NEW STREPTOTHRICIN-GROUP ANTIBIOTICS, AN-201 I, II AND III

II. CHEMICAL STRUCTURES

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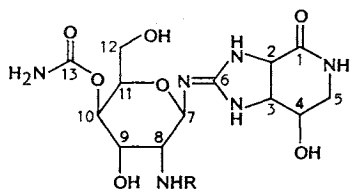
The new, belonging to the streptothricin-group antibiotics AN-201 I, II and III were found to be produced by a soil actinomycete identified as *Streptomyces nojiriensis* C-13. The chemical structures and the physical and spectroscopic properties of these compounds are reported here. On the basis of NMR and fast atom bombardment mass spectrometry (FAB-MS) spectra the antibiotics were identified as N^{β}-acetylated derivatives of streptothricins E, D and F.

In the course of our screening study for antitumor antibiotics, new streptothricin antibiotics, named AN-201 I, II and III, were isolated from the culture broth of *Streptomyces nojiriensis* C-13. Our previous paper[1] was concerned with their production by fermentation, isolation and biological activities. They were effective against both Gram-positive and Gram-negative bacteria, and showed *in vitro* and *in vivo* antitumor activity. The structures of AN-201 I, II and III are determined as the N^{β}-acetylated derivatives of streptothricins E, D and F (Fig. 1). This report describes the structure elucidation by application of NMR and fast atom bombardment mass spectrometry (FAB-MS).

Fig. 1. Structures of AN-201.



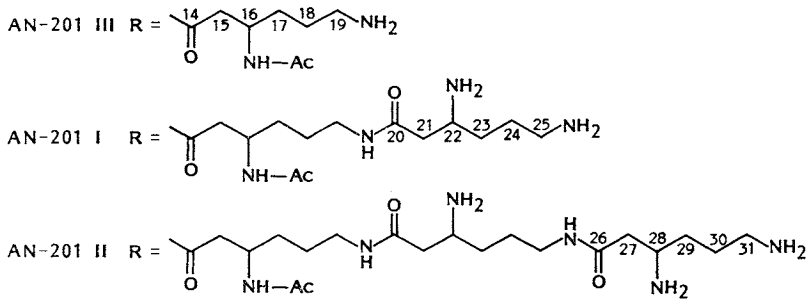


Table 1. ^{1}H NMR parameters for AN-201 components and streptothricins in D_2O.

