by means of a force transducer connected to a two-channel Gemini polygraph.

Guinea Pig Left Atria. The heart of male guinea pigs was rapidly removed, and the right and left atria were excized separately. Left atria were mounted in PSS at 30 °C and stimulated through platinum electrodes by square-wave pulses (1 ms, 1 Hz, 4–7 V). Inotropic activity was recorded isometrically.

Tissues were equilibrated for 1 h, and cumulative dose–response curves to carbachol (0.01–3 μM) were constructed. Following incubation with the antagonist for 30 min, a new dose–response curve to the agonist was obtained.

Guinea Pig Ileum. Twenty-millimeter-long portions of terminal ileum were taken at about 5 cm from the ileum–cecum junction and mounted in PSS at 37 °C. Tension changes were recorded isotonically.

Tissues were equilibrated for 30 min, and dose–response curves to carbachol (0.01–10 μM) were obtained at 30-min intervals, the first one being discarded and the second one taken as control. Following incubation with the antagonist for 30 min, a new dose–response curve to the agonist was obtained.

Determination of Dissociation Constants. The antagonist potency of compounds 1–24 at muscarinic receptors was expressed in terms of their pA_2 values. pA_2 values were estimated by Schild plots[31] constrained to slope −1.0, as required by theory,[32] by calculating the ratio of the doses (DR) of agonist causing 50% of the maximal response in the presence and in the absence of the test compound. The log (DR − 1) was calculated at three antagonist concentrations, and each concentration was tested at least five times. When this method was applied, it was always verified that the experimental data generated a line whose derived slope was not significantly different from unity ($p > 0.05$). For certain compounds pA_2 values were calculated at only one concentration, tested at least five times, according to van Rossum.[33]

Data are presented as means ± SE of n experiments. Differences between mean values were tested for significance by the Student's t test, and a level of $p > 0.05$ was taken as being statistically significant.

Acknowledgment. This work was supported by a grant from the Ministry of Public Education.

Registry No. 1, 104807-40-1; **2**, 115962-36-2; **2** (unreduced), 115962-69-1; **2**·4HCl, 115962-78-2; **3**, 115962-37-3; **3**·4HCl, 115962-79-3; **4**, 115962-38-4; **4**·4HCl, 115962-80-6; **5**, 115962-39-5; **5**·4HCl, 115962-81-7; **6**, 115962-40-8; **6**·4HCl, 115962-82-8; **7**, 115962-41-9; **7**·4HCl, 115962-83-9; **8**, 115962-42-0; **8**·4HCl, 115962-84-0; **9**, 115962-43-1; **9**·4HCl, 115962-85-1; **10**, 115962-44-2; **10**·4HCl, 115962-86-2; **11**, 115962-45-3; **11**·4HCl, 115962-87-3; **12**, 115962-46-4; **12**·4HCl, 115962-88-4; **13**, 115962-47-5; **13**·4HCl, 115981-87-8; **14**, 115962-48-6; **14**·4HCl, 115962-89-5; **15**, 115962-49-7; **15**·4HCl, 115962-90-8; **16**, 115962-50-0; **16**·4HCl, 115962-91-9; **17**, 115962-51-1; **17**·4HCl, 115962-92-0; **18**, 115962-52-2; **18**·4HCl, 115981-85-6; **19**, 115962-53-3; **19**·4HCl, 115981-86-7; **20**, 115962-54-4; **20**·4HCl, 115962-93-1; **21**, 115962-55-5; **21**·4HCl, 115962-94-2; **22**, 115962-56-6; **22**·$4H_2C_2O_4$, 115962-95-3; **23**, 115962-57-7; **23**·$4H_2C_2O_4$, 115962-76-0; **24**, 115962-58-8; **24**·$4H_2C_2O_4$, 115962-77-1; **25**, 115962-59-9; **25** (BOC blocked), 115962-68-0; **26**, 115962-60-2; **27**, 115962-61-3; **28**, 115962-62-4; **29**, 115981-14-1; **30**, 115962-63-5; **31**, 115962-64-6; **32**, 115962-65-7; **33**, 115962-66-8; **34**, 115962-67-9; 1,8-octanedioic acid, 505-48-6; *N*-[(*tert*-butyloxy)carbonyl]-1,5-pentanediamine, 51644-96-3; *N*-[(*tert*-butyloxy)carbonyl]-1,7-heptanediamine, 99733-18-3; *N*-[(*tert*-butyloxy)carbonyl]-1,6-hexanediamine, 51857-17-1; 1,11-undecanedioic acid, 1852-04-6; 1,13-tridecanedioic acid, 505-52-2; 1,14-tetradecanedioic acid, 821-38-5; 2-methoxybenzaldehyde, 135-02-4; 3-methoxybenzaldehyde, 591-31-1; 4-methoxybenzaldehyde, 123-11-5; 2-methylbenzaldehyde, 529-20-4; 4-methylbenzaldehyde, 104-87-0; 2-chlorobenzaldehyde, 89-98-5; 3-chlorobenzaldehyde, 587-04-2; 4-chlorobenzaldehyde, 104-88-1; 2-(methylthio)benzaldehyde, 7022-45-9; 4-nitrobenzaldehyde, 555-16-8; 4-fluorobenzaldehyde, 459-57-4; 2-furancarboxaldehyde, 98-01-1; 5-methyl-2-furancarboxaldehyde, 620-02-0; 2-pyridinecarboxaldehyde, 1121-60-4; 3-pyridinecarboxaldehyde, 500-22-1; benzaldehyde, 100-52-7; *N*,*N*′-bis(6-aminohexanyl)-1,8-octanediamine, 104807-24-1; *N*,*N*′-bis[6-[(2-methoxybenzyl)amino]caproyl]-1,8-octanediamine, 115962-71-5; *N*,*N*′-bis(6-aminocaproyl)-1,8-octanediamine, 115962-70-4; *N*,*N*′-bis[6-[(2-methoxybenzyl)methylamino]-caproyl]-1,8-octanediamine, 115962-72-6; *N*,*N*′-bis[6-[(2-methoxybenzyl)amino]caproyl]-*N*,*N*′-dimethyl-1,8-octanediamine, 115962-73-7; *N*,*N*′-dimethyl-1,8-octanediamine, 33563-54-1; *N*-[(benzyloxy)carbonyl]-6-aminocaproic acid, 1947-00-8; *N*,*N*′-bis(6-aminocaproyl)-*N*,*N*′-dimethyl-1,8-octanediamine, 115962-74-8; *N*,*N*′-bis(6-aminohexyl)-*N*,*N*′-dimethyl-1,8-octanediamine tetrahydrochloride, 115981-84-5; *N*,*N*′-bis(6-aminohexyl)-*N*,*N*′-dimethyl-1,8-octanediamine, 115962-75-9.

Inhibition of ^{125}I-Labeled Ristocetin Binding to *Micrococcus luteus* Cells by the Peptides Related to Bacterial Cell Wall Mucopeptide Precursors: Quantitative Structure–Activity Relationships

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Quantitative structure–activity relationships (QSAR) of *N*-Ac amino acids, *N*-Ac dipeptides, and *N*-Ac tripeptides in inhibition of ^{125}I-labeled ristocetin binding to *Micrococcus luteus* cell wall have been developed to probe the details of the binding between ristocetin and N-acetylated peptides. The correlation equations indicate that (1) the binding is stronger for peptides in which the side chain of the C-terminal amino acid has a large molar refractivity (MR) value, (2) the binding is weaker for peptides with polar than for those with nonpolar C-terminal side chains, (3) the N-terminal amino acid in *N*-Ac dipeptides contributes 12 times that of the C-terminal amino acid to binding affinity, and (4) the interactions between ristocetin and the N-terminal amino acid of *N*-acetyl tripeptides appear to be much weaker than those with the first two amino acids.

For the past 25 years medicinal chemists have sought statistical relationships between the physical and biological properties of molecules. Until recently such quantitative structure–activity relationships (QSAR) were developed without knowledge of the structure of the target of the drug. Hence, most QSAR cannot be checked against a structure of the drug–receptor complex.

We have been involved in an investigation of the complex between the antibiotic ristocetin (Figure 1) and Ac_2-L-Lys-D-Ala-D-Ala, a model of the bacterial cell wall as reported here.

