Structure of the Antibiotic Ristocetin A

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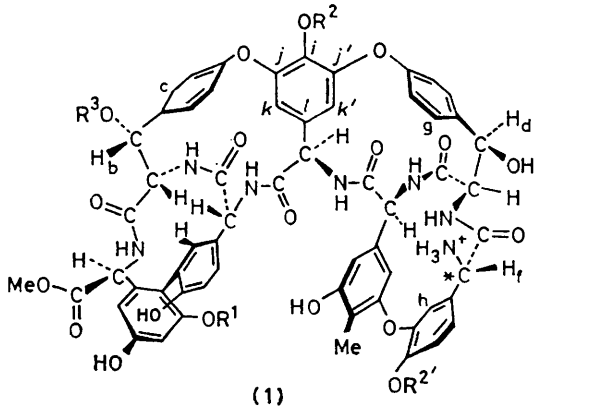
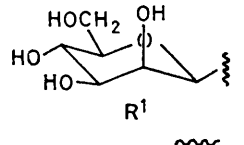
Summary Chemical and n.m.r. experiments, which define the last details of the structure of ristocetin A, are described.

RISTOCETIN A is an antibiotic which is elaborated by the micro-organism *Nocardia lurida* and functions by interfering with cell-wall synthesis.[1] Recent chemical studies[2,3]

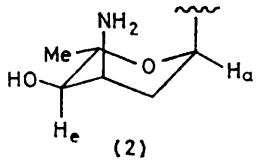
have defined the overall constitution of the antibiotic. Most recently, ^{1}H n.m.r. spectroscopy at 270 and 360 MHz was employed (a) to define the structure of the antibiotic except for three remaining ambiguities and (b) to deduce the molecular basis for the antibiotic action, exercised by binding to cell-wall mucopeptides terminating in the sequence . . . D-Ala-D-Ala.[4] The three remaining ambiguities in the structure (**1**) were: (i) the point of attachment of a glycosidally bound amino-sugar ristosamine (**2**),[5] (ii) the point of attachment of a tetrasaccharide *O*-β-D-arabinopyranosyl-(1 → 2)-*O*-α-D-mannopyranosyl-(1 → 2)-*O*-α-L-rhamnopyranosyl-(1 → 6)-*O*-β-D-glucopyranose (**3**),[4,6] as either R^2 or $R^{2'}$ in (**1**) (the remaining R^2 or $R^{2'}$ group being H), and (iii) the stereochemistry at the carbon atom carrying the free amino group of (**1**) [see asterisk in (**1**)].



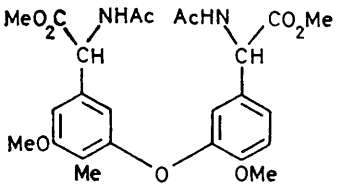
(1)



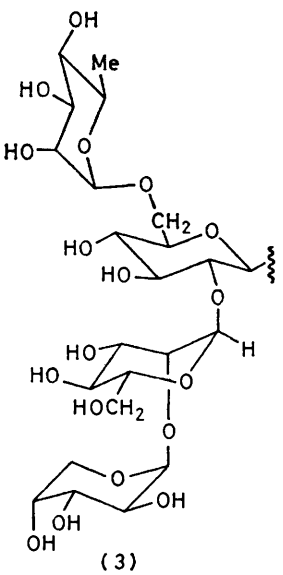
R^1



(2)



(4)



(3)

We now describe experiments which resolve these ambiguities and define the complete structure; the evidence is enumerated in the same order as the ambiguities are listed above. Much of the evidence has been deduced from the study of a ψ-aglycone obtained from ristocetin A by acid hydrolysis (5% HCl in MeOH, under reflux for 1 h). This ψ-aglycone is shown, by complete analysis of its 270 MHz ^{1}H n.m.r. spectrum obtained in $(CD_3)_2SO$ solution, to retain ristosamine (**2**) as the only sugar residue.

(i) The 270 MHz ^{1}H n.m.r. spectrum of the ψ-aglycone (14 mM in CD_3SOCD_3 solution at 30 °C) shows six phenolic OH resonances in the δ 9—10 region. Hence, in this compound, $R^1 = R^2 = R^{2'} = H$, and ristosamine must be attached *via* one of the two aliphatic hydroxyl-groups of the two β-hydroxytyrosine units in (**1**). Ristosamine is in fact attached at R^3 [see (**1**)] since: (a) in the spectrum of the ψ-aglycone, irradiation at the resonance frequency of the anomeric proton *a* of ristosamine (**2**) gives rise to negative nuclear Overhauser effects (n.O.e.s) on both protons *b* and *c* of (**1**); (b) the ψ-aglycone is di-*N*-acetylated (by Ac_2O–MeOH, 1 : 2 room temperature for 5 h) and hexa-*O*-methylated (by CH_2N_2), after which it may be di-*O*-acetylated (by Ac_2O–pyridine, 1 : 1). The final *O*-acetylation step causes downfield shifts (1·0—1·2 p.p.m.) of protons *d* (**1**) and *e* (**2**), but the chemical shift of *b* is unchanged.

