LIPOPOLYSACCHARIDES OF THE PSEUDOTUBERCULOSIS MICROBE *Yersinia pseudotuberculosis*

Yu. S. Ovodov and R. P. Gorshkova

UDC 577.114.5:543.422.25:579.842.23

This review generalizes the results of structural investigations of the polysaccharides of the O-specific side chains, of the core oligosaccharides, and of the lipid A of the pseudotuberculosis microbe Yersinia pseudotuberculosis which causes Far-Eastern scarlatina-like fever. The complete structures of the repeating units of the specific polysaccharides from the lipopolysaccharides of serovars I A, I B, III, IV A, V A, V B, and VI which are responsible for the O-antigenic specificity of the microorganism are given. For the majority of serovars the repeating unit is represented by a branched pentasaccharide. Exceptions are the lipopolysaccharides of serovars I A and III which have a tetrasaccharide repeating unit. The presence of an aminosugar residue at the reducing end, by which the O-specific chain is attached to the oligosaccharide of the core, is common and characteristic for the lipopolysaccharides isolated from all the serovars. The structures of the core oligosaccharide and of lipid A, which are common for all serovars of Yersinia pseudotuberculosis, are given.

The high biological activity of the lipopolysaccharides (LPSs) has led to an intensive study of the properties and structure of these biopolymers. Such investigations are important for medicine and epidemology, assist discrimination in the classification of microorganisms, and open up prospects for understanding the link between the structure of the LPSs and their function in the organism and for elucidating the molecular principles of immunity.

This review describes the structural investigation of the LPSs of the pseudotuberculosis microbe Yersinia pseudotuberculosis (PTM), one of the most studied representatives of the Yersinia genus.

The pseudotuberculosis microbe was discovered in the last century by French workers [1]. It was subsequently found that it is the causative agent of Far-Eastern scarlatine-like fever (SLF) [2-4]. According to the modern classification, the PTM is divided into eight serological variants (serovars), serovars I, II, IV, V having subserovars A and B [5, 6]. It must be mentioned that serovars VII and VIII and serovar II C are new, having been discovered by Japanese workers in 1984 [6].

In human pathology, PTM serovar I is of the greatest importance, being responsible for from 88 to 97.5% of cases of SLF; after this follows serovar III (an average of 10% of cases), and then serovar IV (about 1% of the diseases) [4]. All the serovars of PTM are pathogenic for mice and cause their death in the majority of cases.

Various antigenic complexes have been isolated from PTM for which only preliminary chemical and immunochemical information has been obtained. Thus, in 1957 Sasaki [7] isolated an antigenic complex from untyped strains of PTM with the aid of extraction by trichloroacetic acid and showed that it was common to various species of salmonellas. Glucose, galactose, xylose, arabinose, rhamose, and traces of glucosamine and of ribose were identified in a hydrolysate complex obtained.

In 1958, Davies [8, 9] established that the complex of O-specific antigens of PTM consisted of lipopolysaccharides (LPSs). He isolated the LPSs from five serovars of the S-form and from the R-forms of a number of serovars and showed that the presence of glucosamine and D-glycero-L-mannoheptose residues was common for all the LPSs. In addition, glucose, galactose, mannose, fucose and galactosamine, and also 3,6-dideoxyhexoses (paratose, abequose, tyvelose,

---

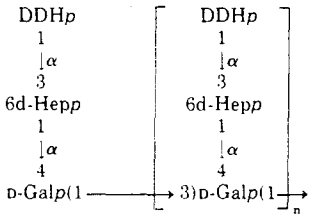
Pacific Ocean Institute of Bioorganic Chemistry, Far Eastern Branch, USSR Academy of Sciences, Vladivostok. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 163-171, March-April, 1988. Original article submittted March 2, 1987; revision submitted August 6, 1987.

0009-3130/88/2402-0135$12.50

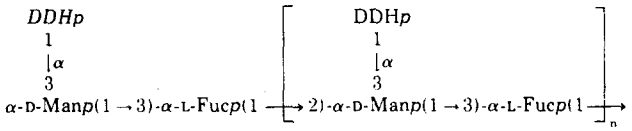
ascarylose), were detected among the monosaccharides and were isolated by preparative paper chromatography [10].

