Deprotection. To a solution of sulfonamide **21** (0.1294 g, 0.148 mmol) in 10 mL of THF at 0 °C was added dropwise a 0.2 M solution of sodium anthracene (3.70 mL, 7.40 mmol). The solution remained blue for 1 min. Water was added (5 mL), the solution was extracted with methylene chloride (3 × 20 mL), and the combined organic extracts were dried over magnesium sulfate. Filtration and removal of volatile components under reduced pressure afforded a crude product, which was purified by column chromatography to yield first anthracene (methylene chloride eluant) and then product **22** (methylene chloride/methanol eluant, 20:1, 0.05058 g, 0.085 mmol, 58%) as a clear oil: R_f 0.44 (silica gel, methylene chloride/methanol, 10:1); $[\alpha]^{23}_D$ −7.75 (*c* 0.004, $CHCl_3$); IR ($CHCl_3$) 3300, 2930, 2850, 1610, 1505, 1450 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.23 (d, 4 H, *J* = 8.32 Hz), 6.91 (d, 4 H, *J* = 8.22 Hz), 4.46 (d of d, 2 H, *J* = 3.8 + 5.9 Hz), 4.59 (d, 2 H, *J* = 6.03 Hz), 4.23 (d of d, 2 H, *J* = 6.80 + 8.30 Hz), 4.11 (d, 2 H, *J* = 14.3 Hz), 3.62 (d, 2 H, *J* = 14.30 Hz), 3.29 (d, 2 H, *J* = 3.37 Hz), 2.37 (m, 4 H), 1.72 (m, 4 H), 1.47 (s, 6 H), 1.38 (m, 4 H), 1.29 (s, 6 H); ^{13}C NMR ($CDCl_3$) δ 156.70, 129.55, 118.81, 112.34, 83.95, 82.58, 81.28, 78.90, 52.10, 45.55, 28.53, 26.25, 25.68, 25.11; exact mass calcd for $C_{34}H_{46}N_2O_9$ 594.3305, found 594.3292.

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Structure of the Antibiotic OA-7653

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The structure of the glycopeptide antibiotic OA-7653 is assigned as **2**. Elucidation of the structure is based primarily on two-dimensional NMR experiments, analogies with other glycopeptide antibiotics of the vancomycin series, and selective degradation studies. Antibiotic OA-7653 contains a heptapeptide aglycon core in which the amino acids *N*,*N*-dimethylalanine and glutamine are encountered as the G and F components of this unit. Mild acid hydrolysis of the antibiotic effects the conversion of the δ-carboxamide of the glutamine residue to yield the carboxylic acid **3** in a reaction that is shown to proceed without rearrangement. This latter conversion to **3** proceeds without effecting cleavage of the β-glycosidic link between the aglycon and glucose. The ^1H spectra of OA-7653 and its derivatives in DMSO at pH 4.0 are shown to represent major and minor conformers that are exchanging at rates comparable to the NMR time scale.

Introduction

Glycopeptide antibiotics are a class of compounds that have received considerable attention recently. The interest, in part, stems from the dramatic increase in the use of vancomycin, the sole clinical representative of this class, for the treatment of methicillin-resistant staphylococcal infections. The isolation and characterization of new members of this series have led to the recognition of four subtypes based upon the nature of the amino acids G and F in structure **1**, which is representative of the heptapeptide core present in the aglycons of all members of this series. With the exception of vancomycin,[1] which is the sole member of its subgroup where G and F represent aliphatic amino acids, all remaining glycopeptide antibiotics whose structures have been described to date belong to the ristocetin (G, F = ArOAr),[2] avoparcin (G, F = ArAr),[3] or synmonicin (G = Ar, F = aliphatic)[4] subgroups. As part of an ongoing program in seeking to identify new members of the class as potential therapeutic agents, we report the results of the elucidation of the structure of the glycopeptide antibiotic OA-7653. The isolation and preliminary characterization of this antibiotic from a strain of *Streptomyces hygroscopicus* was first reported in 1983 by Japanese workers.[5,6] These workers found the compound to be moderately active against Gram-positive bacteria. It was presumed to be related to vancomycin on the basis of physical properties and provisional data summarized as follows. The compound was found to be soluble in aqueous base, sparingly soluble in aqueous acid, and completely insoluble in alcohols, ethyl acetate, and acetone. The isoelectric point was determined to be between 5 and 6 on the basis of paper electrophoresis. No reliable data with respect to molecular weight or elemental composition were provided. In the UV spectrum the compound gave λ_{max} (H_2O) = 278 nm (ε 1% 1 cm 56) and 0.1 N NaOH max = 298 nm (ε 1% 1 cm 100). The shift to longer wavelength in base is consistent with the presence of phenolic chromophores. Analysis of the amino acid composition and the sugar composition revealed that OA-7653 contained glutamic acid and the neutral sugar, glucose.

Experimental Section

NMR Spectroscopy. Proton spectra were obtained on a JEOL GX 500 spectrometer. All 2D NMR spectra were transferred to a VAX 11/780 via magnetic tape and processed with software developed by Hare.[7] T_1 data were determined by the inversion–recovery method using a composite population inversion pulse. The relaxation delay was set to 5 times the longest T_1 value. The T_1 parameters were obtained by nonlinear fitting of peak maxima using the standard software available on the JEOL. Details of the pulse sequences and phase cycling and other relevant details of the experimental parameters used in the various 2D NMR experiments were as described previously.[8,17] Samples for NMR experiments were dried by lyophilization and prolonged

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pumping in the presence of P_2O_5. Spectra on OA-7653 were obtained for solution in DMSO-d_6 on a sample at pH 8.0 containing 6 mg/0.5 mL. Spectra of the hydrolysis product, OA-7653-diacid, were obtained in DMSO-d_6/D_2O (2:3) at 45 °C for a solution containing 5 mg/0.5 mL. Some preliminary spectra were also run at 360 MHz on a Bruker AM 360 instrument.

