3.60, 4.32, 4.43 (4-line AB, 2, OCH_2), 3.63, 3.73, 3.88, 3.98 (4-line AB, 2, ICH_2), 3.8–3.9 (m, 1, OCH).

Anal. Calcd for $C_{30}H_{49}OI$: C, 65.19; H, 8.95. Found: C, 64.97; H, 9.04.

Finally, further elution led to 50 mg of semisolid acetate **18c**: IR ($CHCl_3$) 5.77 (s, C=O) μm; ^1H NMR δ 0.9–1.2 (m, 21, methyls), 2.10 (s, 3, COMe), 4.0–4.2 (m, 1, OCH), 6.40 (s, 1, CO_2CH).

Anal. Calcd for $C_{32}H_{52}O_3$: C, 79.28; H, 10.81. Found: C, 79.16; H, 10.96.

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Iodination of Vancomycin, Ristocetin A, and Ristocetin Pseudoaglycon

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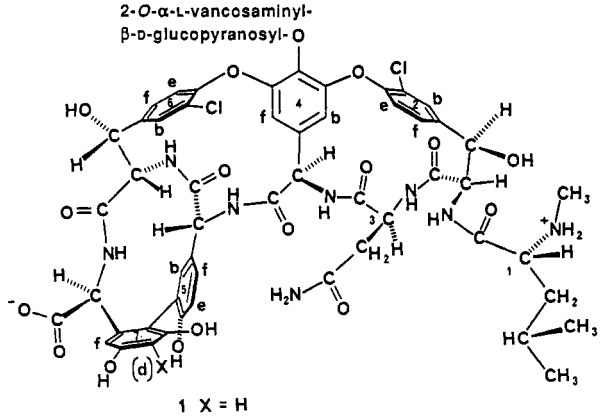
Iodinated derivatives of the glycopeptide antibiotics vancomycin (**1**) and ristocetin A (**2**) have been known for some time; the site(s) of iodination in these compounds have now been determined by NMR and degradation. Vancomycin is iodinated at the para position of the ring in residue 7 (the resorcinol ring of actinoidinic acid). In contrast, ristocetin undergoes iodination predominantly on residue 3 at the ortho position distal to the diphenyl ether linkage. However, if the sugar attached to the aromatic ring of residue 7 is removed as in the pseudoaglycon of ristocetin (Ψ-AGR, **10**) iodination occurs in the same position as in vancomycin, suggesting that in ristocetin the sugar sterically blocks the electronically preferred site of iodination. Peptide binding ability, as measured by K_A determinations for the tripeptide Ac_2-L-Lys-D-Ala-D-Ala, is somewhat diminished by iodination. Iodovancomycin, iodoristocetin, the pseudoaglycon of iodoristocetin and iodinated pseudoaglycon of ristocetin have K_A values ($\sim 10^5$) slightly lower but of the same order of magnitude as the analogous uniodinated compounds.

Vancomycin (**1**) and ristocetin (**2**) are glycopeptide antibiotics, isolated from *Nocardia orientalis* (formerly called *Streptomyces orientalis*) and *Nocardia lurida*, respectively.[1] They are active against Gram-positive bacteria



1 X = H
3 X = I

and the former is used clinically for the treatment of methicillin-resistant *Staphylococcus aureus* infections and *Clostridium difficile* induced pseudomembranous colitis.[2] The antibiotics in this group exert their antibacterial action by binding to peptide intermediates involved in bacterial cell wall synthesis.[3] In vitro these antibiotics bind certain aliphatic peptides, such as *N*-Ac-D-Ala-D-Ala and Ac_2-L-Lys-D-Ala-D-Ala, which are analogues of cell wall precursors.[4] The structures of the glycopeptide antibiotics as well as the nature of the antibiotic–peptide complexes have been the subject of several investigations in recent years.[1,5] High field ^1H NMR studies by Williams,[6] Feeney,[7] and Fesik[8] have revealed many details of the peptide–antibiotic interaction which involves a series of intermolecular hydrogen bonds between the two constituents. It is now of

