

KIBDELINS (AAD-609), NOVEL GLYCOPEPTIDE ANTIBIOTICS

II. ISOLATION, PURIFICATION AND STRUCTURE

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A new glycopeptide antibiotic complex was isolated from the fermentation culture of *Kibdelosporangium aridum* subsp. largum (SK&F AAD-609) by affinity chromatography on a D-alanyl-D-alanine agarose column. The major components of the complex were resolved by preparative reversed-phase HPLC. Mild acid hydrolysis showed that the new antibiotics have the same mannosyl aglycon (**2**) as the aridicins. FAB mass spectrometry, isoelectric focusing, potentiometric titration and carbohydrate and fatty acid analyses were used to determine the structures of the five major components of the complex. These studies showed that the kibdelins differ from the aridicins only in the oxidation level at the C-6 position of the amino sugar. Kibdelin A (**5**), B (**6**), C₁ (**7**), C₂ (**8**) and D (**9**) are a series of *N*-acylglucosamine analogs containing saturated straight and branched chain C₁₀~C₁₂ fatty acids whereas, in kibdelin D the fatty acid component is (*Z*)-4-decenoic acid.

The fermentation of *Kibdelosporangium aridum* subsp. largum (SK&F AAD-609) produces two closely related series of glycopeptide antibiotic complexes, the ratio of which is dependent on the fermentation media and time of harvest¹⁾. Although affinity chromatography has been traditionally reserved for the purification of proteins^{2,3)}, an application of this technique to the rapid screening and purification of glycopeptide antibiotics was recently reported⁴⁾. Using a differential elution technique, the two complexes were separated by affinity chromatography on a D-alanyl-D-alanine agarose column. One series was characterized as the aridicin complex⁵⁾ and the other differed from the aridicins by containing an *N*-acylglucosamine glycolipid moiety. The components of the glycolipid are shown to parallel those found in teicoplanin^{6,7)}. The parent antibiotics, which we have named the kibdelins were tentatively designated as AAD-609 antibiotics in a preliminary report⁸⁾. This paper describes the isolation, physico-chemical properties, and structures (**5**~**9**) of these novel glycopeptide antibiotics.

Materials and Methods

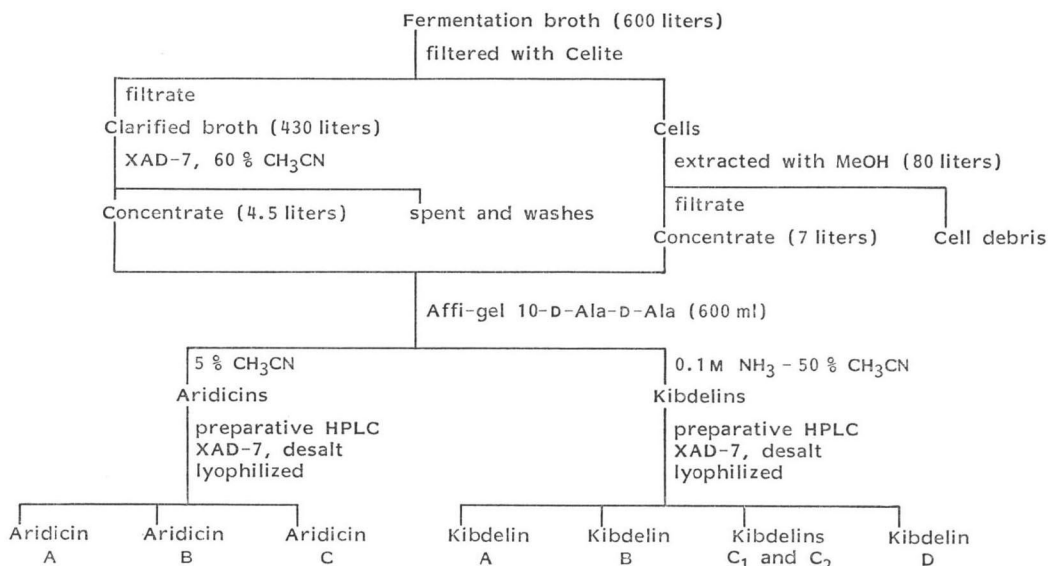
High Performance Liquid Chromatography Assay for Glycopeptide Antibiotics

Broth samples and crude extracts were pretreated using a C₁₈ Sep-Pak cartridge (Waters Associates) and antibiotic quantitated by HPLC as previously described⁹⁾. All other samples were analyzed directly without pretreatment. Authentic samples of aridicins A, B and C were used as standards for quantification of the kibdelins.

Clarification and Extraction of the Crude Antibiotic Complex

Crude antibiotic complex was isolated from 600 liters of fermentation broth as indicated in Scheme 1. The procedures used were as described elsewhere⁵⁾ with some modifications. Following clarification, the broth was adjusted to pH 7 with 2 N HCl and directly applied to Amberlite XAD-7 (Rohm

Scheme 1. Isolation and purification.



and Haas) without a prior acid precipitation step. The 64 liters of XAD-7 eluate was concd to 4.5 liters for further purification and the product yield quantified by HPLC assay.

The cells were extracted with 80 liters of MeOH at room temp, the extract concd to 20 liters at 32°C in a rising film evaporator and then further reduced to 8.25 liters by rotary evaporation. The concentrate was centrifuged at 4°C for 60 minutes at 3,000×*g* and the supernatant (7 liters) filtered through Whatman Number 1 filter paper using precoat filter aid (Hyflo Supercel, Johns-Mansville Products Corporation).

