leading in determining whether the reaction is concerted or stepwise. Successive labeling at various positions of a single substrate is desirable to obtain a firm conclusion.

Acknowledgment. Numerical calculations were carried out at the Computer Center of the Institute for Molecular Science and the Osaka University Computation Center.

Registry No. 1, 71-23-8; 2, 19252-52-9; 3, 17806-70-1; 4a, 17456-36-9; protonated neopentyl alcohol, 18682-92-3.

Structure of the Pseudoaglycon of Actaplanin

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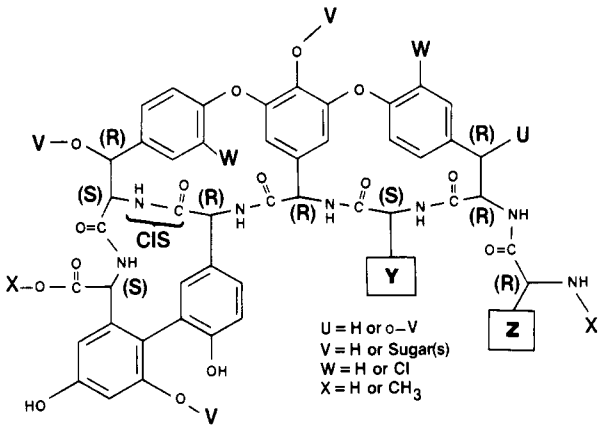
The structure of the pseudoaglycon of actaplanin is described. The proposed structure is based on ^{1}H NMR studies of the ψ-aglycon in dimethyl sulfoxide solution (including observation of negative nuclear Overhauser effects), analogies with the structures of other glycopeptides, and the products of oxidative degradation of the actaplanin aglycon. The actaplanin ψ-aglycon differs from that of ristocetin by the absence of a benzylic OH and the presence of an aromatic Cl.

Introduction

Actaplanin (A4696) is a complex of glycopeptide antibiotics produced by *Actinoplanes missouriensis*;[1] other antibiotics of this class include vancomycin,[2] ristocetin (ristomycin),[3,4] avoparcin,[5] and A35512B.[6] The general structure 1 is shared by all the glycopeptides for which structures have been reported;[2-6] they may be divided into three subclasses, on the basis of the structures of the groups Y and Z in 1.

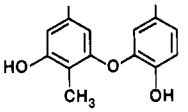


1

(1) Y, Z = Aliphatic. The only reported example of this glycopeptide type is vancomycin, where Y and Z are the side chains of L-asparagine and D-*N*-methylleucine, respectively.[7] The absolute configurations shown in 1 have been determined for a vancomycin degradation product, CDP-I, by X-ray crystallography (based on the known configurations of asparagine and *N*-methylleucine);[2a] the relationship between CDP-I and vancomycin has recently been described by Harris and Harris.[2b] The cis amide linkage shown in 1 is present in the CDP-I crystal structure, and NMR results have supported such a linkage in all other glycopeptides for which spectra have been described.[3a,5c,6c,8]

(2) Y, Z = Aromatic. Two glycopeptide families of this type have been reported, where Y and Z may be a phenylalanine or a *p*-hydroxyphenylglycine side chain: α- and β-avoparcin[5] and actinoidin A and B.[9]

(3) Y, Z = a Diphenyl Ether. The glycopeptide antibiotic that has been studied the most extensively (after vancomycin) is ristocetin A; the ristocetins A and B differ only in the nature of the sugars attached to 1.[10] In the ristocetins[3,4] and in A35512B,[6] the groups Y and Z in structure 1 are linked together by an ether bond; in ristocetin the substituent has the structure 2.[4]



2

The actaplanins are glycopeptides of the ristocetin type; they each contain the diphenyl ether linkage 2. The A4696 complex differs from all previously reported glycopeptides in having only one benzylic hydroxyl group on the aglycon; i.e., U = H in structure 1 for the actaplanins. The actaplanins differ from each other in the nature and distribution of sugars attached to the peptide core;[11] appropriate

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(8) Williamson, M. P.; Williams, D. H. *J. Am. Chem. Soc.* **1981**, *103*, 6580-6585.

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(11) Hunt, A. H.; Elzey, T. K.; Merkel, K. E.; Debono, M. *J. Org. Chem.*; following paper in this issue.

0022-3263/84/1949-0635$01.50/0

hydrolysis conditions remove only the neutral sugars and leave an amino sugar attached to the peptide,[3b] thus producing the pseudoaglycon ("ψ-aglycon"). The structure of the actaplanin ψ-aglycon described in this paper is based on proton NMR studies of the intact ψ-aglycon and isolation of aromatic fragments of the antibiotic following oxidative degradation.