Mass Spectroscopy. FAB mass spectra were obtained on a VG ZAB 1-F-HF mass spectrometer and a DS-2000 data system, as previously described.[9] GC-MS were obtained in the CI mode on a Finnigan 4610 using isobutane as the reagent gas.[10]

Fermentation. A slant of *S. hygroscopicus* sp. (ATCC no. 31613) dispersed in 10 mL of sterile water was used to inoculate 500 mL of seed medium 13 H[11] contained in a 4-L aspirator bottle. This seed culture was incubated at 28 °C for 4 days on a reciprocal shaker at 250 rpm and a 7.5 cm throw. The entire seed was transferred to 9.5 L of production medium E1 (glucose, 20 g/L; soy peptone, 10 g/L; yeast extract, 1 g/L; $CaCO_3$, 1 g/L; $CoCl_2$, 0.001 g/L; distilled water, 1 L) contained in a 14-L New Brunswick Fermentor. The production stage was incubated at 28 °C, aerated at 0.3 VVM, and agitated at 120 rpm. Production of OA-7653 was monitored by analytical HPLC (see below) at various time points. The antibiotic activity against *Staphylococcus aureus* and *Bacillus subtilis* was measured at the same time points by observing zone sizes in disk assays.

High Performance Liquid Chromatography Assay of OA-7653. A C_{18} Sep-Pak cartridge (Waters Associates) was used for pretreatment of broth samples and crude extract. HPLC analyses of samples were conducted on a Beckman 344 binary liquid chromatograph using an Altex Ultrasphere-ODS column (5 μg; 4.6 × 150 mm) with detection at 220 nm. Analyses were performed by using a 12-min gradient of 10–35% CH_3CN in 0.01 N pH 3.2 potassium phosphate buffer at a flow rate of 1.5 mL/min. The antibiotic OA-7653 eluted at 9.8 min under these conditions.

Isolation and Purification. The crude fermentation broth from a 10-L fermentation was clarified by stirring with Supercell and filtered. The filtrate was cooled to 4 °C and hydrochloric acid added until the solution reached pH 7.0. The neutral solution was applied to a column (6 L) of Diaion HP-20 resin. The column was washed with water (10 L) and the antibiotic was eluted with 50% CH_3CN–H_2O (7.2 L). The combined eluates containing the antibiotic (as monitored by HPLC analysis and disk assay against *B. subtilis* and *S. aureus*) were concentrated to 2.5 L at 30 °C on a Rotovap and subjected to affinity chromatography.

Affinity Chromatography. A column containing Affi-gel-10-*d*-Ala-*d*-Ala[12] (125 mL) was preequilibrated in 20 mM sodium phosphate buffer, pH 7. The concentrated extract (500 mL) was applied to the column. The column was then washed with 20 mM sodium phosphate, pH 7 (500 mL); 0.1 N NH_4OH (100 mL); 50% CH_3CN in 0.1 N NH_4OH (v/v, 500 7L); and 0.4 N $NaHCO_3$/30% CH_3CN (v/v, 700 mL). The eluate from each wash was collected separately. The 0.1 N NH_4OH wash and the 50% CH_3CN in 0.1 N NH_4OH contained OA-7653 as judged by HPLC assay. The two solutions were combined and concentrated to yield 752 mg of an off-white powder.

Preparative Reverse Phase HPLC of Affinity Purified Product. Affinity purified OA-7653 (600 mg) was dissolved in 10 mM potassium phosphate pH 6.0 (1 L). The solution was filtered through a 0.45-μm filter and pumped onto a reverse phase column (Whatman, Partisil 10-ODS-3, 21.2 mm × 50 cm) that was preequilibrated with 0.01 N potassium phosphate, pH 6.0. Once all of the sample solution was applied, the column was eluted with 22% CH_3CN in 0.01 N potassium phosphate, pH 6.0. Approximately 100 fractions (22.5 mL) were collected. Those containing OA-7653 as judged by HPLC were pooled and concentrated. The resulting oil was redissolved in water. The solution was applied to a column packed with HP-20 resin (125 mL, 2.5 cm × 25 cm). The column was washed with water (400 mL) and the OA-7653 was eluted with 50% CH_3CN in water (v/v, 400 mL). The solution was concentrated, redissolved in water and lyophilized to an off-white powder (171 mg) that was greater than 95% OA-7653 as judged by the HPLC method above. FAB MS: m/z 1275 (MH^+). An accurate mass measurement gave the value m/z 1275.328, calcd m/z 1275.3328 for $C_{58}H_{61}N_8O_{21}Cl_2$.

Carbohydrate Analysis. Hydrolysis of OA-7653 (1 mg) and conversion of the carbohydrates(s) in the hydrolyzate to the aldol acetate(s) was carried out as previously described.[13] The resulting aldol acetate mixture was dissolved in methylene chloride and compared by capillary GC analysis to a standard mixture of sugar alditol acetate derivatives. Column, OV-17, 15-m capillary column. Temperature program, 180 °C for 5 min, programmed to 270 °C at 2 °C/min, hold for 10 min and then return to initial value (180 °C). Glucose in alditol acetate standards, t_R 13.99 min. Sugar in OA-7653 hydrolysate, t_R 13.98 min, (1 μL injection), t_R 14.00 min (4 μL injection).