Affinity Chromatography

The concd XAD-7 eluate was adjusted to pH 7 with 2*N* HCl and filtered through Whatman Number 1 filter paper using the filter aid. The filtrate was combined with 600 ml of Affi-gel 10-D-Ala-D-Ala (maximum loading capacity, 12 mg/ml)⁴⁾ for 30 minutes in a batch-type procedure, the slurry poured into a 4.5×60 cm glass column fitted with a filter disk and stopcock and the spent collected. The gel was washed with 6 liters of 0.02*M* sodium phosphate at pH 7 followed by 3 liters of 0.5*M* NH₄OAc at pH 7.8. The aridicin complex was specifically eluted using a 5% acetonitrile - H₂O prewash followed by elution of the novel kibdelins with 50% acetonitrile in 0.1*M* ammonia. One liter fractions were collected and assayed by HPLC. Fractions containing the novel glycopeptide antibiotics were pooled and lyophilized to dryness. Following column regeneration with 2 liters of 30% acetonitrile in 0.4*M* sodium carbonate at pH 9.5, the spent material was recycled. Glycopeptide products were purified from the 7 liters of MeOH cell extract as described above with the exception of washing the Affi-gel with 6 liters of 10% MeOH - H₂O prior to product elution.

Preparative Reversed-phase Chromatography

The individual components of the aridicin complex were resolved by HPLC according to the procedure developed by SITRIN *et al.*⁵⁾ using a 22 mm×30 cm prepacked Whatman Magnum-20 10 micron Partisil ODS-3 column equipped with a Beckman 112 preparative pump and ISCO V₄ variable wavelength detector. The chromatography was performed at a flow rate of 15 ml/minute while monitoring the eluate at 300 nm and collecting 25-ml fractions. The sample load was approximately 140 mg dissolved in 15% acetonitrile in 0.1*M* phosphate, pH 6. The step-wise acetonitrile gradient was modified to 28 to 32% acetonitrile in 0.1*M* potassium phosphate at pH 6. The isolated components were desalted on XAD-7 and lyophilized to dryness for further analysis.

The individual kibdelineins were purified from 4.5 g of affinity isolate as described above except that the sample load was increased to 1.0~1.5 g for an individual run and the step-wise gradient extended between the solvent concentrations of 26 through 32%. Components A, B and C were obtained as off-white amorphous powders: MPs <300°C; IR (KBr) cm^{-1} 3400, 2940, 1660, 1595, 1500, 1460, 1420, 1390, 1310, 1290, 1230, 1140, 1060, 1010, 810 and 730; periodate positive; soluble in H_2O , MeOH and DMSO. Each component exhibited well-resolved complex ^1H NMR spectra at 500 MHz (see Results).

General Procedures

Isoelectric focussing was performed as described elsewhere¹⁰⁾ using an LKB Multiphor apparatus and activity against *Bacillus subtilis* as detection. Carbohydrate analysis, potentiometric titration, thermogravimetric analysis and IR and UV spectroscopy were as described⁹⁾ with the following additions. Characterization of the carbohydrates was by GC/CI-MS of mannose and glucosamine as their alditol acetates using ammonia as the reagent gas and a column temperature programmed from 125°C to 310°C at 10°C/minute. FAB-MS spectrometry was carried out as described earlier¹¹⁾.

Identification of the Pseudoaglycon

Solid samples of antibiotics were dissolved in 0.1 M sodium phosphate, adjusted to pH 3.2 at a concentration of 0.2 mg/ml and then heated to 100°C for 15 hours in a sealed vacuum hydrolysis tube. Hydrolysis products were initially identified as the aglycon of the aridicin complex by co-injection with an authentic sample by HPLC. Confirmation of the identity of the mannosyl aglycon was obtained by comparison of the sample FAB-MS with that of the standard.

Lipid Determination

Fatty acid methyl esters were prepared as previously described¹²⁾ and analysis was carried out using a Finnigan 3265 GC-MS operating in the chemical ionization (CI) mode. Separation was achieved using a DB-17 fused silica capillary column (15 m \times 0.25 mm) programmed from 80~250°C at 10°C/minute with either methane or dimethyl ether (DME)* as the reagent gas.

Controlled Methanolysis of Kibdelin D to Glycolipids 2 and 3

A 220-mg sample of kibdelin D (previously dried over P_2O_5 under vacuum for 24 hours) was dissolved in 40 ml of 0.1 N HCl - MeOH and heated at 105°C for 1 hour in a sealed tube. The solution was adjusted to pH 7 with 5% Na_2CO_3 , the solvent removed by rotary evaporation and the white residue redissolved in 10 ml of 15% acetonitrile - H_2O . The sample was initially fractionated by reversed-phase HPLC on a Whatman Magnum 9 ODS-3 column (9.4 mm \times 50 cm) in an aqueous mobile phase containing 37% acetonitrile in 0.05% TFA at a flow rate of 5 ml/minute. The material was further purified on a 2.5 \times 25 cm column of Whatman Partisil ODS-3 media using an aqueous mobile phase of 40% acetonitrile at a flow rate of 15 ml/minute. This yielded two products **2** (18.6 mg) and **3** (6.5 mg).