The first publications from Lindberg's Swedish school [11, 4] on the structural investigation of the LPS from PTM appeared in 1972. On the basis of the results of the methylation of the LPS and of hapten fractions, preliminary structures were established for the repeating unit of the O-specific side chains of the LPSs of serovars IIA and II B (LPS-II A and LPS-II B, respectively). 6-Deoxy-D-mannoheptose was identified for the first time in LPS-II A. In the determination of the structure of the specific polysaccharide of serovar II B (LPS-II B) a disaccharide constructed of 1,3-bound mannose and fucose residues was isolated.

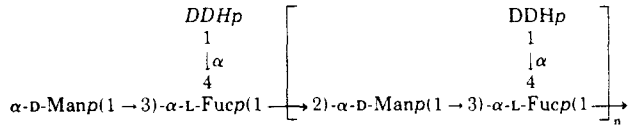
Later [13], solely on the basis of the results of methylation of the LPSs and of hapten fractions, provisional structures were proposed for the O-specific side chains of the LPSs of the following serovars: I A, I B, II A, II B, III, IV A, IV B, V A, and V B – in the form of four types of structures:



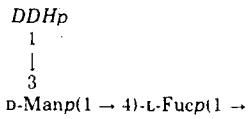
Structure of O-specific side chains of serovars Ia, IIa, and IVB



Structure of O-specific side chains of serovars IB and IIB.



Structure of O-specific side chains of serovars III and IVa.



Structure of O-specific side chains in polysaccharide in serovars VA and VB.

The authors came to the conclusion that each serological group had the same structure of the main carbohydrate skeleton of the O-specific polysaccharide of the LPS; differences within the groups were due to the terminal 3,6-dideoxyhexose residues.

German workers [14], studying the monosaccharide composition of LPSs isolated from serovars I-IV confirmed Lindberg's results for serovars I-III but they did not detect fucose residues in serovar IV, and in serovar VI, in place of colitose they found tyvelose, while serovar V represented the R-form. In all the LPSs isolated an unidentified monosaccharide was found; the hypothesis was expressed that this was 6-deoxy-D-mannoheptose.

The inadequacy of the study of the structure of the LPSs and their specific properties, and also the revelation of SLF as a new clinical form of pseudotuberculosis demanded the careful study of the LPSs isolated from the Far-Eastern strains, their comparison with the LPSs of museum strains, and a systematic investigation of the structures of the LPSs from the PTMs of all known serovars. Since serovar I is the main causative factor of SLF, it appeared of

interest to ascertain the distribution of sub-types I A and I B in the Far East. As a result of a comparative immunochemical investigation of LPS-I A from museum strains and a local strain it was found that the local strain belonged to serovar IB and was, in fact, the main causative agent of SLF [15]. It was established later [16] that, beginning from 1979, in Maritime Territory serovar I A was circulating as well as serovar I B, and strains of both serovars were not infrequently isolated from SLF patients.

A dry erythrocytic diagnostic agent has been developed from LPS-I B [17, 18] which permits the rapid and reliable identification of SLF patients in the early stages of the disease.

As is well known [19], the LPS molecule consists of three main fractions: the polysaccharide of the O-specific side chains, the oligosaccharide of the core, and lipid A.

We have made a structural investigation of the LPSs from the PTMs of serovars I-IV, by a general scheme including complete and partial hydrolysis, methylation, periodate oxidation, and other methods of carbohydrate chemistry. In a number of cases physicochemical methods were used successfully: chromato-mass spectroscopy for the separation and identification of monosaccharides and their derivatives; and ^{13}C NMR for determining more accurately structural features of the polysaccharides and other fragments obtained as a result of the partial hydrolysis of the LPS molecule. On the mild acid hydrolysis of an LPS with dilute acetic acid, lipid A was isolated, and it was then subjected to a further structural study. It was interesting to note that during such hydrolytic cleavage of the LPSs of all PTM serovars a tendency to the splitting out of the O-specific polysaccharides was detected and their isolation in the practically unchanged state facilitated the further study of their structure. The structures of the core polysaccharide and the lipid A have been determined.

