FLUVIRUCINS A_1, A_2, B_1, B_2, B_3, B_4 AND B_5, NEW ANTIBIOTICS ACTIVE AGAINST INFLUENZA A VIRUS

III. THE STEREOCHEMISTRY AND ABSOLUTE CONFIGURATION OF FLUVIRUCIN A_1

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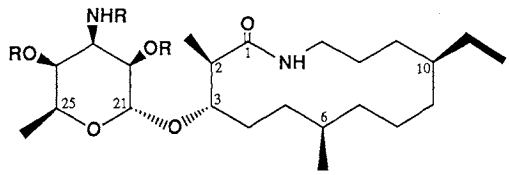
Fluvirucin A_1 was established as (2*R*,3*S*,6*R*,10*S*)-3-[(3-amino-3,6-dideoxy-α-L-talopyranosyl)-oxy]-2,6-dimethyl-10-ethyl-13-tridecanelactam by chemical, spectroscopic, and X-ray crystallographic analyses.

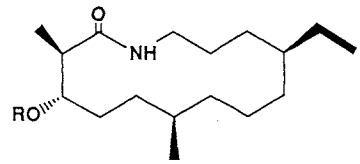
Fluvirucins are new antibiotics isolated from the fermentation broth of unidentified actinomycete species and show potent inhibitory activity against influenza A virus.[1,2] The structure **1** was proposed for the major component, fluvirucin A_1, based on various spectroscopic and chemical properties. The 14-membered macrocyclic lactam was the first such aglycone from a natural source but there remained a question that no direct connectivity between C-11 and C-12 was observed in the 2D-incredible natural abundance double quantum transfer experiment (INADEQUATE) spectrum. The stereochemistry of the sugar part was established as 3-amino-3,6-dideoxy-L-talose but that of the aglycone remained uncertain. We report here several spectroscopic and chemical properties of **1** and a single crystal X-ray analysis of triacetyl derivative **2** of **1** to confirm the structure and the absolute configuration of the 14-membered macrocycle. Hydrolysis of fluvirucin A_1 (**1**) with methanolic hydrogen chloride afforded an aglycone, named fluvirucinine A_1 (**3**) together with two anomeric sugars.[2] When a mesylate **4** derived from **3** was treated with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in *N*,*N*-dimethylformamide at 100°C for 4 hours, three dehydration-products **5**, **6**, and **7** were isolated in 36, 13, and 22%, respectively (Scheme 1). Both **5** and **6** have an olefinic double bond and the structures were easily elucidated by ^{1}H NMR spectroscopy. The remaining **7** contained a β-lactam skeleton (IR 1740 cm^{-1}) and the small coupling constant (J=2.2 Hz) between the adjacent protons on the 4-membered ring requires these two hydrogens to be *trans*. As the β-lactam ring can be considered to derive through an SN_2-displacement mechanism, the stereochemistry of the original C-2 and C-3 substituents is estimated as *trans*. The large coupling constant between C2-H and C3-H in **4** (J=10.3 Hz) or an acetate of **3** (J=9.4 Hz)[2] supports this assignment.

As the C3-OH may be equatorially oriented, application of the MOSHER[3] or TROST method[4] to determine the absolute stereochemistry was investigated. The results, shown in Fig. 2, are in accord with the prediction that the absolute stereochemistry of C-3 is *S*, though most of the protons on the 14-membered ring could not be assigned due to the overlap of the signals. The configuration of remaining C-6 and C-10

Fig. 1. Absolute structures of fluvirucin A_1 (**1**) and fluvirucinine A_1 (**3**), and their derivatives.

