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^{13}C NMR INVESTIGATIONS OF THE NUCLEOSIDE ANTIBIOTIC HIKIZIMYCIN AND ITS CONSTITUENTS

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Abstract—Confirmation of the structure of the nucleoside antibiotic hikizimycin was obtained by comparing the ^{13}C NMR chemical shift data of selected model compounds with hikizimycin and its constituents.

Hikizimycin ($C_{21}H_{37}N_5O_{14}$) is a nucleoside antibiotic which exhibits antifungal activity but possesses little effect on bacterial growth.

Following the preliminary report on its production, isolation procedure and certain biological properties,[1] early structural studies revealed that hikizimycin consisted of a cytosine, a 3-amino-3-deoxy-D-glucopyranose (kanosamine)[2] and an unknown aminosugar component ($C_{11}H_{23}NO_{10}$) named hikosamine which was then shown to be an aminoundecose having formed the 4-amino-4-deoxy-D-glucopyranoside ring within its structure.[3]

Subsequent isolation of D-glycero-D-galactoheptose deriving from the linear part of hikosamine after the oxidative cleavage of the C5–C6 bond led us to propose its structure as 4-amino-4-deoxy-D-glycero-D-galacto-D-glucoundecapyranose.[4]

On the other hand comparative studies on the oxidation behaviour of hikizimycin and its constituents by periodate indicated that the kanosamine was glycosidically bound to the 2-OH group of hikosaminyl cytosine, while NMR and molecular rotation studies suggested the β-configuration for the glycosidic linkages of the two aminosugars. We have thus proposed the total structure of hikizimycin as 1 - N - [2 - O - (3 - amino - 3 - deoxy - β - D - glucopyranosyl) - 4 - amino - 4 - deoxy - β - D - glycero - D - galacto - D - glucoundecapyranosyl]cytosine.[4]

The locus of inhibitory effect of hikizimycin was found to reside on the 50 s unit of the ribosome, inhibiting the peptidyl transferase reaction in the process of protein biosynthesis,[5] which is the common mechanism of action of the so-called aminocyl-4-aminohexosyl cytosine antibiotics.[6,7]

Several attempts have been made to assess the structure-activity relationship of this group of antibiotics.[8-10]

Hikizimycin, with all its identical mode of action and structural analogy to these antibiotics, still possesses distinctive structural features in that it lacks the amino acid components, bears an N-non-substituted 4-aminoundecose instead of an N-substituted 4-aminohexose and contains a second aminosugar, 3-aminohexose.

Having in mind the potential importance of the hikizimycin structure and the uncertainties accompanying the oxidative degradation procedures such as one applied to determine the locus of the glycosidic linkage of the kanosamine, we used ^{13}C NMR as a probing tool in order to support the proposed structure of hikizimycin.

We thus expected to gain unequivocal evidence about the glycosidic bonds involved in the hikizimycin structure and also add knowledge to NMR of some glucose derivatives, which have not yet been scrutinized by ^{13}C NMR.

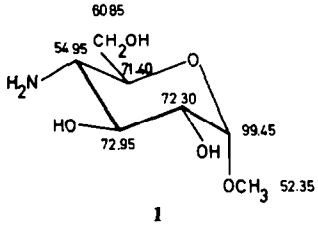
RESULTS AND DISCUSSION

Methyl-4-amino-4-deoxy-α-D-glucopyranoside (**1**) and 3-acetylamino-3-deoxy-D-glucopyranose (**2**) were used as aminoglucose derivatives for data comparison. The latter mutarotates in aqueous solution to an equilibrium mixture of the α- and β-anomer (**2a** and **2b**). The ^{13}C NMR data of α- and β-D-glucopyranose (**3**) and the methyl glycosides as the parent compounds are already assigned.[11,12]

In going from cyclohexanol (**5**) to cyclohexylamine (**6**), an upfield shift of 19·6 ppm is observed for the carbon α to the substituent, and a slight downfield shift of 1 ppm for the β-carbon. The γ- and δ-carbons, however, remain almost unaffected within the limit of $\pm$0·6 ppm.

