The Structure of the Glycolipid Components of the Aridicin Antibiotic Complex

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The structures of the glycolipid components of the glycopeptide antibiotics aridicins A, B, and C have been elucidated. Aridicin A on methanolysis yields 2-deoxy-2-[(1-oxodecyl)amino]-α-D-glucopyranosiduronic acid methyl ester (1) and the related methyl α-D-glucofuranosidurono-6,3-lactone 2. The structure of 1 was confirmed by synthesis. Aridicins B and C are shown to differ from aridicin A in the nature of the *N*-acyl side chain of the 2-amino-2-deoxyglucopyranuronic acid moiety, with aridicins B and C containing 9-methyl-1-oxodecyl and 10-methyl-1-oxoundecyl groups, respectively.

Introduction

Isolation of the antibacterial antibiotic complex known as aridicin from the Actinomycete, *Kibdelosporangium aridum*, an organism of a new genus, has been described recently from our laboratories.[1] These antibiotics possess good antimicrobial activity against gram-positive aerobic and anaerobic bacteria. Subsequently, we have shown[2] that the aridicins are members of the class of glycopeptide antibiotics exemplified by vancomycin[3] and ristocetin[4] which possess similar antibacterial activity. The aridicin complex has been resolved into three major components, A, B, and C, each of which afford the same aglycon after mild acid hydrolysis. The structure of this aglycon, which has been defined recently,[5] includes a cyclic peptide with structural features characteristic of the aglycons found in the vancomycin–ristocetin class of antibiotics. The structural relationships among the three major components A, B, and C, therefore, must reflect the differences within the carbohydrate. This paper will present the chemical and spectral studies on the carbohydrate components of these antibiotics which have led to the elucidation of their structures.

Hydrolysis Products of Aridicin A

Mild acid hydrolysis of aridicin A, typically in 0.5 N HCl at 100 °C, produces an initial cascade of hydrolytic products. The course of the hydrolysis, which is conveniently followed by reverse-phase high-performance liquid chromatography (HPLC), indicates that after 15 min, three major products, pseudoaglycon I, pseudoaglycon II, and an aglycon, are formed. Preparative-scale separations of these hydrolysis products was readily effected by reverse phase HPLC on a C-18 column. Acid hydrolysis of pseudoaglycons I and II also afforded the same aglycon. The fast-atom-bombardment mass spectra (FABMS) of aridicin A and its hydrolysis products in the positive ion mode, obtained under conditions of continuum acquisition, contained a cluster of peaks in the molecular ion region of each compound. The derivation of the molecular weights of aridicin A and the hydrolysis products from observation of the $(M + H)^+$ ions led to the relationships which are

Scheme I. Relationship of Products Produced by Mild Acid Hydrolysis of Aridicin A

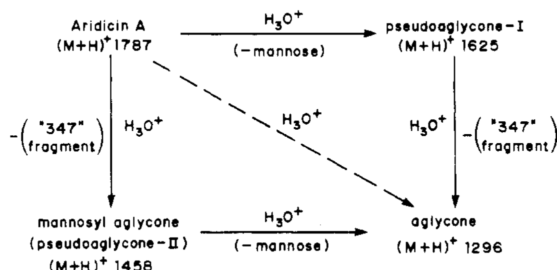

Table I. ^{13}C NMR Carbonyl Chemical Shifts (ppm) for Aridicin A and Its Hydrolysis Products

aridicin A	pseudoaglycon I	mannosyl aglyon	aglycon
177.8	178.2	178.0	178.1
177.6	178.1		
175.6	176.1		
174.6	175.6	174.6	174.6
171.6	171.7	171.7	171.8
170.8	171.0	171.4	170.9
170.4	170.6	171.0	170.9
170.3	170.5	170.1	170.0
169.2	169.3	169.4	169.3

summarized in Scheme I. The mass difference of 162 amu between aridicin A and pseudoaglycon I and between pseudoaglycon II and the aglycon indicated that the hydrolysis reaction in these conversions represents the loss of a hexose unit (M_r = 180). This is consistent with evidence presented earlier that acid hydrolysis of aridicin A and pseudoaglycon II releases mannose.[1] Since pseudoaglycon II is related to the aglycon by the additional presence of a mannose moiety, it will subsequently be referred to as the mannosyl aglycon. Similarly, the mass difference of 329 amu observed in the FABMS between aridicin A and the mannosyl aglycon and likewise between pseudoaglycon I and the aglycon represents the hydrolytic removal of an entity of molecular weight 347. The odd mass value of this latter moiety suggested the presence of nitrogen in its molecular formula. Although this result is reminiscent of the findings reported for other glycopeptide antibiotics[6] of this series which contain amino sugars attached to the central peptide core, attempts to detect the nitrogen-containing carbohydrate fragment released by

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Table II. Apparent pK_a Values of Aridicin A and Its Hydrolysis Products

acidicin A	pseudoaglycon I	mannosyl aglycon	aglycon	assignmts
3.0	3.0	3.3	3.3	carboxyl
4.9	4.3			carboxyl
7.4	7.4	7.1	7.1	ammonium ion
8.4	8.5	8.4	8.3	phenol
10.0	9.9	9.2	9.1	phenol
10.3	10.9	10.0	10.0	phenol
		11.4	11.2	phenol

Table III. N-Acyl Side-Chain ^{13}C Chemical Shifts of Aridicins A, B, and C.

	carbon shift,[a] ppm										
aridicin A	37.3	26.2	29.8	29.7	29.7	29.6	32.2	23.0	14.6		
aridicin B	37.4	26.4	30.3	29.8	29.8	27.9	39.6	28.4	23.3	23.3	
aridicin C	37.4	28.1	30.0	30.0	30.0	30.6	26.4	39.8	28.6	23.5	23.5

[a] Assignments were made by using the equation developed for hydrocarbons by Breitmaier and Voelter.[16]

acid hydrolysis of aridicin A proved unsuccessful. This was surprising since under similar hydrolytic conditions we observed that both vancomycin and ristocetin gave rise to neutral carbohydrates and the amino sugars vancosamine and ristosamine, respectively. These components were all readily characterized by GC–MS as their alditol acetate derivatives.

