s). FD-MS *m/z*: 1234 (M^+). ^{1}H-NMR (in $CDCl_3$) δ: 5.24 (1H, t, *J*=7.0 Hz), 5.04—4.77 (13H, m, protons on C-7, 9, 11, 15, 17, 18, 19, 21, 23, 25, 27, 29 and 30), 3.95 (2H, m, H_2-5), 2.9—0.9 (21H, m, methylene and methine), 2.16—2.00 (42H, 14s, acetyl methyls), 2.20 and 2.18 (3H, 2d, *J*=7.0 Hz, CH_3-49, splitting due to the stereoisomerism at C-30), 0.95 (6H, 2d, *J*=7.0 Hz, CH_3-46 and 47), 0.86 (3H, d, *J*=7.0 Hz, CH_3-48). ^{13}C-NMR (in $CDCl_3$) δ: 170.8—169.9 (14s, acetyl carbonyls), 74.2, 73.7, 72.3, 71.9, 70.5, 70.3, 68.6, 66.8, 66.6 and 66.3 (13d, C-7, 9, 11, 15, 17, 18, 19, 21, 23, 25, 27, 29 and 30), 65.2 (t, C-5), 39.4, 35.1+34.0, 33.4, 31.2, 29.8 and 27.6 (9t, C-8, 12, 13, 16, 20, 22, 24, 26 and 28), 36.2 and 35.9 (3d, C-6, 10 and 14), 21.0 and 20.8 (14q, acetyl methyls), 16.1+15.0 (2q, C-49, splitting due to the stereoisomerism at C-30), 14.8, 13.6 and 9.8 (3q, C-46, 47 and 48).

10b——FD-MS *m/z*: 1237 (M^+). ^{1}H-NMR (in $CDCl_3$): Integration of the multiplet appearing at δ 5.04—4.77 gave 11H, and integration of the signal at δ 3.95 gave 1H. The signals at δ 2.20 and 2.18 each appeared as a singlet.

13a——*Anal.* Calcd for $C_{19}H_{32}O_8$: C, 58.74; H, 8.30. Found: C, 58.60; H, 8.27. $[\alpha]_D^{25}$ +18.6° (*c*=1.1, methanol). EI-MS *m/z* (%): 287 (14), 245 (42), 215 (10), 197 (31), 87 (100). ^{1}H-NMR (in $CDCl_3$) δ: 5.04 (1H, q, *J*=6.8 Hz, H-2), 4.89 (1H, dd, *J*=4.0, 8.0 Hz, H-35), 4.06 (2H, t, *J*=6.2 Hz, H_2-40), 4.05 (1H,

dd, J=4.0, 11.0 Hz, H-33), 3.83 (1H, dd, J=6.2, 11.0 Hz, H-33), 2.13 (1H, m, J=4.0, 6.2, 8.0, 6.5 Hz, H-34), 2.11 and 2.03 (9H, 3s, acetyl methyls), 1.77 (1H, m, J=4.0, 6.5 Hz, H-36), 1.60 (2H, m, H_2-39), 1.50 (3H, d, J=6.8 Hz, H_3-45), 1.38 (3H, m, H-37 and H_2-38), 1.20 (1H, m, H-37), 0.98 (3H, d, J=6.5 Hz, H_3-50), 0.89 (3H, d, J=6.5 Hz, H_3-51). ^{13}C-NMR (in $CDCl_3$) δ: 170.9, 170.8 and 170.5 (3s, acetyl carbonyls), 170.0 (s, C-1), 78.3 (d, C-35), 68.7 (d, C-2), 65.6 (t, C-33), 64.3 (t, C-40), 34.5 (d, C-34), 34.4 (d, C-36), 33.2 (t, C-37), 28.8 (t, C-39), 23.5 (t, C-38), 20.9, 20.8 and 20.5 (3q, acetyl methyls), 17.0 (q, C-45), 14.5 (q, C-50), 13.6 (q, C-51).

13b——EI-MS m/z (%): 289 (22), 247 (68), 217 (15), 199 (54), 198 (44), 55 (100). ^{1}H-NMR (in $CDCl_3$): Integrations of the signals at δ 5.04, 4.06 and 4.05 gave 1H each, and the signal at δ 1.50 appeared as a singlet.