Compound **2** (α -anomer): ^{13}C NMR (CDCl_3), 174.41 (C=O), 131.60 and 127.43 (CH=CH), 98.60 (C-1), 72.86 (C-3), 71.50 (C-4), 70.33 (C-5), 61.30 (C-6), 55.16 (OCH_3), 53.64 (C-2), 36.36 (CH_2), 31.51 (CH_2), 29.30 (CH_2), 27.20 (CH_2), 23.30 (CH_2), 22.57 (CH_2), 14.06 (CH_3); ^1H NMR (CDCl_3) δ 6.16 (1H, d, $J=8.0$ Hz, NH), 5.48 (1H, m, olefinic), 5.35 (1H, m, olefinic), 4.68 (1H, d, $J=4.01$ Hz, 1-H), 4.05 (1H, dd, $J=5.4, 10.8$ Hz, 2-H), 3.50~3.80 (3H, m, 3-H, 4-H, 5-H), 3.38 (3H, s, OCH_3), 2.40 (2H, m, allylic CH_2), 2.31 (2H, m, NHCO , CH_2), 2.05 (2H, m, allylic CH_2), 1.30 (4H, m, CH_2 , CH_2), 0.89 (3H, s, CH_3); carbon-hydrogen coupling (^{13}C - ^1H) for C-1, 169.3 Hz; FAB-MS m/z 346 ($\text{M}+\text{H}$)⁺ and 344 ($\text{M}-\text{H}$)⁻; FT-IR (NHCO) cm^{-1} 1666.

Compound **3** (β -anomer): ^1H NMR (CDCl_3), 5.39 (2H, m, olefinic), 4.32 (1H, d, 1-H), 3.30~3.80 (3H, m, 3-H, 4-H, 5-H), 3.49 (3H, s, OCH_3), 2.32 (2H, m, allylic CH_2), 2.00 (4H, m, NHCOCH_2 and allylic CH_2), 1.30 (4H, m, CH_2 , CH_2), 0.89 (3H, t, CH_3); carbon-hydrogen coupling (^{13}C - ^1H) for C-1,

* DME was used as a CI reagent gas because it has the unique ability to differentiate branched from normal fatty acid methyl esters. With DME, only branched fatty acid methyl esters exhibit intense ($\text{M}-\text{H}$)⁺ ions. Unfortunately, DME does not generate characteristic fragment ions indicative of the branch site. L. B. KILLMER unpublished observation.

Table 1. Glycopeptide yields from fermentation broth^a.

Component	Glycopeptide yields (g)		
	Clarified extract ^b 430 liters	XAD-7 eluate 4.5 liters	Affinity-isolate 1 liter
Kibdelin D	0.90	1.70	1.90
Kibdelin A	1.46	1.61	1.90
Kibdelin B	0.47	0.78	1.16
Kibdelin C	0.52	1.01	0.90
Aridicin A	0.47	—	—
Aridicin B	0.34	—	—
Aridicin C	0.43	—	—

^a Quantitated by analytical HPLC as described in the text.

^b Initial extracts pretreated by using the Sep-pak procedure outlined in the Materials and Methods.

159.3 Hz; ¹³C NMR δ 102.33 (C-1). Other signals closely paralleled those for the α -anomer. FAB-MS m/z 346 (M+H)⁺ and 344 (M-H)⁻.

Compound 2 (3 mg) was dissolved in 1.5 ml of 2 N HCl-MeOH and heated at 105°C in a sealed tube for 4 hours. The solution was adjusted to pH 7 with 2 ml of 2.5% NaHCO₃ and extracted with CH₂Cl₂ (2 × 1 ml). The extract was analyzed by GC-MS following derivatization with dimethyl disulfide according to the method of BUSER *et al.*¹³.

Results and Discussion

Isolation

Previous studies have shown that affinity chromatography on agarose D-Ala-D-Ala is a potential means of purification for the entire family of glycopeptide antibiotics whose mechanism of action is dependent on binding to peptides terminating in the tripeptide sequence L-Lys-D-Ala-D-Ala⁴. In addition to its utility as a means of purifying gram-quantities of glycopeptides the affinity technique combined with discrimination by HPLC analysis⁹ served as a rapid and efficient screening technique for the novel kibdelin antibiotics.

A 600-liter fermentation was required to produce sufficient quantities of the novel antibiotics for subsequent purification and structural and biological analyses¹¹. Although small-scale experiments revealed that pure preparations of kibdelin antibiotics can be made in a one-step affinity isolation procedure from clarified broth, a preliminary chromatography step on XAD-7 was added to preserve the life-time of the affinity support by removing impurities which tended to foul the column during loading. Clarification and XAD-7 chromatography yielded 5 g of the kibdelin antibiotics (Table 1). The yield of glycopeptides prior to affinity chromatography shows some variation due to the Sep-pak pretreatment.

Prior studies determined that elution of affinity-bound glycopeptides is highly dependent on their physical properties, in particular hydrophobicity and isoelectric point⁴. The unusually low isoelectric

Fig. 1. Affinity purification of the kibdelin complex from XAD-7 concentrate on Affi-gel 10-D-Ala-D-Ala (5 × 30 cm).

Glycopeptides were quantitated by analytical RP-HPLC described in Fig. 2.

Represents the kibdelins (—) and represents the aridicins (-----). Eluants were as follows: A; 0.02 M phosphate (pH 7), B; 0.5 M NH₄OAc (pH 7.8), C~E; 5% acetonitrile-H₂O, F; 0.1 M ammonia containing 50% acetonitrile.

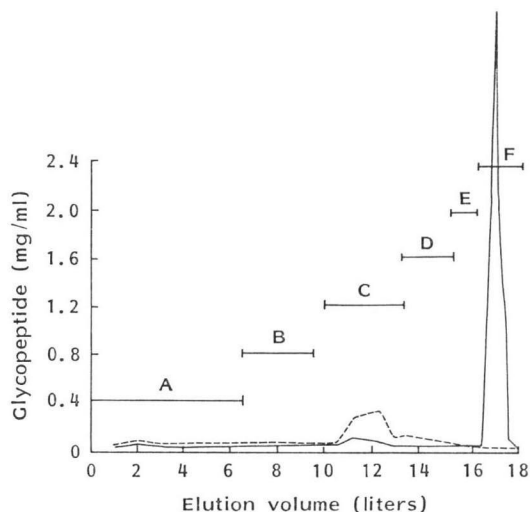


Fig. 2. Analytical chromatograms of affinity purified components.

