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Identification of a novel sugar, 4-amino-4,6-dideoxy-2-*O*-methylmannose in the lipopolysaccharide of *Vibrio cholerae* O1 serotype Ogawa

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ABSTRACT

A novel sugar in the lipopolysaccharide of *Vibrio cholerae* O1 serotype Ogawa has been identified. The sugar was liberated from the lipopolysaccharide when hydrolyzed in 10 M HCl at 90°C for 15 min. The sugar was purified and identified as 4-amino-4,6-dideoxy-2-*O*-methylmannose (2-*O*-methylperosamine). Since it was found only in the lipopolysaccharide of *Vibrio cholerae* O1 serotype Ogawa, it seems that the sugar is one of the specific constituents determining Ogawa serotype specificity.

INTRODUCTION

Vibrio cholerae of serogroup O1 is the most common pathogen causing epidemic cholera. The serogroup O1 is further subdivided into three serotypes, Ogawa, Inaba, and Hikojima[1–3]. Hikojima expresses both Ogawa- and Inaba-specific antigens. The sugar composition and structure of the lipopolysaccharide (LPS) of *V. cholerae* O1 has been investigated. A specific amino sugar, 4-amino-4,6-dideoxymannose (perosamine) was detected as a component[4], and the structure of the O-antigenic side chain was reported to be a regular α-(1 → 2)-linked chain of D-perosamine units[5]. The sugar composition of the O-antigenic polysaccharide of *V. cholerae* O1 of both serotypes has been investigated in order to elucidate the structural difference between the O-antigenic polysaccharides of Ogawa and Inaba serotypes, and it was reported that perosamine, 2-amino-2,6-dideoxy-D-glucose (quinovosamine), glucosamine, glucose, and heptose were the main constituents of these LPSs[6–8]. However, no difference between Ogawa and Inaba serotypes in the sugar composition or in the linkage of sugars has yet been established. In 1975,

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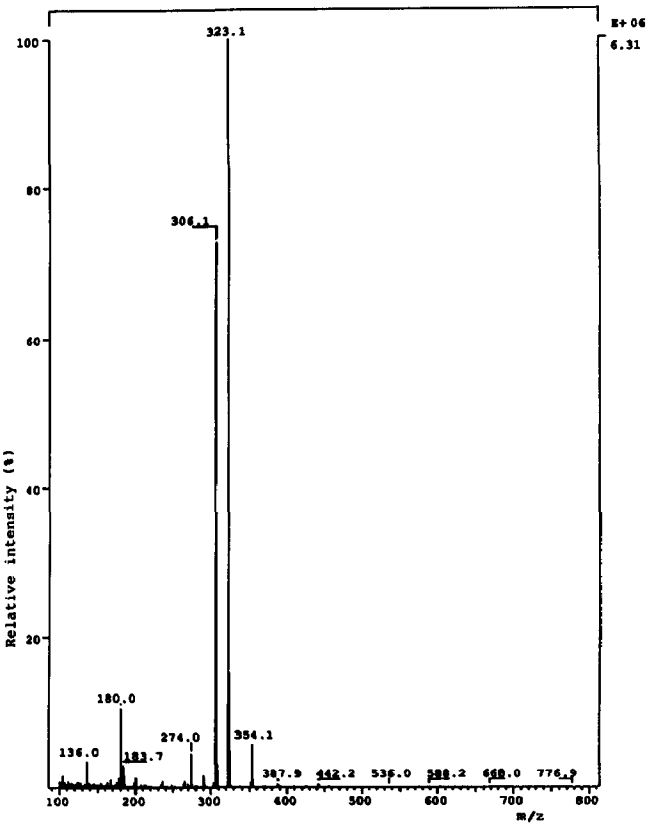
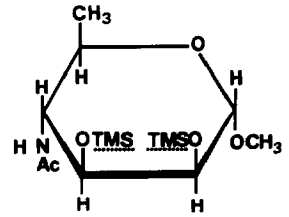
Redmond reported the presence of 4-amino-4-deoxy-L-arabinose in the LPS of *V. cholerae* O1 serotype Ogawa and suggested that this sugar might be a determinant of the Ogawa specific antigen[9]. However, Kabir reported that 4-amino-4-deoxyarabinose was not present in the O-antigenic polysaccharide[8]. Moreover, Hisatsune et al.[6] reported that they could not find 4-amino-4-deoxyarabinose in the LPS of *V. cholerae* O1 serotype Ogawa. Therefore, the possibility that 4-amino-4-deoxyarabinose is a determinant of the Ogawa specific antigen is questionable. In the present report, we have identified a previously undescribed sugar, 4-amino-4,6-dideoxy-2-*O*-methylmannose (2-*O*-methylperosamine) in the LPS of the Ogawa serotype. As the sugar was found only in the LPS and its O-polysaccharide portion of the Ogawa serotype, the sugar seems to be an important constituent of the Ogawa serotype determinant.

