ARIDICINS, NOVEL GLYCOPEPTIDE ANTIBIOTICS

II. ISOLATION AND CHARACTERIZATION

ROBERT D. SITRIN, GEORGE W. CHAN, JOHN J. DINGERDISSEN, WALTER HOLL, JOHN R. E. HOOVER, JOSEPH R. VALENTA, LEE WEBB and KENNETH M. SNADER†

Research and Development Division, Smith Kline & French Laboratories, P.O. Box 7929, Philadelphia, PA 19101, U.S.A.

(Received for publication February 4, 1985)

A new antibacterial antibiotic complex, aridicin, was produced by a new genus, *Kibdelosporangium aridum* (SK&F-AAD-216). The individual factors, aridicins A, B and C, were isolated from the fermentation broth by an Amberlite XAD-7 resin extraction and purified by preparative reversed phase HPLC. The aridicins were found to be novel members of the glycopeptide class of antibiotics as exemplified by ristocetin and vancomycin, based on chemical and spectroscopic data, their molecular weights as determined by FAB mass spectrometry (1,786, 1,800 and 1,814), the detection of actinoidinic acid in their acid hydrolysates, and detailed TLC and HPLC comparisons with representative members of this class.

The aridicins are a new complex of cell-wall active antibiotics produced by fermentation of a new genus *Kibdelosporangium aridum* (SK&F-AAD-216, ATCC 39323), isolated from a soil sample collected near Pima, Arizona[1]. The antibiotic complex was isolated from fermentation broth by acid precipitation and chromatography on Amberlite XAD-7. The individual components were separated and purified by preparative reversed phase high performance liquid chromatography (HPLC). Chemical characterization of the components indicated that they were novel glycopeptide antibiotics[2] related to vancomycin[3,4], ristocetin[5,6], avoparcin[7] and teicoplanin[8]. In this report the isolation and characterization of aridicins A, B and C are reported.

Isolation and Purification

The antibiotic complex was isolated from the fermentation broth as outlined in Scheme 1. Precipitation at pH 3 and redissolution at pH 7 provided a 10-fold volume reduction with high recovery in the preliminary isolation step. This solution was passed through an Amberlite XAD-7 column and eluted with 60% acetonitrile - water to yield a crude extract after concentration *in vacuo*. The overall recovery from broth after these two steps was in excess of 80% with an accompanying volume reduction of 100-fold.

Further purification was effected by preparative reversed phase HPLC. The frequently reported multistep schemes for the purification of polar, water soluble fermentation products often yield products in low recovery and, being limited in capacity, are difficult to scale-up. However, reversed phase HPLC, a useful analytical tool for monitoring antibiotic production, isolation and purification, also has great potential as a preparative tool for isolating and purifying the sesame products, often in one step[9,10,11]. An analytical HPLC chromatogram of the crude extract is shown in Fig. 1, with the major components indicated by peaks A, B and C. This separation was readily scaled-up to preparative level (Fig. 2) on Whatman Partisil Prep 40 ODS-3 packing (37~60 μm)

† Present Address: SeaPharm, P.O. Box 196-A. Ft. Pierce, Florida 33450, U.S.A.

Scheme 1. Isolation scheme for aridicins.

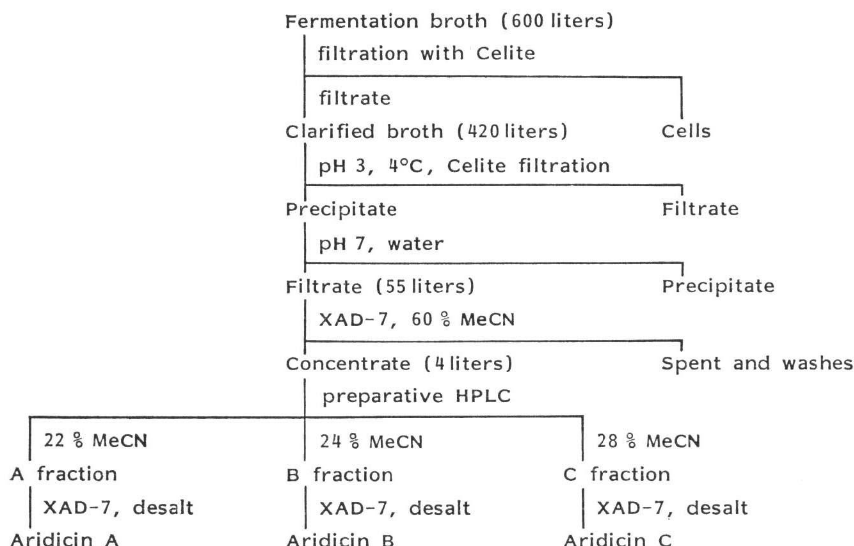


Fig. 1. Analytical chromatogram of crude aridicin complex.

Beckman Ultrasphere ODS, 4.6×150 mm; 27~37% acetonitrile in 0.1 M phosphate, pH 3.2; 1.5 ml/minute; UV at 220 nm.