Similar shielding patterns are expected in comparing the ^{13}C NMR data of 3- and 4-amino-D-glucopyranose derivatives relative to those of α- and β-D-glucopyranose itself. This leads to a straightforward assignment of the carbon shifts measured for methyl-4-amino-4-deoxy-α-D-glucopyranoside (**1**). The signal at 54·95 ppm certainly belongs to C-4 of **1**, the upfield shift relative to C-4 of α-D-glucopyranose (**3a**) and its methyl-glycoside (**4a**) being about 15 ppm. The shifts of all other carbons in **1** are in the order of those reported for corresponding carbons in **3a** and **4a**.[11,12]

In a similar manner, the carbon signals of the anomers of 3-acetylamino-3-deoxy-D-glucopyranose can be assigned. Significant upfield shifts (17·5 to 18·5 ppm) relative to glucopyranose anomers (**3a** and **3b**) are observed for C-3 of **2** (**2a**: 54·00 ppm; **2b**: 57·3 ppm). Since the concentration of the α-anomer is lower than that of the β-anomer in the equilibrium mixture as it was also reported for glucose,[11] the assignments of the carbon signals in the α- and β-anomer are supported by the lower intensities expected for the α-anomer, provided that there is no significant difference of nuclear Overhauser enhancements and spin-lattice relaxation times of ^{13}C nuclei in the



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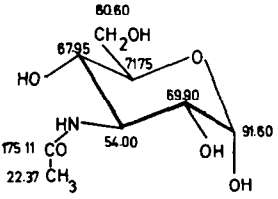
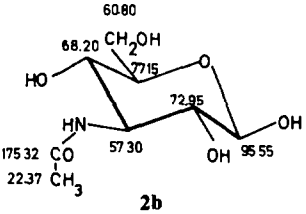


2a



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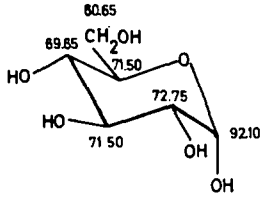
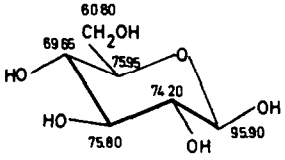
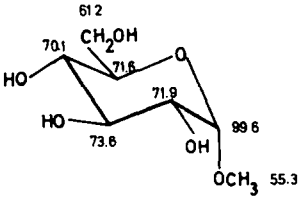


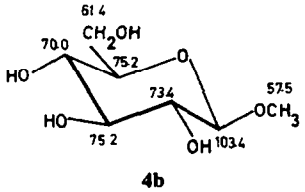
3a



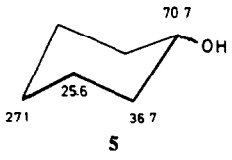
3b



4a



4b



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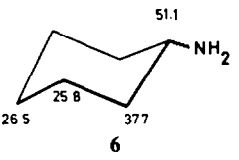

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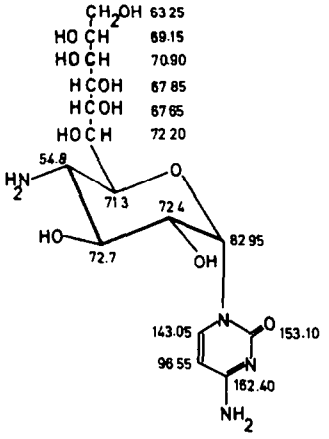

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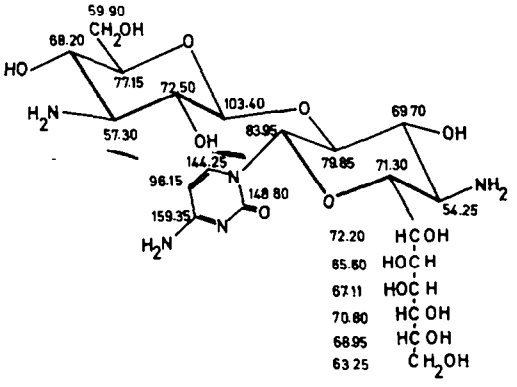

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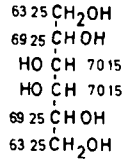

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pyranoside ring. Moreover, the shift differences of corresponding carbons in the anomers have the same directions and equal or similar magnitudes as shown in Table 1.

Using the data assigned so far, the spectra of the isolated nucleosides hikosamine (**7**) and hikizimycin (**8**) can be analysed in order to elucidate the structure.