(a) Sep-pak pretreated XAD-7 concentrate, (b) 5% acetonitrile - H₂O eluate (aricidins) and (c) 0.1 M ammonia - 50% acetonitrile eluate (kibdelins).

The novel components are represented as the shaded peaks. Beckman Ultrasphere ODS, 4.6 × 150 mm; 25 to 60% acetonitrile in 0.1 M phosphate (pH 3.2); 1.5 ml/minute; UV at 254 nm.

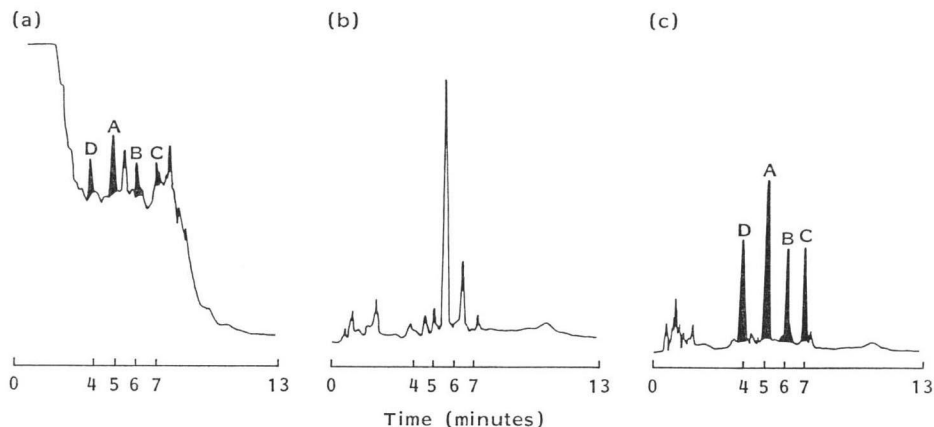
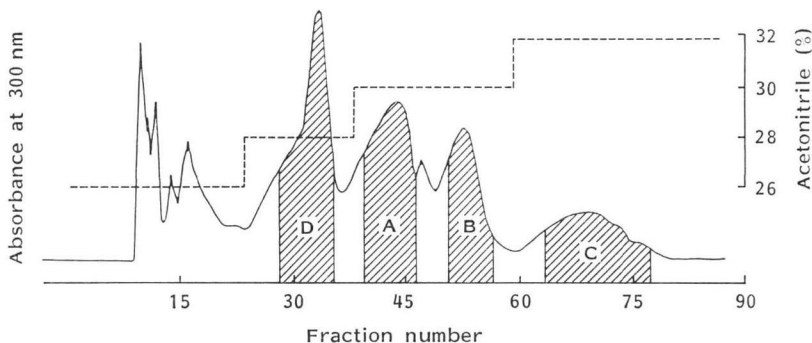


Fig. 3. Preparative chromatogram of the kibdelin complex.

Whatman Partisil Magnum 20 column (2.2 × 30 cm); 26~32% acetonitrile in 0.1 M phosphate (pH 6); 10~15 ml/minute; UV at 300 nm.



point (3.8) of the aricidins results in their elution with combinations of acetonitrile and water in the absence of ammonia. However, vancomycin and ristocetin, which have isoelectric points of 8.2 and 8.4 respectively, require both high pH and organic modifier for good product recovery. Based on these observations, a differential elution procedure was used to separate the two types of glycopeptide complexes produced by the culture AAD-609 from the Affi-gel 10-D-Ala-D-Ala support. After initial washings with phosphate and acetate buffers to remove non-specifically bound contaminants, 5% acetonitrile effectively eluted the bound aricidins with less than 10% loss of the kibdelins (Fig. 1). The latter were eluted in less than 2 column volumes using 50% acetonitrile in 0.1 M ammonia with high recovery (Table 1) and no detectable contamination by the aricidins. The HPLC profiles of the XAD-7 concentrate and the pooled acetonitrile - water and acetonitrile - ammonia eluates are shown in Fig. 2. Because of the similarity in HPLC retention times of the kibdelins and aricidins the pre-fractionation of the two complexes by this selective desorption step greatly facilitated the subsequent fractionation by preparative HPLC. The methanol extract yielded an additional 3 g of

Table 2. Properties of kibdelsins A, B, C and D.

	A	B	C	D
Empirical formula	C ₉₁ H ₉₄ N ₈ O ₂₀ Cl ₄	C ₈₂ H ₉₈ N ₈ O ₂₀ Cl ₄	C ₈₃ H ₉₈ N ₈ O ₂₀ Cl ₄	C ₈₁ H ₈₂ N ₈ O ₂₀ Cl ₄
Molecular weight	1,772	1,786	1,800	1,770
[α] _D ²⁰ (1%, H ₂ O)	-58	-55	-49	-65
UV (E _{1cm} ^{1%})				
λ _{max} 280 nm (0.1 N HCl)	53	53	47	52
λ _{max} 300 nm (0.1 N NaOH)	97	85	78	84
Ignition residue (%)	0.05	0	0.05	0.02
TGA (%)	8.2	6.7	8.1	7.9
Fatty acids	C ₁₀ straight	C ₁₁ branched	C ₁ =C ₁₂ branched C ₂ =C ₁₂ straight	
Carbohydrate	Mannose, 2-amino-2-deoxyglucosamine			

Table 3. Apparent *pKa* values^a.