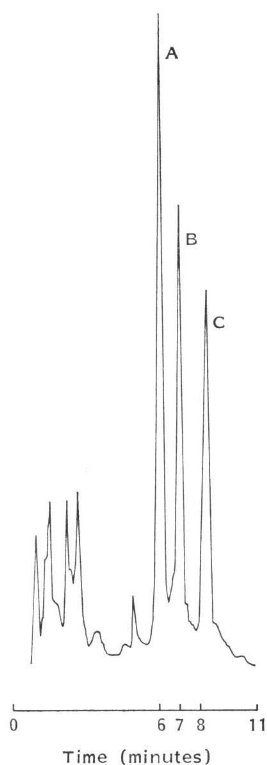
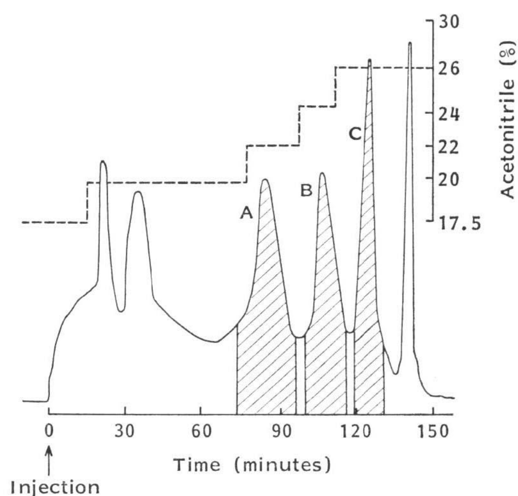


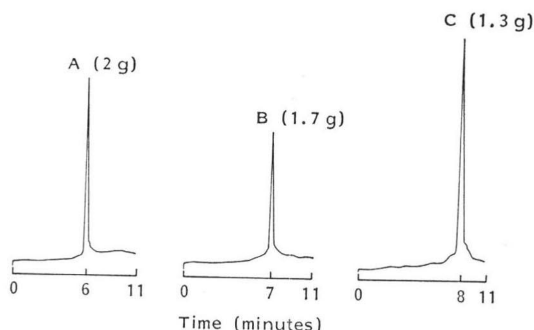
Fig. 2. Preparative chromatogram of crude aridicin complex.

Whatman Partisil Prep 40 ODS-3 in Whatman Magnum Prep 40 column (4.8×50 cm); 18~26% acetronitrile in 0.1 M phosphate, pH 6; 250 ml/minute; UV at 210 nm.



packed in a Whatman Magnum Prep 40 column, using an acetonitrile step gradient. Nearly baseline separation of the three major constituents was obtained as evidenced by the analytical chromatograms (Fig. 3) of the shaded cuts from Fig. 2. It is notable that this process gives in one step pure products with high recoveries

Fig. 3. Analytical chromatograms of pooled fractions of aridicins A, B and C.
Same conditions as Fig. 1.



(>85%) on a multigram scale, starting from a crude isolate. A more detailed description of the preparative liquid chromatography of these antibiotics has been published elsewhere[9].

Characterization

The three major components, labeled A, B and C in order of their elution on reversed phase HPLC, displayed very similar spectroscopic, chemical and biological properties. The IR spectra (Fig. 4) of the three components indicated the presence of hydroxyl (3400 cm^{-1}), amide (1660 cm^{-1}) and aromatic (1600, 1500 and 1450 cm^{-1}) functionalities. The UV spectra displayed maxima at 280 nm ($E^{1\%}_{1cm}$ 54) in acidic solution (pH 2) which shifted to 301 nm ($E^{1\%}_{1cm}$ 68) in base (pH 12), indicating the presence of phenolic groups. The antibiotics were yellow-white powders, displayed decomposition points at 250~350°C and gave no response in their FD or EI mass spectra, implying high molecular weights and/or high polarities. Molecular weights (A, 1,786; B, 1,800; C, 1,814) could be obtained by fast atom bombardment mass spectrometry (FAB-MS)[12]. The empirical formulas for the three antibiotics as listed in Table 1 were obtained from high resolution FAB-MS and are consistent with the combustion data when corrected for water content as determined by thermal gravimetric analysis (TGA) and with carbon counts determined by ^{13}C NMR data. The materials were periodate-positive but ninhydrin-negative, suggesting the presence of a neutral carbohydrate moiety and the absence of an amino sugar. These data indicated that the antibiotics showed spectral similarities to members of the glycopeptide-class exemplified by vancomycin[3,4], ristocetin[5,6], avoparcin[7] and teicoplanin[8].

Glycopeptide antibiotics in general are composed of a peptide aglycone, one or more neutral sugars and frequently an amino sugar[2]. The aglycones consist of heptapeptides of cross-linked unusual aromatic amino acids and, occasionally, conventional amino acids such as aspartic acid and phenylalanine (Table 2). Previous investigators have shown that acid hydrolysis of a glycopeptide antibiotic produces a mixture of amino acids out of which only the conventional (physiological) amino acids as listed in Table 2 can be identified using a typical autoanalyzer system[2]. On alkaline hydrolysis most glycopeptides also produce glycine, a product of retroaldol cleavage of their β-hydroxytyrosine moieties[2].

On acid hydrolysis of the aridicin A, B and C components, none of the amino acids listed in Table 2 could be observed on autoanalyzer analysis, thus, distinguishing the aridicins from actinoidin, mannopeptin, OA-7653, and vancomycin. Glycine, however, could be detected on alkaline hydrolysis, as expected for a typical glycopeptide. Although glycopeptide antibiotics vary considerably in their carbohydrate and amino acid composition, one structural component, namely actinoidinic acid (I), is present in every glycopeptide so far studied.[2] (The only exception to this is A-41030 where **I** appears as a monochloro analog.) The isolation and detection of this amino acid in an underivatized form has historically been a difficult achievement. However, in our hands, this amino acid could readily be detected in aridicin and vancomycin hydrolysates using an alternative HPLC amino acid analysis procedure (0.1% trifluoroacetic acid on reversed phase) developed specifically for this

Fig. 4. IR spectra (KBr) of aridicins A, B and C.