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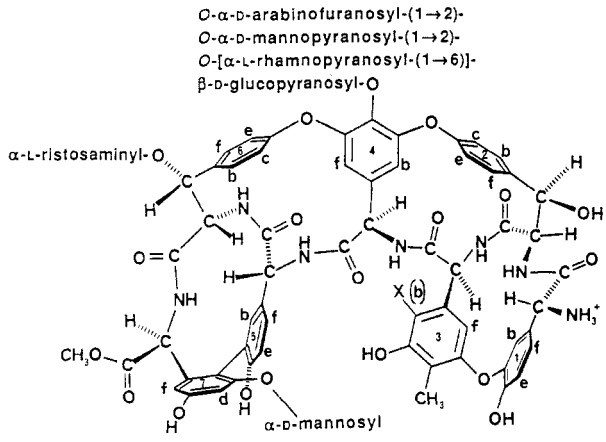
interest to prepare well-characterized derivatives of these antibiotics to examine the effect of structural modification upon their peptide-binding properties. Radioiodinated derivatives of vancomycin and ristocetin were made some time ago, the former for use in following the fate of the antibiotic upon absorption by bacterial cell walls[9] and the latter for study of the role of ristocetin A in blood platelet aggregation.[10] Subsequently ^{125}I-vancomycin has been employed for radioimmunoassay of vancomycin.[11] Nieto and Perkins carried out extensive characterization of iodovancomycin during their early work,[4b] but the structures of both vancomycin and ristocetin were unknown at the time and the sites of iodination were never established. The site of iodination of ristocetin is particularly open to question since it contains two additional activated aromatic rings with potential sites of electrophilic substitution. In this paper we report the structures of iodovancomycin, iodoristocetin, and also that of the product obtained from iodination of the pseudoaglycon of ristocetin.



2 X = H

8 X = I

Experimental Section

General. Vancomycin was obtained as a gift from Eli Lilly and ristocetin A was obtained from Abbott Laboratories. Thin layer chromatography was performed on Merck silica gel plates. HPLC was carried out using a Spectra-Physics SP7000 system or an IBM 9533 ternary liquid chromatograph. A C-18 column (4.6 × 250 mm) was employed with ammonium formate–acetonitrile mixtures or 0.1% TFA–acetonitrile mixtures as eluents.

Preparation of Iodovancomycin (3). Iodovancomycin was prepared by the method of Nieto and Perkins.[4b] A solution of vancomycin (1.0 g in 125 mL of H_2O, pH 6.5) was stirred vigorously with a solution of I_2 in CCl_4 (0.04 M, 20 mL) at room temperature. After 30 min, the iodine color was essentially gone and HPLC (C-18 column, gradient: 5% CH_3CN/0.1 M ammonium formate to 40% CH_3CN/0.1 M ammonium formate, 20 min, 2 mL/min) showed one major peak, eluting later than vancomycin. The CCl_4 and aqueous layers were separated and the aqueous layer was lyophilized. Purification was carried out on an Altex column (15 × 500 mm) packed with Whatman Partisil 40 ODS-3 in 0.1 M ammonium formate. Gradient elution was performed with 0.1 M ammonium formate (350 mL) in one chamber and 15% CH_3CN/0.1 M ammonium formate (350 mL) in the other. Crude iodovancomycin (0.200 g) yielded 0.123 g upon purification. FABMS: calcd for $C_{66}H_{75}Cl_2IN_9O_{24}$ (MH^+) 1574.3, found 1574.

Degradation of Iodovancomycin (3). Iodovancomycin (0.90 g) was treated with 1 N HCl (25 mL) in a boiling water bath for 2 min. The suspension was cooled and the solid removed by filtration. After drying, 0.64 g of aglycoiodovancomycin (AGIV) was obtained.

Acid hydrolysis: AGIV (62 mg) was hydrolyzed with 2 N HCl (6 mL) for 24 h at 107 °C and the hydrolysate was separated by ion-exchange chromatography as described previously.[5c] Actinoidinic acid (**4a**) was identified by TLC and ^{1}H NMR comparison with authentic material. No iodinated species was found.

