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# Studies on Macrocyclic Lactone Antibiotics. I.[1] Physicochemical Properties of Azalomycin $F_{4a}$

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Azalomycin $F_{4a}$ ($C_{56}H_{95}N_3O_{17}$), an antibiotic produced by *Streptomyces hygroscopicus* var. *azalomyceticus*, was purified and its physicochemical properties were determined. Partial structures of this compound were elucidated from its spectral data.

**Keywords**——azalomycin $F_{4a}$; *Streptomyces hygroscopicus* var. *azalomyceticus*; isolation; purification; macrocyclic lactone antibiotics; physicochemical properties; partial structures

The antibiotics azalomycin F, produced by *Streptomyces hygroscopicus* var. *azalomyceticus* and first isolated by Arai in 1959,[2] shows a broad anti-microbial spectrum against gram-positive bacteria, yeast, fungi and protozoa,[3,4] and its clinical utility against vaginal trichomoniasis and candidiasis has also been demonstrated.[4,5] Azalomycin F, which behaved as a single compound on paper chromatography,[3] was later found to be a mixture of at least 5 components,[4] and the three main components, $F_3$, $F_4$ and $F_5$, were isolated from this mixture.[6] Although their physicochemical and biological properties indicated very close structural similarity, their actual structures remained unknown. These facts led us study the structure of $F_4$, a main component of the mixture, and our elucidation of its skeletal structure (shown in Fig. 1) is reported in part 3 of this series.

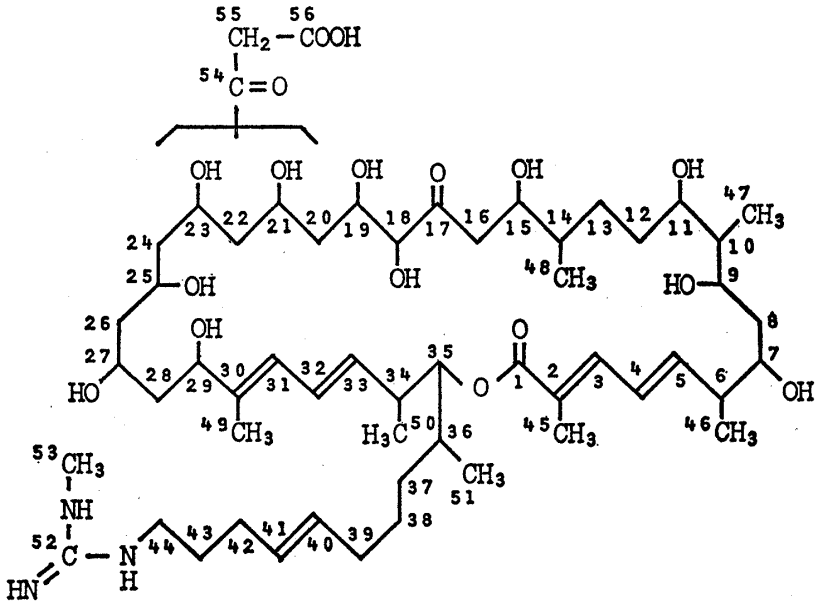

Fig. 1

## Isolation of Azalomycin $F_{4a}$

An azalomycin F complex provided by Sankyo Company was separated by silica gel column chromatography with *sec*-butanol–water (9:1) to give $F_4$, which behaved as a single compound on thin layer chromatography (TLC) developed with *sec*-butanol–water (4:1); however, it was shown to be still a mixture of two compounds, designated as $F_{4a}$ and $F_{4b}$, on TLC developed with butanol–acetic acid–water (5:1:1) which permitted the isolation of these two compounds. $F_{4a}$ isolated in this way was further purified by recrystallization from methanol–water.

$F_{4a}$ and $F_{4b}$ were found to be interconvertible, and their pure methanolic solutions, on standing at room temperature for 7 days or at 50°C for 20 h, gave rise to almost 1:1 mixtures of these compounds from each of them. $F_{4b}$, however, appears to be an artifact, since a 70% aq. acetone extract of cultured mycelia of *S. hygroscopicus* var. *azalomyceticus* initially showed none of this compound on TLC. Consequently, structural study of $F_4$ was carried out using the $F_{4a}$ sample obtained by recrystallization of $F_4$ fraction eluted from column chromatography (see "Experimental").