The difficulty experienced in attempts to isolate the nitrogen-containing carbohydrate fragment suggested that it may differ structurally from vancosamine and other amino sugars previously encountered in this class of antibiotics. Evidence obtained initially from ^{13}C NMR confirmed this suggestion. A comparison of the ^{13}C resonances in the *carbonyl region* of the spectra of aridicin A, pseudoaglycon I, the mannosyl aglycon, and the aglycon (see Table I) revealed that aridicin A and pseudoaglycon I contain two signals which are absent in the mannosyl aglycon and aglycon, indicating that the 347 amu moiety contains two carbonyl functions. Furthermore, determination of the ionizable groups from electrometric titrations of aridicin A and its hydrolysis products (Table II) indicated that both aridicin A and the pseudoaglycon I contain an additional carboxylic acid group with apparent pK_a's of 4.9 and 4.3, respectively, whereas this group is not present in either the mannosyl aglycon or the aglycon. In addition, both the ^1H and ^{13}C NMR spectra (vide infra) of aridicin A and pseudoaglycon I contain signals indicating the presence of a long-chain hydrocarbon fragment which is absent in mannosyl aglycon and the aglycon.

HPLC analysis (Figure 1) of aridicin A and its three aglycons on a reversed-phase C-18 column shed some light on these interrelationships as well. As expected, those transformations involving loss of mannose yield derivatives with higher lipophilicity and longer retention times (cf. aridicin A to the pseudoaglycon I and the mannosyl aglycon to the aglycon). However, the second transformation (aridicin A to the mannosyl aglycon and pseudoaglycon I to aglycon) involves formation of products with shorter retention times reflecting an increase in polarity consistent with the loss of a lipophilic moiety.

The information provided by the foregoing data suggested that aridicin A contains two carbohydrates linked at separate peripheral locations on the central peptide core of the aglycon. One of these carbohydrates is mannose, and the second appears to be a glycolipid containing a carboxyl group and possibly an amide or lactone function based upon the ^{13}C NMR and pK_a data.

Structure of the Glycolipid Component

Despite the difficulty encountered in isolating the glycolipid from hydrolysis of aridicin A, FABMS of the 0.01 N HCl hydrolysate of aridicin A proved to be very in-

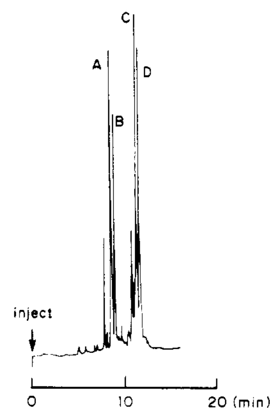
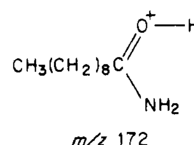


Figure 1. HPLC of aridicin A and its hydrolysis products: column, solvent, Beckman Ultrasphere ODS (4.6 × 150 mm); 7–35% acetonitrile in 0.1 M pH 3.2 phosphate UV 220 mm. Compounds: (A) mannosyl aglycon, 9.2 min; (B) aglycon, 9.6 min; (C) aridicin A, 12.2 min; (D) pseudoaglycon I, 12.5 min.

formative. In addition to the presence of an $(M + H)^+$ ion at m/z 1458 attributable to the pseudoaglycon I, ions associated with the elusive glycolipid were observed at m/z 348 $(M + H)^+$ and 172. High-resolution mass measurements of these latter ions established their elemental compositions as $C_{16}H_{30}NO_7$ and $C_{10}H_{22}NO$, respectively.

Although the occurrence of a triplet methyl signal at δ 0.85 and distorted triplets at δ 2.03 (CH_2CO) and 1.47 (CH_2CH_2CO) together with the appearance of an intense multiplet at δ 1.23 in the 360-MHz ^1H spectrum of the antibiotic was indicative of a long straight chain acyl group, the precise number of methylene groups could not be determined. Fortunately, the high field region of the ^{13}C spectrum of aridicin A provided definitive evidence which permitted the assignment of the hydrocarbon side chain present in the antibiotic as an *n*-decanoyl group (see Table III). This was confirmed by subjecting aridicin A to methanolysis in methanolic HCl to give a product identified as methyl *n*-decanoate by GC–MS. The presence of an *n*-decanoyl residue, implied by this result, allows assignment of the structure of the fragment ion $C_{10}H_{22}NO$ as the protonated *n*-decanamide shown below.



m/z 172

The representation of the glycolipid by the expanded formula $C_5H_8O_4(CO_2H)NHCOC_9H_{19}$ is readily deduced from the foregoing spectral and pK_a data. Furthermore, the formula is consistent with a structure based upon an

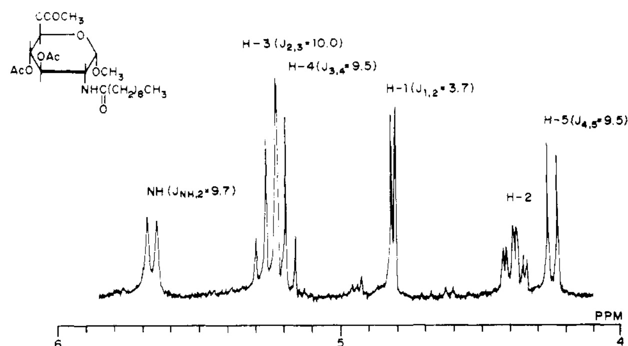
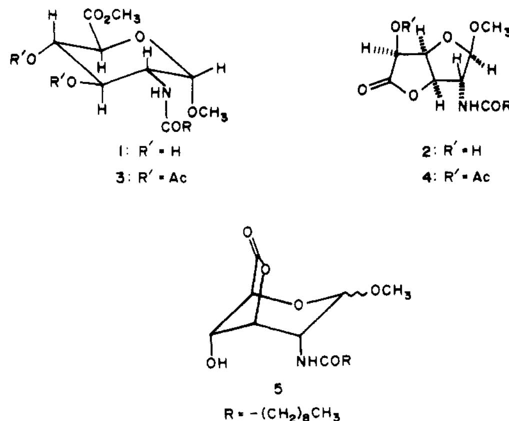


Figure 2. Partial 360-MHz ^{1}H spectrum of 2-deoxy-2-[(1-oxodecyl)amino]-α-D-glucopyranosiduronic methyl ester *O,O*-diacetate (**3**) in $CDCl_3$.

