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CARBON-13 NMR ANALYSIS OF AMIKACIN AND RELATED COMPOUNDS

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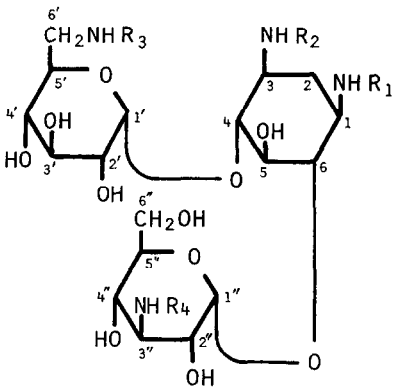
Amikacin[1] is a semisynthetic aminoglycoside antibiotic acylated with L(-)-4-amino-2-hydroxybutyric acid (L-AHBA) at the C-1 amino group of kanamycin A (1). This paper reports the assignment of ^{13}C NMR spectra of amikacin (2), its three positional isomers[2] acylated at the C-3, C-6' and C-3" amino groups (3, 4, 5), and two N-acetyl kanamycin derivatives (1-N-Ac: 6, 6'-N-Ac: 7).



1 : R_1, R_2, R_3 & R_4 = H
2 : R_1 = $COCH(OH)CH_2CH_2NH_2$, R_2, R_3 & R_4 = H
3 : R_2 = $COCH(OH)CH_2CH_2NH_2$, R_1, R_3 & R_4 = H
4 : R_3 = $COCH(OH)CH_2CH_2NH_2$, R_1, R_2 & R_4 = H
5 : R_4 = $COCH(OH)CH_2CH_2NH_2$, R_1, R_2 & R_3 = H
6 : R_1 = $COCH_3$, R_2, R_3 & R_4 = H
7 : R_3 = $COCH_3$, R_1, R_2 & R_4 = H
8 : $H_2NCOCH(OH)CH_2CH_2NH_2$

The ^{13}C NMR chemical shifts of compounds 2 ~ 5 were assigned (Table 1) by comparison with those of 1[3] and L-4-amino-2-hydroxybutyramide (8) and by utilizing a shielding of β-carbon on protonation of amino groups. Omoto et al.[4] reported that the N-acetylation of neamine and ribostamycin caused upfield shifts of the carbons β to the amino groups. On acylation of 1 with L-AHBA or acetic acid, the β-carbons relative to the acylated amino group underwent varied upfield shifts depending upon the site of acylation (Table 2). The chemical shifts of remaining carbons of these compounds were generally in good agreement with those of 1 and 8 except the chemical shift for the carbonyl carbons of 2 ~ 5, which emerged by 2.4 ~ 3.5 ppm higher than that observed with 8.

Nagabhushan and Daniels[5] generalized the correlation of configurations at the anomeric and aglycon carbons with the shifts which took place on protonation in the gentamicins A, B and kanamycin group. According to their