Position	Streptothricin F		Streptothricin E		Streptothricin D		AN-201 III		AN-201 I		AN-201 II	
	δ (ppm)	J (Hz)	δ (ppm)	J (Hz)	δ (ppm)	J (Hz)	δ (ppm)	J (Hz)	δ (ppm)	J (Hz)	δ (ppm)	J (Hz)
2	4.63 (d)	14	4.63 (d)	15	4.63 (d)	14	4.64 (d)	15	4.64 (d)	15	4.65 (d)	14
3	4.09 (d)	14	4.08 (d)	15	4.08 (d)	14	4.24 (m)	—	4.15 (m)	—	4.15 (m)	—
4	4.73 (m)	—	4.73 (m)	—	4.73 (m)	—	4.71 (m)	—	4.73 (m)	—	4.72 (m)	—
5	3.80 (dd)	6, 15	3.80 (dd)	6, 15	3.80 (dd)	6, 15	3.80 (dd)	5, 15	3.81 (dd)	6, 15	3.80 (dd)	6, 15
5	3.40 (d)	15	3.40 (d)	15	3.40 (d)	15	3.40 (d)	15	3.40 (d)	15	3.39 (d)	15
7	5.10 (d)	10	5.10 (d)	10	5.10 (d)	10	5.07 (d)	10	5.09 (d)	10	5.08 (d)	10
8	4.27 (dd)	3, 10	4.26 (dd)	3, 10	4.26 (dd)	3, 10	4.24 (m)	—	4.21 (dd)	3, 10	4.21 (dd)	3, 10
9	4.17 (t)	3	4.16 (t)	3	4.16 (t)	3	4.06 (t)	3	4.07 (t)	3	4.06 (t)	3
10	4.77 (d)	3	4.77 (d)	3	4.77 (d)	3	4.75 (d)	3	4.79 (d)	4	4.76 (d)	4
11	4.33 (t)	6	4.33 (t)	6	4.33 (t)	6	4.32 (t)	6	4.33 (t)	6	4.33 (t)	6
12	3.73 (d)	6	3.73 (d)	6	3.73 (d)	6	3.72 (d)	5	3.71 (d)	5	3.72 (d)	5
12	3.73 (d)	6	3.73 (d)	6	3.73 (d)	6	3.72 (d)	7	3.71 (d)	7	3.72 (d)	7
15	2.81 (dd)	4, 16	2.80 (dd)	5, 16	2.79 (dd)	5, 17	2.54 (dd)	4, 14	2.53 (dd)	5, 14	2.54 (dd)	4, 14
15	2.69 (dd)	8, 16	2.70 (dd)	8, 16	2.69 (dd)	8, 17	2.41 (dd)	9, 14	2.40 (dd)	10, 14	2.42 (dd)	9, 14
16	3.70 (m)	—	3.68 (q)	5	3.66 (m)	—	4.24 (m)	—	4.15 (m)	—	4.15 (m)	—
17	1.79 (m)	—	1.73 (m)	—	1.72 (m)	—	1.68 (m)	—	1.54 (m)	—	1.56 (m)	—
18	1.79 (m)	—	1.66 (m)	—	1.65 (m)	—	1.68 (m)	—	1.54 (m)	—	1.56 (m)	—
19	3.05 (t)	5	3.25 (t)	6	3.26 (t)	7	3.00 (m)	—	3.20 (t)	5	3.21 (t)	5
21			2.76 (dd)	5, 16	2.75 (dd)	5, 17			2.72 (dd)	5, 16	2.75 (dd)	5, 16
21			2.65 (dd)	8, 16	2.64 (dd)	8, 17			2.62 (dd)	8, 16	2.65 (dd)	8, 16
22			3.68 (q)	5	3.66 (m)	—			3.67 (m)	—	3.67 (m)	—
23			1.81 (m)	—	1.72 (m)	—			1.79 (m)	—	1.72 (m)	—
24			1.81 (m)	—	1.65 (m)	—			1.79 (m)	—	1.65 (m)	—
25			3.07 (t)	5	3.26 (t)	7			3.06 (t)	6	3.26 (t)	7
27					2.71 (dd)	5, 16					2.70 (dd)	5, 16
27					2.62 (dd)	9, 16					2.61 (dd)	7, 16
28					3.66 (m)	—					3.67 (m)	—
29					1.81 (m)	—					1.81 (m)	—
30					1.81 (m)	—					1.81 (m)	—
31					3.06 (t)	5					3.07 (t)	6
Acetyl							2.00 (s)		2.00 (s)		2.01 (s)	

—: The J values were not identified for their multiple coupling.

Results and Discussion

The antibiotics, AN-201 I, II and III, were obtained as a white powder, and were soluble in water, methanol and ethanol, but insoluble in 1-butanol, acetone and chloroform. These compounds are positive to ninhydrin reaction. On acid hydrolysis with 6 N HCl at 110°C for 20 hours, AN-201 I, II and III gave β-lysine and other two ninhydrin positive compounds by amino acid analysis. The ^{1}H and ^{13}C NMR spectra of AN-201 I, II and III (Tables 1 and 2) were similar to that of streptothricin F, which is a well-characterized antibiotic containing a β-lysine. Most of its structural features have been known for many years[2,3], and the last structural detail involving the location of the carbamate group on the carbon-10 hydroxyl has been defined[4,5].

