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Identification of a novel sugar 5,7-diacetamido-8-amino-3,5,7,8,9-pentadeoxy-D-*glycero*-D-*galacto*-non-2-ulosonic acid present in the lipooligosaccharide of *Vibrio parahaemolyticus* O3:K6

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Abstract A novel sugar, 5,7-diacetamido-8-amino-3,5,7,8,9-pentadeoxy-D-*glycero*-D-*galacto*-non-2-ulosonic acid (NonlA), has been identified as a component of the oligosaccharide (OS) isolated from the lipooligosaccharide (LOS) of the emerging strain of *Vibrio parahaemolyticus* O3:K6 associated with a global pandemic. In the present study we report the identification and characterization of this novel sugar present in the OS of *V. parahaemolyticus* O3:K6, using chemical analysis, NMR spectroscopy and mass spectrometry.

Keywords *Vibrio parahaemolyticus* O3:K6 · Lipooligosaccharide · Oligosaccharide · 5,7-diacetamido-8-amino-3,5,7,8,9-pentadeoxy-D-*glycero*-D-*galacto*-non-2-ulosonic acid · NonlA

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Introduction

Vibrio parahaemolyticus, a halophilic marine bacterium is commonly associated with seafood borne gastroenteritis. This organism is presently classified into 13 O (somatic) antigen types and 71 different K (capsular) types and serotypes are defined as a combination of both O and K types [1]. Unlike *V. cholerae*, where only two O serogroups (O1 and O139) cause epidemic and endemic cholera, infections by *V. parahaemolyticus* can be caused by any one of the O:K serotypes. However, in February 1996, a unique serotype of *V. parahaemolyticus* namely O3:K6 emerged which accounted for 50–80% of the gastrointestinal infections among patients admitted in Infectious Diseases Hospital in Kolkata, India [2]. In subsequent years, serotype O3:K6 caused food-borne outbreaks in many parts of the world that include Bangladesh, Chile, France, Japan, Korea, Mozambique, Russia, Spain, Taiwan, Thailand and USA [3]. Unlike any of the previously reported serotypes of *V. parahaemolyticus*, the O3:K6 serotype has the ability to rapidly increase hospitalization in areas where it prevails and to become the dominant serotype, supplanting other serotypes of the halophile in a given area [4].

It has been earlier reported [1] that all of the 13 O-serotypes have low molecular weight lipooligosaccharide structure rather than lipopolysaccharide. The structural variation thus, resides mainly in the oligosaccharide portion of the LOSs. Similar types of LOSs are also found in some non-enteric pathogens such as *Neisseria gonorrhoea*, *Neisseria meningitides* and *Haemophilus influenzae* [5]. Out of 13 O-antigenic lipooligosaccharides, structure of *V. parahaemolyticus* O12 has been reported [6] earlier. *Vibrio*

parahaemolyticus O2 has been reported to contain 5,7-diacetamido-3,5,7,9-tetradeoxy-D-*glycero*-D-*galacto*-non-2-ulosonic acid [7], where as, a O-untypable strain KX-V212 contains 5-acetamido-7-(*N*-acetyl-D-alanyl)amido-3,5,7,9-tetradeoxy-D-*glycero*-D-*galacto*-non-2-ulosonic acid [8]. The chemical structure of the lipooligosaccharide of *V. parahaemolyticus* O2 [7] and *V. parahaemolyticus* O-untypable strain KX-V212 [9] have been established, recently.

The distinctive epidemiological attributes of the O3:K6 serotype formed the impetus to study the structure of the O-antigen of this serotype in an effort to understand unusual compositional constituents, which might explain the ability of this serotype to easily transmit and spread. Our studies revealed that the OS of *V. parahaemolyticus* O3:K6 contains D-glucose, D-galactose, L-*glycero*-D-*manno*-heptose and a novel sugar 5,7-diacetamido-8-amino-3,5,7,8,9-pentadeoxy-D-*glycero*-D-*galacto*-non-2-ulosonic acid in the molar proportion of 3:3:3:1. The structure of this OS will be published elsewhere. In this study, we describe the identification of the novel sugar, 5,7-diacetamido-8-amino-3,5,7,8,9-pentadeoxy-D-*glycero*-D-*galacto*-non-2-ulosonic acid, present in the OS of the LOS of *V. parahaemolyticus* O3:K6, by NMR and mass-spectroscopic studies.

Materials and methods

Bacteria and lipooligosaccharide

V. parahaemolyticus O3:K6 strain [KX-V138] was made available from the culture collection of the International Centre for Diarrhoeal Disease Research, Mohakhali, Dhaka-1212, Bangladesh. The strain was cultured in nutrient broth supplemented by 3% NaCl at 37°C for 16 h. LOS was isolated from the acetone dried bacterial cell by hot phenol-water procedure [10], ultracentrifuged thrice at 40,000 ×g at 4 h, 4°C. The precipitate containing LOS was collected and lyophilized. The crude LOS was also purified by enzymatic treatment using DNase, RNase and protease to obtain pure LOS. The UV spectra of purified LOS showed no absorption at 260 and 280 nm indicating absence of nucleic acids and protein respectively.

Preparation of the oligosaccharide

For preparation of the OS, the LOS was treated with 0.1 M NaOAc buffer [11] (pH 4.2) at 100°C for 3 h. After removal of the lipid-A by ultracentrifugation, the supernatant was subjected to gel permeation chromatography on Biogel P-6 (fine) matrix and was eluted with water. The single peak corresponding to OS was collected. The homogeneity of the OS was checked by HPLC using oligo P_W 3000 column (Supelco) with water as eluent (flow 0.5 ml/min).

