^{13}C NMR STUDY OF ACTINOIDINS: CARBOHYDRATE MOIETIES AND THEIR GLYCOSIDIC LINKAGES

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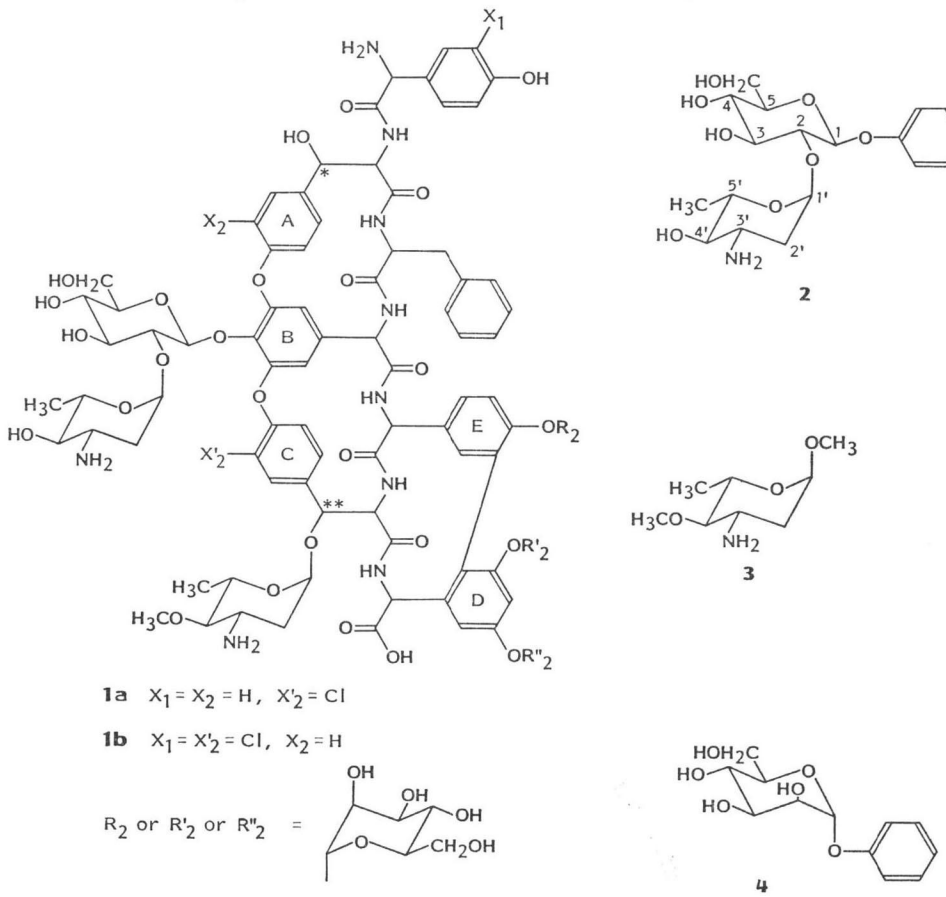
The configuration of the glycosidic linkages and the conformation of the carbohydrate moieties in the molecules of the glycopeptide-type antibiotics actinoidins A and B (**1a**, **1b**) have been determined by means of two-dimensional $^{13}C/^{1}H$ correlation NMR technique and with the application of model compounds **2**~**4**.

Actinoidins A and B (**1a**, **1b**), produced by *Proactinomyces actinoides*, is one of the first-discovered representatives of the vancomycin group of antibiotics[1] (Fig. 1). **1a** and **1b** contains the carbohydrate

Fig. 1. Structure of actinoidins and model compounds.



* Carbon P-1, ** carbon P-2.

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Table 1. ^{13}C NMR shifts (ppm) and $^{1}J_{CH}$ coupling constants (Hz)[a] given in parenthesis.

