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Unusual monosaccharides: components of O-antigenic polysaccharides of microorganisms

N K Kochetkov

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Abstract. The data on new monosaccharides detected in O-antigenic polysaccharides of Gram-negative bacteria have been surveyed. The results of isolation and structure determination of these unusual monosaccharides have been arranged and described systematically. The NMR spectroscopy techniques are shown to be promising for the O-antigenic polysaccharides structure determination. The information about fine structure of monosaccharides which constitute the base of important class of microbial polysaccharides, is of great significance for applied studies, first of all, the design and synthesis of biologically active substances. The bibliography includes 216 references.

I. Introduction

Carbohydrate-containing biopolymers are among the major components of the living cell where they play a role of building blocks and energy donors and provide for the normal course of some highly specific biological processes coupled with the specific properties of the cell surface. Of those, the most abundant ones are polysaccharides (PS) made up of monomeric units, i.e., monosaccharides. The biological functions of PS specifically correlate with their structure. This structure–function relationship is especially well-defined in PS localised on the cell surface of Gram-negative bacteria. These PS determine the specificity of cell interactions with the environment which reflects on the fine immunochemical characteristics of the bacterium (serological reactions), on the specificity of cell interaction with bacteriophages (phage transformation of the microbial cell) and host tissues as well as on other highly specific biological processes.

The biological specificity of PS is determined by the peculiarities of the molecular structure of these biopolymers which, in turn, depends on the structure of the monomers (monosaccharides) in the biopolymer, their sequence in the polymeric chain and the mode of binding of the monomeric units. Whereas the first two factors, the monomeric composition and the sequence of the monomeric units, are not specific for the PS structure only but also for the structure of proteins and nucleic acids, the last factor determines the type of intermonomer binding and is unique to PS. This is due to the multifunctionality of monosaccharide residues containing several hydroxy groups at chiral carbon atoms. The existence of several hydroxy groups provides for the formation of several sterically unique intermonomer glycosidic bonds, thus giving rise to a complex (often branched) polymeric chain and increases manyfold the possibility of variations in the structure of PS built up from these monomers.

Another circumstance which greatly favours the existence in Nature of a broad spectrum of polysaccharide structures is the diversity of the monomeric units which may serve as building blocks for the polymeric carbohydrate chain. Whereas the diversity of nucleic acids is provided by only four nucleotides, and the number of amino acids in most proteins does not exceed 12–15, the diversity of PS is provided by more than a hundred monosaccharides and their derivatives.

Among the immense variety of PS isolated from natural sources, the most diversified (as regards their structure and monosaccharide composition) and most thoroughly studied are the PS of the outer cell membrane of Gram-negative bacteria. The primary structure of many of those has been established. These PS are important constituents of complex biopolymers, the so-called lipopolysaccharides (LPS)[1] localised on the cell surface of Gram-negative bacteria. Lipopolysaccharides consist of the lipid component (lipid A) incorporated into the microbial cell outer membrane, and the polysaccharide chain which, via a short oligosaccharide (so-called 'core') is attached to the lipid. The polysaccharide chain is exposed on the cell surface and is in direct contact with the environment, thereby providing, together with proteins, a unique pattern ('membrane mosaic') which determines the ultimate specific characteristics of the bacterial surface.

These PS are somatic bacterial antigens generally termed O-antigenic polysaccharides. They represent regularly arranged block polymers which only in rare instances occur as homopolymers but are usually composed of repeating oligosaccharide units, each of which contains 2 to 8 monosaccharide residues.[2] It may appear that such block organisation of the biopolymer restricts the number of plausible structures of O-antigenic PS. However, actually, because of the above peculiarities (great diversity of intermonomer glycosidic bonds, possibility to form branched chains and a great number of structurally distinct monosaccharides or their derivatives), the number of plausible structures of oligosaccharide units (and, consequently, of corresponding PS) is enormous amounting to tens of thousands (even when the polymer units are built up of only 3–4 monosaccharides).

It should be stressed that in contrast to PS of plant and animal cells, which are as a rule built up of a limited number of the most

N K Kochetkov Zelinskii Institute of Organic Chemistry, Russian Academy of Sciences, Leninskii prosp. 47, 117913 Moscow, Russian Federation. Fax (7-095) 135 53 28. Tel. (7-095) 137 61 48

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commonly occurring monosaccharides, PS of microbial origin contain a large number of less abundant monosaccharides often detected exclusively in these biopolymers. Very likely, only antibiotics, many of which also contain unusual monosaccharides, may effectively compete with their great diversity.

So far, the biosynthesis of monosaccharides present in PS, including some structurally unusual ones, has been studied in considerable detail. The pathways of formation of various monosaccharide structures from primary photosynthetic products have been elucidated: these shed more light on the diversity of the monomers utilised by microorganisms for the synthesis of the O-antigenic PS chain. These data have been considered in the review of Shibaev.[3]

In many cases structurally unusual monosaccharides or their derivatives play the role of the immunochemical dominant group, i.e., of the component that contributes most to the biological specificity of PS. This becomes especially apparent when an unusual component occupies the position of a side-chain substituent of the main linear chain of the biopolymer.

An example of a rare or even unique monomer of microbial PS may be a monosaccharide containing unusual structural groups. Their representatives in O-antigenic polysaccharides are deoxyhexoses, deoxyheptoses, aminodeoxy sugars, aminouronic acids and nonulosonic acids carrying amino groups. In addition to well-known *O*- and *N*-acetyl monosaccharide derivatives and their *O*-methyl ethers, O-antigenic PS have been found to contain, highly specific for this class of biopolymers, *O*- or *N*-acyl derivatives formed by acylation of monosaccharides by hydroxy or dihydroxy acids and amino acids. Another example of unusual derivatives are amides of uronic acids, including those formed by amino groups of amino acid residue. In current literature such fragments are generally termed 'non-carbohydrate substituents'. The monomers containing such unusual *O*- and *N*-substituents were mostly discovered during the last 10–15 years, and their number is rapidly increasing. Their identification and localisation in the polymeric chain is an indispensable part of the modern-day structural chemistry of polysaccharides.

It is especially worth noting that the vast body of experimental material obtained by analysis of the structure, chemical properties and pathways of synthesis of novel monosaccharides (and their non-carbohydrate substituents) present in O-antigenic PS has been found to be extremely important not only for understanding the nature of the biological specificity of carbohydrate polymers but also for further progress in carbohydrate chemistry in general. The data for novel, unusual combinations of functional groups in the monosaccharide molecules have considerably expanded our concepts of the reactivity and stereochemistry of monosaccharides and culminated in the discovery of some novel reactions, giving a new impetus to the elaboration of new methods of organic synthesis. The availability of a great number of carbohydrates differing in their structure has considerably enriched the methodological arsenal of NMR spectroscopy, for which these derivatives served as perfect model compounds.

The present review is dedicated to the consideration of mostly chemical aspects of the problem: it deals with isolation, determination of structure, identification and chemical properties of novel unusual monosaccharides or their unusual derivatives present in O-antigenic PS as monomeric units. In some instances data are given about the same or structurally related monosaccharides found in microbial PS unrelated to O-antigenic PS of Gram-negative bacteria. Some of the data concern the synthesis of certain monosaccharides. Such syntheses were undertaken with the purpose of confirming their structure or obtaining a corresponding, not easily available monosaccharide or its derivative in larger amounts needed for further analysis and application to the solution of research problems or practical tasks. Of the extensive body of synthetic material, we have focussed our attention only on those syntheses that either have a fundamental importance or are novel, effective and practical.

The data on the structure of PS themselves comprising these monosaccharides have been reviewed in one of the recent reports[2] which also concerns the localisation and type of binding of the monomers in the polysaccharide chain.

Earlier published reviews[4–6] dedicated to O-antigenic PS covered mainly biological and, particularly, microbiological aspects.

II. General observations concerning isolation, determination of structure and identification of monosaccharides and their derivatives present in O-antigenic PS

In order to determine the structure or to identify an unusual component of the polysaccharide chain by comparison with an authentic sample, the component is sometimes isolated in the individual state as such, as one of its standard derivatives (e.g., acetate) or in a modified form (e.g., as a corresponding alditol). However, the use of contemporary physico-chemical methods, and in the first place, NMR spectroscopy, often permits a conclusion about the structure of the unusual monomer directly in the process of establishment of the structure of the PS itself by using a non-destructive method. In recent studies identification of an already known component of a PS is often performed during the spectral analysis of PS by characteristic signals in the NMR spectrum.

1. Isolation of unusual monosaccharides or their derivatives

The initial O-antigenic polysaccharide is obtained by selective degradation of LPS routinely isolated by extraction of the microbial mass with aqueous phenol.[7] This is followed by mild acid hydrolysis of LPS with dilute acetic acid, after which the polysaccharide is isolated by a standard, specific for the given PS procedure (for example, ion-exchange chromatography is effectively used for PS containing acidic groups).†

After establishment of the presence of an unusual component by conventional monosaccharide analysis of PS or by preliminary analysis of its NMR spectrum, the isolation of the monosaccharide in the pure state is achieved by acid hydrolysis of the PS followed by chromatographic separation of the resulting mixture. For a successful solution of this problem, two mutually exclusive conditions often have to be met: (i) cleavage of the glycosidic bond of the monomer to be isolated during its splitting off from the polysaccharide chain, and (ii) avoidance of partial or even complete degradation of the monomer upon hydrolysis. For example, it is well known that monosaccharides containing one or several deoxy units are unstable in acid media and, although the glycosidic bonds of these monosaccharides are readily cleaved, isolation of the corresponding monomers may pose serious problems. At the same time, the glycosidic bonds of uronic acids or amino sugars having a nonacylated amino group are distinguished by increased resistance to hydrolysis which largely prevents the isolation of the corresponding native monomer in the individual state.

This task is further complicated by the situation when a complex monomer, a monosaccharide derivative containing a non-carbohydrate substituent, is to be isolated. Clearly, in this case the hydrolysis must not be accompanied by cleavage, modification, destruction or rearrangement of the substituting group. If after hydrolysis the unchanged non-carbohydrate substituent can be successfully isolated and identified, a new problem arises as to which monosaccharide of the polymeric chain is concerned and how the substituent is bound.

† Examples of standard procedures for isolation and purification of various types of PS can be found in the many-volume monograph *Methods in Carbohydrate Chemistry* Vols I–IX (New York: Academic Press, 1965–1993).

The hydrolytic conditions used to isolate novel components of PS may vary widely depending on the experimental goal. This is pertinent to the choice of the acid (hydrochloric, sulfuric, trifluoroacetic) and its concentration as well as the temperature and duration of hydrolysis. Most commonly, the hydrolytic conditions are selected experimentally, taking into account preliminary data on the monosaccharide composition of the biopolymer. Sometimes, for the isolation of an unusual component, special conditions are applied which may not be suited for achieving complete hydrolytic cleavage of the polysaccharide. In other cases, acetolysis (treatment of PS with an acetic anhydride–acetic acid mixture in the presence of sulfuric or another strong acid, e.g., hydrochloric acid) is used instead of acid hydrolysis; in this case monosaccharides are isolated in the form of full acetates.

Currently, solvolysis with anhydrous HF at reduced or room temperatures is used for depolymerisation of polysaccharides.[8] The distinctive feature of this method is that it cleaves the glycosidic bonds without any effect on bonds cleaved by hydrolysis, e.g., amide bonds. In particular, the method is used effectively to isolate complex derivatives of monosaccharides with their non-carbohydrate substituents attached.

When an unusual monomeric component fails to be isolated, a short oligosaccharide fragment (usually a disaccharide or a trisaccharide) containing this monosaccharide is obtained. For this purpose, in addition to selective acid hydrolysis, other conventional procedures for partial cleavage of the polysaccharide chain are used, such as oxidation with periodic acid (periodate oxidation) with subsequent Smith degradation,[9] and in some cases deamination of an amino sugar present in the PS chain is carried out.[10] This simplifies considerably the determination of the structure of the unusual component of a short oligosaccharide in comparison with its identification within PS, mass spectrometry and NMR spectroscopy being helpful tools in the process.

Chromatographic techniques are now being used almost exclusively to isolate monosaccharides, their derivatives and short oligosaccharide fragments from hydrolysates. Preliminary qualitative estimation of the composition of the hydrolysate and identification of an unusual component are carried out using paper chromatography (PC) or thin-layer chromatography (TLC) and by various modifications of liquid or gas-liquid chromatography. On the basis of the preliminary data, the most appropriate method is selected for preparative isolation of the desired component (a monomer or an oligosaccharide).

2. Chemical methods used to establish the structure

With the development of physico-chemical techniques, classical chemical methods used for establishing the structure of monosaccharides and their derivatives gradually lose their significance, although some of them still remain practicable. If a previously unknown monosaccharide was isolated in the pure state, its structure can be determined by routine approaches commonly employed in monosaccharide chemistry, such as methylation and periodate oxidation, which had especially wide application at early stages of microbial PS analysis. The first of these methods (see Practical Guide [11]) is based on the conversion of free hydroxy groups of a mono-, oligo- or poly-saccharide into *O*-methyl groups. Their localisation in the resulting derivative by using mass spectrometry makes it possible to establish the position of other substituents, deoxy units, and details of the monosaccharide structure.

Oxidation of a monosaccharide with periodate[9] results in the cleavage of the carbon–carbon bonds between adjacent atoms carrying hydroxy groups which prompts a conclusion about their location and the presence of deoxy units in the monosaccharide. Currently, this method is used widely for selective cleavage of the PS chain with the ultimate goal of obtaining short fragments and is followed by mild acid hydrolysis and reduction (Smith degradation) of the oxidised PS.[9]

Other chemical conversions used to establish the monosaccharide structure involve deamination of amino sugars, as a result of which the amino group is first deacetylated and the amino sugar derived is treated with nitrous acid (see review [10]). This reaction is also employed for selective degradation of the PS chain.[12,13] In some cases other reactions of monosaccharides, e.g., conversion of monosaccharides into anhydro sugars, lactonisation of uronic acids, etc., may become helpful.

Sometimes, in order to identify a monosaccharide by comparison with the authentic sample, the former is converted into its derivative, e.g., *O*-acetate or *O*-trimethylsilyl ether. Reduction of a monosaccharide with $NaBH_4$ to the corresponding alditol is especially useful. The latter is converted into the acetate and identified either by direct comparison with an authentic sample or using mass spectrometric analysis.

To establish the absolute configuration of a monosaccharide, this is converted into a glycoside by interaction with an optically active alcohol (most commonly with butan-2-ol or octan-2-ol). The absolute configuration of the initial monosaccharide is inferred from the chromatographic mobility of the glycoside.

Chemical methods used to establish the structure of unusual derivatives of monosaccharides are mostly confined to their degradation into the constituent fragments: a monosaccharide and a non-carbohydrate substituent. To this end, hydrolytic cleavage of a hydroxy group or an amino acid group that is acylating one of the hydroxy groups in the monosaccharide is performed, after which the acid is isolated for further identification. The substitution site in the monosaccharide is determined by one of routine chemical or physicochemical methods.

It should be noted that at the present time such tasks, as well as establishment of the structure of novel monosaccharides, are more easily and effectively accomplished by application of physicochemical methods alone, while chemical methods are used in counterflow syntheses of new monosaccharides or their unusual derivatives for unambiguous and final identification. Synthesis of novel monosaccharides is usually achieved by regio- and stereoselective transformation of one of the available monosaccharides by methods routinely used in carbohydrate chemistry. Examples of different syntheses of this sort will be given below.

3. Mass spectrometric analysis

One of the physicochemical methods used to study the structure of sugars is mass spectrometry. The stipulation of the principles for fragmentation of monosaccharide molecules [14,15] and of alditols derived by their reduction [16,17] using electron impact has made it possible to use mass spectrometry for effective establishment of their structure. Currently, the most commonly used is mass spectrometry of alditols characterised by more simple and unambiguous fragmentation of the molecule.

For this purpose, a monosaccharide or its derivative is reduced with borohydride or, in special cases, with sodium borodeuteride. This results in the formation of an alditol, in which the primary alcohol group (which often comprises deuterium, CHD–OH, providing the 'labelling' of the group essential for further elucidation of the structure on the basis of the mass spectrum) corresponds to the aldehyde group of the original molecule. The alditol thus obtained is converted into the full acetate which is further subjected to mass spectrometry. It should be emphasised that in the majority of cases this method permits the preservation of the non-carbohydrate substituents present in the monosaccharide and makes mass spectrometry a helpful tool for the analysis of complex derivatives of monosaccharides.

Fragmentation of alditols by electron impact has been studied thoroughly.[18] In particular, a correlation has been established between the relative ease of cleavage of the carbon–carbon bonds in alditols and the nature of the substituent at the carbon atom. On the basis of these data, standard procedures have been developed for establishment of the structure of monosaccharide components of PS (see reviews [19,20]).

The mode of degradation of carbon–carbon bonds in alditols by electron impact appears to be especially informative in the analysis of the structure of deoxy and aminodeoxy sugars as well

as of monosaccharides containing non-carbohydrate substituents. Such substituents (e.g., a residue of a hydroxy or an amino acid) are also subject to fragmentation by well-known pathways and easily identified, the fragmentation of the alditol skeleton indicating the position of the non-carbohydrate substituent.

Regretably, mass spectrometric analysis does not allow a conclusion about the stereochemistry of the monosaccharide, since the spatial arrangement of the substituents at the chiral carbon atoms of the monosaccharide skeleton does not appreciably influence the cleavage of the carbon–carbon bonds. Numerous attempts to use mass spectrometry for determination of the configuration of monosaccharides failed and, therefore, NMR spectroscopy is now being used more frequently to establish the polysaccharide structure.

4. NMR spectroscopy

NMR spectroscopy is the major procedure designed to establish the structure of carbohydrates and structurally unusual monomeric components of PS. Sometimes, this method allows a solution of this problem without isolation of monosaccharides in the pure state, directly on the basis of the NMR spectrum of the polysaccharide, and the establishment of the complete structure of PS on the basis of the spectral data alone, i.e., by a non-destructive approach.

Almost all presently known versions of ^{1}H and ^{13}C NMR spectroscopy are used in polysaccharide chemistry; the use of each of those modifications is defined by the specific goal to be sought (see reviews [21–26]). The techniques based on the measurement of nuclear Overhauser effects (NOE) in the ^{1}H NMR spectra, which allows a direct determination of the relative spatial position of protons in the monosaccharide molecule, has acquired special importance in recent years.

Among new approaches expressly designed to solve structural problems of polysaccharide chemistry, special mention should be made of computer analysis of ^{13}C NMR spectra.[24,27] This approach is meant, primarily, for determining the type of binding and the sequence of monosaccharide units in the polysaccharide chain. However, it often appears to be a helpful tool for elucidation of the structure of monosaccharides in PS, particularly, for the determination of their configuration. Thus, this method is now being widely employed for establishment of the absolute configuration of the monosaccharide without its isolation, i.e., directly within the polysaccharide chain.[24] The method is based on the determination of the magnitude of the so-called glycosylation effects, i.e., changes in the chemical shifts of the given carbon atoms in the ^{13}C NMR spectrum depending on the stereochemical features (including the absolute configuration) of the neighbouring monosaccharide unit in the polymeric chain.

5. Other physicochemical methods

An important physicochemical constant used in the structural analysis of sugar derivatives is the specific optical rotation. This value is readily determined with a high degree of accuracy and reproducibility and not only serves as an important criterion of the purity of the preparation obtained but is also routinely used for identification of the compound studied by comparison with an authentic sample. The absolute configuration of the monosaccharide under study as well as of oligosaccharides and other derivatives containing this monosaccharide unit can also be determined from the specific optical rotation; for this purpose, the experimentally determined specific optical rotation is compared with the theoretically calculated value for the given monosaccharide or its derivative using Klein's rule.[28]

In what follows, the description of individual representatives of monosaccharides and their derivatives present in O-antigenic PS will include concrete data on the use of the physicochemical methods mentioned above; this is confined to a brief consideration of only the most important spectral characteristics of the monosaccharide that are critical and sufficient for the elucidation of its structure.

III. Unusual monosaccharides forming a part of O-antigenic PS

1. Pentoses and hexoses

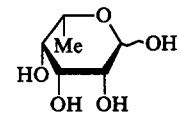
The majority of aldopentoses and aldohexoses widely distributed in Nature are common components of microbial PS. D-Glucose, D-galactose and D-mannose have a peak incidence among hexoses, while D-ribose among pentoses. D-Xylose and D-arabinose are far less abundant.[2]

Aldoses of the L-series are not common in microbial PS. Thus, only PS of two *Pseudomonas* species contain L-xylose.[29,30] This sugar was identified by conventional methods of carbohydrate analysis as the acetate of the corresponding alditol.[29] The absolute configuration of the (−)-2-octyl glycoside derivative of L-xylose was determined by GLC.[30]

2. Deoxyhexoses

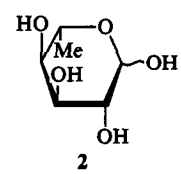
Unlike hexoses which enter into polysaccharides predominantly as D-isomers, deoxyhexoses occur more frequently as L-isomers. Thus, 6-deoxyhexoses, L-rhamnose and L-fucose are common constituents of O-antigenic PS,[2] whereas D-isomers of these monosaccharides are rare. The D-isomers were isolated in the individual state after acid hydrolysis of PS of *Pseudomonas cepacia* or as *O*-methyl ethers after hydrolysis of methylated PS and identified by conventional methods. Their absolute configuration was established either by calculation according to Klein (e.g., see Ref. 31) or by GLC of alkyl glycoside derivatives with optically active alcohols (e.g., see Ref. 32). Sometimes, the absolute configuration was determined by a computer analysis of the structure of PS based on their ^{13}C NMR spectra (e.g., see Ref. 27).

Some PS were found to contain even more rare 6-deoxyaldoses. Thus, 6-deoxy-L-talose (**1**) was detected in PS of several *Pseudomonas* species (*P. fluorescens*,[33] *P. maltophilia*,[34] *P. pseudomallei*[35]) as well as in PS of a strain of *Escherichia coli*.[36] This sugar was isolated in the pure state from acid hydrolysates of PS and identified using PC and TLC by comparison with an authentic sample obtained from glycosides of the terpene series.[37] Its structure was confirmed by mass, ^{1}H and ^{13}C NMR spectra, whereas the L-configuration was evident from the specific optical rotation.



1

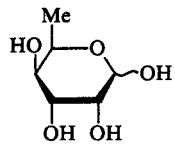
6-Deoxy-L-altrose (**2**) was detected in *Yersinia enterocolitica*[38,39] as a component of a homopolysaccharide composed of the pyranose units of this sugar as well as in PS of *Y. pseudotuberculosis* serovar VB, where its furanose form is present in the side chain.[40] The monosaccharide was identified by direct comparison with the sample isolated earlier from terpene glycosides.[37] Its structure as 6-deoxyaldose was corroborated by mass spectrometry and NMR spectroscopy,[38] whereas its L-configuration was determined from the specific optical rotation.



2

There is evidence[41] that a PS of *Y. enterocolitica* contains 6-deoxy-D-gulose (**3**) which failed to be isolated in a sufficiently pure state. Its structure was established by comparison with an authentic sample[37] using electrophoresis in borate buffer. The stereochemistry of 6-deoxy-D-gulose was determined by analysis of the ^{1}H NMR spectrum of a partially purified sample. This spectrum contained signals corresponding to the CH_3 group of

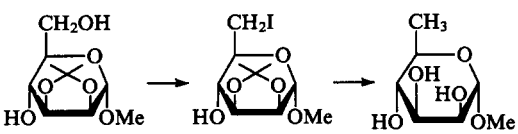
6-deoxyhexose and the coupling constant values ($J_{2,3}$ = 3.5, $J_{3,4}$ = 1.5, $J_{4,5}$ = 2.25 Hz) were consistent with the *gulo*-configuration. The absolute configuration was established from the ^{13}C NMR spectrum of the polysaccharide.



3

6-Deoxyaldoses can be easily synthesised by routine methods, i.e., by introducing a 6-deoxy unit into the corresponding aldoses. An example is provided by the synthesis of rarely occurring D-rhamnose from D-mannose[42] (Scheme 1).

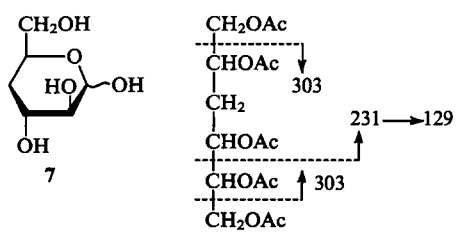
Scheme 1



4 5 6

Methyl 2,3-*O*-isopropylidene-α-D-mannopyranoside (**4**) was converted into the 6-iodo derivative **5** by selective tosylation of the primary hydroxy group and subsequent treatment with NaI. Hydrogenation of the latter over Raney nickel followed by elimination of the isopropylidene group gave the methyl glycoside of D-rhamnose **6**.

Other monodeoxyhexoses have not yet been found in O-antigenic PS. Of particular interest is the fact that 2-deoxyhexoses, widespread in many natural glycosides, are absent from bacterial PS at all. There is also no evidence for the presence of 3-deoxyhexoses in PS. The only representative of 4-deoxyhexoses in O-antigenic PS is 4-deoxy-D-*arabino*-hexose (**7**) detected in some strains of *Citrobacter* (e.g., in *Citrobacter* O36 its pyranose units form a homopolysaccharide,[43] whereas in *Citrobacter* PCM 1487 it is present in the side chain of O-antigenic PS[44]). The monosaccharide **7** is distinguished by very high lability and could not be purified after acid hydrolysis of PS, although the reduction of the hydrolysate gave the alditol **8**. The mass spectrum of the acetate of **8** contained peaks of the fragments with *m/z* 303 and 231 and the corresponding secondary fragments which clearly indicates the presence of a C(4)-deoxy unit. The stereochemistry of **7** was determined on the basis of the ^1H NMR spectrum of the polysaccharide itself; the presence of two signals of H(4) at 1.751 (*a*) and 1.528 (*e*) ppm also corroborates its structure. The D-configuration was assigned to the monosaccharide **7** on the basis of the specific optical rotation.