Experimental Section

NMR Studies. The ψ-aglycon was prepared from the actaplanin complex by acid hydrolysis (5% HCl in refluxing methanol[3b]), followed by precipitation from aqueous acetonitrile as the dihydrochloride salt. Solutions were prepared in either Me_2SO-d_6 or Me_2SO-d_6 containing D_2O; for the latter solvent the ψ-aglycon was lyophilized three times from D_2O before use. Spectra were recorded at ambient temperature (~23 °C) by using a Bruker WH360 spectrometer in the Fourier transform mode. Difference NOE (nuclear Overhauser effect) spectra were obtained by subtracting free induction decays accumulated with the decoupler off-resonance from accumulations with particular resonances irradiated, followed by Fourier transformation of the difference signals. The procedure was not optimized for maximum NOE measurement; the usual irradiation period was 2.0 s, followed by a preaccumulation delay of 0.03 s. Typical decoupler power settings were 16 dB attenuation (0.2-W level) for normal decoupling and 22–26 dB for difference NOE measurements; when overlap of the irradiating frequency to neighboring resonances was suspected, the decoupling was repeated at a lower power level. NOE observations that disappear at lower power levels were not considered in the structure elucidation reported here. The problem of large NOEs arising by spin migration due to the exchange of OH and NH protons[8] has been addressed by doing full decoupling studies in both Me_2SO and Me_2SO/D_2O; NOEs between nonexchangeable protons that are significantly reduced by the additon of D_2O have been discarded. The NOE percentages reported in the text were computed from decreases in peak height (negative NOEs); these values have only qualitative significance, since they were measured under nonoptimized conditions.

Plasma Desorption Mass Spectrometry. PDMS results have been obtained for the actaplanin ψ-aglycon by Prof. R. D. Macfarlane (Texas A and M University): calculated for $C_{66}H_{61}O_{20}N_8Cl$ 1321.7; found 1321.8.

Degradative Studies. A. Actaplanin Aglycon Formation. The actaplanin complex (3.0 g) was dissolved in 25 mL of H_2O, acidified with 3.75 mL of 4 N HCl, and refluxed for 1.5 h. After being cooled, the mixture was evaporated to dryness (40 °C, in vacuo) and redissolved in hot water. The aglycon was precipitated from solution by dropwise addition of 4 N HCl. The precipitate was collected by filtration after cooling and was dried under reduced pressure over P_2O_5 to give 2.0 g of a tan powder. This product was examined by TLC [cellulose/aluminum; butanol:pyridine:acetic acid:water, 15:10:3:12] and was shown by bioautography to consist of two components which retained antimicrobial activity: (a) R_f 0.88 and (b) R_f 0.72. The precipitate was chromatographed twice on 200 g of alumina (acidic, Grade I), which had previously been washed with methanol. Elution with methanol (500 mL) and with 10% H_2O–methanol separated the two components. Bioassay showed (a) to have an antimicrobial assay of 200 units/mg while (b) assayed for 440 units/mg.

B. Potassium Permanganate Oxidation of Actaplanin Aglycon. The actaplanin aglycon (1.94 g) was suspended in methanol (20 mL), and 4.0 g of anhydrous potassium carbonate and 20 mL of methyl iodide were added. The reaction mixture was refluxed for 4 h and concentrated to dryness under reduced pressure. The residue was suspended in the minimum amount of water, filtered, washed with water, and dried under reduced pressure (over P_2O_5) to give 1.0 g of product.

This methylated actaplanin aglycon (1.0 g) was suspended in 75 mL of H_2O and heated to 80 °C (oil bath), and 26.25 mL of 2 N NaOH was added. Potassium permanganate solution (7.5 g in 173 mL of H_2O) was added slowly, and heating was continued at 80 °C for 1 h. The reaction mixture was acidified, and the minimum amount of solid $NaHSO_3$ was added to destroy the MnO_2 that had formed. The reaction mixture was filtered to remove a precipitated solid [MS: M^+ m/e 458, 460 (monochloro)]; treatment of this solid with CH_2N_2 in ether–methanol gave a new substance, **3** [MS: M^+ m/e 500, 502 (monochloro)]. The filtrate of this oxidation reaction was extracted with ethyl acetate (3×); the extract was dried over Na_2SO_4 and evaporated under reduced pressure. The residue was dissolved in methanol and esterified with excess ethereal CH_2N_2. Removal of the solvent gave a syrup, which was chromatographed on 20 g of silica gel in benzene. The elution was carried out with 500-mL portions of benzene–ethyl acetate containing the following fractions of ethyl acetate: 0%, 2%, 5%, 10%, 20%, 50%, and 100%. Fractions were grouped by TLC analysis (silica gel, 30% ethyl acetate in benzene, UV detection), and combinations were analyzed by mass spectrometry (Varian MAT 731). Three major products were revealed by MS analysis: **3** (M^+ m/e 500, 502 (monochloro)); **4** (M^+ m/e 404); and **5** (M^+ m/e 224).

