A35512, A COMPLEX OF NEW ANTIBACTERIAL ANTIBIOTICS PRODUCED BY *STREPTOMYCES CANDIDUS*

I. ISOLATION AND CHARACTERIZATION

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The new antibiotic complex A35512 produced by *Streptomyces candidus* was isolated from the filtered fermentation broth. The individual factors A, B, C, E, and H were separated and purified by column chromatography. A35512B, the major factor, was isolated as the dihydrochloride salt, a white crystalline compound with an approximate empirical formula of $C_{90}H_{101}N_8O_{39}Cl \cdot 2HCl$. The A35512 antibiotics belong to the glycopeptide class of antibiotics and possess high *in vitro* and *in vivo* activity against Gram-positive bacteria.

A new complex of antibacterial antibiotics was detected in the fermentation broth of *Streptomyces candidus* NRRL 8156[1]. The complex was isolated from the fermentation broth by XAD-4 chromatography, and the individual factors A35512A, B, C, E, and H were separated and purified by polyamide and alumina column chromatography[2]. These factors are water-soluble compounds and fall in the general class of glycopeptide antibiotics along with vancomycin, and ristocetin. They possess high *in vitro* and *in vivo* activity against a broad spectrum of pathogenic Gram-positive aerobic and anaerobic bacteria. The A35512 complex effectively promotes growth and increases feed utilization efficiency in animals[3]. A35512B is inhibitory *in vitro* against *Streptococcus aureus* and *Streptococcus faecalis* strains at 4 mcg/ml or less and against *Streptococcus pyogenes* and *Streptococcus pneumoniae* at 2 mcg/ml or less. A variety of species of Gram-positive anaerobic bacteria were inhibited by 4 mcg/ml or less. Subcutaneous doses of 5 mg/kg or less were effective in protecting mice from experimentally induced *Staphylococcus aureus, Streptococcus pyogenes*, or *Streptococcus pneumoniae* infections[4]. In this communication, the isolation and characterization of the complex and the individual factors are reported.

Isolation and Chemical Characterization

Streptomyces candidus was grown under submerged aerobic fermentation conditions[1] and the majority of the antibiotic produced was present in the broth. The procedure for the isolation of the individual factors is shown schematically in Fig. 1 and details are reported in the experimental section.

The five factors A, B, C, E, and H have similar physico-chemical properties. The weakly basic compounds were isolated from the fermentation broth as their mono- or dihydrochloride

Table 1. Yield of each antibiotic factor in percentage of total activity in fermentation broth.

A35512 factors	Approximate % of activity of complex
A35512A·2HCl	10
A35512B·2HCl	80
A35512C·2HCl	2
A35512E·HCl	2
A35512H·HCl	5
A35512F, G	(1)

Note: Factors F and G not isolated, yields are estimated.

Fig. 1. Isolation of A35512 factors A, B, C, E, and H.