Chemical analyses

For sugar analysis, LOS and OS (0.5 mg each) were hydrolyzed with 2 M TFA for 2 h at 120°C. Alditol acetates [12] were prepared by acetylating (0.5 ml Ac_2O, 0.5 ml pyridine, 100°C, 40 min) and analyzed by GLC and GLC-MS on a Hewlett-Packerd 6890 plus series equipped with an HP-5 column (30 m×0.25 μm×0.25 mm) using a temperature program 150°C–5 min–2°C/min–220°C (final temperature). GLC-MS was performed on a Shimadzu model GC-MS-QP-5050A using a program 80°C–2 min–10°C/min–140°C–10 min–5°C/min–250°C–10 min (final temperature) and ZB-5 (30 m×0.25 μm×0.25 mm) column. The absolute configurations of Glc, Gal, GlcN and heptose were determined by GLC and GC-MS as their per acetylated (*S*)-(+)-2-butyl glycosides [13], which were derived by butanolysis with (*S*)-(+)-2-butanol and catalytic amount of trifluroacetic acid at 100°C for 16 h, followed by acetylation using 1:1 Ac_2O-Py. Total phosphate in LOS and OS was estimated by magnesium nitrate–ammonium molybdate colourimetric assay procedure [14].

Methanolysis and acetylation of the OS

Methanolysis of the OS was carried out with 1.1 M dry methanolic hydrogen chloride in a sealed tube at 100°C for 16 h. The excess methanolic hydrogen chloride was removed using stream of nitrogen. Trace of acid was removed by co-distillation with methanol under reduced pressure. The methanolyzed OS was acetylated using 1:1 (v/v) acetic anhydride and pyridine at room temperature for overnight. The excess acetic anhydride and pyridine were removed by co-distillation with toluene under reduced pressure.

Carboxyl reduction and *N*-acetylation of the methanolyzed OS

Carboxyl reduction of the methanolyzed OS was carried out using sodium borohydride in methanol and followed by *N*-acetylation using 1:1 saturated solution of aqueous sodium hydrogen carbonate-acetic anhydride at room temperature for 16 h [15].

Preparations of dephosphorylated OS

The OS was dephosphorylated with 48% HF at 4°C for 48 h [9] and the reaction mixture was diluted five folds with ice-cold water, neutralized slowly with chilled dilute

Table 1 Sugar composition of the LOS and OS isolated from *V. parahaemolyticus* O3:K6

Component sugars as alditol acetates	LOS (%)	OS (%)
Glucose	26.0	33.0
Galactose	23.0	32.0
L-*glycero*-D-*manno*-heptose	25.0	35.0
Glucosamine	26.0	0.0

aqueous NH_3 and dialyzed against distilled water. After lyophilization, the material was purified on the Biogel P-2 (fine) column using water as eluent.

NMR spectroscopy

One-dimensional ^1H, ^{13}C and two dimensional DQF-COSY, TOCSY, NOESY, gHSQC and HMBC NMR spectra of OS (in D_2O) were recorded using a Bruker 600 MHz instrument at 25°C. For the detection of the nitrogen bearing protons, OS was dissolved in DMSO-d_6 and the NMR was recorded at 25°C. Acetone (δ_H 2.225, δ_C 31.45) was used as internal standard.

Electrospray ionization MS and MALDI-TOF MS-MS

Ion cyclotron resonance Fourier transform ESIMS was performed on a Micromass ZQ instrument (Waters). An OS sample was dissolved in methanol–water 1:1 at concentration ~20 ng μL^{-1} and sprayed at a flow rate 2 µl min^{-1}. Capillary entrance voltage was set to 3.0 kV and drying gas temperature to 120°C.

MALDI-TOF MS-MS mass spectra were acquired on Applied Biosystem Voyager MALDI-TOF instrument in linear and delayed mode. DHB (2,5-dihydroxy benzoic acid) was used as matrix and 1:1 mixture of sample to matrix was loaded on the MALDI plate. A 337 nm N_2 laser was used to irradiate the molecule.

Results

Isolation of the lipooligosaccharide and oligosaccharide

The lipooligosaccharide of *V. parahaemolyticus* O3:K6 was isolated from the cultured bacterial cells by hot phenol–water (Westphal) method. The LOS was purified by treatment with enzymes. The OS was prepared from LOS by hydrolyzing with 0.1 M NaOAc buffer (pH 4.2). The OS, after purification using Biogel P-6 (fine) column chromatography, showed presence of D-glucose, D-galactose and L-*glycero*-D-*manno*-heptose as neutral sugars in the molar proportion of 1:1:1 (Table 1). Both LOS and OS contained NonlA which could neither be isolated nor detected by GLC. LOS and OS contained 8% and 10% phosphate respectively. The absolute configurations of glucose and galactose were found to be D- as *S*-(+)-2-butyl glycosides. Heptose was found to be in L-*glycero*-D-*manno* configuration.

Table 2 ^1H NMR data of OS isolated from the LOS of *Vibrio parahaemolyticus* O3:K6

Sugar units	Chemical shift ($J_{H,H}$ Hz) →											
	H-1	H-2	H-3 (ax) ($J_{3ax,4}$)	H-3 (equ) ($J_{3ax,3equ}$)	H-4 ($J_{3equ,4}$)	H-5 ($J_{4,5}$)	H-6 ($J_{5,6}$)	H-7 ($J_{6,7}$)	H-8 ($J_{7,8}$)	H-9 ($J_{8,9}$)	5NAc	7NAc
→4)-α-NonlA-(2→			1.72 (12.0)	2.98 (12.0)	3.65 (4.1)	3.90 (10.7)	3.72 (10.9)	4.19 (2.7)	3.76 (8.8)	1.32 (6.6)	1.94	2.01
→2,3,4)- α-L-D-Hep-(1→	5.12	4.03	4.29		4.13	3.89	nd	3.70				
→2)-α-L-D-Hep-(1→	5.09	4.28	3.98		3.87	3.67	nd	3.54				
α-D-Glc-(1→	5.06	3.52	3.46		3.41	3.89	3.75					
β-D-Gal-(PEtn) $_2$-(1→	4.85	3.45	4.03		3.88	3.65	3.96					
→3)-β-D-Gal-(1→	4.55	3.35	3.67		4.19	3.91	3.54					
→3)-β-D-Gal-(1→	4.49	3.40	4.03		3.60	3.50	4.05					
→4)-β-D-Glc-(1→	4.31	3.54	3.68		3.98	4.02	4.13					
PEtn (A)	4.11	3.27										
PEtn (B)	4.19	3.32										

Sample was dissolved in D_2O, acetone (δ_H 2.225) was used as internal reference, nd—not detected due to complexity of the signals.

