^{13}C NMR SPECTRAL CORRELATION FOR SIMPLIFIED STRUCTURE DETERMINATION OF CURAMYCINS AND RELATED OLIGOSACCHARIDE ANTIBIOTICS[1]

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^{13}C NMR spectral analysis for structure determination of oligosaccharide antibiotics is discussed. The details of the structure of a pentasaccharide segment (pseudo-olgose A) of curamycin A is deduced on the basis of ^{13}C NMR data.

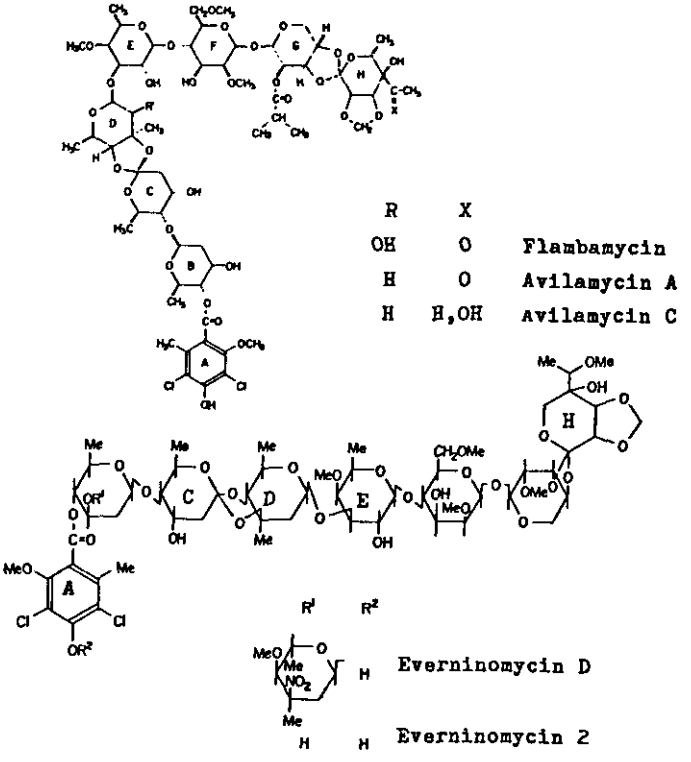
Contents

1. INTRODUCTION

The antibiotics everninomicins, flambamycins, and euramycins constitute a group of oligosaccharides which have close structural resemblance. The first member of this group the structure of which was completely elucidated[2] is everninomicin D, $C_{66}H_{99}O_{35}NCl_2$. The other members of the group are also dichloroisoeverninic acid (A) esters of hexapyranosides (B-C-D-E-F-G) with variations in the substitution pattern.

These compounds are sometimes included in the orthosomycin[3] family of antibiotics because of the presence of two orthoester groups which, incidentally, can be most easily detected from their characteristic chemical shift at 120 ppm in ^{13}C NMR spectra.

This review will consider several aspects of ^{13}C NMR spectra that simplify the structure determination of these oligosaccharides and their degradation products. Previously unpublished ^{13}C NMR spectral data on some members of the everninomicin family will be presented. Structural features of euramycin A will be deduced on the basis of ^{13}C NMR data.



2. NATURE OF ^{13}C NMR DATA

The increased availablity of ^{13}C NMR instrumentation in recent years has led to a growing application of ^{13}C NMR spectral analysis to structural studies on natural products.[4] Many research centers now have instruments capable of observing natural abundance ^{13}C spectra at 20-25 MHz (for example, Varian XL-100, CFT-20, FT-80; JEOL FX-90Q). Some laboratories possess second and third generation FT spectrometers with enhanced capability for conducting various types of spectral measurements, (gated decoupling, selective heteronuclear decoupling, automated T_1 measurements, etc.).

When the solution of a structural problem is undertaken by ^{13}C NMR methods, a variety of instrumental techniques can be used. One technique that is universally employed is complete proton decoupling resulting in a single line for each carbon atom in the molecule. Besides producing a simple spectrum, this technique has the advantage of increased sensitivity due to NOE enhancement of signals inherent in the decoupling process. Most second generation NMR spectrometers are capable of performing gated decoupling experiments which allow coupled ^{13}C NMR spectra to be recorded without loss of NOE but at an appreciable cost in instrument time.

A widely used technique for obtaining additional structural information is SFORD (single frequency off-resonance decoupling) which removes all long range ^{13}C-^{1}H coupling while retaining one bond couplings. Thus the number of protons attached to individual carbon atoms may be readily determined.

Relaxation phenomena may play an important role in structure determination by ^{13}C NMR spectrometry. T_1 values which are useful in studying organic compounds may be measured readily on the newer generation NMR spectrometers. However, a full T_1 study of a complex molecule can often be costly in terms of instrument time, especially if the sample size is small. In such cases a PRFT (partially relaxed Fourier transform) experiment is often desirable. This technique essentially uses a 180-τ-90 pulse sequence for only one or two appropriate values of τ and yields qualitative information about the very slow or very fast relaxing carbon atoms in the molecule.