N-decanoyldeoxyaminoglycuronic acid. The presence of a carboxyl group accounts for the difficulty in detecting the uronic acid in the acid hydrolysates of aridicin A by the GC–MS method. Under the conditions employed in converting the acid hydrolysates to their alditol–acetates ($NaBH_4$, Ac_2O), the carboxyl group in the uronic acid is not reduced, and, consequently, lack of detection by GC is understandable. Before further details of the glycolipid structure could be obtained, it was necessary to devise conditions for its isolation.

Although the course of the acid hydrolysis of aridicin A could be followed successfully by reversed-phase HPLC, an extension of this approach to isolating the glycolipid was unsuccessful. Both mannose and the glycolipid are virtually transparent in the UV region, and their detection at various wavelengths from 190 to 210 nm in the presence of the highly absorbing aglycon coproducts proved an insurmountable impediment to devising a preparative separation procedure.

In view of this difficulty, it was decided that methanolysis of aridicin A might offer the best opportunity to generate separable products from the glycolipid and peptide components of the antibiotic. Thus methanolysis of aridicin A in methanolic HCl (0.1 N) at 90 °C for 90 min, followed by preliminary chromatography on silica to remove the more polar aglycon products provided material which was further purified by reversed-phase HPLC to afford two major products, **1** and **2**, in a 2:1 peak ratio. The



ratio of these two products was found to be dependent on the hydrolysis conditions; when the methanolysis was carried out in 0.01 N HCl, compounds **1** and **2** were found in approximately equal amounts. The IR spectrum of compound **1** contains carbonyl bands at 1750 and 1650 cm^{-1} in its IR spectrum in accord with the presence of ester and amide functions. The molecular formula of **1** ($C_{18}H_{33}NO_7$) was established by elemental analysis and FAB mass spectral data [$(M + H)^+$ and $(M - H)^-$ observed at *m/z* 376 and 374, respectively]. This formula is consistent with compound **1** being the methyl glycoside methyl ester of an *N*-decanoylaminodeoxyglycuronic acid. The assignment of structure **1** to the methanolysis product followed from a detailed study of a 360-MHz ^{1}H NMR spectrum of its diacetyl derivative **3**. In addition to the characteristic peaks associated with the aliphatic side chain and methyl resonances of a methyl ester and a methyl glycoside, well-resolved sugar ring and NH protons were observed in the spectrum of **3** (see Figure 2). The assignment of the signals shown in Figure 2 were made following the assignment of the anomeric proton signal from H-1 at δ 4.81 and with the assistance of spin-decoupling studies. Not only were the structural features of a pyranose ring revealed, but also the stereochemistry was clearly defined as *gluco* as indicated from the large ^3J couplings characteristic of trans-diaxial relationships. The location of the nitrogen at C-2 is established by the mutual coupling of H-1 and the NH protons to H-2 and was confirmed by the spin-decoupling experiments. Therefore, the probable structure of the methanolysis product was 2-deoxy-2-[(1-oxodecyl)amino]-α-D-glucopyranosiduronic acid methyl ester (**1**) or its enantiomer.

The IR spectrum of the second product from the methanolysis has carbonyl bands at 1780 and 1650 cm^{-1} indicative of γ-lactone and amide functions, respectively. Supporting evidence for a lactone was provided by the CI mass spectrum of **2** which shows a $(M + H)^+$ ion at *m/z* 344 in accord with a lactone related to **1** by loss of methanol. Although two structures represented by a glucofurano-6,3-lactone (cf. **2**) or an isomeric glucopyrano-6,3-lactone (**5**) are logical candidates, considerable precedent exists which would suggest that the furano-6,3-lactone **2** is the more likely structure.[7] In the D-glucuronic acid series the furano-6,3-lactone is thermodynamically more stable than the pyrano-6,3-lactone and is readily formed by heating an aqueous solution of the parent acid.[8] Thus, the ^{1}H NMR spectra of this methanolysis product and its *O,O*-diacetate were in full agreement with designation of the *furan* structures **2** and **4**, respectively. The β-configuration of the C-1 substituent is clearly indicated by the very small values of $J_{1,2}$ and $J_{2,3}$ (<0.5 Hz) in both **2** and its acetate **4**. This assignment is in accord with previous observations that on glycosidation the β-anomer is known to be more stable than the α-isomer in bicyclic glucofurano-6,3-lactones.[9] These and the other J values obtained are in good agreement with those expected for methyl β-D-glucofuranosidurono-6,3-lactone.[10]

Confirmation of the structure of the methanolysis product **1** was obtained through synthesis. The syntheses of **1** and its diacetate **3** were undertaken by the route shown in Scheme II and are based upon a similar reaction sequence employed for the syntheses of the related compounds, *N*-decanoyl-D-glucosamine[11] and 2-amino-2-deoxy-D-glucopyranuronic acid.[12]

Two synthetic pathways to the common intermediate **8b** were investigated. A two-step procedure from D-