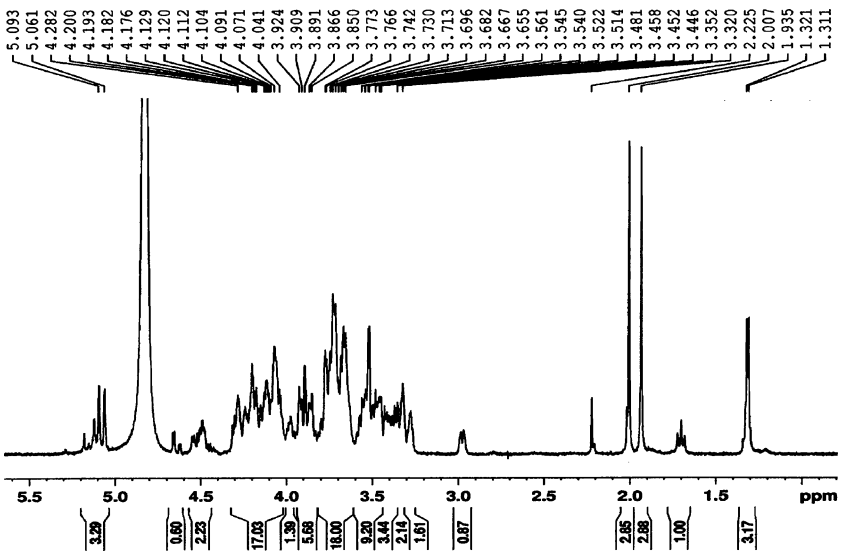

Fig. 1 600 MHz ^{1}H NMR spectrum of OS of *V. parahaemolyticus* O3:K6 at 25°C in D_2O

Characterization of 5,7-diacetamido-8-amino-3,5,7,8,9-pentadeoxy-D-*glycero*-D-*galacto*-non-2-ulosonic acid from the OS of *V. parahaemolyticus* O3:K6 by NMR spectroscopy

^{1}H (Table 2, Fig. 1) and ^{13}C NMR (Table 3, Fig. 2) spectra of OS from *V. parahaemolyticus* O3:K6 were assigned using DQF-COSY, TOCSY, gHSQC, HMBC and NOESY. The characteristic signals of NonlA have been observed in ^{1}H spectrum such as methylene protons at δ_H 1.72 (axial, $J_{3ax,4}$ 12.0 Hz) and 2.98 (equatorial, $J_{3ax,3equ}$ 12.0 Hz), a methyl group at δ_H 1.32 ($J_{8,9}$ 6.6 Hz) and two *N*-acetyl groups at δ_H 1.94 and 2.01.

Apart from the signals from the neutral sugars, characteristic signals of a novel NonlA were observed in the ^{13}C (Table 3, Fig. 2) and DEPT-135 (spectrum not shown) spectra of the OS. The quaternary anomeric carbon at δ_C 100.32 (C-2), one methylene group at δ_C 37.87 (C-3), one methyl group at δ_C 16.43 (C-9), three nitrogen bearing carbons at δ_C 48.65, 50.63, 51.24 and two signals of *N*-acetyl groups (methyl groups at δ_C 22.66 and 23.01) were observed. From the TOCSY, DQF-COSY and gHSQC (Fig. 3) experiments the three nitrogen bearing carbons could be convincingly assigned as C-5 (δ_H 3.90, δ_C 50.63), C-7 (δ_H 4.19, δ_C 51.24) and C-8 (δ_H 3.76, δ_C 48.65). The three carbonyl carbons at δ_C 174.04 (C-1), 174.61 (5-*N*-acetyl), 174.84 (7-*N*-acetyl) were assigned using HMBC experiment (Fig. 4). Thus, the NonlA is a pentadeoxy sugar (C-3, C-5, C-7, C-8 and C-9). The HMBC experiment also showed correlations of H-5 and H-7 of NonlA with the corresponding carbonyl carbons of *N*-acetyl groups, indicating presence of two acetamido groups at C-5 and C-7. Also, the characteristic correlation peak was observed in HMBC spectrum between H-3 (axial, δ_H 1.72) and carbonyl group (C-1, δ_C 174.04).

For determination of the streoisomeric configuration of the NonlA, the ^{1}H and ^{13}C NMR data obtained for OS of *V. parahaemolyticus* O3:K6 were compared in detail with

Table 3 ^{13}C NMR data for OS isolated from the LOS of *Vibrio parahaemolyticus* O3:K6

Sugar units	Chemical shift →										
	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	5NAc	7NAc
→4)-α-NonlA-(2→	174.04	100.32	37.87	72.90	50.63	72.30	51.24	48.65	16.43	22.66 174.61	23.01 174.84
→2,3,4)-α-L-D-Hep-(1→	95.49	76.94	76.29	75.00	67.57	nd	62.38				
→2)-α-L-D-Hep-(1→	102.24	78.88	66.96	67.57	72.10	nd	63.35				
α-D-Glc-(1→	95.49	72.09	72.41	76.29	69.96	61.09					
β-D-Gal-(PEtn) $_2$-(1→	102.24	72.41	77.26	68.53	74.67	62.46					
→3)-β-D-Gal-(1→	103.63	74.03	77.91	68.20	69.82	63.87					
→3)-β-D-Gal-(1→	102.77	73.38	78.23	72.73	75.96	62.71					
→4)-β-D-Glc-(1→	103.63	72.09	74.67	78.56	76.94	62.93					
PEtn (A)	62.70	40.94									
PEtn (B)	63.03	40.94									

Sample was dissolved in D_2O, acetone (δ_C 31.45) was used as internal reference, nd—not detected due to complexity of the signals.

