Tetrahedron Letters Vol. 21, pp 2983 - 2986 0040-4039/80/0729-2983$02.00/0

STRUCTURAL INVESTIGATION OF THE ANTIBIOTIC RISTOMYCIN A: THE ^{13}C-NMR EVIDENCE ON THE CARBOHYDRATE MOIETIES AND THEIR LINKAGES TO THE AGLYCONE

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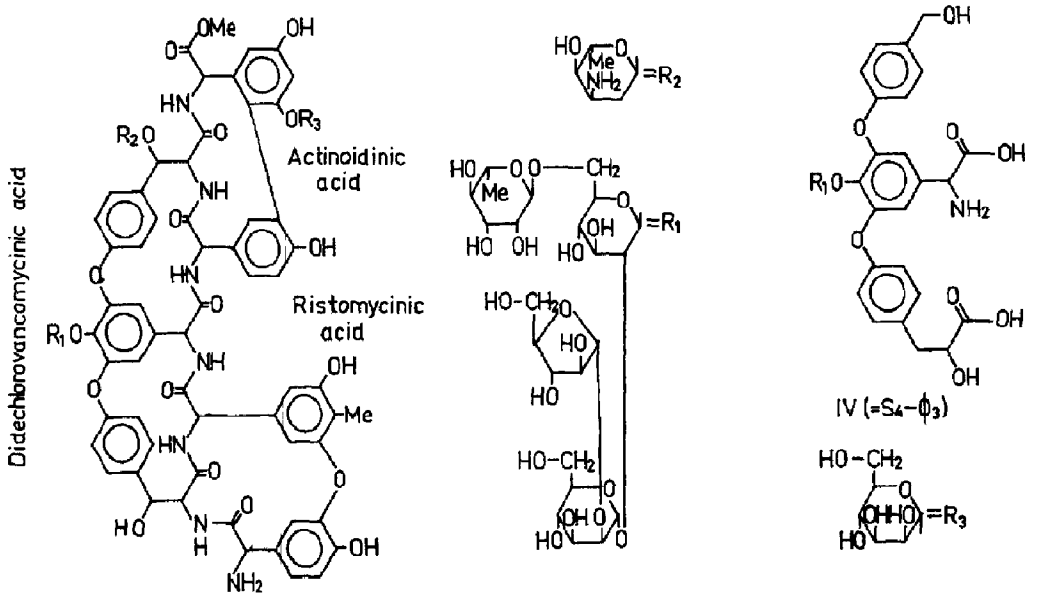
The ^{13}C-NMR experiments, which define the last details of the structure of ristomycin A, are described.

The water-soluble glycopeptide, ristomycin A[1] /I/ belongs to the vancomycin group of antibiotics[2] and it has recently become of great importance in hematology - similarly to ristocetin A - for the indication of von Willebrand's disease[3]. According to earlier structural studies[2,4a-d] I is presumably identical with ristocetin A. In 1979 the same peptide sequence and structure /II/ was proposed for aglycoristocetin A[4d] and aglycoristomycin A[4b]. Very recently Williams et.al.[4e-f] suggested a structure for ristocetin A mainly from ^{1}H NMR studies of this antibiotic and its Ψ-aglycone /III/. The complete structure of the carbohydrate moiety of ristomycin A was established in our laboratory[5a-g] and several papers[4d-f] used and accepted it.

By early 1980 there remained only a few structural details to be elucidated, regarding mainly the aglycone-sugar linkages. We found[5h] that the use of 0.2 M $Ba(OH)_2$ in presence of 0.2 M $NaBH_4$ /48h, under N_2/ is the most suitable hydrolytic reaction for the preparation of didechlorovancomycinic acid fragment containing sugars /IV=$S_4 - \phi_3$/ from O-protected ristomycin A. IV is the main product of base hydrolysis and it includes about half of the antibiotic molecule.

The availability of pure substances of the intact antibiotic and its $S_4 - \phi_3$ fragment /IV/ allowed us to assign most of the lines in their complex ^{13}C NMR spectra and we sum up conclusions for the carbohydrate components only. The C-NMR study was done at three frequencies /20, 25.2 and 90.6 MHz/ including relaxation measurements /Fig.1./.

The tetrasaccharidemoiety. Table 1. contains complete assignments both for the antibiotic molecule and $S_4 - \phi_3$. /i/ Comparison with the model methyl-α-D--arabinofuranoside shows that the terminal unit of the tetrasaccharide is α-D-Araf, i.e. a furanose in contrast to earlier results obtained on the acetolysis product, ristriose[5c]. /ii/ ^{13}C assignments for ristobiose /2-O--α-D-Manp-D-Glcp/ are also included in Table 1. To take into account the



I. R_1= β-ristotetrosyl, R_2= α-L-ristosaminyl, R_3= α-D-Manp. II. R_1=R_2=R_3=H, III. R_1=R_3=H, R_2= α-L-ristosaminyl IV. R_1= β-ristotetrosyl.

effect of the aromatic ring / ϕ_3/ on C-1 of the glucose unit we may consider the anomeric shifts and coupling constants for the phenyl-D-glucosides in D_2O /α=98.2 ppm, 171.9 Hz; β = 101.3 ppm, 162.1 Hz/. Evaluating the effect of the mannose substituent at C-2 to C-1 and the influence of the aromatic ring on C-1 we see that both the shift values and the coupling constants agree only with the assumption of a β -D-Glcp proximal moiety.