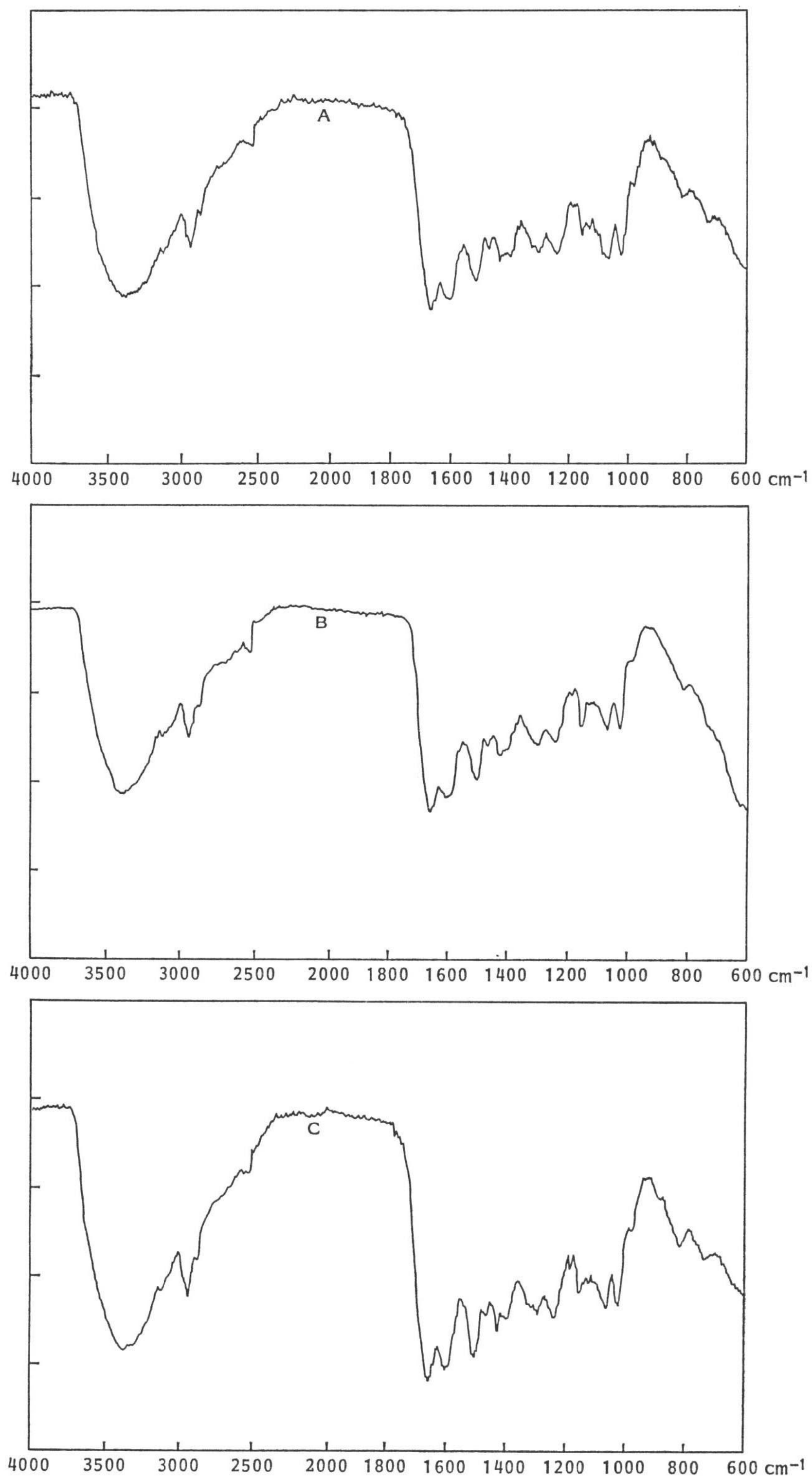


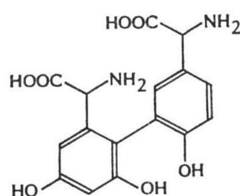
Table 1. Properties of aridicins A, B and C.

	A	B	C
Empirical formula	$C_{81}H_{82}N_8O_{30}Cl_4$	$C_{82}H_{84}N_8O_{30}Cl_4$	$C_{83}H_{86}N_8O_{30}Cl_4$
FAB-MS (MH^+)	1,787	1,801	1,815
UV $\lambda_{max}^{CH_3CN\text{-}H_2O}$ nm ($E_{1cm}^{1\%}$)			
pH 2	280 (51)	280 (55)	280 (51)
pH 12	301 (73)	301 (73)	301 (75)
$[\alpha]_D^{25}$ (c 0.3, H_2O)	$-66°$	$-59°$	$-51°$

Table 2. Physiological amino acid constituents of glycopeptide antibiotics.

A-477[13]	None
A-35512B[14]	None
A-41030A[15]	None
A-47934[16]	None
AB-65[17]	None
Actaplanin[18,19]	None
Actinoidin[2,20]	Phenylalanine
Avoparcin[7]	None
LL-AM374[21]	NR
Mannopeptin[22]	Serine, glycine, tyrosine
OA-7653[23]	Glutamic acid
Ristocetin[5,6]	None
Teicoplanin[8]	None
Vancomycin[3,4]	Aspartic acid, *N*-methyl leucine
Aridicins A, B, C	None

NR: Not reported.