RESULTS

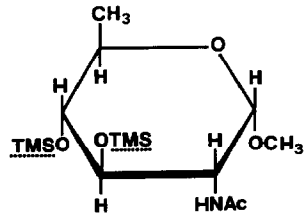
Presence of a novel sugar in LPS of V. cholerae *O1 serotype Ogawa.*—We investigated the sugar composition of lipopolysaccharides of *V. cholerae* O1 strain NIH41 serotype Ogawa and strain NIH35A3 serotype Inaba. Lipopolysaccharides were treated with methanolic 1 M HCl at 80°C for 24 h. After the resulting methyl glycosides had been *N*-acetylated and converted into their trimethylsilyl ethers, they were identified using GLC and GLC–MS. Glucosamine, perosamine, quinovosamine, glucose, heptose, and a small amount of mannose were detected. Among them, a small amount of an unknown sugar was found only in the case of the Ogawa serotype. Fig. 1 shows the CI-mass spectrum of the unknown glycoside. The molecular ion peak at m/z 306 and pseudomolecular ion peak at m/z 323 were observed. The mass number was entirely different from that expected for glucosamine (*M* 451), quinovosamine (*M* 363), or perosamine (*M* 363), well-known components of the LPS of *Vibrio cholerae* O1 (Fig. 1). During a related study to purify perosamine, the LPS of *V. cholerae* O1 strain NIH41 serotype Ogawa was dispersed in 10 M HCl and heated at 90°C for 15 min. The hydrolysate was diluted, then dried in vacuo. The amino sugars were separated by cation-exchange chromatography on Dowex 50 (H^+) by elution with 0.33 M HCl (Fig. 2A). Three main peaks were observed. Fractions corresponding to each peak were concentrated and examined by TLC which showed that the amino sugars eluted in peaks 1 and 2 were glucosamine and perosamine, respectively. However, the amino sugar eluted in peak 3 could not be identified as a known amino sugar in the LPS of *V. cholerae* O1. The R_f value of the sugar (R_{GlcN} 1.91) differed from those of glucosamine (R_{GlcN} 1.00) and perosamine (R_{GlcN} 1.34), but was indistinguishable from that of quinovosamine (R_{GlcN} 1.90). However, the yellowish colour in the ninhydrin reaction indicated that the sugar was not quinovosamine. The sugar was positive in the Elson–Morgan reaction, suggesting that it was a kind of amino sugar.

To investigate the GLC–MS spectrum, the amino sugar eluted in peak 3 was heated at 80°C for 24 h in methanolic 1 M HCl, and converted into the *N*-acetylated

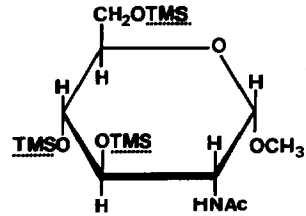




Methyl *N*-acetylperosaminide trimethylsilyl ether (M=363)



Methyl *N*-acetylquinovosaminide trimethylsilyl ether (M=363)



Methyl *N*-acetylglucosaminide trimethylsilyl ether (M=451)
TMS = trimethylsilyl

Fig. 1. CI-mass spectrum of a sugar specifically found in the LPS of the Ogawa serotype, and structures of methyl acetamidodeoxyhexopyranoside trimethylsilyl ethers.