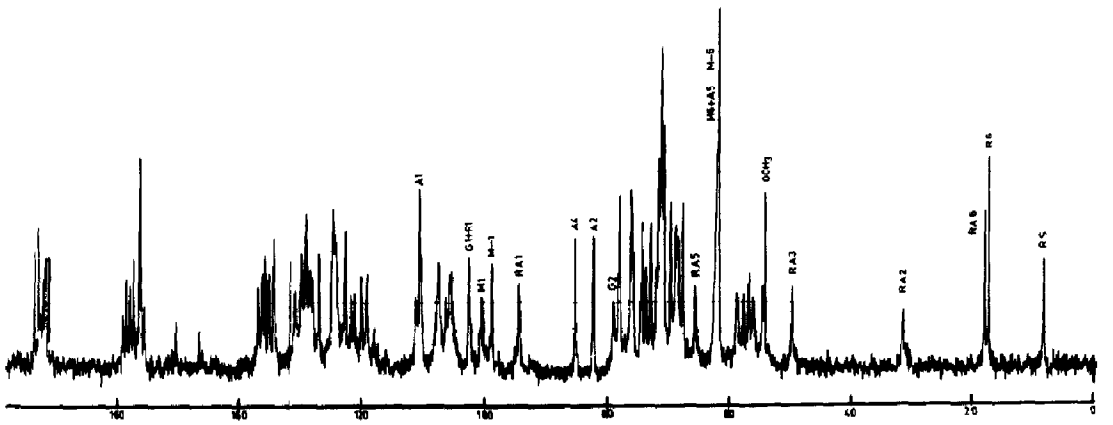


Fig.1. ^{13}C-NMR spectrum taken at 25.16 MHz and 90°C, in D_2O solution /Varian XL-100-FT-15/ Digital resolution: 16 K points in time domaine. For line assignments: RA-ristosamine, RS=ristomycinic acid, R=L-Rhap, A=D-Araf, M=D-Manp, G=D-Glcp in the tetrose unit.M-1= mono D-Manp.

Table 1. ^{13}C-NMR shifts /ppm/ and $^{1}J_{C_1H_1}$ coupling constants /Hz/ given in parenthesis.
Internal reference: dioxane /67.3 ppm/. Interglycosidic linkages are underlined.

Sugar-units	Carbon	Ristomycin-A ·H_2SO_4	S_4-β_3	β-Ristobiose	Me-α-D-Araf.
α-L-Rhap	1	102.3	101.8 (174)		
	2	71.2	71.0		
	3	71.2	70.9		
	4	73.7	73.3		
	5	69.6	69.5		
	6	17.8	17.5		
β-D-Glcp	1	102.3	103.1 (166)	96.9 (162)	
	2	<u>78.9</u>	<u>79.2</u>	<u>80.4</u>	
	3	<u>76.0</u>	75.1	75.4	
	4	70.7	71.0	71.3	
	5	<u>76.0</u>	<u>76.3</u>	76.6	
	6	<u>68.3</u>	<u>69.0</u>	61.7	
α-D-Manp	1	100.1 (176)	100.2 (175)	101.5 (172.4)	
	2	<u>77.9</u>	<u>77.2</u>	71.1	
	3	71.2	71.0	70.7	
	4	67.6	67.7	67.7	
	5	72.9	72.7	73.6	
	6	62.1	61.5	61.9	
α-D-Araf	1	110.3	109.7 (172)		109.3 (172)
	2	82.1	82.0		81.7
	3	77.9	77.5		77.6
	4	85.2	84.4		84.6
	5	61.9	61.5		62.5

Table 2.

Carbon	Ristomycin-A · H_2SO_4	Phenyl-α-D-Manp
1	98.5 (172)	99.3 (172.4)
2	70.7	71.1
3	74.3	74.1
4	67.6	67.5
5	71.6	71.6
6	61.9	61.6

Table 3.

Carbon	Ristomycin-A ·H_2SO_4	Me-α-L-ristosaminide
1	94.1 (172)	97.6 (172)
2	31.8	31.2
3	49.9	49.8
4		68.8
5	65.5	64.7
6	18.5	17.6

For explanation see caption under table 1.

The correct structure of ristotetrose is O-α-D-Araf-/1→2/-O-α-D-Manp-/1→2/-O-[α-L-Rhap]-/1→6/-O-β-D-Glcp in agreement with the original suggestion[5a].

<u>Mannose unit</u>. Table 2. shows shifts and $^{1}J_{C1H}$ values for this monosacharide attached to the actinoidinic acid[5h] subunit of aglycone. Earlier works[4e,f] on ristocetin A were consistent with either an α- or β-linkage. Data for the phenyl-α-D-Manp model and the effect of the aromatic ring on $^{1}J_{C1H}$ and δ_{C1} prove the conclusion that an α-D-Manp unit is present.

<u>Ristosamine unit</u> /Table 3./ We use methyl-α-L-ristosaminide, isolated and synthetised in our lab[5d-f] as a model. /i/ These data and observation of Kasai et. al.[6] show that the linkage to the β-OH of one of tirosine units is responsible for the upfield shift /3.5 ppm/ and therefore α-L-ristosamine is present in I. /ii/ Variable temperature ^{1}H NMR studies in D_2O and DMSO-d_6 at 360 and 500 MHz clearly reveal the equilibrium of $^{1}C_4$ and $^{4}C_1$ conformations of α-L-ristosaminide unit in I.

We have repeated the above procedure with ristocetin A and have obtained the same result.

We thank dr.E.Pretsch /E.T.H.Zurich/, Prof. I.Dabrowski /Max Plank Inst., Heidelberg/ and Dr.V.Formaček /Brucker, Karlsruhe/ for taking high field spectra; dr. A.Lipták /L.K.U. Debrecen/ for submitting model compounds

and prof.G.F.Gause /Moscow/ for the sample of industrial grade ristomycin A.

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(Received in UK 2 June 1980)