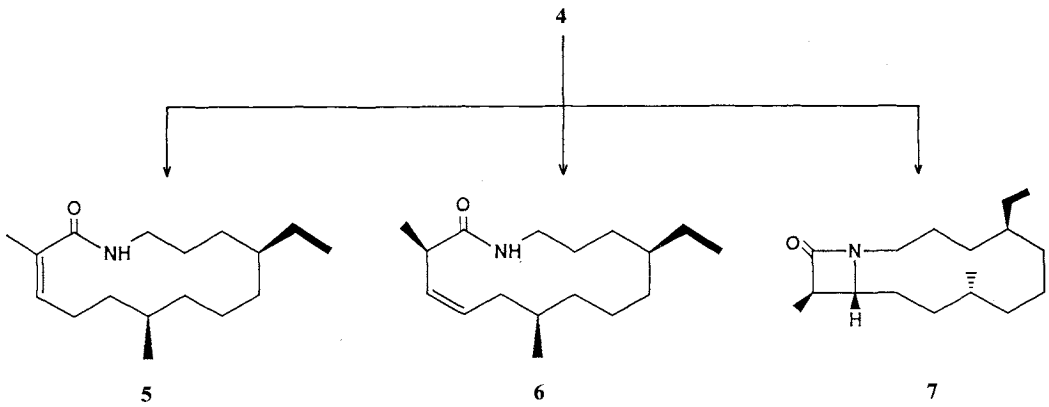


1 R=H
2 R=Ac



3 R=H
4 R=Ms
8 R=(+)-MTPA
9 R=(−)-MTPA
10 R=(−)-MPA
11 R=(+)-MPA

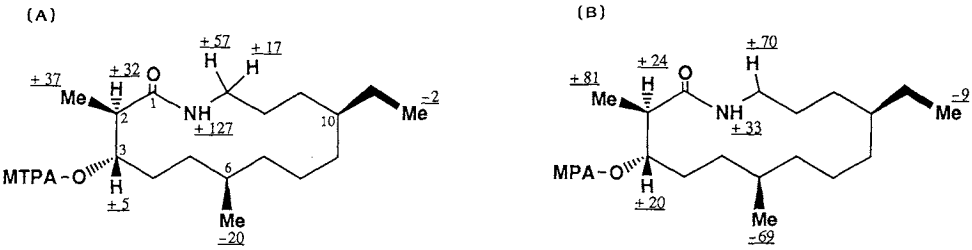
Scheme 1.



5 **6** **7**

Fig. 2. Chemical shifts differences.

R-(+)- and *S*-(−)-MTPA esters: $\Delta\delta$ in Hz (400 MHz)$=\delta(S)-\delta(R)$ (A), *R*-(−)- and *S*-(+)-MPA esters: $\Delta\delta$ in Hz (400 MHz)$=\delta(R)-\delta(S)$ (B).



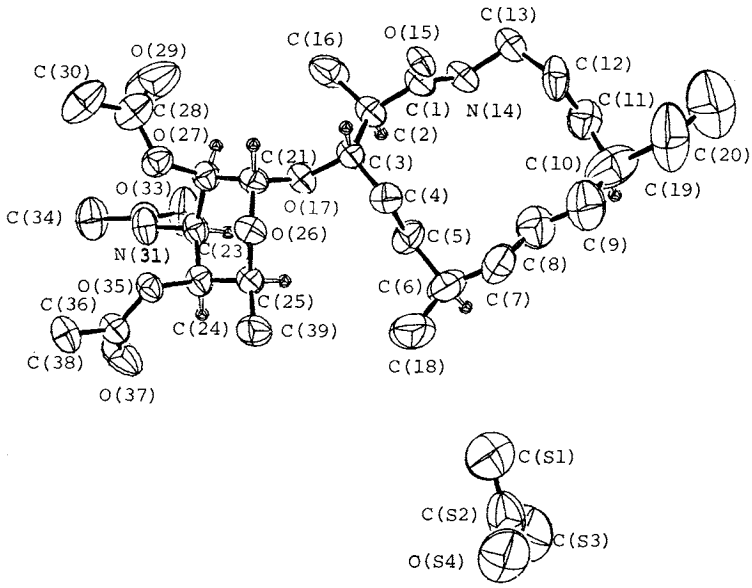
could not yet be clarified by a chemical or spectroscopic method.

We finally tried a single crystal X-ray analysis of **2** to confirm unambiguously the structure and the absolute configuration of the 14-membered macrolactam. **2** was recrystallized from acetone to give colorless rods with one equivalent of acetone as solvent of crystallization. The crystallographic data are summarized as follows: monoclinic, $P2_1$; $a=8.317(1)$, $b=28.472(3)$, $c=8.132(1)$Å, $\beta=110.43(1)°$, $V=1805(5)$Å^3, $Z=2$; $D_m=1.14(1)$, $D_{calc}=1.13$ g/cm^3.