14a——*Anal.* Calcd for $C_{19}H_{32}O_8$: C, 58.74; H, 8.30. Found: C, 59.30; H, 8.31. $[\alpha]_D^{25}$ −33.3° (c=2.0, methanol). EI-MS m/z (%): 287 (15), 245 (30), 215 (13), 197 (33), 196 (24), 101 (100). ^{1}H-NMR (in $CDCl_3$) δ: 5.07 (1H, q, J=7.0 Hz, H-2), 4.91 (1H, dd, J=3.5, 8.0 Hz, H-35), 4.08 (1H, dd, J=4.2, 11.2 Hz, H-33), 4.04 (2H, t, J=6.2 Hz, H_2-40), 3.90 (1H, dd, J=11.2, 6.2 Hz, H-33), 2.16 (1H, m, J=4.2, 6.2, 8.0, 6.8 Hz, H-34), 2.11 and 2.04 (9H, 3s, acetyl methyls), 1.77 (1H, m, J=3.5, 6.5 Hz, H-36), 1.60 (1H, m, H-39), 1.49 (3H, d, J=7.0 Hz, H_3-45), 1.38 (2H, m, H_2-38), 1.31 (1H, m, H-37), 1.15 (1H, m, H-37), 0.98 (3H, d, J=6.8 Hz, H_3-50), 0.87 (3H, d, J=6.5 Hz, H_3-51). ^{13}C-NMR (in $CDCl_3$) δ: 170.9, 170.8 and 170.6 (3s, acetyl carbonyls), 170.0 (s, C-1), 78.4 (d, C-35), 68.7 (d, C-2), 65.7 (t, C-33), 64.2 (t, C-40), 34.6 (d, C-34), 34.3 (d, C-36), 33.3 (t, C-37), 28.8 (t, C-39), 23.5 (t, C-38), 20.9, 20.8 and 20.5 (3q, acetyl methyls), 17.1 (q, C-45), 14.4 (q, C-50), 13.5 (q, C-51).

14b——EI-MS m/z (%): 289 (4), 247 (12), 217 (3), 199 (12), 198 (10), 139 (100). ^{1}H-NMR (in $CDCl_3$): Integration of the signals at δ 5.07, 4.08 and 4.04 gave 1H each, and the signal at δ 1.49 appeared as a singlet.

Alkaline Hydrolysis of F_{4a} and Its Ozonolysis Products 12, 11a, 11b and 2a

Alkaline Hydrolysis of F_{4a}——To F_{4a} (520 mg) in methanol (20 ml) was added aq. 3 N KOH (10 ml) under a nitrogen atmosphere, and the whole was stirred at room temperature overnight. Basic materials were removed by passing the reaction solution through Amberlite IR-120B (50 ml). The eluate was concentrated to dryness, and the residue was extracted with ether to afford crude malonic acid (55 mg).

Alkaline Hydrolysis of 12——Compound **12** (50 mg) in aq. 1 N KOH (10 ml) was stirred overnight at room temperature under a nitrogen atmosphere. The reaction solution was treated with Amberlite IR-120B (20 ml) to remove KOH and with IRA-45 (10 ml) to adsorb malonic acid. Removal of water by evaporation yielded **5a** (35 mg). The column of IRA-45 was eluted with aq. 1 N KOH, and the eluate was treated with IR-120B to remove KOH then concentrated to dryness to yield crude malonic acid (5 mg).

Alkaline Hydrolysis of 11a and 11b——To **11a** (50 mg) in methanol (10 ml) was added aq. 3 N KOH (5 ml) under a nitrogen atmosphere, and the whole was stirred overnight at room temperature. After removal of KOH with Amberlite IR-120B (20 ml) the solution was stirred with IRA-45 (10 ml) to separate acidic material. Concentration of the aqueous solution followed by acetylation of the residue afforded **8a** (30 mg). Acidic material adsorbed on IRA-45 was eluted with aq. 1 N KOH. IR-120B treatment of the aqueous solution to remove KOH followed by removal of water by evaporation yielded crude lactic acid (10 mg).