Sugar			Actinoidin A[b]	Model compounds			Actinoidin B[c]
Unit		Carbon	**1a**	**2**	**3**	**4**	**1b**
β-Acobioside	β-D-Glucopyranoside	1	103.8 (159)	98.7			103.9 (159)
		2	77.9	78.0			78.5
		3	77.0	76.8			77.5
		4	69.6	69.7			69.2
		5	76.1	76.3			75.8
		6	60.8	60.7			60.8
	α-L-Acosaminide	1′	96.6 (173)	96.8			97.7 (173)
		2′	34.5	34.1			35.7
		3′	49.0	49.3			48.4
		4′	73.4	73.4			73.4
		5′	68.4	68.4			
		6′	16.8	16.8			17.2
α-L-Actinosaminide		1	92.7 (168)		97.0		93.1 (171)
		2	34.9		35.6		35.9
		3	46.7		48.0		45.9
		4	83.4		86.4		84.3
		5	67.2		66.9		67.1
		6	17.6		17.1		17.9
α-D-Mannopyranoside		1	99.2 (170)			98.4	98.4 (172)
		2	69.9			70.2	70.1
		3	73.8			73.8	74.0
		4	66.5			66.5	66.7
		5	70.3			70.8	70.5
		6	60.6			60.6	60.8

[a] Recorded at 60°C.
[b] In 50% pyridine-d_5 - D_2O solution.
[c] In D_2O solution slightly alkalized with NaOD.

components (D-glucose, D-mannose, L-acosamine and L-actinosamine) in equal molar ratio. The only difference between them was found[2] in the amino acid composition of the aglycone moieties. The present work was aimed at the determination of the configuration of the glycosidic linkages and the conformation properties of the carbohydrate units attached to the heptapeptide aglycone.

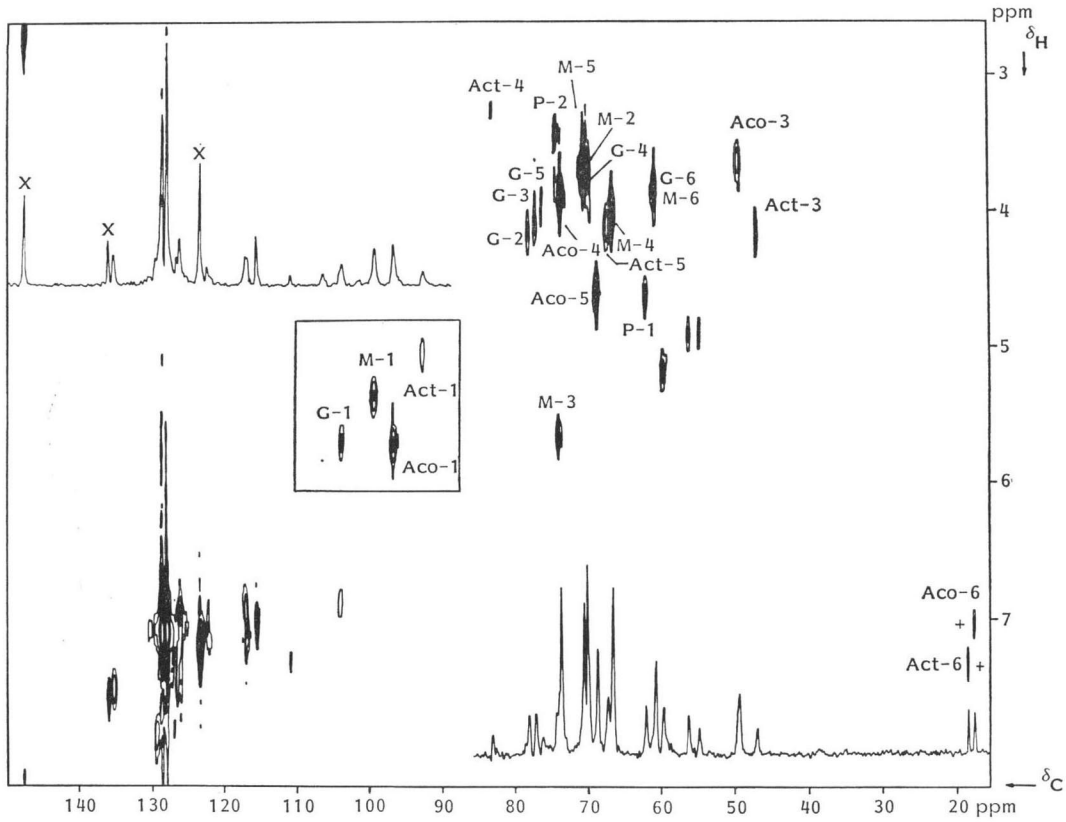
For the measurements phenyl β-acobioside[3] (**2**), methyl α-L-actinosaminide (**3**, unpublished results) and phenyl α-D-mannopyranoside (**4**), each synthesized by our group, were applied as model compounds. For differentiation between the CH_3, CH_2 and =CH carbons the APT and DEPT methods were applied. The most characteristic ^{13}C NMR data obtained for the carbohydrate components of actinoidins A and B, as well as for the model compounds **2**~**4** are summarized in Table 1. In the anomeric-carbon region (93~104 ppm) of the 2D $^{13}C/^{1}H$ NMR correlation map of actinoidin A (Fig. 2) four cross-peaks were observed (the respective proton-shifts were found between 5~6 ppm), proving that the homogeneous intact antibiotic variant contains four saccharide units. On measuring of the $^{1}J_{CH}$ couplings of the anomeric carbons by the gated decoupling technique, the value obtained for C-1 (159 Hz) of D-glucose indicated a β-glycosidic configuration, whereas the additional values (170±