Acid Hydrolysis of OA-7653. OA-7653 (100 mg) was stirred in 2 N HCl (40 mL) at 45–50 °C. After 18 h all of the starting material was converted to a new product as judged by HPLC. The product had also precipitated from the reaction mixture as it formed. Adjusting the pH of the solution to 7 resulted in the redissolution of the product. The product was analyzed by HPLC (Beckman Ultrasphere ODS, 5 μm, 4.6 × 150 mm, 1.5 mL/min, 7–34% CH_3CN in 0.01 N potassium phosphate, pH 3.2 over 12 min; t_R OA-7653, 8.9 min; t_R product, 9.3 min). The solution was concentrated and redissolved in 20% CH_3CN in 0.01 N potassium phosphate, at pH 6.0 (24 mL). The product was purified by semipreparative reverse phase HPLC (Whatman, Partisil-10-ODS-3, 10 μm, 9 mm × 50 cm, 20% CH_3CN in 0.01 N potassium phosphate, pH 6.0). Injections of 2 mL were made and the chromatography was run 12 times. The major peak at 14 min was collected from each run, combined, concentrated, and redissolved in water (20 mL). The solution was applied to an HP-20 column (29 × 2.5 cm). The column was washed with water (300 mL) and the product was then eluted with 50% CH_3CN in water (v/v, 400 mL). The solution was concentrated, redissolved in water, and lyophilized to yield **3** as an off-white powder (48 mg). FAB MS: m/z 1276 (MH^+), indicating conversion of a carboxamide to a carboxylic acid. An accurate mass measurement gave m/z 1276.319, calcd m/z 1276.3168 for $C_{58}H_{60}N_7O_{22}Cl_2$.

Methanolysis of OA-7653. The antibiotic (50 mg) was dissolved in MeOH containing 1.4 N HCl and the solution stirred at 45–50 °C. Aliquots (50 μL) were removed periodically and analyzed by HPLC on an Ultrasphere ODS 5 μm 4 × 150 mm column using 7–34% CH_3CN in 0.01 N KH_2PO_6, pH 3.2 buffer gradient over 12 min. After 5 days, the reaction showed a major product, t_R 13.85 min. The reaction mixture was concentrated to a white solid, redissolved in 5 mL of H_2O, and chromatographed on a Magnum 20 column for preparative HPLC. The column (Partisil 10-ODS-3) was eluted with 25% CH_3CN in 0.01 KH_2PO_4, pH 3.2. The major component was collected as a white solid. It was dissolved in water and desalted by being passed through an HP-20 column previously equilibrated with water. Elution with 50% CH_3CN/H_2O gave the diester **4** (22.3 mg). The FAB MS of the compound contained an MH^+ ion at m/z 1304. The ^1H NMR spectrum showed two methyl singlets at δ 3.71 and 3.75. Complete assignment of all remaining ^1H chemical shifts for this compound appear in the supplementary data (Table 1). This compound was also obtained by reaction of the diacid **3** in CH_3OH/1.4 N HCl under similar conditions. A minor component, t_R 10.8 min, was often present, especially after short reaction times. Isolation of this material by preparative HPLC and subsequent desalting on HP-20, both as described above, gaven an amorphous white solid. The FAB MS of this material contained an MH^+ ion at m/z 1289, in accord with its identity as the methyl ester of OA-7653.

Hydrolysis of OA-7653 in 6 N HCl. The antibiotic (3 mg) was placed in a Pierce hydrolysis tube containing 1 mL of 6 N HCl. The contents were frozen and the tube was purged with N_2 prior to evacuation and sealing. The tube and its contents

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were heated at 125 °C for 15 h. The reaction mixture was lyophilized and the residue was repeatedly (×4) evaporated from water. A small sample (50 μL) was examined by HPLC. Two peaks corresponding to the diastereomers of actinoidinic acid were identified by comparison with an authentic sample obtained by a similar hydrolysis of vancomycin. No other peaks could be identified. The bulk of the solution was lyophilized and analyzed as follows.

(a) A sample (0.625 mg) in 100 μL of water was subjected to HPLC analysis according to the procedure of Jones et al.[14] for amino acid analysis using *o*-phthalaldehyde in a post-column reactor and a Schoeffel F8950 fluorescence detector. Peaks eluting at 3.42 and 10.15 mins were identified as glutamic acid and actinoidinic acid, respectively.

(b) The remaining material was dissolved in 100 μL of 3.5 N 2-butanol/HCl and heated in a sealed tube in vacuo for 5 h at 110 °C. A 1-μL sample of the reaction was subjected to GC-MS-CI analysis using a 30 M DB-1 capillary column temperature programmed 75 °C → 310 °C at 5 °C/min. A component with MH^+ m/z 174 and fragment ions at m/z 118 and 72, identical both in terms of its GC retention time and fragmentation pattern to *N,N*-dimethylalanine 2-butyl ester, was identified.

Synthesis of *N,N*-Dimethylalanine Esters. Sodium cyanoborohydride (70 mg) was added to L-alanine methyl ester hydrochloride (101 mg) in methanol (3 mL) containing formaldehyde (350 μL). After the reaction mixture was stirred for 1 h, the solution was concentrated and then saturated with $NaHCO_3$ before being extracted with CH_2Cl_2 (30 mL). The usual workup provided *N,N*-dimethylalanine methyl ester as an oil (13.4 mg). The GC-CI-MS spectrum showed an MH^+ ion at m/z 132 with fragments at m/z 130 (M − H), 116 (M − Me), and 72 [$(Me_2N{=}CHMe$]. Reaction of the methyl ester with 2-butanol/HCl in vacuo at 100 °C for 2 h, gave the 2-butyl ester. The GC retention time and CI-MS spectrum of this compound were identical with those obtained from the hydrolysis of OA-7653.