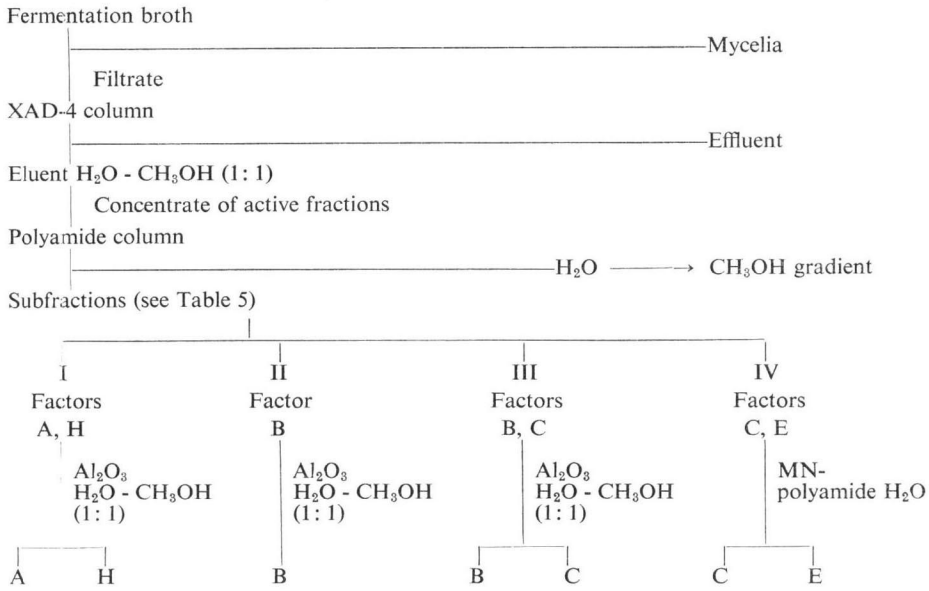


Table 2. Approximate Rf values of the A35512 factors separated by paper chromatography.

A35512 factors	Rf value's
A35512A·2HCl	0.21
A35512B·2HCl	0.34
A35512C·2HCl	0.46
A35512E·HCl	0.64
A35512H·HCl	0.15
A35512F	0.81
A35512G	0.93

Solvent: *n*-butanol - pyridine - acetic acid - water (15 : 10 : 3 : 12).
Sorbent: Whatman Paper No. 1.
Test organism: *Sarcina lutea*.
Development: 20 hours.
Note: Factors F and G were detected by bioautography only.

Table 3. Relative retention times for A35512 antibiotic factors A, B, C, E, and H in high performance liquid chromatography.

A35512 factors	Retention time (seconds)
A	840
B	972
C	984
E	1,230
H	865

Column: Waters semiprep 8 mm × 300 mm silica C-18 (10 micron) Bondapak.
Solvent: (A) 0.1 M ammonium formate.
(B) acetonitrile.
Gradient from 5 % B to 20 % B in A.
Samples: A35512 antibiotic factors A, B, C, E, and H; 20 mcg each.
Flow: 3.3 ml/min.
Detect: at 254 nm.

salt (see Table 1). Analytically, they were separated by paper chromatography (see Table 2) and detected by bioautography, using *Sarcina lutea* as the assay microorganism.

The factors can also be separated by high performance liquid chromatography (HPLC) using reversed phase ODS (octadecyl silane) silica gel as the stationary phase. Retention time and operating conditions are given in Table 3. Known physico-chemical properties of these antibiotic factors are summarized in Table 4. A more detailed description of each compound is found in the experimental section. The infrared spectra of all factors are similar. The spectra of factor B, the major antibiotic, is shown in Fig. 2, while the fingerprint sections of the other factors are shown in Fig. 3.

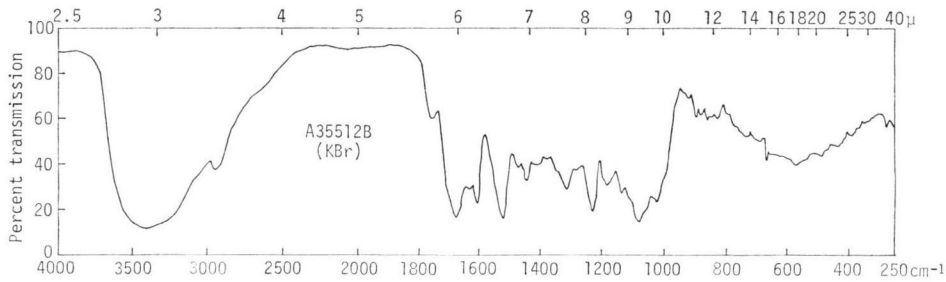
Table 4. Physico-chemical properties of A35512A, B, C, E, and H.

Factor	Empirical formula	MW		$[\alpha]_D^{25}$ (H_2O)	pKa[2]
A	unknown (XCl)·2HCl	2,106	amorphous	−100°	7.35 9.09 10.49 12.44
B	$C_{90}H_{101}N_9O_{39}Cl \cdot 2HCl$	2,027	crystalline[1]	−123°	7.15 8.87 10.30 12.10
C	$C_{83\sim85}H_{95\sim99}N_9O_{37\sim39}Cl \cdot 2HCl$	*ca.* 1,900	amorphous	−161°	7.30 8.92 10.99 >11.50
E	unknown (XCl)·HCl	*ca.* 2,000	amorphous	−108°	6.30 9.09 11.62 >12.50
H	$C_{85\sim87}H_{103\sim107}N_8O_{38\sim40}Cl \cdot HCl$	*ca.* 1,900	amorphous	−123°	5.25 7.60 10.10 12.40 13.02

[1] Analysis and drying experiments indicate the presence of crystal water: $C_{90}H_{101}N_9O_{39}Cl \cdot 2HCl \cdot 3H_2O$.