Similar treatment of **11b** afforded **8b** and d_2-lactic acid.

Alkaline Hydrolysis of 2a——Compound **2a** (20 mg) in aq. 1 N NaOH (10 ml) was heated to reflux (4 h) under gentle bubbling of nitrogen gas, and evolved gas was passed through aq. 1 N HCl to trap the generated amines. The aq. HCl was evaporated off to give methylamine hydrochloride and ammonium chloride (8 mg). The remaining reaction solution was, after cooling to room temperature, treated with benzoyl chloride to afford 10 mg of 4-aminobutanol *N,O*-dibenzoate (**7**).

Periodate Oxidation of 5a and 5b——A solution of **5a** (100 mg) and sodium periodate ($NaIO_4$) (110 mg) in water was stirred for 4 h at room temperature. Excess $NaIO_4$ was decomposed with ethylene glycol. Water was evaporated off *in vacuo*, and the residue was extracted with methanol. The extract, after evaporation of methanol, 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 *in vacuo* to give an oily product mixture (70 mg). Acetylation of the products followed by silica gel column chromatography with benzene–acetone (9 : 1) afforded **15a** (55 mg) and **16a** (40 mg).

Similar $NaIO_4$ oxidation of **5a** (90 mg) followed by $NaBD_4$ treatment gave **15b** (40 mg) and **16b** (30 mg).

Similar $NaIO_4$ oxidation of **5b** followed by $NaBH_4$ treatment yielded **15a** and **16c**.

15a——*Anal.* Calcd for $C_{23}H_{36}O_{12}$: C, 54.75; H, 7.19. Found: C, 55.01; H, 7.28. $[\alpha]_D^{25}$ −1.8° (c=1.7, methanol). EI-MS m/z: 417 ($M^+-CH_2CH_2OAc$). ^{1}H-NMR (in $CDCl_3$) δ: 4.99 (4H, m, H-21, 23, 25 and 27), 4.06 (4H, m, H_2-19 and 29), 2.04—2.00 (18H, 6s, acetyl methyls), 1.88 (4H, m, H_2-20 and 28), 1.82 (6H, m, H_2-22, 24 and 26). ^{13}C-NMR (in $CDCl_3$) δ: 170.8, 170.4 and 170.3 (6s, acetyl carbonyls), 67.5 (2d, C-23 and 25), 66.7 (2d, C-21 and 27), 60.5 (2t, C-19 and 29), 39.6 (t, C-24), 39.1 (2t, C-22 and 26), 33.6 (2t, C-20 and 28), 20.9 and 20.8 (6q, acetyl methyls).

15b——EI-MS m/z: 418 ($M^+-CH_2CH(D)OAc$). ^{1}H-NMR (in $CDCl_3$): Integration of the signal at δ4.06 gave 2H.

16a——*Anal.* Calcd for $C_{28}H_{46}O_{12}$: C, 58.52; H, 8.07. Found: C, 58.58; H, 7.97. $[\alpha]_D^{25}$ +3.9° (c=1.6, methanol). EI-MS m/z: 473 ($M^+-CH(CH_3)CH_2OAc$). ^{1}H-NMR (in $CDCl_3$) δ: 5.00 (1H, ddd, J=8.1, 5.5,

2.8 Hz, H-11), 4.94 (1H, ddd, J=5.1, 6.9, 4.9 Hz, H-7), 4.89 (1H, ddd, J=5.0, 8.0, 5.0 Hz, H-15), 4.80 (1H, ddd, J=7.8, 7.1, 3.9 Hz, H-9), 4.07 (2H, m, H_2-17), 4.02 (1H, dd, J=11.2, 5.9 Hz, H-5), 3.98 (1H, dd, J=11.2, 6.3 Hz, H-5), 2.11 (1H, m, J=5.9, 6.3, 4.9, 7.0 Hz, H-6), 2.16—2.10 (18H, 6s, acetyl methyls), 2.00 (1H, m, H-8), 1.83 (4H, m, H_2-16, H-10 and 8), 1.72 (2H, m, H-14 and 12), 1.40 (1H, m, H-12), 1.32 (1H, m, H-13), 1.10 (1H, m, H-13), 0.97 (3H, d, J=7.0 Hz, H_3-47), 0.97 (3H, d, J=7.0 Hz, H_3-46), 0.89 (3H, d, J=6.8 Hz, H_3-48). ^{13}C-NMR (in $CDCl_3$) δ: 170.6, 170.5, 170.4 and 170.2 (6s, acetyl carbonyls), 74.1 (d, C-15), 72.4 (d, C-7), 72.1 (d, C-11), 72.0 (d, C-9), 65.3 (t, C-5), 61.1 (t, C-17), 39.3 (d, C-10), 36.3 (d, C-14), 36.1 (d, C-6), 33.5 (t, C-8), 29.9 (t, C-12), 29.4 (t, C-16), 28.0 (t, C-13), 21.1, 21.0 and 20.8 (6q, acetyl methyls), 14.8 (q, C-48), 13.6 (q, C-46), 9.8 (q, C-47).

