Tetrahedron Letters No. 10, pp 747 - 750, 1975. Pergamon Press. Printed in Great Britain.

CARBON-13 MAGNETIC RESONANCE SPECTROSCOPY AND ABSOLUTE CONFIGURATION OF ANOMERIC CENTER IN AXIALLY LINKED 4-O- AND/ OR 6-O-GLYCOPYRANOSYL DERIVATIVES OF DEOXYSTREPTAMINE

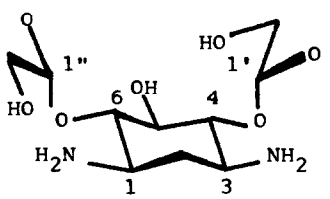
T. L. Nagabhushan* and P. J. L. Daniels

Schering Corporation

86 Orange Street, Bloomfield, N. J. 07003

(Received in USA 8 November 1974; received in UK for publication 28 January 1975)

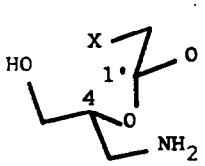
Carbon-13 magnetic resonance spectroscopy is a powerful tool for the elucidation of structures of aminoglycoside antibiotics (1-5). Many of these antibiotics contain two glycopyranosyl units in the $^{4}C_{1}$ conformation linked axially to deoxystreptamine at the 4 and 6-positions. Deoxystreptamine has a σ plane through C-2 and C-5 and hence the carbon nuclei 1,3 and 4,6 are isochronous. As expected, the ^{13}C nmr spectrum of this molecule shows four lines (1). On protonation of the amino groups, the C-4 and C-6 resonances are shielded by the identical magnitudes of 5.2 ppm (3). The spatial disposition of substituents on the asymmetric carbon atoms α and β to the two glycosidic oxygen atoms in the aminoglycoside antibiotics of the type mentioned above present a unique internal diastereoisomeric environment. This situation is illustrated below with the partial structural unit common to the gentamicins A_3, B and kanamycin A. The pairs of deoxystreptamine chiral



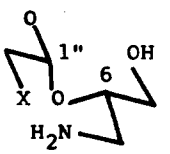
centers C-1, C-3 and C-4, C-6 are no longer enantiotopic. The two anomeric centers are also diastereotopic since they have the same absolute configuration but the chiral aglycon carbon atoms have opposite absolute stereochemistry. Hence, the pairs of nuclei C-1', C-1"; C-1, C-3; and C-4, C-6 are anisochronous regardless of influences from the rest of the molecule. From observations made in this laboratory and published data, it has become apparent to us that the C-4, C-1' and C-6, C-1" chemical shifts are effected substantially differently on protonation of the amino groups. These differences arise mainly from differences in the stereochemistry about the two glycosidic oxygen atoms and therefore can be used diagnostically to determine the unknown anomeric absolute configuration of a 4-O- or 6-O- axially linked deoxystreptamine glycopyranoside. From this knowledge the absolute configuration of the sugar can be deduced if its relative configuration is known. It should be noted that Lemieux, Nagabhushan, Clemetson and Tucker (6) have already demonstrated by pmr that the major factors responsible for the observed differences between the chemical shifts of the two anomeric protons of 4,6-di-O-α-D-glucopyranosyl-deoxystreptamine and related structures come from configurational and conformational asymmetry inherent to these structures.

In Table I, the chemical shifts of the anomeric and aglycon carbon atoms of paromamine and other selected naturally occurring 4,6-di-O-pyranosyl derivatives of deoxystreptamine at a basic and an acidic pH are given. It is readily seen that in all cases, regardless whether there is an amino group or a hydroxy group on C-2', the C-1' resonance is shielded by 3.8 to 4.2 ppm on protonation of the amino groups. Acid shielding of this magnitude appears to be characteristic of a configuration that can be described as the 4-R-1'-R-axial type configuration using partial structures shown below. Also characteristic of this type of a configuration is the shielding of C-4 by 7.4 to 8.8 ppm on acidification. The chemical shift of C-1" either remains, within experimental error, unchanged or the negligible shift is in the opposite direction (deshielded). Although C-6 is also shielded on protonation of the amino groups, the magnitude

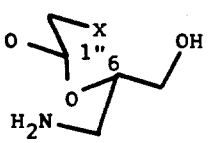
of the shielding (3.4 to 4.1 ppm) is only half as much as that of C-4. These effects seem to reflect a configuration, the 6-S-1"-R-axial type configuration, that is diastereoisomeric with the 4-R-1'-R-axial type configuration. Therefore, by observing the effect of acid on the chemical shifts of the anomeric and aglycon carbon nuclei, the absolute configuration at the anomeric center in new structures



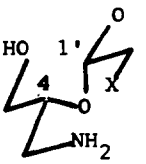
4-R-1'-R-axial



6-S-1"-R-axial



6-S-1"-S-axial



4-R-1'-S-axial

X = OH or NH_2 if 2' and OH if 2"