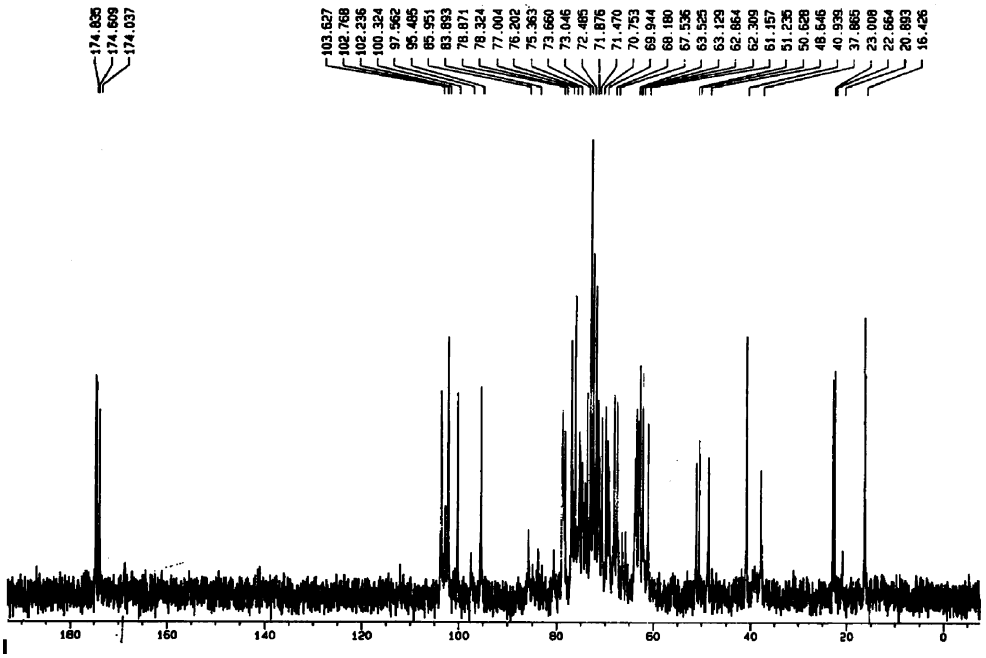

Fig. 2 150 MHz ^{13}C NMR spectrum of OS of *V. parahaemolyticus* O3:K6 at 25°C in D_2O

those reported for nine streoisomers of 5NAc7NAcNonlA [16] as well as with those for legionaminic acid (D-*glycero*-D-*galacto* configuration [17]), isolegionamic acid (D-*glycero*-D-*talo* configuration [16, 17]) and pseudaminic acid (L-*glycero*-L-*manno* configuration) [18, 19]. Relatively large $J_{4,5}$ and $J_{5,6}$ coupling constants of 10.7 Hz and 10.9 Hz, respectively, confirmed the axial orientation of the pyranose ring protons H-4, H-5 and H-6, which could be due to D-*galacto* or L-*altro* configuration [8, 20]. The small $J_{6,7}$ coupling constant of 2.7 Hz indicates the *syn* (gauche) like relationship for H-6 and H-7, which in turn confirms the equatorial orientation of the AcNH-5 group and D-*glycero*-D-*galacto* or L-*glycero*-D-*galacto* configuration of NonlA [16].

The large $J_{7,8}$ coupling constant of 8.8 Hz indicates the *trans* orientation of H-7 and H-8 as reported earlier for α, β-D-*glycero*-D-*galacto* and α, β-L-*glycero*-D-*galacto* ($J_{7,8}$ 7-8.9 Hz) configurations. In case of α, β-D-*glycero*-L-*altro* ($J_{7,8}$ ~1 Hz) and α, β-L-*glycero*-L-*altro* ($J_{7,8}$ 5.8 Hz) configurations, relatively small coupling constant values

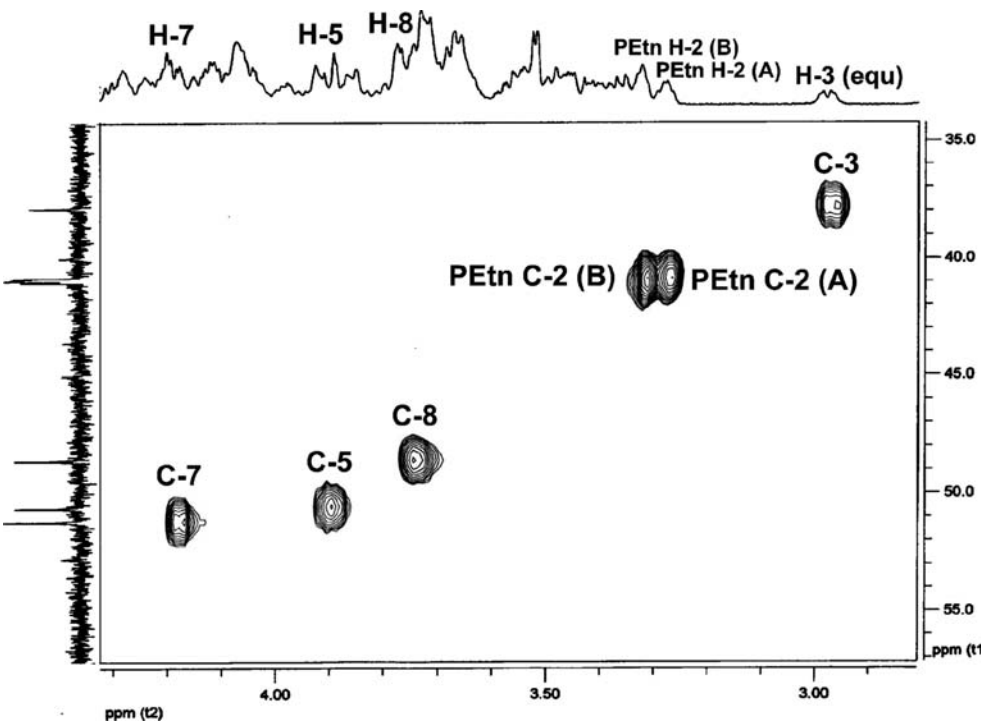

Fig. 3 Selected zone of 600 MHz gHSQC spectrum of OS of *V. parahaemolyticus* O3:K6 at 25°C in D_2O