## Physicochemical Properties of Azalomycin $F_{4a}$

Azalomycin $F_{4a}$, fine needles, mp 131—132°C (dec.), $[\alpha]_D^{22}$ +39° ($c$=1.0, methanol) showed a moleculer ion peak at $m/z$ 1081 in field desorption–mass spectroscopy (FD–MS) and this was confirmed by fast atom bombardment mass spectroscopy (FAB–MS), which showed the M+1 peak at $m/z$ 1082. Its ultraviolet (UV) absorption maxima at 240 nm ($\varepsilon$ 38000) and 268 nm ($\varepsilon$ 23000) indicated the presence fo a conjugated diene and an $\alpha,\beta,\gamma,\delta$-unsaturated acid (or ester) group. Its infrared (IR) spectrum exhibited broad strong bands at 3600—2700 $cm^{-1}$ due to multiple

Table I. $^{13}C$-NMR of Azalomycin $F_{4a}$ (in $^{12}CD_3OD$)

| Signal No. | Chem. shift (ppm) | Multiplicity | Assignment | Signal No. | Chem. shift (ppm) | Multiplicity | Assignment |
|---|---|---|---|---|---|---|---|
| 1 | 10.52 | q | 47 | 28 | 65.62 | d | |
| 2 | 12.88 | q | 45 | 29 | 66.32 | d | 27 |
| 3 | 13.33 | q | 49 | 30 | 69.70 | d | |
| 4 | 14.37 | q | 51 | 31 | 70.69 | d | Bearing Hemiester |
| 5 | 14.87 | q | 48 | | | | |
| 6 | 17.00 | q | 46 | 32 | 72.34 | d | |
| 7 | 17.63 | q | 50 | 33 | 72.40 | d | |
| 8 | 27.89 | t | | 34 | 74.24 | d | 29 |
| 9 | 28.36 | q | 53 | 35 | 75.10 | d | 9 |
| 10 | 29.84 | t | | 36 | 75.78 | d | 7 |
| 11 | 30.57 | t + t | 13 [a] | 37 | 77.27 | d | 18 |
| 12 | 33.57 | t + t | | 38 | 80.77 | d | 35 |
| 13 | 34.47 | t | | 39 | 99.78 | s | 17 (Hemilketal) |
| 14 | 35.16 | d | | 40 | 125.12 | d | 31 |
| 15 | 39.24 | t | | 41 | 126.74 | s | 2 |
| 16 | 40.73 | d | | 42 | 127.57 | d | 4 |
| 17 | 40.90 | d | | 43 | 128.52 | d | 32 |
| 18 | 41.19 | t | | 44 | 130.19 | d | 41 |
| 19 | 41.85 | t | | 45 | 132.51 | d | 40 |
| 20 | 41.98 | t + t | | 46 | 136.14 | d | 33 |
| 21 | 44.04 | t | | 47 | 140.09 | s | 30 |
| 22 | 44.40 | d | | 48 | 140.17 | d | 3 |
| 23 | 44.49 | d | | 49 | 146.07 | d | 5 |
| 24 | 44.58 | t | | 50 | 158.27 | s | 52 |
| 25 | 46.10 | t | 55 | 51 | 170.05 | s | 1 |
| 26 | 46.37 | t | | 52 | 171.60 | s | 54 |
| 27 | 65.53 | d | | 53 | 174.06 | s | 56 |

a) One of these two triplets.

hydroxy groups and at 1700—1500 $cm^{-1}$ due to carbonyl groups. The $^{13}C$-nuclear magnetic resonance ($^{13}C$-NMR) data for this compounds obtained by 100 MHz CMR spectrometer are shown in Table I. Signal assignments were made from the chemical shifts and multiplicities as well as by $^{1}H$–$^{13}C$ selective decoupling experiments. These data revealed that the compound dossesses 56 carbon atoms which are composed of 8 methyl carbons (signals No. 1—7 and 9), 21 methylene and methine carbons (signals No. 8 and 10—26), 12 carbons bearing hydroxy or acyloxy groups (signals No. 27—38), a carbon forming a hemiketal (signal No. 39), 10 olefinic carbons (signals No. 40—49), a guanido carbon (signal No. 50) and 3 acyl carbons (signals No. 51—53). Of its $^{1}H$-NMR signals measured on a 400 MHz PMR spectrometer, those so far assigned from their chemical shifts as well as be extensive decoupling experiments are listed in Table II.