Finally, there are several new and exotic techniques such as tailored excitation[5], J-scaling[6], and 2-D NMR[7] which hold promise for an exciting growth period in ^{13}C NMR spectrometry but involve software and instrumental modifications that are not generally available at present in organic and natural product research centers.

^{13}C NMR SPECTRA OF CARBOHYDRATES

The ^{13}C NMR chemical shifts of carbohydrates are extremely sensitive to substitution, stereochemistry and conformation. For this reason ^{13}C NMR spectra of simple sugars have been studied extensively.[8] Yet at this low level of complexity there was controversy over the chemical shift assignments.[9] Better instrumentation,

study of more compounds and special techniques (e.g. *specific deuteration*[10]) have solved this problem for the most part.

The ^{13}C NMR study of oligosaccharides above the level of complexity of disaccharides has not been well developed, due at least in part to the fact that such compounds are somewhat rare. The compounds that have been studied are ones such as raffinose and stachyose which are composed of very simple sugars.

There has been, however, an increasing amount of ^{13}C NMR analysis of natural products which are glycosides of various unusual sugars.[11] Structural studies on macrolide antibiotics, for instance, have benefited from ^{13}C NMR analysis.[12]

There are some problems associated, however, with ^{13}C NMR studies on oligosaccharides because many of the special techniques may prove to be of limited value - especially if the sample size is small. For instance, SFORD spectra which are useful for structural studies on alkaloids and terpenes[13] are not very helpful for oligosaccharides. This is so because the most difficult to assign carbon atoms in carbohydrates are the large number of methinoxy groups, H-C-O, in rings, all of which appear as doublets in the SFORD spectrum.

Relaxation studies too are of marginal value because the information obtained from such studies is often available by other simpler techniques. Thus, decoupled signals for OMe groups may often be recognized on the basis of their characteristic chemical shift rather than the observation that they are slowly relaxing carbon atoms.

Oligosaccharides are not easy to purify. Often a natural product of this class may be available only in small quantities thereby making SFORD and gated decoupling experiments very costly in terms of spectrometer time. T_1 studies on complex molecules are also often impractical because of the large sample size required and the investment of extensive instrument time.

Even without recourse to these special techniques, simplification of structure determination can be achieved by analyzing just complete proton decoupled ^{13}C NMR spectra of oligosaccharides. Specially valuable are such spectra obtained with a spectrometer which observes ^{13}C nuclei at 60 MHz or higher frequency. Much smaller size samples are needed as higher sensitivity is attained and the chance of overlap of signals which is common with ring carbons is reduced because of the higher frequency used. Such uncrowded spectra permit easy measurement of $J^{13}C\text{-}^{1}H$ of value for determining the stereo- chemistry of anomeric carbons.[14]

The cost of "supercon" NMR spectrometers is still too high for most laboratories. But, regional "High Field NMR. Facilities" have been established in the United States with grants from the National Science Foundation.

An ordinary proton decoupled ^{13}C NMR spectrum may be obtained from one of these laboratories with less than 10 mg size samples of oligosaccharides. Obtaining

a time-consuming T_1 study spectrum, however, may not be particularly easy.

4. A RATIONAL NUMBERING SYSTEM FOR OLIGOSACCHARIDE ANTIBIOTICS

^{13}C NMR was applied very late to the structure determination of oligosaccharide antibiotics. For example, the structure of everninomicin D was first deduced by more classical methods and only then was ^{13}C NMR data used to verify structural features.

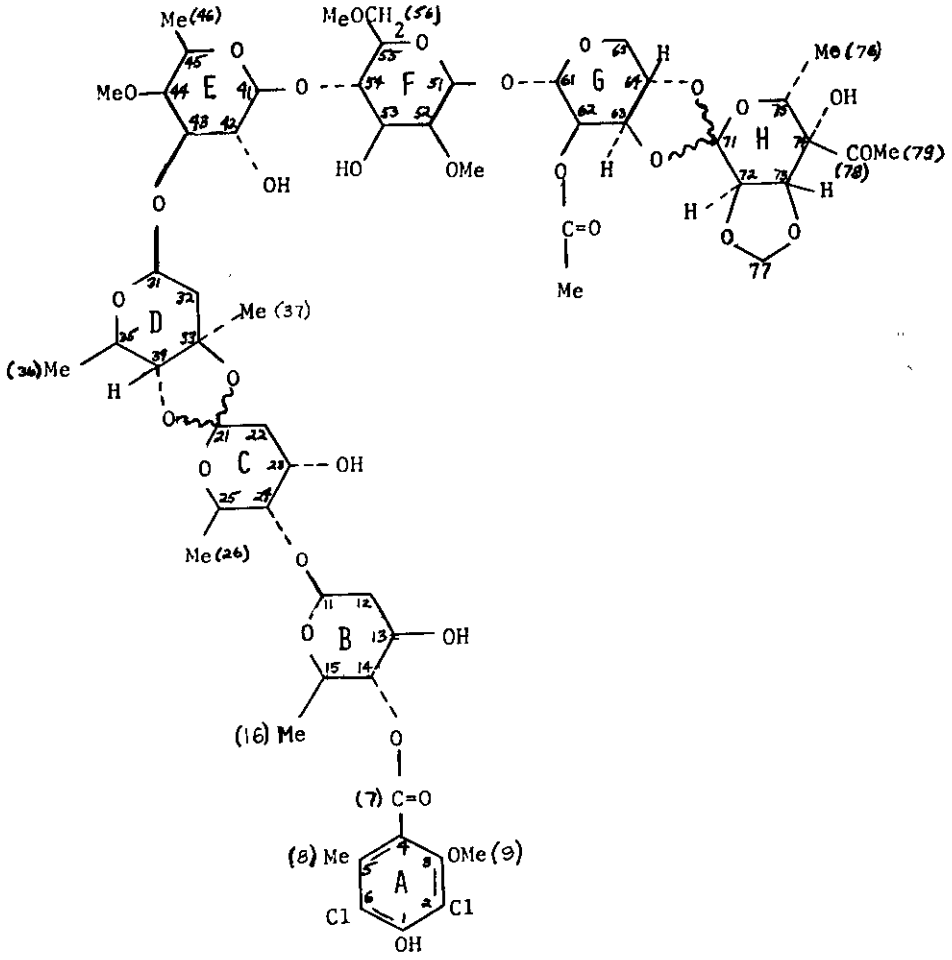
A similar situation existed in the case of flambamycin but in this instance the original structure had to be revised-in part because of discrepancy with ^{13}C NMR spectral data[15]. The structure determination of avilamycins, however, was very dependent on the use of ^{13}C NMR. In fact the final proof of structure[16] relied on a comparison of the ^{13}C NMR data for avilamycins with that of flambamycin.