Table I

^{13}C Chemical Shifts of Anomeric and Aglycon

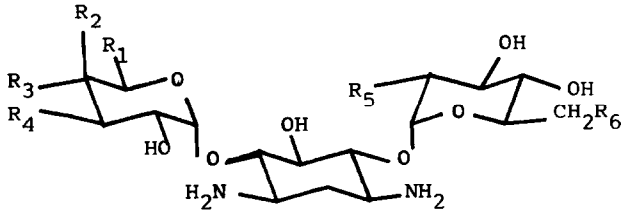
Carbon Atoms of Some Aminoglycoside Antibiotics*

Compound		C-1'	C-4	C-1"	C-6
gentamicin A	pH 9	101.7	88.6	100.8	87.9
	pH 2	97.7	80.9	101.4	84.2
		4.0	7.7		3.7
gentamicin A_3	pH 9	100.4	87.7	101.1	88.1
	pH 2	96.6	79.1	102.0	84.7
		3.8	8.6		3.4
gentamicin A_4	pH 9	101.6	88.5	100.7	88.0
	pH 2	97.8	81.1	101.9	84.2
		3.8	7.4		3.8
gentamicin B	pH 9	100.4	87.5	101.1	88.6
	pH 2	96.5	79.0	102.0	84.6
		3.9	8.5		4.2
paromamine	pH 9	102.0	88.8		78.3
	pH 2	97.9	81.4		74.5
		4.1	7.4		3.8
kanamycin A**	pH 9.6	99.9	87.6	100.4	88.4
	pH 3.6	96.1	78.8	100.7	84.3
		3.8	8.8		4.1
kanamycin B**	pH 10.6	100.5	86.8	100.1	88.2
	pH 5.5	96.3	78.7	100.8	84.2
		4.2	8.1		4.0

* Chemical shifts are in ppm downfield from TMS for solutions in D_2O (1).
** data from R. U. Lemieux and S. Koto, see ref.2.

can readily be determined provided the glycosidic linkage is axial and the position of attachment of the pyranosyl residue to deoxystreptamine is known. This is possible since a configuration of the type 6-S-1"-S-axial is enantiomeric with the type

4-R-1'-R-axial and hence the shielding of the C-6 and C-1" nuclei in the former on protonation of the amino groups would be the same as that of C-4 and C-1' in the latter under similar conditions. Similarly enantiomeric are the 6-S-1"-R-axial and 4-R-1'-S-axial type configurations. Similar general considerations were applied by Lemieux and Koto (2) in the assignment of the position of attachment of the deoxystreptamine residue in the antibiotic apramycin and the related antibiotic which was provisionally named oxyapramycin.



gentamicin A_3: $R_1 = R_3 = H$, $R_2 = R_5 = OH$, $R_4 = NHCH_3$, $R_6 = NH_2$
gentamicin A_4: $R_1 = R_2 = H$, $R_3 = R_6 = OH$, $R_4 = NHCHO$, $R_5 = NH_2$
gentamicin B : R_1=H, R_3=CH_3, $R_2 = R_5 = OH$, $R_4 = NHCH_3$, $R_6 = NH_2$

We have applied the above empirical rule to the determination of the absolute configuration of xylose in gentamicin A_2. A_2 has recently been shown to be 6-O-α-xylopyranosyl-paromamine (7). The C-1" resonance (101.8 ppm) was practically unchanged (101.4 ppm) on protonation of the amino groups. The C-6 signal (87.9 ppm) moved upfield (83.8 ppm) by 4.1 ppm on acidification. The magnitudes of the acid shielding of C-1' and C-4 were 4.2 ppm and 7.2 ppm, respectively. These values are in excellent agreement with those expected for the 6-S-1"-R-axial type configuration which requires xylose to possess D configuration.

Acknowledgements: The authors wish to thank Mr. J. B. Morton and Dr. R. Brambilla for spectral determinations and Drs. J. J. Wright and A. K. Mallams for helpful discussions.

References

1. J. B. Morton, R. C. Long, P. J. L. Daniels, R.W. Tkach and J. M. Goldstein, J. Amer. Chem. Soc., 95, 7464 (1973).
2. R. U. Lemieux and S. Koto, Abstracts 165th National Meeting of the American Chemical Society, Dallas, Texas, 1973, No Medi-022.
3. P. K. Woo and R. D. Westland, Carbohydrate Research, 31, 27 (1973).
4. S. Omoto, R. Inouye, M. Kojima and T. Niida, J. Antibiotics (Japan), 26, 717 (1973).
5. K. F. Koch, J. A. Rhoades, E. W. Hagman and E. Wenkert, J. Amer. Chem. Soc., 96, 3300 (1974).
6. R. U. Lemieux, T. L. Nagabhushan, K. J. Clemetson and L. C. N. Tucker, Can. J. Chem., 51, 53 (1973).
7. T. L. Nagabhushan, P. J. L. Daniels and R. S. Jaret, J. Org. Chem., submitted.