Aridicin Aglycon: Characterization of the Amino Acid Components

Peter W. Jeffs,* George Chan, Luciano Mueller, Charles DeBrosse, Lee Webb, and Robert Sitrin*

SmithKline and French Laboratories, Philadelphia, Pennsylvania 19101

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Hydrolysis of the glycopeptide antibiotic aridicin A in acid under controlled conditions affords (2*R*,2'*S*)-actinoidinic acid (**2**) and the biphenyl ether bis(amino acid) **3**. Isolation of **2** and **3** from the acid hydrolyzate is accomplished by preparative reversed-phase HPLC which affords **2** as a mixture of the atropisomers **2a** and **2b**. The configurations of **2a** and **2b** were established from their CD spectra by application of the exiton chirality rule. A new variation of the COLOC pulse sequence is utilized to define the substitution pattern on the aromatic rings in **3** from chemical shift and long-range $^{13}C-^{1}H$ coupling data. The structure of **3** is fully defined through characterization of the rearrangement product **4** resulting from the reaction of **3** with MeI in the presence of K_2CO_3. Oxidation and basic hydrolysis of the permethylated aglycon of aridicin A affords a tricarboxylic acid which is shown to have structure **6** on the basis of spectral studies of its trimethyl derivative **7**. Consideration of the structures of **2**, **3**, and **6** and comparison with similar structures derived from other members of this family of antibiotics suggests that aridicin aglycon is represented by one of the isomers of structure **8**.

The isolation of the aridicin antibiotic complex from the organism *K. aridum* (SK&F AAD-216) has been described recently and evidence has been presented[1] which demonstrates that these antibiotics belong to the glycopeptide class exemplified by vancomycin,[2] ristocetin,[3] and teicoplanin.[4] The aglycons of glycopeptide antibiotics all contain a heptapeptide nucleus composed of cross-linked aromatic amino acids of general structure **1**. The primary differences between members of this class lie in the structure(s) of the X and Y amino acids although variations exist as well in the number and location of chlorine atoms on the aromatic rings and the presence or absence of one or more of the following groups: a benzylic hydroxyl (C1'), an *N*-methyl, and a methyl ester. The three major components of the aridicin complex have been shown on controlled acid hydrolysis to yield a common heptapeptide aglycon, mannose, and, in addition, an *N*-acylglucuronic acid. Differences in the nature of the *N*-acyl group account for the structural variations between aridicins A–C.[5] It has been shown that these acyl functions are a 1-decanoyl group in aridicin A and 9-methyl-1-decanoyl and 10-methyl-1-undecanoyl groups in aridicins B and C, respectively. The aglycon is obtained by mild acid hydrolysis of the aridicin complex or its components. Its fast atom bombardment mass spectrum (FABMS) exhibits a molecular ion cluster with the M + H ion at 1296.075 which is in agreement with a molecular formula of $C_{59}H_{49}N_7O_{19}Cl_4$.[6] This report described the structure of the component amino acids of heptapeptide aglycon of the aridicins.

Results

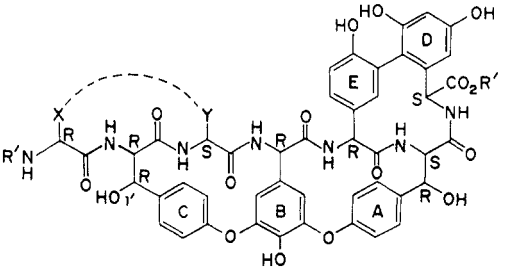
Acid Hydrolysis Products. Previous studies have shown that acid hydrolysis of the vancomycin class of antibiotics produces actinoidinic acid (**2**), the only exceptions being the antibiotics A41030 and A47394 which yield a monochloro analogue of **2**.[7] In addition to the formation of an actinoidinic acid fragment, acid hydrolysis also gives either a diamino acid (X–Y) or two monoamino acids fragments (X,Y) depending on the entities present in structure **1**. Since isolation and characterization of the hydrolysis products provide useful structural information on the aglycons in this class of compounds, particularly with regard to characterization of the X,Y amino acid fragment(s), the approach is valuable. The utility of this method has suffered in the past from difficulties associated with separation and purification of the amino acids by conventional ion-exchange chromatography and the need to employ derivatization of the amino acids for characterization.

To overcome these limitations previously inherent in the method, we investigated the application of reversed-phase high-performance chromatography (RP-HPLC) for the separation and purification of the amino acids present in the acid hydrolyzates of representative examples of these

Table I. HPLC Retention Times[a] for Amino Acids in 6 N HCl Hydrolyzates Derived from Glycopeptide Antibiotics

antibiotic	peak 1 (**2a** + **2b**), actinoidinic acids, min	peak 2, min
Aridicin A	1.4	4.5
Vancomycin	1.4	*b*
Ristocetin	1.4	11.3

[a] Column: Beckman Ultrasphere ODS, 4.6 × 150 mm. Solvent: 0.1% TFA in water for 5 min followed by a gradient to 40% acetonitrile over 10 min. Detection: 220 nm. [b] *N*-methylisoleucine and aspartic acid do not have sufficiently strong chromophores to permit detection at the low concentrations encountered in this experiment.



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(1) Sitrin, R. D.; Chan, G. W.; Dingerdissen, J. J.; Holl, W.; Hoover, J. R. E.; Valenta, J. R.; Webb, L.; Snader, K. M. *J. Antibiot.* **1985**, *38*, 561–571.

(2) (a) Harris, C. M.; Harris, T. M. *J. Am. Chem. Soc.* **1980**, *104*, 4293–4295. (b) Williams, D. H.; Williamson, M. P.; Butcher, D. W.; Hammond, S. J. *J. Am. Chem. Soc.* **1983**, *105*, 1332–1338.

(3) (a) Kalman, J. R.; Williams, D. H. *J. Am. Chem. Soc.* **1980**, *102*, 894–905. (b) Harris, C. M.; Harris, T. M. *J. Am. Chem. Soc.* **1982**, *104*, 363–365. Harris, C. M.; Kopecka, M.; Harris, T. M. *J. Am. Chem. Soc.* **1983**, *105*, 6915–6922.

(4) (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.

(5) Jeffs, P. W.; Chan, G. W.; Sitrin, R. D.; Holder, N.; Roberts, G. D.; DeBrosse, C. *J. Org. Chem.* **1985**, *50*, 1726–1731.

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(7) Hunt, A. H.; Dorman, D. E.; Debono, M.; Molloy, R. M. *J. Org. Chem.* **1985**, *50*, 2031–2035. Hamill, R. L.; Boeck, L. D.; Kastner, R. E., Gale, R. M. Presented at the 23rd Meeting of ICAAC, Oct 1983, Las Vegas; Abstract 443.