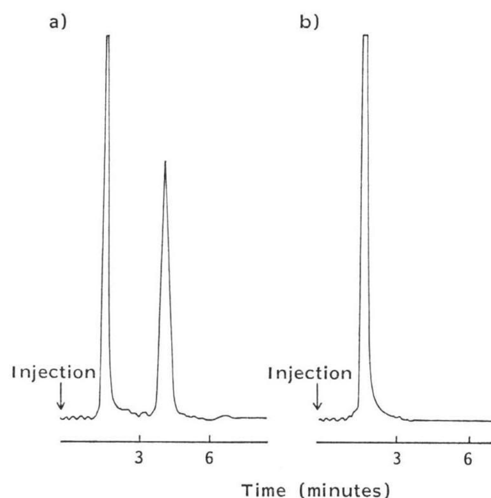


I

Fig. 5. HPLC analysis of antibiotic hydrolysates (6 N HCl, 8 hours).

Beckman Ultrasphere ODS, 4.6×150 mm, 0.1% TFA, 1.5 ml/minute, 220 nm (1.0 AUFS); peak at 1.7 minutes is actinoidinic acid.

a) Aridicin A hydrolysate, b) vancomycin hydrolysate.



purpose (Figs. 5a and 5b). The amino acid eluting at 1.7 minutes was suggested to be **I** since it was present in the hydrolysates of other glycopeptides (ristocetin and teicoplanin) and co-eluted with an authentic sample. This was further confirmed by FAB-MS (MH^+, m/z 349) obtained on a sample of the 1.7-minute peak prepared by preparative HPLC of an aridicin hydrolysate in the same system. This HPLC procedure will be described in further detail in an upcoming structural studies paper. These results as well as biological data previously described[1] confirm that these antibiotics are glycopeptides related to vancomycin and ristocetin.

Confirmation of Novelty

At least fourteen members of the glycopeptide-class have been reported in the literature, most of which appear as complexes with varying carbohydrate content (Table 3). The three aridicin components were found to contain mannose as the only neutral sugar and no detectable amino sugar. This makes them distinct among the antibiotics listed in Table 3, similar only to mannopeptin[22] (which is reported to contain serine, glycine and tyrosine) and perhaps to the incompletely described products

Table 3. Carbohydrate constituents of glycopeptide antibiotics.

	Glucose	Mannose	Rhamnose	Arabinose	Fucose	Galactose	Amino sugar
A-477[13]	NR	NR	NR	NR	NR	NR	NR
A-35512B[14]	1	1	1	0	1	0	*epi*-Vancosamine
A-41030[15]	0	0	0	0	0	0/1/2	0
A-47934[16]	0	0	0	0	0	0	0
AB-65[17]	0	0	0	0	0	0	Unknown
Actaplanin[18,19]	1	1/2/3	0/1	0	0	0	Ristosamine
Actinoidin[2,20]	1	1	0	0	0	0	Acosamine, actinosamine
Avoparcin[7]	1	1	1	0	0	0	Ristosamine (2)
LL-AM374[21]	NR	NR	NR	NR	NR	NR	NR
Mannopeptin[22]	0	1	0	0	0	0	0
OA-7653[23]	1	0	0	0	0	0	0
Ristocetin A/B[5,6]	1	2/1	1	1/0	0	0	Ristosamine
Teicoplanin[8]	0	1	0	0	0	0	Glucosamine
Vancomycin[3,4]	1	0	0	0	0	0	Vancosamine
Aridicins A, B, C	0	1	0	0	0	0	0

NR: Not reported.

Table 4. HPLC retention times for glycopeptide antibiotics.

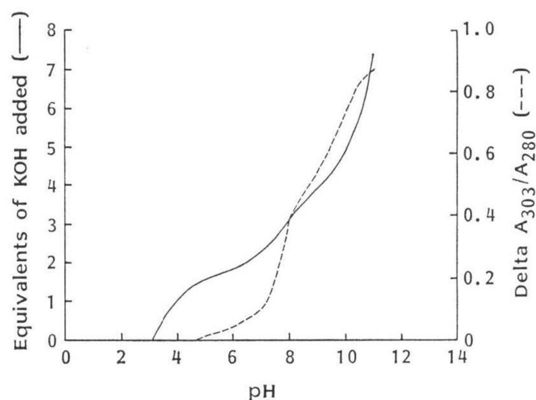
Antibiotics	Retention time (minutes)[a] System A[b]	Retention time (minutes)[a] System B[c]
Aridicin A	15.3	14.5
Aridicin B	15.9	15.2
Aridicin C	17.2	16.1
A-35512B	5.2, 6.2	6.1, 7.2
A-477	11.5, 11.7, 12.1	8.4, 8.9, 10.3
Actaplanin	6.1, 6.3, 6.6, 10.7	7.5, 7.9, 17.2
Actinoidin A/B	5.5, 5.9, 6.1	6.5, 7.1, 8.1
Avoparcin	4.8, 5.8, 6.0	7.6, 7.7
LL-AM374	2.0, 5.1, 6.6	6.8, 10.3
OA-7653	9.4, 9.7, 10.3	9.8, 11.2, 11.8
Ristocetin	3.6	5.4
Teicoplanin	13.1, 13.6, 13.8	13.8, 14.0
Vancomycin	6.0	7.4

[a] Underlined value is the retention time of the major component in multicomponent mixture.

[b] HPLC System A: Column: Ultrasphere ODS, 5 μm, 4.6×150 mm. Solvent: 7~34% acetonitrile (7% for 1 minute, then ramp to 34% over 13 minutes and hold at 34%) in pH 3.2, 0.1 M phosphate. Flow: 1.5 ml/minute. Detection: UV 220 nm.

[c] HPLC System B: Column: Ultrasphere ODS, 5 μm, 4.6×150 mm. Solvent: 5~35% acetonitrile (5% for 1 minute, then ramp to 35% over 13 minutes and hold at 35%) in pH 6.0, 0.025 M phosphate. Flow: 1.5 ml/minute. Detection: UV 220 nm.

