STRUCTURAL INVESTIGATION OF THE ANTIBIOTIC RISTOMYCIN A

^{13}C-NMR SPECTRAL ANALYSIS OF THE INTERGLYCOSIDIC LINKAGES OF THE HETEROTETRASACCHARIDE SIDE-CHAIN

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By ^{13}C-NMR studies on **Ia**, **IIa**, **IIb** and **IVa** obtained by the chemical degradation of ristomycin A and on several synthetic model compounds it has been proved that an O-β-D-arabinopyranosyl-(1→2)-O-α-D-mannopyranosyl-(1→2)-O-[α-L-rhamnopyranosyl-(1→6)]-D-glucopyranosyl heterotetrasaccharide moiety is connected to the aglycone of the antibiotic.

The antibiotic ristomycin, a member of the vancomycin-group[1] of antibiotics, is an oligosaccharide-type compound containing a complex peptide aglycone moiety[2,3] the structure of which has not been completely elucidated as yet. Our earlier investigations[4] have shown that a branched heterotetrasaccharide side-chain containing one molecule of L-rhamnose, D-glucose, D-mannose and D-arabinose is connected to the aglycone moiety of ristomycin A. On acetolysis or partial acid hydrolysis of the antibiotic this tetrasaccharide moiety decomposes into two reducing disaccharides and two reducing trisaccharides. One of the disaccharides had been identified[5,6] with rutinose. The other three compounds have been isolated for the first time by our research group and are named ristobiose, ristotriose and ristriose. The structures of ristobiose and ristotriose were elucidated by chemical degradation experiments and confirmed by syntheses[7].

Amongst the above-mentioned isolated oligosaccharides only ristriose was found to contain D-arabinose and it has been shown by permethylation followed by hydrolysis[4] that D-arabinose is linked at the C-2 position of D-mannose. However neither the ring size of D-arabinose nor the configuration of the glycosidic linkage between D-arabinose and D-mannose in ristriose, *i.e.* at the end of the tetrasaccharide side-chain, were determined. Moreover, the determination of the anomeric configuration of the D-glucopyranose moiety of the reducing end of the degradation products **Ia**, **IIa**, **IIb** and **IVa** required further structural studies.

Results and Discussion

The ^{13}C-NMR spectrum of hepta-O-acetyl-rutinose (**Ia**), isolated from ristomycin A[4], was consistent with the 6-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-1,2,3,4-tetra-O-acetyl-β-D-glucopyranose structure. The value of chemical shift of the anomeric carbon atom of the L-rhamnopyranosyl moiety, as well as of the $^{1}J_{CH}$ coupling constant prove the α-configuration of the interglycosidic linkage of **Ia** (Table 1). These values are in good agreement with that of the synthetic methyl 2,3,4-tri-O-acetyl-α-L-rhamnopyranoside. Comparing the corresponding ^{13}C-NMR data of 1,2,3,4,6-penta-O-acetyl-β-

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D-glucopyranose to those of **Ia** ($\delta_{C-1}=92.0$ ppm; $^1J_{CH}=168.1$ Hz) the β-configuration of the anomeric centre at the reducing end of this disaccharide could also be demonstrated. The 1→6 character of the interglycosidic linkage was supported by the observed downfield glycosylation shift[8] (5.1 ppm) at C-6 and β-alkylation shift[9] at C-5.

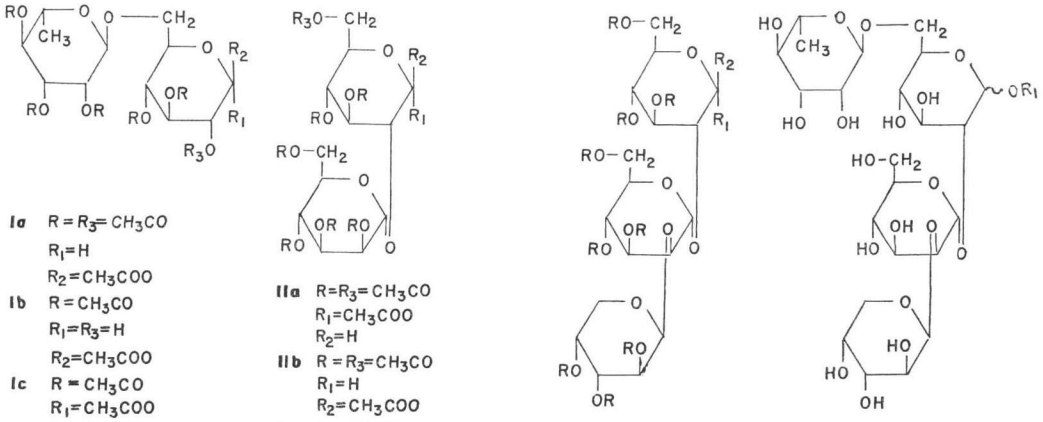
The chemical shifts of the anomeric carbon atom of the non-reducing L-rhamnopyranosyl residues of 1,3,4,2′,3′,4′-hexa-O-acetyl-β (**Ib**) and α-rutinose (**Ic**), previously used as starting materials for the synthesis[7] of ristotriose, were completely identical. Moreover, similar to **Ia** a glycosylation shift of 5.0 ppm was observed at the C-6 atom of both **Ib** and **Ic**. The change of the chemical shift of the other carbon atoms, as compared to those of penta-O-acetyl-β-D-glucopyranose, is not significant; the only exception is the observed downfield shift (3.4 ppm) at C-1 for the β-anomer (**Ib**) caused by the lack of the acetyl group at C-2. The values of the measured $^1J_{CH}$ coupling constants unequivocally prove the assumed configuration of all the glycosidic linkages in these derivatives.

