PREPARATION AND PROPERTIES OF DERIVATIVES AT THE AMINO GROUP OF RISTOCETIN AGLYCONE

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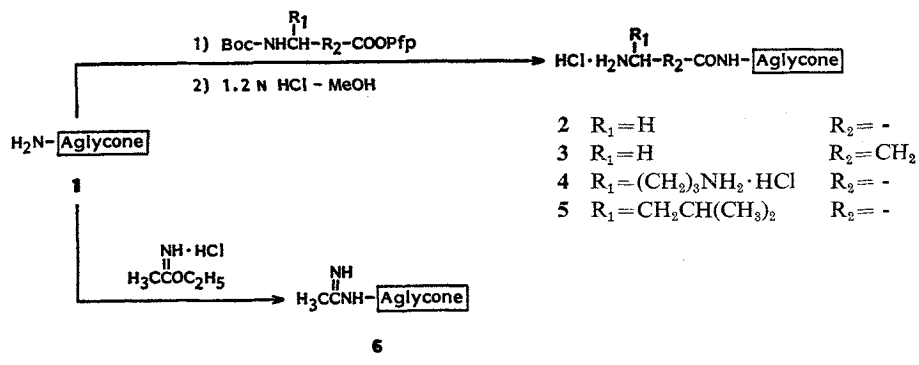
Ristocetin A (synonym of ristomycin A) belongs to the class of glycopeptide antibiotics which act by selective formation of a stable complex with the developing bacterial cell wall peptidoglycan[1]. It is suggested[2,3] that the complex is stabilized by an array of hydrogen, hydrophobic and ionic interactions in particular with participation of the COOH group of the *C*-terminal D-alanine of the growing peptidoglycan and the amino group of the antibiotic aglycone. On the other hand we showed in 1983 that there was no loss of the antimicrobial activity after modification of the ristocetin aglycone amino group[4]. This fact call in question the direct participation of the amino group in the complexation of the antibiotic with the peptidoglycan precursors. Similar results were reported by other authors[5~7]. Aim of this paper is to elucidate the influence of acylation of the amino group of the ristocetin aglycone, the effect of an higher positive charge and of the distance of positive charged group from the molecule nucleus on the antimicrobial activity of the molecule and efficiency of its binding to a biospecific sorbent. A brief communication of this study was published earlier[8]. The ristocetin aglycone (**1**) was prepared as previously described[9]. Aminoacyl derivatives of **1** (**2**~**5**) were obtained by acylation of the amino group with pentafluorophenyl esters of *tert*-butyloxycarbonyl amino acids. The *N*-acetamidoyl-aglycone (**6**) was prepared by reaction of **1** with ethylacetimidate (Scheme 1). Purification of all the derivatives was performed by semipreparative reverse-phase HPLC. The activities were determined by agar serial 2-fold dilution method. Antibacterial activity of all the derivatives is close to that of **1** with the exception of *N*-β-alanylaglycone (**3**) which shows a somewhat lower activity. The MIC of *N*-L-isoleucylaglycone (**5**) containing the moiety of a hydrophobic amino acid does not decrease either. The activity of **6** carrying a strong basic amidine group is practically the same as that of **1** though it was supposed that the activity must have been considerably higher. Antimicrobial activity of *N*-L-ornithylaglycone (**4**) was the same as that of **1**. This means that the additional amino group confers no advantage. Thus, the transfer of the amino group by 2~5 carbon atoms distance from the molecule nucleus of **1** as well as increasing the positive charge or introducing an additional amino group on the *N*-terminus of **1** has no significant effect on the antimicrobial activity. These findings were also confirmed by the data on biospecific chromatography on aminoethylcellulose - D-alanyl-D-alanine. All the prepared derivatives interact with the ligand

Scheme 1.



Boc: *tert*-Butyloxycarbonyl, OPfp: pentafluorophenyl ester

Table 1. Physico-chemical properties and antimicrobial activity of ristocetin aglycone and its derivatives.

	1	2	3	4	5	6
SI-MS m/z $(M+H)^+$	1,174	1,231	1,245	1,287	1,288	1,215
TLC[a] Rf (1)	0.68	0.55	0.53	0.40	0.68	0.67
(2)	0.72	0.43	0.39	0.20	0.66	0.69
Electrophoresis Rm[b]						
(1)	1.00	1.11	1.15	2.00	0.98	0.93
(2)	1.00	1.05	1.06	1.78	0.95	0.98
(3)	1.00	1.09	1.20	2.06	1.09	0.90
HPLC[c] Rt (minutes)	4.47	4.07	4.18	10.03	7.42	5.35
Amino acid analysis[d]	—	7 (1), Gly (1)	7 (1), β-Ala (1)	7 (1), Orn (1)	7 (1), Ile (1)	—
MIC[e] (μg/ml) (1)	1.67	2.5	2.5	1.67	1.67	1.00
(2)	1.25	1.67	2.5	1.25	1.67	0.83

[a] Adsorbent: Silica gel 60 F_{254} plate (Merck); solvent system, (1) BuOH - H_2O - AcOH (3 : 1 : 1), (2) BuOH - EtOAc - AcOH - H_2O (4 : 3 : 1 : 2); detection, ninhydrin and Pauly reagent.

[b] Adsorbent: Filtrak FN-12 paper; conditions, (1) 2 N AcOH, pH 2.4, 600 V, 4 hours, (2) HCOOH - AcOH - H_2O (20 : 24 : 56), pH 1.1, 300 V, 4 hours, (3) pyridine - AcOH - H_2O (4 : 1 : 995), pH 5.6, 4.5 hours; detection, ninhydrin and Pauly reagent. Rm was determined as related to **1**.

[c] Equipment: Liquid chromatograph (Du Pont); column, Zorbax ODS 4.6×250 mm; mobile phase, MeCN - 0.1% aq TFA (30 : 70); column temperature 45°C; detection, UV absorbance at 280 nm; flow rate, 2 ml/minute. Rt: Retention time.

[d] Equipment: Amino acid analyzer 835 (Hitachi), amino acid composition of hydrolysates (6 N HCl, 105°C, 18 hours) was determined as related to ristomycinic acid (**7**).

[e] Organisms: (1) *Bacillus subtilis* ATCC 6633, (2) *Staphylococcus aureus* FDA 209P.

SI: Secondary ion.

and desorb only by 0.25 N NH_4OH in the same way as **1**[10]. It should be noted, that none of prepared derivatives acquire activity against Gram-negative microorganisms.

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