Fig. 6. Titration of aridicin A.
—— Equivalents of KOH added.
------ UV ratio of absorbance at 303 nm/280 nm.



A-477[13] and LL-AM374[21] for which no amino acid or carbohydrate data has been reported.

HPLC analysis provided further support of novelty. Using a gradient program as opposed to isocratic systems previously reported[24] and two different pH's all the glycopeptide antibiotics in our collection could be resolved into distinct peaks (Table 4). Many of the samples displayed more than one component. The aridicin A, B and C components had retention times much longer than any other glycopeptides examined, reflecting a highly lipophilic character. These

Table 5. TLC and paper chromatography of glycopeptide antibiotics.

	TLC (Avicel)*			Paper*	
	A	B	C	D	E
A-477	0.51	0.30, 0.40	0, 0.20, 0.67, 0.79	0.07	0.47
A-35512B	0.14	0.25	0, 0.15	0	0.14, 0.30
Actaplanin	0.16	0.06	0.11	0, 0.01	0.04, 0.12, 0.25
Actinoidin	0.04, 0.24	0.18, 0.43	0.05	0	0.13
Avoparcin	0.15	0.13	0.07	0	0.12
LL-AM374	0.08	0.17	0.05	0	0.08
OA-7653	0.36	0.16	0.62	0.03	0.14
Ristocetin	0.1	0, 0.20	0	0	0.14
Teicoplanin	0.34	0.12, 0.33, 0.47	0.77	0.05	0.24
Vancomycin	0.17	0, 0.35	0.1, 0.6	0	0.22, 0.3
Aridicin A	0.35	0.33	0.83	0.13	0.17
Aridicin B	0.38	0.35	0.83	0.14	0.18
Aridicin C	0.44	0.33	0.81	0.14	0.18

* Rf values; detection: *B. subtilis* activity. Underlined value is major active component.
A. BuOH - AcOH - H_2O (4:1:5); B. PrOH - petroleum ether - conc NH_4OH (4:1:2); C. PrOH - H_2O (6:4); D. BuOH - AcOH - H_2O (4:1:5); E. BuOH - H_2O - pyridine - toluene (10:5:5:1).

results as well as TLC data (Table 5) distinguish aridicins A, B and C from available representative glycopeptides.

In general, glycopeptides are amphoteric molecules containing a variety of charged amino, carboxylic and phenolic functional groups. Structural insights into these molecules are possible using electrophoretic techniques as well as titration data. Evidence of novelty comes from examination of the isoelectric points (pI's) as determined using isoelectric focusing and bioautographic detection using our recently described procedure[25]. Most glycopeptides such as vancomycin and ristocetin are basic with pI's greater than 8.0. This implies the presence of an excess number of cationic groups (peptide *N*-termini plus amino sugars) over anionic centers (carboxylates) or the presence of a carbomethoxy ester group which removes a carboxylate negative charge from the molecule. Some glycopeptides, such as teicoplanin and A-477, have pI's near neutrality[25], indicating an equal number of basic and acidic centers. The aridicins, however, have a low isoelectric point (3.8) indicating an excess number of acidic over basic centers, again distinguishing them from all other reported glycopeptides.

These charged centers are evident as well from the titration data determined using an automated titration procedure[26]. The apparent *pKa* values were obtained on a titration apparatus controlled by an Apple computer (Fig. 6) and calculated on a VAX computer using an iterative computer program[27]. Table 6 lists the apparent *pKa* values for aridicins A, B and C in 30% acetonitrile and those for vancomycin determined in water. Reasonable agreement with literature values[28] was obtained using this technique on vancomycin with deviations perhaps resulting from the use of an incorrect molecular weight for vancomycin in the 1972 paper. The first two *pKa*'s for the aridicins are characteristic of carboxyl groups and are assigned as such. The presence of the more than one carboxylate function has not been reported in any other glycopeptide. The *pKa*'s for the aridicins between pH 7.2 and 7.5 can in principle be assigned to phenolic or amino functions. This was resolved by monitoring changes in the UV spectra during the course of the titrations (see Fig. 6). Ionization of a phenolic

Table 6. Apparent *pKa* values of aridicins A, B, C and vancomycin*.

A	B	C	V	Assignment
3.0	3.0	3.0	2.5 (2.9)[28]	Carboxylate
4.9	4.5	4.2	—	Carboxylate
7.4	7.5	7.2	6.8 (7.2)	Amine
—	—	—	8.6 (8.6)	Amine
8.4	8.5	8.2	9.4 (9.6)	Phenolic
10.0	9.7	9.9	10.3 (10.5)	Phenolic
10.3	10.3	10.3	10.6 (11.7)	Phenolic

* A, B and C refer to aridicins A, B and C in 30% acetonitrile - water, V is vancomycin in water, values in parentheses are literature values.

Table 7. Organic chlorine content of glycopeptide antibiotics.

	Reported %	No. of Cl
A-477[13]	3.5	2
A-35512B[14]	1.7	1
A-41030A[15]	8.6	3
A-47934[16]	8.1	3
AB-65[17]	5.0	2
Actaplanin[18,19]	2.3	1
Actinoidin[2,20]	1.8, 3.6	1, 2
Avoparcin[7]	1.8, 3.6	1, 2
LL-AM374[21]	2.5	1
Mannopeptin[22]	0	0
OA-7653[23]	No data	No data
Ristocetin[5,6]	0	0
Teicoplanin[8]	3.3	2
Vancomycin[3,4]	4.8	2
Aridicins A, B, C	7.0	4

group results in a bathochromic shift from 280 to 303 nm. As can be seen from Fig. 6 a substantial UV shift does not occur until the pH is above this *pKa* value, indicating the *pKa*'s below this region (7.2~7.5) to be amine-associated. Comparable UV data for vancomycin suggests that the *pKa* at 6.8 originally assigned to a phenol[28] should more likely be assigned to an amino group.

