Structure Analysis of the Nucleoside Disaccharide Antibiotic Anthelmycin by Carbon-13 Nuclear Magnetic Resonance Spectroscopy. A Structural Revision of Hikizimycin and Its Identity with Anthelmycin[1]

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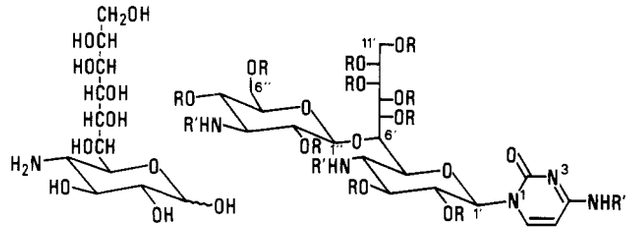
The ^{13}C NMR spectral analysis of the nucleoside disaccharide antibiotic anthelmycin, its derivatives, and several synthetic hexopyranosyl nucleoside models was used to confirm structure **2a** for the antibiotic. The structure of hikizimycin is identical with that of anthelmycin.

Anthemycin and hikizimycin are nucleoside disaccharide $C_{21}H_{37}N_5O_{14}$ antibiotics elaborated by *Streptomyces longissimus*[2] and *Streptomyces* A-5,[3] respectively. Earlier structural studies on these two antibiotics revealed that each is constituted from a 3-amino-3-deoxy-D-glucopyranose (kanosamine) residue β-glycosidically attached to an aminoundecose, $C_{11}H_{23}NO_{10}$, which in turn forms a β-nucleoside linkage with N(1) of a cytosine residue.[4,5] The aminoundecose, hikosamine (**1**), has the 4-amino-4-deoxy-D-glucopyranoside system in its structure.[6] From an evaluation of the periodate oxidation behavior of *N,N'*-diacetylhikizimycin, Uchida suggested the linkage of kanosamine was to position 2′ of the hikosamine moiety of hikizimycin.[7] The polyol side-chain configuration followed from the isolation of D-glycero-D-galactoheptose after oxidative cleavage of the C(4)–C(5) bond of **1**.[7] This structure proposal for hikizimycin also was claimed



1

2a R=R′=H
2b R=R′=Ac
2c R=H, R′=Ac

to be supported by an analysis of the ^{13}C NMR spectrum of the antibiotic.[8]

The possible identity of anthelmycin and hikizimycin was intimated by the presence of common structural features in the two natural products. Comparison of the ^{13}C NMR spectra of anthelmycin and hikizimycin at the same pH as well as of their *N,N'*-diacetyl derivatives now established that the two antibiotics are identical. However, a preliminary analysis of the ^{13}C NMR spectra of anthelmycin and its peracetate (**2b**) excluded position 2′ of the hikosamine moiety as the site of attachment of the 3-amino-3-deoxy-D-glucopyranose unit. The need for an unambiguous structure determination of anthelmycin and hence a structure revision of hikizimycin became mandatory. Recently, our chemical degradative studies performed on anthelmycin combined with mass spectrometry of suitable derivatives thereof resulted in the correct structure assignment for anthelmycin, in which the 3-amino-3-deoxy-D-glucopyranose is linked to position 6′ of the hikosamine moiety as represented by **2a**.[1a] As a consequence, the structure of hikizimycin also is illustrated by **2a**.

The complete assignment of the ^{13}C NMR spectrum of anthelmycin has been performed utilizing appropriate hexopyranosyl nucleoside models and several hydrolysis products and derivatives of the antibiotic. In this communication the ^{13}C NMR analysis of these compounds, independently establishing structure **2a** for anthelmycin, is presented.

The carbon resonances of the cytosyl residue of anthelmycin are recognized easily from cytosyl nucleosides recorded in the literature.[9,10] The signals of the kanosamine moiety can be selected by comparison with the resonances of methyl-3-amino-3-deoxy-β-D-glucopyranoside (**3**). An exact duplication of the resonances of **3** is not observed in the spectrum of anthelmycin (**2a**) in the free amine form (Table II). Deviations which approach 2 ppm are noted for carbons 1″, 2″, and 3″. These differences, however, vanish in strong acid solution, confirming the correct selection of resonances for this residue. The disparity in chemical shifts at high pH reflects probably conformational effects which are disrupted upon amine protonation.