Several direct attempts with various starting phase-sets estimated from a convergence map failed to

Fig. 3. ORTEP drawing[10] of the major conformer of **2** with thermal ellipsoids scaled at the 50% probability level and numbering scheme.

H-Atoms are calculated and are represented by circles of radius 0.1Å.



solve the structure. The RANTAN approach in MULTAN11/82[5] finally succeeded in location of 20 atoms (the sugar part) on the *E*-map. Successive weighted Fourier syntheses revealed the position of the remained non-hydrogen atoms except an ethyl group. The structure was refined by the full-matrix least-squares method (SHELX76).[6] The atomic scattering factors were taken from International Tables for X-Ray Crystallography.[7] All numerical calculations were carried out on a Facom M780/20 computer at the Science Information Processing Center, University of Tsukuba.

After isotropic refinement on 37 atoms, weighted D-Fourier syntheses found the ethyl group and solvent (acetone). Isotropic refinement on all non-hydrogen atoms converged the *R* factor to 0.154; at this stage a disorder was found around the 14-membered ring. Two conformations were estimated with an occupancy of 0.7 and 0.3. Since several bond-lengths in the disordered part, however, had not been converged to the normal values, the bond lengths in the minor part were fixed to 1.540 Å with e.s.d. = 0.001 in the final stage. One reflection (020) considered to suffer to a secondary extinction was omitted. Final blocked full-matrix least squares with anisotropic for non-hydrogen atoms and isotropic for those in the minor disordered part converged the *R* factor to 0.098 ($_wR=0.100$, S=3.72). Final weighting scheme was $\omega=2.9/[\sigma^2(\mathrm{Fo})+0.0012\mathrm{F}^2]$. The ratio of maximum least-squares shift to error was less than 0.1.

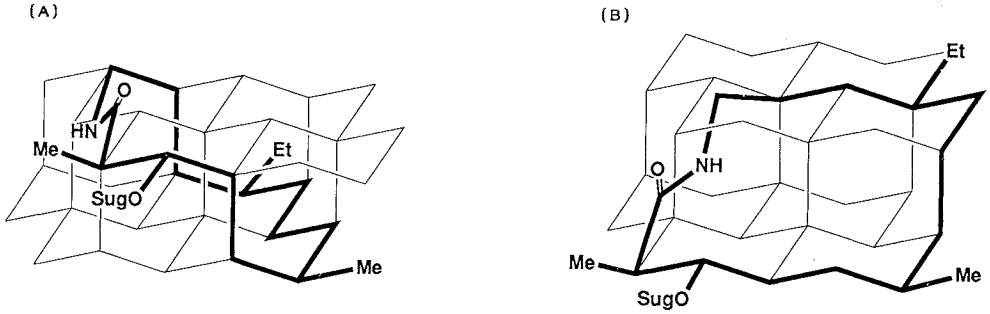
Molecular structure of the major conformer with atomic numbering scheme is shown in Fig. 3.†

The 14-membered lactam skeleton **1** was unequivocally confirmed including the substituents correctly positioned. Since the absolute configuration of the sugar had been elucidated as 3-amino-3,6-dideoxy-L-talose,[2] those on the aglycone were established as 2*R*, 3*S*, 6*R*, 10*S*. The definition of 2*R*, 3*S* is accord with the result obtained by chemical and spectroscopic methods as mentioned above.

† Atomic parameters, selected bond lengths, and tortion angles of the 14-membered lactam and the sugar are deposited in Cambridge Crystalographic Data Centre.

Fig. 4. Diamond-lattice representations.

Major (A) and minor (B) conformations.