Ristocetin is a glycopeptide antibiotic of the vancomycin group[1] isolated from the microorganism *Nocardia lurida*.[2] It is a linear heptapeptide, cross-linked between residues 1 and 2, 2 and 4, and 4 and 6 by diphenyl ether bridges

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0022-2623/89/1832-0084$01.50/0

Table I. Inhibition Constants (K_i) and Physicochemical Parameters Values of Peptides Related to Bacterial Cell Wall Mucopeptide Precursors of Ristocetin Aglycon Binding to *M. luteus* Cell

	compd	K_i, mol	MR_1	MR_3	I_{polar}	I_{aa}	I_{ala}	π_1	π_3
			Set 1: *N*-Ac D-Amino Acids						
1	D-Phe	1.0×10^{-4}	4.948	0.000	0	1	0	1.019	0.000
2	D-Trp	1.6×10^{-4}	6.066	0.000	0	1	0	1.019	0.000
3	D-Met	2.4×10^{-3}	4.171	0.000	0	1	0	−0.401	0.000
4	D-Ile	2.8×10^{-3}	3.828	0.000	0	1	0	0.908	0.000
5	D-Leu	2.8×10^{-3}	3.828	0.000	0	1	0	0.908	0.000
6	D-Val	3.1×10^{-3}	3.365	0.000	0	1	0	0.379	0.000
7	D-Ala	3.2×10^{-3}	2.437	0.000	0	1	0	−0.549	0.000
8	*p*-OH-D-Phg	4.2×10^{-3}	4.638	0.000	1	1	0	0.030	0.000
9[a]	(Cbz)D-Ala	5.1×10^{-3}	2.437	0.000	0	1	0	−0.549	0.000
10	D-Asn	9.0×10^{-3}	3.305	0.000	1	1	0	−1.753	0.000
11	Gly	2.9×10^{-2}	1.973	0.000	1	1	0	−1.078	0.000
			Set 2: Dipeptides (*N*-Ac-Gly-D-amino acid)						
12	Gly-D-Phe	9.8×10^{-6}	4.948	0.000	0	0	0	1.019	0.000
13	Gly-D-Ala	1.3×10^{-4}	2.437	0.000	0	0	0	−0.549	0.000
14	Gly-D-Val	3.4×10^{-4}	3.365	0.000	0	0	0	0.379	0.000
15	Gly-D-Ser	5.4×10^{-4}	2.590	0.000	1	0	0	−1.405	0.000
16	Gly-D-Leu	5.9×10^{-4}	3.828	0.000	0	0	0	0.908	0.000
17	Gly-D-Asn	7.2×10^{-4}	3.305	0.000	1	0	0	−1.753	0.000
18	Gly-D-Asp	1.4×10^{-3}	3.090	0.000	1	0	0	−1.013	0.000
19	Gly-Gly	4.6×10^{-3}	1.973	0.000	1	0	0	−1.078	0.000
20	Gly-D-Thr	4.7×10^{-3}	3.054	0.000	1	0	0	−1.096	0.000
			Set 3: Tripeptides (*N*-Ac-L-amino acid-Gly-Gly)						
21	L-Trp-Gly-Gly	1.2×10^{-3}	1.973	6.066	1	0	0	−1.078	1.019
22	L-Tyr-Gly-Gly	1.3×10^{-3}	1.973	5.101	1	0	0	−1.078	0.352
23	L-Pro-Gly-Gly	1.6×10^{-3}	1.973	3.187	1	0	0	−1.078	−0.168
24	L-Phe-Gly-Gly	1.6×10^{-3}	1.973	4.948	1	0	0	−1.078	1.019
25	D-Leu-Gly-Gly	1.9×10^{-3}	1.973	3.828	1	0	0	−1.078	0.908
26	L-Leu-Gly-Gly	2.0×10^{-3}	1.973	3.828	1	0	0	−1.078	0.908
27	Gly-Gly-Gly	7.1×10^{-3}	1.973	1.973	1	0	0	−1.078	−1.078
			Set 4: Miscellaneous (*N*-Ac-D-Ala-D-amino acid or *N*-Ac-L-amino acid-D-Ala-Gly)						
28	D-Ala-D-Phe	8.7×10^{-6}	4.948	0.000	0	0	1	1.019	0.000
29	D-Ala-D-Ala	7.3×10^{-5}	2.437	0.000	0	0	1	−0.549	0.000
30	D-Ala-Gly	3.1×10^{-3}	1.973	0.000	1	0	1	−1.078	0.000
31	D-Ala-D-Ala-D-Ala	4.2×10^{-5}	2.437	2.437	0	0	1	−0.549	−0.549
32	L-Lys-D-Ala-D-Ala	1.6×10^{-5}	2.437	5.160	0	0	1	−0.549	−1.263
33	D-Leu-Gly	1.1×10^{-2}	1.973	0.000	1	0	0	−1.078	0.000
34	D-Leu-D-Leu	4.5×10^{-3}	3.828	0.000	0	0	0	0.908	0.000

[a] *N*-Cbz derivative instead of *N*-Ac derivative.

and between residues 5 and 7 by a biphenyl bridge. Ristocetin exists as A and B forms that differ only in one of the sugars. The sugar residues in ristocetin are not essential for antibiotic activity: the aglycon exhibits enhanced antibacterial potency.[3]

The three-dimensional structure of ristocetin was elucidated by the use of high-field proton and carbon-13 nuclear magnetic resonance spectroscopy (NMR), numerous classical chemical methods, and mass spectrometry. The X-ray crystallography[4a] structure of the vancomycin degradation product CDP-I, which differs in composition from its parent antibiotic by the hydrolysis of an amide group to a carboxyl group and differs slightly from ristocetin, facilitated in the structural elucidation of ristocetin. The structure of ristocetin currently accepted[1b,5] is shown in Figure 1.

Ristocetin forms a complex with the peptidoglycan precursor of bacterial cell walls, bacterial UDP-*N*-acetylmuramylpentapeptide, thus preventing the enzymatic cross-linking of bacterial cell walls. This leads to a cessation of bacterial growth and the eventual destruction of the cell by lysis. The details of the structure elucidation and mode of action of ristocetin are discussed in recent reviews.[1b,5-7]

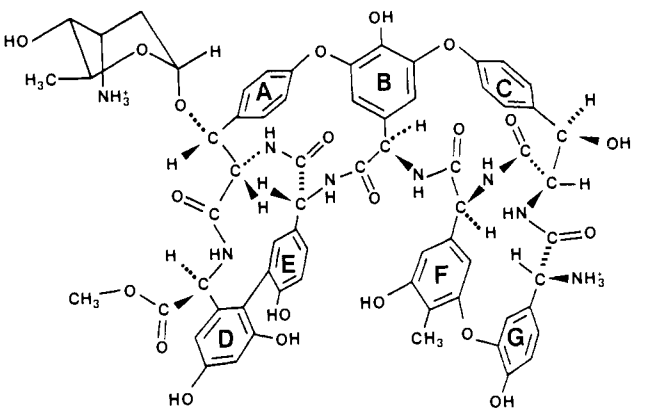

Figure 1. Structure of ristocetin aglycon.

Structures of ristocetin–peptide complexes have been studied previously by proton NMR spectroscopy,[8-12] ultraviolet (UV) spectroscopy,[13] circular dichroism (CD), and

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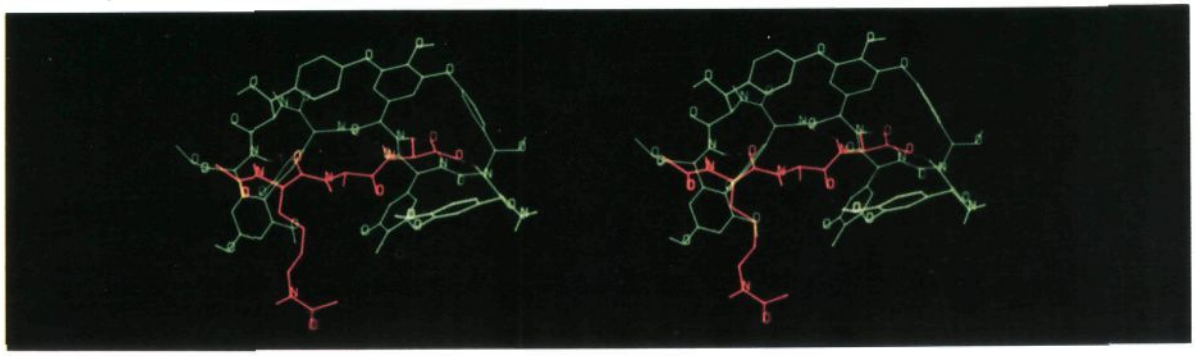

Figure 2. Stereoview of ristocetin–*N*-Ac_2-L-Lys-D-Ala-D-Ala complex.[15a]