POLYSACCHARIDES OF THE O-SPECIFIC SIDE CHAINS

As is well known, the polysaccharides of the O-specific side chains of the LPSs or the O-specific polysaccharides determine the immunological specificity of the LPS molecule. They contain the immunodominant groups and play an important role in the humoral immune response of the human or animal organism to the penetration of the causative agent of the infection and in the development of specific immunity to the given disease.

We have made a detailed investigation of O-specific polysaccharides of PTM serovars I A, I B, III, IV A, V A, V B, and VI [20-36]. Let us consider the results of the investigations performed.

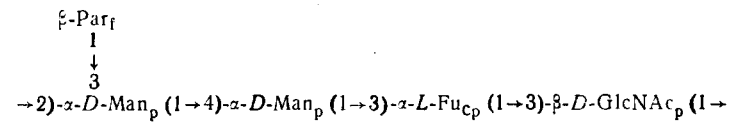
O-Specific Polysaccharide of PTM Serovar I A (PS-I A). The repeating unit of PS-I A consists of a tetrasaccharide containing equimolar amounts of paratose, a 6-deoxyheptose, galactose, and N-acetylglucosamine [28]. The complete structure of the repeating tetrasaccharide unit of PS-I A has been established [21, 28]:

$$\begin{array}{l} \alpha\text{-Par}_p\ (1\rightarrow 3)\text{-}\beta\text{-6d-Hep}_p \\ \qquad\qquad\quad \downarrow 1 \\ \qquad\qquad\quad \downarrow 4 \\ \rightarrow 3)\text{-}\alpha\text{-}D\text{-Gal}_p\ (1\rightarrow 3)\text{-}\beta\text{-}D\text{-GlcNAc}_p\ (1\rightarrow \end{array}$$

As we see, a feature of the structure given is the presence of a 6-deoxyheptose residue in the lateral terminal carbohydrate chain. A similar structure was proposed previously by Lindberg [13], but we have established that a component of the repeating unit is a N-acetyl-D-glucosamine residue. This feature was not observed previously.

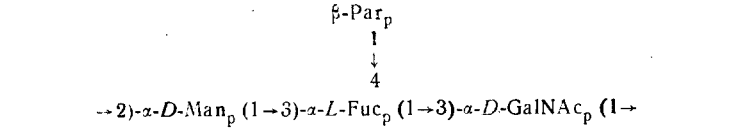
O-Specific Polysaccharide of PTM Serovar I B (PS-I B). On the complete hydrolysis of the LPS-I B of pathogenic strain No. 12 it was shown that its carbohydrate chain included paratose, D-glucose, D-mannose, L-fucose, L- and D-glycero-D-mannoheptose, D-glucosamine, and KDO residues [29]. PS-I B, containing paratose, fucose, mannose, and glucosamine residues could be isolated only by gel filtrations on Sephadex G-10 of the low-molecular-weight fraction obtained on the acetic acid hydrolysis of the LPS. Gel filtration on Sephadex G-50 of the high-molecular-weight-fraction led to the isolation of a polysaccharide containing all the monosaccharides present in the LPS. As a result of the investigation performed, a structure of the pentasaccharide repeating unit of PS-I B was proposed [22, 29, 30].

The molecular weight of PS-I B is 22 kDa [31]. It was shown that to each repeating unit of the specific polysaccharide there is an average of one paratose residue, and the PS-I B molecule consists of 24-26 repeating units. The molar ratio of antigen to antibody in



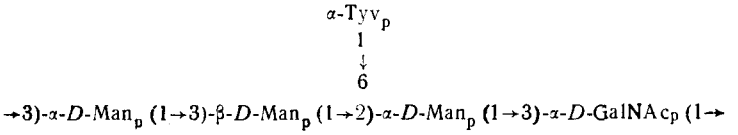
the precipition in the equivalence zone, 1:22, apparently corresponds to the presence of 22 determinant groups [31].