Alkaline hydrolyses: Before alkaline hydrolysis the phenols were protected by methylation. AGIV (0.64 g) was methylated with CH_3I (3 mL) in MeOH (15 mL) containing anhydrous K_2CO_3 (2.5 g). The mixture was refluxed for 16 h, filtered, and evaporated to leave a white solid.

In the first procedure for alkaline hydrolysis, the methylated aglycon was treated with refluxing 4 N KOH containing $NaBH_4$ (1.0 g/30 mL) for 24 h. The hydrolyzed amino acids were converted to *N*-acetyl methyl esters with Ac_2O and CH_2N_2 and the derivatives were separated by column chromatography (TLC silica, elution with $CHCl_3$–MeOH mixtures). Fractions were analyzed by TLC, NMR, and MS; no iodinated species were identified. A second alkaline hydrolysis was carried out using 2 N KOH (30 mL for 0.59 g of protected AGIV) and omitting the $NaBH_4$. Derivatization and chromatography yielded a fraction (0.11 g) which, by NMR, appeared to contain a substituted actinoidinic acid derivative. Further purification by TLC (CH_2Cl_2–MeOH, 92:8) yielded a mixture of stereoisomers of **5** as a white solid. MS: 628 (M^+, 19), 596 (48), 585 (77), 568 (55), 527 (39), 477 (100). The fragmentation is comparable to that seen for uniodinated protected actinoidinic acid.

Degradation of Protected Amino Acid 5. Amino acid **5** (30 mg) was converted to bis(nitrile) **6** by NaOCl treatment as described previously.[12] Purification by TLC (pentane–EtOAc, 8:2) gave 2.9 mg of **6**: ^{1}H NMR (Me_2CO-d_6) δ 7.91 (1 H, dd, J = 8.6 + 2.2 Hz), 7.70 (1 H, dd, J = 2.2 + 0.4 Hz), 7.39 (1 H, dd, J = 8.5 + 0.4 Hz), 7.25 (1 H, s), 4.05 (3 H, br s), 3.93 (3 H, s), 3.44 (3 H, s); MS, m/e 420 (100), 389 (26); HRMS (EI), calcd for $C_{17}H_{13}N_2O_3I$ 419.9949, found 419.9934.

To convert **5** to bis(aldehyde) **7**, protected amino acid **5** (23 mg) was hydrolyzed to the free amino acid with 1 N KOH (reflux, 15 h). The hydrolysate was neutralized with HCl to pH 7.25. NaOCl (0.25 mL) was added dropwise with vigorous stirring over the course of 1 h; the pH was readjusted from 8.3 to 7 during this time. After addition was complete the reaction was stirred for an additional 2.5 h. Excess OCl^- was reduced with Na_2SO_3 and the reaction mixture was extracted with EtOAc and CH_2Cl_2. The organic extracts were combined, dried, and evaporated to give 2.9 mg of bis(aldehyde) **7**: ^{1}H NMR (Me_2CO-d_6) δ 9.99 (1 H, s), 9.61 (1 H, s), 8.06 (1 H, dd, J = 8 + 2 Hz), 7.85 (1 H, d, J = 2 Hz), 7.38 (1 H, d, J = 8 Hz), 7.24 (1 H, s), 4.03 (3 H, s), 3.90 (3 H, s), 3.40 (3 H, s); ^{1}H NMR ($CDCl_3$) δ 9.98 (1 H, s), 9.63 (1 H, s), 8.03 (1 H, d, J = 8 + 2 Hz), 7.86 (1 H, d, J = 2 Hz), 7.27 (1 H, s), 7.16 (1 H, d, J = 8 Hz), 4.03 (3 H, s), 3.88 (3 H, s), 3.43 (3 H, s); MS, m/e 426 (64), 395 (100); HRMS (EI), calcd for $C_{17}H_{15}O_5I$ 425.9874, found 425.9885.

Preparation of Iodoristocetin (8). Ristocetin A (715 mg) in H_2O (50 mL), pH 6.5, was stirred with I_2 in CCl_4 (0.04 M, 10 mL). The color disappeared rapidly but HPLC (C-18, 10.5% CH_3CN/0.1 % TFA) showed primarily starting material; an additional 10 mL of I_2 solution was added and the mixture was stirred overnight. The layers were separated and the aqueous layer was lyophilized to give 0.78 g of **8**.