Methyl 3-[2-chloro-4-(methoxycarbonyl)phenoxy]-5-[4-(methoxycarbonyl)phenoxy]-4-methoxybenzoate (3): mp 105–107 °C (Et_2O); calcd for $C_{25}H_{21}O_9Cl$ 500.0874, found (HRMS) 500.0878; IR (mull) 1720, 1600 cm^{-1}; NMR ($CDCl_3$) δ 6.95 (d, 2 H, J = 9 Hz) and 8.05 (d, 2 H, J = 9 Hz) (A_2B_2 pattern), 6.85 (d, 1 H, J = 9 Hz) and 7.88 (dd, 1 H, J = 9, 2 Hz) and 8.17 (d, 1 H, J = 2 Hz) (ABX pattern), 7.55 (d, 1 H, J = 2 Hz) and 7.67 (d, 1 H, J = 2 Hz) (four CH_3O groups at 3.82, 3.84, 3.89, and 3.90) (s, 3 H each); UV (methanol) λ max (ε) = 206 (60 800) and 253 (45 700).

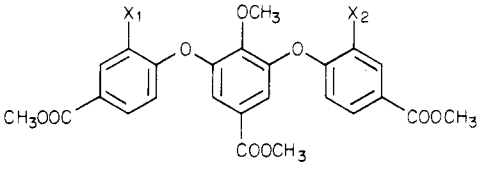
2,5-Bis(methoxycarbonyl)-3-methoxyphenyl 5-(methoxycarbonyl)-2-methoxyphenyl ether (4): mp 126–129 °C; calcd for $C_{20}H_{20}O_9$ 404.1108, found (HRMS) 404.1111 (verified by elemental analysis); NMR ($CDCl_3$) δ 6.90 (d, 1 H, J = 2 Hz), 6.97 (d, 1 H, J = 9 Hz), 7.29 (d, 1 H, J = 2 Hz), 7.67 (d, 1 H, J = 2 Hz), 7.88 (dd, 1 H, J = 9, 2 Hz), five CH_3O groups at 3.82, 3.83, 3.84, 3.88, and 3.89 (s, 3 H each).

Dimethyl 4-methoxyisophthalate (5): mp 95–97 °C (lit.[12] mp 95–96.8 °C); calcd for $C_{11}H_{12}O_5$ 224.0683, found (HRMS) 224.0682; NMR ($CDCl_3$) δ 7.00 (d, 1 H, J = 9 Hz), 8.15 (dd, 1 H, J = 9, 2 Hz), 8.46 (d, 1 H, J = 2 Hz), three CH_3O groups at 3.85 (s, 6 H) and 3.97 (s, 3 H).

Results

Aromatic Fragments of Actaplanin Aglycon. Esterification of the reaction products from $KMnO_4$ oxidation of actaplanin aglycon produced three crystalline products, compounds **3**, **4**, and **5**.

Compound **3** ($C_{25}H_{21}O_9Cl$) has physical properties (see Experimental Section) consistent with the structure



3; X_1 = Cl, X_2 = H
3a; X_1 = X_2 = Cl
3b; X_1 = X_2 = H

The degradation product **3** or closely related products are obtained from other glycopeptides by the same procedures: **3a** from vancomycin,[13] **3b** from ristocetin[4] and A35512B,[6b] and **3** from avoparcin[5b] and actinoidin.[14] These compounds have been shown to arise from complex amino acids in the antibiotic aglycons;[4b,5b] oxidation of amino acid **6** in actaplanin aglycon gives rise to compound **3**.

The numbering system in **6** and subsequent structures is arbitrary; it is included to facilitate discussion of the ψ-aglycon proton NMR studies. The degradation results do not allow the identity of R_1 and R_2 to be specified. In

(12) Smith, K. A.; Williams, D. H.; Smith, G. A. *J. Chem. Soc.; Perkin Trans. 1* **1974**, 2369–2376.
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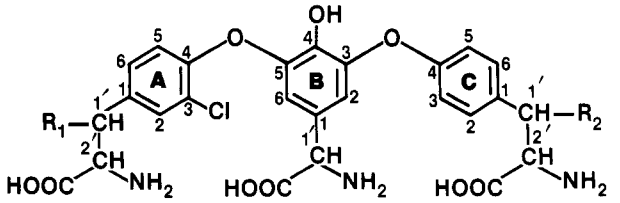
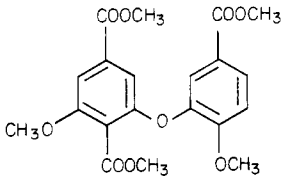


6, R_1, R_2 = H or OH

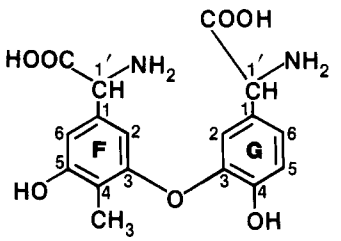
principle, they could be both OH, both H, or one of each; this point will be discussed further below.