The characteristic feature of the crystal structure is that the 14-membered lactam ring is not in a single conformation. The large thermal parameters and abnormal bond lengths of several atoms† show that other conformational isomers are still possible together with disorder of the ethyl group. Two conformations obtained are shown schematically on the diamond lattice in Fig. 4. The C13-N14-C1-C2-C3 regions of both conformers are essentially same with C3-O-sugar in an equatorial position. The difference is detected in three -CH_2-CH_2- units (-C4-C5-, -C7-C8-, and -C11-C12-). The conformations are not in accord with those of the 14-membered lactone antibiotics, erythromycin A[8] or oleandomycin derivatives.[9] The difference may arise by the presence of many substituents in the latter cases, in which the conformation should be fixed by the steric environment of the substituents.

Experimental

The mp's are uncorrected. IR spectra were determined on a Jasco IR-810 spectrometer. ^{1}H NMR spectra were recorded on a Jeol JNM-GX 400 (400 MHz) and chemical shifts were obtained relative to $CDCl_3$ as an internal standard.

Mesylate (**4**)

A mixture of 200 mg of **3** and 1 ml of methanesulfonyl chloride in 10 ml of dry pyridine was stirred at room temperature for 16 hours. After the whole had been poured onto ice-water, products were extracted with EtOAc (70 ml × 2). The organic layer was washed with 1 M HCl, a satd $NaHCO_3$ soln, and water (each 50 ml × 2) and was dried over anhydrous Na_2SO_4. Evaporating the solvent gave 220 mg of **4** as solids. Crystallization from $CHCl_3$ - MeOH afforded a pure **4**. MP 216~217°C; IR ν_{max} (KBr) cm^{-1} 1320, 1160; ^{1}H NMR ($CDCl_3$ - CD_3OD) δ 4.91 (1H, dt, J=10.3 and 4.3 Hz, 3-H), 3.71 (1H, m, 13-H), 3.08 (3H, s, 3-OMs), 2.69 (1H, dq, J=10.3 and 6.8 Hz, 2-H), 2.64 (1H, m, 13-H), 1.27 (3H, d, J=6.8 Hz, 2-CH_3), 0.91 (3H, d, J=6.8 Hz, 6-CH_3), 0.87 (3H, t, J=7.3 Hz, 10-CH_2CH_3).

Anal Calcd for $C_{18}H_{35}NO_4S$: C 59.80, H 9.76, N 3.87, S 8.87.
Found: C 59.55, H 9.77, N 3.74, S 9.21.

DBU Treatment on **4**

To a suspension of 184 mg of **4** in 10 ml of DMF, there was added 1.5 ml of DBU and the mixture was heated at 100°C for 4 hours. After the whole had been cooled to room temperature, EtOAc was added up to 150 ml and the solution was washed with 1 M HCl, a satd $NaHCO_3$ soln, and water (each 100 ml × 2). The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness to give a pale yellow solids. Chromatography on silica gel (toluene - MeOH, 19 : 1) afforded **5** (48.5 mg), **6** (17.7 mg), and **7**

† See p. 758.

(29.5 mg) as white solids.

5: MP 163~165°C; IR ν_{max} (KBr) cm^{-1} 1660; ^1H NMR ($CDCl_3$) δ 5.62 (1H, br s, NH), 5.40 (1H, ddq, J=9.0, 6.4 and 1.3 Hz, 3-H), 3.44 (1H, ddt, J=13.3, 2.1 and 6.8 Hz, 13-H), 3.26 (1H, m, 13-H), 2.20 (1H, ddt, J=13.3, 4.7 and 9.0 Hz, 4-H), 1.94 (1H, m, 4-H), 1.89 (3H, s, 2-CH_3), 0.88 (3H, d, J=6.8 Hz, 6-CH_3), 0.86 (3H, t, J=7.3 Hz, 10-CH_2CH_3). NOESY spectrum showed a cross peak between C2-CH_3 and C3-H.

Anal Calcd for $C_{17}H_{31}NO$: C 76.92, H 11.77, N 5.28.
Found: C 76.97, H 11.95, N 5.25.