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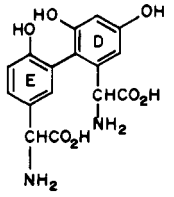
antibiotics and the use of fast atom bombardment mass spectrometry (FABMS) and NMR for their direct characterization.[8]

Aridicin A and other glycopeptide antibiotics including vancomycin and ristocetin were hydrolyzed for 18 h in 6 N HCl at 125 °C in a sealed tube, and, after evaporation of the solution, the residues were analyzed by HPLC on a reversed-phase C-18 column using a gradient of acetonitrile 0.1% aqueous trifluoroacetic acid. In this solvent system, actinoidinic acid elutes at a retention time of 1.4 min as confirmed by analysis of a reference sample kindly provided by Professor Thomas Harris. A peak at this retention time appears in the chromatograms of the hydrolyzates of all the antibiotics examined including aridicin A (See Table I).

The second component of aridicin hydrolyzate, **3**, eluting at 4.5 min, was not present in the hydrolyzates of any other glycopeptides examined.[8] In order to carry out spectroscopic characterization of this key amino acid, the hydrolysis of aridicin A was carried out on a larger scale (200 mg) and afforded samples of both components after isolation by preparative HPLC.

Examination of these amino acids by FABMS showed an $(M + H)^+$ ion at m/z 347 for the short retention time component as expected for actinoidinic acid, whereas the second component when examined under high-resolution conditions contained an $(M + H)^+$ ion at m/z 397.079, in agreement with a molecular formula of $C_{17}H_6N_2O_7Cl$ (calcd m/z 397.080). Furthermore, the FABMS of this second amino acid contained a fragment ion at m/z 366 suggestive of the loss of methylamine whereas the MS of actinoidinic acid showed a strong fragment ion at m/z 331 for $(M + H)^+ - NH_3$.

Preliminary examination of the hydrolysis products **2** and **3** by ^1H NMR indicated both were mixtures of diastereomers. Analytical separation of the individual components in each mixture was accomplished by a second RP-HPLC system using acetonitrile and water containing 0.1% heptafluorobutyric acid (HFBA) as a lipophilic ion pairing agent. The actinoidinic acid fraction was resolved



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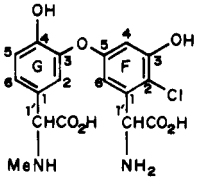


3

into four components, whereas amino acid **3** was resolved into two peaks (see Figure 1a). While the observation of what appeared to be four diastereomers of actinoidinic acid was somewhat unexpected in that it contains only two chiral centers, the potential for an additional element of asymmetry (axial) exists if the biphenyl system has a high barrier to rotation (vide infra).

A study of the hydrolysis conditions using RP-HPLC with this HFBA solvent system to monitor the course of the reaction indicated that complete hydrolysis could be effected in 6 N HCl/125 °C sealed tube with minimal epimerization of the chiral centers in **2** and **3** if the reaction was terminated after 8 h. Under these conditions, the actinoidinic acid consisted of two major components and the amino acid **3** was essentially a single diastereomer (See

(8) The utility of using reversed-phase high-performance chromatography in combination with FAB-MS for isolation and characterization of the amino acids obtained from acid hydrolysis of this class of glycopeptide antibiotic will be the subject of a forthcoming paper.

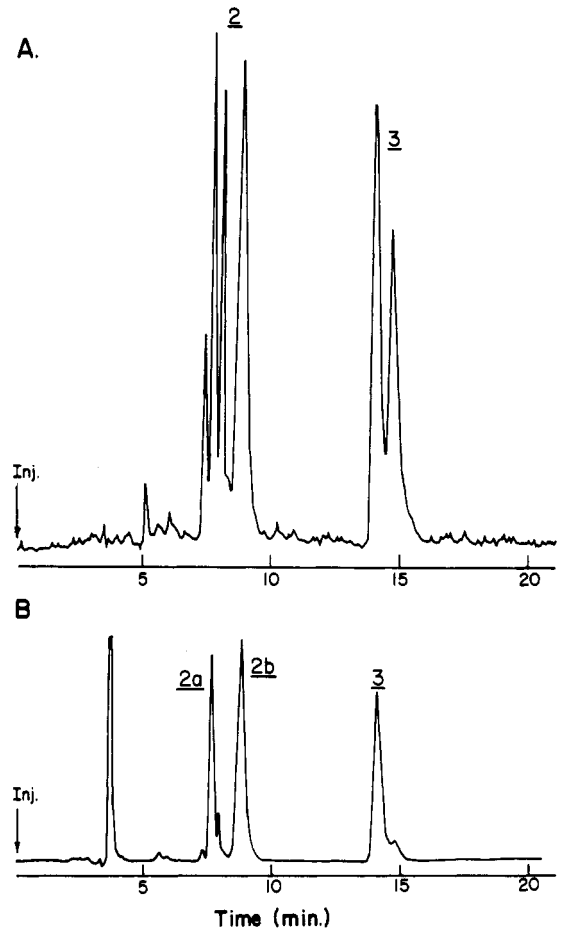


Figure 1. (a) Analytical HPLC chromatogram of product from 6 N HCl hydrolyzate of aridicin A after 28 h: solvent, 6% acetonitrile in 0.1% HFBA, Beckman Ultrasphere ODS 4.6 × 250 mm, 1 mL/min; detection; UV at 254 nm. (b) Analytical chromatogram of product from 6 N HCl hydrolyzate of aridicin A after 8 hr. Column conditions as in Figure 1a.

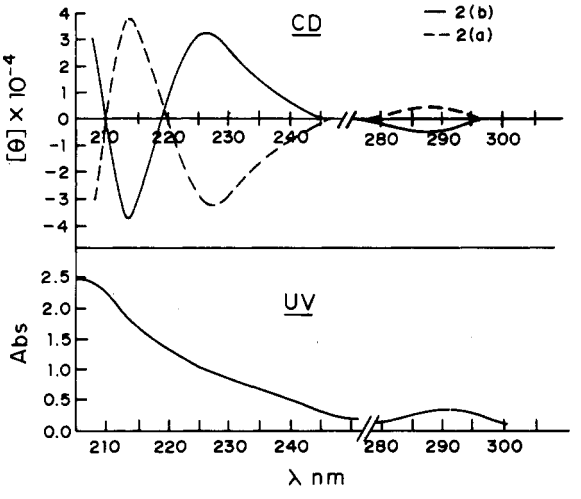


Figure 2. CD and UV spectra of the actinoidinic acid atropisomers **2a** and **2b** in water at pH 5.0.

Figure 1b). In a preparative-scale reaction, aridicin A (1 g) was hydrolyzed and separated by preparative RP-HPLC into two actinoidinic acids [$(M + H^+) = 347$] and a single diastereomer of **3**.