Degradation product 4 ($C_{20}H_{20}O_9$) has properties consistent with the structure



4

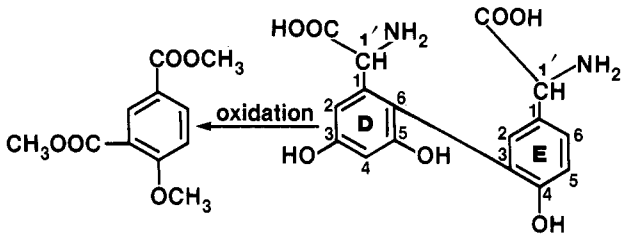
The properties of 4 are identical with those for a product isolated from the degradation of ristocetin A;[15] 4 has been shown to arise from the oxidation of ristomycinic acid (7).[4b]



7

The proton NMR spectra of the actaplanin complex and actaplanin ψ-aglycon both contain an aromatic CH_3 resonance (1.96 ppm, 3 H), indicating the presence of the methylated diphenyl ether linkage (see 2).

Degradation product 5 ($C_{11}H_{12}O_5$) is identical with dimethyl 4-methoxyisophthalate, which has been isolated previously from a number of glycopeptides.[6b,13,16] Compound 5 has been shown to arise from actinoidinic acid (8) during oxidative degradation of glycopeptides.[15]



5 8

Structure of the Actaplanin ψ-Aglycon

Hydrolytic studies have shown that the amino sugar present in actaplanin is L-ristosamine.[1] The major constituents of the actaplanin ψ-aglycon, therefore, are L-ristosamine and the amino acids 6, 7, and 8. The complete assignment of the 360-MHz ^1H NMR spectrum of the ψ-aglycon, together with the observation of a large number of nuclear Overhauser effects, has led to elucidation of the

(15) Harris, T. M.; Fehlner, J. R.; Raabe, A. B.; Tarbell, D. S. *Tetrahedron Lett.* 1975, 2655–2658.
(16) Harris, C. M.; Kibby, J. J.; Harris, T. M. *Tetrahedron Lett.* 1978, 705–708.

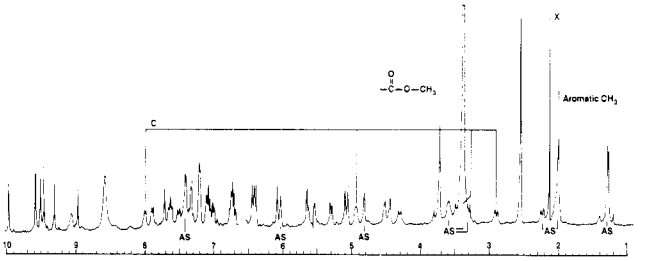


Figure 1. 360-MHz ^1H NMR spectrum of actaplanin ψ-aglycon dihydrochloride (in Me_2SO-d_6, ambient temperature). Peaks marked AS arise from the amino sugar; the resonances marked C are explained in the text. (The peak marked X arises from residual acetonitrile; it disappears after lyophilization.)

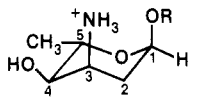
Table I. NMR Parameters for Ristosamine in Actaplanin ψ-Aglycon

proton	chemical shift, δ	remarks
1	4.79	broadened singlet
2a	2.18	broadened doublet (J = 14 Hz)
2e	~2.00	partially buried under aromatic CH_3
3	3.54	broad singlet
NH_3^+	7.40	coupling to 3 observed
4	3.33	overlaps an amino acid aliphatic peak (and HDO)
OH	6.00	coupled to 4 (J = 4 Hz)
5	3.26	multiplet (J = 9, 6 Hz)
CH_3	1.22	doublet (J = 6 Hz)

aglycon structure without ambiguity. Similar studies have led to the structures recently described for ristocetin A,[3a] avoparcin,[5c] and A35512B ψ-aglycon.[6c]

A. General Assignments. The spectrum of actaplanin ψ-aglycon dihydrochloride in Me_2SO-d_6 is shown in Figure 1. Several pieces of structural information may be obtained from this spectrum.

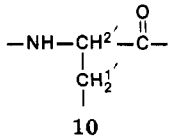
(1) The resonances of the amino sugar (AS) ristosamine are listed in Table I. The coupling of the anomeric proton 1 to either 2a or 2e is small; it is therefore equatorial. The large coupling between protons 4 and 5 indicates that they are both axial and that ristosamine is present in actaplanin as the α anomer 9. The ristosamine OH doublet (6.00



9, ristosamine (α anomer)

ppm, J = 4 Hz) is the only aliphatic hydroxyl resonance in the spectrum of the actaplanin ψ-aglycon.

(2) The ristosamine proton 4 overlaps another resonance at ~3.33 ppm. Irradiation at 3.33 ppm causes a broadened doublet (J = 13 Hz) at 2.85 ppm to collapse and a broad singlet at 4.92 ppm to sharpen; irradiation at 4.92 ppm causes collapse of a doublet at 7.98 ppm. These results suggest a structure such as 10, and they indicate that in



10

amino acid 6 either R_1 or R_2 = H. For simplicity in the subsequent discussion, the resonances in this grouping are labeled C; they are listed in Table II.