Very recently a review[17] has appeared on the ^{13}C NMR spectra of flambamycin and its various degradation products. For convenience in presenting spectral data and comparing corresponding structures in various compounds, Ollis[17] et al. have assigned arbitrary numbers for the 61 carbon atoms of flambamycin. They have also used labels A to H for the eight major residues; A is used for the dichloroisoeverninate residue and B to H for the pyranosides. Corresponding "locants" (numbers) and "residues" (letters) are used in the formulae for analogs, derivatives and degradation products of flambamycin. The same system was used recently for discussing the structure of curamycins.[1]

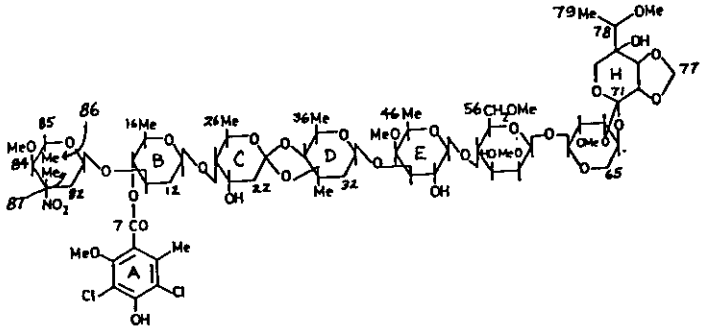
The designation of letters for the various structural elements simplifies the comparison of different antibiotics but the arbitrarily designated numbers are not helpful. Therefore, a more rational system of numbering* is used in the present communication.

The designations for oligosaccharide carbons used here take advantage of the standarized numbering of pyranosides so familiar to carbohydrate chemists. Thus, the anomeric carbon bears the number 1, and the absolute configuration at any ring carbon for D-sugars is indicated by the widely used stereodesignations alpha and beta. The same system is used here except that the relative position in the flambamycin structure (i.e. the locant) is also inherent in the number given to a particular carbon atom: thus, the pyranoside bearing the isoeverninic acid residue (A) is designated as B in the system of Ollis et al.; in the present system all carbons in residue B have locants 11-19; locants 21-29 refer to residue C, locants 31-39 refer to residue D, and so on. Thus, one two digit number is adequate for indicating both the residue (the first digit)

**This numbering system was devised by one of us (A.K.B.) to simplify recognition of various carbons in terms of the carbohydrates on which they are located.*



Curamycin A



Everninomicin D

as the easily recognized position (the second digit) of the particular carbon atom in the pyranoside ring system. In case the absolute configuration of the pyranoside is known, alpha, beta designations could be employed for further characterization of substituents at a specific carbon atom. This numbering system which has been used in this review is illustrated in the stereoformula for curamycin A.

5. ^{13}C NMR Spectra of Oligosaccharide Antibiotics

The regions of interest in the ^{13}C NMR spectrum of oligosaccharide antibiotics can be conveniently divided into seven parts. This arbitrary division is shown in Table I.

Certain areas of this chart are amenable to easier initial interpretation than others. For example, the carbinol region of the spectrum is often very difficult to interpret and assign unequivocally for compounds above the level of complexity of a disaccharide. The carbonyl region on the other hand is often easy to interpret.

Carbonyl Region

Since carbonyls are non-proton bearing, the NOE for small molecules is often small. In addition carbonyls generally have long relaxation time further decreasing their ^{13}C NMR sensitivity. In most cases, for these reasons, carbonyls will appear as small peaks compared to others in the spectrum. The highest field carbonyl signal in the spectrum of the oligosaccharides appears at 166 ppm and is common to all of the oligosaccharide antibiotics. This shift is characteristic of a benzoate carbonyl (165.9 ppm for the C=O of methyl benzoate). Therefore this signal can be assigned to C-7 common to all of these antibiotics.