[2] in 66 % aqueous DMF.

Fig. 2.

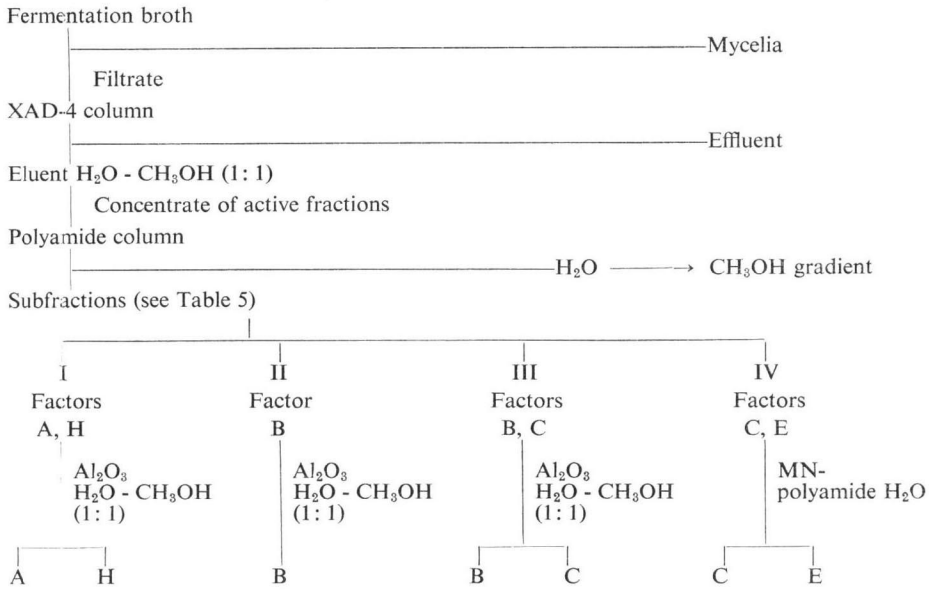


Experimental

Isolation of the A35512 Antibiotic Complex

The fermentation broth (900 liters) was filtered at broth pH (6.9), using a filter aid (Hyflo Super-cel, Johns-Manville Products Corp.). The clear filtrate was passed through a column containing 10 ml of polymeric adsorbent (Amberlite XAD-4, Rohm and Haas, Co.) per 100 ml broth filtrate at a rate of 150 ml/min. Fractions were monitored for biological activity using a standard disc assay against *Sarcina lutea*. The column was washed with water (1/8 of the broth volume) at a rate of 150 ml/min. The filtrate and inactive water wash were discarded.

Fig. 1. Isolation of A35512 factors A, B, C, E, and H.



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A35512C·2HCl	0.46
A35512E·HCl	0.64
A35512H·HCl	0.15
A35512F	0.81
A35512G	0.93

Solvent: *n*-butanol - pyridine - acetic acid - water (15 : 10 : 3 : 12).
Sorbent: Whatman Paper No. 1.
Test organism: *Sarcina lutea*.
Development: 20 hours.
Note: Factors F and G were detected by bioautography only.

Table 3. Relative retention times for A35512 antibiotic factors A, B, C, E, and H in high performance liquid chromatography.

A35512 factors	Retention time (seconds)
A	840
B	972
C	984
E	1,230
H	865

Column: Waters semiprep 8 mm × 300 mm silica C-18 (10 micron) Bondapak.
Solvent: (A) 0.1 M ammonium formate.
(B) acetonitrile.
Gradient from 5 % B to 20 % B in A.
Samples: A35512 antibiotic factors A, B, C, E, and H; 20 mcg each.
Flow: 3.3 ml/min.
Detect: at 254 nm.

salt (see Table 1). Analytically, they were separated by paper chromatography (see Table 2) and detected by bioautography, using *Sarcina lutea* as the assay microorganism.