Evidence that the actinoidinic acids produced from the hydrolysis were indeed the torsional atropisomers **2a** and **2b** was obtained from the following experiments: (1) each isomer on heating to 90 °C for 1 h in water at pH 7.0 afforded an equilibrium mixture (1:2) of the faster eluting

Table II. Thermal Equilibration of Actinoidinic Acid Rotomers Monitored by HPLC[a]

starting rotomer	50 °C 30 min	50 °C 18 h	100 °C 1 hr
compd 2a[b]	98:2	47:53	36:64
compd 2b[b]	1:99	12:88	34:66

[a] Column: Beckman Ultrasphere ODS, 4.6 × 250 mm. Solvent: 6% acetonitrile in 0.1% aqueous heptafluorobutyric acid, 1 mL/min. Detection: 254 nm. [b] Ratio of compound **2a** to **2b**.

component **2a** and the slower eluting component **2b** (see Table II), (ii) the CD spectra of **2a** and **2b** (see Figure 2), although not antipodal, are opposite in sign, (iii) two compounds exhibiting spectroscopic and chromatographic properties identical with those of **2a** and **2b** could be isolated from the acid hydrolyzate of vancomycin by employing the same procedures.

Absolute Configuration of Actinoidinic Acid Atropisomers. The CD spectra of **2a** and **2b** are dominated by a pair of strong exciton-coupled bisignate bands with maxima at 213 and 226 nm associated with the ^1L_a high-energy aromatic absorption. These bands are opposite in sign and different but closely comparable in intensity (see Figure 2). Assignment of the absolute configuration at the α-amino acid centers follows from the identity of these isomers with those obtained from vancomycin which exhibit the same HPLC retention times and CD spectra. The fact that four HPLC peaks were present in the 24-h hydrolyzate where epimerization occured (Figure 1a) assures that the HPLC system is capable of distinguishing between all epimers. To assign the sense of twist of the biphenyl chromophore in these isomers, it is necessary to assign the direction of the electronic transition moment vectors in each of the aromatic rings of **2** originating from the ^1L_a transitions of the two chromophores represented by the (4-hydroxyphenyl)glycine and (3,5-dihydroxyphenyl)glycine rings, respectively. From the work of Mason[9] and others[10] the ^1L_a transitions in these two chromophores should lie on the short axis of the molecule as depicted in Figure 3. Since the thermodynamically less stable isomer with the shorter retention time affords a positive low energy followed by a negative high energy split exciton CD band, the sense of twist of the biphenyl chromophore reflects positive chirality, and consequently it is assigned structure **2a**.[11] It follows that the diastereomer exhibiting negative chirality for the ^1L_a transition has structure **2b**.

Elucidation of the Structure of the Amino Acid 3. The structure of the amino acid **3** was elucidated from comprehensive NMR studies. The ^1H NMR spectrum of **3** contains signals attributable to an NMe at δ 2.42, two one-proton peaks at δ 4.97 and 5.33 from two amino acid α-CHAr systems, two separate aromatic spin patterns characteristic of a meta-coupled pair (AX) at δ 6.80 and 6.40, a three spin AMX pattern representing aromatic hydrogens in a 1,2,4-relationship, and signals from exchange-broadened NH and phenolic OH groups at δ 8.6–9.6.

The foregoing information was consistent with a chlorinated bis(amino acid) containing a diphenyl ether bridge reminiscent of that described by Hunt[12] for the glycopeptide antibiotic A35512B. However, earlier efforts to establish the position of the substituents in this latter compound by ^1H NMR methods led to an erroneous structure[13] which was corrected only after extensive studies involving the synthesis of several postulated degradation products.[14]

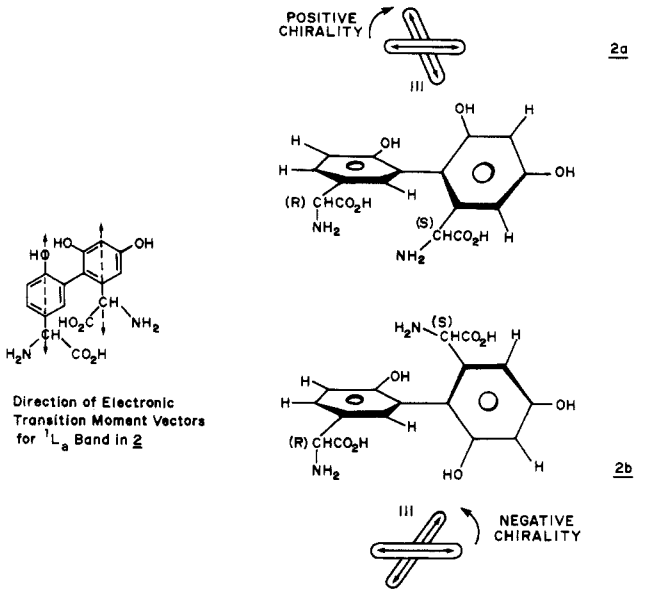
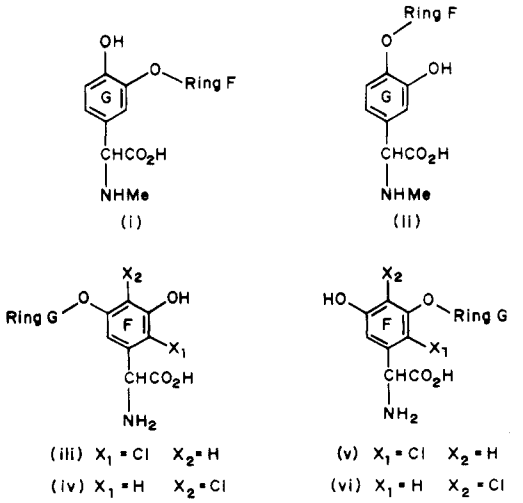


Figure 3. Stereochemical assignments of the actinoidinic atropisomers **2a** and **2b** using the exiton chirality rule.

The problem of determining the structure of highly substituted aromatic rings is still commonly encountered and is often only solved by efforts involving syntheses of several possible targets. An approach which utilizes NMR methods to address this situation has been developed for elucidation of the structure of **3**. Interpretation of preliminary ^1H NMR chemical shift and coupling constant data for compound **3** still leaves six biogenetically plausible structures which are not distinguished by the spectral data.[15] These six structures which arise from two possible substitution patterns for ring G and four for ring F are illustrated by the partial structures i–iv.