The most downfield carbonyl signal at 205 ppm is displayed by flambamycin, curamycin A, and avilamycin A. This area of the carbonyl region is characteristic of saturated aldehydes and ketones (e.g. acetone C=O at 205.1 ppm). This signal is readily assigned to the methyl ketone at C-8 for these compounds. Conversely, the absence of this signal in the spectra of the other compounds (e.g. curamycin B) indicates the absence of this function.

The signal at 175 ppm in flambamycin, avilamycins A and C, and curamycin B is in the region of the spectrum assigned to carboxyl carbons. This particular shift, on the basis of empirical rule, may be assigned to an aliphatic ester, the acyl portion of which bears several carbon atoms (e.g. methyl isobutyrate C=O at 175.7 ppm). Therefore this signal is assigned to the carbonyl of the isobutyrate residue.

An additional signal appears in the spectra of curamycins A and C alone at 169 ppm. This signal also is indicative of an ester carbonyl, but the shift to higher field from

the isobutyrate position indicates possibly an acetate. A shift of 170 ppm is characteristic of acetate carbonyls regardless of the alkoxy substituent of the compound.

TABLE I

Regions of Interest in the ^{13}C NMR Spectra of Oligosaccharides

Region	^{13}C Chemical Shift[a]
Carbonyl	165 to 225
Aromatic (Olefinic)	110 to 160
Orthoester	120
Anomeric	90 to 105
Carbinol	65 to 90
Methoxyl	55 to 65
Methylene	30 to 50
Methyl	10 to 30

[a] ppm from TMS

Aromatic Region

The aromatic or olefinic region of the intact antibiotics contains eight signals. Two of these signals are assigned to the unusual orthoester carbons. Perhaps more than any other single piece of information the recognition of these orthoester functions is uniquely simple using ^{13}C NMR.

The orthoester carbons will generally appear as tall, narrow peaks in a very small area of the spectrum between 118-122 ppm. Aromatic carbons which often fall in this area as well appear as short, broader peaks. The explanation for this difference in appearance lies in relaxation phenomena for these carbons.

Anomeric Region

The anomeric region of the ^{13}C NMR spectrum is probably the single most informative region in the spectrum of carbohydrates. Examination of this region enables one to assess immediately the number of sugar units in a compound. The exact chemical shift may also permit the assignment of stereochemistry for the anomeric linkages.

There are six signals in the anomeric region of the spectra of flambamycin, avilamycins and curamycins but seven in the case of everninomicins. However, there are only six sugars in everninomicins and five sugars each in the others.

The extra signal is for the unusual methylene dioxy function at C-79. This signal can be seen as a triplet in the SFORD spectrum of olgose while anomeric carbon signals appear as doublets.

Carbinol Region

The assignment of all signals for the intact oligosaccharides, at least at fields of 25 MHz, would be nearly impossible. There are approximately 25 carbon atoms in this region of the spectrum for these antibiotics. Many signals are superimposed and alternative interpretations are possible.

Methoxyl Region

This region of the spectrum is readily assignable due to the rather characteristic sharp, tall appearance of the signals and relatively constant chemical shift of the carbons regardless of the number of sugars in the compound studied.
The highest field signal at 62.3 ppm is assigned to the aromatic OMe at C-7. The constancy of the chemical shift ($\pm$0.02 ppm) is testimony to the strength of this assignment.

Although not a great deal of stereochemical or conformational information is available from this methoxyl region, satisfying overall structural similarities among the various compounds are verified easily.

Methylene Region

In the case of carbohydrates this region is not among the most densely populated. However, it is precisely for this reason that this region can be of great structural significance.

The two signals in the range of 30-40 ppm can be assigned to C-12 and C-22.
The highest field signal in the region at 43.8 ppm is a striking point of dissimilarity among the various antibiotics. This signal appears in EVD, curamycins, and avilamycins but is missing in EVB and flambamycin. Accordingly this signal is assigned to C-2 of the 2-deoxy sugar D-evermicose, (C-32), which is common to some of these antibiotics.

Finally there is the signal at 34.0 ppm which appears in flambamycin, avilamycins and curamycin B. This chemical shift is assigned to the methine carbon of the isobutyrate ester, a shift which agrees closely with published values for such an ester (33.5 ppm for methyl isobutyrate)[18].

Methyl Region

The methyl region is the largest of the important functional group regions in terms of total number of carbons and is one of the most difficult to assign unequivocally.

Yet next to the anomeric region it is perhaps the most important to assign because of the wealth of structural detail which it contains. It is this region of the spectrum where untenable assignments were made in the flambamycin and avilamycin series of compounds[17].

The furthest downfield signal in this region which appears at about 25 ppm is assigned to the methyl ketone at C-78 (Cp.28.8 ppm for 2-butanone). This ketone which is also contained in flambamycin and avilamycin A is further confirmed by the keto carbonyl at 205 ppm.

The highest field signal in the curamycins is at about 13.5 ppm. This signal is assigned to the methyl group (C-76) in the side chain.

There is satisfying correlation for these signals in all of the structurally similar compounds curamycins A, B, and C, avilamycins A and C, and flambamycin.