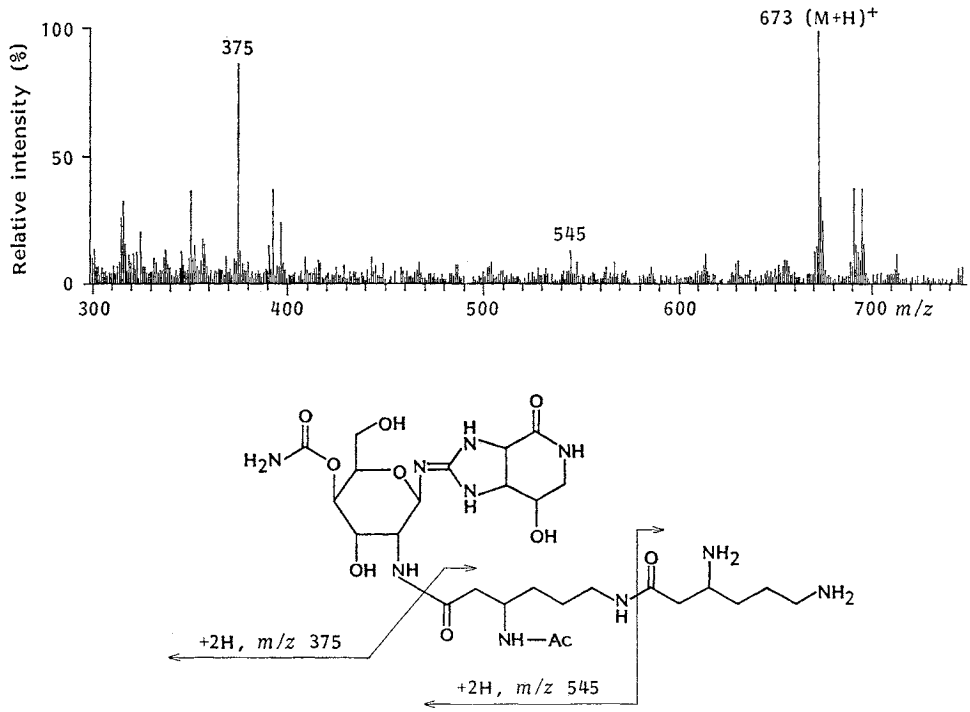
The ^{1}H NMR spectra of AN-201 I, II and III were assigned by the homo-decoupling method and by comparison with the ^{1}H-spectra of streptothricin F[4]. The ^{1}H NMR parameters of these antibiotics are listed in Table 1, where they are compared with those of streptothricins F, E and D. The close similarities of chemical shifts and coupling constants for the compounds listed in Table 1 indicate that

Table 2. Comparisons of ^{13}C NMR chemical shifts (ppm) for AN-201 components and streptothricins.