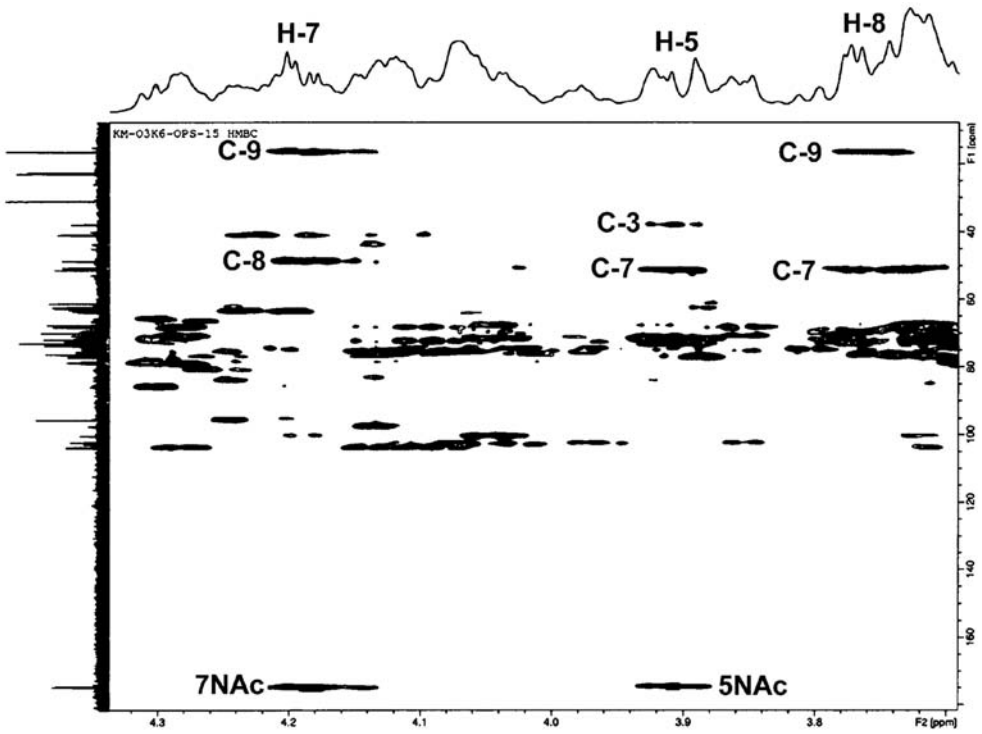

Fig. 4 Selected zone of 600 MHz HMBC spectrum of OS of *V. parahaemolyticus* O3:K6 at 25°C in D_2O

were observed [16]. Therefore, it can be concluded that the NonlA is in either D-*glycero*-D-*galacto* or L-*glycero*-D-*galacto* configuration.

NOESY experiments of the OS revealed strong H-7, H-9; H-9, H-8 (Fig. 5) and medium NH-7, H-9 (Fig. 6) correlations, which are characteristic of D-*glycero*-D-*galacto* configuration. Absence of L-*glycero*-D-*galacto* configuration [16] was evident as no H-9, H-6 correlation was observed.

The NonlA is in the α-anomeric form as is evident from the chemical shift of H-3 (equatorial, δ_H 2.98), which is shifted, downfield by 0.67 ppm compared to the β-anomer [7, 16].

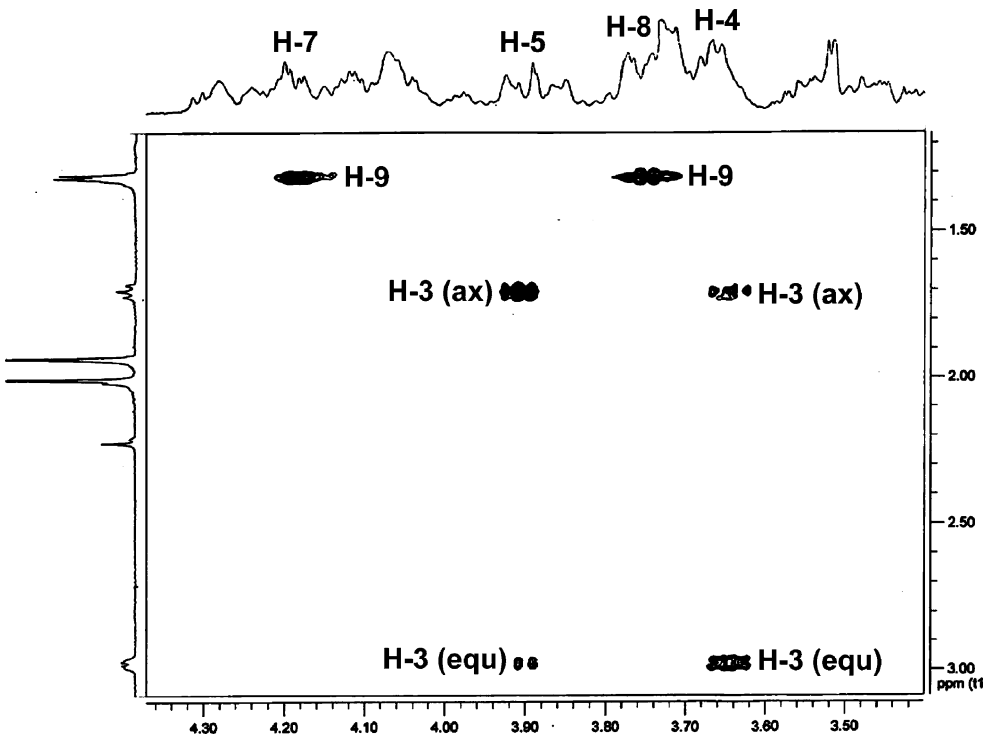

Fig. 5 Selected zone of 600 MHz NOESY spectrum of OS of *V. parahaemolyticus* O3:K6 at 25°C in D_2O