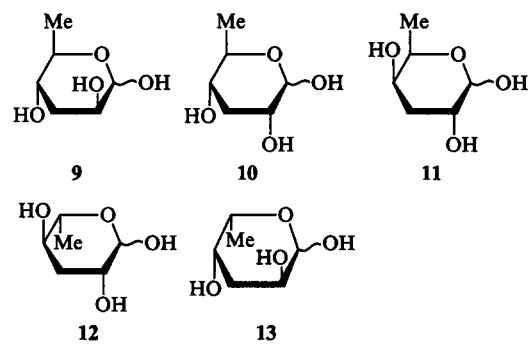


7 8

3. 3,6-Dideoxyhexoses

This type of deoxy sugars is widely spread in PS of Gram-negative bacteria and is typical of PS of the genus *Salmonella*.[2] 3,6-Dideoxyhexoses were detected as components of PS even in pioneer studies of O-antigenic PS of microorganisms. Identification and determination of the structure of 3,6-dideoxyhexoses isolated from various *Salmonella* species demonstrated a direct correlation between the structure of O-antigenic PS of a certain bacterium and its immunochemical properties. These studies, which dated back to the 1950's, had a great historical impact, for they actually opened up a way for extensive systematic chemical investigations into microbial O-antigenic PS.

Up to the present, five representatives of 3,6-dideoxyhexoses of various configurations are known that are present in PS of a rather large number of Gram-negative bacteria. They are: D-*arabino*-hexose (tyvelose **9**), D-*ribo*-hexose (paratose **10**), D-*xylo*-hexose (abequose **11**), L-*arabino*-hexose (ascarylose **12**) and L-*xylo*-hexose (colitose **13**). Among these, the first three monosaccharides, which were first discovered by Westphal and Lüderitz during their study of PS of *Salmonella*, are the most widespread.[45] These compounds are attached as side chains to the main chain of the biopolymer and represent the immunodominant groups, i.e., they determine the immunochemical characteristics of the given microorganism (see review[46]). Thus, PS of *S. paratyphi* contains paratose, PS of *S. typhimurium* abequose, *S. typhi* PS tyvelose.[2,47] The *Salmonella* of serogroups A–D contain as an indispensable component one of these three 3,6-dideoxyhexoses.



9 10 11 12 13

Two species of *Salmonella* were also found to contain colitose[48] first discovered in PS of *Escherichia coli* O111.[49] Later, 3,6-dideoxyhexoses were found in PS of other Gram-negative bacteria. Thus, all the five representatives mentioned above were identified in PS of various strains of *Yersinia pseudotuberculosis*;[50–57] ascarylose was found in PS of *Vibrio cholerae* O3,[58] abequose in PS of *Citrobacter freundii*,[59,60] tyvelose in PS of *Eubacterium saburreum*,[61] ‡ while colitose occurred in PS of one of *Escherichia coli* strains.[62]

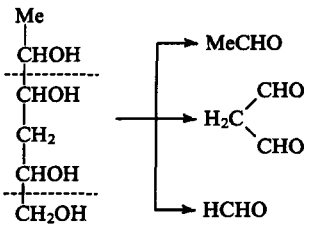
All the 3,6-dideoxyhexoses mentioned above can be isolated in the individual state after acid hydrolysis of the corresponding PS. Their glycosidic bond which, as with other deoxy sugars, is hydrolysed much more easily than typical glycosidic bonds makes possible their selective cleavage. At the same time, they have increased lability towards acids and their isolation presents some difficulties.

Originally, the structure of 3,6-dideoxyhexoses was established by classical methods. An example is abequose (Scheme 2). The positions of its deoxy units were determined by periodate oxidation of the alditol **14** obtained by the reduction of abequose **11** with $LiAlH_4$. Oxidation of the alditol **14** gave formaldehyde, acetaldehyde and malonic aldehyde proving the position of deoxy units at C(6) and C(3) of the hexose skeleton.[46] The establishment of the absolute and relative configurations of abequose, as with other 3,6-dideoxyhexoses, was possible at that time only by comparison with authentic samples. For this purpose, the synthesis of some representatives of 3,6-dideoxyhexoses was carried out.

More recently, mass spectrometry of alditol acetates obtained by reduction of 3,6-dideoxyhexoses came into routine practice as a means for the establishment of the structure of their skeleton. These derivatives exhibit a peculiar fragmentation which indicates

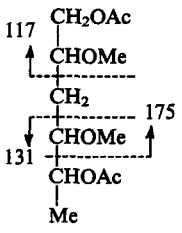
‡ *Eubacterium saburreum* is not a Gram-negative bacterium.

Scheme 2



14

the position of deoxy units. By way of illustration, the mass spectrum of the alditol acetate **15** obtained by reduction of the *O*-methyl ether of abequose isolated from the hydrolysate of methylated PS of *S. typhimurium* contains fragments with *m/z* 175, 131 and 117 indicating the presence of deoxy units at C(6) and C(3).[63]



15

However, the positions of deoxy units in the monosaccharide can be more easily detected by ^{13}C NMR spectroscopy. All the spectroscopic characteristics of 3,6-dideoxyhexoses are now well known and readily detectable directly in the NMR spectra of the corresponding PS: a signal in the region 16.0–18.0 ppm shows the presence of a methyl group at C(6), whereas a signal in the region 33–40 ppm the presence of a deoxy unit at C(3).[64]

The configuration of 3,6-dideoxyhexoses, namely, the stereochemistry of C(2) and C(4) can well be defined by the ^1H NMR spectra, the coupling constant values between the C(3)H_2 group and protons at C(2) and C(4) being the most characteristic ones. According to the general regularities, these constants have higher values (11–12 Hz) for *a,a*-protons and lower values (3–5 Hz) for *a,e*- and *e,e*-protons. Thus, the corresponding values for paratose[10] are $J_{3a,4} \approx J_{2,3a} \approx 11$ Hz and $J_{3e,4} \approx J_{2,3e} \approx 4$ Hz, respectively; those for abequose are $J_{2,3e} \approx 4.5$, $J_{2,3a} \approx 12$, $J_{3a,4} \approx 3$ Hz. Identification of 3,6-dideoxyhexoses is now carried out directly from the NMR spectra of the PS itself.

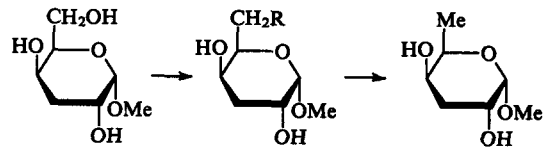
The above data are pertinent to the pyranose form of 3,6-dideoxyhexoses, in which these deoxy sugars are present in almost all bacterial PS. The only exception is PS of *Yersinia pseudotuberculosis* IB, where a 3,6-dideoxyhexose is present in the furanose form.[52]

Several methods of 3,6-dideoxyhexose synthesis have been described; all are based on the introduction of deoxy units into hexoses having a regular structure by classical methods. This synthesis was originally undertaken to establish the structure of dideoxyhexoses isolated from natural PS.

Of considerable historical importance is the synthesis of the abequose derivative **11** from methyl 3-deoxygalactoside **16** (Scheme 3).[65] The latter was selectively tosylated at the primary hydroxy group; the tosylate **17** (R = OTs) was converted into the iodide **17** (R = I), reduction of which gave the methyl abequoside **18**. The same methyl abequoside can also be obtained by direct reduction of the tosylate **17** with $LiAlH_4$. In a similar way, the derivatives of tyvelose (**9**) and paratose (**10**) were synthesised from 3-deoxymannoside and 3-deoxyglucoside,[66] respectively. Later, paratose was synthesised by direct introduction of a halogen atom at C(6) of a 3-deoxyglycoside.[67]

Currently, it has become necessary to develop preparative methods for the synthesis of considerable amounts of 3,6-dideoxyhexoses for the purpose of creating artificial antigens

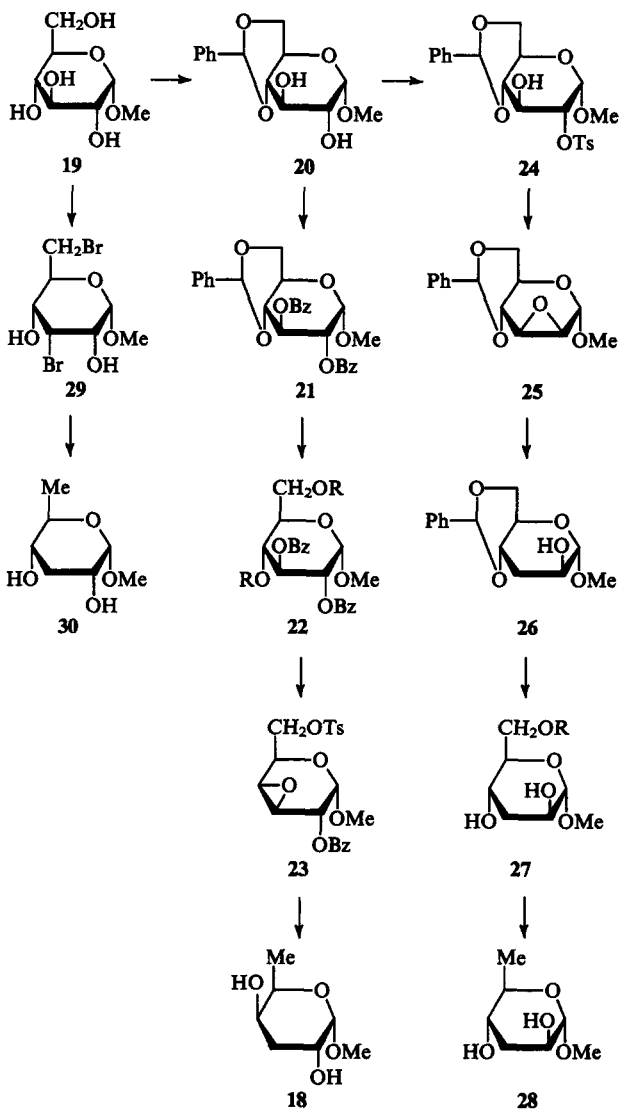
Scheme 3



16 17 18

and diagnostic tools. The synthesis of three most important 3,6-dideoxyhexoses[68] (tyvelose, paratose, abequose) from the most readily available material, the methyl glycoside **19**, is depicted below (Scheme 4).

Scheme 4



To synthesise abequose, methyl α-D-glucopyranoside **19** was converted into the 4,6-*O*-benzylidene derivative **20** and further into its dibenzoate **21**. After elimination of the benzylidene group, the diol **22** (R = H) was converted into the 4,6-di-*O*-tosylate **22** (R = Ts); its treatment with a base gave 6-*O*-tosylate of the 3,4-anhydro sugar **23**. Reduction of the anhydro sugar with $LiAlH_4$ was accompanied by regio- and stereo-specific opening of the epoxide ring and hydrogenolysis of the *O*-tosyl group resulting in the formation of the abequose derivative **18**.

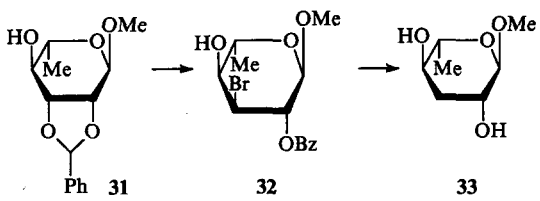
During the synthesis of tyvelose, the benzylidene derivative **20** was subjected to selective tosylation to give the 2-*O*-tosylate **24**. The latter was converted into the 2,3-anhydro derivative **25** which

was converted into the 3-deoxy derivative **26** by the action of $LiAlH_4$. After elimination of the benzylidene group and selective tosylation of the hydroxy group at C(6) of the triol **27** (R = H), the corresponding tosylate **27** (R = Ts) was reduced and the tyvelose derivative **28** was isolated.

Finally, the paratose derivative **30** was obtained by treatment of the glucopyranoside **19** with tribromoimidazole in the presence of triphenylphosphine (cf. published data [69]) followed by reduction of the dibromide **29** by hydrogenolysis over Raney nickel or with Bu_3SnH in the presence of azo-*N,N'*-bis-isobutyronitrile.

A rather simple synthesis [70] of the ascarylose derivative **33** from L-rhamnose is also known (Scheme 5). Methyl 2,3-*O*-benzylidene-α-L-rhamnopyranoside **31** (readily produced by treatment of methyl rhamnoside with benzaldehyde dimethylacetal in the presence of toluenesulfonic acid) interacts with *N*-bromosuccinimide with a regiospecific opening of the benzylidene ring to give the 3-bromo derivative **32**, which is reduced with $LiAlH_4$ into the methyl glycoside of 3,6-dideoxy-L-*arabino*-hexose (ascarylose) **33**. Colitose [71] was synthesised from the 3,4-*O*-benzylidene derivative of α-L-fucose by a similar scheme.

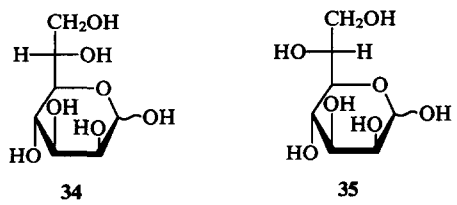
Scheme 5



4. Heptoses

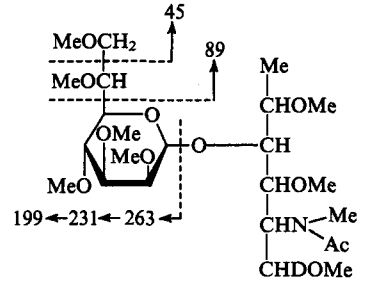
Up to the present, only three heptoses, L-*glycero*-D-*manno*-heptose, D-*glycero*-D-*manno*-heptose and D-*glycero*-D-*altro*-heptose, have been identified as components of O-antigenic PS. The first of these is also a common component of the LPS core of Gram-negative bacteria (see review [72]).

L-*Glycero*-D-*manno*-heptose **34** was isolated [73] after acid hydrolysis of PS of *Pseudomonas cepacia* IMV 673/2 using ion-exchange chromatography in borate buffer. Its structure was established in the following way. The PS was oxidised with a calculated amount of sodium periodate and then reduced with $NaBH_4$. Products of the hydrolysis of the PS thus modified contained mannose resulting from the cleavage of the C(7)–C(6) bond of the heptopyranoside unit. This finding provided evidence for the *manno*-configuration of the C(2)–C(5) fragment of the monosaccharide and was confirmed by ^{13}C NMR spectroscopic data from the original PS. The retention time of the heptose fraction in a sugar analyser and its ^{13}C NMR spectrum were fully consistent with the corresponding parameters for the enantiomeric D-*glycero*-L-*manno*-heptose but differed from that for D-*glycero*-D-*manno*-heptose, thus confirming the relative configuration of the monosaccharide as a whole. Its absolute configuration was evident from the specific optical rotation ($[\alpha]_D = +4.8°$) and confirmed by analysis of the glycosylation effects in the ^{13}C NMR spectrum of the polysaccharide.



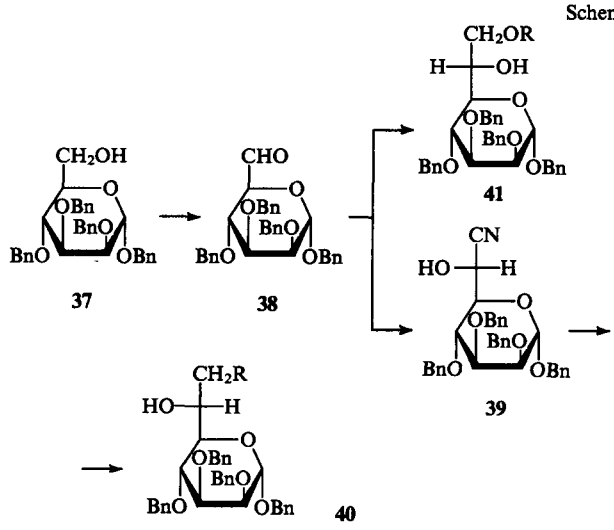
D-*Glycero*-D-*manno*-heptose **35** was found in PS of *Vibrio cholerae* O21 where it is present in the main chain.[74] The monosaccharide **35** was isolated in the pure state from the acid hydrolysate of PS. In the ^1H NMR spectrum of the methyl heptoside pentaacetate, the chemical shifts of the signals and the coupling constants for the protons at C(2)–C(5) practically coincided with those for methyl α-D-mannoside tetraacetate, thus corroborating the configuration of the heptose fragment C(2)–C(5). In GLC analysis the mobility of the heptitol acetate derived from the heptose differed from that of the heptitol obtained from L-*glycero*-D-*manno*-heptose, and the specific optical rotation ($[\alpha]_D = +74°$) was indicative of the D-configuration.

More recently, D-*glycero*-D-*manno*-heptose was also isolated from PS of *Vibrio cholerae* O3.[58] Its presence was demonstrated by conventional monosaccharide analysis of PS, and its structure confirmed by ^1H NMR spectroscopy of the polysaccharide itself as well as by mass spectrometric analysis of the disaccharide fragment **36** (isolated by acid hydrolysis of the methylated PS followed by reduction with $NaBD_4$) which contained a heptose residue and an aminodeoxyalditol residue bound to it. The presence in the mass spectrum of a fragment with *m/z* 263 and the corresponding secondary ions with *m/z* 231 and 199 as well as of fragments with *m/z* 45 and 89 indicated the *O*-methyl ether of a heptose. The final confirmation of the D-*glycero*-D-*manno*-heptose structure was provided by counterflow synthesis.

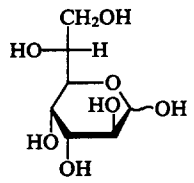


Several procedures for the synthesis of *glycero-manno*-heptose are known which employ conventional methods of synthesis of higher sugars. The most practicable is shown in Scheme 6.[75] Thus, benzyl 2,3,4-tri-*O*-benzyl-α-D-mannopyranoside **37** was subjected to Swern oxidation resulting in the aldehyde **38** whose interaction with HCN gave a mixture of isomers. The cyanohydrin **39** having the D-*glycero*-D-*manno*-configuration was isolated by chromatography and reduced to the amino sugar **40** (R = NH_2) which was further converted into the D-*glycero*-D-*manno*-heptose derivative **40** (R = OH) by treatment with nitrous acid. To obtain the L-*glycero*-isomer, the aldehyde **38** was condensed with allyloxymethylmagnesium bromide and the main isomeric alcohol **41** (R = allyl) having the L-*glycero*-configuration was chromatographically isolated from the resulting mixture; its deallylation gave a derivative of L-*glycero*-D-*manno*-heptose **41** (R = H).

Scheme 6



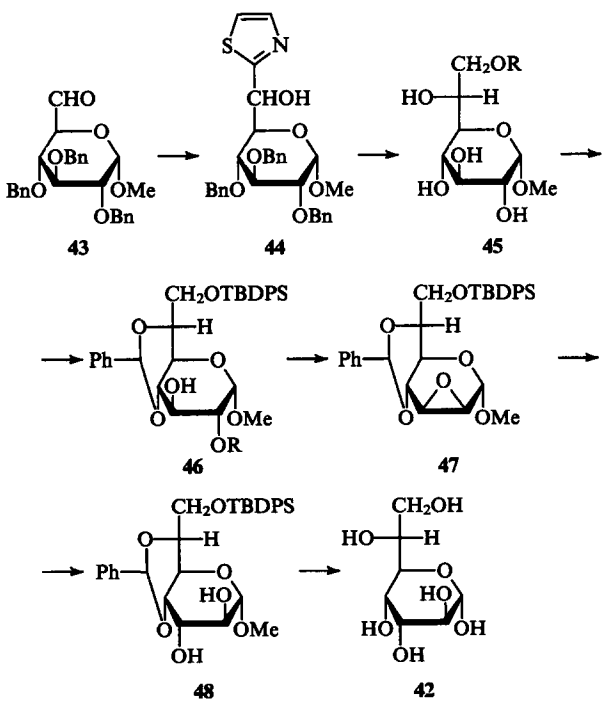
D-*Glycero*-D-*altro*-heptose **42** was found in PS of *Campylobacter jejuni* O23, together with 6-deoxy-D-*altro*-heptose and their 3-*O*-methyl ethers, the relative amount of these components of antigenic PS being dependent on both the bacterial strain and, seemingly, on cultivation conditions.[76] The heptose **42** present in the main chain of PS was isolated after acid hydrolysis of PS in a mixture with its 1,6- and 1,7-anhydro derivatives. The presence in the monosaccharide of the heptose skeleton was confirmed by the mass spectrum of the alditol obtained by reduction. To establish the configuration of the heptose, the polysaccharide was subjected successively to oxidation with a calculated amount of periodate, the glycol system of the C(6)–C(7) fragment is oxidised first, reduction with $NaBH_4$ and methanolysis. As a result, methyl D-altroside was isolated and identified corroborating the configuration of the heptose fragment C(2)–C(5). The configuration of C(6) was established by direct comparison of the corresponding alditol acetate with an authentic sample obtained from synthetic D-*glycero*-D-*altro*-heptose. A similar procedure was used to determine the structure of the 3-*O*-methyl ether of the heptose **42** present in the same PS.[76]



42

The synthesis of D-*glycero*-D-*altro*-heptose was carried out[77] by stereospecific elongation of the carbon chain at C(6) of the glucose derivative **43** by the method of Donadoni[78] with subsequent inversion of configuration at C(2) and C(3) (Scheme 7). The aldehyde **43** obtained by Swern oxidation of methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside was converted into the thiazole derivative **44** by reaction with 2-trimethylsilylthiazole. Treatment of the derivative **44** with formic acid and subsequent debenzylation resulted in a mixture of heptoses epimeric at C(6), from which the isomer having the D-*glycero*-configuration **45** (R = H) was isolated. This was converted into the 7-*O*-*tert*-butyldiphenylsilyl ether **45** (R = TBDPS) and then into the 4,6-*O*-benzylidene derivative **46** (R = H) which was further converted into the corresponding 2-*O*-tosylate **46** (R = Ts). The tosylate **46** was routinely converted into the 2,3-epoxy derivative **47** which after treatment with NaOH and *trans*-diaxial opening of the oxide ring gave the monosaccharide derivative **48** having the *altro*-configuration. Elimination of the protective groups resulted in D-*glycero*-D-*altro*-heptose **42** whose configuration was confirmed by NMR spectroscopic data. The synthetic monosaccharide and that isolated from natural PS were identical.[76]

Scheme 7

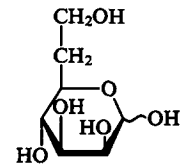


43 **44** **45** **46** **47** **48** **42**

5. Deoxyheptoses

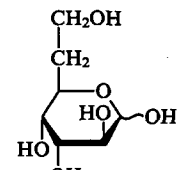
Only two representatives of this class have thus far been detected among monosaccharide components of O-antigenic PS. Both of them are 6-deoxyheptoses.

6-Deoxy-D-*manno*-heptose **49** was detected in PS of *Yersinia pseudotuberculosis* where it is attached as a side chain to the main chain.[54] The presence of a 6-deoxyheptose in PS was first proved by analysis of the ^{13}C NMR spectrum which contains signals characteristic for deoxyaldoses at 34.1 ppm (a methylene group of deoxyaldoses) and 59.0 ppm (a CH_2OH group of deoxyaldoses). The presence in PS of the 6-deoxyheptose **49** was also confirmed by mass spectrometric data of the *O*-methyl ether of the deoxyheptose glycoside obtained in the pure state after methanolysis of methylated PS.[54] This heptose was also found in *Pseudomonas pseudomallei* where it forms a homopolysaccharide.[35] Its structure and stereochemistry were confirmed by ^1H NMR spectroscopic data: in particular, the coupling constant values were consistent with the *manno*-configuration ($J_{2,3}$ = 3.5, $J_{3,4}$ = 9.1, $J_{4,5}$ = 9.5 Hz). The absolute configuration of **49** was established from the specific optical rotation. The structure of the monosaccharide **49** was also corroborated by counterflow synthesis.



49

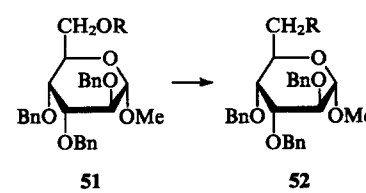
6-Deoxy-D-*altro*-heptose **50** was found in PS of *Campylobacter jejuni* O23A and O23B.[76] It was isolated in a mixture with its anhydro derivative after acid hydrolysis of PS. The structure of the monosaccharide **50** was confirmed by direct comparison with a synthetic sample of the corresponding alditol hexaacetate, and its D-configuration was established by GLC analysis of glycosides with (*R*)- and (*S*)-butan-2-ols.



50

The synthesis of 6-deoxy-D-*altro*-heptose[79] was performed as depicted in Scheme 8.

Scheme 8



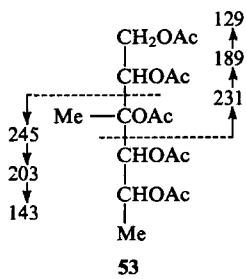
51 **52**

Methyl 2,3,4-tri-*O*-benzyl-α-D-altropyranoside **51** (R = H) was converted via 6-*O*-triflate **51** (R = CF_3SO_2) into the nitrile **52** (R = CN) which, in turn, was converted into a derivative of 6-deoxy-D-*altro*-heptose **52** (R = CH_2OH) by successive reduction with DIBAH, hydrolysis of the resulting imine and reduction of the corresponding aldehyde with $NaBH_4$. The corresponding derivative of 6-deoxy-D-*manno*-heptose was similarly obtained from the mannose derivative.[79]

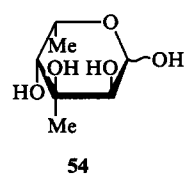
6. Branched monosaccharides

Monosaccharides having a branched carbon chain are a rare class of sugars. These monosaccharides are sometimes found in antibiotics, although some of their representatives were recently detected in O-antigenic PS where they can be localised both in the main chain of the biopolymer and in side chains.