Position	Strepto-thricin F	AN-201 III	$\Delta\delta$*	Strepto-thricin E	AN-201 I	$\Delta\delta$*	Strepto-thricin D	AN-201 II	$\Delta\delta$*
1	172.5	172.5	0.0	172.5	172.5	0.0	172.5	172.5	0.0
2	57.0	57.0	0.0	57.0	57.0	0.0	57.0	57.0	0.0
3	63.5	63.5	0.0	63.5	63.5	0.0	63.5	63.5	0.0
4	63.5	63.5	0.0	63.5	63.5	0.0	63.5	63.5	0.0
5	51.9	51.8	0.1	51.9	51.8	0.1	51.9	51.8	0.1
6	160.5	160.4	0.1	160.5	160.5	0.0	160.5	160.4	0.1
7	81.4	81.4	0.0	81.5	81.5	0.0	81.5	81.5	0.0
8	51.5	51.5	0.0	51.5	51.4	0.1	51.5	51.4	0.1
9	69.1	69.4	−0.3	69.1	69.4	−0.3	69.1	69.4	−0.3
10	72.6	72.6	0.0	72.6	72.6	0.0	72.6	72.6	0.0
11	76.1	76.1	0.0	76.1	76.1	0.0	76.1	76.1	0.0
12	62.9	62.9	0.0	62.9	62.9	0.0	62.9	62.9	0.0
13	165.3	165.2	0.1	165.3	165.2	0.1	165.3	165.2	0.1
14	174.6	176.2	−1.6	174.8	176.1	−1.3	174.8	176.1	−1.3
15	38.8	43.8	−5.0	39.2	43.9	−4.7	39.2	43.9	−4.7
16	50.9	49.5	1.4	51.1	49.8	1.3	51.1	49.7	1.4
17	31.7	33.7	−2.0	32.1	34.1	−2.0	32.1	34.1	−2.0
18	25.5	26.1	−0.6	26.9	27.4	−0.5	26.9	27.5	−0.6
19	41.6	41.8	−0.2	41.4	41.6	−0.2	41.4	41.6	−0.2
20				174.3	174.1	0.2	174.4	174.2	0.2
21				39.2	39.2	0.0	39.2	39.2	0.0
22				51.1	51.1	0.0	51.1	51.1	0.0
23				31.7	31.7	0.0	32.1	32.0	0.1
24				25.6	25.5	0.1	26.9	26.9	0.0
25				41.6	41.6	0.0	41.4	41.4	0.0
26							174.2	174.2	0.0
27							39.4	39.5	−0.1
28							51.3	51.4	−0.1
29							31.7	31.7	0.0
30							25.5	25.5	0.0
31							41.5	41.6	−0.1
C=O (Ac)		176.2			176.4			176.4	
CH_3 (Ac)		24.7			24.7			24.7	

* $\Delta\delta=\delta$ (Streptothricins)$-\delta$ (AN-201 components).

Fig. 2. FAB-MS of AN-201 I and its fragmentation.



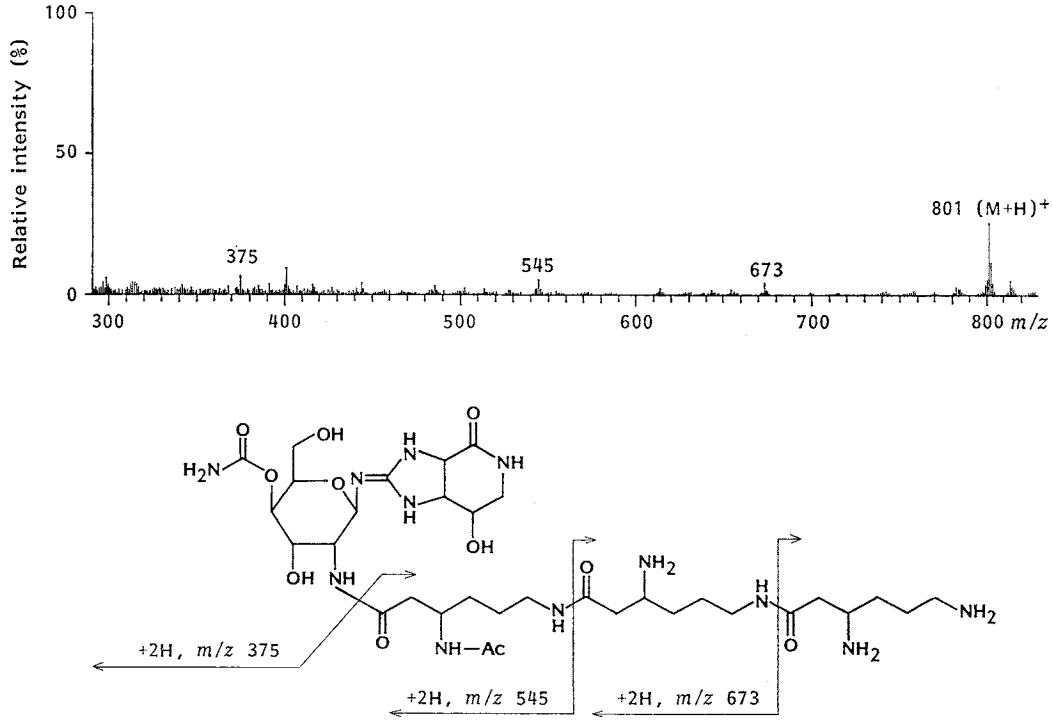
in AN-201 I, II and III the carbons of the streptolidine and gulosamine moieties have the same relative configurations as do the corresponding sites in the other three streptothricins. AN-201 I, II and III contain all the resonances appropriate for streptothricins E, D and F, respectively, plus triply-intense singlet peaks with a chemical shift suggestive of an acetyl group (δ 2.00).