(3) The amino acid structures 6, 7, and 8 contain six phenols, and the spectrum in Figure 1 shows six phenolic resonances (8.97, 9.31, 9.47, 9.51, 9.57, and 9.98 ppm) for

Table II. Proton NMR Assignments[a] for Actaplanin ψ-Aglycon·2HCl Compared with Corresponding Assignments for A35512B ψ-Aglycon·2HCl,[6c] Ristocetin A,[3a] and Vancomycin;[22] Chemical Shifts, δ (J, Hz)

proton	actaplanin	A35512B	ristocetin	vancomycin
A-NH	7.49 (11)	7.10 (11)	7.20 (12)	6.50 (12)
A-2′	4.26 (~10)	4.31 (11)	4.38 (12)	4.22 (12)
A-1′	5.07 (~0)	5.10 (~0)	5.17 (~0)	5.13 (~0)
A-2	7.70	7.50 (8)	7.55 (8)	7.87
A-3	Cl	6.89 (8)	6.88 (8, 2)	Cl
A-5	7.31 (8)	7.15	7.21 (8, 2)	7.28 (8)
A-6	7.33 (8, 1)	7.41 (8)	7.41 (8)	7.48 (8)
B-NH	7.60 (~8)	7.07 (8)	7.76 (8)	8.14 (8)
B-1′	5.61	5.56 (8)	5.65 (8)	5.71 (8)
B-2	5.63	5.84	5.85	5.63
B-4 (OH)	9.47	9.43		
B-6	5.03	5.18	5.38	5.21
C-NH	7.98 (8)	7.74 (8)	7.21 (9)	8.00 (9)
C-2′	4.92	5.10	5.09 (9, 5)	4.86 (4)
C-1′	3.33	5.12	5.19 (5)	5.15 (4)
C-1″	2.85 (13)	OH	OH	OH
C-2	7.85 (8)[b]	7.96 (8)	7.86 (8)	7.57 (8)
C-3	6.90 (8, 2)	7.07	7.25 (8)	7.20 (8)
C-5	7.19 (8)	~7.14	7.29 (8)	Cl
C-6	7.05 (8)	~7.10	7.12 (8, 2)	7.42
D-NH	9.05	9.06	9.26 (5)	8.37 (7)
D-1′	4.41 (5.5)	4.39 (5.5)	4.55 (5)	4.50 (7)
D-2	6.06	6.06	6.32 (2)	6.30 (2)
D-3 (OH)	9.51	9.56		
D-4	6.42 (2)	6.44	6.85 (2)	6.44 (2)
D-5 (OH)	8.97	8.83		
D-$COOCH_3$	3.73	3.70		
E-NH	8.57	8.77	8.58 (6.5)	8.43 (6)
E-1′	4.49 (6)	4.59 (5)	4.73 (6.5)	4.50 (6)
E-2	7.19 (1)	7.26	7.26 (2)	7.19
E-4 (OH)	9.31	9.35		
E-5	6.69 (8)	6.72 (8)	6.77 (8)	6.73 (8)
E-6	6.74 (8, 1)	6.74 (8)	6.84 (8, 2)	6.78 (8, 1)
F-NH	7.62 (10)	7.60 (10.5)	7.41 (10)	6.59 (7)
F-1′	5.27 (10)	5.94 (10.5)	5.25 (10)	4.38 (7)
F-2	6.40	6.54	6.42	
F-4 (CH_3)	1.96	H	2.01	
F-5 (OH)	9.57	10.59		
F-6	6.38	Cl	6.45	
G-NH_3^+	8.57	8.61		
G-1′	5.51	5.50	4.83	
G-2	6.72 (1)	6.63	6.59	
G-4 (OH)	9.98	10.17		
G-5	7.09 (8)	7.14	7.03 (8)	
G-6	7.19 (8)	7.26	7.18 (8, 2)	

[a] Me_2SO solutions; ~28 °C for actaplanin and A35512B, 70 °C for ristocetin and vancomycin. [b] Resonances on ring C are numbered so that the most downfield peak is C-2.

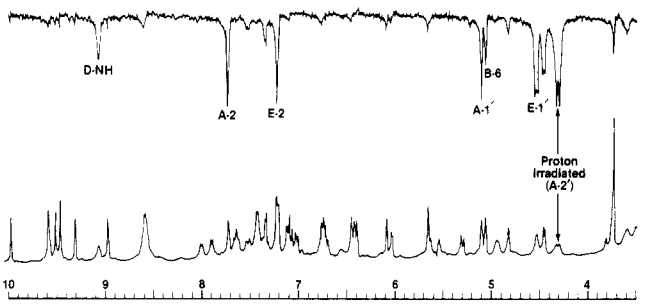
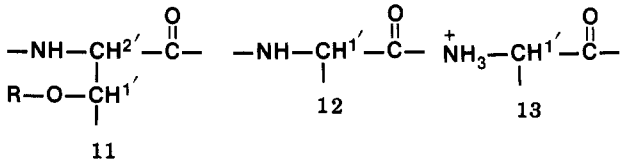


Figure 2. Lower scan, similar to Figure 1; upper scan, difference NOE spectrum produced by irradiating the A-2′ proton (resonance at 4.26 ppm) of the actaplanin ψ-aglycon dihydrochloride.

the ψ-aglycon. The site of ristosamine attachment, therefore, must be an aliphatic hydroxyl group, indicating that either R_1 or R_2 = -O-(ristosamine) in amino acid **6** in the ψ-aglycon. All six of the phenolic resonances have been assigned by observation of negative NOEs produced at adjacent ring proteons when the phenolic peak is irradiated; these assignments are included in Table II. The resonance at 9.47 ppm is the only one of the six for which no NOE is observed, indicating that this peak arises from the phenol of amino acid **6**, which has no adjacent protons.