The remaining resonances arise from the hikosamine moiety of the antibiotic. In view of the 4-amino-4-deoxy-β-D-glucopyranoside substructure in this fragment, an examination of simpler models was undertaken to provide a basis for signal assignment and for the determination of the linkage site between the two sugar residues of anthelmycin. The β-nucleoside compounds **4a**, **5a**, **6a**, **7a**, **8a**, and **9** along with the acetyl derivatives **4b–8b** were synthesized[11] employing standard literature procedures.[12] All δ values of these compounds are listed in Table I.

Internal comparison of compounds **4a–8a** allows a self-consistent assignment of all resonances. The glucopyranose signals of **4a** and **5a** are nearly identical, indicating that β-linked cytosyl and uracyl moieties exert equivalent effects. The 2′-OCH_3 derivative **8a** and the 3′-OCH_3 derivative **6a** differentiate the C(2′), C(3′), and C(5′) resonances of **4a–8a**. Methylation causes downfield shift increments of 9.5 and 9.4 ppm for C(2′) and C(3′) of **8a** and **6a**, respectively, leaving neighboring carbon centers minimally affected.[13] The replacement of the 4′-hydroxy group of **6a** with an amino or

Table I. ^{13}C Chemical Shifts of Glucopyranosyl Nucleosides[a,i]

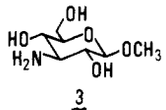
	4a[b]	5a[b]	6a[b]	7a[b]	8a[c]	9[b,f]	4b[d,g]	5b[d,g]	6b[d,g]	7b[e,g]	7c[b,g]	8b[d,g]
C(2)	159.1	153.0	158.8	157.2	152.3	159.1	154.9	150.6	155.4	154.1	158.3	151.3
C(4)	167.1	167.0	166.8	165.2	162.5	167.2	163.0	162.8	163.0	162.4	166.4	162.4
C(5)	98.1	104.0	98.0	97.0	102.2	98.2	97.8	103.9	98.0	96.2	97.4	103.1
C(6)	143.1	143.0	142.9	141.4	140.0	143.2	144.3	139.2	145.0	145.5	142.2	136.9
C(1′)	84.7	83.9	84.6	83.9	83.4	84.8	81.2	80.4	81.4[h]	80.6	84.5	82.4
C(2′)	72.3	72.3	71.8	71.9	81.8	72.3	70.5	69.5	72.3	70.8	72.9	79.1
C(3′)	77.5	77.1	86.9	76.8	78.3	77.4	72.6	72.8	81.9[h]	72.6	75.3	74.7
C(4′)	70.4	70.2	69.7	52.8	71.1	70.4[h]	67.8	67.9	69.2	49.1	52.4	68.3
C(5′)	79.8	79.7	79.6	79.7	80.4	78.9	75.1	74.9	75.1	75.0	79.0	74.6
C(6′)	61.3	61.7	61.5	61.3	62.3	69.9[h]	61.6	61.7	62.1	62.7	61.9	61.9

[a] Chemical shifts expressed on the Me_4Si scale. Secondary references are listed in the Experimental Section. [b] Deuterium oxide solution. [c] Acetone-d_6 solution. [d] Deuteriochloroform solution. [e] Dimethyl-d_6 sulfoxide solution. [f] The resonances of C(1″)–C(6″) of this compound are 103.8, 74.3, 77.2, 70.8, 76.8, and 62.0 ppm, respectively. [g] Carbonyl and methyl resonances of the acetyl function of this substance are not reported. [h] Signals in any vertical column may be interchanged. [i] Registry no.: **4a**, 3319-89-9; **4b**, 3180-75-4; **5a**, 3180-77-6; **5b**, 3180-73-2; **6a**, 62973-63-1; **6b**, 62973-66-4; **7a**, 22212-28-8; **7b**, 22176-13-2; **7c**, 21209-53-0; **8a**, 62973-64-2; **8b**, 62973-67-5; **9**, 62973-65-3.