The factors can also be separated by high performance liquid chromatography (HPLC) using reversed phase ODS (octadecyl silane) silica gel as the stationary phase. Retention time and operating conditions are given in Table 3. Known physico-chemical properties of these antibiotic factors are summarized in Table 4. A more detailed description of each compound is found in the experimental section. The infrared spectra of all factors are similar. The spectra of factor B, the major antibiotic, is shown in Fig. 2, while the fingerprint sections of the other factors are shown in Fig. 3.

Table 6. ^{13}CMR spectrum of A35512A dihydrochloride in D_2O.

No.	PPM	Height (%)	No.	PPM	Height (%)
3	175.3	0.8	28	107.4	1.6
4	173.0	2.1	29	101.7	0.9
5	172.1	2.0	30	77.6	3.8
6	171.4	1.5	31	76.3	4.6
7	170.9	2.7	32	75.5	2.6
8	170.5	2.3	33	74.8	2.5
9	169.5	1.6	34	74.5	2.4
10	159.0	1.3	35	73.4	3.7
11	157.9	2.3	36	72.8	6.0
12	156.2	2.6	37	72.0	4.4
13	155.6	2.3	38	70.9	7.0
14	155.3	2.4	39	69.6	2.8
15	154.4	1.1	40	67.4	90.9*
16	136.3	1.4	41	65.4	1.7
17	136.0	1.0	42	61.7	3.1
18	135.1	1.4	43	56.7	1.7
19	133.5	1.2	44	55.5	1.3
20	129.6	1.6	45	54.7	0.8
21	129.1	1.7	46	24.6	0.9
22	128.7	1.8	47	19.1	1.7
23	127.5	1.0	48	17.9	2.0
24	126.0	1.4	49	17.2	1.9
25	124.3	2.8	50	16.7	3.2
26	122.1	1.6	51	16.2	3.0
27	109.9	1.3			

* dioxane standard.

give 0.3 g of A35512 factor A dihydrochloride.

Antibiotic A35512A dihydrochloride is a white amorphous compound. It is soluble in water and dimethyl sulfoxide, partially soluble in lower alcohols, and insoluble in other less polar solvents. The molecular weight as determined by titration is 2,106; λ_{max} (CH_3OH) 282 nm, acidic and neutral pH (ε 11,700) and λ_{max} 292 nm, basic pH (ε 14,000) calculated using a molecular weight of 2,000, end-adsorption at 225 nm; for IR spectrum (KBr) see Fig. 3.

Anal. Found: C, 51.03; H, 5.10; N, 4.75; O, 34.20; Cl, 4.80;

A ^{13}CMR spectrum in D_2O has the characteristics listed in Table 6.

An acid hydrolyzed sample (6 N HCl, 21 hours, reflux) analyzed by a standard procedure for analysis of amino acid mixtures, indicated the presence of four amino acid residues one of which appears to be glycine. The acid hydrolyzed product also contained carbohydrate components which have not yet been identified.

Ionic-Chlorine-Free A35512A

A35512 factor A dihydrochloride (200 mg) was dissolved in water (10 ml), and passed through a 1.5 $\times$10 cm ion-exchange column (Bio-Rad AG3-4X, in the OH^- cycle) at a flow rate of 0.5 ml/min with deionized water. The initial eluate (10 ml) was discarded. The following eluate (20 ml) was concentrated and lyophilized to give 115 mg of ionic-chlorine-free A35512 factor A.

Anal. Found: C, 54.29; H, 5.19; N, 5.58; O, 33.76; Cl, 1.69.

Purification of A35512B Dihydrochloride

Partially purified A35512B dihydrochloride (400 g) was dissolved in 1.2 liter of 50 aqueous methanol

Table 7. ^{13}CMR spectrum of A35512B dihydrochloride in D_2O.