The FAB-MS data indicate that AN-201 I (m/z 673 $(M+H)^+$), II (m/z 801 $(M+H)^+$) and III (m/z 545 $(M+H)^+$) differ in mass from streptothricins E (m/z 631 $(M+H)^+$), D (m/z 759 $(M+H)^+$) and F (m/z 503 $(M+H)^+$) by the equivalent of one acetyl group, respectively.

The ^{13}C NMR spectra of AN-201 I, II and III have been assigned unambiguously by selective decoupling most of the resonances in the AN-201 I, II and III proton resonances, respectively. These assignments are listed in Table 2, where they are compared with the ^{13}C chemical shifts for the related streptothricins. The ^{13}C NMR spectra of the gulosamine and streptolidine portions of AN-201 are virtually identical[6,7]. The additional peak (δ 24.7) among the resonances which are similar to those in the ^{13}C spectra of streptothricins is a quartet in the off-resonance spectra, confirming the suggestion of an acetyl group. The chemical shift changes listed in Table 2 indicate that AN-201 I, II and III have the structures shown in Fig. 1, where the new acetyl group exists on the β-amino group of the β-lysine residue.

AN-201 I and II have two and three β-lysine residues, respectively. For determining the position of the acetyl group, the FAB-MS of AN-201 I and II (Figs. 2 and 3) were compared with the spectra for the related streptothricins. The fragment ion peaks (m/z 545) for AN-201 I and II have an acetyl group in addition to the fragment (m/z 503) for streptothricins E and D. The m/z 375 ion peaks for AN-201 I and II are deduced from the partial structure of the streptolidyl-gulosamine moiety. These frag-

Fig. 3. FAB-MS of AN-201 II and its fragmentation.



mentations confirm that the additional acetyl groups of AN-201 I, II and III are combined with the β-amino groups of the β-lysine, which is directly connected to gulosamine. Thus, the structures of AN-201 I, II and III are proposed as shown in Fig. 1.

In comparison with known streptothricin antibiotics, AN-201 I and II are new members of streptothricin group of antibiotics. AN-201 III has been prepared by selective acetylation of streptothricin F[8,9]. However, this is the first case of this compound being a microbial metabolite. The structural-bioactivity relationship of these antibiotics are described in a previous paper[1].

Experimental

Materials

Antibiotics AN-201 I, II, III and streptothricins F, E, D were obtained in quantities of 220 mg, 400 mg, 200 mg, 400 mg, 420 mg and 140 mg, respectively, by the procedure described in our previous report[1].

Spectral Data

The ^{1}H and ^{13}C NMR spectra were recorded in D_2O solutions with a Jeol JNM-FX-400 spectrometer at 400 MHz (^{1}H) and a Jeol JNM-FX-100 spectrometer at 25 MHz (^{13}C), respectively. Chemical shifts are expressed in δ values (ppm) with TMS as an external reference and coupling constants are given in Hz (J). The ^{1}H NMR spectra were fully homo-decoupled. The ^{13}C chemical shift assignments were made by selective decoupling and by the off-resonance decoupling method. FAB-MS were recorded with a Jeol JMS-DX-300 apparatus.

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