STRUCTURE OF THE AGLYCONE OF THE GLYCOPEPTIDE ANTIBIOTIC RISTOMYCIN A

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It has been shown previously [1] that the aglycone of the clinical antibiotic ristomycin A contains, in addition to known amino acids (ristomycinic acid (A) [2, 3] and antinoidinic acid (BC) [3, 4]), a new amino acid G (I) — a di-β-hydroxy analog of amino acid E (II). Thus, the complete amino acid composition of the antibiotic has become known, which has enabled us to proceed to an analysis of the structure of its aglycone.

In ristomycin A, one of the free NH_2 groups belongs to a residue of amino acid A [5]. In view of the polyfunctional nature of the aromatic amino acids of the phenol type A, BC, and G, which contain voluminous radicals, and their ready oxidizability, and also the low stability of amino acid G in acid and alkaline media, to analyze the amino acid sequence we used the MTH* variant of the Edman method [6, 7] developed for peptides of β-hydroxy amino acids [8, 9].

Under these conditions, cyclization of the MTC of the aglycone took place by not more than 60%, which made it necessary to separate its MTH with a new NH_2 group from the remaining MTC derivative and the excess of reagent by paper electrophoresis.

Because of the multipoint nature of the binding of amino acids A, BC, and G to one another, the complete splitting off of the MTHs of these amino acids can be expected only after 2-3 cycles of degradation. Consequently, to determine the sequence of cleavage of the peptide bonds in the aglycone we used a new approach — the hydrolysis of the cyclization products with a 57% HI/P mixture [1] followed by an analysis of the change in the amino acid composition of the MTH of the aglycone. At the same time, we developed methods for separating and identifying both the free amino acids and also their MTHs with different degrees of substitution. The results of the analysis are given in Table 1.

An additional check on the course of cleavage was provided by the DNP method, which permitted the identification of the new amino groups arising after a given cycle of cleavage. The previous reduction with sodium tetrahydroborate of the ester group present in ristomycin [10] followed by the performance of acid hydrolysis and mass-spectrometric analysis of the diamino monocarboxylic amino acid formed showed that this group belongs to the dihydroxyphenylglycine residue of amino acid BC which, consequently, is C-terminal in the aglycone.

The splitting off of the di-MTH of amino acid A after the third cycle and of the mono-MTH of amino acid BC after the sixth Edman cycle shows that the amino acids of ristomycin A form a heptapeptide chain composed of alternating residues of amino acids A, G, and BC (formula III), the N-terminal acid being the less substituted hydroxyphenylglycine residue of amino acid A, as was established in a mass-spectrometric investigation of the monoamino analog of amino acid A obtained as the result of the HI/P hydrolysis of N-DNP-ristomycin A.

Thus, we have established a tetracyclic structure of the aglycone of ristomycin A (III) formed by a heptapeptide chain and a series of phenoxy and, in the case of amino acid BC, biphenyl amino acid residues. A similar type of structure is possessed by the antibiotic vancomycin, the structure of which has recently been established by the methods of NMR and x-ray structural analysis [11, 12]. Consequently, ristomycin and vancomycin are representatives of polypeptide antibiotics of the linear-polycyclic group to which nisin [13] and subtilin [14] may also be assigned.

The elucidation of the structure of the aglycone of the glycopeptide ristomycin A opens up routes for the determination of its complete structure which will subsequently enable the mechanism of its action at the molecular level to be established. The experimental results

*Here and below: MTH) methylthiohydantoin; MTC) methylthiocarbamide; DNP) 2,4-dinitrophenyl.

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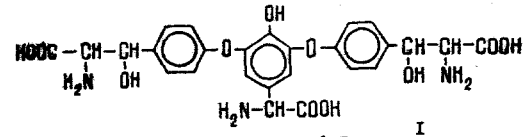
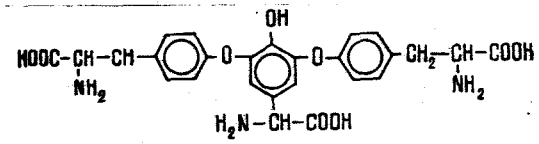
TABLE 1. Change in the Amino Acid Composition of Ristomycin A as a Result of Its Degradation by the Edman MTH Method.

Edman method	Amino acid A			Amino acid E				Amino acid BC		
	free	MTH		free	MTH			free	MTH	
		mono	di		mono	di	tri		mono	di
Initial ristomycin	+			+				±		
1		±		±				+		
2		+			+			+		
3		±	+		±			+		
4		±	±		±	+		+		
5						±		±	+	
6						±	+	±	+*	

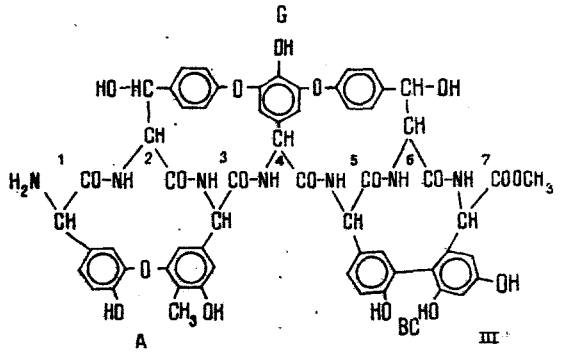
*Detected in the free form after hydrolysis of the product of the sixth Edman cycle in 2N HCl, 106°C, 2 h.



Amino acid G I



Amino acid E II



A III

Scheme 1. Structure of the aglycone of the glycopeptide antibiotic ristomycin A.

on the analysis of the structure of the aglycone of ristomycin A have been given in part in a dissertation [15] and will be published in the near future.

The mass-spectrometric analysis of some derivatives of the amino acids of ristomycin was performed by B. V. Rozynov (M. M. Shemyakin Institute of Bioorganic Chemistry of the Academy of Sciences of the USSR).

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