Two representatives of this monosaccharide class were isolated in the pure state after acid hydrolysis of PS of the Gram-negative bacterium *Coxiella brunetti* phase I.[80] One of those appeared to be identical by its chromatographic characteristics to 6-deoxy-3-*C*-methylgulose (virenose) earlier detected in the antibiotic virenomycin.[81] The structure of the monosaccharide isolated from the O-antigenic PS was also confirmed by the mass spectrum of the fully acetylated alditol **53**. Fragmentation of this compound corresponded to the structure of the parent monosaccharide; in particular, the presence of fragments with *m/z* 231 and 245 and the corresponding secondary fragments indicated the type and the site of the chain branching. Direct comparison of the natural monosaccharide with the samples of the corresponding monosaccharides of the *manno*-, *talo*- and *gulo*-series isolated from other natural products demonstrated its *gulo*-configuration. However, the specific optical rotation of this monosaccharide differed from that of virenose, for which the D-configuration had been established.[81] Based on these data, the monosaccharide from PS of *Coxiella brunetti* was ascribed the structure of 6-deoxy-3-*C*-methyl-L-gulose **54**.

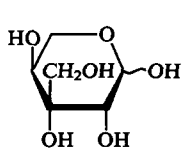


53

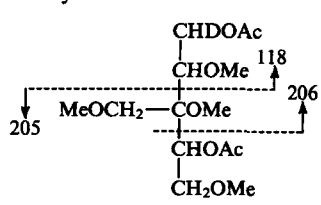


54

The second monosaccharide isolated from O-antigenic PS of *Coxiella brunetti* was assigned the structure of 3-*C*-hydroxymethyl-L-lyxose **55**. Successive methylation of PS, acid hydrolysis of methylated PS, reduction of the resulting mixture of methylated monosaccharides with $NaBD_4$ and their acetylation resulted in a mixture of alditol acetates, from which the acetate of the partially methylated alditol **56** corresponding to the branched monosaccharide was isolated. Its fragmentation in mass spectrometric analysis fully confirmed the structure of the skeleton and the positions of the hydroxy groups in the monosaccharide as can be evidenced, in particular, from the presence of fragments with *m/z* 205, 206 and 118. Determination of configuration of the monosaccharide **55** and its assignment to the L-lyxose series was made by the authors on the basis of synthetic data.

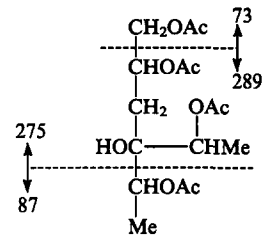


55



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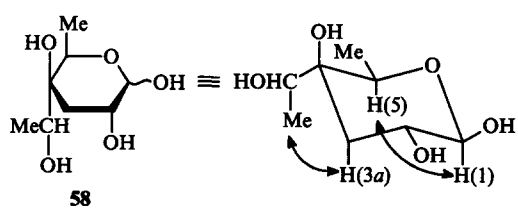
Two other structurally complex monosaccharides belonging to branched octoses were found in O-antigenic PS of several serotypes of *Yersinia*. They were isolated in the individual state after acid hydrolysis of PS and found to be similar in structure with the only difference the configuration of one of the chiral centres. One of those, yersiniose A, was isolated from *Y. pseudotuberculosis* serovar VI,[82] while the second one, yersiniose B from *Y. enterocolitica* serovar O4,32.[83] Reduction of yersinioses A and B with $NaBH_4$ and subsequent acylation resulted in tetraacetates of the corresponding alditols **57**. In this case, one of the hydroxy groups failed to be acetylated under standard conditions that showed its localisation at the tertiary carbon atom. The mass spectra of both alditol tetraacetates appeared to be identical; their fragmentation demonstrated that both yersinioses are 3,6-dideoxy-4-*C*-(1′-hydroxyethyl)hexoses with the identical structure of the carbon skeleton and positions of the hydroxy groups. The same result was obtained by mass spectrometry of methyl glycosides of both monosaccharides.



57

The NMR spectra of yersinioses A and B also appeared to be almost identical. Thus, the ^{13}C NMR spectrum of yersiniose A contained signals at 13.4 and 16.4 (two CH_3 groups), 35.9 (CH_2 group) and 75.8 ppm (tertiary carbon atom). The signal of C(1) with a low-field chemical shift indicated the D-configuration of the monosaccharide. The ^1H NMR spectrum of yersiniose A contained signals at 4.71 [$J_{1,2}$ = 8.25 Hz, H(1)], 3.80 [$J_{2,3e}$ = 5.22 and $J_{2,3a}$ = 11.82 Hz, H(2)], 1.85 [$J_{3a,3e}$ = 13.86 Hz, H(3)], 4.18 [$J_{5,6}$ = 6.63 Hz, H(5)] and 1.30 ppm [H(6)].

Higher values of the coupling constants ($J_{1,2}$ and $J_{2,3a}$) for yersiniose B present in the β-form show the axial orientation of H(2), whereas NOE data revealed interaction between H(1) and H(5) [i.e., the axial position of H(5)] and the proximity of H(3*a*) and the CH_3 group of the side chain, thus indicating the equatorial position of the side chain at C(4). These data suggest that yersiniose B has the *xylo*-configuration. Comparison of the ^{13}C NMR spectra for yersinioses A and B revealed that both of them are 3,6-dideoxy-4-*C*-(1′-hydroxyethyl)-D-*xylo*-hexopyranoses **58**. The only distinction between them is in the stereochemistry of the asymmetric centre C(7) in the side chain. This problem was solved by counterflow synthesis of the yersinioses.



58

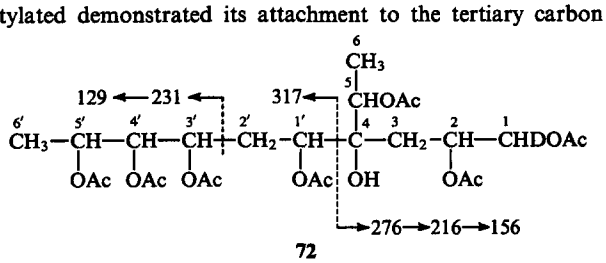
To this end, a general procedure for the synthesis of 3,6-dideoxy-4-C-(1′-hydroxyethyl)hexoses was elaborated which, in principle, permits the synthesis of any kind of branched octoses of this type. Leaving aside the consideration of relationships between different configurations of the chiral centres in isomeric octoses of this series (see Ref. 84), it is worth noting that the solution of the particular problem of determination of the absolute configuration of the chiral centre C(7) of the isomeric yersinioses, required a stereospecific synthesis of compounds having the D-*xylo*-configuration and distinguished by only the geometry of the chiral centre present in the side chain.

This rather complex synthesis [84,85] is depicted in Scheme 9. As the initial compound, 1,6-anhydro-β-D-glucopyranose (levoglucosan **59**) was used, which via a standard set of reactions was converted into 4-*O*-allyl-3-*O*-mesyl-2-*O*-tosyllevoglucosan **60** and then into 1,6:2,3-dianhydro-4-*O*-allyl-β-D-allopyranose **61**. Reduction of the epoxide **61** and subsequent *O*-benzylation gave 1,6-anhydro-4-*O*-allyl-2-*O*-benzyl-3-deoxy-β-D-*ribo*-hexose **62**. Elimination of the allyl protective group and Swern oxidation of the liberated hydroxy group gave the key intermediate of the synthesis, a derivative of the keto sugar **63**. Its reaction with 2-lithium-2-methyl-1,3-dithiane resulted in a mixture of two alcohols **64a** and **64b** epimeric at C(4) which were separated chromatographically. The structure of the isomers differing in only the configuration at C(4) was established by NMR spectroscopy (including NOE measurements). It appeared that the alcohol **64** has the D-*xylo*-configuration of the pyranose ring; namely, this compound was selected for further synthesis.

Treatment of the alcohol **64a** with a mixture of $HgCl_2$ and $CdCO_3$ gave the ketone **65** which after reduction with $NaBH_4$ and subsequent benzylation produced two epimeric (with regard to the chiral centre of the side chain) alcohols **66** and **67** in a 2 : 1 ratio. The absolute configuration of the new chiral centre C(7) in the alcohols **66** and **67** was established by spectroscopic analysis and NOE measurements of isopropylidene derivatives obtained by debenzylation of the initial alcohols **66** and **67**. The side chain of the monosaccharide derivative **67** appeared to have the D-*glycero*-configuration, and that of **66** the L-*glycero*-configuration.

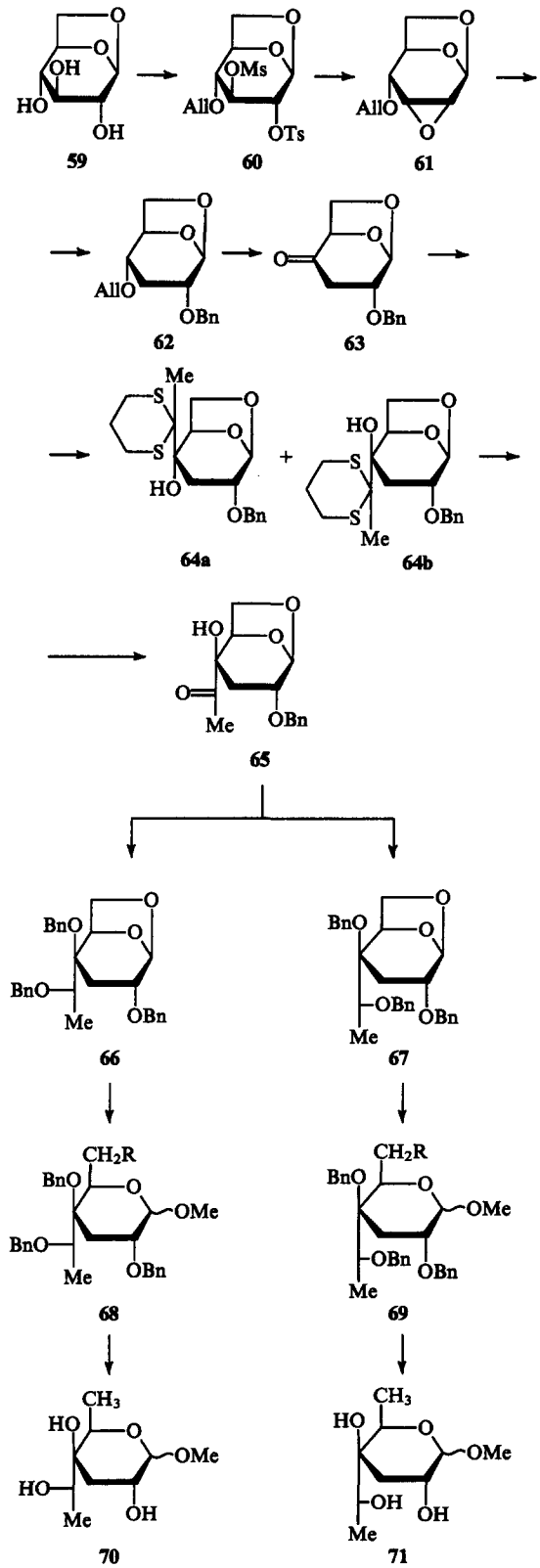
To convert the alcohols **66** and **67** into the corresponding yersinioses, the former were exposed to very mild acetolysis and subsequent methanolysis which opened the 1,6-anhydro ring without any accompanying elimination of the *O*-benzyl groups (which might result in a complex mixture of pyranose and furanose forms of yersinioses). The resulting glycosides **68** and **69** (R = OH) with a free hydroxy group at C(6) were thus obtained. This group was substituted with bromine by treatment with Ph_3P-CBr_4 in pyridine. After separation and catalytic hydrogenolysis with simultaneous removal of the benzyl groups, the resulting bromides **68** and **69** (R = Br) were converted into glycosides of the synthetic yersinioses **70** and **71**. Their comparison with the corresponding derivatives of natural yersinioses isolated from PS using various versions of NMR spectroscopy (of which NOE analysis was the most important one) allowed establishment of the configuration of the chiral centre of C(7) in both monosaccharides. It was found that yersiniose A is 3,6-dideoxy-4-*C*-(L-*glycero*-1′-hydroxyethyl)-D-*xylo*-hexopyranose, while yersiniose B 3,6-dideoxy-4-*C*-(D-*glycero*-1′-hydroxyethyl)-D-*xylo*-hexopyranose.

A structurally unique higher monosaccharide with a branched chain has recently been detected in PS of *Pseudomonas caryophilli*.[86,87] This monosaccharide was isolated in the pure state after acid hydrolysis of PS and called caryophillose. Its ^{13}C NMR spectrum suggested the presence of six CHOH groups (signals around 68–78 ppm), two CH_3 groups (signals in the region 16–17 ppm), two methylene groups (signals in the region 29–32 ppm) and one tertiary carbon atom carrying a hydroxy group (signal at 74 ppm). Reduction of caryophillose with $NaBD_4$ resulted in a branched alditol which upon acetylation gave the heptaacetate **72**. The fact that one of the hydroxy groups was not acetylated demonstrated its attachment to the tertiary carbon



72

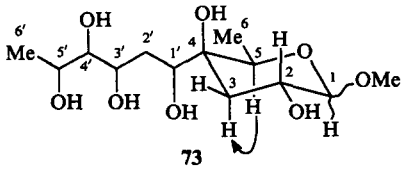
Scheme 9



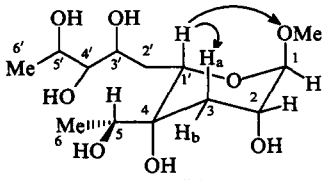
atom. The type of fragmentation in the mass spectrum of the acetate **72** as well as ^{13}C NMR spectroscopic data indicated that caryophillose comprises 12 carbon atoms, two methyl groups and two methylene units, has a branched chain and is a 3,6-dideoxy-4-*C*-(1′,3′,4′,5′-tetrahydroxyhexyl)hexose.

Determination of the stereochemistry of caryophillose was a challenge. This problem was solved using NMR spectroscopy (including NOE measurements).

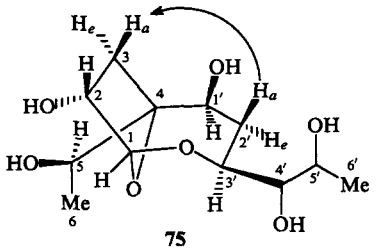
Briefly, the essence of this complex study is as follows. Acid methanolysis of the monosaccharide gave two isomeric methyl glycosides of caryophillose **73** and **74**, and one internal glycoside **75**.



73



74

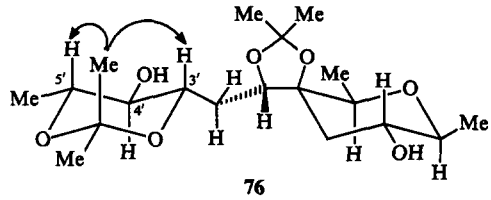


75

The glycoside **73** formed by closing of the pyranose ring via the hydroxy group at C(5) was isolated as a mixture of α- and β-anomers and had the $^4C_1$ conformation of the pyranose cycle (as could be evidenced from NMR spectroscopic data). Its ^{1}H NMR spectrum suggested the lack of protons at C(4), thus confirming the site of branching of the carbon chain determined from the mass spectrum of the alditol **72**. An NOE study revealed interaction between H(5) and H(3*a*) in the glycoside **73** which proved the axial position of H(5). The equatorial position of the aliphatic chain C(1′)–C(6′) was evident from the spatial proximity of H(5) and H(1′) of the side chain. Based on these data, the pyranose ring and, correspondingly, the C(1′)-C(6′) fragment of caryophillose were assigned the *xylo*-configuration. To establish the spatial structure of this aliphatic fragment of caryophillose was a more complicated task which was solved by complex analysis of the NMR spectra of the glycosides **74** and **75** and of some other derivatives.

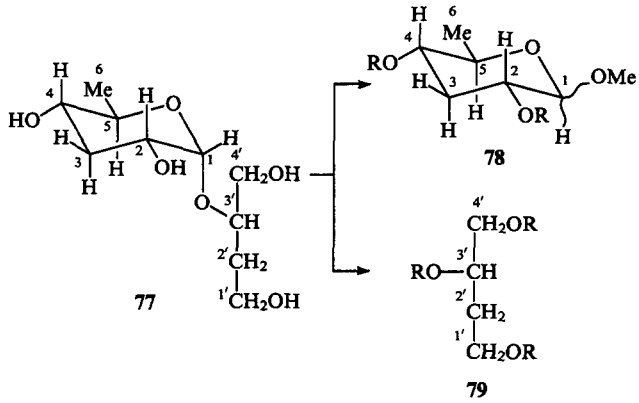
The glycoside **74** isomeric with the glycoside **73** was formed by closing of the pyranose ring via the hydroxy group at C(1′) of caryophillose (this was accompanied by stereospecific formation of the β-glycoside alone). The data of the ^{1}H NMR spectrum suggest the glycoside **74** to be in the $^1C_4$ conformation. NOE measurements with the glycoside **74** and its *O*-acetate reveal that H(1′) is closed to the axial methoxy group and, consequently, occupies the axial position as well. Hence, C(1′) in the C(1′)–C(6′) chain has the (*S*)-configuration.

The glycoside **75**, isolated by acid methanolysis of caryophillose, is formed as a result of intramolecular glycosylation involving the hydroxy group at C(3′). Glycosylation gives a bicyclic system containing a furanose ring. The structure of the glycoside **75** was reliably established: the compound has the composition $C_{12}H_{22}O_7$ (as can be evidenced from the FAB mass spectrum); its ^{13}C NMR spectrum is completely consistent with the structure **75**. In the ^{13}C NMR spectrum of the acetate of **75**, the signal of the proton at C(3′) (3.88 ppm) lacked the characteristic shift which might result from the presence of a heminal acetoxy group; consequently, C(3′) is linked by an acetal (i.e., glycosidic) bond corroborating the accepted structure of the glycoside **75**. The conformation of the glycoside **75** was established by NOE measurements for the protons at C(2) and C(3) as well as at C(1′), C(2′) and C(3′) in the glycoside **75** itself and its *O*-acetate, which suggest, in particular, that C(3′) has the (*S*)-configuration. To establish the relative configuration at C(4′) and C(5′) of caryophillose, the 4,1′:3′,5′-di-*O*-isopropylidene derivative **76** was prepared from the glycoside **73**. NOE measurements for H(3′) and H(5′) in the 3′,5′-*O*-isopropylidene group, forming 1,3-dioxane ring with the chair conformation, show their *cis*-diaxial orientation and *trans*-diaxial position relative to H(4′). Therefore, it was concluded that C(4′) and C(5′) have the (*R*)-configuration.



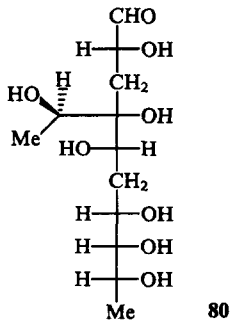
76

The absolute configuration of the C(1)–C(6) and C(1′)–C(6′) fragments of caryophillose was established mainly on the basis of circular dichroism measurements for each of these fragments.[88] Methylation analysis revealed that in the PS chain the neighbouring residues of caryophillose are linked by the glycosidic bonds at the hydroxy group localised at C(3′). Therefore, periodate oxidation of the original PS results in the cleavage of the bonds between C(4′) and C(5′) as well as between C(1′) and C(4). As a result, reduction of the oxidation product gives rise to compound **77**, which is a glycoside of 3,6-dideoxy-α-*ribo*-hexose (paratose), and 1,4-dihydroxybutan-3-ol. The structure of this compound was confirmed by NMR spectroscopy. Methanolysis of the glycoside **77** gives α- and β-glycosides of paratose **78** (R = H) and 1,3,4-trihydroxybutane **79** (R = H). Analysis of the circular dichroism spectra by the method of Nakanishi[88] was carried out for the *p*-bromobenzoates **78** and **79** (R = *p*-BrC_6H_4CO). A change in the Cotton effect as a function of wavelength indicates that C(2) in the glycoside **78** has the (*R*)-configuration, and C(3′) in **79** the (*S*)-configuration.



77 **78** **79**

These data suggested that the caryophillose present in PS is 3,6-dideoxy-4-*C*-(D-*altro*-tetrahydroxyhexyl)-D-*xylo*-hexopyranose **73**, which is a cyclic form of the higher branched sugar, 3,6,10-trideoxy-4-*C*-[(*R*)-1′-hydroxyethyl]-D-*erythro*-D-*gulo*-decaose **80**.



80

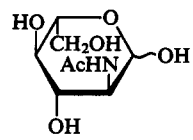
7. Amino sugars

Monosaccharides containing amino groups (amino sugars) of various structure are widespread in bacterial PS. Many of them contain several deoxy units and amino groups in various combinations which contributes largely to the great diversity of known natural amino sugars.

a. Aminodeoxyhexoses

Derivatives of 2-amino-2-deoxyhexoses having the D-*gluco*-, D-*galacto*- or D-*manno*-configuration (glucosamine, galactosamine and mannosamine) are among the most common components of PS of microorganisms. Most often, they are present in the polysaccharide chain as *N*-acetyl derivatives, although *N*-acyl derivatives comprising residues of other organic acids are also known (see Section 4).

At the same time, O-antigenic PS have been found to contain only one representative of 2-amino-2-deoxyhexoses with the L-configuration, namely, 2-acetylamino-2-deoxy-L-glucose **81** detected in PS of *Pseudomonas cepacia* O1.[89]

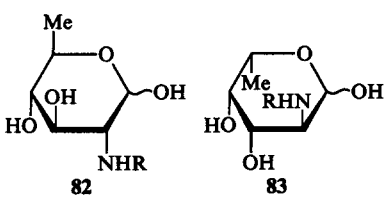


81

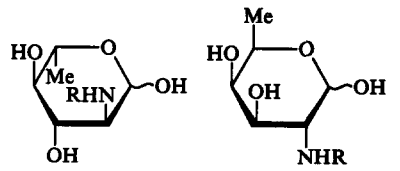
The monosaccharide **81** gave qualitative reactions for amino sugars; the mass spectrum of the aminodeoxyalditol acetate derived was identical to that of the well-known derivative obtained from the D-isomer. The fragmentation observed demonstrated the presence of an acetylamino group at C(2). This finding was also confirmed by ^{13}C NMR spectroscopic data: the spectrum of PS contained a signal of C(2) at 54.23 ppm. Finally, the structure of this amino sugar was also corroborated by analysis of a disaccharide fragment obtained after Smith degradation of PS. The unusual L-configuration of the monosaccharide was established on the basis of GLC of its glycosides with optically active butan-2-ol and octan-2-ol, by its specific optical rotation[80] and by comparison of the circular dichroism spectrum of the derived aminodeoxyalditol with that of the corresponding D-isomer.

b. Aminodideoxyhexoses

Derivatives of 2-amino-2,6-dideoxyhexoses are widespread in O-antigenic PS. Two of these, 2-amino-2,6-dideoxy-D-glucose (D-quinovosamine) **82** and 2-amino-2,6-dideoxy-L-galactose (L-fucosamine) **83** are common components of O-antigenic PS.[2] Their enantiomers, L-quinovosamine **84** and D-fucosamine **85** occur less frequently. L-Quinovosamine was found in PS of *Shigella boydii, Proteus vulgaris, Pseudomonas fluorescens*, etc.,[2] while D-fucosamine in *Pseudomonas fluorescens* and some species of *Pseudomonas aeruginosa*.[2] These 2-amino-2,6-dideoxyhexoses are present in the polysaccharide chains as various *N*-acyl (most frequently *N*-acetyl) derivatives. They can be isolated in the pure state from acid hydrolysates of PS or after solvolysis of PS with liquid HF. Currently, these monosaccharides are identified directly by the NMR spectra of PS themselves.

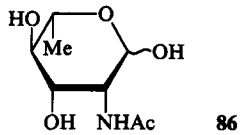


82 **83**



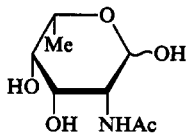
84 **85**

An example of more rare amino sugars of this type present in microbial PS is 2-acetylamino-2,6-dideoxy-L-mannose **86** (L-rhamnosamine) detected in *Escherichia coli* O3.[90] The monosaccharide was isolated in the pure state after acid hydrolysis. Its structure was established by classical methods: periodate oxidation yielded acetaldehyde, thus suggesting the presence of a methyl group (the 6-deoxy fragment); based on different ion-chromatographic behaviour of the monosaccharide and the derivatives of other 2-amino-2,6-dideoxy sugars, the monosaccharide was assigned the structure of an L-rhamnose derivative.



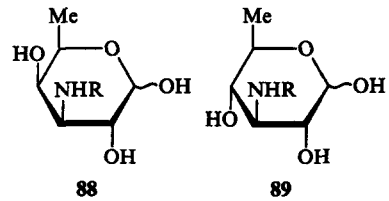
86

It is interesting to note that another very rare monosaccharide of this class, 2-acetylamino-2,6-dideoxy-L-talose **87** (pneumosamine), was identified long ago in a capsular polysaccharide of *Streptococcus pneumoniae* type 5; however, its structure was not strictly confirmed.[91]



87

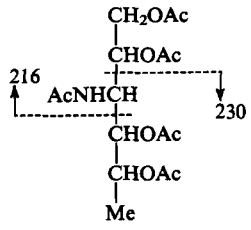
Among other monoaminodideoxyhexoses, O-antigenic PS contain derivatives of 3-amino-3,6-dideoxyhexoses present in the PS chain as *N*-acyl derivatives (most frequently, as acetyl but sometimes also as derivatives of hydroxy and amino acids; see Section 4). Among monosaccharides of this type, derivatives of 3-amino-3,6-dideoxy-D-galactose **88** (3-amino-3-deoxy-D-fucose) are rather common components of many PS.[2] An analogue of 3-amino-3,6-dideoxy-D-galactose, 3-amino-3,6-dideoxy-D-glucose **89** (3-amino-3-deoxyquinovose), was found in O-antigenic PS of several species of Gram-negative bacteria: in *Escherichia coli*,[92] *Hafnia alvei*,[93] *Proteus penneri*,[94] *Pseudomonas fluorescens*,[33] *Vibrio mimicus*.[95] The L-isomer, 3-amino-3,6-dideoxy-L-glucose, was isolated from O-antigenic PS of *Vibrio anguillarum*.[96] This is the only example of 3-amino-3,6-dideoxyhexoses with the L-configuration detected in O-antigenic PS. This sugar was earlier detected in the LPS core[97] of *Aeromonas hydrophila* III.