Fig. 2. Two-dimensional $^{13}C/^{1}H$ correlated NMR-spectrum[a] of actinoidin A[b].

[a] Recorded at 60°C by Bruker WP-200 SY spectrometer 50.3/200.1 MHz.

[b] 250 mg sample dissolved in 2 ml 50% pyridine-d_5 - D_2O.

G=Glucose, M=mannose, Aco=acosamine, Act=actinosamine, X=signals of pyridine (internal references at 148.3 ppm), +=folded methyl signals, P-1=carbon P-1, P-2=carbon P-2.



3 Hz) were characteristic of α-glycosidic linkages. The data of Table 1 show that the signal of the anomeric carbon of D-glucose in the antibiotic components appears with a downfield shift of *ca.* 5 ppm as compared to that of compound **2**. The 3.3~4.0 ppm upfield shift, as compared to the value of **3**, indicated[4,5] that L-actinosamine was linked to the β-hydroxyl group of the tyrosine unit. Conversely, the anomeric signal of compound **4** appears at somewhat lower field than that of the D-mannose unit in actinoidins. At the same time the chemical shift values of the carbohydrate skeleton carbons (60~80 ppm) of both antibiotic components were in good agreement with those of the respective carbons of the model compounds. These results, as well as the data of $^{1}J_{CH}$ couplings, unequivocally prove the structural identity of the compared carbohydrate moieties. The CH carbon of the non-glycosylated β-hydroxytyrosine unit of the aglycone was detected also in this region with a shift of 62 ppm. In the region 40~60 ppm the C-3 atom of the amino sugars as well as the OCH_3 carbon of **3** could be also assigned by comparison with the spectral data of the model compounds **2** and **3**. In the region 34~39 ppm the signal near 38 ppm was identified as the carbon of the CH_2 function in the L-phenylalanines of the aglycones, whereas the additional two signals were assigned to the C-2 carbon of L-acosamine (in compound **2**) and of compound **3**, respectively. The methyl signals of both trideoxyamino hexoses were assigned at 16.8~17.9 ppm.

According to the obtained ^{13}C NMR parameters the conformation of the sugar components of actinoidins A and B, as well as the configuration of the glycosidic linkages of these carbohydrate units, correspond to the structure depicted on Fig. 1.

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