Table II. Assigned $^{1}H$-NMR Signals of Azalomycin $F_{4a}$ (in $CD_3OD$)

| Signal No. | Chem. shift (ppm) | Multiplicity and coupling constant ($J$ in Hz) | Assignment |
|---|---|---|---|
| 1 | 0.87 | d, $J_{=47-10}=7.0$ | $H_3$–47 |
| 2 | 0.90 | d, $J_{48-14}=6.9$ | $H_3$–48 |
| 3 | 0.94 | d, $J_{51-36}=6.6$ | $H_3$–51 |
| 4 | 0.99 | d, $J_{50-34}=6.6$ | $H_3$–50 |
| 5 | 1.10 | d, $J_{46-6}=6.8$ | $H_3$–46 |
| 6 | 1.64 | d, $J_{49-31}=1.6$ (long-range) | $H_3$–49 |
| 7 | 1.82 | m, $J_{36-35}=4.0$, $J_{36-51}=6.6$ | H–36 |
| 8 | 1.91 | d, $J_{45-3}=1.6$ (long-range) | $H_3$–45 |
| 9 | 1.98 | m, | $H_2$–39 |
| 10 | 2.06 | m, | $H_2$–42 |
| 11 | 2.44 | m, $J_{6-5}=8.8$, $J_{6-46}=6.8$, $J_{6-7}=4.8$ | H–6 |
| 12 | 2.55 | m, $J_{34-33}=8.8$, $J_{34-35}=8.0$, $J_{34-50}=6.6$ | H–34 |
| 13 | 2.84 | s, | $H_3$–53 |
| 14 | 3.14 | t, $J_{44-43}=6.8$ | $H_2$–44 |
| 15 | 3.23 | s, | $H_2$–55 |
| 16 | 3.34 | d, $J_{18-19}=10.0$ | H–18 |
| 17 | 3.76 | m, $J_{7-6}=4.8$ | H–7 |
| 18 | 4.02 | m, | H–27 |
| 19 | 4.16 | dd, $J_{29-28}=8.9$ and 3.6 | H–29 |
| 20 | 4.77 | dd, $J_{35-34}=8.0$, $J_{35-36}=4.0$ | H–35 |
| 21 | 5.22 | m, | H–C–O-malonyl |
| 22 | 5.43 | m, $J_{41-40}=14.9$ | H–41 |
| 23 | 5.44 | m, $J_{40-41}=14.9$ | H–40 |
| 24 | 5.44 | dd, $J_{33-32}=14.9$, $J_{33-34}=8.8$ | H–33 |
| 25 | 5.98 | br 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 | 6.43 | dd, $J_{4-3}=11.2$, $J_{4-5}=14.7$ | H–4 |
| 29 | 7.09 | br d, $J_{3-4}=11.2$ | H–3 |

**Partial Structures of Azalomycin $F_{4a}$**

Structure A (C-1—C-7): A UV absorption maximum of $F_{4a}$ appearing at 268 nm indicated the presence of an $\alpha,\beta,\gamma,\delta$-unsaturated acid (or ester) moiety. In its $^{1}H$-NMR spectrum a signal at $\delta$ 7.09 (signal No. 29 in Table II) due to H-3 also suggested the conjugation of the olefinic bond with an acyl carbonyl group. $^{1}H$–$^{1}H$ Spin couplings between vicinal protons of the system, C-3—C-7, and a long-range coupling between H-3 and H-45 were determined by the decoupling technique (see Table II, signals No. 5, 8, 11, 17, 26, 28 and 29). ($E$)-Orientation of the C(4)=C(5) bond was indicated by a large coupling constant (14.7 Hz) between H-4 and H-5. ($E$)-Orientation of the C(2)=C(3) bond could not be elucidated from the NMR data for $F_{4a}$ itself, but it was deduced in a degradation product as described in the following paper.