16b——EI-MS m/z: 474 (M^+—$CH(CH_3)CH_2OAc$). ^{1}H-NMR (in $CDCl_3$): Integration of the signal at δ 4.07 gave 1H.

16c——EI-MS m/z: 474 (M^+—$CH(CH_3)CH(D)$–OAc). ^{1}H-NMR (in $CDCl_3$): Integration of the signal at δ 4.07 gave 1H and integration of the signals at δ 4.02 and 3.98 gave 0.5H each.

Ozonolysis of an Acetylation Product of F_{4a}

Acetylation of F_{4a}——F_{4a} (520 mg) suspended in dioxane (20 ml) was acetylated with acetic anhydride (2 ml) and pyridine (1 ml) in the presence of 4-dimethylaminopyridine (60 mg) by stirring at room temperature for 2 d. The reaction solution was poured into ice-water (100 ml), and the separated oil was extracted with methylene chloride. The extract was washed with water and dried over sodium sulfate. Removal of the solvent by evaporation afforded a product mixture (710 mg). This showed, on TLC, more than two spots but was unstable during chromatographic separation, so it was subjected to ozonolysis without purification. This acetylation product of F_{4a} showed no signal due to hemiketal carbon in its ^{13}C-NMR spectrum, in contrast to that of F_{4a} at δ 99.7 ppm, but instead showed a signal at δ 192.2 ppm indicating generation of an α,β-unsaturated keto group.

Ozonolysis of the Acetylation Product——The product mixture (690 mg), obtained as described above, was ozonized in methanol (30 ml), and the ozonide was decomposed with $NaBH_4$ by addition of 520 mg (in water, 10 ml) initially and 530 mg (in water, 10 ml) after stirring for 10 h, and the solution was further stirred for 16 h. The reaction solution was neutralized with 1 N HCl, and the solvent was removed by evaporation. Reacetylation of the residue followed by column chromatography afforded a product **17** (110 mg) in addition to **6a** (40 mg), **8a** (120 mg) and **9a**.