Aridicin A	Kibdelin A	Kibdelin B	Kibdelsins C ₁ and C ₂	Kibdelin D	Assignment
3.0	3.4	3.5	3.6	3.4	Carboxyl on aglycon
4.9	—	—	—	—	Carboxyl on side chain
7.4	7.1	7.1	7.2	7.0	Amino on aglycon
8.4	8.2	8.1	8.4	8.0	Phenolic hydroxyl
10.0	9.7	9.7	9.9	9.3	Phenolic hydroxyl
10.3	10.4	10.6	10.6	10.2	Phenolic hydroxyl
^b	11.7	^c	11.6	11.6	Phenolic hydroxyl

^a Titration performed in 30% acetonitrile - H₂O.

^b Present program can only calculate 6 *pK* values.

^c Program was unable to fit data to a sixth *pK*.

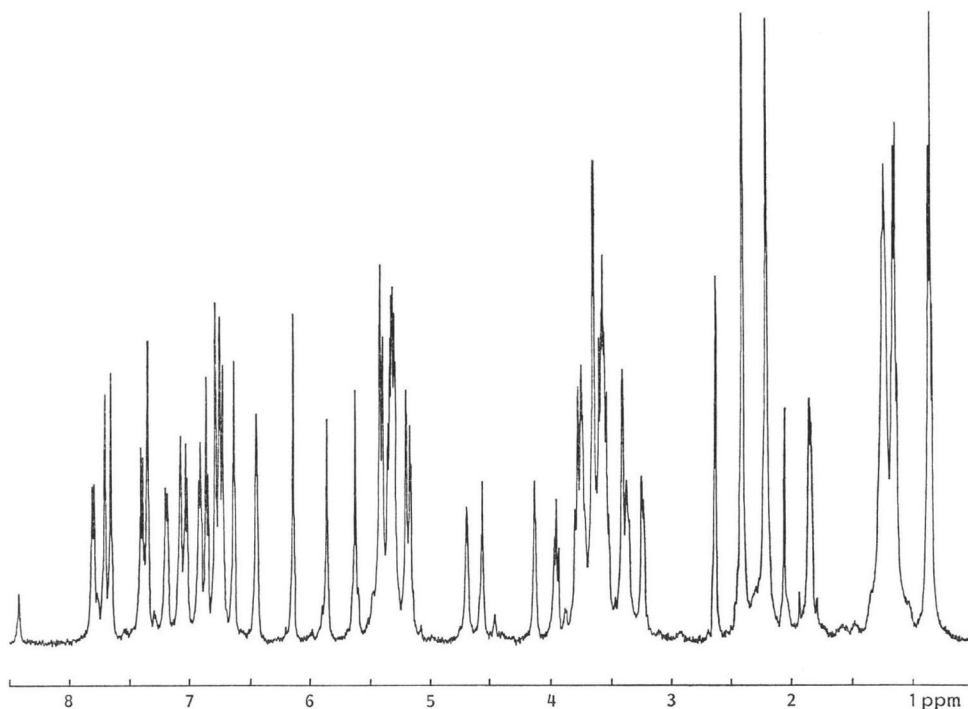
kibdelsins after affinity purification.

The aridicin components were purified to homogeneity in order to firmly establish their identity. Because the 600-liter fermentation had been optimized for novel component production¹⁾, an earlier fermentation, where the aridicins predominated, was used for this purpose. Reversed-phase chromatography on Whatman ODS-3, desalting on XAD-7 and lyophilization, yielded each of the individual components in approximately 73% yield. From 140 mg of affinity isolate was obtained the following: Aridicin A 59 mg, B 22 mg, C₁ 14 mg and C₂ 11 mg. Their identity was confirmed by comparison with authentic standards using HPLC with co-injection, UV spectra and E_{1%} values in 0.1 M HCl and 0.1 N NaOH, production of pseudoaglycon by mild acid hydrolysis, fatty acid and carbohydrate content, *pKa* values, and molecular weight and fragmentation pattern by FAB-MS. In addition, the isoelectric point of the affinity purified aridicin complex was 3.8 as previously reported³⁾.

Purified kibdelin components were obtained by maximizing the sample load on the preparative reversed-phase column to 1.0~1.5 g per injection and pooling only the center portions of each of the resolved peaks (Fig. 3). After desalting and lyophilization, the yields of purified components from 4.38 g of affinity-purified complex were A 550 mg, B 317 mg, C 315 mg and D 560 mg.

Structural Characterization

Chemical and physical properties of the novel components are listed in Table 2. All four

Fig. 4. ^1H NMR spectrum of kibdeline A at 500 MHz.

components have identical UV spectra and exhibit a bathochromic shift from 280 nm, under neutral or acidic conditions, to 300 nm in base. The ^1H NMR spectrum of kibdeline A at 500 MHz (Fig. 4) is representative of the glycopeptide antibiotics with signals attributable to protons associated with aromatic rings, carbohydrates, peptide and fatty acid moieties. The molecular weights of the parent glycopeptides were determined by FAB-MS¹¹. Aside from the m/z protonated molecular ion cluster, the most abundant ion in all four spectra occurs at 1,458 and is identical in mass to the mannosyl aglycon of the aridicin complex. This is in good agreement with results showing that mild acid hydrolysis converts each component of the complex to a single HPLC component which co-elutes on HPLC with authentic aridicin mannosyl aglycon. This data plus the presence of mannose (Table 2) and 7 titratable groups having the same pK_a values as those in the heptapeptide core of aridicin A (Table 3) supports the finding that the glycopeptides have the same mannosyl aglycon (**1**) as the aridicins.