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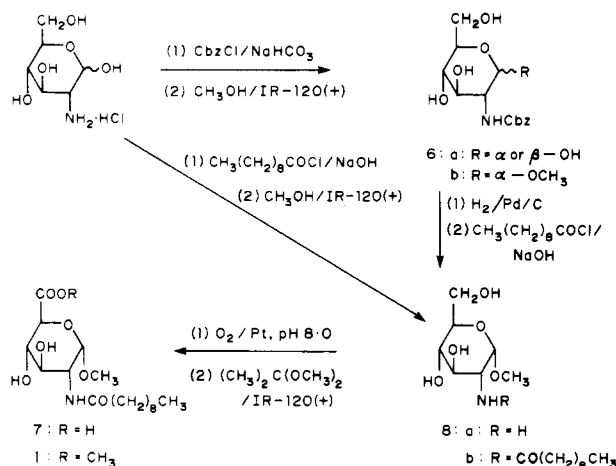
Scheme II. Synthesis of 2-Deoxy-2[(1-oxodecyl)amino]-α-D-glucopyronosiduronic Acid Methyl Ester



glucosamine which involved *N*-acylation with *n*-decanoyl chloride followed by glycosidation in methanol in the presence of acid proceeded to the thermodynamically more stable α-anomer **8b** in good overall yield. Similar yields for this compound were obtained by a more circuitous route employing the *N*-benzyloxycarbonyl intermediate **6a**. Catalytic oxidation of the primary alcohol group in **8b** proceeded smoothly when this compound was heated in the presence of platinum catalyst in aqueous base through which oxygen was continuously passed.[12] The spectral data of compound **7** support the structure and include among the more significant features: (1) an unresolved doublet for the anomeric proton H-1 in accord with the assigned methyl α-glucoside configuration, (2) carbonyl resonances at 170.8 and 172.7 ppm from the carboxylic acid and amide groups, respectively, and (3) a peak at m/z 362 $(M + H)^+$ in the mass spectrum consistent with the molecular formula of $C_{17}H_{31}NO_7$. Also, the pK_a value of 3.4 determined in 30% CH_3CN-H_2O was in good agreement with pK_a's reported for glucuronic acid (pK_a = 3.2).[13] Conversion of the carboxylic acid **7** to its methyl ester was most conveniently effected with acetone dimethyl acetal in the presence of the ion-exchange resin Amberlite IR-120 in its acid form.[14] The ester **1**, mp 135–137 °C and $[\alpha]_D$ +83.4°, was shown to be identical with the glycolipid component obtained from methanolysis of aridicin A by comparison of spectral (^1H NMR, IR, MS) and HPLC data. Furthermore, the CD spectra of the synthetic and natural material were identical which serves to establish that the absolute stereochemistry of **1** is correctly represented by 2-deoxy-2-[(1-oxodecyl)amino]-α-D-glucopyranosiduronic acid methyl ester.

Structures of Glycolipid Components of Aridicins B and C

With the structure of the glycolipid component of aridicin A established, the structures of the analogous fragments in the aridicins B and C were readily determined. The differences in the structures of aridicins A, B, and C are confined to the glycolipid components of these compounds, since selective hydrolysis of the individual antibiotics was shown to give the mannosyl aglycon as a common product.

Corresponding GC–MS analysis of the fatty acid methyl esters derived from aridicin B and C indicated that they were undecanoate and dodecanoate esters, respectively. Unlike the ester derived from aridicin A, they appeared to be branched in that they both displayed shorter GC retention times when compared to the authentic straight chain esters of the same mass.

Examination of the ^{13}C spectra of aridicins B and C provided definitive information on the structural differences which exist within this series. By employing the GASPE ^{13}C editing sequence,[15] aridicin B was shown to contain a fatty acid side chain with an *N*-acyl carbon (37.4 ppm), seven methylene carbons, a methine carbon (24.6 ppm), and two equivalent methyl groups (23.3 ppm) as shown in Table III. This established that the undecanoyl group in aridicin B is present as a 9-methyl-1-(oxodecyl)-*N*-acyl moiety. This result is in accord with the FABMS results which show that aridicin B has a $(M + H)^+$ ion at 14 amu higher than aridicin A. Further evidence supporting this conclusion was provided by the 360-MHz ^1H NMR spectrum of aridicin B which exhibits a six-proton methyl doublet at 0.83 ppm (J = 6.7 Hz) and a methine multiplet at 1.47 ppm.

A similar analysis of the ^{13}C NMR spectra of aridicin C (see Table III) employing the GASPE editing procedure provided evidence for its structure. These latter results together with the GC–MS data on the methanolysis reaction product when taken in conjunction with interlocking results derived from the FABMS of aridicin C, which exhibits an $(M + H)^+$ ion at m/z 1815, clearly indicates that the dodecanoyl group in this antibiotic contains a 10-methyl-1-oxoundecyl side chain.

In summary, the aridicins are members of the vancomycin–ristocetin class of glycopeptide antibiotics containing mannose and novel glycolipids[17] linked to a central peptide core at, as yet, undetermined locations.

Experimental Section

General Methods. Melting points were determined in a Thomas-Hoover capillary melting point apparatus and are uncorrected. Optical rotations obtained in methanol solutions (c = 1) were observed at 589 nm using a Perkin-Elmer 241 MC Polarimeter and elemental analyses were done on a Perkin-Elmer 240 C Elemental Analyzer. Common reagent-grade chemicals were purchased from Aldrich Chemical Co. and were used as received. Samples were dried in a Precision Vacuum Oven at 40 °C for 24 h or under high vacuum at room temperature for 24–48 h.

Routine ^1H NMR spectra were obtained on a 90 MHz Varian EM390 spectrometer, while high field spectra were determined on a JEOL JNM-GX 270 or Bruker WM-360 spectrometer. ^{13}C NMR spectra were recorded on a Varian FT-80 or Bruker WM-360 spectrometer. IR spectra were recorded on a Perkin-Elmer 580

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(18) Borghi, A.; Coronolli, C.; Faniuolo, L.; Allievi, G.; Pallanza, R.; Gallo, G. G. *J. Antibiot.* **1984**, *37*, 615–620.

(19) The three major components of the teichoplanin complex contain *N*-acylamino sugars[20] in which the acyl group which differentiates these antibiotics contain C-10, C-11, and C-12 fatty acids (Hunt, A., Symposium on "Recent Developments on Glycopeptide Antibiotics", 23rd Meeting of ICAAC, Oct 1983, Las Vegas). Following the completion of this paper the exact nature of these fatty acids was published: (a) Hunt, A. H.; Molloy, R. M.; Occolowitz, J. L.; Marconi, G. G.; Debono, M. *J. Am. Chem. Soc.* **1984**, *106*, 4891–4895. (b) Barna, J. C. J.; Williams, D. H.; Stone, D. J. M.; Leung, T.-W.C.; Doddrell, D. M. *J. Am. Chem. Soc.* **1984**, *106*, 4895–4902.