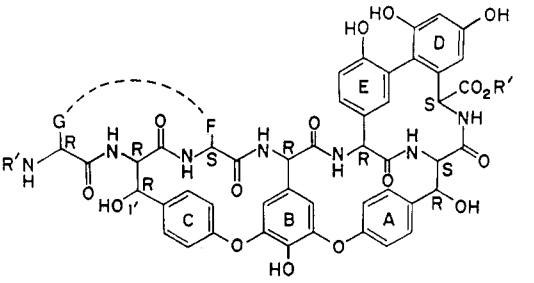
Results and Discussion

Examination of the FAB MS of OA-7653 showed an MH^+ ion at m/z 1275 and a pattern for the molecular ion cluster that suggested that the molecular formula of the antibiotic contained two chlorines. A high resolution mass measurement on the MH^+ ion gave an m/z 1275.334. While this information did not permit an unambiguous assignment of the molecular formula, $C_{58}H_{60}N_8O_{21}Cl_2$ (calculated MH^+ is m/z 1275.333) was selected as a working hypothesis based on the structural patterns established for other members of this antibiotic class.

The heptapeptide core of the aglycone of these antibiotics usually accounts for seven nitrogens in the molecular formula. Nitrogens in excess of this number in the majority of examples are accounted for by the presence of amino sugars. An exception to this is vancomycin, where eight nitrogen atoms are associated with the aglycon by virtue of the presence of the basic amino acid asparagine as one of the seven components of the heptapeptide.

To investigate the origin of the eighth nitrogen in OA-7653, we first sought to confirm the earlier finding where the presence of glucose was suggested on the basis of analyses involving paper chromatography. In support of these previous studies, the FAB spectrum of the antibiotic showed only one major fragment ion at m/z 1113 corresponding to a loss of 162 amu from MH^+, consistent with the loss of a hexose. Confirmation of this identity of the sugar as glucose was provided by GC-CI-MS analysis of the alditol acetates obtained by the appropriate derivatization of the acid hydrolyzate of OA-7653. These data indicate that OA-7653 is comprised of a heptapeptide aglycon containing eight nitrogen atoms which is linked to glucose as the sole carbohydrate component.

Amino Acid Components. The amino acids of the heptapeptide core common to these antibiotics are constituted in an arrangement such that acid hydrolysis leads to the release of the actinoidinic acid unit and the nonconserved amino acids represented by the designations G and F in structure **1**. The triphenyl ether moiety is not usually recovered in this process.[15]



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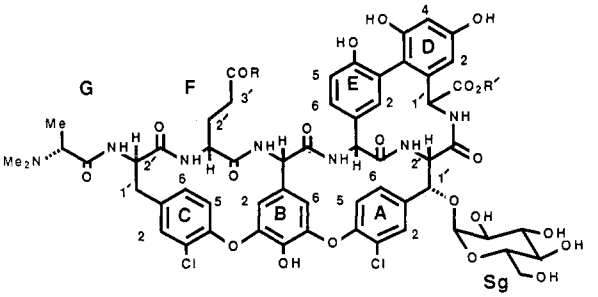
Hydrolysis of the antibiotic in 6 N HCl and analysis of the hydrolyzate by reversed phase HPLC showed the presence of glutamic acid and actinoidinic acid, the latter as a mixture of diastereomers.[15] This left one amino acid component of the heptapeptide to identify. Preliminary examination of the ^1H NMR spectrum of the antibiotic provided a clear indication for the presence of a *C*-methyl doublet at 1.1 ppm and a six-proton *N,N*-dimethyl singlet at 2.25 ppm. This observation suggested that the terminal amino acid in OA-7653 was *N,N*-dimethylalanine. By relying on the existence of a strong analogy with other antibiotics of this class and the molecular formula $C_{58}H_{60}N_8O_{21}Cl_2$, a tentative structure **2** was suggested (no stereochemistry implied) in which the eighth nitrogen was accommodated by incorporating the glutamic acid in a glutamine residue.



2 R = NH_2, R′ = H
3 R = OH, R′ = H
4 R = OMe, R′ = OMe

The presence of *N,N*-dimethylalanine in the acid hydrolyzate of the antibiotic was established by esterification of the lyophilized hydrolyzate with 2-butanol and analysis of the product by GC-CI-MS. A peak could be detected by GC that corresponded in retention times to that of an authentic sample and also showed the expected MH^+ ion at m/z 174 and characteristic fragment ions at m/z 172, 118, and 72 in the CI spectrum.

NMR Studies. Through the use of a variety of 2D NMR experiments in conjunction with computer-assisted molecular modeling and energy refinement methods, we have shown that it usually is possible to derive complete structural information for antibiotics in this series.[17]