The initial experiments utilized were an edited gated-spin–echo-type ^{13}C spectrum and a new two-dimensional

(9) Mason, S. F.; Seal, R. H.; Robert, D. R. *Tetrahedron* **1974**, *30*, 1671–1682.
(10) Platt, J. R. *J. Chem. Phys.* **1949**, *47*, 484–495. Platt, J. R. *J. Chem. Phys.* **1951**, *19*, 263–271.
(11) Harada, N.; Nakanishi, K. In *Circular Dichroic Spectroscopy—Exciton Coupling in Organic Stereo Chemistry*; University Science Books: Mill Valley, CA, 1983.
(12) Hunt, A. H. *J. Am. Chem. Soc.* **1983**, *105*, 4463–4468.
(13) Debono, M.; Molloy, R. M.; Barnhart, M.; Dorman, D. E. *J. Antibiot.* **1980**, *33*, 1407–1416.
(14) Harris, C. M.; Harris, T. M. *Tetrahedron* **1983**, *39*, 1661–1666.
(15) The number is actually 12 if the location of the *N*-methyl group is considered undefined, i.e., at G1′ or F1′. To simplify the argument it is placed at G1′, which was confirmed by subsequent experiments.

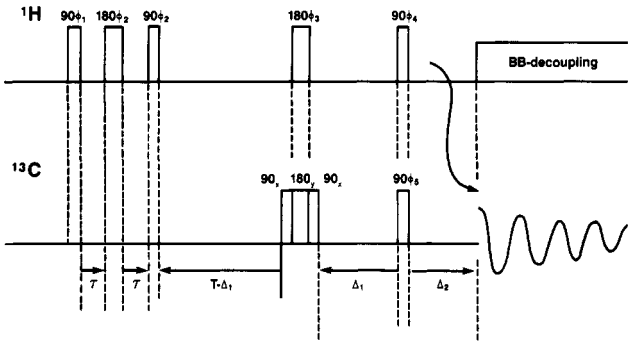


Figure 4. Pulse sequence of the COLOC experiment with J filter added to suppress signals from protonated carbons, $\tau = 1/(4 - J)$ where J is the heteronuclear coupling constant of protonated carbons. $\Delta_1 = n\ \Delta t_1/2$ where $n = 1, 2, \ldots$ NE.

Table III. GASPE Edited ^{13}C NMR Spectrum of Biphenyl Ether 3

	^{13}C shift, ppm	multi
F1	134.2	s
F2	113.7	s
F3	164.7	s
F4	104.0	d
F5	156.9	s
F6	107.5	d
F1′	53.2	d
F2′	168.9	s
G1	123.4	s
G2(5,6)[a]	117.8	d
G3	141.8	s
G4	150.6	s
G5(2,6)[a]	122.8	d
G6(2,5)[a]	126.4	d
G1′	63.6	d
G2′	169.4	s
NMe	30.6	q

[a] G2,5,6 are assigned on the basis of substituent effects on chemical shifts which predicted their order but failed to predict accurate shifts.

^{13}C–^{1}H proton correlation experiment which emphasizes long-range couplings. The results of the 1D edited ^{13}C spectrum of **3** are collected in Table III and show signals consistent with those expected for a substituted bis(amino acid) biphenyl ether as described in general terms above, namely, two carboxyl carbons, two amino acid α-carbons, an N-methyl, and twelve aromatic carbons (five protonated, four bearing oxygens, and three bearing other substituents). Signals from the latter are of particular interest and occur at 113.7, 123.4, and 134.2 ppm; they constitute the aromatic positions that are substituted by a chlorine or one of the two amino acid residues.

The relationship of these three carbons to protons on neighboring carbons to which they are long-range coupled was demonstrated by a modification of the recently described COLOC experiment.[16] The modification employed was designed to suppress selectively signals arising from protonated carbons. The COLOC sequence as described by Kessler et al.,[16] when applied to the model compound, 2-chloro-4-hydroxybenzoic acid, generated extra peaks in the proton frequency domain associated with the carbon resonances from C5 and C6. These extra peaks[17] could be eliminated by suppressing the signal of all protons directly

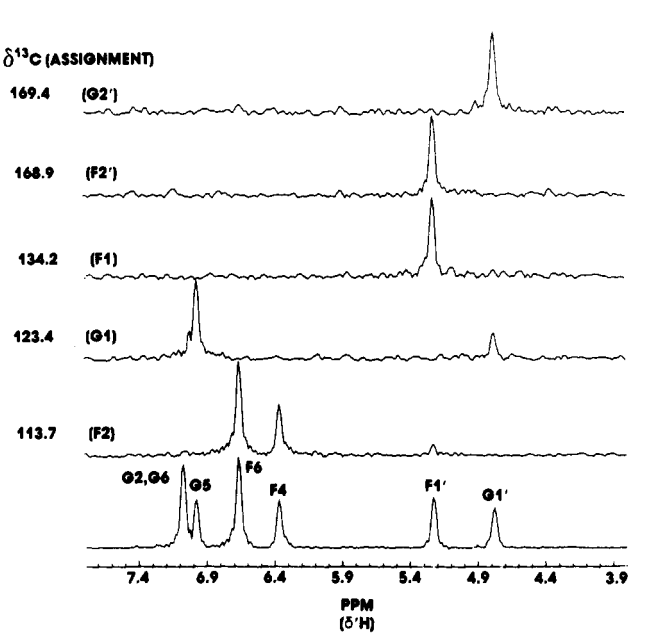


Figure 5. COLOC spectrum of the biphenyl ether **3**. The spectrum was obtained by employing the pulse sequence depicted in Figure 4 with the following parameters: $\tau = 1.75$ ms, $T = 40$ ms, $\Delta_1 = 0.5 + n + 0.227$ ms, where $n = 1, 2, \ldots 170$, $\Delta_2 = 33$ ms, relaxation delay = 2 s.

bound to a carbon-13 nucleus. This was accomplished by adding a J filter in the preparation period as depicted in Figure 4. This sequence, even when used with a single τ value of 1.75 ms to dephase all protons which are coupled to carbon with a J value of 135 Hz, led to spectra with good suppression of all protons directly bound to C13 nuclei.

To examine the structure of **3**, the timing intervals were set to optimize the heteronuclear transfer for $J = 12$ Hz. This setting establishes a linear relationship between the magnitude of J coupling and cross-peak intensities leading to the virtual absence of communication in an *aromatic ring* between protons and carbon separated by two (ortho) and four (*para*) bonds while retaining three bond couplings. It is important to note that under these conditions, two and three bond extraannular couplings between the benzylic protons and ring carbons are still detectable.