The remaining functionalities are phenols in both vancomycin and the aridicins. Unfortunately, because of computer limitations, only six *pKa* values can be simultaneously determined by this procedure. Thus, any phenolics with higher *pKa*'s cannot be detected. These assignments of functionality are consistent with the observed isoelectric points of 3.8 for the aridicins and 8.1 for vancomycin[25].

Combustion analyses indicated the presence of approximately 7% organic chlorine in each of the three aridicin components. Assuming a molecular weight of 1,800 daltons for the aridicins and 10% water content as estimated by thermal gravimetric analysis, four chlorines are calculated to be present per molecule, a finding consistent with FAB-MS peak matching data[12]. No other reported glycopeptide has this many chlorines per molecule (see Table 7).

The above data all support the conclusion that the aridicin components are novel glycopeptides, physically and chemically distinct from all other reported members of this class. Studies on these antibiotics describing their unusual glycolipids[29] and their complete structure determination by degradative and NMR techniques will be published separately.

Experimental

General Procedures

Analytical HPLC was performed on a Beckman 345 gradient HPLC system monitored at 220 nm with a Beckman Model 165 detector and an Altex CR1B integrator. Chromatography was run on a Beckman Ultrasphere ODS column (4.6×15 cm) equipped with a Brownlee guard cartridge containing C-18 packing (Brownlee 18-GU). Phosphoric acid buffer (0.1 M) was prepared from Fisher

HPLC grade phosphoric acid, 85% (6.77 ml/liter, adjusted to pH 3.2 with KOH). Acetonitrile was HPLC grade (UV, Burdick and Jackson).

Amino acid analyses were run on a Beckman Autoanalyzer after 6 N HCl or 1 N NaOH hydrolysis. Actinoidinic acid was detected in an 8-hour 6 N HCl hydrolysate of aridicin A by HPLC using 0.1% (v/v) trifluoroacetic acid in water on a Beckman Ultrasphere ODS-3 column (4.6×250 mm). Isoelectric focusing was carried out on an LKB Multiphor using *Bacillus subtilis* as detection as previously described[25]. IR spectra were run on a Perkin Elmer 299B Spectrophotometer. UV spectra were run on a Beckman DU-7. FAB-mass spectra were run on a VG-ZAB 1F-HF mass spectrometer equipped with a standard FAB ion source. Thermo gravimetric analyses were carried out on a Perkin Elmer TGS 2 instrument.

Carbohydrate Analyses

A sample of antibiotic (2 mg) was heated at 100°C for 2 hours in 1 ml of 2 N HCl in a Pierce reaction tube. The cooled solution was mixed with 3 ml of water and passed through a Waters Sep-Pak C-18 cartridge previously washed with acetonitrile (5 ml) and water (5 ml). The cartridge was eluted with an additional 2 ml of water and the combined eluate and wash lyophilized. The residue was treated with 2 mg of $NaBH_4$ in 1 ml of water for 3 hours at room temperature, quenched with 0.05 ml glacial acetic acid, and repeatedly evaporated (three times) under a nitrogen stream with 4 ml of methanolic HCl (methanol - concentrated HCl, 1,000: 1). The residue was acetylated with 3 ml of acetic anhydride - pyridine (1 : 1) for 1 hour at room temperature, evaporated to dryness with a vacuum pump and dissolved in 0.25 ml of CH_2Cl_2 for GC analysis. GC analysis was performed in a DuPont Model 5790 chromatograph using a Quadrex OV-17 glass silica capillary column, 15 m×0.25 mm I.D., 0.25 μm thickness, with temperature programming of 180~270°C at a rate of 2°C/minute, and FID detection.

Titration Procedures

Samples of aridicins A, B and C (25 mg) were adjusted to pH 3 and lyophilized. Titrations were carried out on samples dissolved in 10 ml of 30% acetonitrile - water (v/v) with the addition of 0.1 N KOH in 0.02 ml increments using a Mettler automatic titration vessel attached to an Apple computer system[26]. Values for multiple *pKa*'s were calculated using an iterative computer program[27].

Samples of A-477, A-35512B and actaplanin were provided by BERNARD ABBOTT, Lilly Research Laboratories; avoparcin and LL-AM374 by DONALD BORDERS, Lederle Laboratories; teichomycin A_2 (teicoplanin) by M. R. BARDONE, Gruppo Lepetit; and actinoidins A and B by G. F. GAUSE, Institute for New Antibiotics, Moscow. Vancomycin and ristocetin were purchased from Sigma. Samples of OA-7653 were obtained by fermentation of *Streptomyces hygroscopicus* ATCC 31613.

Isolation of the Aridicin Complex

The fermentation broth (600 liters) was clarified by rotary drum filtration (Komline-Sanderson, Laboratory Scale Model) using precoat filter aid (Hyflo Supercel, Johns-Manville Products Corp.) at existing broth pH (7.7~8.2). The broth filtrate (420 liters) was chilled to 4°C and adjusted to pH 3.0 by slow addition of concd HCl, with mixing. The resulting precipitate was recovered by rotary vacuum filtration using filter aid as previously described. The product was extracted from the precipitated cake by mixing with deionized water (55 liters), adjusting to pH 7.0 for 10 minutes, and filtering. This filtrate was applied to two Amberlite XAD-7 (Rohm and Haas) resin columns (8.5×110 cm) at a flow rate of 0.5 column volume per hour. After washing with 8 column volumes of deionized water, the desired complex was recovered by elution with acetonitrile - water (60%). The eluate was concentrated at 35°C in a rising film evaporator to yield a crude extract containing 22 g of complex (HPLC assay) and approximately 130 g of other solids in 4 liters of water.