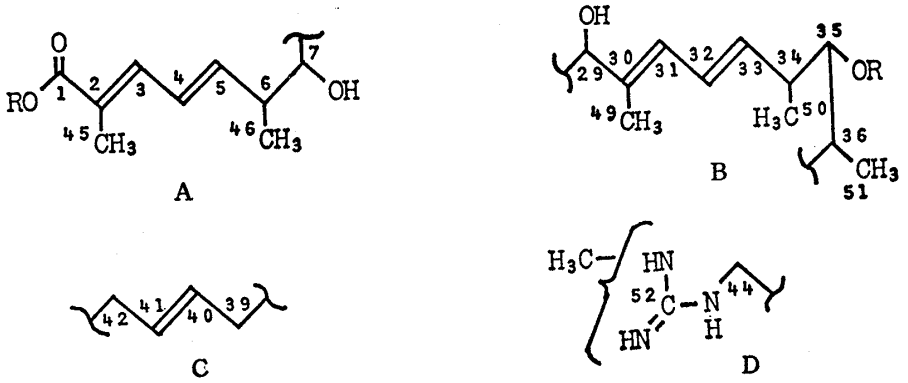


Fig. 2

Structure B (C-29—C-36): A UV absorption maximum at 240 nm is in accord with the conjugated diene structure. In the $^1$H-NMR spectrum of $F_{4a}$, spin coupling between vicinal protons of the system, C-31—C-36, and long-range couplings between H-29 and H-31, and H-49 and H-31 were determined by decoupling experiments (see Table II, signals No. 3, 4, 6, 7, 12, 19, 20, 24, 25 and 27). Since H-33 resonated at a magnetic field overlapping the resonance positions of H-40 and H-41 and its signal could not be resolved in normal spectra, the signal was analyzed by the use of decoupling difference spectra generated by subtraction of the control spectrum from decoupled spectra irradiated at the resonance frequencies of H-32 and H-34. (*E*)-Orientations of the C(30)=C(31) bond and of the C(32)=C(33) bond were elucidated from the long-range coupling constant (1.6 Hz) between H-49 and H-31 and the large coupling constant (14.9 Hz) between H-32 and H-33, respectively.

Structure C (C-39—C-42): $^1$H-NMR signals due to H-40 (signal No. 23 in Table II) and H-41 (signal No. 22) could be resolved by decoupling difference spectra between the control spectrum and the decoupled spectra irradiated at the resonance frequencies of H-39 and H-42, respectively, and the couplings of vicinal protons of the system, C-39—C-42, could be determined.

Structure D: The facts that 3.67% nitrogen content observed on elemental analysis of $F_{4a}$ corresponds to 3 nitrogen atoms on the basis of its molecular weight (1081) determined by FD-MS and that its $^{13}$C-NMR spectrum exhibited a signal due to guanido carbon ($\delta$ 158.27) indicated the presence of a guanidine moiety. Signals due to *N*-methyl and *N*-methylene groups were also observed.

Analysis of $^{13}$C-NMR and $^1$H-NMR data for this compound revealed that the following functional groups are present in this molecule in addition to the partial structures A—D; 8× CH–OH, 1×CH–O-acyl, 2×acyl carbonyl, 13×$CH_2$, 2×CH and 1×C=O as hemiketal.

From all these spectral and analytical data, the molecular formula of this compound was determined as $C_{56}H_{95}N_3O_{17}$.