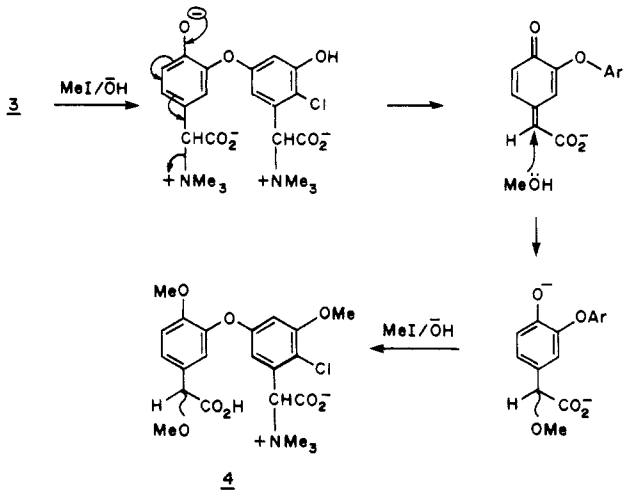
The results obtained from this experiment are presented in Figure 5 in which cross sections through the 2D contour map have been made at positions corresponding to the three aromatic carbons of interest and the two carbonyl signals F2′ and G2′ which are unequivocally assigned by this experiment. However, more importantly the experiment demonstrates that the carbon signals at 113.7 and 134.2 ppm give rise to cross peaks at the position of the benzylic proton at 5.33 ppm and therefore must belong to the same ring (F) and be in an ortho relationship. The carbon signal at 123.4 ppm provides a cross peak at the position of the upfield benzylic proton at 4.92 ppm and therefore must arise from carbon G1. In addition, the carbon signal at 113.7 ppm is further ^{3}J coupled to the F4 and F6 protons, allowing it to be assigned to the carbon bearing the chlorine (F2); this assignment is supported by the absence of cross peaks for F1 arising from its coupling with ring protons. This experiment eliminates structures iv and vi as probabilities and establishes the relationship of the chlorine and amino acid substituents of each of the rings relative to the oxygen atoms as being consistent with isomers i–v.

Two points thus remained concerning the structure of **3**, namely, confirmation of the position of the N-methyl group as suggested by the mass spectral results and the

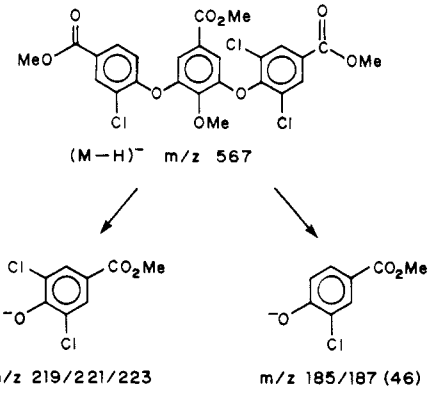
(16) Kessler, H.; Griesinger, C.; Zarbock, J.; Loosli, H. R. *J. Magn. Reson.* **1984**, *57*, 331–336.

(17) These extra peaks are caused by the following phenomenon. Magnetization exchange is induced between H-5 and H-6 by imperfections in the proton 180° pulse for short values of Δ_1 (see Figure 4). This exchange magnetization is finally relayed to a ^{13}C nucleus by direct heteronuclear J coupling in the subsequent mixing period.

Scheme I



Scheme II



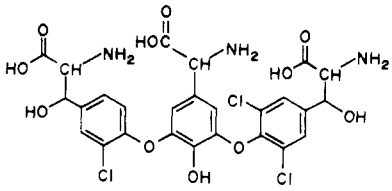
definition of the position of the ether linkage between rings F and G. Location of the *N*-methyl at the G1′ position was readily confirmed by the ^1H COSY spectrum of compound **3** obtained in Me_2SO-d_6 containing 5 equiv of trifluoroacetic acid. Under these conditions it was possible to observe cross peaks between both the upfield (ring G) benzylic proton and the *N*-methyl with the diastereotopic ammonium protons signals at δ 9.35 and 9.45, whereas the ring F benzylic α-CH shows a cross peak with an ammonium proton at δ 8.80.

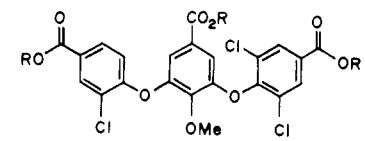
The remaining issue of the disposition of the ether linkage between rings F and G was solved by an NOE experiment on the product obtained from methylation of **3**. Various attempts to effect selective methylation of the phenolic hydroxyls in **3** were unsuccessful. However, reaction of **3** in methanol containing potassium carbonate and methyl iodide led to the formation in good yield in an equimolar mixture of compounds which, from evidence derived from the edited ^{13}C spectrum and the ^1H spectrum, contained three methoxyl groups and a *single* nitrogen. The spectral evidence also showed that the compounds were diastereomers which clearly differed in stereochemistry at a benzylic position.

The ^1H spectrum of the diastereomeric mixture revealed two (partially overlapped) AX spin patterns and a similar pair of AMX spin patterns, arising from the 1,2,3,5-substituent arrangement in ring F and the 1,3,4-trisubstituted ring G, respectively. Irradiation of the methoxyl group at δ 3.90 produced an enhancement only at the doublet at (δ 6.97), which is consistent with the methoxyl (previously the hydroxyl) being ortho to the chlorine. Had the methoxyl been para to the chlorine, NOS's to both the A and X spins would have been expected. In a comparable experiment, irradiating the methoxyl at δ 3.82 resulted in an enhancement of only the A (δ 7.25) doublet (J = 8 Hz) of the AMX pattern on ring G. This argues for the placement of the methoxyl in that ring para to the glycine moiety. If the methoxyl had been meta, a single NOE of the X resonance of the AMX pattern would be predicted. These observations led to the formulation of the methylation product as structure **4**. It follows that the structure of the bis phenolic ether is correctly represented as depicted in **3**. The derivation of compound **4** under the reaction conditions is readily accounted for by a pathway involving the intermediacy of a quinone methide (see Scheme I).

Elucidation of the structure of the aromatic bis(amino acid) **3** is completely rigorous and does not rely on any assumptions based upon structural analogies derived from previous work on related compounds in this series. Furthermore, the utility and efficiency of the NMR methods employed here are clearly evident especially if one considers the extensive degradative and synthetic efforts that were required to establish the structure of a structurally similar fragment from the A35512B antibiotic.[14]

The characterization of **2** and **3** has accounted for 33 of the 59 carbon atoms of the aglycon. The third possible hydrolysis fragment, a ristomycetic acid analogue **5** is expected to be too acid labile to allow its isolation in an intact form.[18] Following an approach successfully used with vancomycin and ristocetin,[18] the permethylated aglycon of aridicin A was oxidatively degraded to trichlororistomyetic acid (**6**), which was isolated and characterized as its trimethyl ester **7**. A peak match measurement (EI)





6 R = H
7 R = Me

on the M^+ peak of the ester defined an exact mass of 568.008, indicating a molecular formula of $C_{25}H_{19}Cl_3O_{19}$ (calcd m/z 568.010) for this component. ^1H NMR and CI mass spectral data on **7** were consistent with a type of structure analogous to those of the corresponding degradation products isolated from other glycopeptides. Prominent fragment ion peaks observed at m/z 185/187 (ratio 100:31) and m/z 219/221/223 (ratio 100:62:11) in the negative ion CI (CH_4) mass spectrum suggested that two chlorines are on a ring at one terminus of the trimeric structures, and the third chlorine is on the ring at the other terminus as shown in structure **7** (see Scheme II).