The differences in mass of 14 amu of the individual kibdeline A~C is in keeping with observations made for the aridicins and the teicoplanins^{6,7}. The individual components in each series is due to the presence of different fatty acid constituents. An analogous situation occurs in the kibdeline. Methanolysis of the complex, or the individual components, was shown by analysis of the resulting methyl esters GC/CI-MS of the components A~C to directly parallel the results obtained with the aridicins⁸ where C-10, C-11 and C-12 fatty acid methyl esters were detected (Table 2)*. Kibdeline D on methanolysis produced a methyl ester with the same retention time on GC as methyl decanoate but the CI-MS of this compound which contains an $(M+H)^+$ ion at m/z 185 indicated it was derived from a C-10 fatty acid with one double bond.

* See the footnote on p. 1397.

Table 4. ^{13}C NMR spectra of **2** and **3**.

Compound	C-1 (ppm)	^{13}C - ^1H Coupling (Hz)	Methyl glycopyranosides literature values ^a	
			C-1 (ppm)	^{13}C - ^1H (Hz)
2 (α -anomer)	98.60	169.3	99.1~106.6	169~171
3 (β -anomer)	102.33	159.3	97.8~103.2	158~162

^a These values were taken from the paper by Bock *et al.*¹⁴⁾

Since the kibelins contain the mannosylated heptapeptide core common to the aridicins (and in the case of components A~C, the same fatty acid components), the aridicins and the kibelins can only differ in the nature of the amino sugar component, which on the basis of mass differences in the two series, must be represented as a deoxyaminohexose. Characterization of this amino sugar as glucosamine was readily achieved by hydrolysis of kibelin D in acid and subsequent treatment of the total acid hydrolysate sequentially with NaBH_4 and acetic anhydride to give a crude product containing the alditol-acetates⁵⁾. The GC retention times of the two major components corresponded to the alditol-acetates of mannose and glucosamine. Furthermore, ions in the CI-MS spectra of the alditol-acetate products using ammonia as a reagent gas were also in agreement with the identification of these carbohydrates as mannose and glucosamine in that strong $(\text{M}+\text{NH}_4)^+$ ions were found at m/z 452 and 451, respectively.

While the foregoing evidence suggests that the kibelins differ from the aridicins only in the oxidation level at the C-6 position of the amino sugar, firm proof was lacking that the glucosamine was *N*-acylated and that both the position of attachment and the stereochemistry of the glycosidic linkage to the heptapeptide core corresponded to that found in the aridicins. To establish these points, we undertook a study of kibelin D. The choice of this particular compound was dictated largely by the fact that its structure had an additional uncertainty in the location of the double bond in the C-10 fatty acid component. Methanolysis of kibelin D under very mild conditions was found to effect selective cleavage at the glycosidic position of the glycolipid component. The methanolysis product was separated into two isomeric compounds with molecular weights of m/z 345 which was ascertained from both positive and negative ion FAB-MS. The ^1H NMR of both isomers corresponded closely with the spectrum of a sample of methyl 2-deoxy-2-[(1-oxodecyl)amino]- β -D-glucofuranoside (**4**)¹²⁾ in the region of the sugar ring protons between δ 2.2~7.0. The similarity of the proton spectra of the two compounds derived from kibelin D suggested that they differed only in configuration at C-1. Unfortunately, assignment of the stereochemistry at this position although clearly suggested from the ^1H NMR spectra could not be ascertained with certainty due primarily to lack of resolution in the spectrum of **3**¹²⁾. The use of ^1H - ^{13}C one-bond coupling constants to determine the anomeric configuration of glycopyranosides may be made with confidence, whereas the use of ^{13}C chemical shifts is somewhat less certain because of some overlap in the range of values found in compounds in the α - and β -anomer series¹⁴⁾. The results of the study of the ^{13}C spectra of compounds **2** and **3** are presented in Table 4 and constitute clear evidence for assigning the stereochemistry as the α - and β -methyl glycosides, respectively.

The location of the acyl substituent on nitrogen was supported by the occurrence of an amide absorption at 1666 cm^{-1} in the FT-IR spectrum of the major product. Location of the position of the double bond in the *N*-acyl substituent in kibelin D was accomplished by employing the method of

Scheme 2.