6: MP 172~174°C; IR ν_{max} (KBr) cm^{-1} 1660; ^1H NMR ($CDCl_3$) δ 5.61 (1H, br s, NH), 5.53 (1H, ddd, J=11.7, 10.3 and 3.8 Hz, 4-H), 5.42 (1H, ddd, J=11.7, 9.0 and 1.9 Hz, 3-H), 3.53 (1H, m, 13-H), 2.97 (1H, m, 13-H), 2.91 (1H, dq, J=9.0 and 6.8 Hz, 2-H), 2.16 (1H, ddd, J=13.7, 4.4 and 3.8 Hz, 5-H), 1.83 (1H, dt, J=13.7 and 10.3 Hz, 5-H), 1.22 (3H, d, J=6.8 Hz, 2-CH_3), 0.93 (3H, d, J=6.8 Hz, 6-CH_3), 0.84 (3H, t, J=7.3 Hz, 10-CH_2CH_3).

Anal Calcd for $C_{17}H_{31}NO$: C 76.92, H 11.77, N 5.28.
Found: C 76.82, H 11.88, N 5.17.

7: MP 77~78°C; IR ν_{max} (KBr) cm^{-1} 1740; ^1H NMR ($CDCl_3$) δ 3.75 (1H, dt, J=14.3 and 8.2 Hz, 13-H), 3.10 (1H, dt, J=8.8 and 2.2 Hz, 3-H), 2.70 (1H, dq, J=2.2 and 7.3 Hz, 2-H), 2.66 (1H, ddd, J=14.3, 7.8 and 3.3 Hz, 13-H), 1.24 (3H, d, J=7.3 Hz, 2-CH_3), 0.89 (3H, d, J=6.6 Hz, 6-CH_3), 0.86 (3H, t, J=7.3 Hz, 10-CH_2CH_3).

Anal Calcd for $C_{17}H_{31}NO$: C 76.92, H 11.77, N 5.28.
Found: C 76.77, H 11.88, N 5.28.

R-(+)- and *S*-(−)-α-Methoxy-α-(trifluoromethyl)phenylacetyl Esters **8** and **9**

A mixture of 13 mg of **3**, 150 μl of α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (*R*-(+)-MTPACl), 1 ml of Et_3N, and 10 mg of 4-dimethylaminopyridine (DMAP) in 2 ml of dichloromethane was stirred at room temperature for 16 hours. EtOAc and ice-water were added to the solution and the whole was stirred for 1 hour. After had been separated, the organic layer was washed with 1 M HCl, satd $NaHCO_3$ soln, and water. After concentrating under reduced pressure, the crude products were purified by preparative TLC (toluene-MeOH (17:3) followed with hexane-acetone, 9:1) and column-chromatography on Sephadex LH-20 (MeOH) to afford 15 mg of **8** as white solids. The *S*-(−)-MTPA ester **9** was prepared as similar procedure.

8: MP 185~187°C; ^1H NMR ($CDCl_3$) δ 7.57 (2H, m), 7.42 (3H, m), 5.45 (1H, dd, J=8.1 and 4.3 Hz, NH), 5.26 (1H, dt, J=3.4 and 8.1 Hz, 3-H), 3.75 (1H, ddt, J=13.7, 1.7 and 8.1 Hz, 13-H), 3.59 (3H, s), 2.50 (1H, m, 13-H), 2.44 (1H, dq, J=8.1 and 7.3 Hz, 2-H), 1.72 (1H, tt, J=13.9 and 3.4 Hz, 4-H), 1.06 (3H, d, J=7.3 Hz, 2-CH_3), 0.85 (3H, t, J=7.3 Hz, 10-CH_2CH_3), 0.84 (3H, d, J=7.3 Hz, 6-CH_3).

9: MP 184~186°C; ^1H NMR ($CDCl_3$) δ 7.54 (2H, m), 7.41 (3H, m), 5.76 (1H, dd, J=8.1 and 4.3 Hz, NH), 5.27 (1H, dt, J=3.4 and 8.6 Hz, 3-H), 3.79 (1H, ddt, J=13.7, 1.7 and 8.1 Hz, 13-H), 3.54 (3H, s), 2.64 (1H, m, 13-H), 2.52 (1H, dq, J=8.6 and 7.3 Hz, 2-H), 1.73 (1H, tt, J=13.9 and 3.4 Hz, 4-H), 1.15 (3H, d, J=7.3 Hz, 2-CH_3), 0.84 (3H, t, J=7.3 Hz, 10-CH_2CH_3), 0.79 (3H, d, J=6.8 Hz, 6-CH_3).