The ^1H NMR spectrum of this triester (see Experimental Section) contained a three-spin AMX system, confirmed by decoupling experiments, associated with the monochloro substituted ring. The lowest field doublet at

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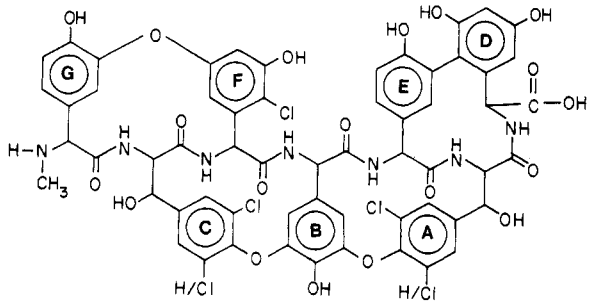
δ 8.18, corresponding to a proton flanked by a chlorine and a carbomethoxy group, is meta coupled (J = 2 Hz) to a second proton (δ 7.88) which is in turn ortho coupled to third proton (δ 6.85). This latter proton signal, being farthest upfield, is typical of a proton adjacent to an oxygen atom in agreement with the substitution pattern assigned to the terminal ring containing a single chlorine. Very similar chemical shifts have been reported for the corresponding fragments isolated from teichoplanin,[20] actinoidin,[21] and vancomycin.[22] In addition the occurrence of a two proton singlet (δ 8.11) indicates a symmetrical disposition of the protons in the dichlorinated ring. Furthermore, the chemical shift of this signal is consistent with the location of the protons adjacent to both a carboxyl group and a chlorine atom. The alternative symmetrical structure, which is unlikely on biogenetic grounds, would require the two protons to be adjacent to the ether oxygen, and the ^{1}H chemical shifts consequently would be expected to occur at higher field. The remaining two protons at δ 6.99 and 7.44 are meta coupled (J = 2 Hz) but, unlike the corresponding protons in the vancomycin derived fragment, are nonequivalent because of the presence of an extra chlorine on one of the terminal rings. Chemical shift arguments which may be made to support this conclusion indicate that they are situated between an oxygen and a carbomethoxy substituents rather than being flanked by oxygen substituents on either side. A combination of the information derived from the MS and NMR data together constitute strong evidence in proposing structure **7** for the triester.

In the summary, the characterization of fragments **2**, **3**, and **6** accounts for 54 of the 59 carbon atoms and 4 of the 7 nitrogen atoms. On the basis of analogy with other work[18] in this class of antibiotics, the missing five carbons and three nitrogen atoms can be accounted for by the fragments lost in the oxidative degradation of **1** to the tribenzoic acid. Consequently, structure **6** suggests that the amino acid from which it is derived is **5**. Reassembling the three amino acid units **2**, **3**, and **5** gives an aglycon with a molecular formula $C_{59}H_{45}Cl_4N_7O_{19}$ which is in agreement with the FABMS high-resolution peak match experiment.

From the foregoing structural information derived from spectral and chemical studies of the aglycon and its constituent amino acids, structure **8** is proposed for the aglycon based upon analogy with the reported structures for ristocetin and A35512B. Furthermore, the presence of two



8

benzylic hydroxyls is supported by the ^{13}C NMR data of the aglycon, which contains two signals at 70.2 and 71.2 ppm, typical of methine carbons linked to oxygen. The molecular weight as defined by FABMS as well as the requirement for 19 oxygen atoms from the FABMS is also in accord with this latter conclusion. Furthermore, the *N*-methyl group is placed at the N-terminus on the basis of the absence of a positive ninhydrin reaction for the aglycon. Incorporation of the amino acid **5** into the intact aglycon may be accomplished in one of two possible ways. The consequence of this is that the location of one chlorine, which must occur either in rings A or C, remains in doubt.[23]

Experimental Section

Vancomycin and ristocetin were purchased from Sigma. Trifluoroacetic, heptafluorobutyric, and constant-boiling hydrochloric acids were purchased from Pierce. Analytical HPLC was run on a Beckman Model 345 chromatograph equipped with a Beckman 165 detector operated at 220 or 254 nm, as indicated. ^{13}C and ^{1}H NMR spectra were obtained with a Bruker Instruments WM 360 spectrometer equipped with a 5-mm C/H probe.

Fast atom bombardment mass spectra were obtained with a VG Analytical ZAB-1F mass spectrometer as previously reported.[6] Electron ionization mass spectra were obtained with a Finnigan MAT CH5-DF mass spectrometer. Chemical ionization mass spectra were obtained with a Finnigan Model 3625 mass spectrometer capable of pulsed positive/negative ion chemical ionization. Both positive and negative CI mass spectra were obtained with methane as the CI reagent gas. Circular Dichroism (CD) spectra were obtained with a Jasco J-200C spectropolarimeter interfaced through an IF-500C converter to an Oki if-800 computer. Samples were run as a solution of approximately 0.5–1 mg/mL in water (pH 5.0). Spectra are the result of at least four computer-averaged scans. Ultraviolet spectra were run on a Perkin-Elmer Lambda 5 UV–vis spectrophotometer.

Small-Scale Acid Hydrolysis Procedure. A suspension of antibiotic (1–5 mg) in 1 mL of Pierce Sequanol grade constant-boiling HCl was frozen in a Pierce vacuum hydrolysis tube in dry ice–acetone. Nitrogen gas was used to displace air, and the tube was evacuated. The contents of the sealed tube were allowed to melt and then heated in a Pierce heating block for 8–24 h. The cooled solution was evaporated to dryness, taken up in 4 mL of water, and filtered through a Millipore Milex-HV 0.45 μm filter. The resulting filtrate was subjected to analysis by RP-HPLC using a system of 0.1% TFA in water on a Beckman Ultrasphere ODS column, 4.6 × 250 mm at 1.5 mL/min for 5 min followed by a linear gradient to 40% acetonitrile over 10 min with detection at 220 nm.