acetamido function (cf. **6a** with **7a** and **6a** with **7c**, respectively) results in well-characterized shift modifications of the relevant carbon resonances.[14,15]

Comparison of the spectra of **4a** or **5a** with that of methyl-β-D-glucopyranoside[16] reveals fair agreement of the shift values of C(2′), C(3′), C(4′), and C(6′). However, aside from the expected upfield shift of the C(1′) resonance in the nucleoside, C(5′) suffers a 3-ppm downfield shift. This behavior, common to all substances in Table I, is analogous to that observed between adenosyl-β-D-xylopyranoside and methyl-β-D-xylopyranoside.[17] The spectra of several adenosyl α- and β-hexopyranosides show that the replacement of a methyl group by the heterobase does not lead only to unique perturbation of C(5′), but also of C(3′) and C(2′) depending on the nature of the sugar residue.[17,18]

The peracetates **4b**–**8b** show regular shift perturbations with respect to the parent nucleosides. Carbons 3′ and 5′ experience shielding increments of 4.3 ± 0.7 and 4.6 ± 0.2 ppm, respectively, from the acetyl functions on flanking carbon centers. Carbons 2′ and 4′ of **4a**, **5a**, and **8a** respond to a single adjacent acetyl group with 2.3 ± 0.5 and 2.6 ± 0.2 ppm shielding increments and the anomeric carbon resonances of **4a**, **5a**, **6a**, and **7a** are shielded 3.3 ± 0.2 ppm by a 2′-*O*-acetyl function. In the conversion of **8a** to **8b** the anomeric carbon is insulated from the direct effect of *O*-acetylation and, therefore, shifts minimally.



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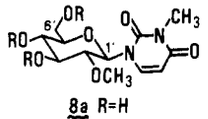
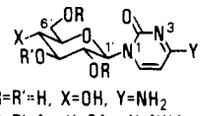
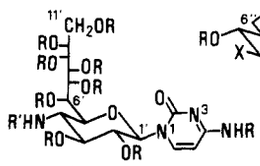


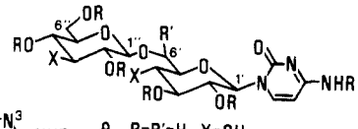
8a R=H
8b R=Ac



4a R=R′=H, X=OH, Y=NH_2
4b R=R′=Ac, X=OAc, Y=NHAc
5a R=R′=H, X=OH, Y=OH
5b R=R′=Ac, X=OAc, Y=OH
6a R=H, R′=Me, X=OH, Y=NH_2
6b R=Ac, R′=Me, X=OAc, Y=NHAc
7a R=R′=H, X=Y=NH_2
7b R=R′=Ac, X=Y=NHAc
7c R=R′=H, X=NHAc, Y=NH_2



11a R=R′=H
11b R=R′=Ac
11c R=H, R′=Ac



9 R=R′=H, X=OH
10a R=H, R′=CH_2OH, X=NHAc
10b R=Ac, R′=CH_2OAc, X=NHAc

The unassigned carbon resonances of **2a** contain signals corresponding closely to those of C(1′), C(2′), C(3′), and C(4′) of 4-amino-4-deoxy-β-D-glucopyranosyl nucleoside **7a**, suggesting the absence of glycosidic substitution at C(2′) and C(3′). A similar shift relationship is observed between *N*,*N*′-diacetylanthelmycin (**2c**) and **7c**. The spectrum of hikosaminylcytosine (**11a**), an acid hydrolysis product of anthelmycin (**2a**), substantiates partially this view by eliminating the possibility of a 3′-O-glycosidic linkage. Equivalent C(1′)–C(4′) resonances of **11a** and **2a** are found at both high and low pH. The large upfield resonance shift of carbon centers situated β to primary amine functions, which are incurred upon N-protonation, identify unambiguously C(3′) and C(5′) of these substances.[19,20] The C(3′) δ value, equal to that of C(3′) in model **7a**, demands an unsubstituted 3′-hydroxyl group.