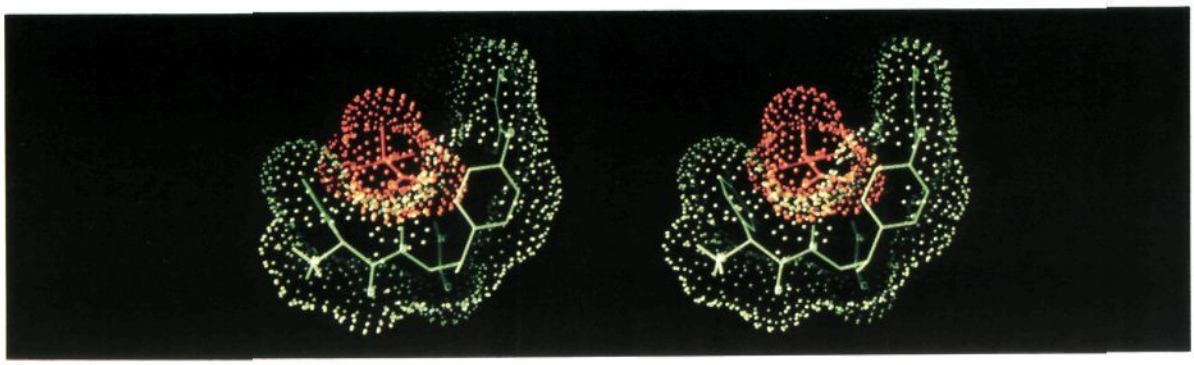

Figure 3. Stereoviews of ristocetin–*N*-Ac_2-L-Lys-D-Ala-D-Ala complex showing that ristocetin does not form an especially close contact with the methyl group of the C-terminal D-Ala.

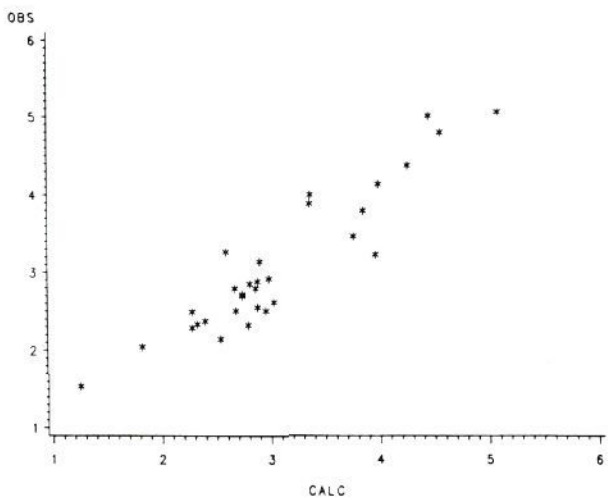

Figure 4. Plot of observed vs calculated log $1/K_i$ using eq 8.

optical rotatory dispersion (ORD).[14a] From these studies the C-terminal D-Ala-D-Ala portion of the growing bacterial cell wall has been identified as the point of interaction with ristocetin.

From extensive NMR and molecular modeling studies a three-dimensional model of the complex between ristocetin and Ac_2-L-Lys-D-Ala-D-Ala was proposed (Figure 2).[10,15] The NMR data indicated a high specificity of binding between ristocetin and the peptide, suggesting that the bound peptide is in an extended conformation.[10] One of the main interactions is proposed to be a polar attraction between the carboxyl group of the peptide and an amino or hydroxyl group of the antibiotic in the hydrophobic environment in the complex.[4b,14] In the complex this polar interaction is insulated from the solvent by the peptide side chain; the effectiveness of this insulation would depend on the length of the peptide and the size of the side chain in relation to the precise geometry of the antibiotic.[14]

The purpose of this work is to quantitatively examine the specificity of ristocetin aglycon for peptides of varying lengths and in which alterations to the side chains have been introduced. We sought to use QSAR to explore the forces that bind ristocetin to Ac_2-L-Lys-D-Ala-D-Ala and to examine whether the QSAR and NMR–molecular modeling models are compatible. Thus, this study was a test case for the validity of using QSAR equations to draw conclusions about the details of intermolecular interactions.

QSAR Methods

The observed inhibition constants and the physicochemical parameters used in this study are listed in Table I. The log $(1/K_i)$ is the reciprocal value of the logarithm of the molar K_i for ristocetin. Thus, the larger log $(1/K_i)$ is, the more strongly it binds to the antibiotic. The error in the experimentally determined K_i values is about ±50% or ±0.30 in logarithm units. Thus, we expected our QSAR equations to fit the data roughly this well.

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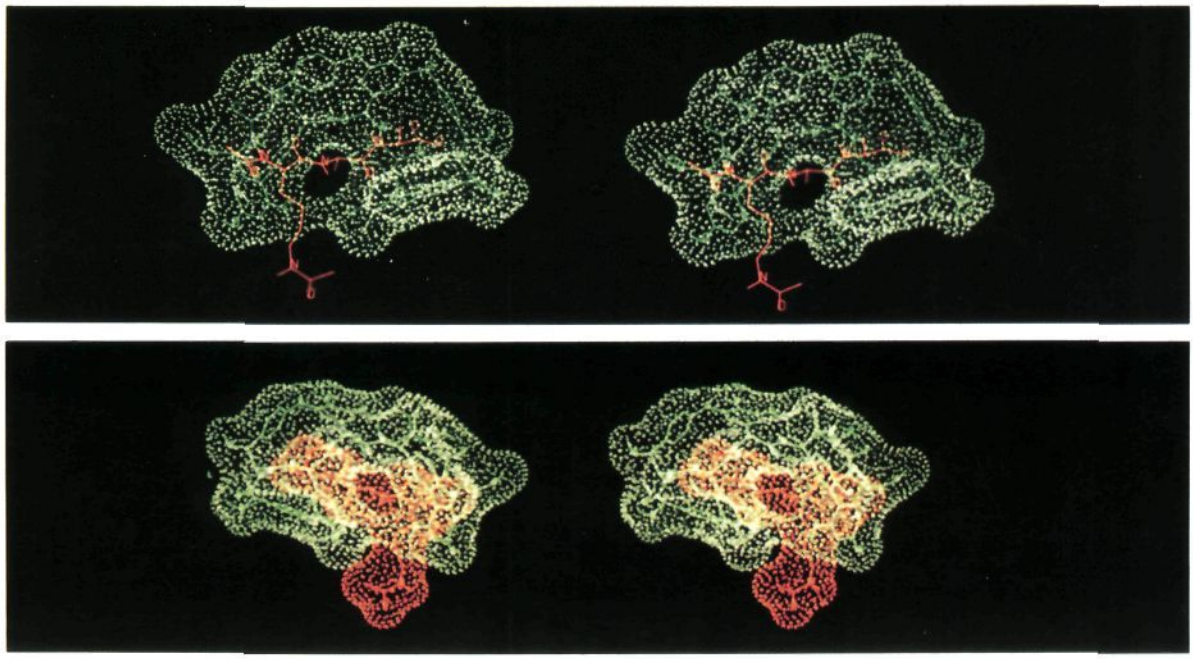

Figure 5. Two stereoviews of ristocetin–*N*-Ac_2-L-Lys-D-Ala-D-Ala complex showing that the fit between the antibiotic and the methyl group of the second amino acid is very precise.

The relative potential of a substituent to participate in dispersion interactions was parameterized with its molar refractivity (MR) value.[16-19] MR values were calculated by the additive–constitutive method.[20,21] MR_1's are for the C-terminal amino acid residue, and MR_3's are for the N-terminal amino acid residue. All MR values used in this study were scaled by 0.1 to make them more nearly equiscalar with the π constant. The octanol/water partition coefficient (log *P*) of each molecule was calculated by using the CLOGP program.[20]

The indicator variable I_{polar} is given the value of 1.0 for analogues that have polar groups in the side chain of the C-terminal amino acid and 0.0 for all other side chains in this position. The indicator variable I_{aa} has the value of 1.0 for the *N*-acetyl amino acids (set 1) and 0.0 for the *N*-acetyl di- and tripeptides.

The regression analyses were performed with the SAS program on a VAX computer. For each data set, all possible linear and quadratic equations were first generated, and then the individual equations were examined. The three subsets were correlated separately, and then all sets were correlated together. In the regression equations shown below, *n* is the number of compounds used in deriving the equation, *s* is the standard error of the calculation from the observed log $(1/K_i)$, and r^2 is the squared correlation coefficient. The standard error of estimation is given in parentheses.