The O-Specific Polysaccharide of PTM Serovar III (PS-III). The structure of the repeating tetrasaccharide unit of PS-III was established by chemical methods using methylation and Smith degradation [23], while, later [32], the ^{13}C NMR spectrum enabled the configuration of the glycoside bonds to be determined accurately and confirmed the results obtained previously:



As can be seen from the structure shown, the immunodeterminant sugar is the paratose residue, as in the case of PS-I A and PS-I B. A special feature consists in the presence of a N-acetyl-D-galactosamine residue as a component of the repeating unit of PS-III.

The O-Specific Polysaccharide of PTM Serovar IV A (PS-IV A). Strains No. 31 D, isolated from a patient in Maritime Territory, and No. 32, a museum strain obtained from France, were used to study and characterize LPS-IV. A comparison of the monosaccharide composition of the lipopolysaccharides with literature information [13] showed that the serovar IV that has been isolated very rarely from patients belongs to subserovar IV A [24]. On the basis of the results of the investigations performed, the following structure of the repeating unit was proposed for PS-IV A [24].



It must be mentioned that this structure differs from the structure proposed by Lindberg [13] by the absence of a fucose residue and the presence of a galactosamine residue in the carbohydrate chain.

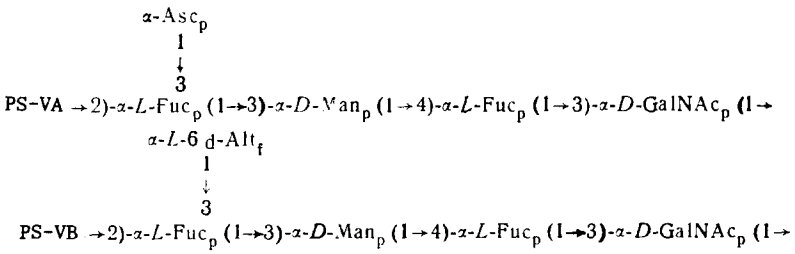
O-Specific Polysaccharides of PTM Serovars V A and V B (PS-V A and PS-V A). To determine the structures of PS-V A and PS-V B, a comparative immunochemical and chemical investigation of the LPSs of various strains of PTM serovars V A and V B, obtained from France and Japan, was carried out [33]. A difference was detected in the monosaccharide compositions of the LPSs of the strains assigned to one and the same serovar in different countries. Each LPS gave a single precipitation band in agar with antiserum to the initial strain. A precipitation band in agar with plague antiserum was given only by the LPS of a R-form (strain No. 2456) which includes the monosaccharides of the "core": glucose, galactose, D- and L-glycero-D-mannoheptose, glucosamine, galactosamine, and KDO.

In the LP of strain V A ≈ 12 (Japan) in addition to the "core" monosaccharides, rhamnose, fucose, and mannose residues were detected; in LPS-V A (strains Nos. 2457 and 52), ascarylose, fucose, and mannose residues; and in LPS-V B-R2 6-deoxy-L-altrose, fucose, and mannose residues [34].

PS-V A (strain No. 2457) [25] and PS-V B (strain R2) [26], were isolated by the gel filtration of the hapten fractions on Sephadex G-50 after acetic acid hydrolysis of the LPS. It was established that the two specific polysaccharides possess identical linear polymeric chains and differ only by the monosaccharide residues on the side chains.

It must be mentioned that 6-deoxy-L-altrofuranose was found in the PS-V B for the first time for PTM, this, like the ascarylose residue, being attached to fucose by 1,3-glycosidic bond.

O-Specific Polysaccharide of PTM Serovar VI (PS-VI). PS-VI is distinguished by a very peculiar feature [35]. In it, a branched monosaccharide, the first representative of natural octoses, has been detected. It has been called yersiniose [36]. The structure of yersiniose was established on the basis of the results of mass spectrometry and ^{1}H and ^{13}C NMR spec-

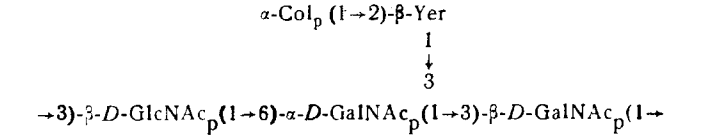


troscopy both of the monosaccahride itself and of its derivatives. It was shown that yersiniose is 4-C-(α-hydroxyethyl)-3,6-dideoxy-D-xylo-hexose. In the specific polysaccharide, colitose, and 2-acetoamido-2-deoxy-D-glucose residues and two 2-acetamido-2-deoxy-D-galactose residues were identified as well as yersiniose.