The 80.2-ppm signal in the spectrum of **2a** is a reasonable value for C(2′), if the 2′ oxygen is involved in a glycosidic linkage [cf. C(2′) of 81.8 ppm in the spectrum of 2′-*O*-methyl nucleoside **8a**]. This possibility may be dismissed by the shift response of C(1′) of the antibiotic **2a**, its *N*,*N*′-diacetyl derivative **2a**, and of the *N*,*N*′-diacetyl derivative of **11a** accompanying peracetylation. The C(1′) resonance is shielded in excess of 3 ppm in each case. This fact, in conjunction with the acetylation results of models **4a**–**8a** summarized above, is incompatible with a 2′-O-glycosidic linkage for anthelmycin. The almost identical C(1′) signals of **2a** and **11a** (and **2c** and **11c**) further refute a 2′ linkage by analogy with the shielding difference of the C(1) resonance of β-D-glucopyranose and 2-*O*-glucopyranosyl-β-D-glucopyranose (β-sophorose).[13]

Upon allocation of the resonances of the C(1′)–C(5′) fragment of **2a**, **11a**, and their corresponding *N*-acetyl derivatives **2c** and **11c** the assignment of the remaining six side-chain carbons of **11a** was achieved by comparison of the shift values of D-mannitol.[21,22] The isolation of D-glycero-D-galacto-heptose[7] dictated the choice of this model, which reflects the polyol side-chain stereochemistry of the undecose moiety of anthelmycin (≡ hikizimycin). The good resonance correlation of the terminal four-carbon residue of **2c** and **11c** with those of D-mannitol and the shielding experienced by the C(5′) signal of **2c** with respect to **11c** are explained best by attachment of the 3-acetamido-3-deoxy-β-D-glucopyranoside substituent to position 6′ in **2c**. This interpretation is corrobo-

Table II. ^{13}C Chemical Shifts of Anthelmycin, Hikosaminylcytosine, and Models[a,b,g]

	2a[c] pH >11	2a[c] pH <1	3[c] pH >11	3[c] pH <1	2b[d]	2c[c]	11a[c] pH >11	11a[c] pH <1	11b[d]	11c[c]	10a[c]	10b[d]
C(2)	157.6	148.8			155.1	157.4	157.8	148.2	155.6	159.2	159.1	155.2
C(4)	165.5	159.3			162.7	165.9	165.7	158.8	163.2	167.2	167.1	162.9
C(5)	96.6	96.1			97.3	98.0	96.6	95.9	97.6	98.2	98.8	98.0
C(6)	141.8	144.2			145.3	143.6	141.9	144.1	145.2	143.0	144.5	144.7
C(1′)	84.1	82.6			80.9	85.3	83.9	82.6	81.6	85.0	84.6	81.2
C(2′)	71.7	70.7			69.6	72.7	71.4[e]	71.9	69.3	72.7	72.8	70.7
C(3′)	77.2	72.3[e]			72.8	75.7	78.0	72.3	73.1	75.7	75.4	72.8
C(4′)	53.0	54.2			49.9	53.4	53.5	54.5	49.6	54.3	53.1	50.2
C(5′)	79.1	72.0[e]			80.6	77.9	78.8	72.3	79.0	79.1	79.3	79.0
C(6′)	80.2	79.5			73.9	80.8	71.2	70.7	68.0[e]	70.5	81.5	76.0
C(7′)	68.2[e]	67.0			67.7[e]	69.2	68.2[f]	69.0	67.5[e]	70.2	61.2	62.8
C(8′)	69.2[e,f]	68.2[f]			67.7[e]	69.6	68.4[f]	67.5[e]	67.2[e]	69.2		
C(9′)	69.5[f]	68.8[f]			67.7[e]	69.8	69.4	67.7[e]	66.9[e]	69.2		
C(10′)	71.3	71.0			67.1[e]	72.0	71.3[e]	71.2	66.0	72.1		
C(11′)	63.1	63.1			61.9	64.5	63.1	63.1	62.1	64.4		
C(1″)	105.0	103.0	103.3	103.0	101.7	106.1					105.0	101.0
C(2″)	74.0	69.5	72.8	69.3	71.4	73.0					73.1	71.9
C(3″)	58.5	57.3	56.9	57.1	53.0	58.0					58.2	53.2
C(4″)	69.8	65.5	69.7	65.8	68.4	69.2					69.3	69.0
C(5″)	77.8	76.8	76.8	76.8	73.6	78.2					78.2	73.4
C(6″)	61.1	59.8	60.9	59.9	61.9	61.9					62.1	62.5