The assignment of the pyrimidine moieties follows directly from comparison with the data reported for cytidine.[11] The carbon shifts found for the 4-amino-4-deoxy-D-glucopyranoside residue in **7** match quite well to those of methyl-4-amino-4-deoxy-α-D-glucopyranoside (**1**). An exception is the anomeric carbon which, of course, becomes significantly shielded when replacing its OH or OMe in **3a** or **4a** by the nucleo-base nitrogen in **7**. It can be

concluded therefore that the 4-amino-4-deoxy-D-glucopyranose residue in **7** is linked in the α-configuration to the nucleo-base. The hexitol residue attached to C-5 of the pyranoside ring is found to be related to galactitol by comparison with the carbon shift data reported for this polyol[11] (**9**) and taking into account the known downfield alkylation shift by about 7–10 ppm to its linking carbon. It was found to be D-glycero-D-galactitol by chemical degradation methods.[4]

Within an error of ±0·4 ppm and less, all carbon resonances found for 3-acetylamino-3-deoxy-β-D-glucopyranoside (**2b**) except those of C-1 and N–COMe can be recognized in the spectrum of hikizimycin (**8**).

Table 1. ^{13}C shift differences of corresponding carbons in the anomers of **2** and **3**

Carbon	**2** $\delta_\beta - \delta_\alpha$ (ppm)	**3** $\delta_\beta - \delta_\alpha$ (ppm)
1	−3·95	−3·8
2	−3·05	−1·45
3	−3·3	−4·3
4	−0·25	0·0
5	−5·4	−4·45
6	−0·2	−0·15

Thus, 3-amino-3-deoxy-β-D-glucopyranoside is attached to **7** by its anomeric carbon. The bond is equatorial as following from comparing the shift of this carbon (103·4 ppm) with that reported for C-1 of methyl-β-D-glucopyranoside (**4b**) and disaccharides of comparable structures.[11] This equatorial bond goes to C-2 of the 4-amino-4-deoxy-D-glucopyranoside residue of **8** since the resonance of C-2 in this moiety is shifted to lower field by about 7·5 ppm relative to that in **7**. The downfield shifts of the anomeric carbon (1 ppm) and the upfield shift of C-3 (3·35 ppm) in the 4-amino-4-deoxy-glucopyranoside residue of **8** may be attributed to a configurational change of the nucleobase from axial in **7** to equatorial in **8**. Further evidence for this explanation are the shift changes of the nucleobase carbons in comparing **7** with **8**. This applies particularly for the carbonyl carbon which might be more strongly affected by H-bonding in **7** (153·1 ppm) than as in hikizimycin (**8**) where the OH hydrogen at C-2 is replaced by the 3-amino-3-deoxy-β-D-glucopyranoside moiety. Thus, in keeping with the results obtained from degradation reactions and from other spectroscopic data, hikizimycin is assigned to be 1 - N - [2 - O - (3 - amino - 3 - deoxy - β - D - glucopyranosyl) - 4 - amino - 4 - deoxy - β - D - glycero - D - galacto - D - glucoundecapyranosyl] - cytosine (**8**).

EXPERIMENTAL

Hikizimycin was prepared as was reported.[1]

Hikosaminyl cytosine was prepared as was reported for the fragment B.[2]

3-Acetamido-3-deoxy-D-glucopyranose (N-acetyl-kanosamine) was prepared from the peracetyl derivative, which was a gift from Prof. Dr. H. Paulsen, Universität Hamburg.

The 3 - acetamido - 1,2,4,6 - tetra - O - acetyl - 3 - deoxy - β - D - glucopyranose (100 mg) was dissolved in anhyd MeOH and to this was added methanolic NaOMe (0·25 M, 0·5 ml) under stirring. The soln was allowed to stand for 30 min at room temp before Dowex 50W (H^+, previously washed with MeOH) was added to neutralize the soln.

The resin was filtered off and washed successively with MeOH and water. The concentration of the combined filtrates and washings yielded the title compound (*ca.* 50 mg).

The proton wide band decoupled ^{13}C NMR spectra were taken at room temp and 22·63 MHz, using the pulse Fourier transform technique as outlined previously.[13] Solns of 50–100 mg (**7** and **8**) in 1 ml of D_2O were used as samples. External 1,4-dioxane was the reference. The ^{13}C shifts obtained were converted to the TMS scale (1,4-dioxane: δ = 66·5 ppm) without making bulk susceptibility corrections.

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