Purification

A sample of the Amberlite XAD-7 isolate, containing 25 g dry weight in 4 liters of 18% acetonitrile - 0.1 M potassium phosphate at pH 6.0 (prepared from Fisher HPLC grade KH_2PO_4 and adjusted to pH 6.0 with KOH before addition of acetonitrile) was pumped at a rate of 200 ml/minute onto a Waters Prep-500A system equipped with a Whatman Magnum 40 (4.8×50 cm) column dry packed

with Whatman Partisil Prep 40 ODS-3 (37 ~ 60 μm) mounted in place of the radial compression chambers. Eluates were monitored using a Gow Mac Model 80-850 variable wavelength detector equipped with a 210 nm filter. Stepwise elution with 22, 24 and 26% acetonitrile in buffer (Fig. 2) yielded fractions homogeneous in aridicins A, B and C, respectively (see Fig. 3). The homogeneous antibiotic fractions were desalted by passing through a column (500 ml) of Amberlite XAD-7 resin, followed by eluting with water and 40 to 60% acetonitrile - water to yield, after lyophilization, 2.0, 1.7 and 1.3 g of aridicins A, B and C respectively. Aridicins A, B and C were obtained as off-white powders: mp's >300°C (dec); IR ν_{max}^{KBr} 3400, 2920, 1660, 1510, 1460, 1430, 1390, 1320, 1300, 1240, 1150, 1060 and 1020 cm^{-1}; periodate positive, ninhydrin negative; soluble in H_2O, MeOH, DMSO, insoluble in EtOH, acetonitrile, acetone, ether and aliphatic hydrocarbons.

Aridicin A
Anal Calcd for $C_{81}H_{82}N_8O_{30}Cl_4 \cdot 12H_2O$: C 48.40, H 5.33, N 5.58, Cl 7.07, TGA 10.7%
Found: C 48.20, H 5.01, N 5.20, Cl 6.43, TGA 10.80, Ash 0.53%

Aridicin B
Anal Calcd for $C_{82}H_{84}N_8O_{30}Cl_4 \cdot 10H_2O$: C 49.65, H 5.29, N 5.65, Cl 7.15, TGA 10.0%
Found: C 49.67, H 5.07, N 5.19, Cl 6.70, TGA 10.8%, Ash Negligible

Aridicin C
Anal Calcd for $C_{83}H_{86}N_8O_{30}Cl_4 \cdot 10H_2O$: C 49.91, H 5.35, N 5.61, Cl 7.10, TGA 9.9%
Found: C 47.89, H 5.09, N 4.95, Cl 6.39, TGA 8.2, Ash 3.68%
(Corrected: C 49.72, H 5.28, N 5.13, Cl 6.63, TGA 8.5%)

Acknowledgments

We would like to acknowledge the able technical assistance of JOHN HOBDELL and RAY POLACHEK in the isolation work, GEORGE UDOWENKO and PETER DEPHILLIPS in purification and analysis work, EDITH REICH for analytical data, GERALD ROBERTS for FAB-MS data, ANTHONY GILLETTO and LEWIS KILLMER for carbohydrate analyses, Dr. JOEL HENNER for carrying out isoelectric focusing, Prof. THOMAS HARRIS (Vanderbilt University) for a sample of actinoidinic acid, Prof. JAMES TRAVIS (Univ. Georgia) for autoanalyzer amino acid studies, and CHARLES PAN, MICHAEL POLANSKY and SUE SPETH for supplies of fermentation broths.

References

1) SHEARER, M. C.; P. ACTOR, B. A. BOWIE, S. F. GRAPPEL, C. H. NASH, D. J. NEWMAN, Y. K. OH, C. H. PAN & L. J. NISBET: Aridicins, novel glycopeptide antibiotics. I. Taxonomy, production and biological activity. J. Antibiotics 38: 555 ~ 560, 1985
2) WILLIAMS, D. H.; V. RAJANANDA, M. P. WILLIAMSON & G. BOJESEN: The vancomycin and ristocetin group of antibiotics. Top. Antibiotic Chem. 5: 119 ~ 158, 1980
3) WILLIAMS, D. H.; M. P. WILLIAMSON, D. W. BUTCHER & S. J. HAMMOND: Detailed binding sites of the antibiotics vancomycin and ristocetin A: Determination of intermolecular distances in antibiotic/substrate complexes by use of the time-dependent NOE. J. Am. Chem. Soc. 105: 1332 ~ 1339, 1983
4) HARRIS, C.; H. KOPECKA & T. HARRIS: Vancomycin: Structure and transformation to CDP-1. J. Am. Chem. Soc. 105: 6915 ~ 6922, 1983
5) WILLIAMS, D. H.; V. RAJANANDA, G. BOJESSEN & M. WILLIAMSON: Structure of the antibiotic ristocetin A. J. Chem. Soc. Chem. Commun. 1979: 906 ~ 908, 1979
6) KALMAN, J. R. & D. H. WILLIAMS: An NMR study of the structure of the antibiotic ristocetin A. The negative nuclear Overhauser effect in structure elucidation. J. Am. Chem. Soc. 102: 897 ~ 905, 1980
7) MCGAHREN, W. J.; J. H. MARTIN, G. O. MORTON, R. T. HARGREAVES, R. A. LEESE, F. M. LOVELL & G. A. ELLESTAD: Avoparcin. J. Am. Chem. Soc. 101: 2237 ~ 2239, 1979
8) BARDONE, M. R.; M. PATERNOSTER & C. CORONELLI: Teichomycins, new antibiotics from *Actinoplanes teichomyceticus* nov. sp. II. Extraction and chemical characterization. J. Antibiotics 31: 170 ~ 177, 1978
9) SITRIN, R. D.; G. CHAN, P. DEPHILLIPS, J. DINGERDISSEN, J. VALENTA & K. SNADER: Preparative reversed phase HPLC as a recovery and purification process for nonextractable polar antibiotics. *In* Purification of Fermentation Products. ACS Symposium Series. No. 271, *Eds.*, D. LEROITH, *et al.*, pp. 71 ~ 89, Amer. Chem. Soc., Washington, D.C., 1985