Table 1 ^{13}C-Chemical shifts of compounds 1 ~ 8

Carbon	1		2		3		4		5		6		7	
	δ*	Δδ**	δ	Δδ	δ	Δδ	δ	Δδ	δ	Δδ	δ	Δδ	δ	Δδ
C-1	51.2	0.6	50.4	0.9	51.2	0	51.2	0.6	51.2	0.5	50.1	0.8	51.2	0.6
C-2	36.1	7.8	35.1	4.2	34.9	4.2	36.3	7.8	36.4	8.1	35.1	4.2	36.4	7.9
C-3	49.7	1.3	49.4	0.7	48.6	0.5	50.2	1.0	49.8	1.3	49.4	0.6	50.2	1.0
C-4	87.3	8.4	87.6	7.8	81.6	2.7	88.4	8.2	88.1	9.4	87.6	7.6	88.4	8.1
C-5	74.9	1.4	75.4	2.2	76.1	0.4	75.1	1.4	74.9	1.0	75.2	2.1	75.1	1.4
C-6	88.5	3.9	81.2	0	88.0	3.4	89.0	4.8	88.2	3.7	82.7	0.4	89.5	5.3
C-1'	99.8	3.5	99.2	3.0	99.5	0.9	100.7	1.9	100.3	4.1	99.5	3.3	100.6	1.8
C-2'	72.6	0.9	72.7	1.0	72.5	0.4	72.7	0.7	72.7	1.0	72.7	1.0	72.7	0.6
C-3'	73.6	0.6	73.7	0.6	73.3	-0.1	73.5	0	73.7	0.7	73.7	0.6	73.7	0
C-4'	71.9	0.3	71.8	0.1	72.3	0.8	71.9	-0.1	71.8	0.2	71.9	0.2	72.0	0
C-5'	72.9	3.4	73.7	4.2	73.0	4.0	72.0	0.8	73.7	4.3	73.5	4.0	72.0	0.7
C-6'	42.1	0.9	42.4	1.2	42.8	1.6	40.7	0.7	42.4	1.1	42.3	1.1	41.0	0.4
C-1"	100.8	-0.4	100.3	1.6	100.8	-0.3	101.1	0	100.7	-0.9	100.2	1.2	101.4	0.3
C-2"	72.6	3.7	72.5	3.7	72.5	3.5	72.7	3.7	70.8	0.3	72.4	3.7	72.7	3.7
C-3"	55.1	-0.7	54.9	-1.3	55.0	-0.8	55.0	-0.9	55.0	0.4	54.9	-1.2	55.1	-0.8
C-4"	70.0	3.6	70.1	3.7	70.1	3.8	70.1	3.8	68.1	0.3	70.2	3.7	70.3	4.0
C-5"	72.6	-0.4	72.8	0.1	73.0	-0.1	72.9	-0.1	73.3	0.3	72.9	0.1	73.0	-0.1
C-6"	61.1	0.2	61.2	0.5	61.1	0.4	61.1	0.4	61.0	-0.4	61.2	0.5	61.3	0.6
	8													
C=O	180.7	1.4	177.2	1.1	177.6	1.8	177.6	1.0	178.3	0.9	174.7	-0.2	175.1	-0.4
C-α	70.4	0.3	70.7	0.2	70.3	-0.1	70.5	0.2	70.6	0.1				
C-β	36.8	5.2	36.5	4.9	36.4	4.4	36.6	4.9	36.6	4.9				
C-γ	37.9	0.3	38.1	0.2	37.8	0.4	37.8	0.2	37.6	0.1				
$C-CH_3$											23.1	-0.1	22.8	0

* Chemical shifts of free base in ppm downfield from TMS (in D_2O)
** Acid shift (pD <2.0) in ppm

empirical rule, characteristic acid shifts ($\Delta\delta$) are to be observed for C-1', C-4, C-1" and C-6 by 3.8 ~ 4.2, 7.4 ~ 8.8, 0 ~ small negative value, 3.4 ~ 4.1, respectively, provided that these anomeric and aglycon carbons bear 4R-1'R-axial and 6S-1"R-axial type configurations. As shown in Table 3, the acid shifts observed for C-1', C-4, C-1" and C-6 of 1 agreed well with the reported values[5]. In 2, a derivative of 1 acylated with L-AHBA at the C-1 amino function, the C-1' and C-4 resonances were shielded by 3.0 and 7.8 ppm on protonation, the shifts being similar to those of 1. On the other hand, the C-1" signal of 2 showed an unexpected upfield shift by 1.6 ppm and the C-6 signal unchanged. In 3, the 3-N-acylated derivative of 1, the C-1' and C-4 resonances showed much smaller shifts (0.9 and 2.7 ppm) than those of 1. The 6'-N-acylkanamycin derivative, 4, the acid shift for C-1' was upfield only by 1.9 ppm in contrast to the reported acid shift for the 1'-carbon of 3.8 ~ 4.2 ppm[5], while the shifts for other anomeric and aglycon carbons were fairly close to those of 1. Finally, the acid shifts of the anomeric and aglycon carbons of 5, the 3"-N-AHBA derivative of 1, were in the range of same magnitude as those of 1. This last observation is consistent with the reported acid shift data for gentamicin A and its 3"-formyl derivative (gentamicin A_4)[5]. The decreased acid shifts observed with C-6 of 2 and C-4 of 3 are apparently due to the location of these carbons β to the acylated amino groups. However, the abnormal acid shifts noted for C-1" of 2 (shielded) and C-1' of 3 and 4 in the opposite direction (deshielded) cannot be interpreted by the established rules.