No.	PPM	Height (%)	No.	PPM	Height (%)
2	173.0	4.1	32	120.7	3.3
3	171.9	3.7	33	116.5	2.7
4	171.6	3.3	34	109.5	0.8
5	171.0	5.8	35	108.2	1.1
6	170.8	5.0	36	107.7	2.7
7	169.6	3.6	37	104.5	1.7
8	159.0	4.1	38	101.8	2.9
9	157.9	4.4	39	100.9	1.6
10	157.5	3.7	40	98.2	1.0
11	156.6	4.8	41	76.9	1.2
12	155.6	6.1	42	76.1	1.8
13	155.3	4.2	43	74.1	2.0
14	154.9	3.3	44	73.5	2.7
15	154.3	4.2	45	72.7	2.4
16	151.7	3.3	46	72.3	4.0
17	144.3	3.1	47	71.0	7.1
18	136.7	3.5	48	70.3	2.5
19	136.2	4.9	49	69.7	2.5
20	135.4	4.0	50	67.4	74.7*
21	135.2	4.4	51	64.6	1.2
22	133.6	4.2	52	62.0	1.5
23	133.3	4.1	53	58.0	1.3
24	129.8	1.7	54	56.8	1.7
25	129.3	3.0	55	55.4	3.9
26	128.8	2.6	56	54.3	2.5
27	127.6	1.5	57	24.5	2.0
28	126.1	3.9	58	17.9	3.0
29	124.2	5.6	59	17.2	2.0
30	122.4	1.4	60	16.3	2.5
31	122.0	4.4			

* dioxane standard.

and chromatographed on a glass column having a diameter of 13.5 cm. The column was packed with acidic aluminum oxide (10 kg, M. Woelm) and washed with 50% aqueous methanol until a clear effluent was obtained.

The solute was loaded onto the column and the column eluted with 50 aqueous methanol at a flow rate of 8~10 ml/min. The fractions (240~300 ml each) were monitored by thin-layer bioautography as described above, combined, concentrated under reduced pressure and lyophilized to yield the following purified A35512B dihydrochloride preparations.

Fractions	17~21	22~29	30~37
Weight	9.6 g	72.0 g	117.0 g

A35512B dihydrochloride precipitated in crystalline form from super-saturated solutions prepared from these fractions in 50% aqueous methanol when left at 4°C for several hours.

Antibiotic A35512B dihydrochloride is a white crystalline compound. It is soluble in water and dimethyl sulfoxide, partially soluble in lower alcohols, and insoluble in other less polar solvents. Although A35512B dihydrochloride is hygroscopic and does not exhibit a distinct melting point, a thermogram showed weight loss beginning at 25°C, resulting in a 7.4% loss at 121°C; at 135°C another loss

occurred, resulting in decomposition. The molecular weight as determined by plasma desorption mass spectrometry is 1,954±2; λ_{max} (CH_3OH) 282 nm, acidic and neutral pH (ε 12,000) and λ_{max} 292 nm, pH 11~12 (ε 14,000) calculated using a molecular weight of 2,000, end absorption at 225 nm; for IR spectrum (KBr) see Fig. 2.

Anal. Calcd for $C_{90}H_{101}N_3O_{39}Cl \cdot 2HCl \cdot 3H_2O$: C, 51.93; H, 5.28; N, 5.38; O, 32.29; Cl, 5.11.
Found: C, 52.39; H, 5.38; N, 5.28; O, 32.28; Cl, 5.07.

A ^{13}CMR spectrum in D_2O has the characteristics shown in Table 7.

A35512 factor B dihydrochloride, crystallized from methanol - water, has the following characteristic X-ray powder diffraction pattern (Cu^{++} radiation, 1.5405 λ, nickel filter, d=interplanar spacing in Angstroms) (Table 8).

Table 8. X-Ray powder diffraction pattern of A35512B dihydrochloride.

d	Relative intensity
17.15	100
12.90	80
10.85	70
9.25	70
8.87	60
8.22	50
7.86	50
6.93	40
6.20	40
5.62	40
5.04	05
4.02	02
3.54	02

An acid hydrolyzed sample (6 N HCl, 21 hours reflux) analyzed by a standard procedure for analysis of amino acid mixtures, indicated the presence of four amino acid residues one of which appears to be glycine. The acid-hydrolyzed product also contained carbohydrates. Their identification is reported by DEBONO *et al.*[5]. This antibiotic has at least one hydroxyl group which can be esterified with acetic anhydride in pyridine at room temperature.