The signal at 16.1-16.4 ppm can be assigned to C-46 since it is the only signal that occurs in this area and is common to all compounds.

The assignment of the other methyl groups is less straightforward.

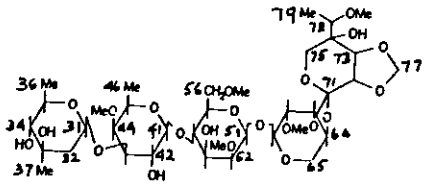
6. ^{13}C NMR SPECTRAL ASSIGNMENT FOR OLGOSE

Olgose (1) is a degradation product of everninomicin D, that figured prominently in the structure determination of everninomicins.[2] Analogous degradation products are found in flambamycin, avilamycins, and curamycins. The most significant structural variation among these various antibiotics occur in the olgose type sugars as well. Thus the complete assignment of the ^{13}C NMR spectral data is of significance in soloving structures of oligosaccharide antibiotics.

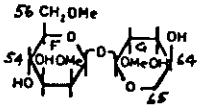
Table II contains ^{13}C NMR data for olgose and its various degradation products along with ^{13}C NMR data for several model compounds.

The complete ^{13}C NMR chemical shift assignment for olgose is made by studying and assigning the ^{13}C NMR data for the di, tri, and tetrasaccharide moieties of olgose. This was done by reference to appropriate monosaccharide model compounds, chemical shift rules, and SFORD techniques.

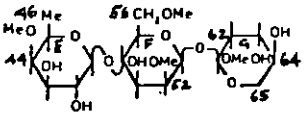
The disaccharide everninose (2) is the starting point for this discussion. Everninose is composed of 2,6,di-O-methyl-D-mannose and 2-O-methyl-L-lyxose, which are connected



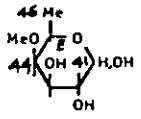
(1) Olgose



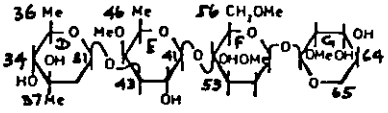
(2) Everninose



(3) Evertriose



(4) Curacose



(5) Evertetrose

TABLE II

^{13}C NMR Assignments for Everninomicin Oligosaccharides*

Carbon #	Compounds							
	olgose	ever-tetrose	ever-triose	ever-ninose	β-methyl-D-curacose	β-methyl-D-mannose[1]	α-D-lyxose[2]	β-methyl-L-mycarose[3]
61	95.0	94.8	94.7	94.8			95.0	
62	79.3[a]	80.0[d]	80.0[f]	80.0			71.5	
63	79.7[a]	70.7[e]	70.7[g]	70.5			71.1	
64	75.2	66.8	66.8	66.6[h]			68.5	
65	63.1	63.7	63.7	63.6			63.9	
62-O-Me	58.4	58.8	58.7	58.7				
51	96.0	95.7	95.7	95.9		102.2		
52	79.4[a]	79.8[d]	79.8[f]	80.9		71.5		
53	71.1[b]	70.8[e]	70.6[g]	73.2		74.2		
54	77.7	77.8	77.5	67.1[h]		68.0		
55	74.8	74.5	74.4	76.2		77.5		
56	72.2	71.8	71.7	71.9		62.3		
52-O-Me	58.4	58.4	58.3	58.4				
56-O-Me	60.9	60.9	60.8	60.9				
41	103.9	103.5	103.6			104.3		
42	70.2	69.4	70.4[g]			70.5		
43	82.1	82.0	73.8			74.1		
44	81.3	81.1	81.5			81.8		
45	69.8	70.0	70.2			69.7		
46	16.0	16.0	16.1			16.4		
44-O-Me	60.9	60.9	61.4			61.3		
31	101.3	101.1						99.4
32	45.8	45.7						43.3
33	70.9	70.6						70.9
34	77.8	78.9						76.7
35	70.9[b]	70.7[e]						70.3
36	18.7	18.7						18.1
33-α-Me	20.6	20.5						27.3
71	120.0							
72	80.5[c]							
73	76.8[c]							
74	75.2							
75	69.2							
76	--							
77	95.7							
78	73.0							
79	13.6							
78-O-Me	56.7							

*Chemical shifts are expressed in ppm from TMS. All spectra were determined for DMSO-d_6 solutions unless otherwise noted.

[a-h]Assignments in the same vertical column may be reversed.

[1]Data taken from Perlin *et al.*[8]

[2]Data taken from Dorman and Roberts.[20]

[3]Data taken from Lukacs *et al.*[12]

through their anomeric centers. Suitable model compounds for these two sugars are alpha-methyl-D mannose and beta-D-lyxose.

The three furthest upfield signals are easily assignable to the O-methyl groups since they appear as quartets in the SFORD spectrum.

The signals which appear as triplets in the SFORD at 63.6 ppm and 71.9 ppm are assigned by reference to the model compounds to C-65 and C-56 respectively.

The remainder of the signals in the 60-80 ppm region are not as easily assigned since they all appear as doublets in the SFORD.