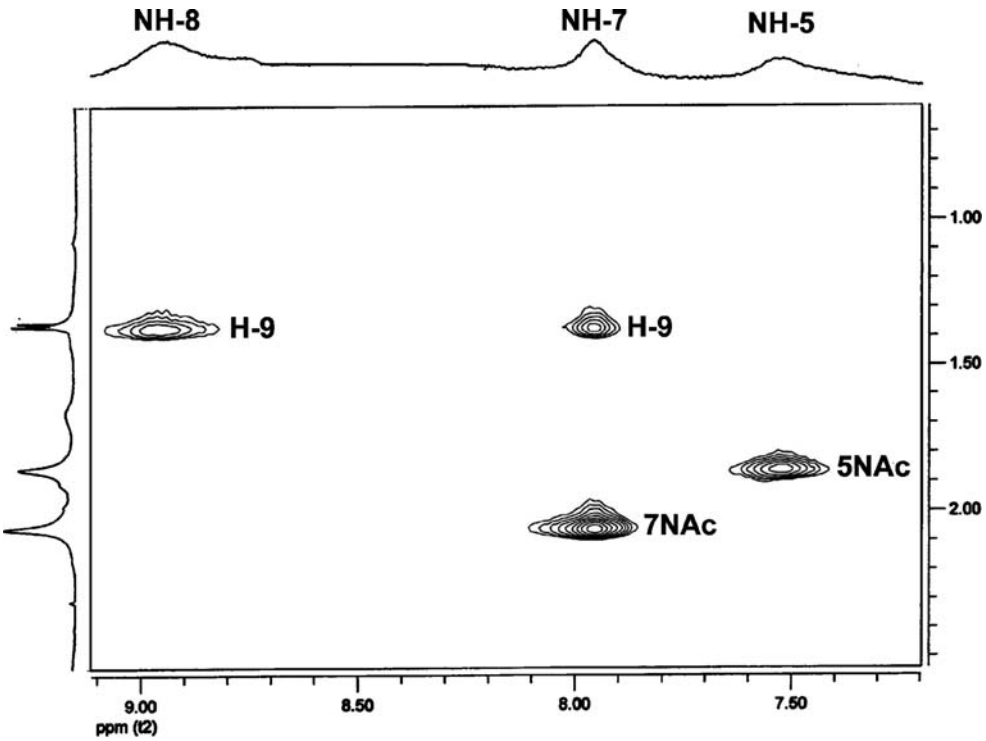

Fig. 6 Selected zone of 600 MHz NOESY spectrum of OS of *V. parahaemolyticus* O3:K6 at 25°C in DMSO-d_6

The NOESY spectrum of the OS (DMSO-d_6) showed strong correlation of C-5 amide proton (δ_H 7.52) with the methyl protons of 5-*N*-acetyl group. The C-7 amide proton (δ_H 7.95) showed strong correlation with the methyl protons of 7-*N*-acetyl group and expected medium correlation with C-9 methyl protons. The C-8 free amine protons (δ_H 8.95) showed correlation with C-9 methyl protons (Fig. 7).

Therefore, the NonlA has a novel structure; 5,7-diacetamido-8-amino-3,5,7,8,9-pentadeoxy-D-*glycero*-D-*galacto*-non-2-ulosonic acid.

Characterization of 5,7-diacetamido-8-amino-3,5,7,8,9-pentadeoxy-D-*glycero*-D-*galacto*-non-2-ulosonic acid from the OS of *V. parahaemolyticus* O3:K6 by mass spectrometry.

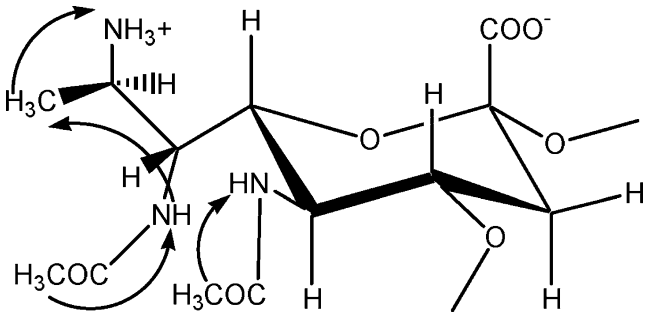

Fig. 7 NOE correlations of the nitrogen bearing protons in 5,7-diacetamido-8-amino-3,5,7,8,9-pentadeoxy-D-*glycero*-D-*galacto*-non-2-ulosonic acid

Methanolysis of the OS using 1.1 M dry methanolic hydrogen chloride produced a methyl ester methyl glycoside of a disaccharide and a mixture of methyl glycosides of monosaccharides. The ESIMS analysis (Fig. 8) of the methanolyzed products showed the methyl ester methyl glycoside of the disaccharide comprising of 5,7-diacetamido-8-amino-3,5,7,8,9-pentadeoxy-D-*glycero*-D-*galacto*-non-2-ulosonic acid and a hexose having molecular ion peak at *m/z* 524, attributed to $[M+H]^+$. The molecular ion peak at *m/z* 362 $[M+H]^+$ is that of the methyl ester methyl glycoside of 5,7-diacetamido-8-amino-3,5,7,8,9-pentadeoxy-D-*glycero*-D-*galacto*-non-2-ulosonic acid.

The methanolyzed products were acetylated using 1:1 (v/v) acetic anhydride and pyridine, 16 h at room temperature. The ESIMS analysis of the acetylated products (spectrum not shown) showed the molecular ion peaks as sodium adduct of methyl 2,3,4,6-tetra-*O*-acetyl-D-hexopyranoside (*m/z* 385), methyl 2,3,4,6,7-penta-*O*-acetyl-L-*glycero*-D-*manno*-heptopyranoside (*m/z* 457), methyl (methyl 5,7,8-triacetamido-4-*O*-acetyl-3,5,7,8,9-pentadeoxy-D-*glycero*-D-*galacto*-non-2-ulopyranosid)onate (*m/z* 468) and acetylated methyl ester methyl glycoside of the disaccharide (*m/z* 756) as mentioned earlier. The molecular ion peak (*m/z* 734) corresponds to $[M+H]^+$ of the same disaccharide.