88 **89**

Derivatives of these aminodideoxyhexoses including those of glucose with the L-configuration were isolated from PS in the individual state after acid hydrolysis or solvolysis with liquid HF and, to simplify analysis, converted into *N*-acetates. To this end, they were deacylated (the *N*-acyl substituent was eliminated) and then *N*-acetylated by a routine procedure. The structure of the 3-acetylamino-3,6-dideoxyhexoses thus obtained was established by physicochemical methods and confirmed by counterflow synthesis.

An example is the establishment of the structure of 3-acetylamino-3,6-dideoxy-D-glucose.[95,98] The mass spectrum of the full acetate of the aminodeoxyalditol **90** derived from the amino sugar by reduction and subsequent acetylation contained fragments with *m/z* 230 and 216 which uambiguously indicated the position of the acetylamino group at C(3) and of the deoxy unit (CH_3 group) at C(6). This finding demonstrated that the parent sugar has the 3-acetylamino-3,6-dideoxyhexose structure.



90

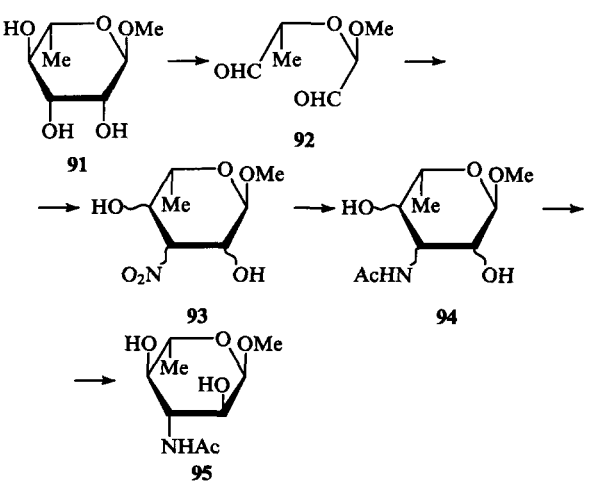
This conclusion was also confirmed by the ^{13}C NMR spectrum containing signals at 18 ppm (CH_3 group) and 55–58 ppm [NHAc-substituent at C(3)]. The ^1H NMR spectrum of the full acetate of the amino sugar glycoside was characterised by the coupling constant values of $J_{2,3}$, $J_{3,4}$ and $J_{4,5}$ exceeding 10 Hz, thus suggesting the *gluco*-configuration of the monosaccharide. Finally, the specific optical rotation ($[\alpha]_D = +26°$) showed that it belongs to the D-series.

The structure of 3-acetylamino-3,6-dideoxy-D-galactose[99] was also confirmed by analysis of the mass spectrum of the corresponding aminodeoxyalditol acetate which exhibited characteristic fragmentation. The ^{13}C NMR spectrum of this compound contained signals at 17 and 52 ppm, and the ^1H NMR spectrum demonstrated the *galacto*-configuration (the coupling constant values were: $J_{2,3} = 10$, $J_{3,4} = 4$, $J_{4,5} = 1$ Hz). The D-configuration was established by comparison with a synthetic authentic sample and confirmed by the specific optical rotation. It is interesting to note that initially this monosaccharide was erroneously assigned the D-*gluco*-configuration,[100] and this error was corrected later.[98]

The structure of 3-acetylamino-3,6-dideoxy-L-glucose, the only monosaccharide in this group having the L-configuration,[96,97] was established by the same methods, and its absolute configuration determined by circular dichroism.

Several methods for the synthesis of 3-amino-3,6-dideoxyaldoses are known. These compounds were obtained by the classical Fischer–Bayer method (condensation of dialdehydes prepared by oxidation of 6-deoxyhexose glycosides with nitromethane followed by reduction of the nitro group). As an example, we refer to the synthesis of a derivative of 3-acetylamino-3,6-dideoxy-L-glucose (Scheme 10).[101]

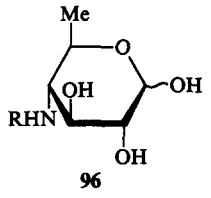
Scheme 10



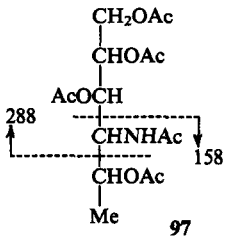
Methyl-α-L-rhamnopyranoside **91** was oxidised by periodate, and the dialdehyde **92** derived was condensed with nitromethane in the presence of sodium methoxide. The resulting isomeric 3-nitrohexoses **93** were reduced over Raney nickel; the corresponding amino sugars were *N*-acetylated, and then methyl 3-acetyl-amino-3,6-dideoxy-α-L-glucopyranoside **95** was isolated from the resulting mixture of the amino acetates **94** by chromatography in a 26% yield. Other aminodeoxyhexoses can be synthesised by conventional methods based on inversion of the configuration at certain carbon atoms (e.g., see Ref. 102).

The third group of amino sugars present in O-antigenic PS are derivatives of 4-amino-4,6-dideoxyhexoses, although they seem to be less common than their 3-amino analogues. By now, three hexoses of this type having the D-*gluco*-, D-*manno*- or D-*galacto*-configuration have been identified. The amino groups in these monosaccharides are acylated (usually, acetylated).

4-Amino-4,6-dideoxy-D-glucose **96** (4-amino-4-deoxy-D-quinovose, viosamine) was first identified as the *N*-acetyl derivative in PS of *Escherichia coli* O7 where it is present in the main chain.[103] As an *N*-acyl derivative carrying the *N*-acetylglycine residue, it was also found in PS of *Shigella dysenteriae* 7,[104,105] and as the *N*-formyl derivative in PS of *Francisella tularensis* 15 (tularemia pathogen).[106] Recently this sugar was also detected in PS of *Vibrio anguillarum*[107] and in O-antigenic PS of *Hafnia alvei* 1205 having an unusual polysaccharide chain similar to that of teichoic acids.[108]



96

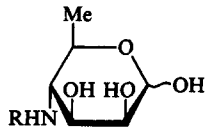


97

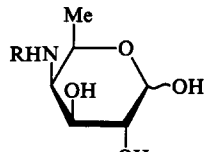
The monosaccharide **96** was isolated as a structurally complex *N*-acylated derivative after treatment of PS with liquid HF and, to simplify analysis, further converted into the *N*-acetyl derivative. Its structure was studied by physicochemical methods. The mass spectum of the fully acetylated aminodeoxyalditol **97** derived contained fragments with *m/z* 288 and 158 as well as the corresponding series of secondary ions indicating the presence of a deoxy unit at C(6) and of an acetylamino group at C(4). Such a structure was confirmed by ^{13}C NMR spectroscopic data [signals at 18.0 ppm for a CH_3 group and at 58.2 ppm for C(4)]. The *gluco*-configuration of the monosaccharide was established by ^1H NMR spectroscopic data, and the absolute configuration was confirmed by the specific optical rotation ($[\alpha]_D = +17°$) which almost coincided with the corresponding value for the synthetic sample (see below).

At present, identification of this aminodeoxy sugar (as well as of its isomers) is performed almost exclusively on the basis of NMR spectral data of the initial PS themselves.

4-Amino-4,6-dideoxy-D-mannose **98** (4-amino-4-deoxy-D-rhamnose, perosamine) was first detected as an *N*-acyl derivative with 2-deoxy-L-*glycero*-tetronic acid in O-antigenic PS of *Vibrio cholera* Inaba (Asian cholera pathogen) where it forms a homopolysaccharide.[109] Later, it was found as the *N*-formyl derivative in a homopolysaccharide in O-antigenic PS of *Brucella melitensis* (brucellosis pathogen).[110] As some other *N*-acyl derivatives, it is present in PS of several species of *Salmonella*,[111,112] *Escherichia coli*,[113,114] *Yersinia*[115] and *Xanthomonas*.[116] Since 4-amino-4,6-dideoxyhexoses are very unstable in acid media, they were isolated as *N*-acyl derivatives exclusively by solvolysis of PS with liquid HF.



98



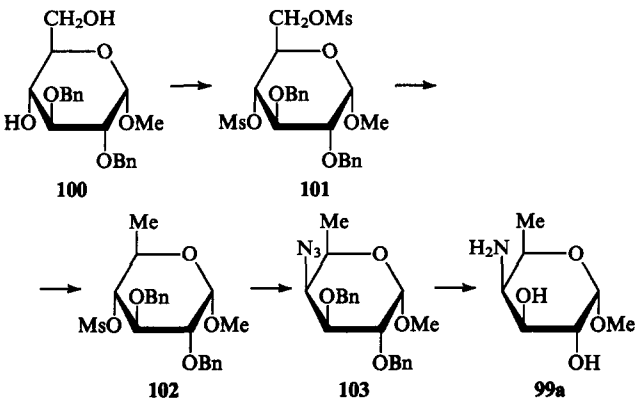
99

The monosaccharide **98** was identified by the mass spectrum of the corresponding aminoalditol acetate which appeared to be identical with that of the alditol derived from the *gluco*-analogue. That this monosaccharide belongs to the *manno*-series was first demonstrated by deamination with nitrous acid of deacetylated PS: hydrolysis of deaminated PS revealed the presence of D-rhamnose and 6-deoxy-D-allose which could be formed only when the original monosaccharide contained the NH_2 group at C(4) and had the *manno*-configuration.[109] Later, it was confirmed by ^1H NMR spectroscopic analysis of the PS itself that this monosaccharide belongs to the *manno*-series (the coupling constant values were: $J_{2,3} = 4.5$, $J_{3,4} = 10$, $J_{4,5} = 10$ Hz). The absolute configuration of the monosaccharide was established by its conversion into a glucoside with (−)-butan-2-ol.

4-Amino-4,6-dideoxy-D-galactose **99** (4-amino-4-deoxy-D-fucose, tomosamine) occurs less frequently and has thus far been found only in O-antigenic PS of *Escherichia coli* O10[117] and *Pseudomonas fluorescens*.[118] In the former PS the amino sugar is *N*-acylated by the residue of 3-hydroxybutyric acid and localised in the side chain attached to the main chain of the PS; in the latter case the PS is *N*-acetylated. The amino sugar was isolated after solvolysis of PS with liquid HF; its structure was established by ^1H NMR spectroscopic data: the position of the *N*-acyl group was poved by a low-field shift of H(4), and the *galacto*-configuration was concluded from the coupling constant values: $J_{2,3} > 10$, $J_{3,4} \sim 4.5$, $J_{4,5} \sim 1.8$ Hz.[118]

Synthesis of several 4-amino-4,6-dideoxyhexoses was carried out by using a general approach well-known in sugar chemistry: introduction of an amino function into a neutral monosaccharide via the corresponding azide. By illustration, let us consider the synthesis of tomosamine and viosamine[119] derivatives from glucose (Scheme 11).

Scheme 11



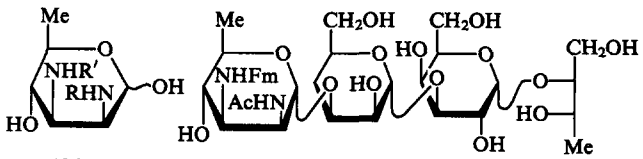
100 101 102 103 99a

Methyl-2,3-di-*O*-benzyl-α-D-glucopyranoside **100** was converted into the dimesylate **101**, which after interaction with KI, hydration of the corresponding monoiodide and repeated mesylation gave the 4-*O*-mesylate of 6-deoxyglucose **102**. The reaction of the compound **102** with sodium azide accompanied by inversion of the configuration gave the azide **103** hydration of which was accompanied by removal of the benzyl groups to yield the glycoside of tomosamine **99a**. To obtain the *gluco*-analogue, double inversion at C(4) was used: the mesylate **102** was converted into a galactose derivative by reaction with sodium benzoate. The derivative of 4-amino-4,6-dideoxyglucose **96** (R = H) (viosamine) was obtained via the corresponding azide after debenzoylation and mesylation of the galactose derivative.

c. Diaminotrideoxyhexoses

PS of some Gram-negative bacteria were found to contain amino sugars of unusual structure, which belong to 6-deoxyhexoses containing two amino groups at C(2) and C(3) or at C(2) and C(4) and only one hydroxy group. Diaminotrideoxyhexoses are distinguished by high lability to acids and usually cannot be isolated in the pure state. Their structure was mostly established by NMR spectroscopic and mass spectrometric data for oligosaccharide fragments isolated by partial cleavage of the corresponding PS.

The only representative of 2,3-diamino-2,3,6-trideoxyhexoses in O-antigenic PS is a derivative of 2,3-diamino-2,3,6-trideoxy-D-mannose **104** (2,3-diamino-2,3-dideoxy-D-rhamnose) containing *N*-formyl and *N*-acetyl substituents at the amino groups at C(3) and C(2), respectively.



104 105

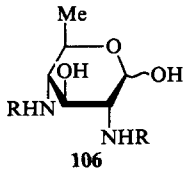
This monosaccharide is a component of PS of *Escherichia coli* O119, where it occupies the position of a lateral substituent of the polysaccharide chain and is an immunochemical determinant.[120] Its structure was established on the basis of the following data. The PS was oxidised two times with periodate, and the subsequent Smith degradation resulted in the oligosaccharide **105** containing a diaminotrideoxyhexose residue. This finding was confirmed by mass spectrometric data: the FAB mass spectrum of the PS contained a fragment with *m/z* 215 suggesting the presence of a diaminotrideoxyhexose residue carrying *N*-formyl and *N*-acetyl substituents as well as the corresponding secondary fragments, thus indicating the localisation of the formyl residue at the amino group at C(3). The complex NMR spectra of the tetrasaccharide **105** contained signals of the diaminotrideoxyhexose residue. In the ^{13}C NMR spectra the signals of C(2) and C(3) (52.11 and 53.03 ppm) demonstrated the presence of the acylamino groups at these atoms. The signals at 165 and 168 ppm indicated the presence of a formylamino group, and the signal at 176.36 the presence of an acetylamino group. The signal at 17.86 ppm corroborated the presence of the CH_3 group at C(6). Application of the INEPT technique to NMR spectroscopic analysis revealed localisation of the *N*-acetyl group at C(2) and the *N*-formyl group at C(3).

In the ^1H NMR spectra the coupling constant values ($J_{2,3} = 4.0$, $J_{3,4} = 10.3$, $J_{4,5} = 9.5$ and $J_{5,6} = 6.1$ Hz) suggested the *manno*-configuration for the monosaccharide.

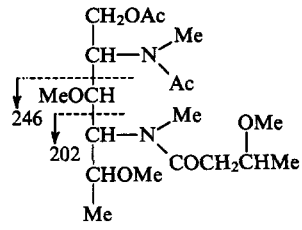
The absolute configuration of the monosaccharide **104** was evident from the NOE data: irradiation of H(1) of this sugar caused a strong effect on the adjacent H(2) in the chain of the oligosaccharide **105** mannose residue. This finding suggested that both monosaccharides have the same absolute configuration. Since mannose had the D-configuration, the novel monosaccharide was also assigned the D-configuration. Therefore, the novel sugar was identified as a derivative of 2,3-diamino-2,3,6-trideoxy-D-mannose.

2,4-Diamino-2,4,6-trideoxyhexoses are more common in PS. Thus, derivatives of 2,4-diamino-2,4,6-trideoxy-D-glucose **106** (bacillosamine) were first detected in PS of the cell wall of *Bacillus licheniformis*[121] and, later, in O-antigenic PS of several species of *Pseudomonas aeruginosa*,[122] *Pseudomonas aurantiaca*,[123] *Vibrio cholerae* O3[58,124] and *Fusobacterium necroforum*.[125] A derivative of this amino sugar with the acetylated amino group at C(2) and the amino group at C(4) acylated with 3-hydroxybutyric acid was isolated in the pure state from PS of *P. aeruginosa* O3 only after

solvolysis with liquid HF.[122] The ^{13}C NMR spectrum of the monosaccharide indicated the presence of a CH_3 group (18.3 ppm) at C(6) and of amino groups at C(2) and C(4) (57.6 and 56.8 ppm). High coupling constant values for the ring protons ($J_{2,3}$, $J_{3,4}$ and $J_{4,5} \approx 10$ Hz) demonstrated unambiguously the *gluco*-configuration of this monosaccharide. The absolute D-configuration was evident from the specific optical rotation ($[\alpha]_D = +36°$). All the characteristic signals of the NMR spectra of the monosaccharide **106** mentioned above were also found in the spectra of the PS itself and of its fragments.



106

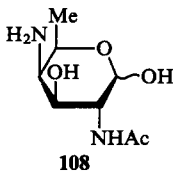


107

For final establishment of the position of the amino groups and localisation of the acyl substituents by routine degradation of PS, the alditol **107** was obtained by solvolysis with anhydrous HF followed by reduction with $NaBH_4$ and methylation involving the NH groups. The mass spectrum of the alditol **107** contained fragments with *m/z* 202 and 246 which confirmed the presence of an *N*-acetyl group at C(2) and of a *N*-hydroxybutyryl group at C(4).[122]

In subsequent studies, this diamino sugar was not isolated and its identification was carried out by NMR spectroscopy of the initial PS. Mass spectrometry of PS fragments obtained by their cleavage made it possible to establish the structure and localisation of *N*-acyl groups. For instance, it was found that bacillosamine in PS of *Vibrio cholerae* O3 is acetylated at the amino group at C(2) and acylated by the residue of 3,5-dihydroxyhexanoic acid at the amino group at C(4) (see Section 4).[58]

2,4-Diamino-2,4,6-trideoxy-D-galactose (**108**), a monosaccharide isomeric with bacillosamine and having an acetylated amino group at C(2), was found in O-antigenic PS of *Shigella sonnei*.[126] The monosaccharide **108** failed to be isolated in the pure state even by solvolysis with HF, and its structure was determined on the basis of the ^{13}C NMR spectrum of PS which contained signals similar to those observed for its *gluco*-isomer: 17.3 (CH_3 group), 56.6 and 53.0 ppm [C(2) and C(4) substituted by the amino groups]. The D-configuration was assigned to the monosaccharide based on the results of deamination and subsequent hydrolysis of PS. The formation as the main product of 2-acetylamino-2,6-dideoxy-D-glucose and (after reduction) of small amounts of a mixture of 2-acetylamino-2,4,6-trideoxyhexoses allowed a conclusion that the monosaccharide has the *galacto*-configuration.



108

No more unambiguous proof of the amino sugar configuration is given. The presence in the ^1H NMR spectrum of PS (at pH 10) of a signal at 3.15 ppm with small coupling constant values also confirmed the axial position of the free amino group at C(4) and, correspondingly, the *galacto*-configuration of the monosaccharide.

8. Hexuronic acids

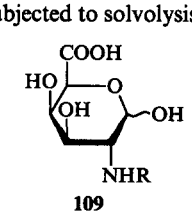
Many O-antigenic PS contain acidic monosaccharides. The presence of such components in PS localised on the surface of the microbial cell confers specific properties on the bacterial envelope. In some cases it protects the cell from external agressive factors and offers an alternative to the microbial capsule which fulfils a similar protective function in encapsulated bacteria. At the same time, the presence of acidic components in O-antigenic polysaccharides strongly influences the character of the somatic antigen, since it is precisely the acidic monosaccharide that often plays the role of the immunodominant group.

The most common acidic monosaccharides present in the polysaccharide chain of O-antigenic PS are hexuronic acids. Two of those, D-glucuronic and D-galacturonic acids are widespread in nature and are common components of microbial PS of a number of Gram-negative bacteria belonging to different species. Procedures for their identification have been well developed and standardised and their chemistry has been studied in a sufficiently great detail. Uronic acids bearing one or two amino groups occur as well.

a. 2-Amino-2-deoxyhexuronic acids

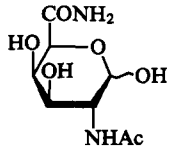
O-Antigenic PS have been found to contain some representatives of 2-amino-2-deoxyhexuronic acids with the D-*galacto*-, D-*manno*-, L-*galacto*-, L-*gulo*- and L-*altro*-configuration. In an effort to isolate and to establish the structure of aminuronic acids at various stages of analysis of microbial PS, different approaches were employed depending upon the degree of sophistication of the physicochemical approaches developed at the time when the particular study was carried out. Recent trends are towards the use of NMR spectroscopy of PS for establishment of the structure of aminuronic acids and their fragments and of mass spectrometry of alditols derived by their reduction. Of chemical methods, the most often used is the reduction of the carboxy group of a uronic acid resulting in formation of the corresponding easily identified 2-acylamino-2-deoxyhexose. This allows a direct conclusion about the structure of the initial aminuronic acid including its absolute configuration.

2-Amino-2-deoxy-D-galacturonic acid **109** in the form of its derivatives is found in O-antigenic PS more frequently than other sugars of this class. It is detected in PS of *Shigella dysenteriae* 7,[105] some serotypes of *Pseudomonas aeruginosa*,[128-130] *Salmonella arizonae*[127] and *Vibrio anguillarum*[107] as well as in some capsular PS. A homopolysaccharide built up of residues of this aminuronic acid has long been known as the Vi-antigen of Gram-negative bacteria.[131] The amino group of this and other aminuronic acids is usually acylated (most commonly acetylated), while the carboxy group is free. In PS of some bacteria the carboxy group is present as an amide; sometimes the same PS contains various proportions of both free acid and its amide. 2-Acetylamino-2-deoxy-D-galacturonic acid and its amide as well as other aminuronic acids can be isolated in the pure state using various procedures for PS cleavage: solvolysis with liquid HF, acid hydrolysis, methanolysis, etc.[127,128] To separate the amide of *N*-acetylaminogalacturonic acid from the free acid and to isolate it in the pure state, the following approach was used: the PS was first reduced with $NaBH_4$ in the presence of a water-soluble carbodiimide (the uronic acid residue was thereby converted into 2-acetylamino-2-deoxygalactose, while the amide residue was not reduced) and subjected to solvolysis with liquid HF.

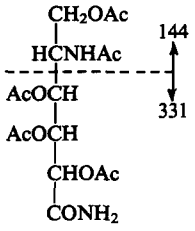


109

To determine the structure of aminogalacturonic acid derivatives, they were converted into the acetates of the corresponding alditols, and the amides of galacturonic acid into the acetates of the aldonic acid amides. Then, their structure was established by mass spectrometry. Significantly, this approach made it possible to keep the *N*-acyl group residues unchanged and to determine their structure. For example, the presence in the mass spectrum of the acetate of the amide of 5-acetylamino-5-deoxygalactonic acid **111** (obtained by reduction and acetylation of the amide of 2-acetylamino-2-deoxygalacturonic acid **110**) of fragments with *m/z* 331 and 144 suggests the presence of an *N*-acetylamino group in the amide **110** and reveals its position.



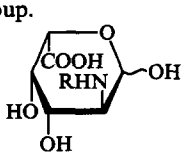
110



111

The structure of 2-amino-2-deoxygalacturonic acid can be established on the basis of NMR spectroscopic data. Thus, the ^{13}C NMR spectrum contains a signal due to the carboxy group at 173–176 ppm and a signal of C(2) substituted by the amino group at 49–51 ppm. The assignment of the acid to the *galacto*-series was done on the basis of the ^1H NMR spectrum: the coupling constant values for the ring protons were: $J_{2,3} = 11$, $J_{3,4} = 3$ and $J_{4,5} = 1.5$ Hz. The specific optical rotation ($[\alpha]_D = +17.9°$) corroborated the absolute configuration of the monosaccharide.

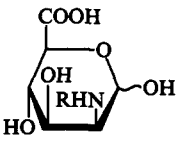
Polysaccharides of some other serotypes of *Pseudomonas aeruginosa* were found to contain the L-isomer of the acid **109**, 2-acetylamino-2-deoxy-L-galacturonic acid **112**,[122,132] which, like its D-isomer, is a component of the main chain of PS. The presence in PS of the uronic acid was also evident from spectral data: the IR spectrum contained an absorption band at 1740 cm^{-1} and the ^{13}C NMR spectrum a signal at 175 ppm belonging to the carboxy group.



112

Reduction of PS with $NaBH_4$ and subsequent hydrolysis resulted in isolation and identification of 2-acetylamino-2-deoxy-L-galactose (*N*-acetyl-L-galactosamine), thus confirming finally the structure of the monosaccharide **112** including its absolute configuration.

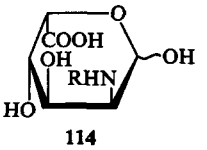
A derivative of 2-amino-2-deoxy-D-mannuronic acid **113** was found in O-antigenic PS of *Vibrio cholerae* O5.[124] The acid **113** was not isolated in the pure state, and its structure was established by a non-destructive method based on a detailed analysis of NMR spectra of the PS itself. Thus, the ^{13}C NMR spectrum of the aminomannuronic acid residue contained a carboxy group signal at 160–170 ppm; in addition, the presence of the carboxy group of the uronic acid was evident from the absence of H(6) signals in the ^1H NMR spectrum in combination with a characteristic pH dependence of the H(5) proton shift. For confirmation of the acid **113** structure, the polysaccharide was reduced with $NaBD_4$ and hydrolysed. As a hydrolytic product, a derivative of 6-[$^2H_2$]-mannosamine was isolated and identified by mass spectrometry as the acetate of the corresponding alditol. The *manno*-configuration of the acid was corroborated by NMR spectroscopic data of the initial PS. The absolute configuration was suggested on the basis of the glycosylation effects determined from the ^{13}C NMR spectrum taking into account the type of substitution of the adjacent unit in the polysaccharide chain.