The signals at 66.6 ppm and 67.1 ppm are assigned to C-64 and C-54 respectively by comparison to the models and by observation that in evertriose a signal at 67 ppm is absent because C-54 is glycosylated in the triose.

The signals at 70.5, 73.2 and 76.2 ppm are assigned to C-63, C-53, and C-55 respectively by comparison to the model compounds.

The remaining two signals at 80.0 and 80.9 ppm are assigned to C-62 and C-52, respectively, but distinction between the two is difficult.

The anomeric carbons, C-61 and C-51, are assigned to signals at 94.6 and 95.9 ppm; respectively.

Having assigned the spectrum of everninose, the assignment of ^{13}C NMR data for evertriose (3) is simplified but still difficult. ^{13}C NMR spectral data for beta-methyl-D-curacose (4), the next sugar in the growing chain of olgose, is of great utility in assigning the spectrum of evertriose.

The lone methyl carbon of evertriose is C-46 and its signal appears at 16.1 ppm. The extra O-methyl group at C-44 of the D-curacose residue is assigned at 61.4 ppm since this signal should not shift much from its position in the monosaccharide.

C-64 and C-65 likewise would not be expected to shift very much from everninose to evertriose and are assigned at 63.7 ppm and 66.8 ppm, respectively.

There are four signals in the region of 70.2 70.6 ppm and although individual assignments are difficult, C-42, C-45, C-63, and C-56 are included in this group.

The signal at 71.7 ppm is assigned to C-53 which has been shielded slightly (-1.5 ppm) from its position in everninose by glycosylation.

The signal at 73.8 ppm is assigned to C-43 of the new D-curacose residue by comparison to the model compound.

The signal at 74.4 ppm is assigned to C-55 which also has been shifted upfield (-1.8 ppm) by glycosylation at C-54. C-54 has been shifted downfield considerably to 77.5 ppm by glycosylation.

Neither C-52 or C-62 would be expected to be affected very much from everninose to evertriose and thus are assigned to the signals at 79.8 and 80.0 ppm.

This leaves the signal at 81.5 ppm for C-44 of the D-curacose residue in very close agreement with its position at 81.8 ppm in methyl curacoside.

The assignment of the anomeric region is straightforward with C-61 and C-51 in nearly the same position as in everninose at 94.7 and 95.7 ppm. The anomeric carbon C-41 appearing far downfield at 103-6 ppm.

The assignment of ^{13}C- NMR data for evertetrose (5) is a more difficult problem due to the increased complexity of the molecule and the absence of data for an exactly analogous model compound for the new sugar residue D-evermicose. The model compound which is used, beta-methyl-L mycaroside, however, is important in fixing some assignments in evertetrose.

The use of this model compound is critical in assigning the methyl region of evertetrose. The most upfield signal at 16.0 ppm is assigned to C-46 by comparison to its position in evertriose.

The signal at 18.7 ppm is assigned to C-36. This assignment is made because the chemical shift of this carbon should not change very much from its position in the mycaroside at 18.1 ppm to the evermicoside.

This leaves the signal at 20.5 ppm for the alpha-methyl carbon at C-33. In the model compound, L-mycarose, this carbon appears at 27.3[15] ppm. However, the orientation of this methyl shifts from equatorial in the mycaroside to axial in the evermicoside accounting for the increased shielding of this carbon that is observed.

The only methylene carbon in evertetrose at C-32 appears at 45.7 ppm in the ^{13}C-NMR spectrum. The assignment of the methoxyl region in evertetrose follows directly from the assignment for evertriose.

The assignment of signals at 63.7 and 66.8 ppm also are straightforward for C-65 and C-64 by reference to evertriose.

The signal at 69.4 ppm is assigned to C-42 is assigned to C-42 of the D-curacose residue. This particular carbon which appeared in the area of 70.2-70.6 ppm in evertriose would be expected to shift upfield about 1.0 ppm by the glycosylation at C-43 in evertetrose.

The signal at 70.0 ppm is assigned to C-33 of the D-evermicose residue. The assignment for this carbon is made on its appearance in the ^{13}C- NMR spectrum. It appears as a tall peak in the spectrum which is a consequence of the fact that as a quaternary carbon atom it has relatively long relaxation time compared to protonated ring carbons.

Three signals which are left individually unassigned appear from 70.6 - 70.8 ppm; these signals correspond to C-53, C-35 and C-63.

The signals at 71.8, 74.5 and 77.8 ppm are assigned to C-56, C-55, and C-54, respectively, since their position would not be expected to change very much from the triose to the tetrose.

A new signal which appears at 78.9 ppm is assigned to C-34 by comparison to the L-mycaroside model.

The signals at 79.8, 80.0 and 81.1 ppm are assigned to C-52, C-62, and C-44 by comparison to the triose. The signal at 82.0 ppm is assigned to C-43 which has been shifted downfield considerably from its position in the triose by its glycosylation.

The new signal in the anomeric region is assigned to C-31 of the D-evermicose residue.

There remains now the assignment for olgose itself. This problem is difficult for the same reasons that assignment of evertetrose was difficult, except that in olgose there was no suitable example for the side chain at all.

The assignment of the methyl region, however, is easy with the furthest upfield signal at 13.6 ppm assigned to C-78 of the side chain.