Ionic-Chlorine-Free A35512B

Purified A35512B dihydrochloride (1 g) was chromatographed over weakly basic ion exchange resin, using the procedure described above to give 0.76 g of ionic-chlorine-free A35512B as a white powder which contained 1.59% chlorine.

A35512B is a white, amorphous, basic compound, soluble in water and aqueous methanol mixtures; a 5% solution in 66% aqueous DMF has a pH of 9.13; $[\alpha]^{25}$ −123° (*c* 1.0, H_2O), $[\alpha]_{365}$ −446° (*c* 1.0, H_2O); pKa's 7.15, 8.81, 10.20, 12.00 and the possible presence of another group with a pKa value greater than 13.5; λ_{max} (CH_3OH) 282 nm, acidic and neutral pH (ε 15,000) and λ_{max} (CH_3OH) 292 nm, basic pH (ε 16,000), calculated using a molecular weight of 2,000; the IR spectrum (KBr) is essentially identical with the spectrum of A35512B dihydrochloride.

Anal. Calcd. for $C_{90}H_{101}N_3O_{39}Cl$ (MW 1,954): C, 55.31; H, 5.21; N, 5.73; O, 31.93; Cl, 1.82.
Found: C, 54.22; H, 5.15; N, 5.50; O, 31.59; Cl, 1.74.

The ^{13}CMR spectrum in D_2O was essentially identical with the spectrum of A35512B dihydrochloride; an acid hydrolyzed sample showed the same amino acid and sugar constituents as described under A35512B dihydrochloride.

Purification of A35512C Dihydrochloride

Partially purified A35512 factor C dihydrochloride (15 g) from subfraction III, Table 5, was dissolved in deionized water (40 ml). This solution was applied to a 4×115 cm polyamide column (MN, 0.07 nm, Brinkman Instruments, Inc.); packed in water and washed overnight with water. The column was eluted with deionized water at a flow rate of about 3 ml/min. The first effluent (250 ml) was discarded; thereafter, 24-ml fractions were collected.

The fractions were monitored by thin-layer bioautography. Cellulose tlc plates (on aluminum sheets; E. Merck, W. Germany), *sec*-butanol - pyridine - acetic acid - water (10: 10: 3: 8) as a solvent system, and *Bacillus subtilis* as a detection organism were used. Fractions 1 through 33 were combined, concentrated under vacuum to a volume of 150 ml and lyophilized.

Two more columns were carried out, using the same conditions. These three lyophilized samples were combined to give 12.3 g of partially purified A35512C dihydrochloride.

This preparation was then further purified by chromatography over another polyamide column as described above, at a flow rate of 1 ml/min and 15-ml fractions were collected. The column was again

Table 9. ^{13}CMR spectrum of A35512C dihydrochloride in D_2O.

No.	PPM	Height (%)	No.	PPM	Height (%)
1	172.9	2.5	23	118.0	1.7
2	172.2	2.0	24	116.5	1.2
3	171.5	2.2	25	107.8	2.7
4	171.0	3.9	26	104.5	1.9
5	169.6	2.0	27	101.7	1.9
6	158.6	1.8	28	94.5	1.0
7	157.8	3.0	29	75.9	3.0
8	156.5	2.1	30	74.3	2.0
9	156.1	2.8	31	73.4	2.3
10	155.6	4.2	32	72.1	3.5
11	154.6	3.9	33	70.9	4.3
12	151.1	1.5	34	68.7	2.9
13	143.3	1.4	35	67.4	71.7*
14	136.0	3.2	36	64.3	1.3
15	135.4	2.7	37	62.2	1.6
16	133.2	3.7	38	56.1	1.4
17	128.7	2.3	39	55.2	3.5
18	126.5	3.1	40	54.2	2.1
19	124.6	2.1	41	24.3	1.9
20	129.3	2.7	42	17.9	2.2
21	121.5	2.2	43	17.1	2.0
22	120.1	1.2	44	16.2	2.0

* dioxane standard.

monitored by tlc bioautography and fractions 36 through 58 were combined, concentrated under vacuum to a volume of 150 ml and lyophilized to give 5.3 g of A35512C dihydrochloride.