Preparation of the Pseudoaglycon of Iodoristocetin (9). The pseudoaglycon of **8** was prepared by refluxing a solution of **8** (300 mg) in 5% HCl/MeOH for 1 h.[5e] The solvent was evaporated and the resulting solid was suspended in a small volume of water, cooled, and filtered to give, after lyophilization, 130 mg of Ψ-AGIR (**9**). The crude product showed two peaks (9:1 ratio) by HPLC. The major (slower moving) fraction was purified by chromatography on Whatman Partisil 40 ODS-3 with elution by

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20% CH_3CN/0.1 M ammonium formate. Fractions were analyzed by HPLC (C-18, 20% CH_3CN/0.1% TFA). FABMS: calcd for $C_{66}H_{63}IN_8O_{21}$ (MH^+) 1430.3, found 1430. The minor product had the same retention time as product **11** described below.

Degradation of 9. Degradation of **9** was carried out on crude material. After protection of the phenols by methylation with CH_2N_2, **9** was degraded to the bis(nitrile) stage by the procedure described above for vancomycin. The only detectable iodinated product was a trace of bis(nitrile) **6**, arising from iodination of the actinoidinic acid in the minor product **11**.

Preparation of Iodinated Pseudoaglycon of Ristocetin (I-Ψ-AGR 11). Iodination of the pseudoaglycon of ristocetin was carried out as for ristocetin except that the reaction was worked up after 4 h. HPLC analysis was performed similarly. Compound **11** eluted somewhat before **9**. FABMS: calcd for $C_{66}H_{63}IN_8O_{21}$ (MH^+) 1430.3, found 1430. Methylated (CH_2N_2) **11** was degraded to the bis(nitrile) stage as above to give bis(nitrile) **6** as the only isolable iodinated product.

UV Difference Spectroscopy. Binding constants of Ac_2-L-Lys-D-Ala-D-Ala with vancomycin, ristocetin, and their derivatives were measured essentially as described by Nieto and Perkins[4b,c] except that the tandem arrangement of cells was not required, since the peptides used in this work had no significant absorption in the range 250–320 nm. Cells with a 5-cm light path were used. Solutions (15 mL) containing antibiotic (0.08–0.1 mg/mL in 0.02 M sodium citrate buffer, pH 5.1) were placed in the sample and reference cells and the difference in absorbance which developed upon addition of peptide (5–100 μL of 0.015 M solution) to the sample cell and buffer to the reference cell was measured at 282–285 nm or 292–294 nm for vancomycin and iodovancomycin and at 242 nm for ristocetin and its derivatives. The difference in extinction was measured with an accuracy of ±0.0003 absorbance units. The temperature was 25 ± 2 °C. Association constants were determined by means of a nonlinear least-squares program or by Scatchard plots, based on the assumption of a simple V + P ⇄ VP equilibrium. Binding constants of 10^4 or less were obtained with a standard deviation of ±5–10%; because of the steepness of the curve for peptides with association constants of 10^5 or greater, the standard deviation in these cases was greater, ranging from 10 to 20%. Antibiotic concentrations were determined by UV, using a value of $\epsilon^{1\%}$ 45.0 at 280 nm for vancomycin and 44 for iodovancomycin[4b] and $\epsilon^{1\%}$ 49 at 280 nm for ristocetin B[4d] (values for other ristocetin derivatives were extrapolated from these data).

