ALTEMICIDIN, A NEW ACARICIDAL AND ANTITUMOR SUBSTANCE

II. STRUCTURE DETERMINATION

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The structure of altemicidin, a new acaricidal and antitumor agent, was determined to be (1*R*,2*S*,3a*R*,7a*S*)-4-carbamoyl-2-hydroxy-6-methyl-1-(sulfamoylacetamido)-2,3,3a,6,7,7a-hexahydro-6-azaindene-1-carboxylic acid by a combination of spectroscopic and X-ray crystallographic analysis of its derivatives. Altemicidin is a monoterpene alkaloid.

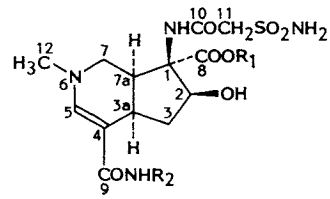
Altemicidin, isolated from a culture broth of an actinomycete strain identified as *Streptomyces sioyaensis* SA-1758, is a new compound having the acaricidal and antitumor activities. We have reported the fermentation, isolation, biological properties and physico-chemical properties of altemicidin in the preceding paper[1].

In this report, we describe the structure determination of altemicidin.

Results and Discussion

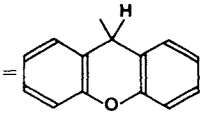
Altemicidin (**1**, Fig. 1) was obtained as white powder. Its molecular formula was established to be $C_{13}H_{20}N_4O_7S$ as described in the preceding paper[1]. The IR spectrum of **1** showed the presence of amide group (1650 and 1550 cm^{-1}) and sulfonyl group (1345 and 1165 cm^{-1})[1]. In the ^{13}C NMR spectrum (in D_2O) of **1**,thirteen carbon signals were observed at δ 179.7, 174.2, 164.4, 147.3, 97.1, 76.0, 69.1, 60.3, 45.5, 43.2, 41.4, 40.8 and 31.7. The signal at δ 60.3 gradually changed to be small and broad. In the ^1H NMR spectrum (Table 1), an olefinic proton at δ 7.39 and *N*-methyl protons at δ 2.98 were readily assignable. Furthermore, the isolated methylene protons were observed at δ 4.29 and 4.82 which were easily deuterated in D_2O resulting in a half intensity of the proton signals within 1 hour.

Fig. 1. Structures of altemicidin (**1**) and its derivatives (**2** and **3**).



Altemicidin (**1**) $R_1=R_2=H$

2 $R_1=CH_3$ $R_2=H$

3 $R_1=CH_3$ $R_2=$ 

Treatment of **1** with diazotrimethylsilylmethane in methanol gave the methyl ester (**2**, secondary ion mass spectrum (SI-MS) *m/z* 391 (MH^+)). The data

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of ^{1}H and ^{13}C NMR spectra (in C_5D_5N) are listed in Tables 1 and 2. The multiplicities of carbon signals were determined by the distortionless enhancement by polarization transfer (DEPT) experiment. The correlation between protons and carbons was obtained by the ^{1}H-^{13}C heteronuclear shift correlation spectroscopy (COSY) experiment. The results of these NMR experiments indicated the presence of following fragments in **2**, that is, NCH_3, =CH, $CH_2CH<$, $>CHCH_2CH$-O, $COOCH_3$, the isolated CH_2, $CONH_2$, NHCO, =C< and >C<. The ^{1}H-^{13}C long range connectivities were determined by the ^{1}H-detected heteronuclear multiple-bond ^{1}H-^{13}C correlation spectroscopy (HMBC) experiment. The olefinic proton (δ 7.64) attached directly to C-5 (δ 143.7) showed connectivities to an *N*-methyl carbon (δ 42.2), a quaternary olefinic carbon (C-4, δ 102.2) and a primary amide carbonyl carbon (C-9, δ 171.0). The primary amide protons (δ 7.11) were coupled to C-4. Although the methine proton of C-3a showed no cross preak in the ^{1}H-^{1}H shift COSY spectrum with the methine proton of C-7a, the HMBC spectrum showed a connectivity between 3a-H (δ 3.64) and a methine carbon (C-7a, δ 41.1). Furthermore, 3a-H was coupled to two methylene carbons (C-3, δ 43.8, C-7, δ 45.4), two olefinic carbons (C-4 and C-5) and a primary amide carbonyl carbon (C-9). The quaternary carbon (C-1, δ 67.3) showed connectivities to a lower field proton at δ 3.19 of the nonequivalent methylene proton of C-3, a methine proton (7a-H, δ ~3.6) and a secondary amide proton (1-NH, δ 8.93). The proton of the oxygen-bearing methine (2-H, δ 4.50) was coupled to an ester carbonyl carbon (C-8, δ 174.6). The structure of the remaining part of sulfamoylacetyl moiety was determined as follows; the isolated and deuterium-exchangeable methylene protons (11-H, δ 4.77, 4.89) was coupled to an amide carbonyl carbon (C-10, δ 164.2) and C-10 also was coupled to an amide proton (1-NH) in the HMBC spectrum.