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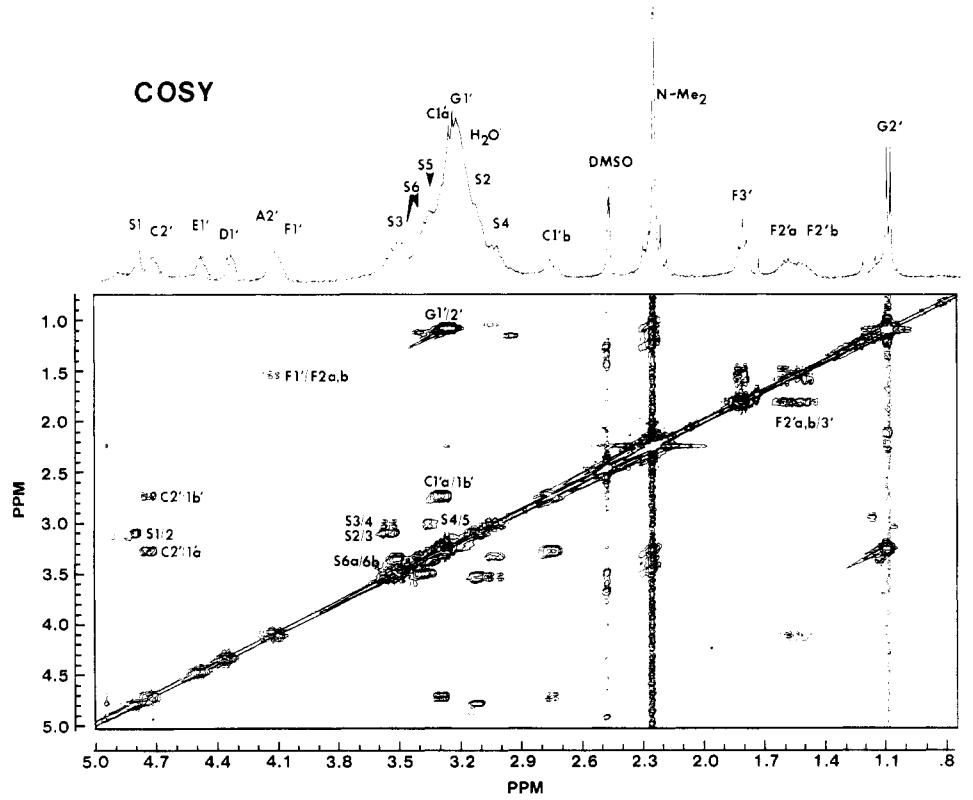

Figure 1. COSY spectrum of antibiotic OA-7653 in 1:1 DMSO/D_2O at pH 8.0 and 40 °C.

In attempting this approach with OA-7653, difficulties were encountered initially in that under a variety of conditions of solvent, pH, and temperature, the ^1H NMR spectra showed considerable line broadening (>3 Hz). Since there are examples where the NMR spectra of the corresponding aglycons give better resolution, we attempted to remove the glucose moiety from the antibiotic by acid hydrolysis. In our hands, this reaction could not be accomplished successfully. Conditions that led to hydrolysis of the sugar inevitably were accompanied by reactions that caused disruption of the heptapeptide core. Attempts to remove the glucose by incubation with α- or β-glucosidase were also unsuccessful, as only starting material was recovered from these reactions.

Despite these initial difficulties, useful information was obtained from spectra obtained in DMSO-d_6 at pH 8.0 at 40 °C, and the assignments discussed below were made in the most part from these spectra. In the course of these experiments, the origin of the line broadening as a consequence of the presence of two (or more) conformations in slow exchange in solution became evident from the occurrence of exchange crosspeaks in the NOESY spectra of OA-7653 and its derivatives **3** and **4**. The same phenomenon has been observed recently in the spectra of teicoplanin.[8] In latter experiments we were able to obtain spectra with improved line widths by the addition of H_2O to DMSO solutions. The effect of the H_2O was apparently to reduce the concentration of the minor conformer. Further studies with the diacid derivative **3** in DMSO and DMSO–H_2O showed that the major conformers in these solvents are equivalent.

The 1D ^1H NMR spectrum of OA-7653 at 500 MHz at DMSO showed, in addition to the characteristic features mentioned previously for the *N,N*-dimethylalanine resonances, a pair of one-proton multiplets at 1.60 and 1.51 ppm and a two-proton triplet at 1.82. These latter signals are reasonably assigned to the side-chain protons in the glutamine residue.

A complete assignment of the remaining spin systems in the ^1H spectrum was made from the result of the 2D COSY and double quantum coherence (DQCE) spectra. All five aromatic spin systems (three ABX and two AB) were readily identified (spectra not shown). On the basis of previous ^1H NMR spectral analysis of other glycopeptides, cf. vancomycin, the frequencies representing the aromatic protons in the two AB spins (rings B and D) and one of the ABX spins (ring E) could be confidently assigned (chemical shifts are listed in Table 1, provided as supplementary data). This left the assignment of the remaining two ABX spins to rings A and C. Since rings A and C yield ABX spin patterns, it was concluded that each ring contained one chlorine.

Six amide NH to $C^{\alpha}H$ coupling crosspeaks were identified. Three of these involved α protons which showed no other couplings to aliphatic signals and so could be collectively assigned as beloning to B1′, D1′, and E1′. The B1′ signal was assigned on the basis of the detection of long-range COSY crosspeaks to the ortho aromatic spins B2 and B6. Similarly, the F1′ signal in glutamine residue was identified from its coupling to the methylene multiplets at 1.60 and 1.15 ppm, which in turn were coupled to the triplet at 1.82 ppm. In the terminal *N,N*-dimethylalanine residue, G1′ was identified from its strong coupling crosspeak to the methyl doublet at 1.10 ppm. The two remaining spin patterns, one AMX and one ABXY, were associated with the *J* networks incorporating A2′ and C2′.

The specific assignment of aromatic protons in rings A and C and α protons A1′, C2′, D1′, and E1′ relied upon unique dipolar couplings observed in the NOESY spectra. All glycopeptides analyzed to date yield the same intense 2D NOE fingerprint involving the cluster of protons A2,