NMR Studies. NMR experiments were performed on 10 mg/mL solutions in Me_2SO-d_6. Spectra were recorded on a Nicolet NT-360 or Bruker AM-400 NMR spectrometer using 16K data points with a spectral width of 4000 Hz. NOE difference spectra were obtained by subtraction of the FIDs acquired with preirradiation (0.25 s) on the peak of interest as previously described.[8] COSY experiments were performed by using a $(90°\text{–}t_1\text{–}45°\text{–acquire})_n$ pulse sequence. The transmitter was placed at the center of the spectrum and 256 × 1K FIDs were collected. F1 data were zero-filled once before Fourier transformation. Sine-bell multiplication was employed in both dimensions and the transformed data were symmetrized. Pure absorption phase 2D NOE data sets were acquired using a $(90°\text{–}t_1\text{–}90°\text{–}\tau_m\text{–}90°\text{–acquire})_n$ pulse sequence with the carrier frequency placed to one side of the spectrum. Typically, 512 × 1K FIDs were collected using a spectral width of 6666 Hz and a delay of 1.5 s between acquisitions. To process the data, the FIDs were multiplied by a sine-bell shifted (45°) window function, zero-filled to 2K data points, and Fourier transformed. In F1, a real Fourier transformation was performed after applying a sine-bell shifted (45°) filter.

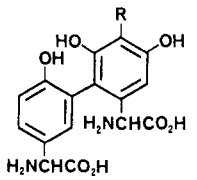
Results and Discussion

Nieto and Perkins prepared iodovancomycin by two routes.[4b,9a] The method which involves stirring an aqueous solution of the antibiotic with 1 equiv of I_2 in CCl_4[4b] leads cleanly to a monoiodo product which can be further purified by reverse-phase chromatography. ^1H NMR was used to determine the site of iodination of iodovancomyin prepared in this manner. The ^1H NMR resonances of iodovancomycin were assigned by identifying the scalar (through bond) coupled protons from COSY experiments and the dipolar (through space) coupled protons from absorption phase 2D NOE experiments. The ^1H NMR assignments for the aromatic ring protons of iodovancomycin and, for comparison purposes, vancomycin appear in Table I. In the proton NMR spectrum of iodovancomycin the two doublets (J = 2 Hz) at 6.32 and 6.34 ppm corresponding to the 7f and 7d protons of vancomycin are absent. Instead, a sharp singlet at 6.49 ppm is observed, indicating that one of the protons in ring 7 has been substituted by an iodine. Since the resonance at 6.49 ppm in iodovancomycin exhibits an NOE to the α-proton of residue 7, this resonance has been assigned to 7f. Thus, 7d is the site of iodination as shown in **3**. To confirm this conclusion chemical degradation was carried out on iodovancomycin. The aglycon was prepared by brief treatment with acid. In the first experiment the aglycon was hydrolyzed with 2 N HCl for 24 h. The hydrolysate was separated by ion-exchange chromatography. On the basis of the NMR results, we had expected to find iodinated actinoidinic acid (**4b**). However, only uniodinated actinoidinic acid (**4a**) was isolated. The acid hydrolysis conditions evidently led to displacement of the iodine from the highly activated aromatic ring.

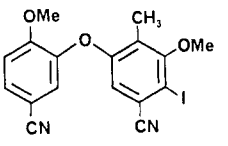
Table I. Chemical Shifts of Aromatic Protons in Vancomycin and Iodovancomycin[a]

proton	vancomycin (1)	iodovancomycin (3)
2b	7.32	7.49
2e	7.23	7.23
2f	7.50	7.50
4b	5.50	5.62
4f	5.22	5.15
5b	7.16	7.15
5e	6.68	6.75
5f	6.73	6.83
6b	7.89	7.79
6e	7.32	7.33
6f	7.44	7.46
7d	6.34	*b*
7f	6.32	6.49

[a] Spectra were run in Me_2SO-d_6 at 298 K. [b] Site occupied by iodine.



4a R = H
b R = I



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The second hydrolysis was performed on *O*-methylated aglycon using 4 N KOH/$NaBH_4$. The amino acids were isolated as *N*-acetyl OMe methyl esters. Examination of column chromatography fractions by NMR and MS again showed no iodinated species—only uniodinated protected actinoidinic acid was isolated. In this case apparently the iodine had been removed by nucleophilic attack by the BH_4^-, deiodination of aromatic rings by sodium borohydride has been observed previously.[13]

A second base hydrolysis was then performed, omitting the borohydride. Chromatography of the protected amino acids yielded a fraction with NMR and MS properties compatible with an iodinated actinoidinic acid. Because