All results of HMBC experiments of **2** were shown in Fig. 2. Thus, the planar structure of **2** was proposed as methyl 4-carbamoyl-2-hydroxy-6-methyl-1-(sulfamoylacetamido)-2,3,3a,6,7,7a-hexahydro-6-azaindene-1-carboxylate.

To determine the absolute structure of **1**, X-ray crystallographic analysis was performed. Many efforts to obtain the single crystal of **1** or its derivatives were made. Compound **2** was treated with 9-hydroxyxanthene in acetic acid to yield 9-*N*-(9-xanthenyl) derivative (**3**) which gave crystals suitable for

Table 1. ^{1}H NMR data of altemicidin (**1**) and its methyl ester (**2**).

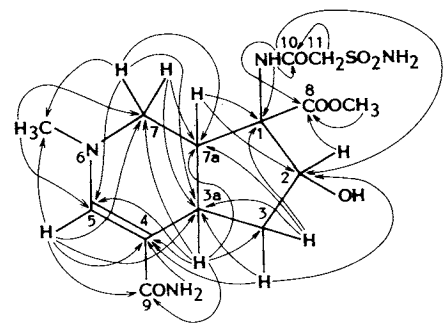
Proton	Chemical shifts (δ value in ppm) and coupling constants (Hz)					
	1			**2**		
2-H	4.28	dd	J=7.6, 9.0	4.50	br dd	J=7.2, 9.0
3-H_a	1.26	dt	J=9.0, 12.6, 12.6	1.77	dt	J=9.0, 12.7, 12.7
3-H_b	2.67	dt	J=7.6, 7.6, 12.6	3.19	dt	J=7.2, 7.2, 12.7
3a-H	~2.93	m		3.64	q	J=7.2, 12.7
5-H	7.39	s		7.64	s	
7-H_a	~2.86	m		3.10	t	J=12.0
7-H_b				3.30	dd	J=5.7, 12.0
7a-H	~2.86	m		~3.6	m	
11-H_a	4.29	d	J=14.0	4.77	d	J=14.0
11-H_b	4.38	d	J=14.0	4.89	d	J=14.0
NCH_3	2.98	s		2.54	s	
1-NH				8.93	br s	
9-NH_2				7.11	br s	
$COOCH_3$				3.57	s	

1: In D_2O (ref DOH; δ 4.80), **2**: in C_5D_5N (ref TMS).

Table 2. ^{13}C NMR data for altemicidin methyl ester (**2**) in C_5D_5N.

Carbon	Chemical shift (ppm)
C-1	67.3
C-2	75.1
C-3	43.8
C-3a	32.7
C-4	102.2
C-5	143.7
C-7	45.4
C-7a	41.1
C-8	174.6
C-9	171.0
C-10	164.2
C-11	60.9
$COOCH_3$	52.4
NCH_3	42.2

Fig. 2. Proton-carbon correlation map of altemicidin methyl ester (**2**) by HMBC experiment.



X-ray experiment. Compound **3** was labile in protic solvent.