The final purification of A35512C dihydrochloride was carried out by chromatography on a 5× 41 cm acidic aluminum oxide (M. Woelm) column. The A35512C sample (5.3 g dissolved in 30 ml of 50% aqueous methanol) was applied to the column, and eluted with 50% aqueous methanol at a flow rate of 1 ml/min. The fractions (12 ml) were monitored by tlc bioautography. Fractions 22 through 74 were combined, concentrated under vacuum to a volume of 250 ml and lyophilized to give 3.86 g of purified A35512C dihydrochloride.

Antibiotic A35512C dihydrochloride is a white, amorphous compound. It is soluble in water and DMSO, partially soluble in alcohols, and insoluble in other less polar solvents. The molecular weight as determined by titration is 1,982; λ_{max} (CH_3OH) 282 nm, acidic and neutral pH (ε 14,600) and λ_{max} 292 nm, basic pH (ε 16,400) calculated using a molecular weight of 2,000, end absorption at 225 nm; for IR spectrum (KBr) see Fig. 3.

Anal. Calcd. for $C_{83\sim85}H_{95\sim99}N_8O_{37\sim39}Cl \cdot 2HCl$: C, 52.2; H, 5.1; N, 5.8; O, 31.5; Cl, 5.4.
Found: C, 51.76; H, 5.07; N, 5.61; O, 31.29; Cl, 4.88.

A ^{13}CMR spectrum in D_2O has the characteristics listed in Table 9.

An acid hydrolyzed sample (6 N HCl, 21 hours, reflux) indicated at least four amino acid residues, one of which appears to be glycine; the acid hydrolyzed product also contained carbohydrate components which have not yet been identified.

Ionic-Chlorine-Free A35512C

A35512C dihydrochloride (200 mg) was chromatographed over weakly basic ion exchange resin, using the procedure described above to give 156 mg of ionic-chlorine-free A35512C. This material contained 1.90% chlorine.

Anal. Calcd. for $C_{83\sim85}H_{95\sim99}N_8O_{37\sim39}Cl$: C, 54.13; H, 5.35; N, 6.01; O, 32.61; Cl, 1.90.
Found: C, 53.93; H, 5.15; N, 5.80; O, 32.35; Cl, 1.90.

Purification of A35512E Hydrochloride

Partially purified A35512E hydrochloride (8.1 g) was dissolved in deionized water (40 ml) and applied to a 5×110 cm polyamide column (MN, <0.07 nm, Brinkman Instruments, Inc.), prepared in and washed with water overnight. The column was eluted with deionized water at a flow rate of 20 ml/15 min, collecting 20 ml fractions. At fraction 118, the eluting solvent was changed to 50% aqueous methanol.

Fractions 148 through 195 contained A35512E as determined by bioautography. These fractions were combined, concentrated under reduced pressure to a volume of 150 ml, and lyophilized to give 2.7 g of A35512E hydrochloride.

A portion of this partially purified A35512E hydrochloride (615 mg) was dissolved in 50% aqueous methanol (5 ml) and applied to a 1.5×50 cm acidic aluminum oxide (M. Woelm) column. The column was eluted with 50% aqueous methanol at a flow rate of 1 ml/min., and 10 ml fractions were collected. The fractions were monitored by bioautography. Fractions 5~8 were combined, concentrated to a volume of about 10 ml, added to 50 ml of water, and lyophilized to give 480 mg of A35512E hydrochloride.

Antibiotic A35512E hydrochloride is a white amorphous compound. It is soluble in water and DMSO, partially soluble in alcohols, and insoluble in other less polar solvents. The molecular weight as determined by titration is 2,018; λ_{max} (CH_3OH) neutral 270 nm (sh) and 359 nm (ε 16,200); λ_{max} (CH_3OH), acidic, 286 nm (ε 18,000) and 310 nm (sh); λ_{max} (CH_3OH), basic 270 nm (sh), 300 nm (ε 16,200) and 354 nm (ε 17,600) calculated using a molecular weight of 2,000, end absorption at 225 nm; for IR spectrum (KBr) see Fig. 3.

Anal. Found: C, 52.67; H, 4.59; N, 5.55; O, 33.51; Cl, 3.62.