O-trimethylsilylated methyl glycosides. The CI-mass spectrum showed the molecular ion peak at m/z 306 and a pseudomolecular ion peak at m/z 323. The mass number was exactly the same as that of an unknown glycoside from the LPS of *V. cholerae* O1 strain NIH41 shown in Fig. 1.

To investigate the occurrence of the sugar in the LPS of other strains of *V. cholerae* O1 serotype Ogawa or in the LPS of serotype Inaba, we hydrolyzed several kinds of LPSs in 10 M HCl at 90°C for 15 min, and the amino sugars were compared (Fig. 2B–H). The sugar (peak 3) was liberated from the LPSs of *V. cholerae* O1 strain NIH41 (Ogawa), 47-4041 (Ogawa), P1418 (Ogawa), and K20 (Ogawa). However, this amino sugar was not liberated from the LPSs of *V. cholerae* O1 strain NIH35A3 (Inaba), 47-4041-9 (Inaba), P1418-201 (Inaba), or 78-651 (Inaba) (Fig. 2).

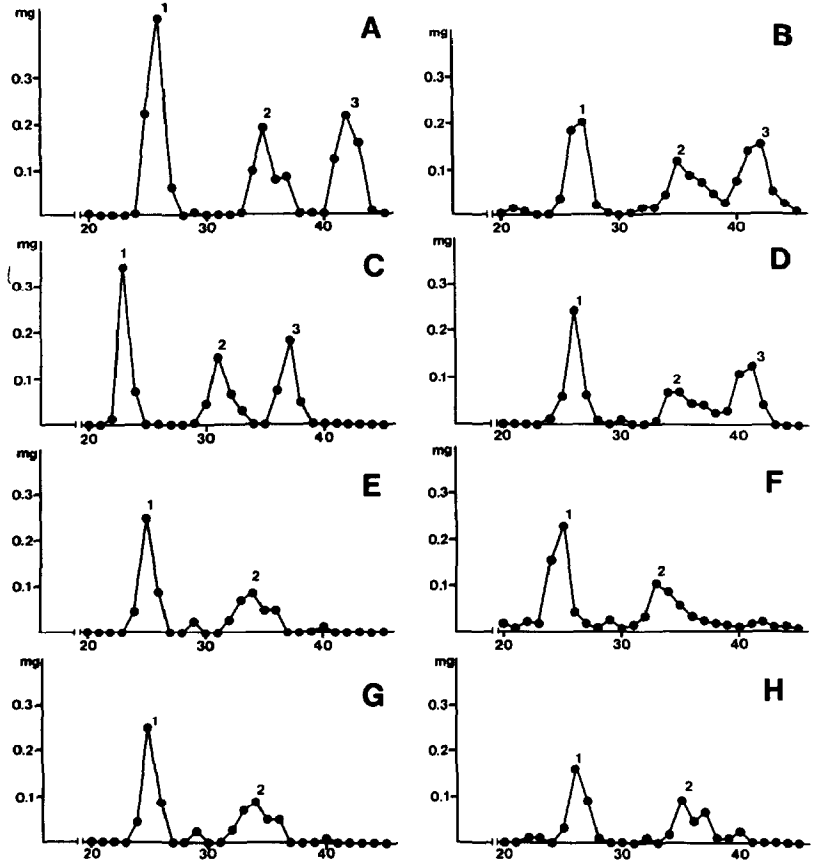


Fig. 2. Chromatography on Dowex 50(H^+) of the hydrolysates of the LPSs of *Vibrio cholerae* O1 of various strains. Amino sugars were eluted from the column (1.4×45 cm) with 0.33 M HCl (flow rate of ca. 10 mL/h; 9.6-mL fractions). The ordinate indicates the amount of amino sugar measured by the Elson–Morgan reaction, using glucosamine as a standard; the abscissa indicates the fraction number. The LPSs of the following *V. cholerae* strains were used: A, NIH41 (Ogawa); B, K20 (Ogawa); C, 47-4041 (Ogawa); D, P1418 (Ogawa); E, NIH35A3 (Inaba); F, 78-651 (Inaba); G, 47-4041-9 (Inaba); H, P1418-201 (Inaba).