17——*Anal.* Calcd for $C_{24}H_{40}O_{10}$: C, 59.00; H, 8.25. Found: C, 59.20; H, 8.34. $[\alpha]_D^{25}$ −4.1° (c=1.7, methanol). EI-MS m/z: 387 (M^+—$CH(CH_3)CH_2OAc$). ^{1}H-NMR (in $CDCl_3$) δ: 5.02 (1H, ddd, J=8.1, 5.5, 2.8 Hz, H-11), 4.94 (1H, ddd, J=5.1, 4.9, 6.9 Hz, H-7), 4.80 (1H, ddd, J=7.8, 7.1, 3.9 Hz, H-9), 4.02 (1H, dd, J=11.2, 5.9 Hz, H-5), 3.98 (1H, dd, J=11.2, 6.3 Hz, H-5), 3.91 (1H, dd, J=11.0, 6.1 Hz, H-15), 3.88 (1H, dd, J=11.0, 6.6 Hz, H-15), 2.11 (1H, m, J=5.9, 6.3, 7.2, 4.9 Hz, H-6), 2.10, 2.08 and 2.05 (15H, 5s, acetyl methyls), 2.00 (1H, m, H-8), 1.86 (2H, m, H-8 and 10), 1.78 (1H, m, H-14), 1.67 (1H, m, H-12), 1.46 (1H, m, H-12), 1.34 (1H, m, H-13), 1.14 (1H, m, H-13), 0.97 (3H, d, J=7.2 Hz, H_3-47), 0.96 (3H, d, J=7.2 Hz, H_3-46), 0.93 (3H, d, J=7.0 Hz, H_3-48). ^{13}C-NMR (in $CDCl_3$) δ: 171.0, 170.8, 170.5, 170.3 and 170.2 (5s, acetyl carbonyls), 72.2 (d, C-7), 71.8 (2d, C-9 and 11), 68.8 (t, C-15), 65.2 (t, C-5), 39.1 (d, C-10), 35.9 Hz, H_3-46), 0.93 (3H, d, J=7.0 Hz, H_3-48). ^{13}C-NMR (in $CDCl_3$) δ: 171.0, 170.8, 170.5, 170.3 and 170.2 (5s, acetyl carbonyls), 72.2 (d, C-7), 71.8 (2d, C-9 and 11), 68.8 (t, C-15), 65.2 (t, C-5), 39.1 (d, C-10), 35.9 (d, C-6), 33.3 (t, C-8), 32.4 (d, C-14), 29.5 (t, C-12), 29.1 (t, C-13), 21.0 and 20.8 (5q, acetyl methyls), 16.8 (q, C-48), 13.5 (q, C-46), 9.7 (q, C-47).

Ozonolysis of a Partial Hydrogenation Product of F_{4a}——F_{4a} (1.02 g) was hydrogenated over 10% palladium-on-charcoal, and the reaction was stopped when *ca.* 3 molar equivalent volumes of hydrogen had been absorbed. The product (920 mg) was then ozonized and treated with $NaBH_4$ as in the case of F_{4a} (ozonolysis 1). The reaction solution was passed through a column of Amberlite IR-120B (60 ml) to trap basic fragments, and the column was eluted with aq. 1 N HCl (500 ml). The eluate, after neutralization, was concentrated to dryness. The residue was dissolved in 3 N KOH (ethanol–water, 1: 1, 6 ml) and the solution was heated in a sealed tube at 120°C for 16 h. The reaction solution, after neutralization, was concentrated and the residue was acetylated with acetic anhydride in pyridine to give a mixture (110 mg) which was separated by GC (10% OV-1, 1.5 m, 250°C) affording **18** (20 mg), **19** (35 mg) and **20** (15 mg).

18—*Anal.* Calcd for $C_{20}H_{37}NO_5$: C, 64.66; H, 10.04; N, 3.77. Found: C, 64.36; H, 10.09; N, 3.61. $[\alpha]_D^{25}$ 0.0° (c=0.9, methanol). EI-MS m/z: 371 (M^+). ^{1}H-NMR (in $CDCl_3$) δ: 4.83 (1H, dd, J=4.0, 8.0 Hz, H-35), 4.01 (1H, dd, J=4.5, 11.0 Hz, H-33), 3.92 (1H, dd, J=6.0, 11.0 Hz, H-33), 3.22 (2H, d t, J=6.0, 6.0 Hz, H_2-44), 2.11 (1H, m, H-34), 2.05 and 1.99 (9H, 3s, acetyl methyls), 1.72 (1H, m, H-36), 1.48 (2H, m, H_2-43), 0.97 (3H, d, J=6.8 Hz, H_3-50), 0.86 (3H, d, J=7.0 Hz, H_3-51). ^{13}C-NMR (in $CDCl_3$) δ: 170.9, 170.7 and 170.0 (3s, acetyl carbonyls), 77.6 (d, C-35), 66.1 (t, C-33), 39.7 (t, C-44), 34.5 (d, C-34), 34.3 (d, C-36), 33.8, 29.6, 29.4, 29.2, 27.0 and 26.9 (7t, C-37, 38, 39, 40, 41, 42 and 43), 23.2 and 20.8 (3q, acetyl methyls), 14.5 (q, C-50), 13.6 (q, C-51).