## 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), the maxima are given in nm (extinction $\varepsilon$). IR spectra were measured on a Japan Spectroscopic Co. apparatus (model IR-S) and are recorded in cm$^{-1}$. $^1$H-NMR spectra and $^{13}$C-NMR spectra were measured on JEOL JNM 4H-100 ($^1$H: 100 MHz), JNM FX-100 ($^1$H: 99.95 MHz, $^{13}$C: 25.05 MHz) and JNM FX-400 ($^1$H: 400.5 MHz, $^{13}$C: 100.7 MHz) machines; chemical shifts are given in ppm (in $\delta$) relative to TMS (=0 ppm) as an internal or external standard and coupling constants are recorded in Hz ($J$). Mass spectra were measured on a Shimadzu LKB-9000 apparatus (EI-MS), on a JEOL JMS-01SG-2 apparatus (FD-MS) or on a JEOL JMS-DX-300 apparatus (FAB-MS). Optical rotation was measured on a Japan Spectroscopic Co. apparatus (model DIP-181).

Thin layer chromatography 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 machines. High perfor-

mance liquid chromatography was performed on a Shimadzu apparatus (model LC-830).

Azalomycin F complex was provided by Sankyo Co.

**Column Chromatography of Azalomycin F Complex**——A typical procedure was as follows: 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 12 l eluate gave foreruns containing $F_3$ and $F_4$, and the next 6 l eluate afforded almost pure $F_4$ fraction containing *ca.* 5 g of a mixture of $F_{4a}$ and $F_{4b}$ (more than 70% of $F_{4a}$). After further elution with this solvent mixture (2—3 l) 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 $F_5$. The mixture of $F_{4a}$ and $F_{4b}$ obtained from column chromatography was recrystallized from methanol–water to give a mixture containing more than 80% of $F_{4a}$ and this was normally used for degradation reactions.

**Separation of $F_{4a}$ and $F_{4b}$**——The $F_4$ mixture (10—15 mg) obtained from column chromatography 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 16—30 h. During the developing time the top of the plate was exposed to air to allow the solvents to evaporate. $F_{4a}$ and $F_{4b}$ fractions, obtained by extraction of the plate sicica gel, were each filtered to remove insoluble materials. Crude $F_{4a}$ thus obtained was recrystallized from methanol–water to give fine needles; *Anal.* Calcd for $C_{56}H_{95}N_3O_{17}$: C, 62.14; H, 8.85; N, 3.88; O, 25.13. Found: C, 61.78; H, 8.80; N, 3.67; O, 25.23, mp 131—132°C (dec.), $[\alpha]_D^{22}$ +39° ($c$=1.0, methanol), FD-MS $m/z$: 1081 ($M^+$), UV $\lambda_{max}^{MeOH}$ nm ($\varepsilon$): 240 (38000), 268 (23000), IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3600—2700 (br, strong), 1700—1500 (br, strong), $^{13}$C-NMR: see Table I, $^1$H-NMR: see Table II.

Attempts to recrystallize $F_{4b}$ have so far been unsucoessful, but $F_{4a}$ and $F_{4b}$ separated by TLC each gave a single peak on HPLC (silica gel packed column, Zorbax SIL, 2.1 mm × 25 cm) eluted with *sec*-butanol–water (9: 1) at a rate of 0.14 ml/min (100 kg/cm$^2$); $v_R$: 4.72 ($F_{4a}$), 5.46 ($F_{4b}$).

**Interconversion of $F_{4a}$ and $F_{4b}$**——1) Methanol solutions of $F_{4a}$ and of $F_{4b}$ were allowed to stand at room temperature. Conversions of one to the other were observed in both solutions by TLC analysis after 3 d, and after 7 d both solutions had yielded almost 1: 1 mixtures of the two compounds. Decomposition of these compounds was also observed.

2) Such solutions, on standing at 50°C, yielded almost 1: 1 mixtures of the two compounds after 20 h. Decomposition of these compounds was also observed.

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## References and Notes

1) The structure of azalomycin $F_{4a}$ was preliminarily reported in the preceding papers; *a*) M. Namikoshi, K. Sasaki, M. Amano, Y. Koiso, S. Iwasaki, S. Okuda, S. Nozoe, and K. Fukushima, The Proceedings of the 23rd Symposium on the Chemistry of Natural Products, Oct., 1980 in Nagoya, p. 600; *b*) The dissertation of M. Namikoshi, Graduate School of Pharmaceutical Sciences, The University of Tokyo, 1981.
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