Results

The different amino acid residues have been numbered as shown below to facilitate the description and discussion of the results:

Ac-L-Lys-D-Ala-D-Ala-OH
3 2 1

Set 1: Variation in the Side Chain of *N*-Acetyl Amino Acids, AcNHCHXCOOH. Equation 1 is the "best" two variable equation for set 1. When MR_1^2 was used instead of MR_1, a slightly statistically better correlation was obtained. However, since the squared correlation coefficient between MR_1 and MR_1^2 is very high (r^2 = 0.975), a simple model of MR_1 is used at the present time.

$$\log 1/K_i(r) = 0.42\ (\pm 0.09)MR_1 - 0.62\ (\pm 0.23)I_{polar} + 1.22\ (\pm 0.37) \quad (1)$$

$$n = 11,\ s = 0.334,\ r^2 = 0.821,\ F_{(2,8)} = 18.33,\ p < 0.001$$

Equation 1 indicates that compounds with a large MR_1 value bind to ristocetin more strongly than those with smaller MR_1 values. Since the coefficient of I_{polar} is negative, nonpolar groups in the side chain are preferred to polar groups. All compounds except one (1) fits this equation well. Compound 1, *N*-Ac-Phe, binds about 5 times less strongly than predicted. The use of octanol/water partition coefficients (π_1) did not improve the correlation, nor did log *P* substitute for MR_1 or I_{polar} (r^2 = 0.55 for the π_1 and I_{polar} two variable equation).

This result suggests that dispersion interactions are involved in binding to the side chain of the first amino acid and confirms the qualitative observation that ristocetin can accommodate groups much larger than methyl at this position. From the other point of view, ristocetin does not form an especially close contact with the methyl group of the C-terminal D-alanine of the bacterial cell wall precursor (see Figure 3).

Set 2: Variation in the Side Chain of the C-Terminal Amino Acid of *N*-Ac-Gly Dipeptides, $AcNHCH_2CONHCHXCOOH$. Equation 2 is the most important two-variable equation for set 2. Again, MR_1 is the most significant physicochemical property of the substituent. As in set 1, octanol/water partition coeffi-

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cients (log P) did not improve the correlation. In this set, five out of the nine compounds have a polar group in the variable side chain, and the addition of the variable, I_{polar}, to the singly variable equation explains an additional 18% of the variance of the activity.

$$\log 1/K_i(r) = 0.41\ (\pm 0.27)MR_1 - 0.77\ (\pm 0.44)I_{polar} + 2.40\ (\pm 1.02) \quad (2)$$

$$n = 9,\ s = 0.562,\ r^2 = 0.649,\ F_{(2,6)} = 5.54,\ p < 0.04$$

Equation 2 suggests that binding is enhanced by nonpolar substituents with a large MR_1 value: this is consistent with the results of set 1. The essentially identical coefficients of MR_1 and I_{polar} in eq 2 to those in eq 1 are especially significant and the equation is worth presenting in this respect, even if the standard error is larger than others.

Combination of Sets 1 and 2: Variation in the Side Chain of the C-Terminal Residue of *N*-Acetyl Amino Acids and Dipeptides. Assuming that the compounds in sets 1 and 2 bind to ristocetin A in a similar manner, one would expect the regression equations to have similar coefficients for both the MR_1 and I_{polar} variables. Indeed, the coefficients of MR_1 and I_{polar} of the eq 1 and 2 are essentially identical.

The combination of sets 1 and 2 resulted in eq 3, which is the most significant three-variable equation. In addition

$$\log 1/K_i(r) = 0.42\ (\pm 0.10)MR_1 - 0.69\ (\pm 0.21)I_{polar} - 1.09\ (\pm 0.20)I_{aa} + 2.33\ (\pm 0.39) \quad (3)$$

$$n = 20,\ s = 0.419,\ r^2 = 0.777,\ F_{(3,16)} = 18.53,\ p < 0.0001$$

to the two variables MR_1 and I_{polar}, an indicator variable I_{aa} is included to describe the difference in intercept between set 1 ($I_{aa} = 1$) and set 2 ($I_{aa} = 0$). The coefficient of the indicator variable I_{aa} shows that *N*-acetyl dipeptides bind about 12 times more strongly than *N*-acetyl amino acids, other factors being equal. This is interpreted to mean that the terminal CH_3CONH contributes 1.5 kcal/mol (= 1.4 kcal × 1.09) to the binding of ristocetin to the *N*-Ac dipeptide.

The strength of binding increases with increasing MR of the side chain of the first amino acid, and analogues with nonpolar side chains bind more strongly than those with polar side chains. The fact that set 1 and set 2 could be combined into one equation without losing the quantitative information strongly indicates that the compounds in both sets bind to ristocetin with essentially identical geometry.

Set 3: Variation in the Side Chain of the N-Terminal Amino Acid of *N*-Ac Tripeptides, AcNHCHX-$(CONHCH_2)_2COOH$. Equation 4 is the most significant single-variable equation for set 3. It suggests that peptides with a large MR_3 value bind more strongly to ristocetin than those with smaller MR_3 values. The addition of MR_3^2 to eq 4 improved the correlation significantly (eq 5). The

$$\log 1/K_i(r) = 0.16\ (\pm 0.05)MR_3 + 2.05\ (\pm 0.20) \quad (4)$$

$$n = 7,\ s = 0.155,\ r^2 = 0.704,\ F_{(1,5)} = 11.90,\ p < 0.018$$

$$\log 1/K_i(r) = 0.17\ (\pm 0.05)MR_3 - 0.06\ (\pm 0.03)MR_3^2 - 1.19\ (\pm 0.41) \quad (5)$$

$$n = 7,\ s = 0.115,\ r^2 = 0.871,\ F_{(2,4)} = 13.48,\ p < 0.017$$

$$\text{optimum } MR_3 = 5.40$$

small standard error in eq 5 may be due to the small number of compounds included in deriving this equation (two variables with seven compounds). However, eq 5 may also be a statistical artifact[22] because it lowers the standard deviation to 0.115; this is very small compared to the average experimental error of about 0.3. In addition, the significant improvement of the parabolic equation (eq 5) over eq 4 is due to one compound, Ac-Gly-Gly-Gly (compound **27**). Without this compound, only the singly variable equation (eq 6) is significant.

$$\log 1/K_i(r) = 0.07\ (\pm 0.03)MR_3 + 2.51\ (\pm 0.12) \quad (6)$$

$$n = 6,\ s = 0.061,\ r^2 = 0.617,\ F_{(1,4)} = 6.44,\ p < 0.06$$

In set 3, all but one compound (compound **25**; Ac-D-Leu-Gly-Gly) are L isomers. Excluding compound **27**, which has the smallest MR_3, and compound **25**, which is the only D analogue, an essentially identical result was obtained, indicating that the D analogue fits eq 6 well. However, this result may be a fortuitous one, and it should be confirmed by the analysis of additional D isomers.

As in sets 1 and 2, the octanol/water partition coefficients (log P) did not correlate with binding, alone or in combination with MR. Thus, as previously observed, dispersion interactions between the side chain of the N-terminal amino acid of the peptide and antibiotic are indicated by the QSAR.

Combination of Sets 1, 2, and 3. Because compounds in sets 1 and 2 could be successfully combined into one QSAR equation, an attempt was made to combine all the 27 compounds in sets 1, 2, and 3 into one equation. All the compounds in set 3 have Gly-Gly for the first and second amino acids and varied only in the third amino acid residue. The coefficient of MR_1 in eq 3 and that of MR_3 in eq 4 are different because they interact at different regions in space. Equation 4 does not have the variable I_{polar} because all the compounds in set 3 have an identical amino acid in the C-terminal position.

Equation 7 is the "best" four-variable equation obtained with all 27 compounds. When MR_3^2 was used instead of MR_3 in eq 7, a slightly improved correlation was obtained.

$$\log 1/K_i(r) = 0.43\ (\pm 0.09)MR_1 - 0.71\ (\pm 0.19)I_{polar} - 1.08\ (\pm 0.18)I_{aa} + 0.08\ (\pm 0.05)MR_3 + 2.28\ (\pm 0.34) \quad (7)$$

$$n = 27,\ s = 0.371,\ r^2 = 0.771,\ F_{(4,22)} = 18.50,\ p < 0.0001$$

The coefficients of MR_1, I_{polar}, and I_{aa} of eq 7 are essentially those of eq 3. The quality of eq 7 is equally as good as eq 3.

The fact that in the combined sets the binding between ristocetin and the peptides correlates with MR_1 strongly indicates that the side chain of the C-terminal amino acid of the *N*-acetyl amino acids and di- and tripeptides forms similar dispersion interactions with ristocetin. The side chain of the third amino acid of the *N*-Ac tripeptides interacts with ristocetin at an additional site. The small coefficient of MR_3 in eq 7 suggests that the dispersion interactions at this site are much weaker than those that bind the side chain of first amino acid. The weakness of the former interaction may explain why the interaction between the side chain of the third amino acid and ristocetin was not experimentally observed in the UV,[9] and why it was difficult to define the position of the third amino acid residue by NMR.[12]

Refinement of the QSAR and Receptor Mapping around the Side Chain of the Second Amino Acid. All the *N*-Ac dipeptides and *N*-Ac tripeptides included in the

(22) Kim, K. H.; Martin, Y. C.; Otis, E. R.; Mao, J. C., in preparation.