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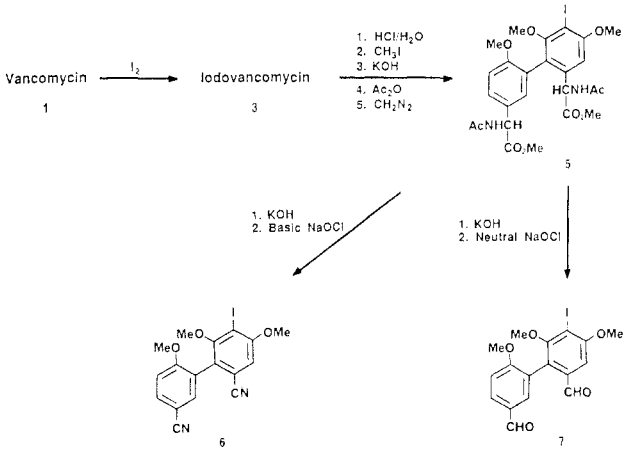
Scheme I



of the complexity of the NMR spectrum caused by multiple stereoisomers, protected amino acid **5** was degraded with NaOCl, first to bis(nitrile) **6** and second to bis(aldehyde) **7** (Scheme I).

The ^{1}H NMR spectrum of bis(nitrile) **6** showed three methoxyls at 3.44, 3.93, and 4.05 ppm, a singlet at 7.25 and three doublets, 7.39 (J = 8.5 + 0.4 Hz), 7.70 (2.2 + 0.4 Hz), and 7.91 (8.6 + 2.2 Hz), verifying that iodination had occurred on the resorcinol ring. Irradiating the singlet at 3.93 ppm sharpened the doublet at 7.39 ppm, hence the singlet must be the 4-OCH_3; irradiation at 4.05 ppm sharpened the singlet at 7.25 ppm; unambiguous assignment of the 4.05 ppm signal could not be made, however.

Bis(aldehyde) **7** was prepared so that NOE effects involving the aldehyde protons could be investigated. Bis(aldehyde) **7** was obtained by treatment of OMe amino acid **4b** with NaOCl at pH 7. The product was obtained in low yield but sufficient purity to use in NMR studies. In Me_2CO-d_6, compound **7** showed singlets at 3.40, 3.90, and 4.03 ppm for the three methoxyls, a singlet at 7.24 ppm, doublets at 7.38 and 7.85 ppm, and a doublet of doublets at 8.06 ppm for the aromatic protons and singlets at 9.61 and 9.99 ppm for the aldehydic protons. The singlet at 7.24 ppm is the lone remaining proton on the resorcinol ring and the other three aromatic signals arise from the 5, 2, and 6 protons, respectively, on the other ring. Irradiation at 9.99 ppm noticeably sharpened the doublet of doublets at 8.06 ppm, hence the 9.99 ppm signal must be the CHO on the para-hydroxylated ring; however, irradiating the other CHO proton produced no detectable effect on the singlet at 7.24 ppm, leaving the assignment on the resorcinol ring still in doubt. However, use of the Eu(fod)$_3$ shift reagent resolved the ambiguity. This reagent, which could be expected to complex with the aldehyde groups, caused a marked downfield shift of the singlet at 7.24 ppm allowing the assignment of this proton unequivocally to the 6′ position rather than the 4′ position, thus confirming the site of iodination as being at 4′ as had been deduced from the high field NMR studies on intact antibiotic.

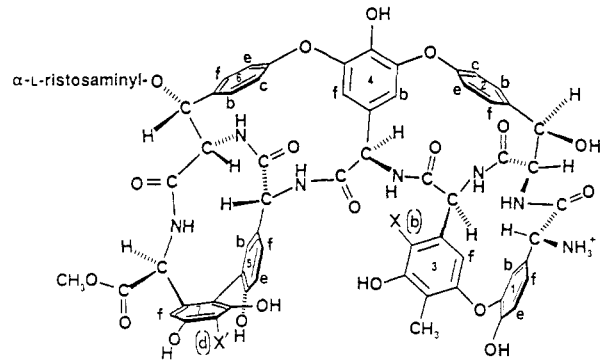
A similar approach was taken to study the iodination of ristocetin. Ristocetin was iodinated under the same conditions as vancomycin; HPLC indicated that iodination was much slower, taking more than a day to reach completion. ^{1}H NMR studies of the major product (IR, **8**) (Table II) showed that ring 3 now had only one proton signal, at 6.31 ppm. To determine whether the remaining proton was 3b or 3f, a 2D NOE experiment was performed which clearly showed an NOE between 1b and 3f. Iodination had occurred primarily, therefore, at position 3b. This result was in marked contrast to the result obtained with vancomycin but can be accounted for by the presence of a mannosyl residue on ring 7 in ristocetin sterically inhibiting the iodination reaction at position d in this ring.