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or 299-B infrared spectrophotometer or on a Nicolet 6000 FT-IR spectrophotometer. Mass spectra were obtained either with a VG Analytical ZAB-IF mass spectrometer equipped with a high field magnet and operated in the fast-atom-bombardment mode with glycerol as solvent matrix or with a Finnegan 3600 spectrometer operated in the CI mode using methane or dimethyl ether as the reagent gas.

High-performance liquid chromatography (HPLC) analyses of samples were determined on a Beckman 345 or Spectra Physics 8100/4000 liquid chromatographs. Analyses were performed with HPLC system A: Beckman Ultrasphere ODS; 18–33% acetonitrile in 0.1 M pH 3.2 phosphate buffer; flow at 1.5 mL/min; detection at 220 nm. Analyses of glycolipids were performed with HPLC system B: Beckman Ultrasphere ODS, 5 μm, 4.6 × 150 mm; 40–50% acetonitrile in water; flow at 1.5 mL/min; detection at 205 nm.

Preparative liquid chromatography (LC) was performed by using a Beckman 112 delivery system equipped with a preparative head and a glass Altex column (25 × 500 mm) packed with Merck Silica Gel 60 (230–400 mesh) or Whatman reversed-phase packing (Partisil 40, ODS-3, 37–63 μm).

Hydrolysis of Aricidin A. Aridicin A (200 mg) was heated at 100 °C for exactly 15 min in 20 mL of 0.5 N HCl. To the resulting solution was added 30 mL of a solution of 20% acetonitrile in phosphate buffer (pH 3.2, 0.1 M), and the mixture was filtered. The filtrate was pumped on to a reversed-phase column (2.5 × 50 cm Whatman ODS-3) which had been preequilibrated with 10% acetonitrile in phosphate buffer (pH 3.2, 0.1 M), and the column was eluted with a step gradient (15–25%) of acetonitrile in the phosphate buffer. The UV-absorbing (254 nm) fractions were assayed by HPLC using system A. The fractions containing pure aglycon (retention time 5.5 min) were combined and lyophilized. The residue was dissolved in 60 mL of 5% aqueous acetonitrile and then desalted by passing solution through a XAD-7 resin column (100 mL). After being washed with five column volumes of water, the residue was eluted with four column volumes of acetonitrile. The organic eluates were combined and lyophilized to give 26 mg of aglycon. With the same desalting procedure, pseudoaglycon I (retention time 10.2 min) and the mannosyl aglycon (retention time 4.4 min) were also isolated (62 and 36 mg, respectively).

Conversion of Pseudoaglycon I to Aglycon. Pseudoaglycon I (0.2 mg) was heated in 0.3 mL of 0.1 N HCl at 100 °C for 10 min. The mixture was assayed by analytical HPLC (system A) and showed only one major product which had a retention time identical with that of the aglycon (5.5 min).

Conversion of Mannosyl Aglycon to Aglycon. Mannosyl aglycon (0.2 mg) was heated in 0.3 mL of 0.1 N HCl at 100 °C for 10 min. The mixture was assayed by analytical HPLC (system A) and showed only a major product which had a retention time identical with that of the aglycon (5.5 min).

Sugar Determination. A. Neutral Sugar. Neutral and basic carbohydrates were assayed as described previously.[1]

B. Glycolipid. A solution of aridicin A (200 mg) in 200 mL of acetonitrile–0.01 N hydrochloric acid (20:80, v/v) was heated at reflux for 20 h. Analysis of the solution by HPLC (system A) showed the complete absence of starting material and the presence of mannosyl aglycon (70%) and an unidentified product (30%). The solution was evaporated in vacuo. FAB mass spectrometry of the residue showed an intense ion peak at m/z 1458 and a weak ion peak at m/z 348. A high-resolution measurement on the latter peak gave m/z 348.203 [calcd for $C_{16}H_{36}NO_7$ $(M + H)^+$ 348.202].

Methanolysis of Aridicin A. A solution of 1.5 g of aridicin A in 30 mL of 0.1 N methanolic hydrochloric acid was heated in a sealed tube to 110 °C for 30 min after which all the aridicin A was gone (HPLC, system A). The solution was neutralized to pH 7, and the solvents were removed by lyophilization. Medium-pressure chromatography of the lyophilizate in petroleum ether–acetone (2:8) on a silica gel column (25 × 500 mm) gave 130 mg of the glycolipid mixture eluted in two column volumes. Final purification was effected on a Whatman ODS-3 column (25 × 500 mm) eluted with a step gradient of 20%, 30%, 40%, and 60% acetonitrile in water. Fractions were combined according to analytical HPLC (system B) and lyophilized to give two products, ester **1** (32 mg) and lactone **2** (19 mg). Lactone **2**: mp 80–82 °C; IR (KBr) 3500–3400 (OH) 2950–2850 (CH_2), 1780 (δ-lactone C=O), 1650 (amide CO) cm^{-1}; ^1H NMR (360 MHz, acetone-d_4, partial assignment) δ 4.39 (t, 1 H, H-5), 4.57 (d, 1 H, $J_{2,NH}$ = 6.7 Hz, H-2), 4.93 (d, 1 H, $J_{3,4}$ = 5.0 Hz, H-3), 4.96 (dd, 1 H, H-4), 4.98 (s, 1 H, H-1), 5.56 (d, 1 H, $J_{NH,2}$ = 6.7 Hz, NH); ^{13}C NMR ($CDCl_3/CD_3OD$) δ 108.80 (C-1), 55.77 (C-2), 82.45 (C-3), 77.50 (C-4), 69.27 (C-5), 174.21 (C-6), 175.25 (CONH), 59.38 (OCH_3), 36.13–22.74 (8 CH_2) 14.12 (CH_3); mass spectrum (CI, reagent gas, CH_4), m/z 344 $(M + H)^+$, 312 $(M + H - CH_3OH)^+$, 172 $(C_9H_{19}CONH_2 + H)^+$. Anal. Calcd for $C_{17}H_{29}NO_6$: m/z 343.199. Found: m/z 343.199. Compound **4** was obtained as an oil after **2** (2 mg) was allowed to stand for 18 h with acetic anhydride (0.05 mL) in pyridine (0.15 mL): ^1H NMR (360 MHz, acetone-d_6, partial assignment) δ 4.64 (d, 1 H, $J_{2,NH}$ = 7.8 Hz, H-2), 4.92 (s, 1 H, H-1), 4.94 (d, 1 H, $J_{5,4}$ = 5.2 Hz, H-5), 5.12 (dd, 1 H, $J_{4,3}$ = 6.5, $J_{4,5}$ = 5.2 Hz, H-4), 5.22 (d, 1 H, $J_{3,4}$ = 6.5 Hz, H-3), 5.37 (d, 1 H, $J_{NH,2}$ = 7.8 Hz, NH).