Table II. Observed and Calculated log $1/K_i$ Values from Eq 1, 2, 4, and 8

compound	log $1/K_i$ obsd	calcd[a]	dev	calcd[b]	dev
1 D-Phe	4.000	3.373	0.626	3.298	0.701
2 D-Trp	3.795	3.858	−0.062	3.767	0.028
3 D-Met	2.619	3.036	−0.416	2.972	−0.352
4 D-Ile	2.552	2.887	−0.334	2.828	−0.275
5 D-Leu	2.552	2.887	−0.334	2.828	−0.275
6 D-Val	2.508	2.686	−0.178	2.633	−0.125
7 D-Ala	2.494	2.284	0.210	2.244	0.250
8 *p*-OH-Phe-Gly	2.376	2.404	−0.027	2.545	−0.169
9 (Cbz)D-Ala	2.292	2.284	0.008	2.244	0.047
10 D-Asn	2.045	1.826	0.219	1.986	0.059
11 Gly	1.537	1.248	0.289	1.427	0.110
12 Gly-D-Phe	5.008	4.457	0.551	4.435	0.573
13 Gly-D-Ala	3.886	3.367	0.518	3.400	0.485
14 Gly-D-Val	3.468	3.770	−0.301	3.782	−0.314
15 Gly-D-Ser	3.267	2.599	0.668	2.698	0.569
16 Gly-D-Leu	3.229	3.971	−0.741	3.973	−0.744
17 Gly-D-Asn	3.142	2.909	0.233	2.992	0.149
18 Gly-D-Asp	2.853	2.816	0.037	2.904	−0.050
19 Gly-Gly	2.337	2.331	0.005	2.444	−0.106
20 Gly-D-Thr	2.327	2.800	−0.472	2.889	−0.561
21 L-Trp-Gly-Gly	2.920	2.991	−0.070	3.019	−0.099
22 L-Tyr-Gly-Gly	2.886	2.886	−0.000	2.865	0.021
23 L-Pro-Gly-Gly	2.795	2.869	−0.073	2.840	−0.044
24 L-Phe-Gly-Gly	2.795	2.678	0.117	2.557	0.238
25 D-Leu-Gly-Gly	2.721	2.747	−0.026	2.660	0.060
26 L-Leu-Gly-Gly	2.698	2.747	−0.048	2.660	0.038
27 Gly-Gly-Gly	2.148	2.546	−0.397	2.362	−0.214
28 D-Ala-D-Phe	5.060	5.089	−0.028		
29 D-Ala-D-Ala	4.136	3.999	0.136		
30 D-Ala-Gly	2.508	2.963	−0.455		
31 L-Lys-D-Ala-D-Ala	4.795	4.560	0.235		
32 L-Ala-D-Ala-D-Ala	4.376	4.264	0.112		

[a] Calculated using eq 8. [b] Equation 1 is used for compounds 1–11, eq 2 for **12–20**, and eq 4 for **21–27**.

above correlation equations have Gly in the second position. The CPK model[7] and the molecular modeling[15] of the complex between ristocetin and Ac_2-L-Lys-D-Ala-D-Ala indicate that there is just enough space for the D-methyl group where the side chain of the second amino acid interacts with ristocetin. Equation 8 was developed by including three additional dipeptides [Ac-D-Ala-D-Phe (**28**), Ac-D-Ala-D-Ala (**29**), Ac-D-Ala-Gly (**30**)] and two additional tripeptides [Ac_2-L-Lys-D-Ala-D-Ala (**31**) and Ac-D-Ala-D-Ala-D-Ala (**32**)] with the original set of compounds. These compounds take the value of 1.0 for I_{ala}, indicating the presence of Ala in the second amino acid position.

$$\log 1/K_i(r) = 0.43\ (\pm0.08)MR_1 - 0.84\ (\pm0.17)I_{polar} - 1.08\ (\pm0.17)I_{aa} + 0.11\ (\pm0.04)MR_3 + 0.63\ (\pm0.21)I_{ala} + 2.31\ (\pm0.31) \quad (8)$$

$$n = 32,\ s = 0.363,\ r^2 = 0.860,\ F_{(5,26)} = 31.95,\ p < 0.0001$$

The quality of eq 8 is very good, and the positive coefficient of I_{ala} suggests that ristocetin binds more strongly to peptides with D-Ala in the second amino acid position. Perhaps the more important point is that the coefficients of all other variables are essentially the same as those in eq 7. Changing this side-chain substituent from H to Me increases the strength of interaction by 0.9 kcal/mol (= 1.4 kcal × 0.63). This large value indicates that the fit between the antibiotic and the methyl group is very precise. As one might expect, compounds that have a side chain larger than D-Ala bind less strongly than predicted by these equations. Indeed, Ac-D-Leu-Gly is less active than Ac-D-Ala-Gly, and even weaker than Ac-Gly-Gly. Ac-D-Leu-D-Leu is also weaker than Ac-Gly-D-Leu, but no direct comparison could be made with Ac-D-Ala-D-Leu. Although I_{ala} is a crude descriptor for the side chain of the second amino acid compared to the MR_1 for those of the first or MR_3 for those of the third, it was chosen because there are only three kinds of amino acids (Gly, Ala, and Leu) at this position.

Equations 9–12 are the stepwise development of eq 8. Table II lists the observed and calculated activity values with eq 8 as well as the eq 1, 2, and 4 for the individual sets. Table III lists the mean and standard deviations and Pearson correlation coefficients of the parameters examined in this study.

$$\log 1/K_i(r) = -0.96\ (\pm0.27)I_{polar} + 3.55\ (\pm0.19) \quad (9)$$

$$n = 32,\ s = 0.754,\ r^2 = 0.304,\ F_{(1,30)} = 13.07,\ p < 0.001$$

$$\log 1/K_i(r) = -1.32\ (\pm0.20)I_{polar} - 1.15\ (\pm0.22)I_{aa} + 4.12\ (\pm0.17) \quad (10)$$

$$n = 32,\ s = 0.545,\ r^2 = 0.648,\ F_{(2,29)} = 26.73,\ p < 0.0001$$

$$\log 1/K_i(r) = 0.34\ (\pm0.09)MR_1 - 1.02\ (\pm0.19)I_{polar} - 1.38\ (\pm0.19)I_{aa} + 3.03\ (\pm0.32) \quad (11)$$

$$n = 32,\ s = 0.450,\ r^2 = 0.769,\ F_{(3,28)} = 31.02,\ p < 0.0001$$

$$\log 1/K_i(r) = 0.36\ (\pm0.08)MR_1 - 0.80\ (\pm0.18)I_{polar} - 1.19\ (\pm0.18)I_{aa} + 0.63\ (\pm0.23)I_{ala} + 2.67\ (\pm0.32) \quad (12)$$

$$n = 32,\ s = 0.404,\ r^2 = 0.820,\ F_{(4,27)} = 30.75,\ p < 0.0001$$

Figure 4 is the plot of the observed vs calculated values with equation 8. A summary of the QSAR results is shown in Scheme I.