113

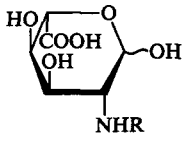
The derivatives of the two other 2-amino-2-deoxyuronic acids, 2-acetylamino-2-deoxy-L-guluronic acid and 2-acetylamino-2-deoxy-L-altruronic acid, are very rare in occurrence and have thus far been found only in O-antigenic PS.

2-Acetylamino-2-deoxy-L-guluronic acid **114** was found in PS of *Alteromonas macleodi* 2MM.[133] Its structure was established by a non-destructive method. The presence of the uronic acid residue was confirmed by the signals corresponding to the carboxy group (177.7 ppm) and C(2) substituted by an amino group (52.4 ppm) in the ^{13}C NMR spectrum of the polysaccharide and was also corroborated by a low-field shift of the H(2) signal in the ^1H NMR spectrum (4.38 ppm). The *gulo*-configuration of the acid is evident from the ^1H NMR spectrum of the polysaccharide, in which the coupling constant values for the ring protons are characteristic of the *gulo*-series ($J_{2,3} = 9$, $J_{3,4} = 4.0$, $J_{4,5} = 2$ Hz). The absolute L-configuration of the acid was suggested on the basis of NOE measurements for PS and construction of molecular models.



114

2-Acetylamino-2-deoxy-L-altrouronic acid **115** was found in PS of *Shigella sonnei*, phase I.[126,134] It was isolated in the pure state by acid methanolysis of PS followed by *N*-acetylation. Its reduction with $NaBH_4$ gave 2-acetylamino-2-deoxy-L-altrose identified by comparison with an authentic sample using the specific optical rotation.[134] More recently,[126] the ^{13}C NMR spectrum of the acid **115** containing, in particular, signals at 175.6 (COOH) and 56.8 ppm [C(2)] was recorded. The L-configuration of the acid was confirmed by the fact that ninhydrin oxidation of 2-acetylamino-2-deoxyaltrose derived by its reduction gives L-ribose.[126]

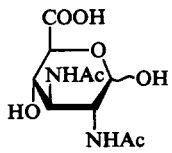


115

b. 2,3-Diamino-2,3-dideoxyhexuronic acids

These structurally unique acidic monosaccharides are rare in occurrence and have thus far been detected only in O-antigenic PS of several strains of *Pseudomonas aeruginosa* and one of their representative has been found in PS of *Bordetella*. These monosaccharides are unstable in acid media and not all of them could be isolated in the pure state; determination of their structure posed considerable difficulties which could be obviated by structural analysis of oligosaccharide fragments obtained by partial degradation of PS in combination with a thorough analysis of the NMR spectra of the PS themselves.

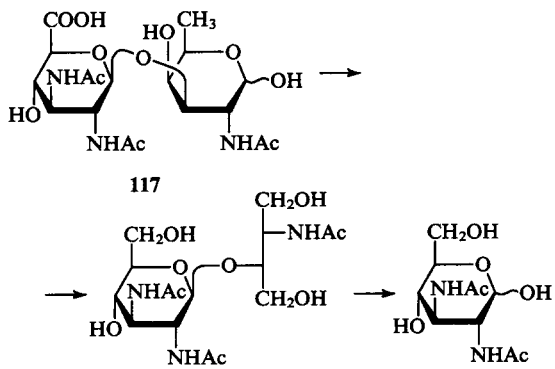
Currently, derivatives of 2,3-diamino-2,3-dideoxyuronic acids having the D-*gluco*-, D-*manno*-, D-*galacto*- and L-*gulo*-configurations are known.



116

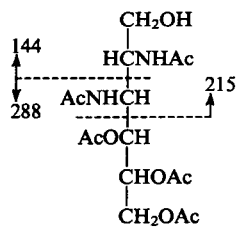
2,3-Acetylamino-2,3-dideoxy-D-glucuronic acid **116** was found in PS of *P. aeruginosa* O6.[135,136] Using this acid as an example, a scheme for structural analysis of these unusual monosaccharides has been elaborated (Scheme 12). The presence in PS of a free carboxy group was confirmed by the presence in the IR spectrum of a band at 1740 cm^{-1}. Solvolysis of PS with liquid HF performed at room temperature gave the disaccharide **117** containing a uronic acid. Successive reduction of the disaccharide with $NaBH_4$ followed by oxidation with HIO_4, repeated reduction with $NaBH_4$ and carboxyl reducion resulted in the compound **118**. Strong acid hydrolysis of **118** and its subsequent *N*-acetylation gave 2,3-diacetylamino-2,3-dideoxyhexose **119**. The structure of the acid **119** was established by mass spectrometric analysis of the alditol acetate **120** derived from **119**.

Scheme 12



117 118 119

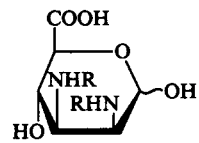
The mass spectrum of the compound **120** contained fragments with *m/z* 288, 215 and 144; such fragmentation, which is characteristic of 2,3-diacetylamino-2,3-dideoxyhexitol acetates, was previously observed for synthetic samples.[137]



120

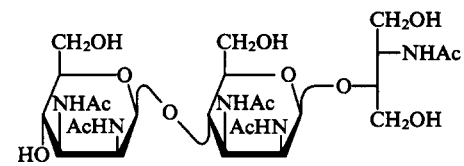
The ^{13}C NMR spectrum of the disaccharide **117** contained signals belonging to the carboxy group (176 ppm) and C(2) and C(3) substituted by the amino group (54.9 and 56 ppm). High coupling constant values of the ring protons in the ^1H NMR spectrum of the monosaccharide **116** ($J_{2,3}$ = 11.5, $J_{3,4} = J_{4,5}$ = 10.0 Hz) indicated its *gluco*-configuration. The acid **116** was assigned to the D-series on the basis of direct comparison of the diacetyldiaminohexose **119** derived by reduction of **116** with a synthetic sample of 2,3-diacetylamino-2,3-dideoxy-D-glucose.[138]

Derivatives of 2,3-diamino-2,3-dideoxy-D-mannuronic acid **121** were found in PS of several serotypes of *Pseudomonas aeruginosa* where they are present in the main chain as one or two residues.[139,140] The amino groups of the acid appeared to be substituted by acetyl and acetimidoyl (acetamidino) groups. It should be noted that the latter is unique for carbohydrate derivatives and was first detected in O-antigenic PS. The presence of an acetimidoyl group offered some difficulties in elucidation of the structure of diaminomannuronic acid derivatives and originally prompted an erroneous conclusion about the presence in PS of a monosaccharide containing a condensed pyrano-imidazoline system.[139,140] Subsequently this error was corrected.[141] Therefore, to simplify the structural analysis, the polysaccharide or its fragments were treated with triethylamine to convert the acetimidoyl group into a more common acetyl group.



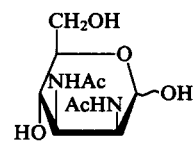
121

The structure of the acid **121** was established by the scheme described above for the *gluco*-analogue. Evidence for the presence of a carboxy group was derived from the band at 1725 cm^{-1} in the IR spectrum of PS. Successive solvolysis of PS with liquid HF, reduction with $NaBH_4$, oxidation with HIO_4, repeated reduction with $NaBH_4$, treatment with triethylamine (conversion of the *N*-acetimidoyl into the *N*-acetyl group) and carboxyl reduction gave the trisaccharide derivative **122**.



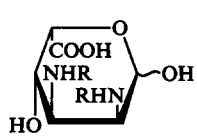
122

Drastic hydrolysis of **122** afforded 2,3-diacetylamino-2,3-dideoxy-D-mannose **123** whose structure was corroborated by mass spectrometry of the corresponding diacetylaminoalditol acetate. Fragmentation of this derivative proceeds by the same pathway as that of the alditol obtained from the *gluco*-analogue. The parameters of the NMR spectra of the diaminuronic acid residue present in the acetate of the trisaccharide **122** confirmed its structure: the ^{13}C NMR spectrum contained signals of C(2) and C(3) carrying NH groups at 52.3 and 52.5 ppm, and the coupling constant values of the ring protons in the ^1H NMR spectrum were consistent with the *manno*-series ($J_{2,3}$ = 3.9, $J_{3,4} \approx J_{4,5}$ = 9.8 Hz). Finally, the absolute configuration of the diacetylaminodideoxyhexose obtained from PS was confirmed by direct comparison with a synthetic sample of 2,3-diacetylamino-2,3-dideoxy-D-mannose.[143]



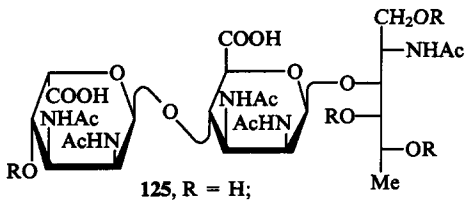
123

The third isomer of diaminuronic acids of this series, a derivative of 2,3-diacylamino-2,3-dideoxy-L-guluronic acid **124** containing an *N*-acetimidoyl group, was found in PS of several subtypes of *Pseudomonas aeruginosa* O3[140,143] and *Pseudomonas aeruginosa* immunotype 7.[143,144] Some of these PS also contained a residue of 2,3-diamino-2,3-dideoxy-mannuronic acid that posed some problems while establishing the structure of the *gulo*-isomer, since interpretation of the NMR spectra of fragments containing both isomeric diaminouronic acids was quite complicated.



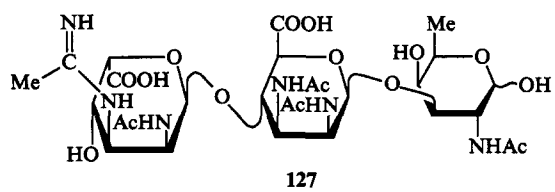
124

The structure of 2,3-diacetylamino-2,3-dideoxy-L-guluronic acid in PS of *Pseudomonjas aeruginosa* O3 was established using the same scheme as for its *gluco*- and *manno*-analogues. After detection in the IR and NMR spectra of PS of signals for carboxy groups, the polysaccharide was subjected to solvolysis with liquid HF and subsequent treatment as described above. This resulted in a derivative of the trisaccharide **125** containing residues of diaminouronic acids with the *manno*- and *gulo*-configurations that was corroborated by NMR spectroscopy.



125, R = H;
126, R = Ac

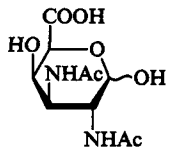
Regretfully, not only the derivative of the diaminoguluronic acid itself but also the product of its reduction, 2,3-diacetylamino-2,3-dideoxy-L-gulose, appeared to be rather unstable in acid media. The latter also failed to be isolated and identified. For this reason the structure and the configuration of the diaminoguluronic acid was established by analysis of the complex NMR spectra of the trisaccharide derivative **125** and its acetate **126** (their composition was confirmed by mass spectrometry) using special versions of NMR spectroscopy. It was found that, in addition to the carboxy group, the novel monosaccharide contains two acetylamino groups at C(2) and C(3) and has the *gulo*-configuration as evidenced by the coupling constant values for the ring protons in the ^{1}H NMR spectrum ($J_{2,3}$ = 4.5, $J_{3,4}$ = 3.0 and $J_{4,5}$ = 3.0 Hz). This configuration was finally corroborated by comparison of signals of the carbon atoms belonging to the residue of the acid **124** in the ^{13}C NMR spectrum of the derivative **125** with the spectral characteristics of synthetic samples of all isomeric 2,3-diacetylamino-2,3-dideoxyhexoses.[145] This configuration was also established for the oligosaccharide **127**, one of the intermediate products of selective cleavage of PS from *Pseudomonas aeruginosa* immunotype 7, during the analysis of the NMR spectra.[144]



127

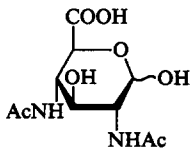
The absolute L-configuration of diaminoguluronic acid was evident from the specific optical rotation of the oligosaccharide **125**. It was finally confirmed by analysis of the ^{13}C NMR spectrum of the trisaccharide **127** using the dependence of the glycosylation effects on the absolute configuration of the neighbouring monosaccharide units. A negative β-effect of glycosylation (0.9 ppm) on C(3) of the diaminomannuronic acid (for which the D-configuration had been established previously) attached to the diaminoguluronic acid residue in **127** by the α-glycosidic bond at position C(4) demonstrated, unambiguously, the L-configuration of 2,3-diacetylamino-2,3-dideoxyguluronic acid.

Recently, the fourth isomer of diaminodideoxyhexuronic acids, 2,3-diacetylamino-2,3-dideoxy-D-galacturonic acid (**128**), was identified. This acid is a component of O-antigenic PS of the Gram-negative bacteria *Bordetella bronchiseptica* and *Bordetella parapertussis*.[146] Both PS are homopolysaccharides built up of diaminogalacturonic acid residues, the immunochemical difference between the antigens being exclusively due to variations in the structure of the core of the corresponding LPS. The structure of the novel monosaccharide was established without any difficulty by a non-destructive method using NMR spectroscopy. The presence of signals of the carboxy group (175 ppm) and of C(2) and C(3) bearing amino groups (46.6 and 49.1 ppm) in the ^{13}C NMR spectra and the coupling constant values for the ring protons in the ^{1}H NMR spectrum characteristic of the *galacto*-series ($J_{2,3}$ = 10.8, $J_{3,4}$ = 3.0 and $J_{4,5}$ = 3.0 Hz), fully confirmed the structure of 2,3-diacetylamino-2,3-dideoxygalacturonic acid. The absolute D-configuration was evident from the specific optical rotation of the PS.



128

Recently, the first representative of 2,4-diamino-2,4-dideoxyuronic acids has been identified. 2,4-Diacetylamino-2,4-dideoxy-D-glucuronic acid **129** was found in O-specific PS of *Thiobacillus* IFO 14570.[147] The structure of this PS was established by a non-destructive method using a complex analysis of PS with the help of several versions of NMR spectroscopy, including NOE measurements. This study is an exellent demonstration of contemporary structural analysis of a structurally complex PS using NMR spectroscopy alone. Signals of the diaminoglucuronic acid residue identified in the two-dimensional heteronuclear ^{1}H–^{13}C NMR spectrum corroborated the structure of the molecular skeleton: the ^{13}C NMR spectrum contained a signal at 173.0 ppm [COOH group at C(6)] and signals at 55.3 and 56.5 ppm [the carbon atoms at C(2) and C(4) containing NHAc substituents]. Large coupling constant values for the ring protons in the ^{1}H NMR spectrum (9–10 Hz) showed the *gluco*-configuration of the acid **129**, and the data obtained during NOE measurements proved the D-configuration.



129

To the above statements concerning hexuronic acids it must be added that sometimes these monosaccharides are present in PS as amides. This fact may have a considerable influence on the immunochemical specificity of the whole biopolymer, since amides, in contrast to the parent acids, are neutral and their presence alters the distribution of charged groups along the polysaccharide chain. In this context, identification of a uronic acid amide and its localisation in the chain are integral parts of structural analysis of O-antigenic PS.

Up to the present, both unsubstituted glycuronamides and more complex compounds of this series with the carboxy group amidated, e.g., by an amino acid, have been found in PS (see Section 5).

Unsubstituted amides of uronic acids were detected in polysaccharides of various classes. For the first time, they were found in PS of several serotypes of *Pseudomonas aeruginosa*,[128] which contain 2-acetylamino-2-deoxy-D-galacturonamide. More recently, this same hexuronamide was detected in PS of *Shigella dysenteriae* 7[105] and *Vibrio anguillarum*.[107]

Since identification of unsubstituted hexuronic acids is now being carried out by a non-destructive method on the basis of NMR spectra, and the spectra of the acid and its amide differ insignificantly, the presence of an amide group is usually established by the pH dependence of the position of the H(5) signal in the ^{1}H NMR spectrum: for an amide the chemical shift of this signal does not depend on pH, while in case of an acid the signal is shifted down-field with a decrease in pH (due to the effect of the adjacent carboxy group).[148] Thus, it was shown that the PS chain

of *Shigella dysenteriae* 7 contains two adjacent units, 2-acetylamino-2-deoxy-D-galacturonic acid and its amide: with a drop in pH from 9 to 1 the signal of H(5) of the acid residue is shifted from 4.40 to 4.63 ppm, whereas the chemical shift of the amide signal remains unchanged.

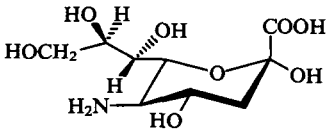
In conclusion, it should be noted that amidation of hexuronic acid residues in the PS chain may also be non-stoichiometric. In this case the same PS may comprise both a free acid and its amide, their quantitative ratio being different for PS of related serotypes or subtypes. This phenomenon was studied for several subtypes of PS of *Pseudomonas aeruginosa* O4 containing residues of both 2-deoxy-2-formylamino-D-galacturonic acid and its amide. By using anion-exchange and reversed-phase chromatography for separation of the trisaccharide fragments obtained from PS after solvolysis with liquid HF and study of the pH dependence of their ^{1}H NMR spectra, it was found that the degree of amidation of this uronic acid in subgroups O4a,4b, O4a,4d and O4a,4c is 10%, 20% and 0%, respectively.[148] Derivatives of more complex amides of uronic acids containing 'non-carbohydrate substituents' will be considered in Section 5.

9. Glyculosonic acids

Glyculosonic acids are α-keto acids of the carbohydrate series. These acidic monosaccharides which have a peculiar structure are found in various PS of microbial origin; however, the main source of glyculosonic acids is extracellular and capsular PS. The chain of natural glyculosonic acids may comprise 5 to 9 carbon atoms; some of those acids contain deoxy units and amino groups.

Most widespread representative of glyculosonic acids in the microbial world is 3-deoxy-D-*manno*-octulosonic acid (KDO), an indispensable component of the inner core of all Gram-negative bacteria.[149] This acid, like some hexulosonic acids as well, was found in capsular PS of *Klebsiella* and some other bacteria (see review[6]).

In the present review we shall restrict our consideration to only some representatives of this class of acidic monosaccharides detected in O-antigenic PS of Gram-negative bacteria. Of those, most widespread is 5-amino-3,5-dideoxy-D-*glycero*-D-*galacto*-nonulosonic acid **130** more commonly known as neuraminic acid. Derivatives of this acid having the common name of sialic acids are especially widely distributed in carbohydrate-containing biopolymers of animal tissues where they enter into the carbohydrate chains of glycoproteins and glycolipids and play a crucial role in the determination of specific biological properties and conversions of these biopolymers.[150]



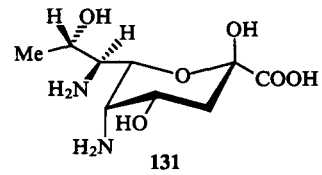
130

Residues of neuraminic acid and other glyculosonic acids are present in the PS chain in the pyranose form **130** and have a deoxy unit adjacent to the anomeric centre. Derivatives of neuraminic acid are distinguished by high acid lability and are destroyed during hydrolysis of the carbohydrate-containing biopolymer. Perhaps, for this particular reason, derivatives of neuraminic acid were first identified in O-antigenic PS not long ago.

So far, *N*-acetyl derivatives of neuraminic acid have been found in O-antigenic PS of several bacteria: *Salmonella arizonae*,[151,152] *Citrobacter freundii*,[152] *Escherichia coli*,[152–156] *Hafnia alvei*[152,157] and *Vibrio cholerae*.[158] They are also present in some extracellular PS. One of such polysaccharides, the long known colaminic acid, is a homopolysaccharide composed of residues of *N*-acetylneuraminic acid.[159]

Because of the wide occurrence of neuraminic acid derivatives in glycoproteins and glycolipids, their chemical properties and spectroscopic characteristics have been studied in great detail and are comprehensively discussed in reviews.[150,160] In this connection, identification of a neuraminic acid residue (or its derivatives) in O-antigenic PS is now being achieved by a non-destructive method based on the NMR spectroscopic analysis of the PS itself or of its oligosaccharide fragments.

Recently, another two structurally unique representatives of aminodeoxynonulosonic acids have been identified in O-antigenic PS. Derivatives of one of them, 5,7-diamino-3,5,7,9-tetradeoxy-L-*glycero*-L-*manno*-nonulosonic acid **131** (pseudaminic acid), were detected in PS of several serotypes of *Pseudomonas aeruginosa*,[161–163] *Shigella boydii* 7[161,162,164] and *Vibrio cholerae* O2.[165]

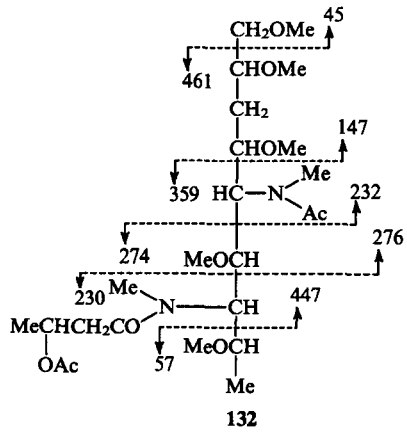


131

In the monosaccharides of these PS (with the exception of *V. cholerae* PS) the amino group at C(5) is acetylated, while the amino group at C(7) carries a formyl group or a residue of 3-hydroxybutyric acid. In the case of *V. cholerae* PS, the monosaccharide contains an acetylated amino group at C(7) and an acetimidoyl group at the amino group at C(5).

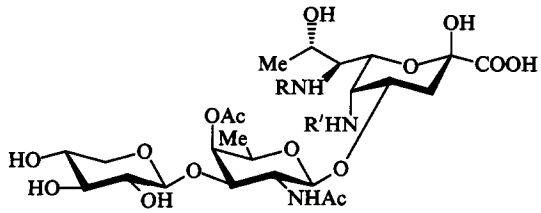
Originally, the presence in these PS of a pseudaminic acid residue was established by a qualitative reaction for *N*-acetylneuraminic acid with the resorcinol reagent.[166] Furthermore, the ^{13}C NMR spectra of the fragments obtained by acid hydrolysis of PS contained signals characteristic of a 3-deoxyaldulosonic acid.

In view of the high lability of pseudaminic acid derivatives towards acids, the establishment of their structure met considerable difficulties. The main approaches[161,162,164] used for determination of their complete structure can briefly be summarised as follows. Carboxy groups of PS isolated from *S. boydii* 7 and *P. aeruginosa* O10 were first reduced, and then the PS were subjected to hydrolysis. This was accompanied by cleavage of the glycosidic bonds of the reduced pseudaminic acid residues; however, the monosaccharide residues themselves that are deprived of their carboxy groups were not destroyed. The products of the hydrolysis were then treated with $NaBH_4$. After methylation of the hydroxy groups, the oligosaccharide was solvolysed with liquid HF. The resulting mixture of partially methylated monosaccharides (in which the *N*-acylated amino groups were also *N*-methylated) was reduced with $NaBH_4$ and acetylated. Chromato-mass spectrometry of the mixture of partially methylated alditol acetates obtained after degradation of PS of *P. aeruginosa* O10 revealed the presence of the alditol **132** containing nine carbon atoms and corresponding to the residue of pseudaminic acid. Its fragmentation allowed establishment of the structure of the skeleton and localisation of deoxy units [at C(3) and C(9)] and aminoacyl substituents [at C(5) and C(7)].

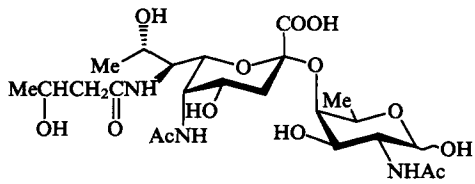


132

These data were confirmed by NMR spectroscopy of several oligosaccharides containing residues of pseudaminic acid, which were obtained by degradation of PS. For example, the ^{13}C NMR spectrum of the oligosaccharide **133** obtained by very mild acid hydrolysis of PS from *P. aeruginosa* O5 contained signals belonging to the residue of pseudaminic acid: at 99.7 ppm [C(2)] and 34.8 ppm [a deoxy unit at C(3)]. The ^{13}C NMR spectrum of the oligosaccharide **134** obtained by solvolysis of PS from *P. aeruginosa* O10a with liquid HF contained signals at 37.1 ppm [a deoxy unit at C(3)], 49.1 ppm [C(5) carrying an aminoacyl substituent], 54.7 ppm [C(7) carrying an aminoacyl substituent] and 17.7 ppm [a CH_3 group at C(9)]. A slight difference between the chemical shifts in the ^{13}C NMR spectra of the compounds **133** and **134** is attributed to the difference in the substituents of the amino groups of pseudaminic acid in the fragments obtained from different bacteria. The ^1H NMR spectra of the oligosaccharides **133** and **134** fully corroborate the structure described above.

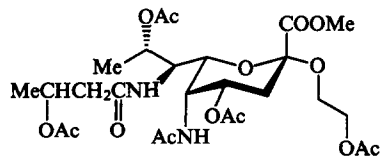


133



134

The configuration of pseudaminic acid was established by analysis of the ^1H NMR spectra of the fragment **135** obtained from PS of *S. boydii* 7 by three successive Smith degradations followed by its conversion into the methyl ester and acetylation. The spectrum of the compound **135** is characterised by the following coupling constant values for the ring protons: $J_{3a,4} = 13$, $J_{4,5} = 3.5$, $J_{5,6} = 1.6$ Hz, that indicate the axial orientation of H(4) and the equatorial orientation of H(5). The equatorial orientation of the substituent at C(6) was confirmed by NOE measurements which indicated the proximity of H(7) and of the NH proton at C(5).