Ester 1: mp 123–125 °C (solvate); $[\alpha]^{25}$ +80.0° (MeOH); CD (*c* 0.01, MeCN), 25°; $[\theta]_{204}$ 0; $[\theta]_{212}$ −46000; $[\theta]_{253}$ 0; IR (KBr) 3500–3400 (OH), 2950–2850 (CH_2), 1750 (ester C=O), 1650 (amide CO) cm^{-1}; ^1H NMR (270 MHz, pyridine-d_5) δ 0.84 (t, 3 H, CH_3), 1.05–1.39 (m, 12 H, 6 CH_2), 1.84 (m, 2 H, CH_2), 2.56 (t, 2 H, CH_2), 3.40 (s, 3 H, OCH_3), 3.78 (s, 3 H, $COOCH_3$), 4.45–4.55 (m, 2 H, H-4, H-3), 4.66 (m, 1 H, H-5), 4.87 (m, 1 H, H-2), 5.3 (d, 1 H, $J_{1,2}$ = 4 Hz, H-1); mass spectrum (CI, methane), m/z 376 $(M + H)^+$, 344 $[(M + H) - CH_3OH]^+$, 326 $(344 - H_2O)^+$, 316 $(M - COOCH_3)^+$, 172 $(C_9H_{19}CONH_3)^+$, 155 $(172 - NH_3)^+$. Anal. Calcd for $C_{18}H_{33}NO_7$: m/z 375.226. Found: m/z 375.224.

Preparation of *O,O*-Diacetyl Derivative 3 of the Ester 1. A solution of 2 mg of the ester **1** in 0.05 mL of dry pyridine and 0.15 mL of acetic anhydride was stored at room temperature for 18 h. The mixture was evaporated in vacuo to dryness to give the *O,O*-diacetyl compound **3**. It's ^1H NMR spectrum was identical with that of the compound obtained by synthesis.

Characterization of Fatty Acid Methyl Esters from Methanolysis of the Aridicins. The antibiotic (3 mg) was dissolved in 1 mL of 2 N methanolic HCl and heated in a sealed Pierce side-arm flask at 100–110 °C for 4 h. After the reaction mixture was allowed to cool to room temperature, 2 mL of 5% (w/v) aqueous $NaHCO_3$ was added and the resulting mixture was extracted twice with 0.5-mL portions of pentane. The combined pentane extracts after being dried over anhydrous Na_2SO_4 were filtered through a small plug of cotton in a Pasteur pippette and injected directly into a DB-17 capillary column (15 m × 0.25 mm i.d.) interfaced with a Finnigan 3600 mass spectrometer operating in the CI mode with methane as the reagent gas and helium as the carrier gas. The column was temperature programmed from 80 to 250 °C at a rate of 10 °C/min. Characterization of the esters were made by comparisons with the GC–MS data obtained from authentic samples of the straight chain methyl esters as follows: aridicin A fatty acid ester (11.47 min, $(M + H)^+$ m/z 187), aridicin B (12.45 min, $(M + H)^+$ m/z 201), aridicin C (14.07 min, $(M + H)^+$ m/z 215), methyl *n*-decanoate (11.47 min, $(M + H)^+$ m/z 187), methyl undecanoate (13.13 min, $(M + H)^+$ m/z 201), and methyl dodecanoate (14.72 min, $(M + H)^+$ m/z 215).

Methyl 2-Deoxy-2-[[(phenylmethoxy)carbonyl]amino]-α-D-glucopyranoside (6b). To a solution of 2-deoxy-2-[[(phenylmethoxy)carbonyl]amino]-D-glucopyranose (**6a**)[21] (25.0 g, 0.08 mol) in 750 mL of dry methanol was added 50.0 g of dried Amberlite IR-120(H^+) resin. The mixture was stirred and heated at reflux temperature for approximately 24 h. The reaction was monitored by HPLC (μ Bondapak Carbohydrate column (10 μm, 3.9 × 300 mm); mobile phase, water/acetonitrile (10/90); flow rate 1.0 mL/min; detector UV, 220 nm; sample 10 μL at concentration ~1 mg/mL; temperature, 25 °C). When the reaction was complete, the mixture was filtered, the solvent was removed under vacuum, and the product was crystallized from ethanol to give 19.5 g (75%) of **6b**: mp 157–159 °C; $[\alpha]^{25}$ +95.2° (*c* 1, MeOH); IR (KBr) 3320 (OH), 1685, 1545 (NC=O) cm^{-1}; ^1H NMR [$(CD_3)_2SO$] δ 7.35 (s, 5 H, C_6H_5), 7.00 (d, 1 H, $J_{NH,2}$ = 9 Hz, NH), 5.05 (s, 2 H, CH_2Ph), 4.95 (1 H, H-1), 3.29 (s, 3 H, CH_3); ^{13}C NMR δ 98.08 (C-1), 54.33 (C-2), 70.75 (C-3), 70.65 (C-4), 72.68 (C-5), 60.86 (C-6), 156.12 (CONH), 55.93 (OCH_3); mass spectrum (FAB), m/z 328 $(M + H)^+$, 326 $(M - H)^-$. Anal. Calcd for $C_{15}H_{21}NO_7$:

(21) Chargraff, E.; Bovarnick, M. *J. Biol. Chem.* **1937**, *118*, 421–426.