Table II. ^{1}H Chemical Shifts of Aromatic Protons in Ristocetin A and Derivatives[a]

proton	RC (2)	I-RC (8)	Ψ-AGR (10)	Ψ-AGIR (9)	I-Ψ-AGR (11)
1b	6.55	6.47	6.57	6.50	6.56
1e	7.02	6.95	6.94	6.96	6.96
1f	7.05	7.02	7.08	7.10	7.08
2b	7.08	7.05	7.05	7.07	7.06
2c	7.27	7.28	7.14	7.17	7.18
2e	7.10	7.22	7.14	7.12	7.12
2f	7.90	7.69	7.80	7.76	7.80
3b	6.37	*b*	6.37	*b*	6.34
3f	6.42	6.31	6.42	6.53	6.37
4b	5.78	6.05	5.65	5.79	5.67
4f	5.20	5.21	5.22	5.20	5.16
5b	7.15	7.18	7.19	7.21	7.23
5e	6.74	6.70	6.64	6.66	6.69
5f	6.80	6.78	6.71	6.73	6.80
6b	7.38	7.44	7.47	7.46	7.47
6c	6.83	6.80	6.81	6.85	6.88
6e	7.19	7.16	7.14	7.12	7.12
6f	7.35	7.38	7.36	7.36	7.37
7d	6.75	6.76	6.42	6.40	*b*
7f	6.25	6.22	6.03	6.04	6.28

[a] Spectra were determined in Me_2SO-d_6 at 291–315 K. [b] Site occupied by iodine.

The NMR spectrum of the pseudoaglycon obtained by removal of the neutral sugars from iodoristocetin (Ψ-AGIR, **9**) provided confirmation of the result deduced from the



9 X = I; X′ = H
10 X = X′ = H
11 X = H; X′ = I

studies of iodoristocetin. The chemical shifts of the ring 7 protons were unchanged from uniodinated pseudoaglycon **10** and only one proton, which had moved downfield, was found for ring 3. Iodination at 3b also affects 4b, which moves downfield 0.14 ppm, and most markedly, causes the α-proton of residue 3 to move downfield to 5.95 ppm. This observation is in accord with the NOE from this α-proton to 3b seen in the uniodinated compound and is a further indication of the closeness of this proton and the substituent at 3b in the solution conformation of these compounds. A similar downfield shift is seen in the ^{1}H NMR spectrum of the antibiotic A35512B which has a chlorine at the 3b position.[14]

For comparison a study of the iodination of the pseudoaglycon of ristocetin (Ψ-AGR, **10**) was undertaken. Ψ-AGR has only one sugar remaining—the ristosamine on the benzylic OH group of residue 6. Iodination of **10** proceeded much more quickly than of ristocetin itself and gave a product which was different by HPLC from the

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Table III. K_A Values for Binding of Iodovancomycin and Iodinated Ristocetin Derivatives to Ac_2-L-Lys-D-Ala-D-Ala[a]

compound	K_A
vancomycin (1)	$1.5 \pm 0.1 \times 10^6$
iodovancomycin (3)	$3.4 \pm 0.3 \times 10^5$
ristocetin (2)	$2.6 \pm 0.1 \times 10^5$
iodoristocetin (8)	$1.8 \pm 0.1 \times 10^5$
Ψ-AGIR (9)	$1.7 \pm 0.2 \times 10^5$
Ψ-AGR (10)	$3.4 \pm 0.6 \times 10^5$
I-Ψ-AGR (11)	$2.1 \pm 0.2 \times 10^5$

[a] K_A values were determined by UV difference spectroscopy at 298 K. Solutions were prepared at pH 5.1 in 0.02 M sodium citrate buffer.

pseudoaglycon of iodoristocetin (Ψ-AGIR, 9); by HPLC it was identical with the minor constituent seen in crude 9.