A partial hydrolysis of this polysaccharide gave for the first time a disaccharide containing a 3,6-dideoxyhexose residue:

$$\text{Col}(1\rightarrow2)\text{-Yer},$$

where Yer represents a yersiniose residue. The following structure has been put forward for the repeating unit of PS-VI [28]:



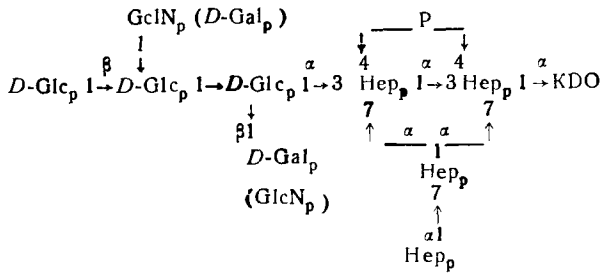
As the structures of the specific polysaccharides of the pseudotuberculosis microbe show, for the majority of serovars the repeating unit is represented by a branched pentasaccharide, but serovars I A and III are exceptions, having tetrasaccharide repeating units. The only common feature is the presence of an aminosugar residue at the reducing end of the repeating unit. In general the presence of an aminosugar residue at the end to which the O-specific chain is attached to the core oligosaccharide is characteristic of the LPSs isolated from all serovars.

The linking unit between the specific polysaccharide and the core oligosaccharide is probably galactosamine, but PS-I A and PS-I B, having glucosamine residues at the end of the repeating units, form exceptions.

THE CORE OLIGOSACCHARIDE

Information on the structure of the core of the PTM was obtained in an investigation of two mutants of PTM serovar V A (strain No. 2456) [37]. LPS-R I contains D-galactose, D-glucose, 2-acetamido-2-deoxy-D-glucose, and 2-keto-3-deoxyoctonic acid (KDO) residues, and, in addition, D-glycero-D-manno- and L-glycero-D-mannoheptose residues in a ratio of 1:3. LPS-R II differed only by the absence of a D-galactose residue.

Mild acid hydrolysis followed by gel filtration of the carbohydrate component (after the elimination of lipid A), gave the corresponding oligosaccharides of the core (OSC-I and OSC-II). OSC-I consisted of D-galactose, D-glucose, two heptose, and D-glucosamine residues in a ratio of 1:2.5:4:1, respectively, and OSC-II consisted of D-glucose, a heptose, D-glucosamine residues in a ratio of 1:2.5:0.2. The configurations of the glycosidic bonds were determined on the basis of the oxidation of the acetylated glycosides with chromium trioxide [38].



As a result of the structural investigations of OSC-I, OSC-II, LPS-R I, and LPS-R II the structure on previous page of the core oligosaccharide of the LPS from PTM has been proposed.

It may be assumed that the LPS of all the serovars have cores of similar structure or, in any case, the same heptose regions since identical heptose derivatives from the fully methylated LPSs were identified.

LIPID A

Lipid A is a component of the LPS and is responsible for its endotoxic properties. As has been shown [39], in the majority of Gram-negative bacteria the attachment of lipid A to the oligosaccharide core is effected by an acid-labile glycosidic bond of KDO.