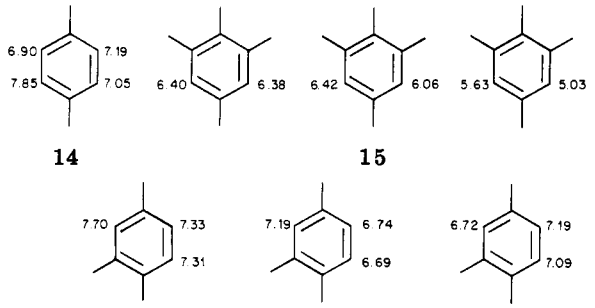
(4) The ψ-aglycon dihydrochloride spectrum contains two NH_3^+ resonances, one of which arises from the amino sugar. The second (8.57 ppm) is from the amino terminal of the peptide core. The presence of a singlet of intensity 3 at 3.73 ppm in the spectra of both the ψ-aglycon and the intact antibiotic indicates that actaplanin, like ristocetin[3] and A35512B,[6] has a methyl ester at its carboxy terminal.

B. Specific Assignments. Decoupling experiments show that the actaplanin ψ-aglycon contains (in addition to **10**) one substructure of type **11**, four of type **12**, and one of type **13**. The resonances arising from these groups have



been assigned on the basis of NOE observations to be discussed below and on chemical shift comparisons with data from other glycopeptides, as illustrated in Table II.

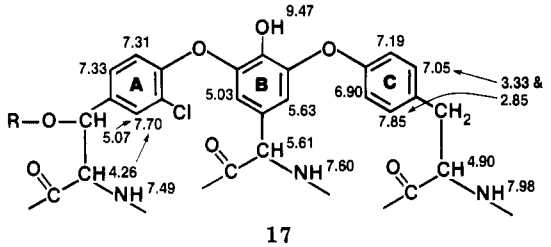
The substructures are labeled as follows in Table II: 11 (A), 12 (B, D, E, F,), and 13 (G). The peaks remaining to be classified into substructure groups all arise from protons on aromatic rings. Decoupling experiments in both Me_2SO and Me_2SO/D_2O have shown the presence of one para-disubstituted aromatic ring (14), three rings having two meta protons and four other substituents (15), and three trisubstituted rings with protons at positions 2, 5, and 6 (16). During the course of the spin-decoupling experiments a large number of negative nuclear Overhauser effects (NOEs) were observed, arising from the close proximity of many of the protons in the glycopeptide core. Consideration of the spacial requirements imposed by the NOEs allows the complete structure of the actaplanin ψ-aglycon to be derived.



14 15 16

(1) Amino Acid 6. The tetraproton structure 14 obviously corresponds to ring C in amino acid 6. The aromatic doublet at 7.05 ppm experiences a negative NOE when the resonance at 3.33 ppm (Figure 1) is irradiated; a similar NOE occurs at 7.85 ppm when the peak at 2.85 ppm is irradiated. These NOEs indicate that R_2 in structure 6 is H, and thus that 10 is attached to ring C. From this it follows that R_1 in structure 6 is OH [or -O-(ristosamine) in the ψ-aglycon] and that 11 is attached to ring A. The aromatic singlet at 7.70 ppm experiences negative NOEs from the resonances at 5.07 and 4.26 ppm in structure 11; this allows the assignment of the aromatic resonances at 7.31, 7.33, and 7.70 ppm to ring A. (The 7.70-ppm singlet experiences a number of other NOEs as well; several of these are listed in Table III and discussed below.)

The chemical shift comparisons of the various glycopeptides listed in Table II indicate that the aromatic protons on ring B occur at unusually high field, while the B-1′ proton occurs at low field for an α-CH. The resonances from the central portion of amino acid 6 are assigned on this basis and are shown in 17, which contains



17

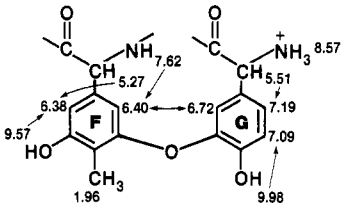
the complete assignment of resonances from the triphenyl amino acid fragment in actaplanin ψ-aglycon; NOEs are shown as arrows (R = ristosamine).