10) STAMPWALA, S. S.; R. H. BUNGE, T. R. HURLEY, N. E. WILLMER, A. J. BRANKIEWICZ, C. E. STEINMAN, T. A. SMITKA & J. C. FRENCH: Novel antitumor agents CI-920, PD 113,270 and PD 113,271. II. Isolation and characterization. J. Antibiotics 36: 1601~1605, 1983
11) BERAN, M.; J. TAX, V. SCHON, Z. VANER & M. PODOJIL: Preparative reversed-phase high-performance liquid chromatography of monensin A and B sodium salts. J. Chromatogr. 268: 315~320, 1983
12) ROBERTS, G. D.; S. A. CARR, S. ROTTSCHAEFER & P. W. JEFFS: Structural characterization of glycopeptide antibiotics related to vancomycin by fast atom bombardment mass spectrometry. J. Antibiotics 38(6): 1985, in press.
13) HAMILL, R. L.; M. K. HANEY & W. S. STARK (Eli Lilly & Co.): Antibiotic A477 and process for preparation thereof. US 3,780,174, Dec. 18, 1973
14) DEBONO, M.; R. M. MOLLOY, M. BARNHART & D. E. DORMAN: A35512, a complex of new antibacterial antibiotics produced by *Streptomyces candidus*. II. Chemical studies of A35512B. J. Antibiotics 33: 1407~1416, 1980
15) EGGERT, J. H.; K. H. MICHEL, L. D. BOECK, W. M. NAKATSUKASA & R. E. KASTER: A41030, a complex of novel glycopeptide antibiotics. Discovery, fermentation, isolation and characterization. 23rd Intersci. Conf. Antimicrob. Agents Chemother., Abstract No. 440, Las Vegas, 1983
16) HAMILL, R. L.; L. D. BOECK, R. E. KOSTNER & R. M. GALE: A47934, a novel glycopeptide-aglycone antibiotic: Fermentation, isolation and characterization. 23rd Intersci. Conf. Antimicrob. Agents Chemother., Abstract No. 443, Las Vegas, 1983
17) TAMURA, A. & I. TAKEDA: Antibiotic AB-65, a new antibiotic from *Saccharomonospora viride*. J. Antibiotics 28: 395~397, 1975
18) DEBONO, M.; K. E. MERKEL, R. M. MOLLOY, M. BARNHART, E. PRESTI, A. H. HUNT & R. L. HAMILL: Actaplanin, new glycopeptide antibiotics produced by *Actinoplanes missouriensis*. The isolation and preliminary chemical characterization of actaplanin. J. Antibiotics 37: 85~95, 1984
19) HUNT, A. H.; M. DEBONO, K. E. MERKEL & M. BARNHART: Structure of the pseudo-aglycone of actaplanin. J. Org. Chem. 49: 635~640, 1984
20) SZTARICSKAI, F.; C. M. HARRIS & T. M. HARRIS: Investigation of the antibiotic actinoidin: Identification of the Tris(amino acid). Tetrahedron Lett. 1979: 2861~2864, 1979
21) KUNSTMAN, M. P. & J. N. PORTER (American Cyanamid): Animal feed composition containing antibiotic AM374 as a growth promoter. US 3,803,306, Apr. 9, 1974
22) HAYASHI, T.; Y. HARADA & K. ANDO: Isolation and characterization of mannopeptins, new antibiotics. J. Antibiotics 28: 503~507, 1975
23) KAMOGASHIRA, T.; T. NISHIDA & M. SUGAWARA: A new glycopeptide antibiotic, OA-7653, produced by *Streptomyces hygroscopicus* subsp. *hiwasaensis*. Agric. Biol. Chem. 47: 499~506, 1983
24) SZTARICSKAI, R.; J. BORDA, M. M. PUSKÁS & R. BOGNÁR: High performance liquid chromatography (HPLC) of antibiotics of vancomycin type. Comparative studies. J. Antibiotics 36: 1691~1698, 1983
25) HENNER, J. & R. D. SITRIN: Isoelectric focusing and electrophoretic titration of antibiotics using bioautographic detection. J. Antibiotics 37: 1475~1478, 1984
26) HOLL, W. & L. WEBB: An automated potentiometric titrations procedure. in preparation.
27) ALBERT, A. & E. P. SERJEANT: The Determination of Ionization Constants. p. 35. Chapman and Hall Ltd., London, 1971
28) NIETO, M. & H. P. PERKINS: Physicochemical properties of vancomycin and iodovancomycin and their complexes with diacetyl-L-lysyl-D-alanyl-D-alanine. Biochem. J. 123: 773~788, 1971
29) JEFFS, P. W.; G. CHAN, R. SITRIN, N. HOLDER & C. DEBROSSE: The structure of the glycolipid components of the aridicin antibiotic complex. J. Org. Chem., in press.