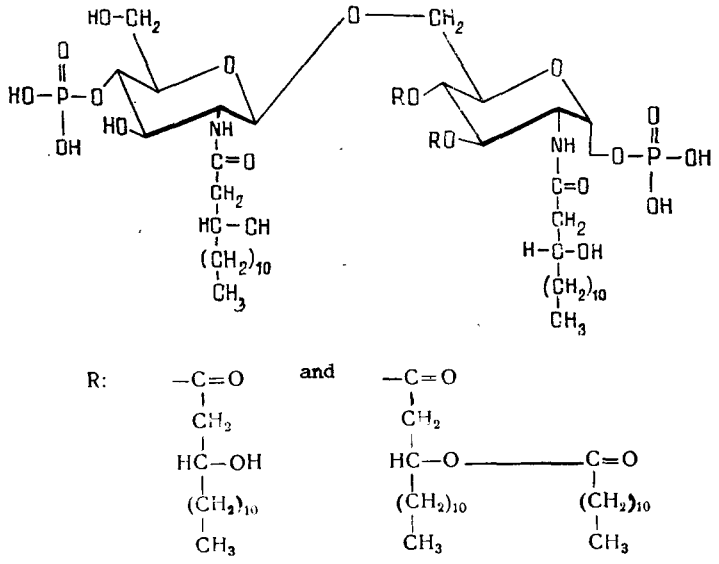
Lipid A was isolated from LPS-I B on acetic acid hydrolysis. The purified lipid A formed a quarter of the LPS molecule and had a molecular weight of 2900 Da [40]. The presence of dodecanoic and β-hydroxytetradecanoic acids in a ratio of 1:3.6 was established by GLC and chromato-mass spectrometry.

To determine the hydrocarbon skeleton of lipid A it was subjected to mild acid hydrolysis and was reduced and deacylated with anhydrous hydrazine. As a result, a degraded lipid A was obtained which had the mobility of a glucosaminobiose and contained no phosphorus and fatty acids. It included glucosamine and glucosaminitol residues in a ratio of 1:1.

The interpretation of the ^{13}C NMR spectra of the degraded lipid A and of a sample of β-1,6-glucosaminobiose showed their complete identity and the presence of a β-1,6-bond between the glucosamine residues [41]. The ^{31}P NMR spectrum of lipid A showed two signals of equal intensity corresponding to monophosphates.

To determine the nature of the bond of the fatty acid residues with the carbohydrate skeleton we used mild alkaline agents hydrolyzing ester bonds. Analysis of the fatty acids (the yield of which was about 40%) showed that dodecanoic and 3-hydroxytetradecanoic acid were present in a ratio of 1:2. In addition, 3-dodecanoyloxytetradecanoic acid, a component of the fatty acids of lipid A, was isolated and characterized by chromato-mass spectrometry and ^{13}C NMR spectroscopy for the first time [42].

The complete structure of lipid A was established in an interpretation of the ^{13}C NMR spectrum of lipid A previously methylated at the phosphate group. The ^{13}C NMR spectra of synthesized analogs of lipid A were used to interpret the spectrum [43, 44]. Thus, lipid A consists of β-1,6-bound glucosaminobiose with two phosphoric acid residues, one of which is present at the reducing end of the molecule. The amino group of the glucosamine residue is acrylated by 3-hydroxytetradecanoic acid and some hydroxy groups are substituted by residues of 3-hydroxytetradecanoic and dodecanoic acids.



It has been established that lipid A of PTM is immunogenic, its immunodeterminant group being a glucosamine residue acylated in the amino group by 3-hydroxytetradecanoic acid [45].

The study of Y. pseudotuberculosis lipids A from the LPSs of other serovars and their common characteristics confirm that they have approximately the same qualitative and quantitative compositions.

Thus, the complete structure of the LPS has been established for the pseudotuberculosis microbe, the structures of the core oligosaccharides and of the lipids probably being the same but the structure of specific polysaccharides being characterized by great diversity.