The green crystals of **3** were grown in acetone-methanol solution. A small crystal with approximate dimensions $0.3 \times 0.25 \times 0.2$ mm was chosen for X-ray work. The crystal data and intensity data were collected by graphite monochromated CuKα radiation. The crystal data are as follows: Acetone solvate of **3**; $C_{27}H_{30}N_4O_8S \cdot (CH_3)_2CO$, FW = 628.7. Orthorhombic, space group $P2_12_12_1$. Cell dimensions, $a = 14.419(8)$, $b = 24.426(13)$, $c = 8.678(5)$ Å, $U = 3056$ Å^3. $Z = 4$, $D_x = 1.366$ gcm^{-3}, μ for CuK$\alpha = 14.1$ cm^{-1}.

Intensities of 2,624 independent reflections were measured out of 3,319 possible reflections in the 2θ range of 6° through 156°. In addition, 942 Friedel pairs (hk$l$ and $\bar{h}$kl) were carefully measured for the layers $l = 1$ through 5 in the 2θ range of 30° through 90° with the scan speed of 2°θs^{-1}. The first set of intensity data were used for the structure determination and refinement and the second set for the determination of absolute configuration.

The crystal structure was solved by the direct method based on MULTAN[2] and the atomic parameters were refined by the block-diagonal-matrix least squares methods. The final R value for 2624 reflections was 0.062 including 44 C, N, O and S and 36H atoms. Of the 36 hydrogen atoms, 9 atoms of xanthene nucleus, 1 atom of ester methyl group and 4 atoms of acetone molecule were not found on the difference electron density map and therefore they were located at the calculated positions. The dispersion corrections for C, N, O and S atoms for CuKα radiation were taken into account in the final refinement assuming the absolute configuration determined as described below.

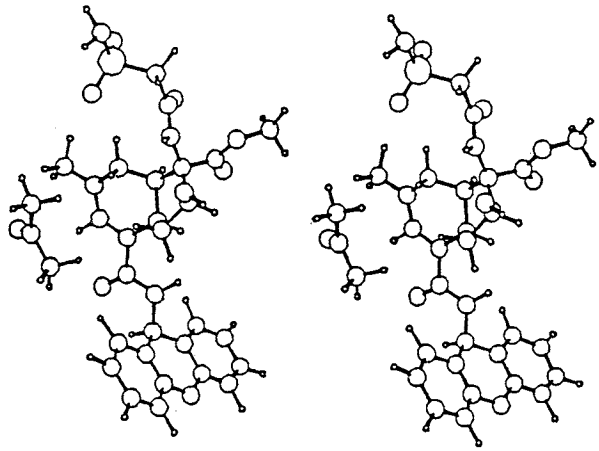
Of the 199 Friedel pairs which showed the difference of observed structure amplitudes between Friedel reflections greater than twice of their standard deviations, $||F_{obs}(hkl)| - |F_{obs}(\bar{h}k\bar{l})|| \geq 2\sigma[F_{obs}(hkl)]$, and the ratios of their calculated structure amplitudes differ more than 3% from unity, $|F_{calc}(hkl)|/|F_{calc}(\bar{h}k\bar{l})| < 0.97$ or $|F_{calc}(hkl)|/|F_{calc}(\bar{h}k\bar{l})| > 1.03$, 188 pairs showed the absolute configuration as Fig. 3. The stereo view of the crystal structure of **3** is shown in Fig. 3 which is drawing by PLUTO[†]. One equivalent mol of acetone was included in the crystal as a solvent crystallization.

The final atomic parameters and the bond lengths have been deposited at the crystallographic data center.

From the above spectroscopic analysis of **1** and **2** and X-ray crystallographic analysis of **3**, the absolute

[†] Cambridge Crystallographic Data Base, The Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2, 1EW, England, 1983.

Fig. 3. A stereoscopic drawing of **3**.



structure of altemicidin was determined to be (1*R*,2*S*,3a*R*,7a*S*)-4-carbamoyl-2-hydroxy-6-methyl-1-(sulfamoylacetamido)-2,3,3a,6,7,7a-hexahydro-6-azaindene-1-carboxylic acid (Fig. 1).