Preparative Hydrolysis of Aridicin to 2 and 3. Aridicin A (1 g) was suspended in 30 mL of 6 N hydrochloric acid and transferred to four Pierce vacuum hydrolysis tubes. The tubes were frozen under nitrogen and evacuated. The contents were allowed to melt and the sealed tubes heated in an oil bath at 120 °C for 8 h. The contents of the tubes were combined and evaporated to dryness in vacuo. The residue was twice suspended in 30 mL of water and evaporated to dryness in vacuo. The remaining material was dissolved in 100 mL of 0.1% trifluoroacetic acid and filtered through a glass-fiber filter. The filtrate was loaded on to a glass column (2.5 × 50 cm, Altex Beckman) which had been dry packed with Whatman Partisil ODS 3 (40–60 μm) and preequilibrated with 0.1% trifluoroacetic acid–water. The column was eluted with 0.1% trifluoroacetic acid. Fractions containing the front-running HPLC peak (t_r 1.4 min, TFA system, Table I) were pooled and lyophilized to yield 210 mg of actinoidinic rotamers **2a** and **2b** as a tan solid. Treatment of fractions containing the second peak (t_r 4.5 min, TFA system) in a similar manner provided 134 mg of the amino acid **3** as a yellow powder: UV λ_{max} (H_2O, pH 2.0) 283 nm (ε 3971), (pH 11.0) 300 (5559); ^{1}H NMR (Me_2SO-d_6/TFA) δ 2.37 (s, 3 H, NCH_3), 4.67 (br dd, 1 H, G1′), 5.08 (br, 1 H, F1′), 6.45 (d, 1 H, J = 2.6 Hz, F4), 6.68 (d, 1 H, J = 2.6 Hz, F6), 7.05 (d, 1 H, J = 6.7 Hz, G2), 7.07 (d, 1 H, J = 1.6 Hz, G5), 7.11 (dd, 1 H, J = 1.6 Hz, J = 6.7 Hz, G6), 8.82 (br d, 3 H, F-NH_3), 9.4 (br dq, 2 H, G-NH_2), 10.3 (OH); FAB-MS;

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(21) Sztarcskai, F.; Harris, C. M.; Harris, T. M. *Tetrahedron Lett.* **1979**, 2861–2864.
(22) Smith, K. A.; Williams, D. H.; Smith, D. H. *J. Chem. Soc., Perkin Trans. 1* **1974**, 2369–2376.
(23) It has been shown subsequently by independent 2D ^{1}H NMR studies[19] on the intact aridicin aglycon that it is correctly represented by the structure in which ring A gbears the extra chlorine.

calcd for $C_{17}H_6N_2O_2Cl$ m/z 397.080, found m/z 397.079.

Separation of Actinoidinic Acid Rotamers. Analytical separation of **2a** and **2b** was run on a Beckman Ultrasphere ODS column, 4.6 × 250 mm, eluted with 6% acetonitrile in 0.1% heptafluorobutyric acid, at 1 mL/min with detection at 254 nm (see Figure 1). Preparatively the mixture of actinoidinic acids (**2a** and **2b**) was chromatographed in five 20-mg batches on a 10-μm Whatman Partisil ODS-3, 9.4 × 500 mm column, with 0.5% v/v heptafluorobutyric acid, yielding, after lyophilization, 23 mg of **2a** (t_r 8 min, HFBA system): ^{1}H NMR (10:1 Me_2SO-d_6–TFA) δ 7.24 (br, 1 H), 7.17 (br, 1 H), 6.91 (br, 1 H), 6.44 (br, 1 H), 6.40 (br, 1 H), 4.85 (q, 1 H), 4.37 (q, 1 H); ^{13}C NMR 170.4 (s), 170.0 (s), 158.1 (s), 157.5 (s), 157.0 (s), 133.8 (s), 133.3 (d), 128.6 (d), 123.5 (s), 123.0 (s), 116.7 (s), 116.0 (d), 104.2 (d), 103.7 (d), 55.4 (d), 53.2 (d) ppm; FAB-MS, m/z 347 [(M + H)$^+$]; UV λ_{max} 287 nm (ε 498), 220 sh (ε 2346); CD (H_2O) $[\theta]_{315}$ 0, $[\theta]_{287}$ +5800, $[\theta]_{260}$ 0, $[\theta]_{227}$ −33 800, $[\theta]_{218}$ 0, $[\theta]_{212}$ +39 000, and $[\theta]_{209}$ 0. **2b** (27 mg) was also obtained (t_r 9 min): ^{1}H NMR (10:1 Me_2SO-d_6–TFA) δ 7.28 (dd, 1 H, J = 2.2, 8.5 Hz), 7.24 (d, 1 H, J = 2.2 Hz), 6.99 (d, 1 H, J = 8.5 Hz), 6.48 (d, 1 H, J = 2.1 Hz, 6.37 (d, 1 H, J = 2.1 Hz), 4.94 (q, 1 H, J = 4.8 Hz), 4.32 (q, 1 H, J = 4.8 Hz); ^{13}C NMR 170.4 (s), 170.0 (s), 158.1 (s), 157.5 (s), 157.0 (s), 133.2 (s), 132.1 (d), 128.8 (d), 123.6 (s), 123.0 (s), 117.4 (s), 116.0 (d), 104.4 (d), 103.7 (d), 55.6 (d), 53.0 (d); FAB-MS, m/z 347 [(Me + H)$^+$]; CD (H_2O) $[\theta]_{315}$ 0, $[\theta]_{287}$ −6300, $[\theta]_{260}$ 0, $[\theta]_{228}$ +34 200, $[\theta]_{220}$ 0, $[\theta]_{213}$ −39 500, and $[\theta]_{209}$ 0.

Equilibration Studies on 2a and 2b. Sealed tubes containing aqueous solutions of **2a** or **2b** (0.2 mg/mL) were heated in separate experiments in a Pierce reaction block at (1) 50 °C for 30 min, (2) 50 °C for 18 h, and (3) 100 °C for 1 h. The cooled solutions were assayed by using the heptafluorobutyric acid HPLC system (Figure 1) and area ratios compared as shown in Table II.

Hydrolysis of Vancomycin to Actinoidinic Acids 2a and 2b. Vancomycin (350 mg) was hydrolyzed in 10 mL of 6 N hydrochloric acid in the same manner as described for aridicin. After chromatography on a reversed-phase column (Whatman Partisil ODS 3, 40–60 μm, 2.5 × 50 cm), the fractions which contained the 1.4-min HPLC peak were pooled and lyophilized to give 55 mg of the actinoidinic rotamers **2a** and **2b**. The two rotamers were separated as was described for the aridicins on the Whatman Patisil ODS-3 (9.4 × 500 mm) column to yield 22 mg of **2a** and 25 mg of **2b**, identical in HPLC and CD with **2a** and **2b** derived from aridicin A.