The ESIMS analysis of the methanolyzed *N*-acetylated products of the OS showed molecular ion peak (*m/z* 426) of methyl (methyl 5,7,8-triacetamido-3,5,7,8,9-petadeoxy-D-*glycero*-D-*galacto*-non-2-ulopyranosid)onate $[M+Na]^+$

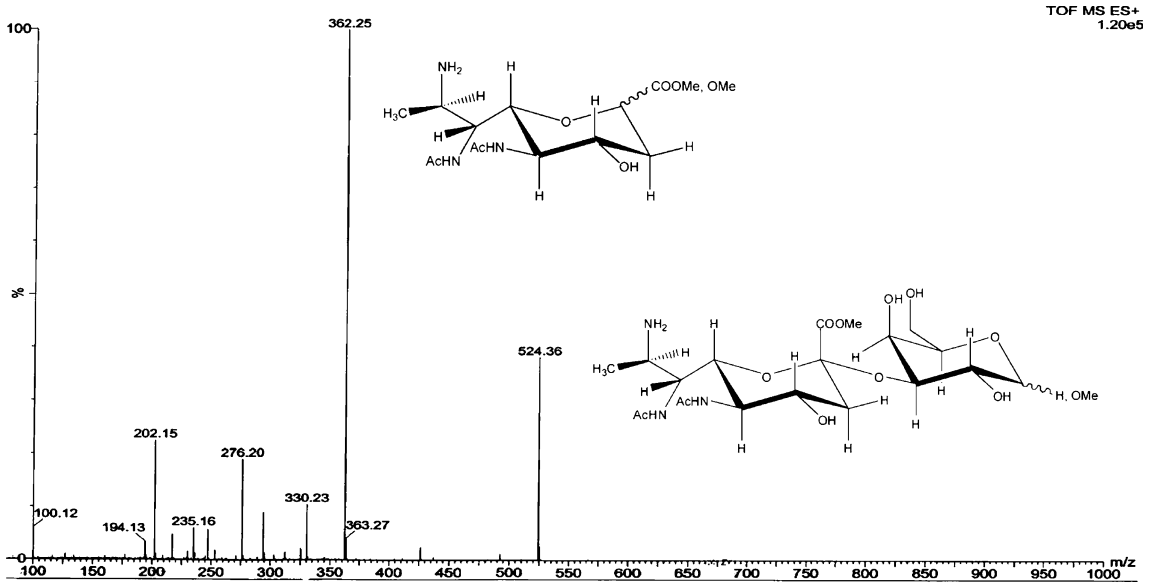

Fig. 8 ESIMS spectrum of the methyl glycosides of sugars present in OS

(spectrum not shown). Futhermore, the methanolyzed OS was carboxyl reduced with sodium borohydride and then *N*-acetylated. The ESIMS analysis of the methanolyzed carboxyl reduced *N*-acetylated products (Fig. 9) showed the molecular ion peak as sodium adduct of methyl 5,7,8-triacetamido-3,5,7,8,9-pentadeoxy-D-*glycero*-D-*galacto*-non-2-ulopyranoside (*m*/*z* 398) and the corresponding carboxyl reduced *N*-acetylated methyl glycoside of the same disaccharide (*m*/*z* 560).

MALDI-TOF MS^2 analysis of the dephosphorylated OS showed a daughter ion peak at *m*/*z* 640, which on further MS^3 corresponds to a trisaccharide sugar backbone containing two residues of hexoses and one residue of 5,7-diacetamido-8-amino-3,5,7,8,9-pentadeoxy-D-*glycero*-D-*galacto*-non-2-ulosonic acid as in Fig. 10. All these results confirm the structure of the novel NonlA.

Discussion

In the present study, a novel NonlA, 5,7-diacetamido-8-amino-3,5,7,8,9-pentadeoxy-D-*glycero*-D-*galacto*-non-2-ulosonic acid, has been identified as a constituent of the oligosaccharide of the lipooligosaccharide from *V. para-*

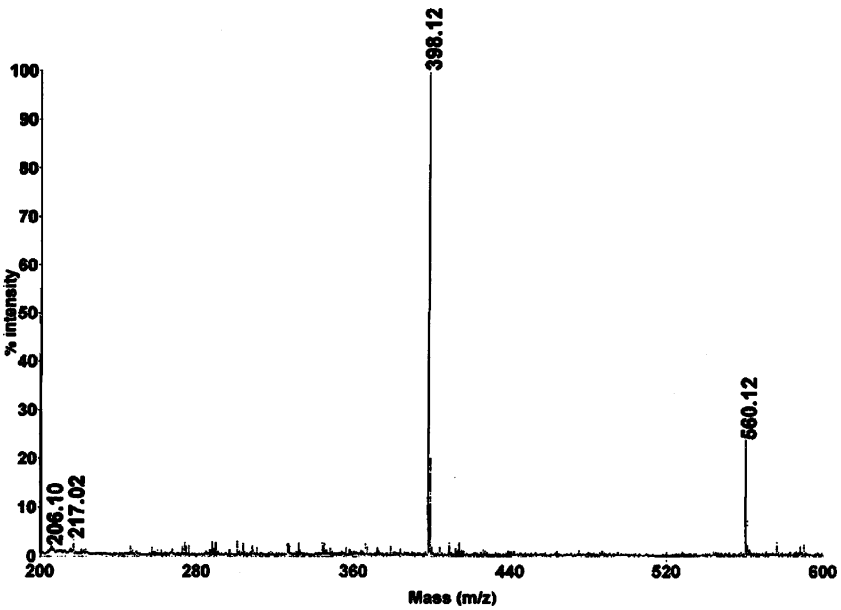

Fig. 9 ESIMS spectrum of the carboxyl reduced *N*-acetylated methyl glycosides of sugars of the OS