Recently with the methanolysis of ristomycin A we have succeeded in isolating methyl-α-D-mannopyranoside in addition to methyl-α-L-ristosaminide[10]. Acetylation of the former in pyridine-acetic anhydride gave methyl-2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside.

A comparison of the spectral data of this latter to those of 1,3,4,6-tetra-O-acetyl-2-O-methyl-α-D-glucopyranose demonstrate the structure of octa-O-acetyl-ristobiose (**IIa**) to be 2-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-1,3,4,6-tetra-O-acetyl-α-D-glucopyranose. This structure has been corroborated by definitive synthesis[7], as well. The 5.8 ppm difference between the chemical shift of the C-2 carbon atom of the 2-O-methyl-D-glucose derivative as a model compound, and that of **IIa** can be attributed to a downfield methylation shift.

On the other hand, according to the spectral data summarized in Table 1, octa-O-acetyl-ristobiose, formed on the hydrolysis of the antibiotic[4], proved to be an anomeric mixture containing **IIa** and **IIb** in a ratio of 5 : 2.

As a result of the different anomeric configuration of the D-glucopyranose moiety and also of the 1→2 linkage, a ~2.6 ppm downfield shift was observed at the C-1 carbon atom of the non-reducing D-mannopyranosyl residue. A similar effect has been observed for the first time by Usui *et al.*[10] in



the case of kojibiose and sophorose.

Besides **Ia** and **Ib**, methyl 3,4-di-O-acetyl-2,6-di-O-methyl-α-D-glucopyranoside and methyl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside proved, to be useful model compounds for the structure elucidation of synthetic deca-O-acetyl-ristotriose (**IIIa**). Amongst these monosaccharide derivatives the former was suitable for the comparison of the alkylation shifts, whereas the latter made the identification of the branching C-2 D-mannopyranosyl moiety possible.

The value of chemical shift of the C-6 atom of the D-glucopyranose residue at the reducing end of **IIIa** was identical with that of the same carbon atom of compound **Ia**. On the other hand, the shift of the C-2 atom was lower than that of methyl 3,4-di-O-acetyl-2,6-di-O-methyl-α-D-mannopyranoside. This significant difference is due to the well-known alkylation shift[9]. These data and also the other spectral data summarized in Table 1 unequivocally prove the 6-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-[(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)]-1,3,4-tri-O-acetyl-β-D-glucopyranose structure of **IIIa** which has been supported by definitive synthesis[7], as well.

Table 1. ^{13}C-NMR-spectrum data of di- and tri-saccharide acetates

Sugar units	^{13}C-NMR								
	L-Rhamnose						D-Glucose		
Carbon atoms	1	2	3	4	5	6	1	2	3
Methyl-2,3,4-tri-O-acetyl-α-L-rhamnopyranoside[s]	98.8 (171.2Hz)	69.4	70.0	71.3	66.4	17.5			
1,2,3,4,6-Penta-O-acetyl-β-D-glucopyranose[s]							91.9 167.8Hz)	70.7	72.9
Hepta-O-acetyl-β-rutinose[i] **(Ia)**	98.8 (171.1Hz)	69.3	70.0	71.4	67.4	17.5	92.0 (168.1Hz)	70.9	73.1
Hexa-O-acetyl-β-rutinose[s] **(Ib)**	98.4 (172.3Hz)	69.4	69.4	71.3	67.8	17.4	95.4 (158.7Hz)	73.1	73.3
Hexa-O-acetyl-α-rutinose[s] **(Ic)**	98.4 (172.3Hz)	69.4	69.9	71.3	67.8	17.4	90.0 (173.8Hz)	71.6	69.9
Methyl-2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside[i]									
Octa-O-acetyl-α-ristobiose[i] **(IIa)**							88.5 (174.6Hz)	72.5	70.8
Octa-O-acetyl-β-ristobiose[i] **(IIb)**							93.3		
1,3,4,6-Tetra-O-acetyl-2-methyl-α-D-glucopyranoside[s]							88.9 (175 Hz)	78.3	71.8
Deca-O-acetyl-β-ristotriose[s] **(IIIa)**	98.4	69.3	70.1	71.3	66.8	17.4	93.7	74.5	72.3
Methyl-3,4-di-O-acetyl-2,6-di-O-methyl-α-D-glucopyranoside[s]							97.6 (168.6Hz)	79.4	71.6[a]
Deca-O-acetyl-α-ristriose[i] **(IVa)**							88.5 (175 Hz)	75.3	71.0
Benzyl-2,3,4-tri-O-acetyl-β-L-arabinopyranoside[s]									
Methyl-2,3,5-tri-O-acetyl-α-D-arabinofuranoside[s]									