19——*Anal.* Calcd for $C_{21}H_{39}NO_5$: C, 65.42; H, 10.20; N, 3.63. Found: C, 65.12; H, 10.17; N, 3.45. $[\alpha]_D^{25}$ −2.5° (c=2.1, methanol) EI-MS m/z: 385 (M^+). ^{1}H-NMR (in $CDCl_3$) δ: 4.82 (1H, dd, J=4.5, 6.5 Hz, H-35), 4.10 (2H, m, H_2-32), 3.22 (2H, d t, J=6.0, 6.0 Hz, H_2-44), 2.09, 2.04 and 1.98 (9H, 3s, acetyl methyls), 1.87 (1H, m, H-34), 1.73 (3H, m, H-36 and H_2-33), 1.49 (2H, m, H_2-43), 0.92 (3H, d, J=6.8 Hz, H_3-50), 0.85

(3H, d, J=6.8 Hz, H_3-51). ^{13}C-NMR (in $CDCl_3$) δ: 170.9 and 170.0 (3s, acetyl carbonyls), 80.4 (d, C-35), 62.7 (t, C-32), 39.6 (t, C-44), 34.2 (d, C-36), 31.7 (d, C-34), 30.8 (t, C-33), 33.8, 29.7, 29.6, 29.4, 29.2 and 26.9 (7t, C-37, 38, 39, 40, 41, 42 and 43), 23.1 and 20.9 (3q, acetyl methyls), 16.3 (q, C-50), 13.9 (q, C-51).

20—*Anal.* Calcd for $C_{22}H_{41}NO_5$: C, 66.13; H, 10.34; N, 3.51. Found: C, 66.21; H, 10.31; N, 3.33. $[\alpha]_D^{25}$ −4.1° (c=1.2, methanol). EI-MS m/z: 399 (M^+). ^{1}H-NMR (in $CDCl_3$) δ: 4.71 (1H, dd, J=4.5, 7.5 Hz, H-35), 4.02 (2H, m, H_2-31), 3.22 (2H, d t, J=6.0, 6.0 Hz, H_2-44), 2.06, 2.04 and 1.99 (9H, 3s, acetyl methyls), 1.73 (3H, m, H-32, 34 and 36), 1.48 (3H, m, H-32 and H_2-43), 0.88 (3H, d, J=7.0 Hz, H_3-50), 0.85 (3H, d, J=7.0 Hz, H_3-51). ^{13}C-NMR (in $CDCl_3$) δ: 170.9 and 169.9 (3s, acetyl carbonyls), 80.3 (d, C-35), 64.6 (t, C-31), 39.7 (t, C-44), 34.2 (2d, C-34 and 36), 34.0, 29.6, 29.4, 29.2, 28.2, 27.0, 26.9, 26.0 and 23.2 (9t, C-32, 33, 37, 38, 39, 40, 41, 42 and 43), 20.8 (3q, acetyl methyls), 16.0 (q, C-50), 13.7 (q, C-51).

Periodate Oxidation of F_{4a} and Ozonolysis of an Oxidation Product 21——To F_{4a} (1.02 g) in methanol (40 ml) was added $NaIO_4$ (1.1 g) in water (20 ml) under a nitrogen atmosphere, and the whole was stirred for 3 d at room temperature. Excess $NaIO_4$ was decomposed with ethylene glycol. The solvent was evaporated off, and the residue was extracted with methanol. The extract was reduced with $NaBH_4$ by addition of 1.2 g (in water, 10 ml) initially and 1.1 g (in water, 10 ml) after stirring for 10 h, and the solution was further stirred for an additional 14 h. The reaction solution, after neutralization with 1 N HCl, was concentrated to dryness and the residue was extracted with methanol to remove salt. This process was repeated several times. The obtained product mixture was treated with 1 N KOH (methanol–water 2: 1; 120 ml) under a nitrogen atmosphere. The solution, after neutralization, was concentrated, and the residue was extracted with methanol to separate the products from salt. The product mixture was suspended in dioxane and acetylated with acetic anhydride-pyridine in the presence of 4-dimethylaminopyridine. The obtained acetylation product mixture was treated with diazomethane, and the mixture (1.25 g) was separated by silica gel column chromatography to afford a guanidine derivative 21 (35 mg) and an $\alpha, \beta, \gamma, \delta$-unsaturated ester derivative **22** (20 mg) together with **15a** and another guanidine derivative, as well as an ester derivative.