Purification of the sugar.—The LPS of the strain NIH41 was hydrolyzed in 10 M HCl for 15 min. After dilution with water, hydrolysates were dried in vacuo to remove HCl and subjected to chromatography on a Dowex 50 (H^+) column. The column was washed with water and the amino sugars were eluted from the resin with 0.33 M HCl (Fig. 3A). The sugar was separated from the amino acids by selective adsorption of the amino sugar onto Dowex 50 (K^+) resin (Fig. 3B). After the amino acids had been washed off in the effluent, the column was further washed with water, and the sugar was eluted from the resin with 0.1 M potassium acetate buffer (Fig. 3B). The sugar was rechromatographed on Dowex 50 (H^+) to remove salts (Fig. 3C). Finally, the sugar was purified by chromatography on Amberlite CG-50, eluting with aqueous ammonia. The final yield was 8.7 mg.

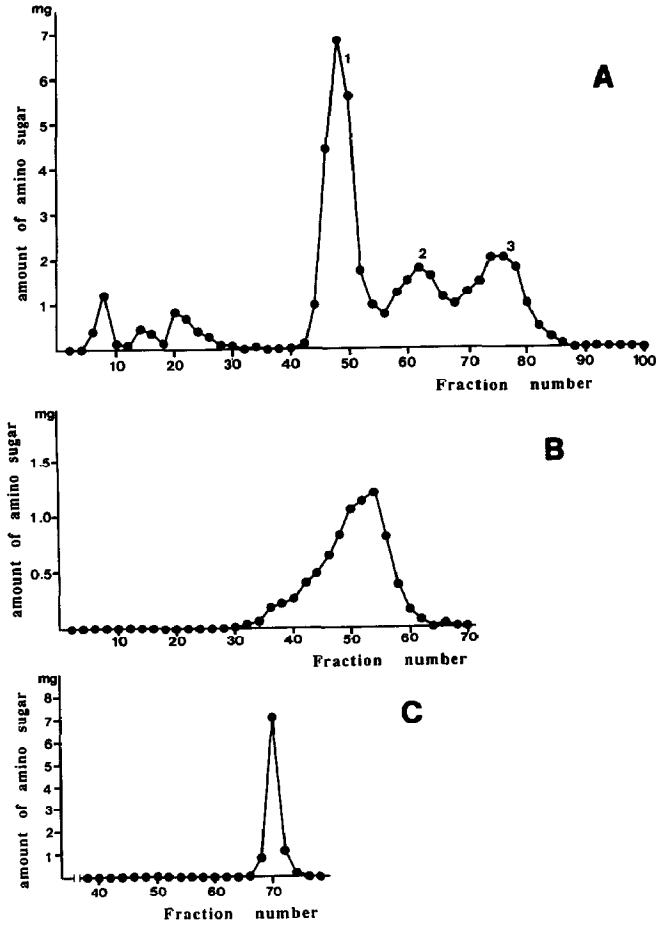


Fig. 3. Purification of the sugar. A, Chromatography on Dowex 50 (H^+) of the hydrolysates of the LPS of strain NIH41 (Ogawa): amino sugars were eluted from the column (4.4×15.5 cm) with 0.33 M HCl (flow rate of 40 mL/h; 18.4-mL fractions). B, Chromatography on Dowex 50 (K^+) of the sugar (peak 3 in A): amino sugars were eluted from the column (2.5×12.5 cm with 0.1 M potassium acetate (flow rate of 29 mL/h; 9.5-mL fractions). C, Chromatography on Dowex 50 (H^+) of the sugar after elution from the Dowex 50 (K^+) column: amino sugars were eluted from the column (1.4×45 cm) with 0.33 M HCl (flow rate of 16.7 mL/h; 9.5-mL fractions). The ordinate indicates the amount of amino sugar measured by the Elson–Morgan reaction, using glucosamine as a standard; the abscissa indicates the fraction number.