(2) Amino Acid 7. The structure 13 is the amino terminal of the aglycon dihydrochloride, and it must be attached to ring G in amino acid 7. Irradiation of the G-1′ resonance (5.51 ppm in Me_2SO; shifts to 5.38 ppm in Me_2SO/D_2O after lyophilization of the aglycon from D_2O three times) causes reduction of the aromatic doublet at 7.19 ppm (shifts to 7.14 ppm in Me_2SO/D_2O), allowing the assignment of the aromatic resonances at 7.19, 7.09, and 6.72 ppm in 16 to ring G. The meta-coupled resonances 6.38 and 6.40 ppm (15) arise from ring F of amino acid 7; the 6.38-ppm peak is reduced by irradiation of either the 9.57-ppm phenolic resonance or the F-1′ doublet at 5.27 ppm, while the 6.40-ppm peak experiences NOEs from F-NH (7.62 ppm) and G-2 (6.72 ppm). The complete assignment of the biphenyl ether fragment in the actaplain ψ-aglycon is therefore as shown in 18.

Table III. NOEs[a] for Actaplanin ψ-Aglycon·2HCl, A35512B ψ-Aglycon·2HCl,[6c] Ristocetin A,[3a] and Vancomycin[8]

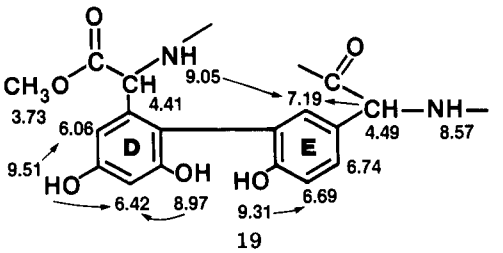
proton irradiated	resonances reduced (%)			
	actaplanin	A35512B	ristocetin	vancomycin
A-1′	A-2 (40)		A-2 (15)	A-2 (49)
A-2′	A-1′ (27)[b]	A-1′ (21)[b]	A-1′ (10)	A-1′ (32)
A-2′	A-2 (37)	A-2 (25)	A-2 (20)	A-2 (40)
A-2′	D-NH (31)	D-NH (46)		D-NH (43)
A-2′	E-1′ (41)	E-1′ (57)	E-1′ (60)	E-1′ (35)
A-2′	E-2 (27)[b]	E-2 (27)	E-2 (25)	E-2 (48)
C-1′	C-6 (33)[b]		C-6 (20)	
C-1″	C-2 (30)[b]			
C-NH	F-2 (12)	F-2 (55)[c]		
C-NH	F-NH (40)			
D-NH	A-2′ (22)	A-2′ (30)		A-2′ (11)
D-NH	A-2 (20)	A-2 (13)		A-2 (8)
D-NH	E-1′ (12)	E-1′ (28)		E-1′ (2)
D-NH	E-2 (6)	E-2 (27)		E-2 (6)
E-1′	A-2′ (50)	A-2′ (50)	A-2′ (40)	A-2′ (56)
E-1′	A-2 (34)	A-2 (19)		A-2 (33)
E-1′	B-6 (10)	B-6 (26)		B-6 (30)
E-1′	D-NH (25)	D-NH (31)		D-NH (38)
E-1′	E-2 (31)[b]	E-2 (27)	E-2 (30)	E-2 (56)
E-NH	B-1′ (43)	B-1′ (31)	B-1′ (25)	B-1′ (53)
F-1′	F-6 (28)		F-6 (40)	
F-2	G-2 (24)			
F-NH	F-2 (36)	F-2 (55)[c]	F-2 (20)	
F-NH	C-NH (13)			
G-1′	G-6 (28)[b]	G-6 (15)[b]	G-6 (15)	
G-2	F-2 (20)			

[a] Spectra recorded in Me_2SO at ~23 °C for actaplanin and A35512B ψ-aglycons, variable temperatures for ristocetin A, and 35 °C for vancomycin. [b] Measured in Me_2SO/D_2O. [c] Both C-NH and F-NH irradiated.



18

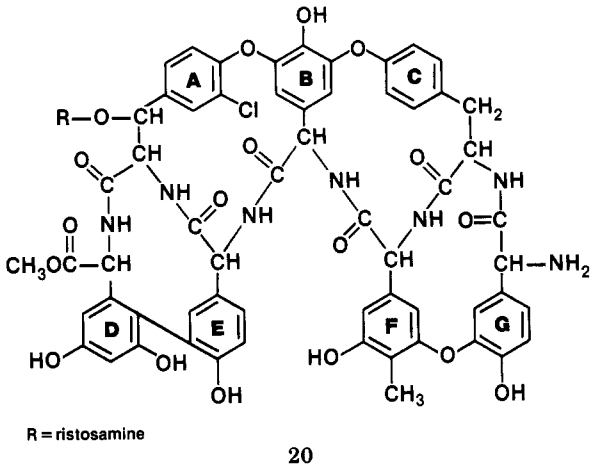
(3) Amino Acid 8. The remaining resonances arise from the biphenyl fragment containing rings D and E; they may be assigned by comparison with the data for other glycopeptides in Tables II and III, as shown in 19.