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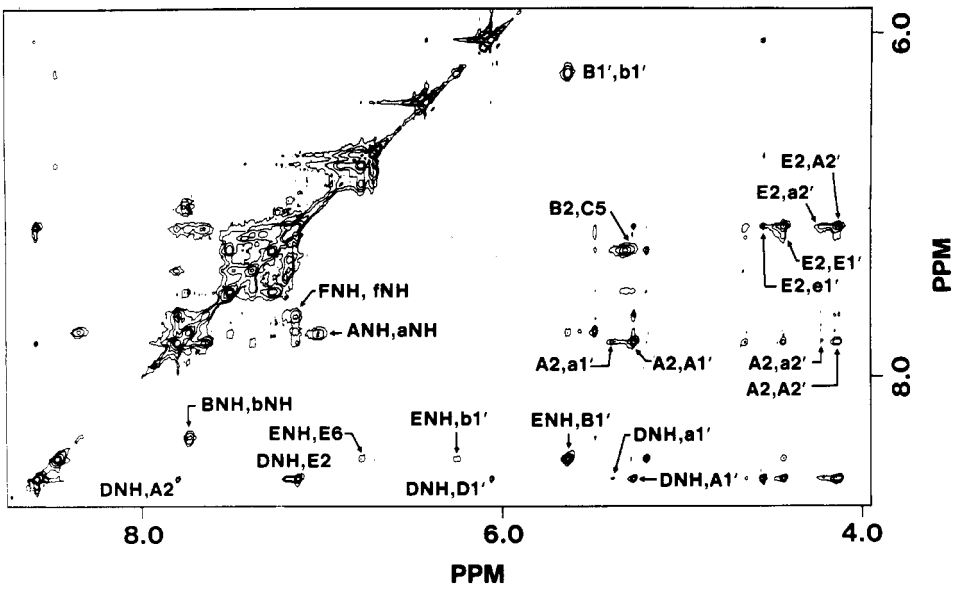

Figure 2. Computer model of the structure of antibiotic OA-7653 generated from interactive modeling using the distance information derived from quantitative NOE intensities and subsequent energy minimization employing semiempirical force field calculations.

A1′, A2′, B6, E2, E1′, and DNH (see Figure 2). The NOE map positively assigned A1′ to the AMX spin system and A2 to one of the aromatic ABX spin systems. The fact that the A2 signal occurs as a broad singlet places one chlorine substituent at the A3 position. This chlorine is located on the "inner" face of the glycopeptide, as indicated from the NOESY crosspeaks originating from the adjacent A2 proton.

From the assignment of B6, one can move through the B ring and orient the protons in the C residue relative to B2 and the BNH/FNH/CNH binding pocket. Weak NOE crosspeaks between B2 and the protons tentatively assigned as C2 and C5 indicated which of the ABX signals were closest to the B ring. In addition, CNH showed an NOE interaction with C6 but none to C2, which placed C6 toward the CNH/FNH/BNH binding pocket. Since C2 is a broad singlet, the remaining chlorine substituent can be placed at C3. This chlorine is therefore positioned on the "outer" face of the glycopeptide. Finally, NOEs observed between C2, C6, and C1′a and b clearly link the C ring protons with the ABXY spin system and confirm that C1′ is a methylene group. The COSY, DQCE, and NOESY patterns observed for the A and C residues are, in fact, almost identical with those obtained from teicoplanin A_2.

The frequencies for the sugar substituents are readily assigned on the basis of the COSY and DQCE spectra. As seen in Figure 1, the anomeric proton in the glucose residue occurs at 4.81 ppm. This resonance gives a strong coupling crosspeak to S2. Then by walking through the COSY map, all six protons can be specifically assigned. From the NOESY data, information regarding the location of the sugar and the stereochemistry of the anomeric proton was obtained. Through-space coupling is observed between S1 and A1′ and A2. This clearly places the sugar at A1′. The anomeric proton also shows a strong NOESY crosspeak to S2, but none to S3 and S5. This can only occur with glucose attached as an α-glucopyranoside.

The stereochemistry of the backbone of the heptapeptide core and the chiral center at A1′ was derived by using distance information obtained from quantifying the volume integrals of the NOESY crosspeaks in conjunction with interactive computer modeling using methods that have been previously described.[17] The model obtained from this approach corresponded in its primary stereochemical features to the stereochemistry that has been found to exist in all other antibiotics of the series in those members in which it has been determined. In deriving the model, the conformational mobility exhibited through local motions in the vicinity of the N-terminus precluded obtaining the necessary distance information to permit the unambiguous assignment of stereochemistry at the G1′ center. The stereochemistry depicted in structure **2** is based upon that found in other members of the series. A representation of the three-dimensional solution structure

Table I. Spin-Lattice T_1 Relaxation Times (s) for OA-7653 and Its Derivatives 3 and 4

residue	assignmnt	OA-7653	OA diacid	OA diester
A	ANH	1.77	1.32	(BNH/CNH)
	A2′	1.59	1.21	1.07
	A1′	1.89	1.42	1.72
	A2	1.94 (CNH)	1.64	1.52
	A5	3.72	2.57	2.87
	A6	2.91	1.95	1.95
B	BNH	1.47 (CNH)	1.23	1.15 (A/CNH)
	B1′	2.90	2.04	1.97
	B2	4.12	2.37	2.99
	B6	4.24	2.75	3.28
C	CNH	(BNH)	0.80	(ANH/BNH)
	C2′	1.56 (sgl)	0.95	1.42 (sgl)
	C1′a	0.63 (G1′)		
	C1′b	0.72	0.54	0.65
	C2	2.00	1.49	1.77
	C5	2.22	1.59	1.89
	C6	1.91	1.37	1.36
D	DNH	1.42	1.12	1.04
	D1′	4.19	1.61	4.16
	D2	2.25	1.04	2.85
	D4	0.86	0.92	1.36
	OCH_3			2.17
E	ENH	1.63	1.47	1.25
	E1′	1.73	1.34	1.15
	E2	1.75	1.39	1.20 (FNH)
	E5	2.83	1.65	1.98
	E6	3.30	1.96	2.42
F	FNH	1.33	0.98	(E2)
	F1′	1.41	1.11	1.21
	F2′a	0.68	0.61	0.57
	F2′b	0.70	0.66	0.58
	F3′	0.62	0.54	0.62
	OCH_3			1.28
G	G1′	(C1′a)[a]	*a*	*a*
	G2′	0.76	0.68	0.76

[a] Obscured by H_2O peak.