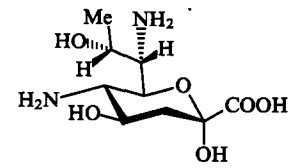


135

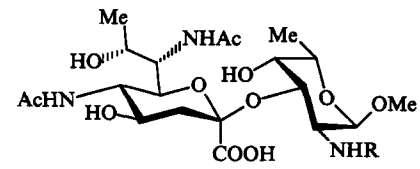
These data suggest that the C(4)–C(5)–C(6) fragment of pseudaminic acid has the *lyxo*-configuration. A large coupling constant value ($J_{6,7} = 10$ Hz) and a strong NOE on H(5) and H(6) caused by preiirradiation of the NH proton at C(7) proved the *erythro*-configuration of the C(6)–C(7) fragment. Finally, the L-*erythro*-configuration of the C(7)–C(8) fragment of pseudaminic acid was established by comparison of the glycosylation effects on C(9) of pseudaminic acid and on C(1) of the glucosamine residue (linked by the glycosidic bond to C(8) of pseudaminic acid in the polysaccharide chain) as well as by analysis of the ^{13}C NMR spectra of model compounds, glycosylated derivatives of D- and L-threonine and of D- and L-*allo*-threonine. These data suggested that the C(7)–C(8)–C(9) fragment of pseudaminic acid is homomorphous with the fragment of L-*allo*-threonine, i.e., that the C(7)–C(8) fragment has the L-*erythro*-configuration. Therefore, pseudaminic acid has the L-*glycero*-L-*manno*-configuration **131**.[161]

Later, identification of pseudaminic acid derivatives in PS was performed using ^1H and ^{13}C NMR spectroscopy (including NOE measurements) taking into account the above data.[163,165]

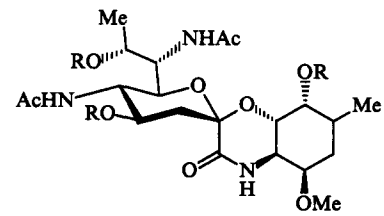
Derivatives of a pseudaminic acid isomer, 5,7-diacylamino-3,5,7,9-tetradeoxy-D-*glycero*-L-*galacto*-nonulosonic acid **136**, were found in O-antigenic PS of *Salmonella arizonae* O61,[167] *Pseudomonas aeruginosa* O13,[168,169] *Vibrio alginolyticus*[170] and *Legionella pneumophila* 1.[171] The amino groups of this nonulosonic acid were acylated by various acids. A derivative was also found which contained an *N*-acetamidino group.[171] The complete structure of this acid was established by the same methods as described for pseudaminic acid.[168,169] Solvolysis of PS of *P. aeruginosa* O13 with an HF solution in methanol resulted in the disaccharide **137** containing residues of the nonulosonic acid **136** and fucosamine. After treatment with acetic anhydride in pyridine, the disaccharide **137** underwent intramolecular cyclisation to give the lactam **138**.



136



137



138

The composition of the compounds **137** and **138** was confirmed by determination of their molecular weights using FAB mass spectrometry. The ^{13}C NMR spectra of the compounds **137** and **138** contained signals at 19.7 ppm [a CH_3 group at C(9)], at 54.4 and 53.8 ppm [C(7) and C(5) carrying acylamino groups] and 42–41 ppm [a deoxy unit at C(3)]. The data obtained by ^1H NMR spectroscopy were fully consistent with this structure of the nonulosonic acid skeleton. The presence in the ^1H NMR spectra of characteristic coupling constant values for the ring protons ($J_{3a,3e} = J_{3a,4} = 12$, $J_{3e,4} = 4.2$, $J_{4,5} = J_{5,6} = 10$ Hz) demonstrated the axial orientation of the protons in the C(4)–C(5)–C(6) fragment of the nonulosonic acid and, consequently, its *arabino*-configuration. A small coupling constant value ($J_{6,7} = 2.5$ Hz), which is close to the corresponding value for *N*-acetylneuraminic acid (1.5 Hz) but different from that for pseudaminic acid (10 Hz), indicates the *threo*-configuration of the C(6)–C(7) fragment. Finally, the C(7)–C(8) fragment was also assigned the D-*threo*-configuration which, as in the previous case, was established on the basis of glycosylation data for the nonulosonic acid **136** and model glycosides of *N*-acetylgalactosamine, D- and L-threonine, D- and L-*allo*-threonine.

Therefore, the nonulosonic acid **136** was ascribed the D-*glycero*-L-*galacto*-configuration. Later, this monosaccharide containing various *N*-substituents was detected in PS of other bacteria.[167,170,171] Its identification was carried out mainly by non-destructive methods on the basis of NMR spectral data, some of which were partly listed above. Analysis of oligosaccharide

fragments obtained by partial cleavage of PS was performed only in a few particular cases.

Among biopolymers containing the nonulosonic acid **136**, PS of *Legionella pneumophila* (the pathogen of a severe lung disease, so-called 'Legionnaires disease') is of special interest.[171] This PS is a homopolysaccharide built up of residues of a derivative of 5,7-diamino-3,5,7,9-tetradeoxy-D-*glycero*-L-*galacto*-nonulosonic acid with acetamidino and *N*-acetylamino groups at C(5) and C(7), respectively, and is the first example of a polysaccharide of this type. It should be stressed that identification of this nonulosonic acid and establishment of the structure of the whole PS was carried out by a non-destructive method on the basis of NMR spectral data alone.

In conclusion, it should be noted that to finally solve the question concerning the structure and, especially, the configuration of the representatives of this novel class of monosaccharides, it is most desirable to carry out counterflow syntheses of both 5,7-diamino-3,5,7,9-tetradeoxynonulosonic acids **131** and **136**.

IV. Non-carbohydrate substituents in monosaccharide units

As indicated in the Introduction, the second factor determining the differentiation of monomeric components in O-antigenic PS and the diversity of their structures is the presence in PS of so-called 'non-carbohydrate' substituents. The presence of such substituents markedly and, sometimes, dramatically alters the spatial structure of the polysaccharide chain and the general 'topography' of the polysaccharide molecule by exposing on its surface additional polar and hydrophobic groups which eventually determine the specificity of its interaction with the protein (antibody) surface, i.e., its immunologic specificity.

It should be noted that, although the presence of some non-carbohydrate substituents in polysaccharides of various origin, e.g., in plant PS, is well known, these are microbial PS and, particularly, O-antigenic PS of Gram-negative bacteria that contain an especially great variety of such substituents. These substituents can modify hydroxy groups of monosaccharides, amino groups of amino sugars and carboxy groups of acids of the carbohydrate series.

O-Substituted monosaccharides may include ethers or esters, the latter being far more widely spread. Also known are monosaccharides carrying an acetal group linked to two hydroxy groups of a monosaccharide unit.

Exclusively *N*-acylated derivatives are characteristic for amino sugars, whereas *N*-alkylated monosaccharides are practically unknown in O-antigenic PS. The carboxy groups of uronic acids appear to be most frequently substituted to give an amide group, both unsubstituted amides and more complex derivatives, including, in particular, an amino acid residue being known. Finally, residues of phosphoric acid and some of its derivatives have also been detected in O-antigenic PS as non-carbohydrate substituents.

Establishment of the structure and localisation of a non-carbohydrate substituent in a carbohydrate unit of PS presents a separate and often complicated problem. Since in recent years the number and diversity of non-carbohydrate substituents detected in O-antigenic PS is growing fast, the search for the most effective ways to solve these problems is becoming more and more important in the chemistry of O-antigenic PS. One of these consists in selective cleavage of the non-carbohydrate substituent with subsequent determination of its structure by conventional methods; however, the problem of its localisation in the polysaccharide chain cannot be solved by this approach. Another approach is confined to isolation of the corresponding substituted monosaccharide followed by its structural analysis. Regretfully, both pathways are not always practicable, since attempts to cleave the non-carbohydrate substituent or to isolate the substituted monosaccharide often result in irreversible changes or even complete destruction of the compounds being isolated. For this reason, the problem of elucidation of the structure is now being solved using physicochemical methods. This approach permits determination of the nature and localisation of the non-carbohydrate substituent directly by analysis of PS or oligosaccharide fragments obtained by its specific degradation.

1. *O*-Alkyl substituents

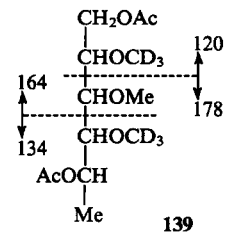
Substitution of hydroxy groups in monosaccharide units of O-antigenic PS with formation of the ether bond occurs rarely. Only *O*-methyl and 1-carboxyethyl ethers are now known.

a. *O*-Methyl ethers

Partially methylated monosaccharides are rather widely distributed in plant PS. Among microbial PS, they are mostly found in extracellular and capsular PS as well as in PS of photosynthetic bacteria (see review[6]); in O-antigenic PS they were detected only in a few cases. It is believed that methylation of a monosaccharide hydroxy group takes place during one of the stages of PS biosynthesis, the SMe group of methionine being the donor of the methyl group.

3-*O*-Methyl-L-xylose was found in PS of *Pseudomonas maltophilia* NCTC 10257,[172] where it occupies the terminal position in the side chains of the branched PS. This sugar was isolated after acid hydrolysis of PS and identified by routine methods (comparison with an authentic sample).

3-*O*-Methyl-L-rhamnose was isolated from O-antigenic PS of *Klebsiella* O10[173] by acid hydrolysis. This monosaccharide occupies the terminal position at the non-reducing end of the polysaccharide chain. To establish its structure, the PS was first deuteromethylated with CD_3I and then hydrolysed. The resulting monosaccharide was converted into the alditol acetate **139** and analysed by mass spectrometry. Its mass spectrum contained peaks corresponding to the fragments with *m/z* 164, 134, 178 and 120 which corroborated unambiguously the structure of 3-*O*-methylrhamnose.



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It is likely that the presence of both methylated monosaccharides at the ends of the polysaccharide chains serves as a signal for termination of further elongation of this chain during biosynthesis.

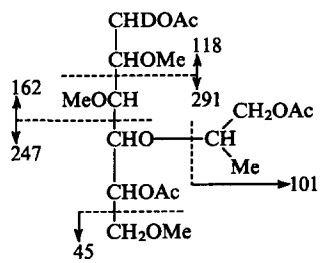
3-*O*-Methyl-D-*glycero*-D-*altro*-heptose and 3-*O*-methyl-6-deoxy-D-*altro*-heptose were found in some PS of *Campylobacter jejuni* where they partially substitute hydroxy groups.[76] Interestingly, the ratio of methylated to non-methylated monosaccharides in PS is determined by the cultivation conditions of these bacteria, although the actual reason for this phenomenon still remains to be elucidated. The structure of both methylated heptoses was established by mass spectrometry of partially methylated alditol acetates derived from PS after deuteromethylation, hydrolysis and reduction (see above).

b. 1-Carboxyethyl ethers (glycolactylic acids)

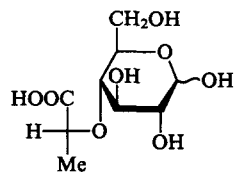
Glycolactylic acids present in O-antigenic PS contain a residue of lactic acid linked by the ether bond to the sugar molecule. Compounds of this type were first detected in peptidoglycan of the bacterial cell wall (muramic acid and its *manno*-isomer[174,175]). Later, they were also found in O-antigenic and some other microbial PS (exocellular PS, capsular PS and PS of photosynthetic bacteria). Thus, glycolactylic acids are a rather large group of acidic monosaccharides.

The ether bond in these acids is rather stable; therefore, they can be isolated in the pure state after acid hydrolysis of PS using ion-exchange chromatography. Representatives of this class differ from one another by the nature of the parent monosaccharide, the absolute configuration of the lactic acid residue and its localisation. So far, the following representatives of glycolactylic acids have been isolated from O-antigenic PS of Gram-negative bacteria: derivatives of D-glucose, L-rhamnose and 2-acetylamino-2-deoxy-D-glucose usually containing a residue of (*R*)-lactic acid, although the derivatives of the (*S*)-isomer are also known. In addition, PS of other microorganisms contain derivatives of D-mannose [176] and D-glucuronic acid.[177]

Initially, the structure of glycolactylic acid was established by classical chemical methods and mass spectrometry. However, more recent studies employed NMR spectroscopy for this purpose. An illustrative example of the classical approach is the establishment of the structure of glucolactylic acid isolated from PS of *Shigella dysenteriae* 3.[178,179] The substituted monosaccharide was isolated in the pure state from the acid hydrolysate of PS; its ^{1}H NMR spectrum contained a characteristic signal of a methyl group CH_3-CH at 1.4 ppm (d, J = 6.0 Hz). Its treatment with BCl_3 resulted in cleavage of the ether bond to form D-glucose. To establish the site of attachment of the lactic acid residue to the glucose residue, the polysaccharide was methylated, reduced with $LiAlH_4$ and hydrolysed. The resulting mixture of monosaccharides was converted into alditol acetates by reduction with $NaBD_4$ and subsequent acetylation. Chromato-mass spectrometry of the alditol acetate mixture revealed the tetraacetate **140** whose fragmentation provided evidence for its structure: a fragment with *m/z* 101 showed the presence of a propanediol residue (formed by reduction of the lactic acid carboxy group), and fragments with *m/z* 118, 291 and 162 and 247 indicated the site of its binding to C(4) of glucose. Based on these data, the original monosaccharide was ascribed the structure of 4-*O*-(1′-carboxyethyl)-D-glucose **141**.



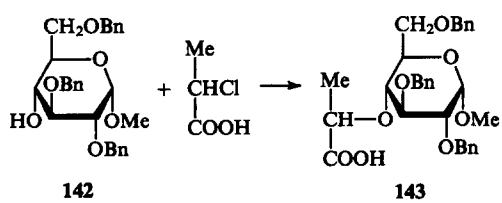
140



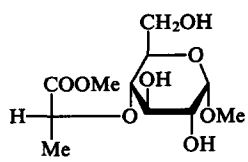
141

The absolute configuration of the lactic acid residue was established by counterflow synthesis of glucolactylic acid [179] using condensation of methyl 2,3,6-tri-*O*-benzyl-α-D-glycopyranoside **142** with (*S*)-2-chloropropionic acid (obtained from L-alanine) in the presence of sodium hydride (Scheme 13). Treatment of the condensation product **143** with diazomethane and elimination of the benzyl groups gave the methyl glycoside of the methyl ester of the glucolactylic acid **144** whose physicochemical characteristics (including the specific optical rotation) were identical to those of the natural product. Thus, in the natural compound the residue of lactic acid has the (*R*)-configuration, and this glycolactic acid is 4-*O*-[(*R*)-1′-carboxyethyl]-D-glucose **141**.

Scheme 13



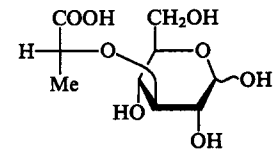
142 **143**



144

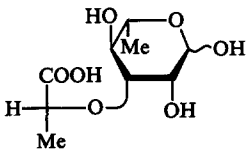
The glycolactylic acid **141** was also found in PS of an immunochemically related to *Shigella dysenteriae* 3 bacterium *Escherichia coli* O124 and identified in the same way. Moreover, the PS structures in both bacteria, *S. dysenteriae* 7 and *E. coli* O124, appeared to be identical. This fact provided the first experimental proof that bacteria with identical immunochemical specificity, even those belonging in biological classification to different species, contain the structurally identical PS.[180] Notice that another glycolactylic acid, 4-*O*-[(*S*)-1′-carboethoxyl]-D-glucose, isomeric by the lactic acid residue to the glycolactylic acid **141**, was isolated from an extracellular PS of the bacterium *Aerococcus viridans*.[6]

An isomeric glucolactylic acid, 3-*O*-[(*R*)-1′-carboxyethyl]-D-glucose **145**, has recently been isolated in the pure state from PS of *Aeromonas haloplanktis* [181] after acid hydrolysis of PS with trifluoroacetic acid. Its structure was established by NMR spectroscopy. The ^{1}H NMR spectrum contained signals characteristic of a lactic acid residue: at 1.45 (d, 7.0 Hz, a CH_3 group) and 4.41 ppm (q, a CH group) as well as signals characteristic of the glucose residue. The ^{13}C NMR spectrum also contained signals characteristic of the lactic acid residue: at 19.6 (CH_3 group) and 79.5 ppm (CH group). The signal of C(3) of the glucose residue was shifted down-field to 83 ppm in comparison with the signal of unsubstituted glucose, indicating the binding of lactic acid residue to this atom. This structure was also confirmed by full coincidence of the NMR spectra for the acid **145** and the glucolactylic acid isolated from a capsular poylsaccharide of *Pseudomonas fragi*, for which the same structure had been established.[182] The D-configuration of the glucose residue was evident from the regularities in the glycosylation effects determined from the ^{13}C NMR spectrum of the original PS; the (*R*)-configuration of the lactic acid residue was suggested on the basis of the specific optical rotation of the acid **145**.

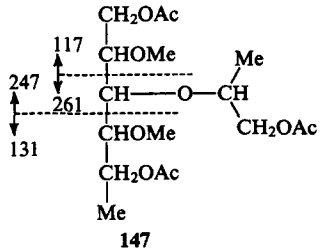


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The structure of other glycolactylic acids was established in a similar way (using the methods available at the time of the investigation). Thus, the 3-*O*-[(*R*)-1′-carboxyethyl]-L-rhamnose **146** (rhamnolactylic acid) isolated from *Shigella dysenteriae* 5,[183] after treatment with BCl_3 gave L-rhamnose, and the mass spectrum of the partially methylated alditol **147** derived from PS revealed the presence of a lactic acid residue bound to C(3) of rhamnose. The (*R*)-configuration of the lactic acid residue was established by counterflow synthesis. More recently, this acid was isolated from PS of *Vibrio fluvialis* [184] and its structure was confirmed by NMR spectroscopy. The ^{1}H NMR spectrum contained signals at 1.42 and 4.33 ppm (a lactic acid residue) and a set of characteristic signals of the rhamnose residue. The conclusion about the (*R*)-configuration of the lactic acid residue was made on the basis of comparison of the chromatographic behaviour and optical rotation of the sample tested with synthetic samples of the (*R*)- and (*S*)-isomers of this rhamnolactylic acid.[170]

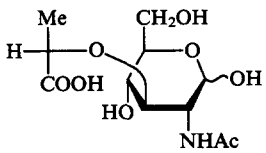


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A glycolactylic acid derived from glucosamine, 2-acetylamino-3-*O*-[(*S*)-1′-carboxyethyl]-2-deoxy-D-glucose **148**, was found in O-antigenic PS of *Proteus penneri* 62.[185] Its structure was established by spectral data. The ^1H and ^{13}C NMR spectra of the polysaccharide contained signals characteristic of the lactic acid residue [1.31, (d, J = 7 Hz) and 4.05 ppm (q) (^1H NMR) and, correspondingly, 20.3, 80.6 and 182.9 ppm (^{13}C NMR)] and of the glucosamine residue. The ^{13}C NMR spectrum revealed a characteristic down-field shift of the C(3) signal, indicating substitution of the corresponding hydroxy group. The monosaccharide was isolated in the pure state after solvolysis of PS with liquid HF. Its spectrum coincided with that of 2-acetylamino-3-*O*-[(*S*)-(1′-carboxyethyl)]-2-deoxy-D-glucose but differed from that of the well-known muramic acid[174] with the (*R*)-configuration of the lactic acid residue. On the basis of these data and using the specific optical rotation differing markedly from the corresponding value for muramic acid, the novel glycolactylic acid was ascribed the (*S*)-configuration. This compound is known as isomuramic acid.



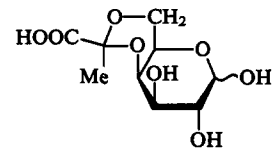
148

Recently, PS of another serotype of the same species of *Proteus penneri* 35 was found to contain an isomer of isomuramic acid, 2-acetylamino-4-*O*-[(*S*)-1′-carboxyethyl]-2-deoxy-D-glucose, whose structure was established by similar methods.[186]

Apart from the compounds described above, some other glycolactylic acids were identified. Thus, a capsular PS of *Klebsiella* K37 was found to contain 4-*O*-[(*S*)-1′-carboxyethyl]-D-glucuronic acid,[177] whereas an extracellular PS of *Mycobacterium lacticolum* 121 4-*O*-[(*S*)-1′-carboxyethyl]-D-mannose;[176] their structures were established by the approaches described above.[187]

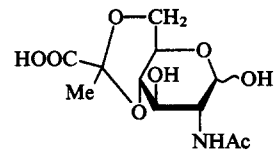
2. Acetal (carboxyethylidene) groups

Monosaccharides carrying acetal groups with two hydroxy groups of the monosaccharide simultaneously substituted by a pyruvic acid residue (carboxyethylidene groups) are rather widely spread in exocellular and capsular PS. Methods have been developed for determination of the structure of their derivatives present in PS. In O-antigenic PS of Gram-negative bacteria, monosaccharides carrying carboxyethylidene groups have thus far been detected only in PS of *Shigella dysenteriae* 9[188] and *Escherichia coli* O149.[189] NMR spectroscopy in combination with chemical methods is used to identify this group. Thus, the ^1H NMR spectrum of PS of *S. dysenteriae* 9 contains a signal at 1.60 ppm that is characteristic of the methyl group of the pyruvic acid residue. This group is not affected by basic treatment of PS but gradually disappears during treatment of PS with dilute acetic acid, thus suggesting the binding of the pyruvic acid residue as an acetal. The nature of the monosaccharide unit carrying this substituent and the substitution site were established by comparing the results of PS methylation before and after its deacetalation with acetic acid: in the former case hydrolysis of methylated PS resulted in 2-*O*-methylgalactose, while in the latter case, in 2,4,6-tri-*O*-methyl-D-galactopyranose. It was thus demonstrated that the carboxyethylidene group modified the hydroxy groups at C(4) and C(6) of galactose, and the monomeric unit of PS is 4,6-*O*-(1′-carboxyethylidene)-D-galactose **149**.



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The ^1H NMR spectrum of *E. coli* PS also contains a signal at 1.50 ppm, and the ^{13}C NMR spectrum has signals at 25.4 and 101.3 ppm characteristic of the pyruvic acid residue. As in the previous case, the position of this residue was established by comparison of the products of methylation of the native and deacetalised PS which revealed that the carboxyethylidene group binds to C(4) and C(6) of *N*-acetylglucosamine. Therefore, the substituted monosaccharide has the structure of 2-acetylamino-4,6-*O*-(1′-carboxyethylidene)-2-deoxy-D-glucose **150**.

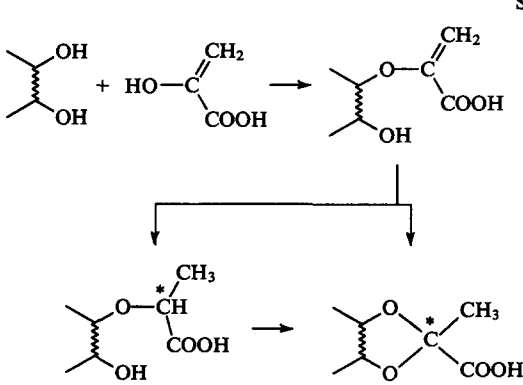


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Introduction of the carboxyethylidene group into the monosaccharide results in the formation of a new chiral centre whose configuration can be easily established by ^{13}C NMR.[190] Depending on the conformation of the dioxane ring of the 4,6-di-*O*-substituted monosaccharide, the methyl and carboxy groups can have either axial or equatorial orientation that can be readily determined by the values of the chemical shifts of the corresponding signals. With the known conformation of the dioxane ring (defined by the structure of the parent monosaccharide), the relative position of the CH_3 and COOH groups determined from the NMR spectra provides direct evidence for the (*R*)- or (*S*)-configuration of the central carbon atom of the acetal group.[190] This approach was used to establish the (*S*)-configuration of the pyruvic acid residue in PS of *E. coli* O149.

The acetal group, like the lactic acid residue in glycolactylic acids, is introduced into PS in the course of PS biosynthesis in the cell by reaction of the corresponding monosaccharide with the enol form of pyruvic acid catalysed by a specific enzyme. The ester formed is probably reduced to give the (*R*)- or (*S*)-isomer of a glycolactylic acid, which, in the case of a suitable spatial arrangement of the adjacent hydroxy group, can further be converted into the corresponding acetal. An alternative pathway, namely, direct binding of the hydroxy group to the double bond of the intermediate enol (Scheme 14), is also not excluded.

Scheme 14



3. *O*-Acyl substituents

Substitution of hydroxy groups in monosaccharide residues of PS by residues of aliphatic acids is common for plant and microbial polysaccharides. Usually, *O*-acetylation takes place but acylation with other aliphatic (including higher fatty acids) and hydroxy acids is also known. Thus, the aliphatic acids $C_{12}-C_{16}$ and hydroxycarboxylic acids of this series are indispensable components of the lipid part of LPS (the so-called lipid A) where they acylate glucosamine residues, thereby providing the high lipophilic character of LPS needed for its fixation in the outer cellular membrane of Gram-negative bacteria.[1]

O-Acetylation of particular hydroxy groups in certain monosaccharide units of the O-antigenic polysaccharide chain is essential for the determination of immunochemical specificity of PS. To fully characterise this epitope of the O-antigen, it is necessary to establish the precise localisation of the *O*-acetyl group. The assignment of the *O*-acetyl group to a certain monosaccharide unit in the PS chain is a routine task which can readily be solved using ^1H and ^{13}C NMR spectroscopy.

Evidence for the presence of other *O*-acyl groups in O-antigenic PS is almost absent. The only experimental proof seems to be the presence of the *O*-propionyl group in PS of *Vibrio anguillarum*.[96]

The paucity of variation of *O*-acyl groups seems to be remarkable, especially in comparison with the great diversity of aliphatic acids acylating amino groups of monosaccharides in O-antigenic PS.

4. *N*-Acyl substituents

The occurrence of monosaccharides with acylated amino groups in various natural compounds is a common but not compulsory phenomenon, whereas polysaccharides almost always contain residues of amino sugars with acylated amino groups. Most frequently, PS comprise *N*-acetyl derivatives, while derivatives of other organic acids are far less common. The exception is O-antigenic PS of Gram-negative bacteria, where the diversity of acids acylating amino groups is rather high and includes simplest aliphatic acids, hydroxy and polyhydroxy acids, and amino acids. Monosaccharides containing two amino groups can be acylated by the same or by two different acids (this is also true of the amino groups in different monosaccharide units of PS).