LITERATURE CITED

1. L. Malassez and W. Vignal, Arch. J. Phys., 2, 369 (1983).
2. I. I. Grunin, G. P. Somov, and I. Yu. Zalmover, Voenno-med. Zh., 8, 62 (1960).
3. V. A. Znamenskii and A. K. Vishnyakov, Zh. Mikrobiol., 2, 125 (1967).
4. G. P. Somov, Far-Eastern Scarlatina-like Fever [in Russian], Meditsina, Moscow (1979).
5. M. Tsubokura, K. Itagakii, and K. Kamamura, Jpn. J. Vet. Sci., 33, 137 (1971).
6. M. Tsubokura, K. Otsuki, Y. Kawaoka, H. Fukushima, K. Ikemura, and Y. Kanazawa, Curr. Microbiol., 11, 89 (1984).
7. T. Sasaki, Nature (London), 179, 920 (1957).
8. D. A. L. Davies, J. Gen. Microbiol., 18, 118 (1958).
9. M. J. Crumpton, D. A. L. Davies, and A. M. Hutchison, J. Gen. Microbiol., 18, 129 (1958).
10. D. A. L. Davies, Nature (London), 191, 43 (1961).
11. G. G. Hellerqvist, B. Lindberg, K. Samuelsson, and R. Brubaker, Acta Chem. Scand., 26, 1389 (1972).
12. G. G. Hellerqvist, B. Lindberg, K. Samuelsson, and R. Brubaker, Acta Chem. Scand., 26, 1394 (1972).
13. K. Samuelsson, B. Lindberg, and R. Brubaker, J. Bacteriol., 117, 1010 (1974).
14. G. Seltmann, W. Beer, and E. Thal, Z. Allg. Microbiol., 14, 73 (1974).
15. N. N. Besednova, G. P. Somov, R. P. Gorshkova, S. V. Tomshich, N. F. Timchenko, T. A. Sharapova, and Yu. S. Ovodov, Zh. Mikrobiol., 3, 27 (1975).
16. T. A. Kuznetsov, N. N. Besednova, T. A. Sharapova, R. P. Gorshkova, N. A. Komandrova, and N. F. Timchenko, Zh. Mikrobiol., 5, 111 (1981).
17. N. N. Besednova, G. P. Somov, R. P. Gorshkova, S. V. Tomshich, T. A. Sharapova, and Yu. Ovodov, Zh. Mikrobiol., 7, 48 (1974).
18. N. N. Besednova, R. P. Gorshkova, S. V. Tomshich, Yu. S. Ovodov, and G. P. Somov, Lab. Delo., 2, 119 (1976).
19. O. Lüderitz, A. N. Staub, and O. Westphal, Bacteriol. Rev., 30, 192 (1966).
20. Yu. S. Ovodov, R. P. Gorshkova, and S. V. Tomshich, Immunochemistry, 11, 777 (1974).
21. N. A. Komandrova, R. P. Gorshkova, V. V. Isakov, and Yu. S. Ovodov, Bioorg. Khim., 10, 232 (1984).
22. S. V. Tomshich, R. P. Gorshkova, Yu. E. Elkin, and Yu. S. Ovodov, Eur. J. Biochem., 65, 193 (1976).
23. R. P. Gorshkova, N. A. Komandrova, A. I. Kalinovsky, and Yu. S. Ovadov, Eur. J. Biochem., 107, 131 (1980).
24. R. P. Gorshkova, V. A. Zubkov, V. V. Isakov, and Yu. S. Ovodov, Bioorg. Khim., 2, 1401 (1983).
25. R. P. Gorshkova, N. I. Korchagina, and Yu. S. Ovodov, Eur. J. Biochem., 131, 345 (1983).
26. N. I. Korchagina, R. P. Gorshkova, and Yu. S. Ovodov, Bioorg. Khim., 8, 1666 (1982).
27. R. P. Gorshkova, V. A. Zubkov, V. V. Isakov, and Yu. S. Ovodov, Bioorg. Khim., 9, 1068 (1983).
28. N. A. Komandrova, R. P. Gorshkova, and Yu. S. Ovodov, Khim. Prir. Soedin., 231 (1983).
29. S. V. Tomshich, R. P. Gorshkova, Yu. N. El'kin, and Yu. S. Ovodov, Khim. Prior. Soedin., 563 (1975).
30. V. V. Isakov, R. P. Gorshkova, S. V. Tomshich, Yu. S. Ovodov, and A. S. Shashkov, Bioorg. Khim., 7, 559 (1981).
31. T. F. Solov'eva, G. M. Frolova, O. P. Sanina, and Yu. S. Ovodov, Mikrobiol. Zh., 47, 66 (1985).
32. V. V. Isakov, N. A. Komandrova, R. P. Gorshkova, and Yu. S. Ovodov, Bioorg. Khim., 9, 1565 (1983).
33. R. P. Gorshkova, N. I. Korchagina, T. A. Medonova, E. N. Kalmykova, N. N. Besednova, and Yu. S. Ovodov, Carbohydr. Res., 84, 237 (1980).