Discussion

Only qualitative observations were made from the earlier structure–activity relationships[13,14] and most NMR-mod-

Table III. Pearson Correlation Coefficients, Mean, and Standard Deviation of Parameters Used in the Correlation

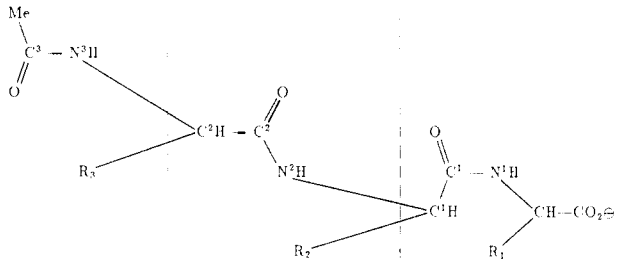
1. Pearson Correlation Coefficient

	MR_1	MR_1^2	MR_3	I_{polar}	I_{aa}	I_{ala}	π_1	π_3
MR_1	1.00							
MR_1^2	0.98	1.00						
MR_3	−0.51	−0.45	1.00					
I_{polar}	−0.50	−0.47	0.34	1.00				
I_{aa}	0.43	0.41	−0.42	−0.32	1.00			
I_{ala}	−0.07	−0.07	0.08	−0.25	−0.31	1.00		
π_1	0.76	0.75	−0.35	−0.76	0.35	0.04	1.00	
π_3	−0.12	−0.09	0.28	0.32	−0.05	−0.36	−0.12	1.00

2. Mean and Standard Deviation of Parameters

variable	mean	SD	variable	mean	SD
MR_1	3.050	1.134	I_{aa}	0.343	0.482
MR_1^2	10.554	8.187	I_{ala}	0.156	0.368
MR_3	1.141	1.979	π_1	−0.434	0.894
I_{polar}	0.500	0.508	π_3	0.035	0.471

Scheme I. Summary of QSAR Results



eling studies[9-12] dealt with ristocetin itself or complexes with one peptide to study the ristocetin binding sites.[1b,14b] The overall size and shape of the carboxylate binding pocket of ristocetin was suggested to be very important for antibacterial activity.[14c] An ionic interaction is not critical for binding, but the possibility of a dipole–dipole or hydrogen-bonding interaction stabilizing the complex was proposed.[14c] The present QSAR study not only confirms these earlier qualitative observations but also describes such interactions quantitatively.

Contribution of the Carboxylate and the First Peptide Bond to Binding. Earlier structure–activity[14] and NMR-modeling studies[10,12,15] highlighted the essential role of the first peptide bond of the peptide in binding to ristocetin. Structure–activity analyses also revealed the critical role of the C-terminal carboxylate group.[14] It was postulated that an electrostatic interaction between the carboxylate anion of the peptide and the protonated amino terminus of ristocetin was important in the complex formation. However, the importance of this electrostatic interaction was questioned since a series of ristocetin analogues lacking positively charged amino group were found to bind to Ac_2-L-Lys-D-Ala-D-Ala and had antibacterial activity.[14c,15] QSAR analyses of these observations are not available since the necessary compounds are not included.

Contribution of the Side Chain at Position 1 to Binding. Although previous workers suggested that the minimum requirement for binding of a peptide to ristocetin is an *N*-acetyl dipeptide with a free C-terminal carboxylate,[1,13] we observed weak but definite binding of N-acetylated amino acids.

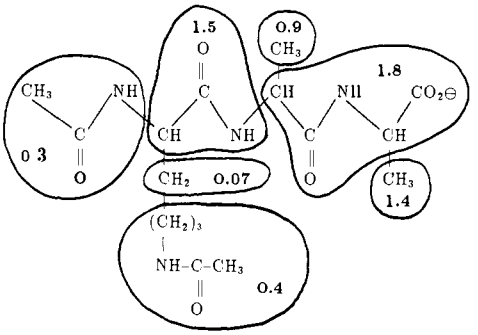
Earlier structure–activity observations indicated that ristocetin will bind to peptides in which the C-terminal D-amino acid has a side chain larger than methyl.[14] A NMR–modeling study of the structure of the ristocetin–Ac_2-L-Lys-D-Ala-D-Ala complex (Figure 3) shows that this methyl group lies over the face of one of the aromatic rings in the antibiotic in a relatively open space that can accomodate larger groups. Our QSAR results have extended and quantified these observations. From the point of view of antibiotic design, the QSAR suggests that improvements could be made in the specificity of the binding pocket for the methyl group of the C-terminal D-amino acid of the bacterial cell wall. This pocket in vancomycin, a related antibiotic, is more specific.[22] However, increased specificity sacrifices binding energy since the contribution to binding of this methyl group is 1.5 kcal/mol for ristocetin and 0.3 kcal/mol for vancomycin.[22]

Nieto and Perkins observed little binding to ristocetin of *N*-acetyl dipeptides in which the side chain bears a positive charge.[14] They attributed this to an electrostatic repulsion of the positively charged amino group in the antibiotic. In a polar environment electrostatic interactions are less strong than in a hydrophobic environment. The importance of an electrostatic interaction is supported by the statistical significance in the QSAR equations of the negative contribution of polar side chains at position 1, and could be related to the adjacent hydrogen bonds of the ristocetin–peptide complex shown in Figure 2.

Contribution of the Side Chain at Position 2 to Binding. Earlier studies revealed the importance of a D-amino acid at the second position and that substituents larger than methyl are not accomodated.[9] These results are substantiated by the present study. Both dipeptides with D-leucine, Table I, bind less strongly than the corresponding dipeptides with glycine, and when glycine was replaced by D-alanine, the resulting dipeptides bind more strongly than the original. These effects are quantitatively described in eq 8 with the indicator variable I_{ala}: the introduction of a methyl group improved the binding by an average of 5-fold. An explanation for the interactions responsible for this rather large increase in binding affinity by the addition of a methyl group is not revealed by the QSAR, but it is clear when one examines a molecular model of a ristocetin–peptide complex[15a] (Figure 5). One can easily see that there is just enough space for the size of a methyl group where D-alanine of position 2 interacts with ristocetin. Hydrophobic interactions between this methyl group and the hydrocarbon portion of the ristocetin certainly help to improve the binding.

There are only two compounds with D-Leu in position 2; they were not included in the correlation equation. This

Scheme II.[a] The Contribution of Portions of the *N*-Ac_2-L-Lys-D-Ala-D-Ala Peptide Molecule to Binding to Ristocetin



[a] The values in kcal/mol are calculated from the coefficients of eq 8 or the calculated log $1/K_i$ values from eq 8: (from right to left) 1.8 = Gly (1.25) × 1.4; 1.4 = [D-Ala (2.28) − Gly (1.25)] × 1.4; 0.9 = I_{ala} (0.63) × 1.4; 1.5 = I_{aa} (1.08) × 1.4; 0.07 = $MR_{(CH_2)}$ (0.46) × MR_3 (0.11) × 1.4; 0.4 = [L-Lys-D-Ala-D-Ala (4.56) − L-Ala-D-Ala-D-Ala (4.26)] × 1.4; 0.3 = [GlyGlyGly (2.55) − Gly-Gly (2.33)] × 1.4.

is so because it is conceivable that any substituent larger than D-Ala would reduce the binding. Perhaps, a parabolic relationship could be obtained with additional compounds in this position. Equation 13 is the correlation equation with all 34 compounds in Table I.

$$\log 1/K_i(r) = 0.43\ (\pm 0.09)MR_1 - 0.87\ (\pm 0.17)I_{polar} - 0.87\ (\pm 0.20)I_{aa} + 0.13\ (\pm 0.05)MR_3 + 0.82\ (\pm 0.25)I_{ala} + 2.08\ (\pm 0.38) \quad (13)$$

$$n = 34,\ s = 0.454,\ r^2 = 0.779,\ F_{(5,28)} = 19.79,\ p < 0.0001$$

Early work suggested that a charged amino acid at position 2 decreased the binding affinity.[14] Because only two different amino acid residues are included in this study, it is not possible to quantify the electrostatic effects of the second residue with the present set of data.

Contribution to Binding of the Third Amino Acid Peptide Bond. The QSAR equation does not reveal the nature of the interaction between ristocetin and the second peptide bond, but it does quantitate by the indicator variable I_{aa} that it contributes to binding by an average of 1.5 kcal/mol.

The amino acid residues in the third position were found to bind better with a long-chain L substituent, but detailed changes were not so important and even a D substituent (as in Ac-D-Ala-D-Ala-D-Ala) did not prevent binding.[3] When the side chain at position 3 was varied, comparatively few qualitative changes in the UV difference spectrum were produced,[9] and the binding with the third amino acid residue is thus considered weak.[8] The small coefficient of MR_3 in eq 8 suggests weak interaction of this region with ristocetin and supports these previous experimental observations.

Summary of the Quantitative Contribution of Portions of Ac_2-L-Lys-D-Ala-D-Ala to Binding to Ristocetin. Equation 8 can be used to evaluate the contribution of each part of the model peptide to binding to the antibiotic. This was done by calculating the change in the predicted binding constant that corresponds to the structural change of interest. This change in equilibrium constant is then multiplied by 1.4 to convert to kcal/mol. The binding of N-acetylated-D-Ala was used as the reference point.