An acid hydrolyzed sample (6 N HCl, 21 hours, reflux) indicated six as yet unidentified amino acid residues, the acid hydrolyzed product also contained carbohydrate components which have not yet been identified.

Ionic-Chlorine-Free A35512E

A35512E hydrochloride (200 mg) was chromatographed over weakly basic ion-exchange resin, using the procedure given above to give 170 mg of ionic-chlorine-free A35512E. This material contained 1.72% chlorine.

Anal. Found: C, 54.84; H, 4.73; N, 5.26; O, 32.67; Cl, 1.72.

Purification of A35512H Hydrochloride

Partially purified A35512H hydrochloride (30 g) was dissolved in a minimal amount of methanol - water (7: 3). The solution was adsorbed on an acidic aluminum oxide column (3×60 cm; Woelm; packed in methanol and eluted with methanol until the effluent was clear). The column was then eluted with methanol at a flow of 4 ml/min, and 24 ml fractions were collected. The eluting solvent was changed at fraction 59 to methanol - water (1: 1).

The fractions were monitored by thin-layer chromatography using a chloroform - methanol - ammonium hydroxide (2 : 3 : 1) solvent system and *Bacillus subtilis* bioautography at alkaline pH. Fractions 51 through 118 were combined and evaporated under vacuum to give 6.4 g of A35512H hydrochloride.

Antibiotic A35512H hydrochloride is a white, amorphous compound. It is soluble in water and DMSO, partially soluble in alcohols, and insoluble in other less polar solvents; λ_{max} (CH_3OH) 282 nm, acidic and neutral (ε 12,500) and λ_{max} 292 nm, basic pH (ε 14,000) calculated using a molecular weight of 2,000, end absorption at 225 nm; for IR spectrum (KBr) see Fig. 3.

Anal. Calcd. for $C_{85\sim87}H_{103\sim107}N_8O_{39\sim41}Cl \cdot HCl$: C, 52.62; H, 5.35; N, 5.71; O, 32.63; Cl, 3.61.
Found: C, 53.86; H, 5.47; N, 5.46; O, 29.93; Cl, 3.62.

Weight loss on drying *ca.* 10%; a ^{13}CMR spectrum in D_2O has the characteristics listed in Table 10.

An acid hydrolyzed sample (6 N HCl, 21 hours, reflux) indicated at least four amino acid residues, one of which appears to be glycine; the acid hydrolyzed product also contained carbohydrate components which have not yet been identified.

Table 10. ^{13}CMR spectrum of A35512H hydrochloride in D_2O.

No.	PPM	Height (%)	No.	PPM	Height (%)
2	177.2	2.7	21	124.2	6.9
3	171.6	5.2	22	122.6	4.1
4	170.9	5.8	23	107.6	2.7
5	169.6	4.7	24	101.8	1.8
6	158.9	3.1	25	76.2	2.8
7	157.6	4.3	26	73.5	4.4
8	156.6	3.8	27	72.3	7.4
9	155.6	4.1	28	71.0	12.2
10	155.4	3.8	29	69.7	4.6
11	154.3	2.4	30	67.4	73.1*
12	151.3	1.6	31	61.6	3.5
13	137.7	2.0	32	56.8	1.8
14	136.7	2.2	33	55.4	2.8
15	136.0	4.0	34	55.0	1.5
16	135.3	1.9	35	24.5	2.6
17	133.5	5.0	36	17.9	4.6
18	129.4	3.7	37	17.2	2.6
19	127.3	1.3	38	16.3	3.6
20	126.1	3.2			

* dioxane standard.

Ionic-Chlorine-Free A35512H

A35512H hydrochloride (200 mg) was chromatographed over weakly basic ion-exchange resin using the procedure given under ionic-chlorine-free A35512A to give 143 mg of ionic-chlorine-free A35512H. This material contained 1.59% chlorine.

Anal. Calcd. for $C_{85\sim87}H_{104\sim106}N_8O_{39\sim41}Cl$: C, 53.7; H, 5.5; N, 5.8; O, 33.2; Cl, 1.8.
Found: C, 53.76; H, 5.32; N, 5.53; O, 33.48; Cl, 1.59.

Weight loss on drying (Block): 5.7.

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