The great diversity of acids acylating amino groups in O-antigenic PS has become especially apparent recently, mainly due to the rapid progress in NMR spectroscopy. At present, the nature of *N*-acyl substituents and their position in PS is almost exclusively established by NMR spectroscopy of the PS themselves or of their fragments obtained by their specific degradation. Mass spectrometry is also used for this purpose.

a. *N*-Acylation by aliphatic acids

The most common substituents in O-antigenic PS are *N*-acetyl groups. Their identification is a routine task which is most often solved during interpretation of the NMR spectra of PS (by the presence of characteristic signals of the $NHCOCH_3$ group). For example, in the ^{13}C NMR spectrum the signals at 22.0–24.0 (CH_3 group) and 174–177 ppm (CO group) correspond to this group.

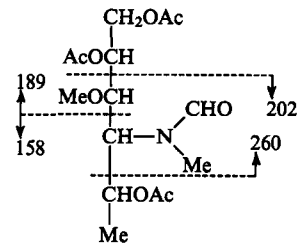
Localisation of the *N*-acetyl group is evident from the characteristic shift caused by this substituent in the neighbouring carbon atom and proton: in the ^{13}C NMR spectrum, it is a signal in the region 45–58 ppm; in the ^1H NMR spectrum, the signal of a ring proton in the region 3.8–4.3 ppm These data, after reliable assignment of the signal, allow both simultaneous identification of the monosaccharide unit carrying this substituent and localisation of the substituent in this unit.

Substitution of the amino group by a residue of formic acid occurs less frequently. *N*-Formyl groups were found in PS of *Escherichia coli* O119,[120] *Yersinia enterocolitica* O9,[115] *Pseudomonas aeruginosa* O4[128] and related serotypes as well as in PS of *Vibrio anguillarum*,[107] *Brucella*[110,191] and *Francisella tularensis*[106] where they are localised in residues of 2,3-diamino-2,3,6-trideoxy-D-mannose,[120] 4-amino-4,6-dideoxy-D-mannose,[110,115] 2-amino-2-deoxy-D-galacturonic acid[107,128] and 4-amino-4,6-dideoxy-D-glucose.[106]

The presence of an *N*-formyl group can be readily established by the presence in the ^{13}C NMR spectrum of a signal in the 166–169 ppm region.

Since rotation around the CO–NH bond is restricted, and the NHCHO group may exist in stable *cis*- or *trans*-conformations, a splitting of the proton signals is characteristic of this group. By comparing the intensities of these signals, one can draw a conclusion about the ratio of both conformers.[192] Localisation of the *N*-formyl group in PS is also evident from the NMR spectral data. Thus, a characteristic shift of the signal of the C atom carrying the NHCHO group (45–55 ppm) is observed in the ^{13}C NMR spectrum.

Sometimes, the presence and localisation of the *N*-formyl group is determined by mass spectroscopic analysis. Thus, the aminoalditol **151**, whose mass spectrum clearly indicated the presence of an *N*-formyl group and its localisation at C(4) of 4,6-dideoxy-4-(*N*-formylamino)-D-mannose,[110] was isolated from PS of the bacterium *Brucella melitensis* after methylation (involving the NH group of the formamide residue), solvolysis with liquid HF and subsequent conversion of the degradation products into acetates of partially methylated alditols.



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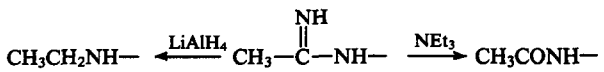
The *N*-formyl group is fully cleaved by mild acid treatment of PS to give a PS with free amino groups. At the same time, this group is stable towards solvolysis with liquid HF and retaind in fragments formed by degradation of PS that is employed in their structural analysis.

One of unusual *N*-acylating groups thus far found only in O-antigenic PS is the *N*-acetimidoyl (amidino) group, $CH_3C(=NH)-$. It was first detected in PS of several species of *Pseudomonas*,[143,144,193] and, later, in PS of *Vibrio cholerae*,[165] *Salmonella arizonae*[151,167] and *Legionella pneumophila*[171] where it acylates the amino groups of aminuronic and aminononulosonic acids to form the corresponding amidines.

The *N*-acetimidoyl group has a basic nature and it confers it on the whole PS. Its presence is readily detectable spectroscopically. Thus, the ^{13}C NMR spectrum contains signals at 19.5–21 ppm (CH_3 group) and 167–169 ppm (N=C–N group), whereas the signal of the carbon atom carrying the *N*-acetimidoyl group has a chemical shift of 45–57 ppm; the ^1H NMR spectrum contains a singlet at 2.2–2.4 ppm.

The amidino group is not cleaved by treatment with liquid HF; however, upon basic treatment (e.g., with triethylamine) or reduction with $LiAlH_4$, it is converted into the *N*-acetylamino group or the *N*-ethylamino group, respectively (Scheme 15). This property is often used in structural analyses of PS or their fragments, since these conversions of the amidino group are readily controlled by NMR spectroscopy. Thus, transformation of the amidino group to the *N*-acetylamino group is accompanied by the appearance of a signal near 2 ppm in the ^1H NMR

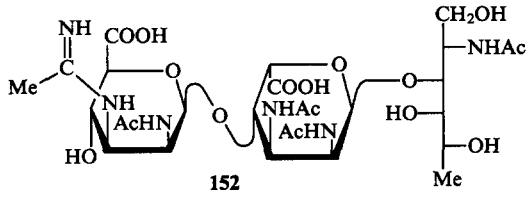
Scheme 15



spectrum and signals near 23 and 176 ppm in the ^{13}C NMR spectrum. When the amidino group is reduced to the *N*-ethylamino group, the changes are even more apparent: the ^{1}H NMR spectrum becomes a triplet at 1.35 ppm (J = 7.5 Hz, CH_3 group) and a quadruplet at 3.2–3.4 ppm (CH_2 group), and the ^{13}C NMR spectrum signals at 11–13 (CH_3 group) and 42–45 ppm (CH_2 group). In the process, the signal of the corresponding C atom of the pyranose ring is shifted to 62 ppm, and that of the attached proton from 3.86 to 3.62 ppm. In the spectra of a specially synthesised model compound, methyl 3-acetimidoyl-3,6-dideoxy-α-L-glucopyranose, the changes were essentially the same.[194]

As an example of identification and localisation of the acetimidoyl group, we may refer to the analysis of PS isolated from *Pseudomonas aeruginosa* O2.[143]

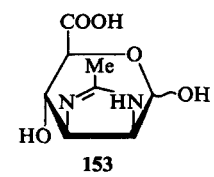
The ^{13}C NMR spectrum of this PS contained signals at 19.9 and 167.3 ppm indicating the presence of an acetimidoyl group. Solvolysis of PS with liquid HF and subsequent reduction of degradation products with $NaBH_4$ gave a derivative of the trisaccharide **152** containing an acetimidoyl group. The composition of the trisaccharide **152** was corroborated by an analysis of the FAB mass spectrum. The ^{13}C NMR spectrum of the compound **152** contained signals characteristic of an acetimidoyl group localised in a residue of 2,3-diamino-2,3-dideoxy-D-mannuronic acid. The signals of the ring carbons, C(2) and C(3), at 50.9 and 55.7 ppm showed the presence of two *N*-containing substituents, one of which was an amidino group. Upon reduction of the trisaccharide **152** with $LiAlH_4$, the amidino substituent was converted into the *N*-ethylamino group, corroborated by the corresponding changes in the NMR spectra (see above). Thus, the signal of the ring carbon C(3) was shifted from 57.7 to 61.4 ppm, while after treatment of **152** with triethylamine the amidino group was converted into the *N*-acetylamino group, and the signal of C(3) shifted to 54.4 ppm. In both cases, the position of the C(2) signal did not change. These data suggest unambiguously that in the PS studied the amidino group is located at C(3) of the diaminomannuronic acid residue.



152

Similar methods were used to reveal the presence of the *N*-acetimidoyl substituent at the amino group of 2-amino-2,6-dideoxy-D-galactose in PS of *S. arizonae* O21,[151] *S. arizonae* O61,[167] *P. aeruginosa* O12 and at the C(5) amino groups of 5,7-diamino-3,5,7,9-tetradeoxy-L-*glycero*-L-*manno*-nonulosonic acid in PS of *Vibrio cholerae*[165] and its D-*glycero*-L-*galacto*-isomer in PS of *Legionella pneumophila*.[171]

It is interesting to note that originally the monosaccharide forming a part of PS of several serotypes of *P. aeruginosa* and simultaneously containing an *N*-acetimidoyl and an *N*-acetyl group, was erroneously ascribed, on the basis of spectral data, the structure **153** containing a bicyclic pyrano-imidazoline system (which is quite admissible because the substituents at the C(2) and C(3) atoms were in the equatorial-axial position).[139,140] This conclusion was revised[141] on the grounds that the FAB-mass spectra of fragments obtained by degradation of PS lacked molecular ions corresponding to the imidazoline group but contained ions corresponding to the acetimidoyl and acetylamino groups which were localised by the methods indicated above.



153

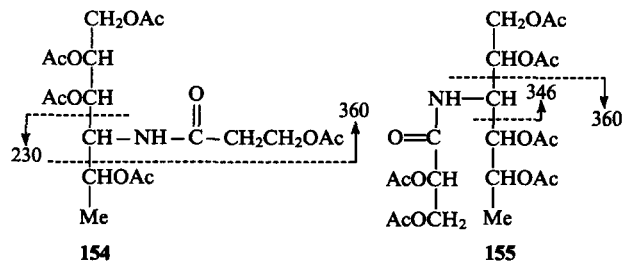
b. *N*-Acylation by hydroxy- and polyhydroxy-acids

Apart from amino sugars *N*-acylated by unsubstituted aliphatic acids, O-antigenic PS often contain derivatives of hydroxy and polyhydroxy acids of the aliphatic series; most commonly, these are C_2–C_6 acids having one or two hydroxy groups (3-hydroxypropionic, L-glyceric, 3- and 4-hydroxybutyric, 2,4-dihydroxybutyric and 3,5-dihydroxyhexanoic acids). The presence of hydroxy acid residues in the polysaccharide chain confers peculiar characteristics on its peripheral surface by simultaneously exposing both hydrophobic and hydrophilic regions that markedly increases the selectivity of its contacts (e.g., with the surface of an immunoglobulin during antigen-antibody interaction).

Establishment of the structure of hydroxy acids acylating amino groups of monosaccharides and determination of the site of their attachment to the monosaccharide is usually performed simultaneously with establishment of the structure of the monosaccharide itself (most often, using physicochemical methods).

Thus, PS of *Vibrio cholerae* 1875 was found to contain 3-hydroxypropionic acid[195] which acylates the amino group of 4-amino-4-deoxy-D-rhamnose (perosamine). The *N*-acylated monosaccharide was isolated after solvolysis of the polysaccharide with HF. Its structure, including the presence of a residue of 3-hydroxypropionic acid was established by NMR spectroscopy and confirmed by mass spectrometry. In particular, the ^{1}H NMR spectrum of its acetate contained signals at 4.58 (NH proton), 4.23 and 1.98 ppm (CH_2O and CH_2 groups of the hydroxyacyl residue). The presence and localisation of the 3-hydroxypropionyl residue was corroborated by mass spectrometry of alditol acetate **154**.

L-Glyceric acid was found in PS of *Citrobacter freundii* O32. This biopolymer can only conventionally be regarded as a polysaccharide, because its main chain contains alternating glycosidic and amidic bonds.[196] Glyceric acid acylates the amino group of 3-amino-3,6-dideoxy-D-fucose whose C(2′)-hydroxy group is linked by the glycosidic bond to the adjacent residue of the same monosaccharide; as a result, a polymeric chain with alternating bond types is formed. Hydrolysis of the biopolymer with trifluoroacetic acid and subsequent reduction and acetylation of the resulting mixture of fragments gave a derivative of the corresponding alditol **155** whose mass spectrum revealed the presence of a monosaccharide *N*-acetylated by glyceric acid. The presence of the glyceric acid residue in the biopolymer was fully confirmed by characteristic signals in the ^{13}C NMR spectrum: at 173 [C(1)], 80.2 [C(2)] and 62.8 ppm [C(3)]. Glyceric acid was isolated from the acid hydrolysate of the polymer using paper electrophoresis; its specific optical rotation ($[\alpha]_D$ = −5.5°) proved the L-configuration.



154 **155**

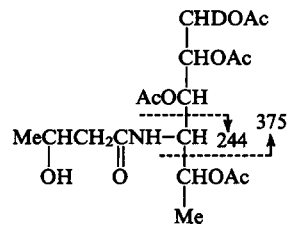
(*R*)-3-Hydroxybutanoic (β-hydroxybutyric) acid was detected in a number of O-antigenic PS: *Escherichia coli* O10 (where it acylates the amino group of 4-amino-4-dideoxy-D-fucose),[117] *Yersinia aldovae*[197] and *Proteus penneri* 16 (where it acylates the amino group of 3-amino-3-deoxy-D-fucose),[198] as well as in PS of several serotypes of *Hafnia alvei* (where it acylates the amino group of glucosamine or of 3-amino-3-deoxy-D-fucose),[199,200] *Pseudomonas aeruginosa* O5 and O10, *Shigella boydii* O7 [where it acylates the amino group at C(5) or C(7) of 5,7-diamino-3,5,7,9-

tetradeoxy-L-*glycero*-L-*manno*-nonulosonic acid],[162,163] and *Vibrio mimicus* 26768 (where it acylates the amino group of 3-amino-3-deoxy-D-quinovose).[95]

The (*S*)-isomer of 3-hydroxybutanoic acid occurs less frequently: it was found in PS of *P. aeruginosa* O1 [where it acylates the amino group at C(4) of 2,4-diamino-2,4,6-trideoxy-D-glucose].[122]

Identification of a 3-hydroxybutanoic acid residue and determination of the site of its attachment to the corresponding monosaccharide in the polysaccharide chain are usually performed by isolation of the *N*-substituted monosaccharide and its analysis by physicochemical methods. In some cases the PS is methylated, and an *O*-methyl derivative of the corresponding *N*-substituted monosaccharide is isolated and analysed. As a typical example, we may refer to identification of the residue of (*R*)-3-hydroxybutanoic acid in PS of *Escherichia coli* O10.[117]

Solvolysis of PS with liquid HF and subsequent mild acid hydrolysis gave, among other fragments, an *N*-acyl derivative of 4-amino-4-deoxy-D-fucose. This derivative was reduced with $NaBD_4$ and then acetylated. The mass spectrum of the acetate of the corresponding alditol **156** revealed unambiguously the presence and localisation of the residue of 3-hydroxybutanoic acid in the derivative of 4-amino-4,6-dideoxyhexose. Similar results were obtained in analysis of an *O*-methylated derivative of this alditol obtained after degradation of the methylated PS. The absolute (*R*)-configuration of this residue was established by its oxidation with specific D-3-hydroxybutyrate dehydrogenase in the presence of nicotinamide adenosine diphosphate.

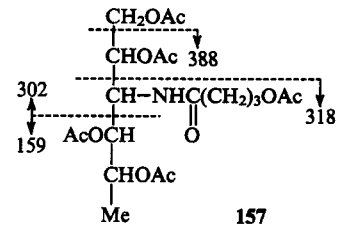


156

In other cases, e.g., in studies of PS from *P. aeruginosa* and *S. boydii* 7,[162] 3-hydroxybutanoic acid was isolated in the individual state and its structure was confirmed by spectroscopic data. The ^1H NMR spectrum of 3-hydroxybutanoic acid contained signals at 4.25 [H(3′), sextet; $J_{2,3} = J_{3,4} = 6$ Hz], 2.50 [H(2′), doublet] and 1.27 ppm [H(4′), doublet, $J = 6$ Hz]; the ^{13}C NMR spectrum contained signals at 180.0 [C(1′)], 44.8 [C(2′)], 66.2 [C(3′)] and 23.5 ppm [C(4′)]. These characteristic signals were also detected in the NMR spectra of PS themselves containing residues of 3-hydroxybutanoic acid and are therefore employed for direct spectroscopic identification of this residue. If this acid is isolated in the pure state, its (*R*)- or (*S*)-character can be readily established from the specific optical rotation.

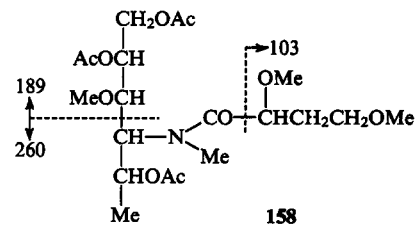
Usually, a repeating unit of O-antigenic PS contains not more than one residue of 3-hydroxybutanoic acid; other amino groups are acylated by other acids, most commonly, by acetic acid.[162] Also, PS are known where the same amino group is partially substituted by an acetic acid residue and partially by a 3-hydroxybutanoic acid residue, as in PS of *E. coli* O10 where 60% of the amino groups in the 4-amino-4-deoxy-D-fucose residue are acylated by acetic acid and 40% by 3-hydroxybutanoic acid.[117] This makes the polysaccharide chain irregular as could be evident from the NMR spectrum. The question still remains open as to whether such substitution has a heterogeneous character, that is, the same poylsaccharide chain contains both substituents, or, in contrast, there exist two different polysaccharide chains, each of which is substituted only by the same residue. Because of the structural similarity of both chains and their rather high molecular mass, they cannot be separated by the currently available methods.

4-Hydroxybutanoic (γ-hydroxybutyric) acid has thus far been found only in O-antigenic PS of *Alteromonas macleodii* 2MM6, where it acylates the amino group of 3-amino-3-deoxy-D-fucose.[133] Hydrolysis of PS resulted in an *N*-acyl derivative of this monosaccharide. The ^1H NMR spectrum of this derivative contains, in addition to signals of the monosaccharide unit, characteristic signals of a residue of 4-hydroxybutanoic acid: at 2.37 [H(2′), triplet, $J_{2,3} = 8.0$ Hz], 1.85 [H(3′), multiplet, $J_{3,4} = 7.0$ Hz] and 3.62 ppm [H(4′), triplet]. The ^{13}C NMR spectrum contained signals at 33.3 [C(2′)], 28.7 [C(3′)] and 61.8 ppm [C(4′)]. The mass spectrum of the alditol acetate **157** derived from this monosaccharide confirmed the presence of a 4-hydroxybutanoic acid residue and its localisation at the amino group at C(3) of the monosaccharide.



157

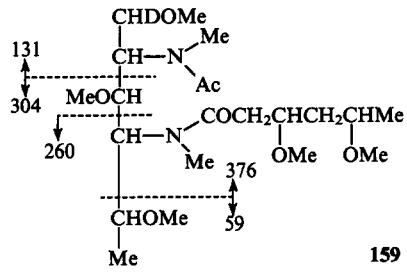
(*S*)-2,4-Dihydroxybutanoic (3-deoxytetronic) acid was found in O-antigenic PS of *Vibrio cholerae* O1 where it acylates the amino group of 4-amino-4,6-dideoxy-D-mannose (perosamine).[109] This acid was isolated in the pure state after hydrolysis of PS with dilute trifluoroacetic acid and identified by NMR spectroscopy. The ^{13}C NMR spectrum of this acid contained signals at 182.8 (CO group), 71.0 (CHOH group), 37.7 (CH_2 group) and 59.8 ppm (CH_2OH group). The ^1H NMR spectrum also fits this structure. The structure and localisation of the residue of (*S*)-2,4-dihydroxybutanoic acid was established using the mass spectrum of the acetate of the partially methylated alditol **158** obtained by solvolysis of methylated PS with liquid HF and subsequent reduction and acetylation of the corresponding *N*-acyl derivative of perosamine.



158

The (*S*)-configuration (L-*glycero*-configuration) of the acid residue was evident from the specific optical rotation ($[\alpha]_D = -8°$) and confirmed by the identity of its ester with (*R*)-octan-2-ol to the corresponding synthetic sample.

3,5-Dihydroxyhexanoic acid was detected in PS of *Vibrio cholerae* O3 where it acylates the amino group at C(4) of 2,4-diamino-2,4,6-trideoxy-D-glucose (bacillosamine).[58] The other amino group of this monosaccharide is *N*-acetylated. The presence of 3,5-dihydroxyhexanoic acid in PS was corroborated by the presence in the ^1H NMR spectrum of signals characteristic of the fragment $CH_3-CHOH-CH_2-CHOH-CH_2CO$. The structure of the acid residue was also confirmed by the ^{13}C NMR spectrum which contained signals at 45.0 [C(2′)], 66.7 [C(3′)], 46.1 [C(4′)], 65.3 [C(5′)] and 23.7 ppm [C(6′)]. The mass spectrum of the methylated alditol **159** derived from PS by a conventional method also confirmed the presence and localisation of this acid residue at the corresponding amino group of bacillosamine. These conclusions are consistent with the results of mass spectrometric analysis of disaccharide fragments obtained by partial degradation of PS.



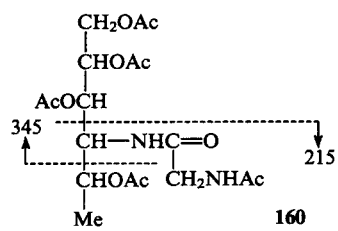
159

c. *N*-Acylation by amino acids

Among amino acids acylating an amino group of an amino sugar, mainly α-amino acids and some other more complex proline derivatives are known. Most commonly, PS contain only one amino acid residue per one repeating unit.

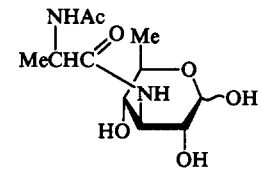
The finding of α-amino acids in PS of microbial origin presents substantial interest since it demonstrates the relationship between the two most important classes of carbohydrate-containing biopolymers: glycoproteins as typical representatives of the animal world and polysaccharides as classical biopolymers of microorganisms. Further expansion of our knowledge in this area may disclose interesting regularities in variations in biosynthetic pathways in the course of evolution.

An example of *N*-acylation of the chain of O-antigenic PS by an amino acid is acylation of the amino group of 4-amino-4,6-dideoxy-D-glucose (4-amino-4-deoxy-D-quinovose) by glycine, the amino group of the glycine itself being acetylated. This derivative was detected in PS of *Shigella dysenteriae* 7.[105] The residue of *N*-acetylglycine was first identified in PS using spectroscopic analysis: the ^{13}C NMR spectrum contained a characteristic signal of C(2) of glycine at 44.1 ppm. Hydrolysis of PS with dilute HCl resulted in a derivative of 4-amino-4-dihydroxyquinovose; conventional analysis of the hydrolysate using an amino acid analyser revealed glycine. The mass spectrum of the corresponding alditol acetate **160**, derived by a routine procedure, indicated unambiguously the position of the glycine residue.



160

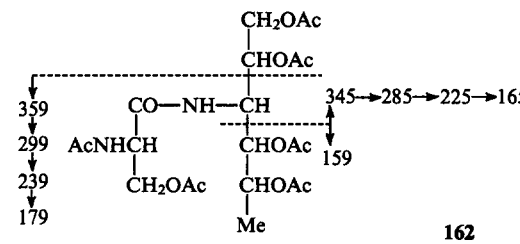
PS of *Proteus penneri* 14 was found to contain residues of D- and L-alanine. The former acylated the amino group of 3-amino-3,6-dideoxy-D-glucose (3-amino-3-deoxy-D-quinovose), while the L-isomer amidated the carboxy group of D-galacturonic acid.[94] This was the first example of a PS containing two different amino acid residues linked by different bonds to different monosaccharide residues. Solvolysis of PS with liquid HF resulted in the derivative **161** which contained a residue of *N*-acetyl-D-alanine linked to 3-amino-3-deoxyquinovose. The structure of this compound was confirmed by the mass spectrum of the derived alditol acetate as well as by NMR spectroscopy of the PS itself. The ^{13}C NMR spectrum contained signals characteristic of D-alanine at 18.4 [C(2′)], 51.8 [C(3′)] and 178.3 ppm (CO), and the position of the C(3) signal of the monosaccharide residue (58.4 ppm) proved unambiguously the presence of an acylamino group at this atom.



161

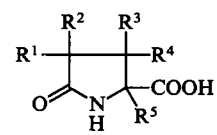
A residue of *N*-acetyl-L-serine was found in PS of *Escherichia coli* O114[92,98] where it acylated the amino group of 3-amino-3-deoxyquinovose. The corresponding *N*-acylated monosaccharide was obtained after solvolysis of PS with liquid HF, from which L-serine was isolated after acid hydrolysis and identified by conventional amino acid analysis. Its L-configuration was established by GLC using an optically active stationary phase.

A routine procedure was used to convert the monosaccharide derivative into the alditol acetate **162** whose mass spectrum revealed fragmentation indicating the presence of a serine residue and its localisation in the monosaccharide (secondary fragments were especially characteristic). The structure of this monosaccharide was also confirmed by ^{13}C NMR spectral data.



162

Several O-antigenic PS were found to contain structurally more complex *N*-acyl substituents related to 5-oxoproline derivatives of the type **163**. They can be regarded as lactams of substituted glutamic acids. Elucidation of their structure required a great deal of effort and was performed with the help of mass spectrometric analysis and NMR spectroscopy. The first substituent of the oxoproline type was detected in PS of *Pseudomonas fluorescens*[201] where it acylates the amino group of 3-amino-3-deoxyquinovose. The derivative of the *N*-acylated amino sugar was isolated from the acid hydrolysate of PS and was neutral. Its IR spectrum contained bands at 1660 and 1730 cm^{-1} which demonstrated the presence of amide and lactam groups in this compound. The molecular weight of the *N*-acyl substituent, as determined from the FAB mass spectrum, was 156 Da corresponding to the compound of the type **163** containing two methyl groups and one hydroxy group.