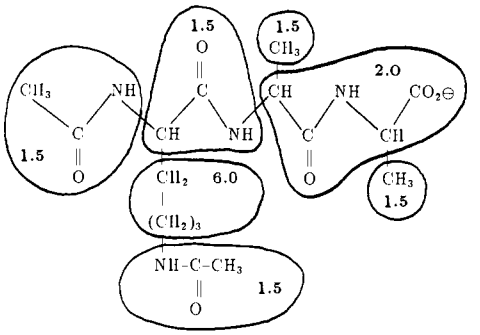
The results of these calculations are shown in Scheme II and Table IV. Notice that the energy of binding the second peptide bond is much larger than the energy of binding the third peptide bond. It is interesting to compare the results in Table IV with the results for the contribution in the free energies for the combination of ristocetin with the peptide obtained by UV difference spectroscopy.[13b,14a] The contribution of the methyl group of the first amino acid residue on the binding energy is 1.95,[14a] 2.13,[14a] and 1.9[4b] [= Ac_2-L-Lys-D-Ala-D-Ala (7.8) − Ac_2-L-Lys-D-Ala-Gly (5.9)]. Scheme II and Table IV show the average contribution is 1.4 kcal/mol. The contribution of the methyl group of the second amino acid residue is 0.78,[14a] 0.21,[14a] and 0.7[4b] [= Ac_2-L-Lys-D-Ala-D-Ala (7.8) − Ac_2-L-Lys-Gly-D-Ala (7.1)]. Our results show the average contribution is 0.9 kcal/mol. The antibiotic achieves maximal interactions with only a part of the peptide. Scheme III illustrates an extrapolation from these results. It represents the maximum expected interaction energy of each part of the model peptide. Notice that the antibiotic achieves only a small part of the theoretical maximum binding energy. It is not possible to estimate how much of the theoretical maximum could be realized in any compound.

Table IV. Contribution of the Portion of the Peptide to Binding Energy[a]

		UV spectroscopy method[b]	QSAR method[c]
methyl	(residue 1)	1.95,[13] 2.13,[13] 1.9[29] kcal	1.4 kcal
	(residue 3)	0.78,[13] 0.21,[13] 0.7[29] kcal	0.9 kcal

[a] See the reference section for the corresponding references. [b] Values at 26 °C, pH = 5.1[13] or pH = 10.[29] [c] Values at 4 °C, pH = 6.5.

Scheme III. Estimated Maximum Contribution to Binding of Each Fragment of *N*-Ac_2-L-Lys-D-Ala-D-Ala to a Hypothetical Antibiotic[a]



[a] The values are taken as the maximum contribution of the particular fragment. For example, since the methyl group to binding to ristocetin of the C-terminal D-Ala contribute 1.5 kcal/mol, each methyl or methylene is assumed to have a potential to contribute 1.5 kcal/mol to binding.[1]

In summary, the QSAR equations give a great deal of insight into the details of the interaction between ristocetin and peptides. Specifically, they suggest a dispersion interaction in a relative open space for the side chain of the first amino acid residue, a close-fitting pocket for the methyl group of the second amino acid residues, specific interactions with the peptide bond to the third amino acid residue, and relatively little specific interaction between the side chain of the third residue and the antibiotic. These insights would be helpful in molecular modeling of the proposed complex in the absence of detailed NMR data. They also highlight the fact that ristocetin is not the optimum compound for binding to Ac_2-L-Lys-D-Ala-D-Ala.

The present study demonstrate that the classical QSAR studies of the Hansch analysis can be used to describe the ligand–macromolecule interactions quite well, and the results are consistent with those of qualitative observations

Table V. Physical Properties of N-Acetylated Amino Acids and Peptides

compound	mp, °C	$[\alpha]_D^{25}$ (c, %)[d]	formula	anal.	R_f[r]
1[a] Gly	206–208 dec		$C_4H_7NO_3$	C,H,N	0.14[s]
2[b] (Cbz)D-Ala[c]	83–85	15.9 (1)	$C_{11}H_{13}NO_4$	H,N;C[f]	0.57[s]
3[a] D-Ala	125–127	63.7 (1)	$C_5H_9NO_3$	H,N;C[g]	0.23[s]
4[b] D-Trp	216–218 dec	−27.0 (1)	$C_{13}H_{14}N_2O_3$	H,N;C[h]	0.21[s]
5[b] D-Phe	170–172	−39.1 (1)	$C_{11}H_{13}NO_3$	C,H,N	0.22[s]
6[a] D-Met	100–102	19.1 (4)	$C_7H_{13}NO_3S$	H,N;C;S[i]	0.25[s]
7[a] D-Leu	183–184	21.4 (2)	$C_8H_{15}NO_3$	C,H,N	0.45[s]
8[b] D-Val	152–155	−10.6 (1)	$C_7H_{13}NO_3$	C,H,N	0.45[s]
9[a] D-Ile	156–157	−19.6 (2)	$C_8H_{15}NO_3$	C,H,N	0.49[s]
10 *p*-OH-Phe-Gly			$C_{10}H_{11}NO_4$	C,H,N	0.65
11[a] D-Asn	167–169	−13.6 (2)	$C_6H_{10}N_2O_4$	C,H,N	0.04[s]
12 Gly-D-Ala	178–180 dec	26.5 (2)	$C_7H_{12}N_2O_4$	C,H,N	0.13
13 Gly-Gly	178–180 dec		$C_6H_{10}N_2O_4$	C,H,N	0.32
14 Gly-D-Ser	hygroscopic	−8.4 (1)	$C_7H_{12}N_2O_5$	C,H,N	0.03
15 Gly-D-Phe	hygroscopic	−44.6 (1.1)	$C_{13}H_{16}N_2O_4$	C,H,N	0.38
16 Gly-D-Leu	hygroscopic	18.8 (1.1)	$C_{10}H_{18}N_2O_4$	H,N;C[j]	0.27
17 Gly-D-Asp	hygroscopic	−20.6 (1)	$C_8H_{12}N_2O_6$	C,H,N	0.02
18 Gly-D-Val	hygroscopic	2.5 (1)	$C_9H_{16}N_2O_4$	C,H,N	0.28
19 Gly-D-Asn	163–165 dec	−12.6 (2)	$C_8H_{13}N_3O_5$	C,H,N	0.04
20 Gly-D-Thr	hygroscopic	−1.8 (2)	$C_8H_{14}N_2O_5$	C,H;N[k]	0.22
21 Gly-Gly-Gly	211–213 dec		$C_8H_{13}N_3O_5$	H;C;N[l]	0.18
22 L-Leu-Gly-Gly	hygroscopic	−24.9 (2)	$C_{12}H_{21}N_3O_5$	C,H,N	0.37
23 L-Pro-Gly-Gly	72–74	−89 (1)	$C_{11}H_{17}N_3O_5$	C,H;N[m]	0.36[s]
24 L-Phe-Gly-Gly	86–90	18.4 (1)	$C_{15}H_{19}N_3O_5$	H,N;C[n]	0.58
25 L-Tyr-Gly-Gly	hygroscopic	19.6 (2)	$C_{15}H_{19}N_3O_6$	C,H,N	0.40
26 L-Trp-Gly-Gly	101–103	20.0 (0.92)	$C_{17}H_{20}N_4O_5$	H;C;N[o]	0.17
27 D-Leu-Gly-Gly	hygroscopic	24.3 (2)	$C_{12}H_{21}N_3O_5$	C,H,N	0.37
28 D-Ala-D-Ala		78 (1)[e]	$C_8H_{14}N_2O_4$	C,H,N	0.57
29 D-Ala-D-Phe	215–217 dec	6.0 (0.65)[e]	$C_{14}H_{18}N_2O_4$	C,H,N	0.63
30 D-Ala-Gly	165–168 dec	53.5 (4)	$C_7H_{12}N_2O_4$	C,H,N	0.17
31[b] L-Lys-D-Ala-D-Ala	207–208 dec	38 (2)	$C_{14}H_{26}N_4O_5$	C,H;N[p]	0.34
32 D-Ala-D-Ala-D-Ala	216–218 dec	111.1 (1.5)	$C_{11}H_{19}N_3O_5$	H,N;C[q]	0.19[s]
33 D-Leu-Gly		44.2 (2.1)	$C_{10}H_{18}N_2O_4$	C,H,N	0.25
34 D-Leu-D-Leu	186–188	54.8 (0.9)[e]	$C_{14}H_{26}N_2O_4$	C,H,N	0.95

[a] Sigma Chemical Co. [b] Serva Feinbiochemica. [c] *N*-Cbz derivative instead of *N*-Ac derivative. [d] Water, except *e*. [e] 50% MeOH in water. [f] C: calcd, 59.19; found, 58.65. [g] C: calcd, 45.80; found, 45.28. [h] C: calcd, 63.40; found, 55.88. [i] C: calcd, 43.96; found, 43.19. S: calcd, 16.77; found, 17.82. [j] C: calcd, 52.15; found, 51.58. [k] N: calcd, 12.84; found, 12.32. [l] C: calcd, 41.56; found, 39.60. N: calcd, 18.17; found, 17.28. [m] N: calcd, 15.49; found, 14.76. [n] C: calcd, 56.08; found, 55.08. [o] C: calcd, 56.66; found, 54.68; N: calcd, 15.55; found, 14.88. [p] N: calcd, 16.96; found, 14.71. [q] C: calcd, 48.34; found, 47.12. [r] MeOH/HOAc/CH_2Cl_2 (10:5:85) unless otherwise noted. [s] EtAc/DMF (9:1).

obtained from UV, CD and ORD, and NMR experiments.