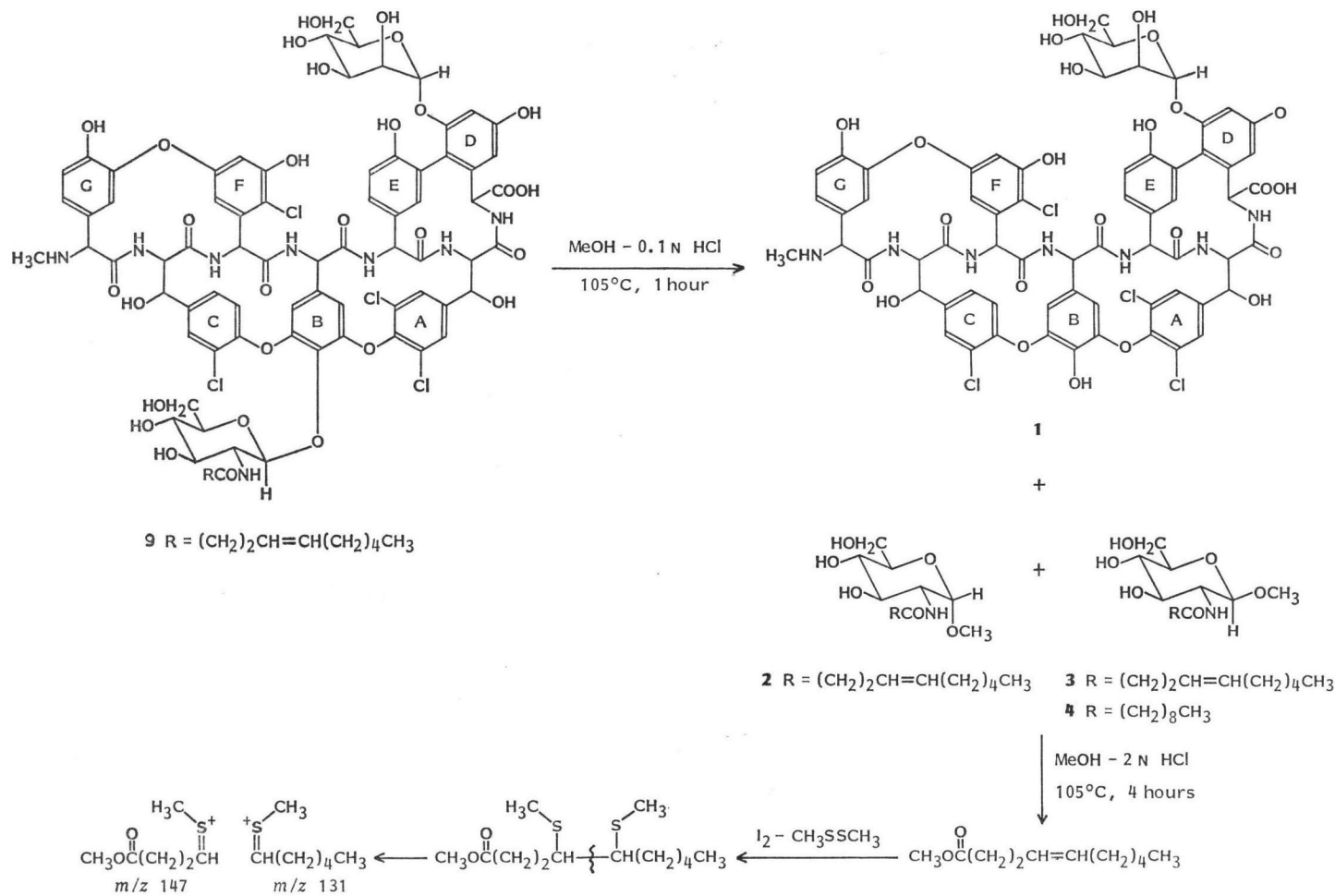
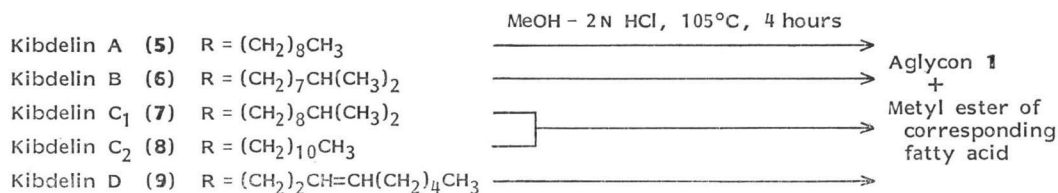
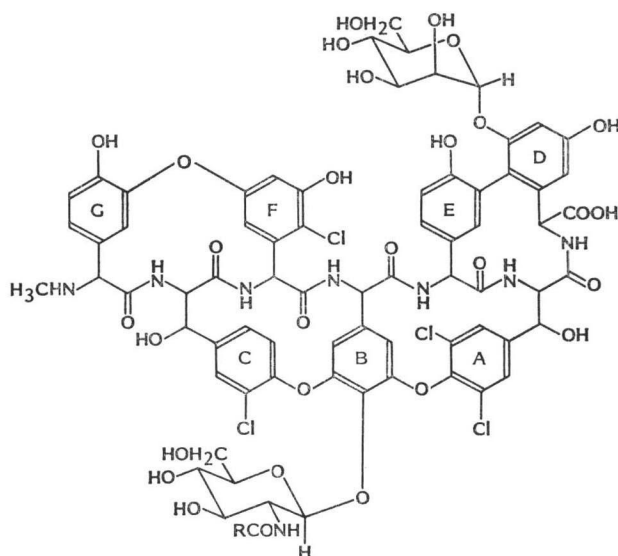


Fig. 5. Structures of the kibdeline A~C₁, C₂ and D.

BUSER *et al.*¹³⁾. The major isomer from the methanolysis was subjected to further methanolysis to afford the methyl ester of the fatty acid. Treatment of the fatty acid with dimethyl disulfide in the presence of iodine gave the dimethyl disulfide adduct. The methane CI-MS of the derivatized sample shows an (M+H)⁺ ion at *m/z* 185 and more importantly two fragment ions at *m/z* 147 and 131 corresponding to the fragments shown in Scheme 2. These results indicate that the ester derived from the methanolysis is methyl-4-decenoate. Furthermore, a study of the reaction of a number of unsaturated fatty acids of known configuration indicates that the stereochemistry of the double bond may be inferred from the rate of formation of the dimethyl disulfide adduct. Under the standard conditions reported by BUSER and co-workers we have found that after 15 hours at 30°C, the addition of dimethyl disulfide to mono-unsaturated fatty acid methyl esters with an *E*-configuration the reaction is complete, whereas the corresponding *Z*-isomers reacts more slowly and undergo only 50~70% conversion to product after this time. A comparison of the rates of dimethyl disulfide addition to 4-methyl decanoate obtained from kibdeline D compare well with those obtained for unsaturated fatty acid methyl esters in the *Z*-series. It follows that the structure of the products derived from the initial methanolysis of kibdeline D are represented by the α - and β -pyranosides as 2 and 3, respectively in which the double bond has the *Z*-configuration.