R-(−)- and *S*-(+)-α-Methoxyphenylacetic Acid (MPA) Esters **10** and **11**

A mixture of 14 mg of **3**, 53 mg of *R*-(−)-MPA, 10 mg of DMAP, and 1-ethyl-3-(3-diethylaminopropyl)carbodiimide hydrochloride in 5 ml of dichloromethane was stirred at room temperature for 16 hours. After concentrating under reduced pressure, 40 ml of EtOAc was added and the whole was washed with a 5% citric acid soln, water, a 5% $NaHCO_3$ soln, and water (each 30 ml × 2). After evaporating the solvent, the residue was purified by preparative TLC (toluene-MeOH, 17:3) and column-chromatography on Sephadex LH-20 (MeOH) to afford 11.5 mg of **10** as white solids. *S*-(+)-MPA ester **11** was prepared by a similar procedure.

10: MP 220~221°C; ^1H NMR ($CDCl_3$) δ 7.44 (2H, br d, J=7 Hz), 7.35 (3H, m), 5.70 (1H, br dd, J=7.3 and 4.7 Hz, NH), 5.05 (1H, dt, J=2.7 and 8.8 Hz, 3-H), 4.77 (1H, s), 3.63 (1H, m, 13-H), 3.43 (3H, s), 2.83 (1H, dddd, J=13.5, 7.3, 4.7 and 2.1 Hz, 13-H), 2.39 (1H, dq, J=8.8 and 6.8 Hz, 2-H), 1.09 (3H, d, J=6.8 Hz, 2-CH_3), 0.83 (3H, t, J=7.3 Hz, 10-CH_2CH_3), 0.62 (3H, d, J=6.8 Hz, 6-CH_3).

11: MP 250~252°C; ^1H NMR ($CDCl_3$) δ 7.45 (2H, br d, J=7 Hz), 7.37 (3H, m), 5.62 (1H, br dd,

J=7.3 and 4.7 Hz, NH), 5.00 (1H, dt, J=2.6 and 6.8 Hz, 3-H), 4.78 (1H, s), 3.65 (1H, ddt, J=13.3, 2.6 and 7.3 Hz, 13-H), 3.42 (3H, s), 2.66 (1H, dddd, J=13.3, 8.6, 4.7 and 2.1 Hz, 13-H), 2.33 (1H, quint, J=6.8 Hz, 2-H), 0.88 (3H, d, J=6.8 Hz, 2-CH_3), 0.86 (3H, t, J=7.3 Hz, 10-CH_2CH_3), 0.79 (3H, d, J=7.3 Hz, 6-CH_3).

Crystallographic and Diffraction Data Collection of **2**

The crystals were grown from an acetone solution as colorless rods. MP 246~247°C (changed to opaque above 148°C due to the solvent loss).

Anal Calcd for $C_{29}H_{50}N_2O_8 \cdot C_3H_6O$: C 62.72, H 9.21, N 4.57.
Found: C 62.21, H 9.18, N 4.57.

The densities (D_m) were measured by flotation in aqueous KI solution. The unit-cell dimensions were refined by least-squares refinement from 25 reflections with $11° < \theta < 13°$ on an Enraf-Nonius CAD4 automated kappa-axis diffractometer with graphite monochromated Mo Kα radiation (50 kV and 26 mA). The diffraction intensities were measured for a single crystal of about $0.4 \times 0.3 \times 0.3\,mm^3$ on the diffractometer up to $2\theta = 60°$; $2\theta - \omega$ scan, the scan rate with $4° min^{-1}$ in θ, the ω scan width = $0.6 + 0.35\ tan\ \theta$. Three standard reflections were measured after every 2 hours. The intensities were corrected for Lorentz, polarization, and decay (−1.2% in intensity) but not for absorption. Systematic absence (k=2n) indicated unambiguously the space group $P2_1$. Of 5,345 unique reflections, 3,453 of independent structure factors with $|Fo| > 3\sigma$ (Fo) were used for structure determination.

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