Experimental Section

Ristocetin was purchased from H. Lundbeck, Copenhagen, Denmark and U.S. Biochemical Corp., Cleveland, OH. Iodine-125 (specific activity 17 Ci/mg) was purchased from New England Nuclear, Boston, MA. Radioactivity was determined with an ANSR γ-counter from Abbott Laboratories, Abbott Park, IL. *N*-Acetyl D-amino acids were purchased either from Sigma Chemical Co. or from Serva Feinbiochemica, Garden City Park, NY. Dipeptides and tripeptides were obtained from Vega Biochemicals, Tucson, AR, and Serva. Peptides were acetylated by the following procedure.

Peptides were dissolved in a minimum amount of saturated $NaHCO_3$ solution. Twice molar equivalents of acetic anhydride were added. After 10 min at room temperature, the reactant was heated to boiling for 3 min in order to destroy the unreacted acetic anhydride. The acetylated peptides were purified by Dowex-50 column chromatography. After the reactant was absorbed into the column, it was washed with water. The first column volume of elutant was discarded, and the next 1.5 column volume elutant was collected and lyophilized. Yields of the products were in the ranges between 45 and 90%. The purity of all the compounds was determined by TLC on silica gel using the solvent system (a) MeOH/HOAc/CH_2Cl_2 (10:5:85) or (b) EtAc/DMF (9:1). Melting points were determined on a Mettler FP5 melting point apparatus and were uncorrected. Optical rotation was measured in a Perkin-Elmer Model 241 polarimeter. Table V lists the physical properties of the N-acetylated amino acids and peptides.

Micrococcus luteus cells were purchased from Miles Laboratories, Elkhart, IN. The lyophilized cells (2.4 g) were suspended in 150 mL of 1% sodium lauryl sulfate and heated to boiling, then washed four times with equal volume of distilled water by centrifugation, and finally were suspended in 240 mL of distilled water and stored at 4 °C.

Iodination of ristocetin was accomplished by the following procedure. To 10 mg of ristocetin dissolved in 1 mL of 0.1 M sodium phosphate buffer, pH 6.5, were added 0.1 mL of iodine-125 (5 mCi) and 1.25 mg of I_2 in 0.5 mL of carbon tetrachloride. The reaction mixture was shaken vigorously until the purple color disappeared and then 10 mL of distilled water was added with mixing. The water phase was separated and applied to a CM-cellulose column (1.5 × 20 cm) equilibrated with 0.01 M ammonium acetate, pH 6.8. The CM-cellulose column was eluted with the same solution. The radioactivity of each fraction was monitored by the γ-counter. After about 100 mL were collected, the concentration of ammonium acetate was increased to 0.1 M. A main radioactive peak was collected which contained material having antibacterial activity and a UV spectrum similar to that of ristocetin.

The dissociation constants (K_d) of ^{125}I-labeled ristocetin for *M. luteus* cells were found to be 1.035 μmol determined by Scatchard analysis. Affinity of *N*-acetyl peptides to ^{125}I-labeled ristocetin was determined by measuring the inhibition of the complex formation between bacterial cells and ^{125}I-labeled ristocetin by the *N*-acetyl peptide. The assay, in duplicates, contained 50 μg of *M. luteus* cells, 1 μg of radioactive antibiotic, and various amounts of *N*-acetyl peptide in 50 mmol of sodium citrate buffer, pH 5.1, in a volume of 0.2 mL. The binding is completed immediately at room temperature. Tubes were centrifugated at 10000*g* for 10 min. One-tenth milliliter of the supernatants was transferred to counting tubes and radioactivity was determined with an ANSR γ-counter. These data were fed directly into a computer. Based on the Cheng–Prusoff equation,[23] a computerized procedure was used to calculate the inhibition constant (K_i) for the specific *N*-acetyl peptide.

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Molecular Representation. Figures 2, 3, and 5 were generated on the Abbott CAMD computer graphics system[24] using the Cartesian coordinates of Fesik et al.[15a] The molecular surface was calculated by using Connolly's MS surface program.[25] The pictures were taken directly from the Evans & Sutherland MPS terminal screen connected to a VAX 11/785 computer.

Acknowledgment. We express sincere thanks to T. J. O'Donnell for the ristocetin–peptide complex coordinates.

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Synthesis and Anticonvulsant Properties of 2,3,3a,4-Tetrahydro-1*H*-pyrrolo[1,2-*a*]benzimidazol-1-ones

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A series of 2,3,3a,4-tetrahydro-1*H*-pyrrolo[1,2-*a*]benzimidazol-1-ones were synthesized and evaluated for anticonvulsant activity in DBA/2 mice against sound-induced seizures and in rats against maximal electroshock-induced seizures. Most of the derivatives showed an anticonvulsant effect better than that of valproate, a commonly used anticonvulsant drug. Compound **3** possessed an anticonvulsant activity comparable to that of diphenylhydantoin in both tests and was selected for further studies. Structure–activity relationships are discussed.

The need for improved agents for the treatment of seizure disorders is widely recognized. The therapeutic efficacy of available antiepileptic drugs cannot be defined as totally satisfactory, and moreover the most marketed anticonvulsants possess a broad range of undesiderable side effects. This warrants the continuing research for antiepileptic drugs with more selective anticonvulsant activity and lower toxicity.

Drugs clinically active against epilepsy include derivatives with structural similarities. The most common structural elements appear to be a nitrogen heteroatomic system and at least one carbonyl group. Most of them also have at last one phenyl group and either another phenyl ring or an alkyl substituent attached to the heteroatomic system.

As part of our program on the chemistry of heteropolycyclic systems[1-4] as potential pharmacological agents, we reported[2,3] the synthesis and identification of a series of 2,3,3a,4-tetrahydro-1*H*-pyrrolo[1,2-*a*]benzimidazol-1-ones in which all of the above-mentioned structural characteristics were present.

We now report here the synthesis of new derivatives and the evaluation of anticonvulsant activity of all hitherto synthesized pyrrolo[1,2-*a*]benzimidazolones. This study was performed in an effort to elucidate the relationship between the pyrrolobenzimidazolone structure and the anticonvulsant activity and to determine the optimal substitution pattern in the tricyclic system. To our knowledge no member of this chemical class has been tested as an anticonvulsant agent.

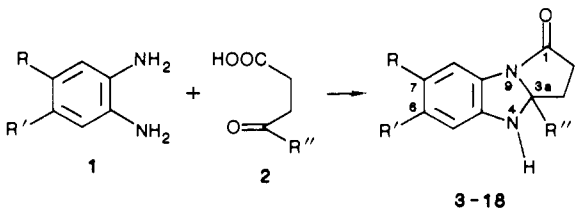
The title compounds were evaluated for anticonvulsant activity in DBA/2 mice against sound-induced seizures and in rats against maximal electroshock-induced seizures (MES).

Results and Discussion

The compounds employed in this study were prepared according to reported procedures[2,3] (Scheme I).

Synthesis of the new compounds **5** and **6** was carried out by reacting 1,2-phenylenediamine **1** with 3-acylpropionic acids **2** in boiling toluene with azeotropic removal of water. The obtained products were isolated by flash chromatography and characterized by spectroscopic methods.

Scheme I



1 2 3-18

	R	R′	R″
3	H	H	CH_3
4	H	H	C_6H_5
5	H	H	4-Cl-C_6H_4
6	H	H	4-F-C_6H_4
7	H	Cl	CH_3
8	Cl	H	CH_3
9	H	Cl	C_6H_5
10	Cl	H	C_6H_5
11	H	Cl	4-Cl-C_6H_4
12	Cl	H	4-Cl-C_6H_4
13	H	Cl	4-F-C_6H_4
14	Cl	H	4-F-C_6H_4
15	H	CH_3	CH_3
16	CH_3	H	CH_3
17	H	NO_2	CH_3
18	NO_2	H	CH_3

In the ^1H NMR spectra of the synthesized compounds (Table I), the alicyclic region was characterized by an ABCD-like system analyzed by means of the LAOCN3 program.[5] Protons at the C-2 atom were assigned as the nearest to the carbonyl group on the basis of their smaller

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