The highest field signal in the methoxyl region is assigned to the O-methyl at C-77. The signal at 69.2 ppm is assigned to C-75 on chemical shift grounds.

Signals in the region 70.2 - 74.8 ppm are assigned by precedent to the earlier degradation products. The signal at 70.9 ppm is assigned with some certainty to the quaternary carbon C-33 due to its characteristic appearance.

One of the peaks at 75.2 ppm is assigned as quaternary carbon C-74 due to its characteristic appearance. The second signal at 75.2 ppm is assigned to C-64 shifted downfield 8.4 ppm by inclusion in the orthoester linkage.

The signals at 77.7 and 77.8 ppm are assigned to C-54 and C-34 respectively by comparison to evertetrose.

Signals at 73.0, 76.8 and 79.4 ppm are assigned to C-77, C-72, and C-73, respectively, but are not certain due to lack of a suitable model compound.

Assignment of the anomeric region is unremarkable except for the signal for the methylene dioxy carbon, C-79, at 96.0 ppm.

Finally perhaps the most significant aspect of the ^{13}C- NMR spectrum of olgose is the signal at 120.0 ppm. This signal is assigned to the orthoester carbon, C-71. This ^{13}C-NMR signal was the most conclusive piece of evidence for the existence of this unusual functionality in the oligosaccharide antibiotics before the x-ray analysis of olgose.[19]

This completes the assignment of the ^{13}C spectral data for olgose. There are remarkably few ambiguities in this final assignment and the solution of this problem will indicate directly where the differences and similarities may lie in similar degradation products of the other oligosaccharide antibiotics. An example of this will be given in the next section with a discussion of the ^{13}C- NMR spectrum of pseudo olgose A, a degradation product of curamycin A.

7. ^{13}C NMR STRUCTURAL STUDIES ON pseudo-OLGOSE A

pseudo-Olgose A(6) is a degradation product of curamycin A[1]. The structure of the pseudo olgose A was a critical piece of information in solving the structure of curamycins. Since a limited amount of pseudo-olgose A was available, the structural proof had to rely heavily on spectral analysis.

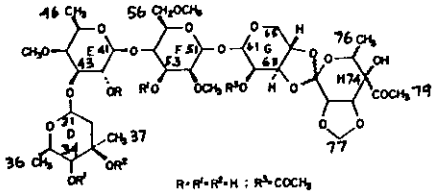
The complete proton noise decoupled ^{13}C NMR spectrum of pseudo-olgose A was determined in $CDCl_3$ and compared to that of olgose (1) which had been determined in DMSO-d_6. Using the assignments derived for the ^{13}C NMR data of olgose and cognizant of solvent effects on the chemical shift, a structure of pseudo- olgose A was deduced from its ^{13}C NMR data.

Table III contains a ^{13}C NMR chemical shift map for olgose and olgose A, where the regions are divided according to the various sugar residues.

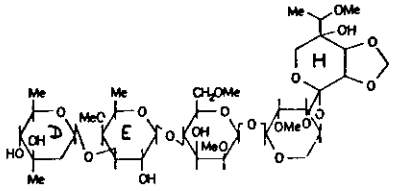
pseudo-Olgose A exhibited a ^{13}C NMR spectrum in $CDCl_3$ in which 36 resolved signals were observed two of which accounted for two carbons each due to overlap. In interpreting the structure of pseudo-olgose A by comparison to olgose, perhaps the most convenient area to consider first is the anomeric region.

Examining the chemical shifts of the various anomeric carbons, C-31, C-41, C-51 and C-61, a close correspondence between olgose and pseudo olgose A is observed. Thus the information indicates in a very preliminary fashion that the same order and stereochemistry of sugar residues exist in olgose and pseudo- olgose A. The presence of the unusual methylene-dioxy carbon, C-77, is suggested by the signal at 96.3 ppm.

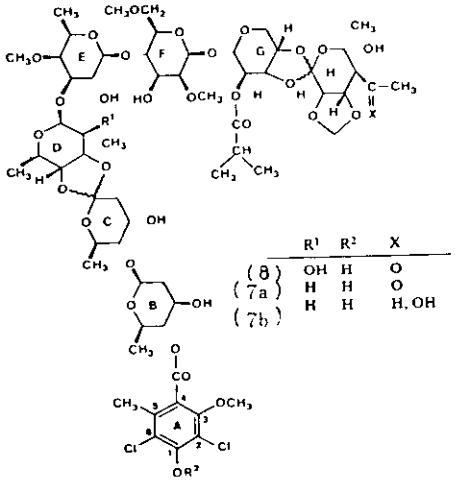
The signal at 119.1 ppm in the spectrum of pseudo-olgose A indicates the presence of the orthoester carbon, C-71. Thus the overall sequence of five sugar residues with one orthoester linkage is the same in pseudo-olgose.