Altemicidin is the first isolated monoterpene alkaloid having a 6-azaindene skeleton as a metabolite of microorganisms. A few compounds having this skeleton were known in plants, such as skytanthine from *Skytanthus acutus* Meyen[3], dinklageine and strychnovoline from *Strychnos dinklagei*[4], tetrahydrocantleyine (artifact of cantleyine) from *Lasianthera austrocaledonica*[5] *etc.*

Experimental

General

MP was determined with a Yazawa mp apparatus and was uncorrected. Optical rotation was measured with a Perkin-Elmer model 241 polarimeter. IR and UV spectra were recorded with a Hitachi 260-10 IR spectrophotometer and a Hitachi 220S spectrophotometer, respectively. The ^{1}H and ^{13}C NMR spectra were measured with a Jeol JNM-GX400 spectrometer. The MS were recorded with a Hitachi M-80H mass spectrometer. TLC was performed on a silica gel plate (Kieselgel 60 F_{254}, Merck).

Methylation of Altemicidin

To a solution of **1** (50 mg) in MeOH (15 ml) was added excess diazotrimethylsilylmethane. The solution was stirred for 30 minutes at room temperature and then concentrated to 1 ml. The concentrate was purified on preparative silica gel TLC ($CHCl_3$ - MeOH - conc NH_4OH, 20 : 15 : 8) to afford 25.4 mg of white powder (**2**): MP 135~140°C; SI-MS *m/z* 391 (MH^+); $[\alpha]_D^{29}$ −30.4° (*c* 1.0, water); UV λ_{max} (pH 6.8, phosphate buffer) nm (*ε*) 298 (21,600), λ_{max} (0.1 M HCl) nm (*ε*) 306 (13,800), λ_{max} (0.1 M NaOH) nm (*ε*) 299 (22,100); IR (KBr) cm^{-1} 1730, 1640, 1540, 1440, 1400, 1340, 1290, 1240, 1210, 1160, 1130, 1080, 1000, 940, 810.

Xanthenylation of Altemicidin Methyl Ester

To a solution of xanthydrol (23 mg) in AcOH (0.35 ml) was added **2** (23 mg). The solution was vigorously stirred and then allowed to stand at room temperature for 30 minutes. The reaction mixture was purified on preparative silica gel TLC (EtOAc - MeOH, 9 : 1) to afford 22.5 mg of greenish solid. The solid was further purified by recrystallization in acetone - MeOH solution to give 9.8 mg of greenish crystals (**3**): SI-MS *m/z* 571 (MH^+); $[\alpha]_D^{29}$ −18.0° (*c* 0.2, DMSO); mp 166~168°C; IR (KBr) cm^{-1} 1740, 1700, 1640, 1560, 1500, 1480, 1460, 1330, 1300, 1260, 1190, 1160, 1120, 1090, 1040, 1000, 940, 900, 760.

References

1) TAKAHASHI, A.; S. KURASAWA, D. IKEDA, Y. OKAMI & T. TAKEUCHI: Altemicidin, a new acaricidal and antitumor

substance. I. Taxonomy, fermentation, isolation and physico-chemical and biological properties. J. Antibiotics 42: 1556~1561, 1989

2) MAIN, P.; S. E. HULL, L. LESSINGER, G. GERMAIN, J.-P. DECLERCQ & M. M. WOOLFSON: MULTAN78, A System of Computer Programs for the Automatic Solution of Crystal Structures from X-ray Diffraction Data. University of York (England) and Louvain (Belgium), 1978
3) DJERASSI, C.; J. P. KUTNEY & M. SHAMMA: Alkaloid studies. XXXII. Studies on *Skytanthus acutus* Meyen. The structure of the monoterpenoid alkaloid skytanthine. Tetrahedron 18: 183~188, 1962
4) MICHEL, S.; A. LEANDROS, F. TILLEQUIN & M. KOCH: Alkaloids from leaves of *Strychnos dinklagei*. J. Nat. Prod. 48: 86~92, 1985
5) SEVENET, T.; A. HUSSON & H.-P. HUSSON: Alkaloids artefact of *Lasianthera austrocaledonica*. Phytochemistry 15: 576~577, 1976