[a] Chemical shifts expressed on the Me_4Si scale. Secondary references are listed in the Experimental Section. [b] The carbonyl and methyl resonances of the acetyl derivatives are not individually assigned and are not reported. [c] Deuterium oxide solution. [d] Deuteriochloroform solution. [e,f] Assignments in any vertical column may be interchanged. [g] Registry no.: **2a**, 12706-94-4; **2b**, 62990-71-0; **2c**, 62990-72-1; **3**, 14133-36-9; **10a**, 63018-04-2; **10b**, 62973-68-6; **11a**, 58976-11-7; **11b**, 58933-70-3; **11c**, 63016-79-5.

rated by the observed 0.9-ppm upfield shift of the C(5′) resonance in the 6′-O-substituted nucleoside **9** (synthesized from β-gentiobiose) with respect to C(5′) of **4a**. Consequently, *N,N′*-diacetylanthelmycin is represented by structure **2c** and anthelmycin by **2a**. This conclusion is supported further by the isolation of **10a** as one of the products of periodate oxidation of **2c** and subsequent borohydride reduction. The carbon shifts of **10a** and its peracetate **10b**, listed in Table II, are consistent with this structure.

The ^{13}C NMR data of hikizimycin (≡ anthelmycin) (**2a**) and hikosaminylcytosine (**11a**) reported by Uchida et al.[8] are in reality the values corresponding to their hydrobromide[23] and hydrochloride[23] salts, respectively. Spectral comparisons between neutral and protonated amine species seem, in part, to be the reason for the previous erroneous structure assignment.[8]

Experimental Section

The ^{13}C NMR spectra of compounds reported in Table I and **2a**, **10a**, **11b**, and **11c** of Table II were recorded on a Bruker HX-90E spectrometer operating at 22.63 MHz or a Bruker WP-60 spectrometer operating at 15.08 MHz in the Fourier transform mode. Me_4Si as internal standard was used for spectra taken in deuteriochloroform solution and Me_4Si as external standard for the spectra recorded in deuterium oxide solution (0.05–0.50 M).

The spectra of **10b** and **11a** were recorded on a JEOL PFT-100 spectrometer operating at 25.03 MHz in the Fourier transform mode. Compound **11a** was examined in deuterium oxide solution containing 2% dioxane as internal reference and **10b** in deuteriochloroform solution. The spectra of **2a**, **2b**, and **3** were recorded on a Varian DP-60 spectrometer operating at 15.08 MHz in the Fourier transform mode. Compounds **2a** and **3** were examined in deuterium oxide solution containing dioxane as internal reference; **2b** was recorded in deuteriochloroform solution. All chemical shifts are expressed on the Me_4Si scale by the use of the relationship: $\delta_{Me_4Si} = \delta_{p\text{-dioxane}} + 66.3$ ppm = $\delta_{CDCl_3} + 76.9$ ppm.

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References and Notes

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(22) Though the spectrum of D-mannitol has been reported,[21] the C(2), C(3) assignments presented have not been unambiguously established. A more recent investigation has necessitated the reassignment of the spectrum of galactitol,[15] indicating the chemical shift correlation procedure employed in the earlier analysis is suspect.
(23) These compounds were prepared by Dr. K. Uchida in the laboratory of one of the present authors (B. C. Das).