34. R. P. Gorshkova, N. I. Korchagina, V. V. Isakov, and Yu. S. Ovodov, Khim. Prir. Soedin., 3 (1983).
35. R. P. Gorshkova, V. A. Zubkov, and Yu. S. Ovodov, Immunochemistry, 13, 581 (1976).
36. R. P. Gorshkova, V. V. Isakov, and Yu. S. Ovodov, Carbohydr. Res., 126, 308 (1984).
37. S. V. Tomshich, R. P. Gorshkova, and Yu. S. Ovodov, Khim. Prir. Soedin., 751 (1985).
38. H. Hoffman, B. Lindberg, and K. Jann, Eur. J. Biochem., 56, 41 (1975).
39. O. Lüderitz, M. A. Freudenberg, C. Galanos, V. Lehmann, E. Th. Rietschel, and D. H. Shaw, Curr. Topics Membrane Transport, 17, 79 (1982).
40. I. N. Krasikova, V. I. Gorbach, T. F. Solov'eva, and Yu. S. Ovodov, Eur. H. Biochem., 89, 287 (1978).
41. I. N. Krasikova, V. I. Gorbach, V. V. Isakov, T. F. Solov'eva, and Yu. S. Ovodov, Eur. H. Biochem., 126, 249 (1982).
42. I. N. Krasikova, P. A. Luk'yanov, V. I. Gorbach, T. F. Solov'eva, and Yu. S. Ovodov, Experientia, 40, 709 (1984).
43. V. I. Gorbach, P. A. Luk'yanov, T. F. Solov'eva, and Yu. S. Ovodov, Carbohydr. Res., 101, 335 (1982).
44. V. I. Gorbach, E. V. Ivanchina, V. V. Isakov, P. A. Luk'yanov, T. F. Solov'eva, and Yu. S. Ovodov, Bioorg. Khim., 8, 1670 (1982).
45. V. I. Gorbach, I. N. Krasikova, P. A. Luk'yanov, O. Yu. Razmakhnina, T. F. Solov'eva, and Yu. S. Ovodov, Eur. J. Biochem., 98, 83 (1979).

INTERACTION OF PECTIN SUBSTANCES WITH COPPER, MERCURY, ZINC, AND CADMIUM SALTS

G. N. Katseva, E. Kukhta
É. P. Panova, and V. Ya. Chirva

UDC 547.917:543.257.5

The interaction of the sage, mint, apple, and ginseng pectins, isolated from tissue culture wastes and purified with copper, mercury, zinc, and cadmium salts, has been studied by the amperometric method with two metal indicator electrodes. The optimum conditions of titration have been determined: pH 3.5-5.0; concentration of pectin substances $5\cdot10^{-5}$-$1\cdot10^{-3}$ g/ml of solution. It has been established by graphical and mathematical methods that the interaction is accompanied by the formation of compounds with a ratio of the carboxy groups of pectins to the metal cation of two. The IR spectra of sage pectin and of copper and mercury pectinates are given.

The wastes from various enterprises of the foodstuff and pharmaceutical industries contain pectin substances (PSs) which are natural ion-exchangers capable of replacing the hydrogen of the carboxy groups by cations of multivalent metals. This property is used in the prophylaxis of poisoning by heavy-metal salts [1-7]. The inclusion of sugar beet pectin in the food ration of people coming into contact in their work with lead and other poisonous metals is obligatory. The use in various ways of radioactive isotopes in the national economy has expanded the limits of the protective action of pectin substances still more widely [9, 10].

Up to the present, there has been no single opinion on the mechanism of the action of pectins and the nature of the compounds formed with metal cations. This is due to the fact that, depending on the raw material source and the method of isolation, the PSs have different chemical structures. Some authors consider that the binding of cations by polygalacturonic acids takes place through the carboxy and hydroxy groups [4]. According to investigations by G. B. Aimukhamedova et al., Mn^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+}, and Zn^{2+}form with pectins compounds in which, in addition to the carboxy groups, the hydroxy groups of the macromolecules participate [10].

M. V. Frunze, Simferopol' State University. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 171-175, March-April, 1988. Original article submittted July 28, 1987.

 0009-3130/88/2402-0142$12.50