C, 55.05; H, 6.42; N, 4.28. Found: C, 54.96; H, 6.41; N, 4.18.

Methyl 2-Amino-2-deoxy-α-D-glucopyranoside (8a). Into a 500-mL high-pressure bottle were placed **6b** (17.0 g, 0.05 mol), 2.0 g of 10% palladium on active carbon, and water (240 mL), and the mixture was shaken under a hydrogen atmosphere at 35 psi for 6 h at room temperature in a Parr shaker. The mixture was filtered through Celite, and the residue was washed with water (4 × 10 mL). A second filtration using a 0.45-m Nylon-66 filter was employed to remove residual catalyst. The aqueous filtrate was lyophilized, and the residue (8.7 g, 87%) was crystallized from water/methanol to give **8a**: mp 57–60 °C; $[\alpha]^{25}$ +145.6° (*c* 1, MeOH); IR (KBr) 3400 (OH, NH_2), 1580 (amine) cm^{-1}; ^1H NMR $[(CD_3)_2SO]$ δ 4.56 (b s, 1 H, H-1), 3.28 (s, 3 H, OCH_3), 3.00–4.20 (m, 4 H, H-2, H-3, H-4, H-5); ^{13}C NMR δ 100.12 (C-1), 54.34 (C-2), 73.08 (C-3), 70.47 (C-4), 75.12 (C-5), 61.06 (C-6), 56.35 (OCH_3); mass spectrum (FAB), *m/z* 194 $(M + H)^+$, 192 $(M - H)^-$. Anal. Calcd for $C_7H_{15}NO_5$: C, 43.52; H, 7.83; N, 7.25. Found: C, 43.29; H, 7.62; N, 6.91.

Methyl 2-Deoxy-2-[(1-oxodecyl)amino]-α-D-glycopyranoside (8b). Method a. Decanoyl chloride (15.0 g, 0.08 mol) was slowly added over 15 min to a cold stirred solution of methyl 2-amino-2-deoxy-α-D-glucopyranoside (**8a**) (10 g, 0.05 mol) in 170 mL of water. The mixture was stirred at ∼-10 °C for an additional 2 h during which time the pH was maintained at 8.0 with 5% NaOH solution. The reaction mixture was stored at 4 °C for 48 h, after which the mixture was filtered and the product washed with water and dried in a vacuum oven to yield 13.5 g (75%) of crude solid. This was triturated with hot ether, the mixture was filtered, and the residue was washed with ether and dried in a vacuum oven to give **8b**: mp 149–151 °C; $[\alpha]^{25}$ +95.5° (*c* 1, MeOH); IR (KBr) 3300 (OH), 1650, 1550 (amide) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.24 (d, 1 H, $J_{NH,2}$ = 8.8 Hz, NH), 4.68 (d, 1 H, $J_{1,2}$ = 3.7 Hz, H-1), 3.98–4.03 (m, 1 H, H-2), 3.40–3.60 (m, 3 H, H-3, H-4, H-5), 3.36 (s, 3 H, OCH_3), 2.24 (t, 2 H, $NCOCH_2$), 1.62 (m, 2 H, CH_2), 1.26 (m, 12 H, 6 CH_2), 0.88 (t, 3 H, CH_3); ^{13}C NMR δ 98.00 (C-1), 53.82 (C-2), 70.89 (C-3), 70.73 (C-4), 72.75 (C-5), 60.93 (C-6), 172.60 (amide), 54.38 (OCH_3) 22.13–35.24 (8 CH_2), 13.95 (CH_3); mass spectrum (FAB), *m/z* 348 $(M + H)^+$. Anal. Calcd for $C_{17}H_{33}NO_6$: C, 58.77; H, 9.57; N, 4.03. Found: C, 58.34; H, 9.40; N, 3.84.

Method b. 2-Deoxy-2-[(1-oxodecyl)amino]-D-glucopyranose (0.25 g) was dissolved in dry methanol (25 mL). To this solution was added 0.50 g of dried Amberlite IR-120(H^+) resin, and the mixture was stirred at reflux temperature for approximately 30 h. The mixture was filtered, and the solvent was removed under vacuum leaving a solid residue (0.2 g) whose ^1H NMR spectrum was the same as that recorded for **8b** obtained in method a.

2-Deoxy-2-[(1-oxodecyl)amino]-α-D-glucopyranosiduronic Acid (7). To methyl 2-deoxy-2-[(1-oxodecyl)amino-α-D-glucopyranoside (**8b**) (15.00 g, 0.04 mol) in a 2-L four-neck round-bottom flask equipped with an overhead stirrer, refluxed condenser, thermometer, and an oxygen inlet was added a suspension of 5.2 g of freshly prepared platinum catalyst in 500 mL of water. The resulting mixture was heated at 90 °C with stirring while oxygen was slowly bubbled into the solution; during the reaction the pH was maintained at 8.0 with solid sodium bicarbonate. After ∼2 h the oxidation was complete (TLC). The solution was passed through Nylon-66 0.45-m pore size filter. The filtrate was lyophilized, and the residue was redissolved in 300 mL of water. After the solution was adjusted to pH 2 with 12 N HCl, the flask was placed in a refrigerator for 18 h. The product was collected by filtration, washed with water, and dried in a vacuum oven to afford 14.0 g (90%). An analytical sample was obtained by triturating the product with hot ether: mp 153–155 °C; $[\alpha]^{25}$ +88.2° (*c* 1, MeOH); p*K* = 3.4 (30% CH_3CN in H_2O); IR (KBr) 3300 (OH), 1710 (COOH), 1645, 1550 (amide) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.58 (d, 1 H, $J_{NH,2}$ = 8.1 Hz, NH), 4.79 (br, 1 H, H-1), 4.20–3.60 (m, 4 H, H-2, H-3, H-4, H-5), 3.42 (s, 3 H, OCH_3), 2.24 (t, 2 H, $NCOCH_2$), 1.62 (m, 2 H, CH_2), 1.26 (m, 12 H, 6CH_2), 0.89 (t, 3 H, CH_3); ^{13}C NMR δ 99.64 (C-1), 58.48 (C-2), 72.27 (C-3), 71.84 (C-4), 70.21 (C-5), 170.15 (C-6), 172.67 (amide), 54.95 (OCH_3), 35.19–22.11 (8 CH_2), 13.96 (CH_3); mass spectrum (FAB), *m/z* 362 $(M + H)^+$, 360 $(M - H)^-$. Anal. Calcd for $C_{17}H_{31}NO_7$: C, 56.51; H, 8.59; N, 3.88. Found: C, 56.27; H, 8.82; N, 3.81.