The two remaining issues concerning the site and stereochemistry of attachment of the C-1 position of the *N*-acylglycopyranoside moiety to the heptapeptide nucleus of the mannosyl aglycon were subsequently resolved by the observation that ¹⁴C-labelled kibdeline A~C serve as biosynthetic pre-

cursors of the corresponding aridicins in *K. aridum* (ATCC 39922)¹⁵⁾. The structures of the kibdelins A~C₁ and C₂ are established by these biosynthetic conversions as indicated by the representations 5~8 shown in Fig. 5, respectively and by analogy kibdelin D is represented as structure 9. The kibdelins thus join the teicoplanins as the second example of glycopeptide antibiotics containing *N*-acyl- β -glucosamine moieties β -linked through the phenolic oxygen in ring B to the parent heptapeptide core of the aglycon.

Conclusion

This study describes the first use of affinity chromatography for the rapid discrimination of novel glycopeptides and resolving structurally distinct classes of these molecules by varying elution conditions from the D-Ala-D-Ala affinity support. The chemical and spectroscopic data supports the conclusion that the kibdelins A, B, C₁, C₂ and D are novel glycopeptides with the structures illustrated in Scheme 2.

Acknowledgments

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References

- 1) SHEARER, M. C.; A. J. GIOVENELLA, S. F. GRAPPEL, R. D. HEDDE, R. J. MEHTA, Y. K. OH, C. H. PAN, D. H. PITKIN & L. J. NISBET: Kibdelins, novel glycopeptide antibiotics. I. Discovery, production, and biological evaluation. *J. Antibiotics* 39: 1386~1394, 1986
- 2) NISHIKAWA, H.: Introduction to affinity purification of biopolymers. *In Solid Phase Biochemistry*. Ed., W. H. SCOUTEN, pp. 17~78, John Wiley & Sons, New York, 1983
- 3) LOWE, C. R. & P. D. G. DEAN: *Affinity Chromatography*. John Wiley & Sons, New York, 1974
- 4) FOLENA WASSERMAN, G.; R. D. SITRIN, F. CHAPIN & K. M. SNADER: Affinity chromatography of glycopeptide antibiotics. *J. Chromatogr.*, to submitted
- 5) SITRIN, R. D.; G. W. CHAN, J. J. DINGERDISSEN, W. HOLL, J. R. E. HOOVER, J. R. VALENTA, L. WEBB & K. M. SNADER: Aridicins, novel glycopeptide antibiotics. II. Isolation and characterization. *J. Antibiotics* 38: 561~571, 1985
- 6) HUNT, A. H.; R. M. MOLLOY, J. L. OCCOLOWITZ, G. G. MARCONI & M. DEBONO: Structure of the major glycopeptide of the teicoplanin complex. *J. Am. Chem. Soc.* 106: 4891~4895, 1984
- 7) BARNA, J. C. J.; D. H. WILLIAMS, D. J. M. STONE, T.-W. C. LEUNG & D. M. DODDRELL: Structure elucidation of the teicoplanin antibiotics. *J. Am. Chem. Soc.* 106: 4895~4902, 1984
- 8) FOLENA-WASSERMAN, G.; B. L. POEHLAND, L. B. KILLMER, E. W.-K. YEUNG, P. W. JEFFS, J. J. DINGERDISSEN, M. C. SHEARER, S. F. GRAPPEL, C. H. PAN & L. J. NISBET: Affinity isolation and characterization of new glycopeptide antibiotics from SK&F AAD-609. Program and Abstracts of the 25th Intersci. Conf. on Antimicrob. Agents Chemother., No. 793, p. 238, Minneapolis, Sept. 29~Oct. 2, 1985
- 9) PAN, C. H.; G. CHAN, J. L. NISBET, M. J. POLANSKY, S. VOGT-SPETH & R. D. SITRIN: Analytical HPLC of the aridicin glycopeptide complex and its application to fermentation development. *J. Indust. Microbiol.*, 1986, in press
- 10) HENNER, J. & R. D. SITRIN: Isoelectric focusing and electrophoretic titration of antibiotics using bioautographic detection. *J. Antibiotics* 37: 1475~1478, 1984
- 11) 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: 713~720, 1985
- 12) JEFFS, P. W.; G. CHAN, R. SITRIN, N. HOLDER, B. D. ROBERTS & C. DEBROSSE: The Structure of the glycolipid components of the aridicin antibiotic complex. *J. Org. Chem.* 50: 1726~1731, 1985
- 13) BUSER, H.; H. ARN, P. GUERIN & S. RAUSCHER: Determination of double bond position in mono-un-

- saturated acetates by mass spectrometry of dimethyl disulfide adducts. *Anal. Chem.* 55: 818~822, 1983
- 14) BOCK, K.; I. LUNDT & C. PEDERSEN: Assignment of anomeric structures to carbohydrates through geminal ^{13}C - ^1H coupling constants. *Tetrahedron Lett.* 1973: 1037~1040, 1973
 - 15) CHUNG, S. K.; P. TAYLOR, Y. K. OH, C. DEBROSSE & P. W. JEFFS: Biosynthetic studies of aridicin antibiotics. I. Labeling patterns and overall pathways. *J. Antibiotics* 39: 642~651, 1986