In consideration of the spatial disposition on the acyl substituents and anomeric carbons in 2, 3 and 4, each of the acylamino groups is located at the δ-position relative to the respective anomeric carbons with the same sequence of intervening atoms : (glycosidic O)-(C-6)-(C-1) in 2, (glycosidic O)-(C-4)-(C-3) in

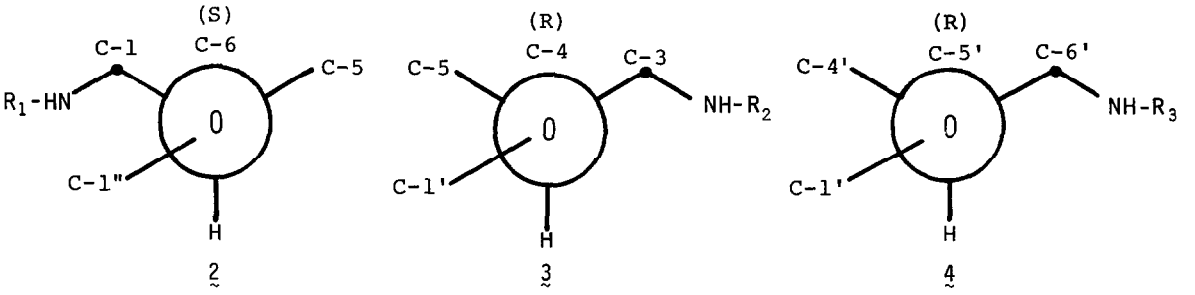
Table 2 β-Carbon shifts by N-acylation ($\Delta\delta$ in ppm compared with 1)

Compound	Carbons	$\Delta\delta$ from 1
2	C-2	1.0
	C-6	7.3
3	C-2	1.2
	C-4	5.7
4	C-5'	0.9
5	C-2"	1.8
	C-4"	1.9
6	C-2	1.0
	C-6	5.8
7	C-5'	0.9

Table 3 Acid shift ($\Delta\delta$ in ppm) for anomeric and aglyconic carbons

Compound	C-1'	C-4	C-1"	C-6
1	3.5	8.4	-0.4	3.9
2	3.0	7.8	1.6	0
3	0.9	2.7	-0.3	3.4
4	1.9	8.2	0	4.8
5	4.1	9.4	-0.9	3.7
6	3.3	7.6	1.2	0.4
7	1.8	8.1	0.3	5.3

3 and (ring O)-(C-5')-(C-6') in 4. The partial structural units relevant to the anomeric carbons are depicted below using the Newman projection :



2 3 4

The acylamino group in 2, located δ to the anomeric carbon C-1", is disposed to the left side in the above diagram (chirality at C-6 is S). The acylation effect brought about in such spatial disposition is a significant upfield shift of C-1" (Δδ: 1.6 ppm) in contrast to the slight downfield shift observed with 1 (-0.4 ppm) on acidification. On the other hand, the acylamino group in 3 and 4 lies on the right side in the diagrams (chirality at C-4 and C-5' is R). Characteristic of this type of a configuration is the decreased shielding effect on acidification of the anomeric carbon which is located δ to the acylamino group. As shown in Table 3, the acid shifts of C-1' of 3 (Δδ: 0.9 ppm) and 4 (1.9 ppm) are approximately 1/4 and 1/2, respectively, of that of 1 (3.5 ppm).

In order to confirm the validity of the above findings, 6 and 7 were synthesized by selective acetylation of 1. The latter compound, 7, is also known to be produced enzymatically by a kanamycin-resistant organism[6]. The ^{13}C NMR chemical shifts of 6 and 7 are shown in Table 1. The acid shift values of 6 and 7 observed for the anomeric and aglyconic carbons (Table 3) are in good agreement with those of 3 and 4, respectively.

The characteristic shielding and deshielding effects on anomeric carbons arising from N-acylation described in this paper should be useful in identifying the structure of modified aminoglycoside antibiotics.

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