Table II. Chemical Shifts of Protons in OA-7653 and Its Derivatives 3 and 4 Reflecting the Existence of Major and Minor Conformations[a] in Slow Exchange

		OA-7653		OA diacid		OA diester	
residue	assignmnt	major	minor	major	minor	major	minor
A	ANH			7.64		7.75	7.00
	A2′	4.01		4.10		4.12	4.22
	A1′			5.38		5.27	5.39
	A2	7.80	7.64	7.81	7.65	7.80	7.64
B	BNH	7.79		7.78		7.73	8.35
	B1′	5.64	6.33	5.66	6.32	5.63	6.25
	B2	5.49	5.60	5.50	5.62	5.48	5.57
	B6	5.20	5.32	5.21	5.32	5.19	5.28
C	C2	7.23	7.45	7.22	7.47	7.19	7.42
	C5	7.20		7.20		7.17	7.32
	C6	7.32	7.39	7.32	7.47	7.38	7.48
E	E1′	4.50		4.47		4.42	4.47
	E2	7.02		7.11		7.13	7.20
	E5	6.69	6.82	6.69	6.86	6.71	6.82
	E6	6.75	6.90	6.77	6.93	6.77	6.88
F	FNH	7.20	7.62	7.18		7.14	7.65
	F1′	4.13	4.85	4.24	4.86	4.21	4.86
	F3′	1.83		1.93		2.03	2.11
G	G2′	1.10	1.24	1.09	1.25	1.08	1.22
D-glucose	Sgl	4.80	4.87	4.73	4.82	4.63	4.72

[a] All spectra were obtained for DMSO solution at 40 °C for compounds recovered by lyophilization of aqueous solutions which had been adjusted to pH 4.0.

derived from the distance data and molecular modeling is shown in Figure 3.

A clear indication of the occurrence of increased local motion of both the *N,N*-dimethylalanine and glutamine residues relative to the overall correlation time of the molecule was provided by the NOESY data where the calculated proton–proton distances within these residues were comparable but larger than predicted. The enhanced local motion in these two residues was confirmed by measurement of proton T_1 relaxation times. The T_1 values obtained for G2′, F2′a, F2′b, and F3′ show that these protons relax twice as fast as found for the other comparable (CH or CH_2) backbone protons (see Table I).

Structure of the Acid Hydrolysis Product and Its Methyl Ester. During studies of attempts to remove the glucose residue using acid hydrolysis, it was found that the antibiotic was rapidly converted to a single major product under mild acid conditions. The FAB MS of the product differed by +1 Δ amu from the parent in accord with the transformation of a primary amide to a carboxylic acid. While the most reasonable explanation of this transformation could be attributed to the conversion of the glutamine residue to glutamic acid represented by the structural change from **2** to **3**, the analogous change involving an asparagine residue in vancomycin is accompanied by a rearrangement of the peptide backbone. Although the propensity for glutaminyl peptides to undergo rearrangement is much less common than their asparaginyl counterparts, it is not without precedent.[18] However, the subsequent studies reported for the diacid **3** and its dimethyl ester **4**, the latter obtained from **2** by methanolysis,

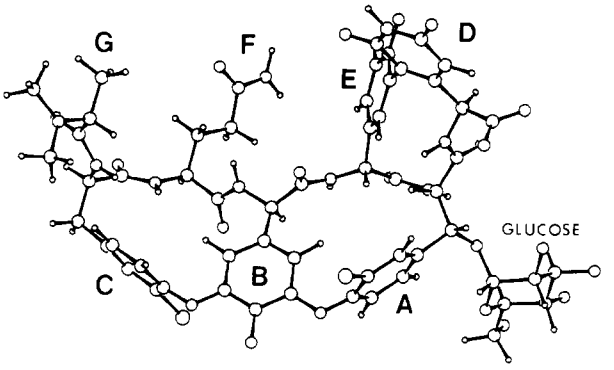

Figure 3. Section of the NOESY spectrum of the diester **4** in $DMSO/H_2O$ at pH 4.0 and 40 °C illustrating the presence of exchange NOEs indicative of the presence of a major and a minor conformation.

established that the reactions did not involve a rearrangement. It is of interest to note that both the acid hydrolysis and methanolysis reactions proceeded without cleavage of the usually sensitive glycosidic bond to glucose which, however, in this series seems to be remarkably resistant.

The general features of the NMR spectra of the diacid and diester showed a very close resemblance to that of the parent antibiotic. The similarity of the spectra for all three compounds in the region between δ 1.4 and 2.4 was particularly relevant, since the characteristic patterns of the β-CH_2 and γ-CH_2 protons that originate from the glutamine residue in this region of the spectrum of OA-7653 also occur in the spectra of **3** and **4** (see supplementary material). It seems very unlikely that the predicted rearrangement product in which the β- and γ-CH_2 groups originating from glutamine ring are constrained in a macrocyclic ring, would show the same J couplings as the analogous side-chain methylenes in OA-7653. To support this argument, the T_1 relaxation times of the protons of **3** and **4** were obtained and compared with the T_1's for OA-7653. The values reported in Table I are essentially the same for all three compounds.

Further evidence for structures **3** and **4** was provided by the distance data calculated from quantitative NOE measurements, which are readily accommodated by the

(18) (a) Battersby, A. R.; Reynolds, J. J. *J. Chem. Soc.* **1961**, 542–530. (b) For a general discussion, see: Bodansky, M.; Martinez, J. In *The Peptides*; Gross, E., Meinhofer, J., Eds.; Academic Press: New York, 1979; Vol. 5, pp 111–216.