163

The ^{13}C NMR spectrum of this derivative contained signals confirming the presence of two methyl groups (18.9 and 23.5 ppm), one methylene group (45.8 ppm), two tertiary carbon atoms (71.6 and 78.2 ppm) and two carbonyl groups (175.7 and 179.7 ppm). The ^{1}H NMR spectrum revealed signals of two isolated methyl groups (1.37 and 1.49 ppm) and a methylene group with nonequivalent protons localised near the carbonyl group [2.45 and 2.65 ppm (AB-system, J_{AB} = 15 Hz)] as well as two NH groups (7.26 and 7.57 ppm), one of which was linked to

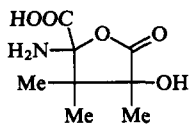
the carbon atom carrying no protons. NOE measurements indicated the *trans*-orientation of the methyl groups. The whole body of experimental evidence suggested that the acylating amino acid can be assigned the structure of 3-hydroxy-2,3-methyl-5-oxoproline **163** ($R^1 = R^2 = H$, $R^3 = R^5 = CH_3$, $R^4 = OH$).

A structurally related substituent (also a derivative of 5-oxoproline) was identified in PS of *Vibrio cholerae* O5,[124] where it acylates the amino group of 3-amino-3-deoxyfucose. The structure of this amino acid was established exclusively on the basis of the NMR spectra of the PS itself. The ^{13}C NMR spectrum of the polysaccharide (and of the disaccharide fragment derived) contained signals of the methyl (23.4 ppm), methine (69.6 ppm), methylene (45.4 ppm) and CO groups (180.2 ppm). This corresponded to the following signals in the ^1H NMR spectrum: 1.37 (methyl), 2.38 and 2.64 (methylene, $J_{H,H} = 17.4$ Hz) and 4.19 ppm (methine) as well as a singlet at 8.62 ppm (NH group). These data which were fully confirmed by several correlations and NOE measurements suggested that the amino acid has the structure of 3-hydroxy-3-methyl-5-oxoproline **163** ($R^1 = R^2 = R^5 = H$, $R^3 = CH_3$, $R^4 = OH$).

This structure of the *N*-acyl substituent was fully confirmed by FAB mass spectrometry of some oligosaccharide fragments obtained by PS degradation. The configuration of the substituent was established by NOE measurements: a strong interaction between the NH proton and H(6) in the monosaccharide residue showed the (*R*)-configuration of C(2′) of the proline ring, and an interaction between the CH_3 group of the amino acid and H(4) of the adjacent monosaccharide (2,4-diamino-2,4-dideoxyquinovose), indicated the (*R*)-configuration of C(3′) of the proline ring.

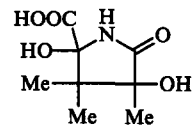
The third representative of *N*-acyl substituents of this type, 2,4-dihydroxy-3,3,4-trimethyl-5-oxoproline, was detected in PS of *Vibrio anguillarum*,[107] where it acylates the amino group of 4-amino-4-deoxyquinovose (viosamine). Selective cleavage of PS by solvolysis with liquid HF and by two successive Smith degradations gave several oligosaccharide fragments. Their ^1H and ^{13}C spectra contained signals corresponding to a substituted 5-oxoproline residue which was attached to the amino group of 4-amino-4-deoxyquinovose as shown by the position of the signal for C(4) of this monosaccharide (57.4 ppm).

A special study was undertaken to establish the structure of this complex *N*-acyl substituent.[202] A derivative of this amino acid was isolated from a complex mixture of fragments obtained by solvolysis of PS with liquid HF and subsequent acid hydrolysis. This derivative displayed an IR spectrum with a band at 1792 cm^{-1} and gave a positive reaction with ninhydrin corresponding to the structure of a lactone carrying a free amino group. This compound was assigned the structure **164**. Its ^{13}C NMR spectrum revealed the presence of eight carbon atoms, two of which belonged to carbonyl groups (171.5 and 172.0 ppm), three other, to methyl groups (15.2, 18.0 and 19.7 ppm) and three more carbon atoms were localised in the ring. Analysis of the ^1H NMR spectrum (signals at 1.62, 1.68 and 1.69 ppm) confirmed the presence of three methyl groups. Esterification and subsequent *N*-acylation of the lactone **164** gave a methyl ester whose ^1H NMR spectrum contained signals of NH proton (6.14 ppm), OH proton (3.97 ppm) and a methyl group of the ester (3.82 ppm) as well as signals of three methyl groups (1.18, 1.59 and 1.75 ppm). Thus, the lactone **164** appeared to be a butyrolactone containing one amino, one hydroxy, one carboxy and three methyl groups.



164

Mild basic treatment of the butyrolactone **164** resulted in its conversion into a lactam, which, on the basis of the NMR and mass spectra of its *O*-methyl derivative, was assigned the structure **165** correlating with the structure of the lactone **164**. Thus, the *N*-acyl substituent present in the PS chain is 2,4-dihydroxy-3,3,4-trimethyl-5-oxoproline **163** ($R^2 = R^3 = R^4 = CH_3$; $R^1 = R^5 = OH$) (2,4-dihydroxy-3,3,4-trimethylpyroglutamic acid).[202] The stereochemistry of this compound still remains unknown.

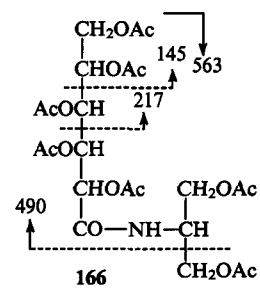


165

5. *N*-Substituents of hexuronic acid amides

As mentioned above, uronic acids occur occasionally in the polysaccharide chain as amides. Only one example of a glycuronic acid amide is known that is formed by an amino alcohol, 2-amino-1,3-dihydroxypropane. This compound was found in PS of *Shigella boydii* 8[203] and *Vibrio cholerae*.[158] The amino alcohol was isolated by paper electrophoresis after strong acid hydrolysis of the PS and identified mass spectrometrically as the *N*-acetate. Its structure was confirmed by counterflow synthesis.

To determine the position of the *N*-substituent, the PS was subjected to solvolysis with liquid HF resulting in isolation of a small amount of an *N*-substituted amide of galacturonic acid. Its structure was established by mass spectrometry of the corresponding alditol acetate **166** derived by a routine procedure. A peak of the molecular ion with *m/z* 563 confirmed its molecular weight, and a fragment with *m/z* 490 the presence of a CH_2OH group.



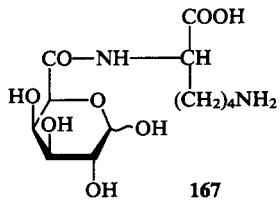
166

α-Amino acids linked by the amide bond to hexuronic acids (*N*-glycuronoyl amino acids) are more widespread. They were found in bacteria of the genus *Proteus*.[204]

So far, the bacteria *Proteus* have been shown to contain L-alanine (PS of *P. mirabilis* O27 and *P. penneri* 14), L-serine (PS of *P. mirabilis* O28), L-threonine (PS of *P. penneri* O12)[205] and L-lysine (PS of *P. mirabilis* O28 and *P. mirabilis* 1959) acylated by D-galacturonic acid, L-lysine being acylated at the α-amino group. In the PS of *P. mirabilis* O27, an L-lysine residue is acylated by D-glucuronic acid.

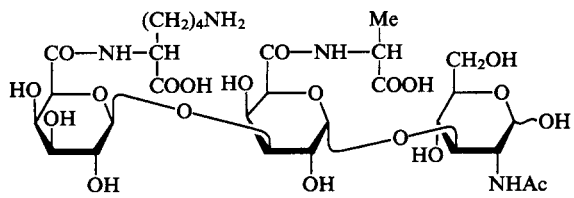
The presence of a uronic acid amide is revealed by the primary analysis of the ^{13}C NMR spectrum which showed a characteristic signal at 170–172 ppm corresponding to C(6) of uronic acid and signals of an amino acid residue. The presence of an amino acid is confirmed by its isolation using strong acid hydrolysis of PS and conventional analysis on an amino acid analyser. Its configuration was routinely established by the specific optical rotation or by esterification with (*R*)- or (*S*)-butan-2-ol.

To obtain final evidence for the amide structure, it was isolated in the pure state or within a short oligosaccharide after solvolysis with liquid HF and subjected to mass spectrometric and NMR analyses. Thus, the glycuronamide **167** whose ^1H NMR spectrum contained characteristic signals of galacturonic acid and lysine residues was isolated by ion-exchange chromatography from the PS of *P. mirabilis* 1959.[206] Notably, the signal of the C(2) proton of lysine appeared to be shifted compared to that of unsubstituted lysine (from 3.20 to 4.35 ppm) suggesting the acylation of the α-amino group of the amino acid.

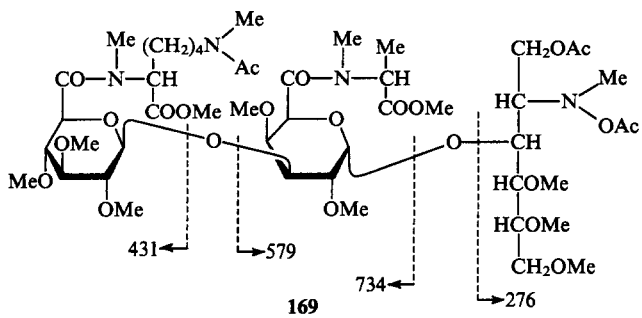


167

A similar approach was used to solve a more complex problem, namely, to determine the structure of two different amino acids and the site of their attachment to the corresponding monosaccharide units of the chain. Thus, after solvolysis with liquid HF, the trisaccharide **168** was isolated from the PS of *P. mirabilis* O27[207] containing L-alanine and L-lysine residues attached to residues of D-galacturonic and D-glucuronic acids; reduction with $NaBH_4$ and subsequent methylation of this trisaccharide gave the oligosaccharide **169**. The presence in its FAB mass spectrum of fragments with *m/z* 431, 579, 734 and 276 demonstrated unambiguously that the residue of glucuronic acid at the non-reducing end of the oligosaccharide **169** was amidated by the lysine residue, and the penultimate unit of galacturonic acid by the alanine residue.

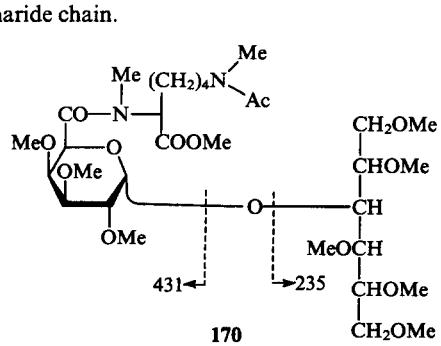


168

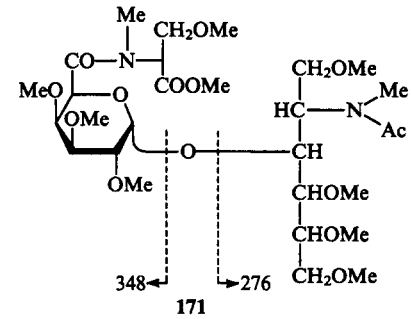


169

Similarly, localisation of L-lysine and L-serine residues in the PS of *P. mirabilis* O28 was established.[208] After mild acid hydrolysis of the PS two disaccharides, one of which contained lysine and the other a serine residue, were isolated from a complex mixture. The acetates of the glycosyl alditols **170** and **171** derived by reduction and methylation were analysed by mass spectrometry. Fragmentation of both compounds clearly demonstrated that the lysine residue was attached to the galacturonic acid bound to the galactose residue and the alanine residue to the galacturonic acid adjacent to the *N*-acetylglucosamine residue in the polysaccharide chain.



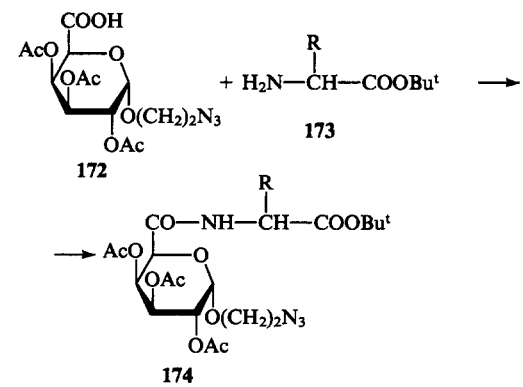
170



171

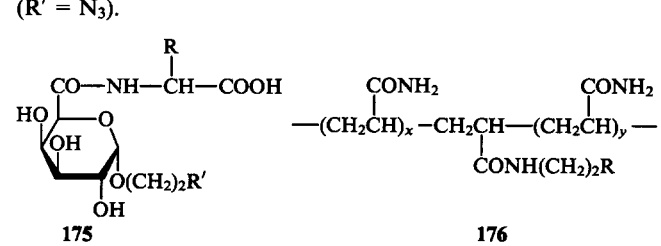
Amino acid residues in PS localised in side chains often represent immunodominant groups, i.e., they define the immunological specificity of the biopolymer. If the same PS contains residues of two different amino acids, an interesting question may arise: which particular amino acid residue plays the role of the determinant? To answer this question is essential from the viewpoint of creating diagnostic tools based on synthetic antigens. To this end, a synthesis of galacturonic acid amides with different amino acids was undertaken with their subsequent conversion to synthetic antigens by immobilisation on a polyacrylamide matrix using copolymerisation.[209,210] The synthesis of these amides was carried out in accordance with Scheme 16.[211]

Scheme 16



172 173 174

A uronic acid was first converted into the glycoside of 2-azidoethanol the residue of which was further converted into a synthetic antigen. 2′-Azidoethyl-2,3,4-tri-*O*-acetyl-α-galactopyranosiduronate **172** was condensed with the *tert*-butyl ester of the amino acid (in which the functional groups were protected) **173** [R = Me, R = $(CH_2)_4NHCOOBu^t$, R = CH_2OBu^t, R = $CH(Me)OBu^t$] in the presence of ethyl 2-ethoxy-1,2-dihydroxyquinoline-1-carboxylate. Deprotection of the condensation products **174** gave the corresponding derivatives of galacturonoyl-D- and -L-alanines, -L-serine, -L-threonine, -D- and -L-lysine **175** (R′ = N_3).



175 176

The azides **175** (R′ = N_3) were hydrogenated to give the amines **175** (R′ = NH_2) which were further *N*-acylated by acryloyl chloride. The resulting derivatives of the acrylic acid **175** (R′ = $NHCOCH=CH_2$) were copolymerised with acrylamide (R is a residue of a galacturonoyl amino acid) to give the synthetic antigens **176**.[211]

Comparison of the immunological specificity of the artificial antigens thus obtained with that of natural LPS isolated from bacteria allowed identification of the immunodominant group.[212] Thus, in the PS of *P. penneri* 12 this group is the L-threonine residue, since of all synthetic antigens studied, only the antigen containing this particular amino acid appeared to be active.

6. Phosphate groups

A unique position among substituents affecting the general characteristics of the polysaccharide chain is held by groups containing residues of inorganic acids, such as sulfate and phosphate groups. These groups create fragments with localised charges along the whole length of the biopolymer chain which influence not only the general properties of the polymeric chain but also its determinant sites. There is still no compelling evidence for the presence of sulfate groups in O-antigenic PS, although these groups are widely distributed in other PS, especially in PS of marine organisms, such as algae; evidently this is related to the peculiarities of the mineral composition of their environment.

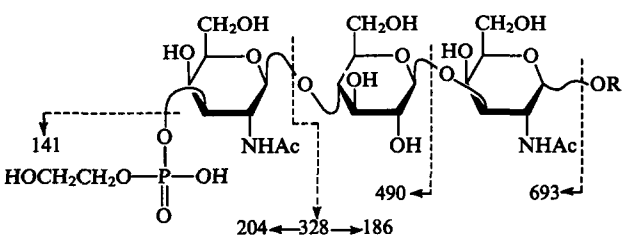
Phosphate groups were detected in many natural PS, including O-antigenic PS; the number of known phosphorylated O-antigenic PS is rapidly increasing. A distinction needs to be drawn between the two modes of incorporation of phosphorus-containing groups into PS. They can be present as substituents attached to one of the hydroxy groups of monosaccharide units in the polysaccharide chain or incorporated into the main skeleton of the polysaccharide linking together, in place of the glycosidic bond, monosaccharide or oligosaccharide fragments of the main chain of the biopolymer. The latter case may be considered as a new type of biopolymers which is not actually a polysaccharide, because its main chain consists of both glycosidic and phosphodiester bonds. Thus, this biopolymer occupies an intermediate position between PS and teichoic acids.

Biopolymers of this type, as well as lipid A, where the phosphate groups are present as components of a disaccharide,[1] are not discussed in the present review.

The presence of *O*-phosphate substituents in the chains of O-antigenic PS is rather well known. Usually they form phosphodiester groups esterifying a monosaccharide unit and a residue of an alcohol or an amino alcohol.

The presence of the phosphodiester group in PS was confirmed (apart from analysis of PS for phosphorus) by ^{31}P NMR spectroscopic data. The nature of the second non-carbohydrate substituent in the phosphodiester group can also be found from NMR spectroscopic data and is finally established during localisation of the phosphodiester group in the PS chain using various approaches as required by the particular experimental goal.

A polysaccharide of the bacterium *Citrobacter* O16[213] was found to contain a glycerol phosphate group substituting a hydroxy group of an *N*-acetylgalactosamine residue. This polysaccharide contained 3% of phosphorus, and its ^{31}P NMR spectrum revealed a signal of a phosphodiester group at 0.48 ppm. The presence of glycerol was established by GLC of the acid hydrolysate of the PS. To localise the glycerophosphate group, the PS was subjected to Smith degradation followed by mild acid hydrolysis to afford the oligosaccharide **177** containing the phosphate group.



177

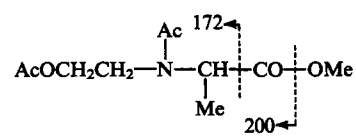
The FAB mass spectrum of the oligosaccharide **177** contained a characteristic fragment with *m/z* 328 which indicated the presence of a phosphodiester group at the *N*-acetylgalactosamine residue. This finding was also confirmed by the presence of a fragment with *m/z* 141 corresponding to the residue of the phosphodiester group modified by oxidation with HIO_4 (the glycerol residue was converted into the ethyleneglycol residue). The presence of fragments with *m/z* 490 and 693 demonstrated localisation of the phosphorylated monosaccharide at the reducing end of the oligosaccharide **177**; this determined its position in the PS chain established by the structural analysis of PS. These data were corroborated by ^{13}C NMR spectroscopic data which suggested also that the glycerol phosphate group substitutes the hydroxy group at C(3) of galactosamine since the signal of C(3) is shifted down-field in comparison with the signal of the unsubstituted monosaccharide (72.4 → 76.4 ppm).

The glycerol phosphate group was also found in the PS of *E. coli* O100. This PS has previously been studied by classical methods.[214] Substitution of one of the hydroxy groups of the monosaccharide chain was demonstrated by cleavage of glycerol phosphate by alkaline hydrolysis of the PS without cleavage of the polymeric chain of PS. The binding of the substituent to one of the hydroxy groups of the rhamnose residue was evident from the stability of the residue of this monosaccharide in the native PS towards periodate oxidation, whereas in the dephosphorylated PS it was destroyed.

In some PS the phosphodiester group contained an ethanolamine residue, as in the PS of *Proteus mirabilis* O27.[207] The ^{31}P NMR spectrum of the polysaccharide containing 2.5% phosphorus includes a signal at 1.33 ppm which indicates the presence of a phosphodiester group. Ethanolamine was identified after acid hydrolysis of PS by routine methods; its presence was confirmed by the presence in the ^{13}C NMR spectrum of signals at 63.2 (CH_2OH) and 41.5 ppm (CH_2NH_2). Both signals, as well as the signals of C(5) and C(6) of *N*-acetylglucosamine present in the PS, were split due to coupling to the neighbouring phosphorus atom, thus suggesting that the phosphodiester group is linked to C(6) of *N*-acetylglucosamine. Elimination of the ethanolamine residue upon dephosphorylation of the PS confirmed its attachment to the phosphate group. The phosphodiester group appeared to substitute in the PS only 80% of the monosaccharide residues as determined by analysis of the ^{13}C NMR spectrum: it contained two sets of signals for C(4), C(5) and C(6) of glucosamine, of which 80% belonged to the phosphorylated monosaccharide and 20% to the unsubstituted one.

A 2-aminoethyl phosphate group was found in the PS of *P. penneri* 8 where its localisation at C(6) of *N*-acetylglucosamine was demonstrated using similar approaches.[205]

A more complex phosphodiester substituent containing a residue of *N*-(2-hydroxyethyl)-D-alanine was found in PS of *P. mirabilis* O3.[215] The polysaccharide containing 2.5% of phosphorus was dephosphorylated with concentrated aqueous HF. The resulting amino acid was isolated from the hydrolysate by chromatography and converted into the methyl ester of the *N,O*-diacetate **178** whose mass spectrum contained ions with *m/z* 200 $(M-OCH_3)^+$ and *m/z* 172 $(M-CH_3OOC)^+$ characteristic of fragmentation of the molecular ion of this kind.



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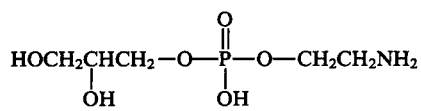
The presence in the ^{13}C NMR spectrum of the amino acid of signals at 15.4 (CH_3), 48.8 (CH_2N), 58.2 (CHN), 56.9 (CH_2O) and 172.5 ppm (COOH) correlated with the structure ascribed to this amino acid. Its binding to the phosphate group was confirmed by the fact that signals of CH_2N and CH_2O groups are shifted from 48.3 and 54.7 ppm in the free amino acid to 47.5 and 62.3 ppm in

the PS and were split due to C–P coupling. The binding of this complex phosphodiester substituent to the hydroxy group at C(6) of a galactose unit in the PS chain is evident from the fact that in the ^{13}C NMR spectrum of the native PS the C(5) signal of this monosaccharide is split and shifted up-field as compared with the signal in the dephosphorylated PS (71.8 → 70.6 ppm), whereas the C(6) signal is shifted down-field (62.2 → 66.0 ppm).

Sometimes, the same PS contains two distinct phosphodiester groups linked to different monosaccharide units. An example is a PS of *P. mirabilis* D52 containing residues of 2-aminoethyl phosphate and ribitol phosphate.[216]

This PS was investigated using classical methods. Apart from phosphorus, it contained 14.5% ribitol and 2.6% ethanolamine which, after strong acid hydrolysis, were isolated and identified by routine methods. Both residues could also be cleaved by alkaline hydrolysis without any cleavage of the polysaccharide chain, thus suggesting their localisation in the side chain but not in the main skeleton of the biopolymer. Reaction of the amino group of ethanolamine with 2,4-dinitrofluorobenzene showed that this group is unsubstituted and that the amino alcohol is phosphorylated at the hydroxy group. The ribitol residue was fully oxidised with HIO_4 to give formaldehyde and formic acid, thus providing a proof for ribitol phosphorylation at one of the primary hydroxy groups.

Localisation of the phosphodiester substituents in the PS chain was determined during establishment of the PS structure, for which purpose the PS was cleaved by acid hydrolysis or several repeated Smith degradations. The resulting oligosaccharides were analysed by classical methods. Thus, analysis of one of the fragments obtained by several repeated Smith degradations of the PS revealed the 2-aminoethyl phosphate group to be localised at C(6) of one of the two galactose residues. This is evident from the structure of the fragment **179** containing the 2-aminoethyl phosphate substituent at the glycerol residue, which is the end product of Smith degradation of the galactose unit.



179

Localisation of the ribitol phosphate group in the second galactose unit followed from the fact that in the native PS this unit is not oxidised with HIO_4, whereas in the dephosphorylated PS it undergoes oxidation. The reason is that dephosphorylation results in formation of a vicinal glycol group, and, consequently, the ribitol phosphate group is localised at C(3) of galactose. Since localisation of these galactose units was determined in the course of structural analysis of the PS chain, the distribution of both phosphodiester groups in the chain is also established.

While considering this relatively complex example of structural analysis of a PS, it should be noted that the use of NMR spectroscopy offers a rapid, simple and reliable tool to solve similar problems.

V. Conclusions

In this review data are given on novel, scantily known monosaccharides present in O-antigenic polysaccharides of Gram-negative bacteria, many of which have thus far been detected only in this particular class of natural compounds. These data which were mainly obtained at the Laboratory of Carbohydrate Chemistry, Institute of Organic Chemistry (Russian Academy of Sciences), demonstrated that the old classical concepts of monosaccharides as a small group of structurally related organic substances are contrary to fact.

Monosaccharides represent a wide class of organic compounds integrated by one common property, namely, the ability to form cyclic oxygen-containing systems existing in a tautomeric equilibrium with the corresponding polysubstituted hydroxy aldehydes or ketones. By appending to these data an ample body of experimental evidence on numerous monosaccharides found in antibiotics and some other natural substances, it may be well to notice that we deal with a large class of compounds whose carbon skeleton comprises very diverse functional groups and substituents, the number of individual representatives increasing largely due to their stereochemical differences determined by the presence of a large number of chiral centres. It should also be remembered that the number of novel monosaccharides carrying more than six carbon atoms (including those with a branched carbon chain) is rapidly increasing.

Such a dramatic widening of the class of monosaccharides calls for new methods and techniques for determination of their structure, conducting stereospecific syntheses and elaboration of new concepts of the spatial structure of their molecules.

The present review shows how important is the application of new methods for establishment of the structure and identification of hitherto unknown monosaccharides and their derivatives. The prominent role of physicochemical studies, without which further progress in this complex branch of organic chemistry would be impossible, becomes especially apparent.

It is quite impossible (and not necessary) to give all the details of structural analysis in a short review like this. An effort has just been made to outline the basic route and general logic which are significantly different in studies of structurally distinct monosaccharides. The syntheses of some representatives of monosaccharides described above illustrate the peculiarities of the stereospecific arrangement of rather complex compounds which may be useful in consideration of pathways of synthesis of other polychiral systems.

It should again be noted in conclusion that the data cited here are essential for development of molecular concepts in related biological sciences: biochemistry, immunochemistry, immunology and molecular biology.

A knowledge of the fine structure of monosaccharides making up the bulk of the important class of microbial polysaccharides, aside from its fundamental natural-scientific significance, is of prime importance for solution of applied tasks, firstly, in the design and synthesis of a new generation of biologically active compounds, especially of medicinal drugs used in chemotherapy and reliable diagnostics of infectious diseases.

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