19

C. Linkage of Fragments. The glycopeptide antibiotics act by binding to cell-wall mucopeptides containing

the terminal dipeptide D-alanyl-D-alanine; this interaction inhibits cross-linking of the bacterial cell wall and leads to eventual lysis of the organism.[9] Vancomycin,[17] avoparcin,[18] ristocetin,[19] and A35512B[20] all form complexes with the mucopeptide analogue Ac-D-Ala-D-Ala, and Williams and co-workers have shown that the regions of vancomycin[17d] and ristocetin A[19b] which take part in complex formation with Ac-D-Ala-D-Ala are contained in the common structure 1. Many of the protons in the binding-site region are held in close proximity, and extensive "nests" of NOEs involving hydrogens or substituents on rings A, D, and E have been observed for vancomycin,[10] ristocetin A,[3a] and A35512B ψ-aglycon.[6c] A similar NOE pattern occurs for the actaplanin ψ-aglycon, involving the following resonances in particular: A-2′, A-2, D-NH, E-1′, and E-2. The large number of NOEs that may be observed from irradiation of a single resonance is illustrated in Figure 2, where the A-2′ resonance is irradiated. The difference spectrum display gives emphasis to the rather large effects produced; these and other NOE observations from the actaplanin ψ-aglycon are compared with analogous results from other glycopeptides in Table III. The close similarities of both chemical shift and coupling constant data (Table II) and NOE results (Table III) between actaplanin and other members of the glycopeptide class indicate that amino acids **6**, **7**, and **8** are linked to form the actaplanin ψ-aglycon **20**. The structure **20** differs



20

from the ψ-aglycon of ristocetin by having Cl at position A-3 (rather than H) and having CH_2 at C-1′ (rather than CHOH). The molecular formula for **20** is $C_{66}H_{61}O_{20}N_8Cl$ (M_r 1321.7); this formula is confirmed by plasma desorption mass spectrometry;[21] M_r found, 1321.8. The cis peptide bond in **1** has been shown to be required by the intense NOE between A-2′ and E-1′ in vancomycin (NOE ~56%, E-1′ → A-2′; half-time for maximum NOE buildup = 0.14 s),[8] granted that the general connectivity is as shown in **1** and that the dihedral angle between the A-2′ and A-NH protons is ~180° ($J_{A2'-NH}$ = 12 Hz for vancomycin[22]). The presence of the same cis bond in the other glycopeptides is required by their NMR data as well (see Tables II and III): for actaplanin, NOE A-2′ → E-1′ = 41%, NOE E-1′ → A-2′ = 50%; $J_{A2'-NH}$ = 11 Hz.

Williamson and Williams have shown that the correct relative stereochemistry can be deduced for vancomycin, on the basis of NMR data, for the carbons bearing the protons A-1′, A-2′, B-1′, D-1′, and E-1′.[8] The similar "nests" of NOEs (Table III) and the similarities in chemical shifts and coupling constants (Table II) have been taken as indications that vancomycin and the other reported glycopeptides (ristocetin,[3a] avoparcin,[5c] and A35512B[6c]) share the same relative configurations at their chiral centers or the same absolute stereochemistry when their ability to form complexes with Ac-D-Ala-D-Ala is considered. Although the binding of Ac-D-Ala-D-Ala to actaplanin has not been examined, the same comparisons in Tables II and III indicate that structure **20** and the general structure **1** share indentical relative configurations at their asymmetric centers. The similarities of antibiotic activity within the glycopeptide class suggest that **20** and **1** share identical absolute configurations as well, rather than being mirror images. If this is the case, the configurations for the actaplanin centers that carry the protons A-1′, A-2′, B-1′, C-2′, *D*-1′, and E-1′ are *R*, *S*, *R*, *R*, *S*, and *R*, respectively.[2a] The configurations of sites where Y and Z are attached in **1** (F-1′ and G-1′) have been determined by Harris and Harris for the diphenyl ether type glycopeptide A35512B: *S* at F-1′ and *R* at G-1′;[23] the comparisons in Tables II and III suggest that actaplanin, ristocetin, and A35512B are identical at these sites as well.

Conclusion

The structure of the ψ-aglycon of actaplanin, based on proton NMR studies and the products of oxidative degradation of the antibiotic, is **20**. Ristosamine is attached to the aglycon at A-1′. The structure **20** differs from the ψ-aglycon of ristocetin A by having a chlorine atom at position A-3 and by the absence of a hydroxyl group at C-1′; the relative configurations of the asymmetric centers in **20** and ristocetin ψ-aglycon are identical. Ristocetin and actaplanin differ, however, in the identity and distribution of the neutral sugars attached to their aglycons.[3b,8,11]

Acknowledgment. We thank Prof. D. H. Williams (Cambridge University) for providing copies of ref 3a and 19b before their publication. We thank Dr. D. E. Dorman of these laboratories for several useful discussions, and we are especially grateful to Mr. T. K. Elzey for obtaining all NMR spectra of the ψ-aglycon.

Registry No. **3**, 70734-92-8; **4**, 57466-21-4; **5**, 22955-73-3; **20**, 88288-92-0.

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