(ii) Deprotonation of the NH_3^+ group of (**1**) causes an upfield shift of protons *f*, *g*, and *h* by 0·57, 0·34, and 0·19 p.p.m., respectively. CPK models of (**1**) establish that the *S* absolute stereochemistry at C* [as represented in (**1**)] places the amino group close to *g*. The R configuration at C* would not result in a large change in the chemical shift of *g* upon deprotonation of the NH_3^+ group.

The *S* configuration at C* was confirmed by the ^{1}H n.m.r. spectrum of the di-*N*-acetyl-hexa-*O*-methyl-ψ-aglycone in $(CD_3)_2SO$ at 30 °C. In this spectrum, irradiation of the N*H*COMe proton derived from the *N*-terminal amino group of (**1**) led to a negative n.O.e. on proton *g*. The *S* configuration places these two protons in close proximity but they are remote in the *R*-configuration.

(iii) Nuclear Overhauser effects have earlier been used to establish the site of attachment of β-D-mannose (see R^1) in (**1**).[4] Furthermore, n.O.e.s to assigned benzene ring protons have established the locations of the three phenolic OH groups of ristocetin A indicated in (**1**). By elimination, a fourth phenolic OH resonance identified in the ^{1}H spectrum of ristocetin A must be due to the OR^2 or $OR^{2'}$ group.

To resolve this ambiguity, ristocetin A was di-*N*-acetylated (Ac_2O–MeOH) and then methylated (CH_2N_2). Complete methylation of the phenols in the latter reaction was established by the lack of a bathochromic shift in the u.v. spectrum of the product in base. After acid hydrolysis (6N HCl; 9 h) of this material, and derivatisation (i, 5% HCl in MeOH; ii, Ac_2O–MeOH) of the hydrolysis products, a fraction (R_F 0·4) was isolated by preparative t.l.c. (silica G_{254}, developed in 10% MeOH in $CHCl_3$). By n.m.r. and mass spectrometry, this product was shown to be the fully protected diphenyl ether (**4**). These experiments establish that $R^{2'}$ in (**1**) is H, and therefore that R^2 is the tetrasaccharide (**3**).

The attachment of the tetrasaccharide to the trioxygenated benzene ring was confirmed by ^{13}C n.m.r. spectroscopy. Aglucovancomycin[7] and ψ-aglycone are structurally identical up to 3 σ-bonds distant from carbons *i*, *j*, *j'*, *k*, *k'*, and *l* [see (**1**), R^2 = H]. Vancomycin differs from aglucovancomycin only insofar as the carbon atom analogous to *i* bears a 2-(vancosaminyl)-β-D-glucosyl residue rather than an OH group.[7] The changes in ^{13}C shifts caused by attachment of this disaccharide are given in the Table, and compared with the corresponding changes observed on passing from ψ-aglycone to ristocetin A.

TABLE. Changes in ^{13}C chemical shifts observed for the changes aglucovancomycin (AGV) → vancomycin (VANCO) and ψ-aglycone (ψAG) → Ristocetin A (RISTO)[a]

Nucleus	*i*	*j*, *j'*	*k*, *k'*	*l*
δ(VANCO) − δ(AGV)	+2·7	+3·2, +3·7 (or +2·3, +4·6)	+ ⩽0·4	*ca.* 0
δ(RISTO) − δ(ψAG)	+2·6	+3·6, +4·0 (or +3·4, +4·2)	0·0, +0·8	−0·1

[a] Positive differences indicate high field shifts upon removal of sugar residues.

The close correspondences of the chemical shift changes (Table) provide confirmatory evidence that R^2 is (**3**).

In conclusion, the structure of ristocetin A is (**1**), in which R^1 is β-D-mannosyl, R^2 is the tetrasaccharide (**3**), $R^{2'}$ is H, and R^3 is the α-L-ristosaminyl residue (**2**). The *S* configuration at the N-terminus of the antibiotic enables the NH_3^+ group to participate (in co-operation with other NH-groups[4]) in binding mucopeptides terminating in . . . D-Ala-D-Ala *via* a salt bridge to their carboxylate anion terminus.

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