Compound **21** (90 mg) was ozonized in methanol (20 ml) and treated with $NaBH_4$ as in the case of F_{4a} (ozonolysis 1). After acetylation the product mixture (120 mg) was separated by silica gel column chromatography to give crude **23** (25 mg), in addition to **6a** (5 mg), **8a** (10 mg) and ethylene glycol diacetate (**9a**). Compound **23** was purified by GC (10% OV-1, 1.5 m, 240°C).

22——UV λ_{max}^{MeOH} nm (ε): 262 (19000). ^{1}H-NMR (in $CDCl_3$) δ: 7.16 (1H, dd, J=10.8, 1.4 Hz, H-3), 6.38 (1H, dd, J=10.8, 15.1 Hz, H-4), 5.96 (1H, dd, J=8.5, 15.1 Hz, H-5), 4.99 (1H, m, H-11), 4.89 (2H, m, H-7 and 15), 4.76 (1H, m, H-9), 4.07 (2H, m, H_2-17), 3.77 (3H, s, O-CH_3), 2.61 (1H, m, H-6), 2.06—2.00 (18H, 6s, acetyl methyls), 1.95 (3H, d, J=1.4 Hz, H_3-45), 1.82 (4H, m, H_2-8 and 16), 1.72 (1H, m, H-10), 1.70 (1H, m, H-14), 1.34 (4H, m, H_2-12 and 13), 1.04 (3H, d, J=7.2 Hz, H_3-46), 0.92 (3H, d, J=7.2 Hz, H_3-47), 0.89 (3H, d, J=7.2 Hz, H_3-48). ^{13}C-NMR (in $CDCl_3$) δ: 170.9, 170.5, 170.4 and 170.3 (6s, acetyl carbonyls), 168.8 (s, C-1), 142.0 (d, C-5), 138.0 (d, C-3), 127.3 (d, C-4), 126.3 (s, C-2), 74.1 (d, C-15), 73.6 (d, C-7), 72.0 (d, C-11), 71.7 (d, C-9), 61.1 (t, C-17), 51.7 (s, O–CH_3), 40.9 (d, C-6), 39.3 (d, C-10), 36.3 (d, C-14), 34.3 (t, C-8), 29.8 (t, C-12), 29.3 (t, C-16), 28.1 (t, C-13), 21.1, 21.0 and 20.9 (6q, acetyl methyls), 16.5 (q, C-46), 14.8 (q, C-48), 12.6 (q, C-45), 9.9 (q, C-47).

23——^{1}H-NMR (in $CDCl_3$) δ: 5.00 (6H, m, H-21, 23, 25, 27, 29 and 30), 4.18 (2H, t, J=7.5 Hz, H_2-19), 2.05—2.00 (18H, 6s, acetyl methyls), 1.92 (1H, m, H-20), 1.77 (8H, m, H_2-22, 24, 26 and 28), 1.20+1.22 (3H, 2d, J=7.0 Hz, H_3-49).[5] ^{13}C-NMR (in $CDCl_3$) δ: 170.3 and 170.1 (7s, acetyl carbonyls), 70.6, 70.5+70.4,[5] 67.5, 67.0, 66.8 and 66.6+66.5[5] (6d, C-21, 23, 25, 27, 29 and 30), 60.6 (t, C-19), 39.5, 39.2, 35.2+34.2[5] and 33.6 (5t, C-20, 22, 24, 26 and 28), 16.1+15.0[5] (q, C-49).

References and Notes

1) Part I: M. Namikoshi, K. Sasaki, Y. Koiso, K. Fukushima, S. Iwasaki, S. Nozoe, and S. Okuda, *Chem. Pharm. Bull.*, **30**, 1653 (1982).
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3) Numbering of the carbons in fragmentation products follows that given for F_{4a} (see Fig. 1 in ref. 1).
4) Unless otherwise specified, NMR data recorded in this paper were measured on a JEOL JNM FX-400 apparatus (^{1}H: 400.5 MHz, ^{13}C: 100.7 MHz).
5) Split signal because of the streoisomerism at C-30.