i: Isolated from ristomycin A. s: Synthetic compound. a: Assignment may be interchanged.

All ^{13}C-NMR-spectra were measured in $CDCl_3$ solutions at 25.16 MHz and at room temperature using

The other trisaccharide isolated after the acetylosis of ristomycin A was found to contain[4] D-arabinose. Its monosaccharide composition differed from those of **IIIa**; thus it was called deca-O-acetyl-ristriose (**IVa**).

The value of chemical shift (88.5 ppm), as well as the value of the observed $^1J_{CH}$ constant (175 Hz) of the C-1 atom of the reducing D-glucopyranose moiety of **IVa** verifies the α-character of its anomeric center. Depending on the conditions of isolation the β-anomer of **IVa** ($R=CH_3CO$; $R_2=CH_3COO$; $R_1=H$) could also be observed in a few instances in a proportion not more than 20%. The α-anomeric configuration of C-1 of the D-mannopyranosyl residue may be deduced on the basis of its 96.1 ppm chemical shift and the corresponding $^1J_{CH}$ constant (174 Hz). These values are in good agreement with that of compounds **IIa** and **IIb**.

Until now some chemical and enzymatical evidences were available for the determination of the ring size and the anomeric configuration of the D-arabinose moiety in **IVa**. The values of chemical shift and the geminal $^1J_{CH}$ coupling constant (96.1 ppm and 174 Hz) of the anomeric carbon atom of the

isolated from ristomycin A and several synthetic model compounds.

shifts (δ_{ppm}^{TMS})													
D-Glucose			D-Mannose						D-Arabinose				
4	5	6	1	2	3	4	5	6	1	2	3	4	5
68.9	72.9	61.8											
69.3	74.2	66.9											
68.6	73.6	66.8											
68.6	70.3	66.8											
			98.8 (172.5Hz)	69.3	68.8	66.6	69.8	62.8					
69.8	68.3	61.8	95.8 (175.4Hz)	70.2	68.8	66.3	69.8	62.5					
			98.4										
69.8	68.3	61.8											
68.8	73.3	66.0	98.1	—	69.0	66.4	70.7	62.5					
69.9	72.4[a]	68.6											
68.3	69.4	61.8	96.1 (174 Hz)	70.6	68.1	66.4	70.5[a]	62.1	94.4 (171 Hz)	69.3	70.4	68.0	62.1
									95.9 (171.6Hz)	69.4[a]	69.9	68.6	60.7
									107.1 —	80.6	77.7	81.6	63.4

a Varian XL-100-FT-15 spectrometer. Signal to noise ratio was always better than 40 : 1, the digital resolution used were in the range of 0.625~1.25 Hz/point.

D-arabinose moiety of **IVa** were identical with that of the same atom of benzyl 2,3,4-tri-O-acetyl-β-L-arabinopyranoside, a model compound, which is the enantiomer of the corresponding D-analogue. The measured chemical shift is different from that of methyl-2,3,6-tri-O-acetyl-α-D-arabinofuranoside ($\delta_{C-1}=107$ ppm). These results and the other data represented in Table 1 unequivocally prove the O-(2,3,4-tri-O-acetyl-β-D-arabinopyranosyl)-(1→2)-O-(3,4,6-tri-O-acetyl-α-D-mannopyranosyl)-(1→2)-(1,3,4,6-tetra-O-acetyl-α-D-glucopyranose) structure of **IVa**, where D-arabinose is present as a $^1C_4$ (D) conformer.

According to the results of the chemical degradation[4] of ristomycin A, the synthetic studies[7] of the identified oligosaccharide fragments, as well as to the present ^{13}C-NMR results of compounds **Ia**, **Ib**, **Ic**, **IIa**, **IIb**, **IIIa** and **IVa** it has been proved that an O-β-D-arabinopyranosyl-(1→2)-O-α-D-mannopyranosyl-(1→2)-O-[α-L-rhamnopyranosyl-(1→6)]-D-glucopyranosyl heterotetrasaccharide side-chain is connected to the aglycone of the antibiotic. The place of linkage of this side chain at the aglycone moiety and the anomeric configuration of the D-glucose residue are still unknown.

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