(19) Although it is clear that the major course of the hydrolysis of OA-7653 proceeds to give the unrearranged product **3**, the HPLC profile of the reaction shows the presence of several minor components that occur during the hydrolysis. Since no attempt was made to isolate and characterize these compounds, we cannot rule out that they may represent products in which the peptide backbone has undergone rearrangement and/or compounds where the orientation of the C ring differs in topology, analogous to the CDP-1 isomers of vancomycin.[20]

(20) Harris, C. M.; Kopecka, H.; Harris, T. M. *J. Am. Chem. Soc.* **1983**, *105*, 6915–6922.

proposed structures and could not be reconciled with models generated for the rearrangement products.

Conformational Exchange. In the course of examining the 1D spectrum of the diester 4 in DMSO-d_6 at pH 4.0, a number of extraneous peaks were present which we first assumed to originate from minor impurities. However, two subsequent observations indicated that these extra peaks originated not from impurities but were due to a minor conformational isomer that under these conditions was in slow exhange. First, the signals associated with what was assumed to originate from a second component essentially disappeared on addition of D_2O or H_2O to the sample and were not observed when the same sample was run at pH 6.0 in DMSO. Convincing evidence that the additional peaks originated from a second component in equilibrium with the major conformer was obtained from the NOESY spectrum for a DMSO solution at pH 4.0. This NOESY spectrum contained a large number of exchange crosspeaks between signals of the major and minor components, clearly indicating that the two species in solution are in equilibrium. A region of the NOESY spectrum of the diester is shown in Figure 2 and clearly reveals the increased intensity of the exchange crosspeaks, cf. (ANH, aNH), (BNH, bNH), (B1′, b1′), and (FNH, fNH), relative to the crosspeaks observed for dipolar couplings. A comparison of the relative intensities of peaks from the major conformer with those in the minor conformer in the 1D spectrum indicated that the concentration of minor conformer under these conditions (DMSO at pH 4.0) is <10%.

Following the observations of exchange NOEs in the spectrum of the diester, the NOESY spectra of both OA-7653 and the diacid 3 in DMSO solution at pH 4.0 were also observed to show exchange crosspeaks consistent with the existence of two observable conformations, one major and the second occurring at the level of ≃10% of the other. Unfortunately, it was not possible to derive any details of the structure of the minor conformation which could be used to derive information on the structural differences between the major and minor conformations. However, the pattern of chemical shift differences between the major and minor components is the same for all three compounds (see Table II). The rather significant difference in chemical shift of the B1′ resonance in the major and minor conformations throughout the series is noteworthy.

The existence of slow conformational exchange in teicoplanin and in OA-7653 and its derivatives in DMSO solution suggests that the occurrence of this phenomena in this class of antibiotics should be considered along with other reasons such as aggregation as a source of line-broadening in the ^1H spectra of these antibiotics.

Conclusions. The structure of the antibiotic OA-7653 places it with vancomycin and its analogues[21] in a subgroup where the variable G and F residues both originate from aliphatic amino acids. A further similarity with vancomycin is that the F residue in OA-7653 is the amino acid glutamine, whereas this position in vancomycin is occupied by asparagine. The glutamine residue is shown to undergo facile acid hydrolysis without rearrangement in providing the diacid 3. Like teicoplanin, OA-7653, the diacid 3 and its dimethyl ester 4 are each shown to exist in DMSO solutions as a pair of conformers that exchange at a rate comparable to the NMR time scale.

Acknowledgment. We are indebted to C. Pan and J. Dingerdissen for carrying out the fermentations and biological assays and providng assistance with the isolation. The FAB MS and GC-CI-MS data were kindly provided by G. Roberts and L. Killmer.

Supplementary Material Available: Figure showing a comparison of the 500-MHz 1D spectra of compounds 2, 3, and 4, which illustrate the signal profiles of the glutamate side chains in these compounds; table of complete ^1H chemical shift assignments for OA-7653 and its derivatives 3 and 4; table identifying proton pairs involved in through-space connectivities from the NOESY spectra of OA-7653, the diacid 3, and ester 4; and a table showing distance data derived from the computer model of OA-7653 with that obtained from the values calculated from quantitative NOE intensities for OA-7653 and compounds 3 and 4 (10 pages). Ordering information is given on any current masthead page.

(21) (a) Hunt, A. H.; Marconi, G. G.; Elzey, T. K.; Hoehn, M. *J. Antibiot.* **1984**, *37*, 917-919. (b) Boeck, L. D.; Marconi, G. G.; Hoehn, M. M. U.S. Patent 4 558 008.10, Dec 1985. We are indebted to a referree for drawing our attention to the vancomycin analog described in these references.

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A Formal Total Synthesis of (±)-Laurenene

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The tetracyclic α,β-unsaturated ketone 2, a key intermediate in the Itô and Crimmins syntheses of racemic laurenene, is prepared in nine steps from the known functionalized triquinane 3. Despite the presence of considerable steric congestion, 3 smoothly undergoes conversion to the silylated cyanohydrin and subsequently to nitrile 6. In the key step, the anion of 6 is efficiently alkylated from its β face to give only 7. Following reduction of the nitrile function to an angular methyl group, the keto aldehyde 9 is elaborated and cyclized effectively under acidic conditions. Neither the structurally related methyl ketone 14 nor the intermediates 16 and 17 could be made to undergo analogous ring closure under a variety of reaction conditions.

In 1979, Corbett and co-workers reported the isolation of a new diterpene hydrocarbon from the volatile oil of *Dacrydium cupressinum*.[1] This levorotatory substance, for which the name laurenene was proposed, was identified as Nature's only known fenestrane molecule, chiefly on the strength of X-ray crystallographic analysis of a brominated derivative.[2] The Otago, New Zealand group headed by

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(2) Corbett, R. E.; Couldwell, C. M.; Lauren, D. R.; Weavers, R. T. *J. Chem. Soc., Perkin Trans. 1* **1979**, 1791.

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