(6) pseudo-Olgose



(1) Olgose



	R^1	R^2	X
(8)	OH	H	O
(7a)	H	H	O
(7b)	H	H	H, OH

(8) Flambamycin

(7a) Avilamycin A

(7b) Avilamycin C

TABLE III

^{13}C NMR Spectral Correlation for Olgose and pseudo-Olgose A*

Carbon #	Olgose	pseudo-Olgose A
61	95.9	94.9
62	79.3[b]	81.6[a]
63	79.7[b]	79.5
64	75.2	73.0
65	63.1	63.4
62-O-Me	58.4	-
62-O-CO-CH_3	--	169.3
62-O-CO-CH_3	--	20.6
51	96.0	97.0
52	79.4[b]	81.3[a]
53	71.1[c]	72.0
54	77.7	78.4
55	74.8	72.8
56	72.2	71.3
52-O-Me	58.4	59.3
56-O-Me	60.9	61.8
41	103.9	104.6
42	70.2	70.0
43	82.1	82.4
44	81.3	81.7
45	69.8	69.8
46	16.0	16.2
44-O-Me	60.9	62.1
31	101.3	100.8
32	45.8	45.3
33	70.9	72.8
34	77.8	78.4
35	70.9[c]	70.9
36	18.7	18.5
33-O-Me	20.6	20.4
71	120.0	119.1
72	80.5[d]	79.5[e]
73	76.8[d]	73.7[e]
74	75.2	79.5
75	69.2	70.4
76	--	13.6
77	95.7	96.3
78	73.0	205.9
79	13.6	25.2
78-O-Me	56.7	-

**Olgose spectrum was determined in DMSO-d_6 and pseudo-olgose A spectrum was determined in $CDCl_3$. All chemical shift data are presented in ppm from TMS.*

[a-e]Assignments in vertical column may be reversed, although those given are preferred.

The most striking initial difference between the ^{13}C NMR spectra of olgose and pseudo-olgose A is in the carbonyl region. There are two carbonyls in pseudo- olgose A but none in olgose.

Although the exact identity and location of the carbonyls cannot be deduced based on their chemical shift alone, some information may be inferred. The signal at 205.9 ppm is in an area where generally aliphatic ketones occur. The signal at 169.3 ppm is characteristic of an ester carbonyl.

The methoxyl region of the ^{13}C NMR spectrum also illustrates significant differences between olgose and pseudo olgose A. Olgose exhibits five methoxyl carbons and pseudo-olgose A only three. Furthermore, it would appear based on the comparative chemical shifts that it is the two highest field methoxyls, C-62-methyl and C-78-0 methyl, in olgose that are absent in pseudo-olgose A.

There is a single peak in the methylene region of the ^{13}C NMR spectra of olgose and pseudo-olgose A at 45 ppm. This indicates that both compounds contain the D-evermicose residue if confirmatory evidence is available in the methyl region.

The assignment of the ^{13}C NMR methyl region is critical to the solution of the structure of pseudo-olgose A. Comparison of the data for olgose and pseudo- olgose A at first would indicate that four of the methyl groups are identical due to their similar chemical shifts and that pseudo-olgose A has two extra methyl groups which must be assigned.

The chemical shift of the two extra methyl carbons, 24.2 ppm and 20.6 ppm gives a clue to their identity. Since both are somewhat downfield for aliphatic methyl groups and there are two carbonyl functionalities yet to be accounted for in pseudo olgose A, the signal 25.2 ppm is assigned to a methyl ketone and the signal 20.6 ppm is assigned to the acetate.

The location of the functionalities is more difficult to determine until reference is made to some other oligosaccharide antibiotics. Ollis and Keller-Schierlein have deduced the structure of avilamycin A and C relying heavily on the comparison of ^{13}C-NMR data between avilamycins (7) and flambamycin (8).[16]

In the structure of these compounds the olgose side chain is modified to include a methyl ketone comprising C-78 and C-79 and an additional methyl group at the 6 position of this residue i.e., C-76. Recently Keller-Schierlein et al. have verified this structural functionality as well as determined some undisclosed features of stereo-chemistry by X-ray analysis of a degradation product of avilamycin A.[21]

The chemical shift of the methyl group attached to the ketone in avilamycin A is 25.4 ppm[16] in close agreement with that of pseudo olgose A. The chemical shift of the C-76 methyl in avilamycin A is 13.5 ppm also in close agreement with that of pseudo-olgose A.

The remaining three methyl carbons of pseudo olgose A are assigned to C-45, C-36, and C-33- alpha methyl and the signal at 18.5 ppm is assigned to C-36. This assignment was reversed by Ollis et al[16] but based on the ^{13}C-NMR data for olgose and L-mycaroside model compound[12] this new assignment seems more suitable.

At this point the structures of olgose and olgose A seem identical to the first sugar residue (D-E-F). Differences occur in the L-lyxose moiety (substitution of an O-methyl by an acetate) and olgose side chain (ring H).

Examination of the methinoxy region of the ^{13}C NMR for the two compounds confirms this. There is close correspondence between olgose and pseudo-olgose A for the carbons C-31 through C-56. In the area of C-61 through C-79 there are significant differences, as to be expected.

The structure of pseudo-olgose A as (6) is supported strongly by the ^{13}C NMR data. Although this spectral correlation is by no means a complete proof of the structure of pseudo-olgose A, a correlation of this kind represents a significant step in the structural elucidation process and taken together with other readily available methods of spectral analysis can lead to a convenient means of structure determination for members of this complex family of antibiotics.

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