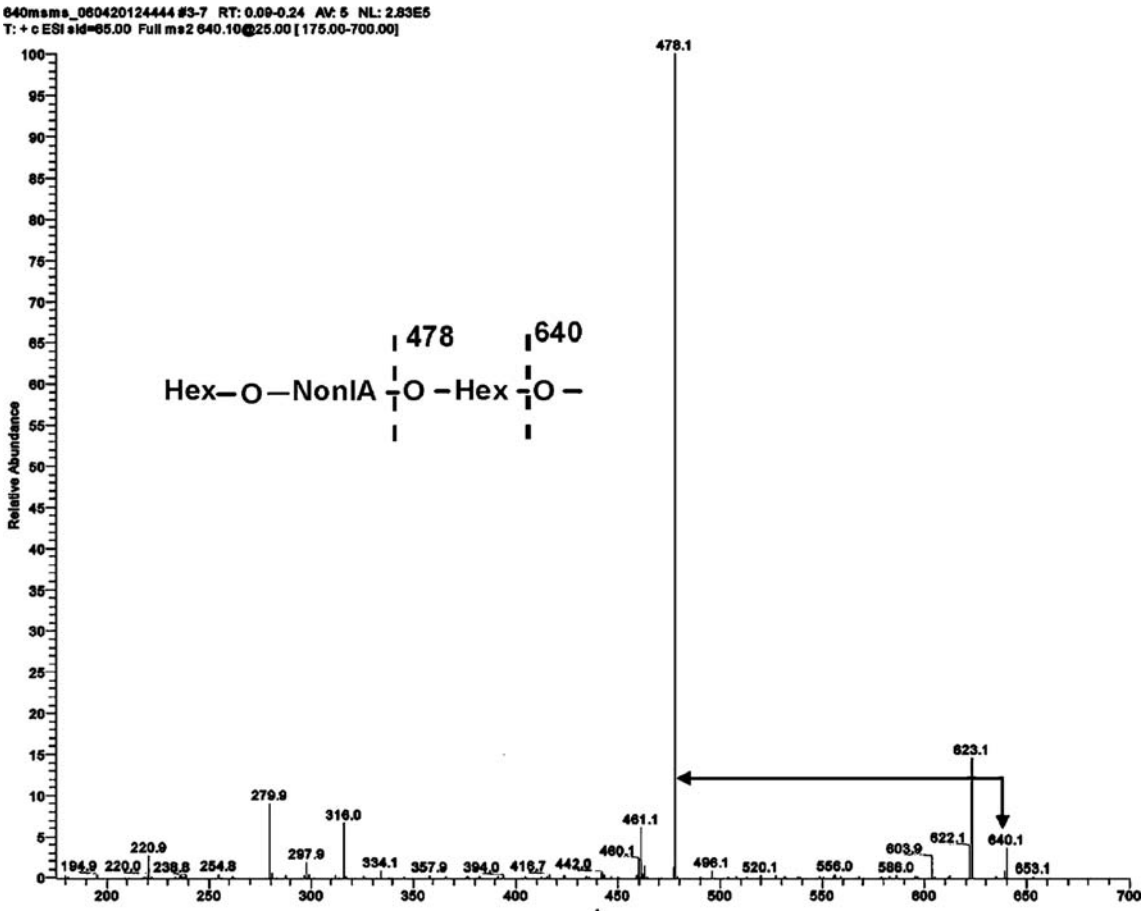

Fig. 10 MALDI-TOF MS^3 spectrum of the dephosphorylated OS of *V. parahaemolyticus* O3:K6

haemolyticus O3:K6. The structure was established by NMR and mass spectrometric analysis of the OS of *V. parahaemolyticus* O3:K6 as the NonlA could not be isolated. The stereo-configuration of the NonlA was established to be D-*glycero*-D-*galacto* based on ^{1}H, ^{13}C, 2D NMR spectroscopic data as well as by comparison with the data reported for synthetic 5,7-diacetamido-3,5,7,9-tetradeoxy-non-2-ulosonic acids [16].

The 5,7-diacetamido-3,5,7,9-tetradeoxy-non-2-ulosonic acids present in Gram-negative bacteria [21] can be classified into four groups depending on the configuration *i.e.*, those containing D-*glycero*-D-*galacto*, L-*glycero*-D-*galacto*, D-*glycero*-D-*talo* and L-*glycero*-L-*manno* isomers [16]. The NonlA in *V. parahaemolyticus* O3:K6, as demonstrated here, belongs to the first group.

5,7-diacetamido-3,5,7,9-tetradeoxy-D-*glycero*-D-*galacto*-non-2-ulosonic acid has been described to be a constituent of *V. parahaemolyticus* O2 [7]. Several isomers of NonlA are found to be constituents of many other Gram-negative bacteria genera such as, *Shigella* [18], *Providencia stuartii* [20], *Pseudomonas* [22], *Vibrio* [23], *Salmonella* [24], *Proteus* [25] and *Legionella* [26]. Although in most cases, the amino groups are substituted with acetyl groups, different *N*-substitutions are also observed. To mention a few, 5-acetamido-7-(*N*-acetyl-D-alanyl)-amido-3,5,7,9-tetradeoxy-D-*glycero*-D-*galacto*-non-2-ulosonic acid has been found in O-untypable strain KX-V212 [8], 5-acetamido-3,5,7,9-tetradeoxy-7-[(*R*)-3-hydroxybutyramido]-L-*glycero*-L-*manno*-nonulosonic acid [27] and 5-acetamido-3,5,7,9-tetradeoxy-7-formamido-L-*glycero*-L-*manno*-nonulosonic acid [28] has been reported as constituent sugar of the LPSs of *P. aeruginosa* O10 and O5 respectively. A novel representative of NonlA such as 5,7-diamino-5,7,9-trideoxy-non-2-ulosonic acid [29] from phytopathogenic *Pseudomonas* lipopolysaccharide has also been reported.

This study demonstrates a new derivative of NonlA having three amino groups at positions 5, 7 and 8, of which amino groups at 5 and 7 positions are acetylated, whereas, the amino group at the 8 position is free. To the best of our knowledge this is the first report of this sugar.

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