Preparation of the Permethyl Derivative 4 of *N*-Methylchlororistomycinic Acid 3. A mixture of compound **3** (30 mg, 0.09 mmol), methyl iodide (1.5 mL, 24 mmol), and potassium carbonate (390 mg, 2.8 mmol) in 7 mL of 90% aqueous methanol was heated at 60 °C for 15 h in a sealed tube. After cooling to 0 °C, the mixture was adjusted to pH 7.0 and then evaporated in vacuo to dryness. The residue was dissolved in 1.5 mL of 10% aqueous acetonitrile and chromatographed on a reversed-phase column (Beckman Ultrasphere ODS, 5 μm 10 × 250 mm) and eluted with an acetonitrile gradient (0–30%) in 0.5% of trifluoroacetic acid. The fractions containing the major reaction product (t_r of 12.4 min by analytical HPLC using the TFA system) were pooled and lyophilized to give 17 mg of **4** as a pale yellow solid: FAB-MS, m/z 506 [(M + K)$^+$], 490 [(M + Na)$^+$], 468 [(M + H)$^+$]; ^{1}H NMR (D_2O/DCl, sample was an equimolar mixture of diastereomers, which caused several of the resonances to split; therefore average chemical shifts are reported) δ 3.20 (s, 9 H, NMe_3), 3.31 (s, 3 H, G1′-OCH_3), 3.76 (s, 3 H, G-OCH_3), 3.83 (s, 3 H, F-OCH_3), 4.86 (s, 1 H, G1′), 5.69 (s, 1 H, F1′), 6.57 (d, 1 H, J = 2.5 Hz, F6), 6.93 (d, 1 H, J = 2.5 Hz, F4), 7.12 (d, 1 H, J = 2.2 Hz, G2), 7.20 (d, 1 H, J = 8.5 Hz, G5), 7.33 (dd, 1 H, J = 2.2 Hz, J = 8.5 Hz, G6); ^{13}C NMR (D_2O/DCl) [INEPT protonated carbon signals only] (CH) 123.25, 122.90, 116.55, 112.35 (112.25), 107.66, 83.44, 74.6, (CH_3) 59.32, 48.59, 54.64 (2) ppm; FT-IR (KBr) 3420, 3300–2400 (OH), 1741 (CO), 1615, 1596 (Ar) 1164 cm^{-1} (C–O).

Preparation of Trimethyl Ester Derivative 7. Aridicin aglycon (450 mg, 0.348 mmol) was dissolved in 100 mL of absolute methanol. The solution was treated with 80 mL of 0.2 M ethereal diazomethane. After 1 h, the yellow solution was evaporated to dryness in vacuo. The crude permethylated product showed no base shift in its UV spectrum. The permethylated aglycon (45 mg) was hydrolyzed in 6 mL of 3 N KOH at 110 °C for 18 h in a sealed tube. The hydrolysis mixture was adjusted to pH 8.0 and then evaporated to dryness in vacuo. The residue was then treated with a $KMnO_4$ solution (240 mg of $KMnO_4$ and 150 mg of $CaSO_4 \cdot 2H_2O$ in 5 mL of aqueous *tert*-butyl alcohol). After heating at 90 °C for 3 h, the excess $KMnO_4$ was destroyed by addition of 5 mL of isopropyl alcohol containing 800 mg of sodium metabisulfite. The solution was acidified to pH 1.5 and extracted with 3 × 50 mL of ethyl ether. The combined organic extract was evaporated to dryness to give a residue (15 mg), which was chromatographed on a reversed-phase column (Whatman Partisil ODS-3, 10/50, Magnum-9) with an acetonitrile gradient (20–50%) in 0.1% trifluoroacetic acid. The column fractions containing the major product with a 254-nm adsorption were pooled and lyophilized to dryness to give 3 mg of the triacid **6**, which was methylated with diazomethane to provide the ester **7**: mass spectrum (EI), calcd for $C_{25}H_{19}O_9Cl_3$ m/z 568.009, found m/z 568.008, m/z 569 [(M + H)$^+$], 536 [(M + H) − CH_3OH)$^+$]; ^{1}H NMR ($CDCl_3$) δ 3.80 (s, 3 H, OCH_3), 3.93 (s, 3 H, OCH_3), 3.98 (s, 3 H, OCH_3), 4.02 (s, 3 H, OCH_3) 6.85 (d, 1 H, J = 8.6 Hz, C5), 6.99 (d, 1 H, J = 2.0 Hz, B6), 7.44 (d, 1 H, J = 2.0 Hz, B2), 7.88 (dd, 1 H, J = 2.0 Hz, J = 8.6 Hz, C6), 8.11 (s, 2 H, A2, A6), 8.18 (d, 1 H, J = 2.0 Hz, C2).

Long-Range ^{13}C–^{1}H Correlation Spectrum (COLOC) of 3. The ^{13}C correlation spectrum of a solution of 35 mg of **3** in 0.5 mL of Me_2SO-d_6 (99.95% *d*) and 25 μL (5 equiv) of TFA was measured by using the pulse sequence described in Figure 4, with τ = 1.75 ms, Δ_2 = 33.0 ms, and T_1 = 40 ms (initially). ^{13}C spectra of 4K data points were collected, each consisting of 512 scans, thus t_{total} was 33.6 ms (170 × δ_1 + 2(0.5 ms)); Δ_1 was incremented in each successive spectrum so as to provide a spectral width in the proton (F1) dimension of 2200 Hz. Sensitivity was enhanced by the use of appropriate Gaussian weighting functions prior to double Fourier transformation. The temperature was maintained at 60 °C during the experiment, in order to achieve optimally resolved ^{13}C signals.

^{1}H COSY and Experiments with Compound 3. Compound **3** (40 mg) was dissolved in 0.5 mL of Me_2SO-d_6 with 10% TFA added. A ^{1}H COSY experiment was carried out with the standard Bruker "COSYN" command sequence, with the following conditions: 512 2K spectra with spectral width 4200 Hz were measured, with increments such as to give a sweep width in the F_1 dimension ±2100 Hz. Sixteen scans were collected per increment, with a 2-s relaxation delay. The data were zero-filled before processing, so the resulting transform was 1 MWord. The data were resolution enhanced by sinebell multiplication in both dimensions, and the transform was symmetrized. The temperature of the sample was maintained at 19 °C throughout the experiment.

NOE Experiments with the Permethylated Compound 4. Approximately 7 mg of **4** was dissolved in deuterium oxide with 20 μL of 0.1 M DCl added. The NOE difference spectra were measured by alternatively adding and subtracting groups of 64 scans with low power irradiation at the frequency of interest, then the same number with the irradiation offset 1800 Hz. The cycle was repeated eight times. The irradiation time was 5 s prior to each aquisition, which was then gated off for 20 ms before applying a 30° pulse. After the acquisition an extra 0.2 s delay was inserted. The irradiation power was set as low as possible (50L) consistent with saturation of the methyl groups. The difference spectra thus contained 512 scans which were Fourier transformed by using an exponential broadening factor of 0.5 Hz to improve subtraction. All experiments were run at ambient temperature.

Acknowledgment. The able technical assistance of George Udowenko is gratefully acknowledged. Analytical data were provided by Edith Reich.

Registry No. 2a, 103531-61-9; **2b**, 103531-62-0; **3**, 103478-54-2; **4** (diastereomer 1), 103478-55-3; **4** (diastereomer 2), 103531-63-1; **6**, 103478-56-4; **7**, 103478-57-5; **8**, 103498-83-5; **8** (permethylated), 103498-82-4; aridicin A, 95935-21-0; vancomycin, 1404-90-6.