2-Deoxy-2-[(1-oxodecyl)amino]-α-D-glucopyranosiduronic Acid Methyl Ester (1). Methyl 2-deoxy-2-[(1-oxodecyl)-amino]-α-D-glucopyranosiduronic acid (**7**) (0.2 g, 0.5 mmol) was dissolved in 0.1 mL of 2,2-dimethoxypropane (98%), and 0.2 g of dry Amberlite IR-120(H^+) resin and 0.01 mL of methanol were added. The mixture was stirred at room temperature, and 0.1-mL aliquots of 2,2-dimethoxypropane were added at 20-min intervals. After 2 h TLC indicated the reaction was complete. Crystallization from ethyl acetate/petroleum ether (bp 30–60 °C) yielded 0.15 g (74%) of the ester **1**: mp 135–137 °C; $[\alpha]^{25}$ +83.4° (*c* 1, MeOH); CD (*c* 0.01, CH_3CN) 25°; $[\theta]^{202}$ 0; $[\theta]_{213}$ -50000; $[\theta]_{253}$ 0; IR ($CHCl_3$) 3300–3500 (OH), 1744 (ester CO), 1664, 1530 (amide) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.24 (d, 1 H, J_{NH2} = 7.9 Hz, NH), 4.77 (d, 1 H, $J_{1,2}$ = 2.9 Hz, H-1), 4.09 (d, 1 H, $J_{5,4}$ = 8.9 Hz, H-5), 3.84 (s, 3 H, $COOCH_3$), 3.41 (s, 3 H, OCH_3), 3.80–3.60 (m, 3 H, H-2, H-3, H-4), 2.24 (t, 2 H, $NCOCH_2$), 1.62 (m 2 H, CH_2), 1.26 (m, 12 H, 6CH_2), 0.88 (t, 3 H, CH_3); ^{13}C NMR δ 99.31 (C-1), 54.02 (C-2), 72.79 (C-3), 72.28 (C-4), 70.64 (C-5), 170.35 (C-6), 173.14 (CONH), 55.64 (OCH_3), 52.53 ($COOCH_3$), 35.73–22.68 (8 CH_2), 14.53 (CH_3); mass spectrum (FAB), *m/z* 376 $(M + H)^+$, 374 $(M - H)^-$. Anal. Calcd for $C_{18}H_{33}NO_7 \cdot {}^1/_4H_2O$: C, 56.90; H, 8.89; N, 3.69. Found: C, 56.81; H, 8.68; N, 3.70.

2-Deoxy-2-[(1-oxodecyl)amino]-α-D-glucopyranosiduronic Acid Methyl Ester 3,4-Diacetate (3). A solution of the methyl ester **1** (0.250 g, 0.0007 mol) in 5 mL of pyridine and 1.0 mL of acetic anhydride was stirred for 3 h at 25 °C. The solvent was evaporated under vacuum, and the residual pyridine was removed by codistillation with toluene leaving a gummy residue which was chromatographed on a silica column. Elution with 10% ethyl acetate in petroleum ether (30–60 °C) gave **3** which crystallized from ethyl acetate/petroleum ether (30–60 °C) (95 mg): mp 75–76 °C; $[\alpha]^{25}$ +84.8° (*c* 1, MeOH); IR (KBr) 1753 (ester C=O), 1652, 1553 (amide) cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.66 (d, 1 H, $J_{NH,2}$ = 9.7 Hz, NH), 5.30–5.16 (m, 2 H, $J_{3,4} = J_{4,5}$ = 9.5, $J_{2,3}$ = 10.0 Hz, H-3, H-4), 4.81 (d, 1 H, $J_{1,2}$ = 3.7 Hz, H-1), 4.42–4.34 (s, 1 H, $J_{2,1}$ = 3.7, $J_{2,3}$ = 10.0, $J_{2,NH}$ = 9.7 Hz, H-2), 4.25 (d, 1 H, $J_{5,4}$ = 9.5 Hz, H-5), 3.75 (s, 3 H, $COOCH_3$), 3.45 (s, 3 H, OCH_3), 2.20 (m, 2 H, $NCOCH_2$), 2.0 (s, 3 H, $COCH_3$), ∼2.0 (s, 3 H, $COCH_3$), 1.75 (bs, 2 H, CH_2), 1.55 (m, 2 H, CH_2), 1.35 (m, 10 H, 5CH_2), 0.90 (t, 3 H, CH_3); ^{13}C NMR δ 98.35 (C-1), 50.33 (C-2), 67.89 (C-3, C-4), 69.97 (C-5), 169.46 (C-6), 172.75 (CONH), 169.11, 167.85 ($OCOCH_3$), 55.47 (OCH_3), 52.43 ($COOCH_3$), 20.33, 20.19 ($OCOCH_3$) 34.25–22.68 (8 CH_2), 13.86 (CH_3); mass spectrum (FAB), *m/z* 460 $(M + H)^+$. Anal. Calcd for $C_{22}H_{37}NO_9 \cdot {}^1/_2H_2O$): C, 56.42; H, 8.17; N, 2.99. Found: C, 56.42, H, 7.97; N, 2.98. Further elution of the column with 50% ethyl acetate/petroleum ether (30–60 °C) gave fractions which were mixtures.

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