To facilitate the structure assignment of the new iodinated pseudoaglycon, designated at I-Ψ-AGR, a comparison with 9 and 10 was made by NMR (Table II), with particular attention being paid to the aromatic region.

The NMR spectrum of iodinated isomer, I-Ψ-AGR, was found to be remarkably similar to that of 10 with the exception of the signals of ring 7. Analogous to the case of vancomycin, a new peak is present at 6.28 ppm in the spectrum of I-Ψ-AGR which has been assigned as 7f on the basis of an NOE observed to the α-proton of residue 7. Ring 3 is essentially unchanged. Removal of the mannose, therefore, causes the site of iodination to shift from predominantly 3b to 7d, the same site as in vancomycin, and the structure of I-Ψ-AGR can be assigned as 11.

Chemical degradation of 9 and 11 was carried out as for vancomycin. From the degradation of crude 9 a trace of bis(nitrile) 6 was isolated, indicating that iodination, albeit very slowly, occurs to some extent on ring 7 in the parent antibiotic. No product from iodination of ring 3 (such as bis(nitrile) 12) could be isolated; possibly it could not survive the highly basic hydrolysis conditions. We think it is more likely, however, that the iodination product escapes detection because the iodo substituent sterically inhibits both methylation of the phenol and hydrolysis of the peptide bond. Degradation of 11 gave the expected bis(nitrile) 6.

Peptide Binding. To determine if iodination causes changes in the affinity of these modified antibiotics for peptides, association constants for the binding of the tripeptide, Ac_2-L-Lys-D-Ala-D-Ala, were measured by UV difference spectroscopy (Table III). For vancomycin it can be seen that iodination causes a modest (less than an order of magnitude) decrease in binding affinity, which is in accord with the earlier results of Nieto and Perkins.[4b] In the current model for the tripeptide–antibiotic complex (in aqueous media) the lysyl side chain lies over ring 7;[6f,8] iodine at position 7d may perturb this orientation, leading to less effective binding. A similar but less marked effect is seen with ristocetin and its iodinated derivatives—the K_A for 8 is slightly less than that for 2 and compounds 9 and 11 bind less effectively than 10 but overall, the effect of iodination is relatively minor.[15] Examination of molecular models reveals that neither iodination at 3b, as in 8 and 9, nor iodination at 7d, as in 11, significantly alters the binding pocket.

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(15) The adjective "minor" used to describe the difference in K_As may be applicable in a chemical sense but not when translated into biological activity. When assayed for antibiotic activity against *B. subtilis*,[16] compound 3 showed about 50% the activity of the parent compound; i.e., twice the quantity of 3 is required for the same area of inhibition. This reduction in activity is roughly comparable in magnitude to the decrease seen in K_A but from a therapeutic viewpoint may be considered a major decrease. A strict correlation between binding constants and antibiotic activity would not be expected, given the complexity of the interaction between antibiotic and microorganism.

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Molecular Mechanics Studies on the Supra Annular Effect of 3-Cyclohexene-1-carboxaldehyde Compounds

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There is little experimental information available on the conformations of Δ^3-cyclohexene compounds. In the early sixties, it was proposed based on models that these systems adopt a conformation with the electronegative group axial. The stability of this arrangement was attributed to an intramolecular carbonyl–double bond interaction. Examining those structures with MMP2 force field and ab initio calculations, we have determined that this resonance effect is unimportant, and in fact there is no preference for an axial aldehyde group.

There has been a vast amount of stereochemical and conformational data obtained for cyclohexane derivatives.[1] The corresponding cyclohexene compounds, however, have not received as much attention.[1] In fact, little work has been reported on the 3-cyclohexene-1-carboxaldehyde series.[2,3] The purpose of this study was (1) to establish the

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