Structural analysis of the sugar, using NMR spectroscopy.—The structure of the sugar was analyzed by NMR. The one-dimensional (1D) ^1H NMR spectrum of the sugar in D_2O exhibited 14 main peaks (Fig. 4), named a–n for convenience; the complexity is due to a mixture of anomers. The chemical shift values indicated that peaks a and b were due to anomeric protons (H-1). The direct coupling between protons was elucidated by DQF-COSY spectra (Fig. 5) with the aid of HOHAHA. Consideration of the DQF-COSY plot (Fig. 5) and the HOHAHA plot (data not

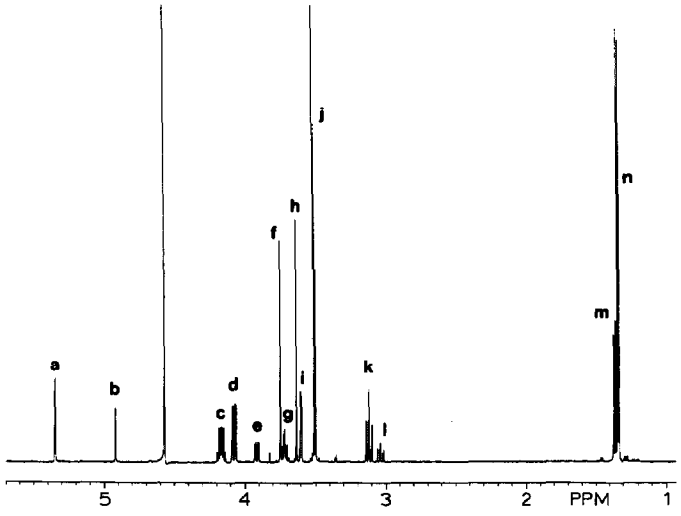


Fig. 4. The 1D 500-MHz ^{1}H NMR spectrum of the sugar.

shown) allowed us to find two series of proton sequence. The order of protons was defined as follows: a-i-d-k-c-n (series 1) and b-g-e-l-g′-m (series 2). In series 2, the intensity of g was higher than b or e which were regarded as one-proton signals. It was reasonable to infer that the signal was due to two protons. Therefore, the peak at the g position was called peak g plus peak g′. The two series of signals (series 1 and 2) were quite similar in their patterns and chemical shifts. The ratio of the relative intensity of the corresponding peaks in series 1 and 2 was ca. 3:1, without exception. It was concluded that the sample was a mixture of α and β anomers. Resonances m and n were assigned to C-6 protons which were recognized as methyl protons from their intensity (3 protons) and high-field chemical shift. These data indicated that the sugar was a 6-deoxyhexose.

The ^{13}C NMR spectra of the sugar showed 14 main signals (Fig. 6). Direct bonding of proton and carbon pairs was determined from the HMQC spectra (Fig. 7). The signals in the 1D ^{13}C NMR spectra were named in accordance with the proton which was bonded to the carbon according to the HMQC spectra. The correlation of a proton resonance with a carbon resonance which is 2–4 bonds distant was observed by HMBC spectra (Fig. 8). The cross-peaks of H-1 and C-5 indicated that the protons at C-1 and C-5 were bonded via oxygen. These data indicated that the sugar is in the pyranoid form. More importantly, distant spin couplings between j and I(C-2), and h and G(C-2) were observed. These data suggested that there were series of carbons such as I(C-2)–X–J and G(C-2)–X–H (X indicates any unknown atom). Since the intensity of the peak indicated that peaks h and j were protons of an isolated methyl group residue, the partial structure of C-2–X–CH_3 was predicted. The relative stereochemistry of the sugar was deduced by a NOESY NMR experiment and coupling constant data. Pairs of

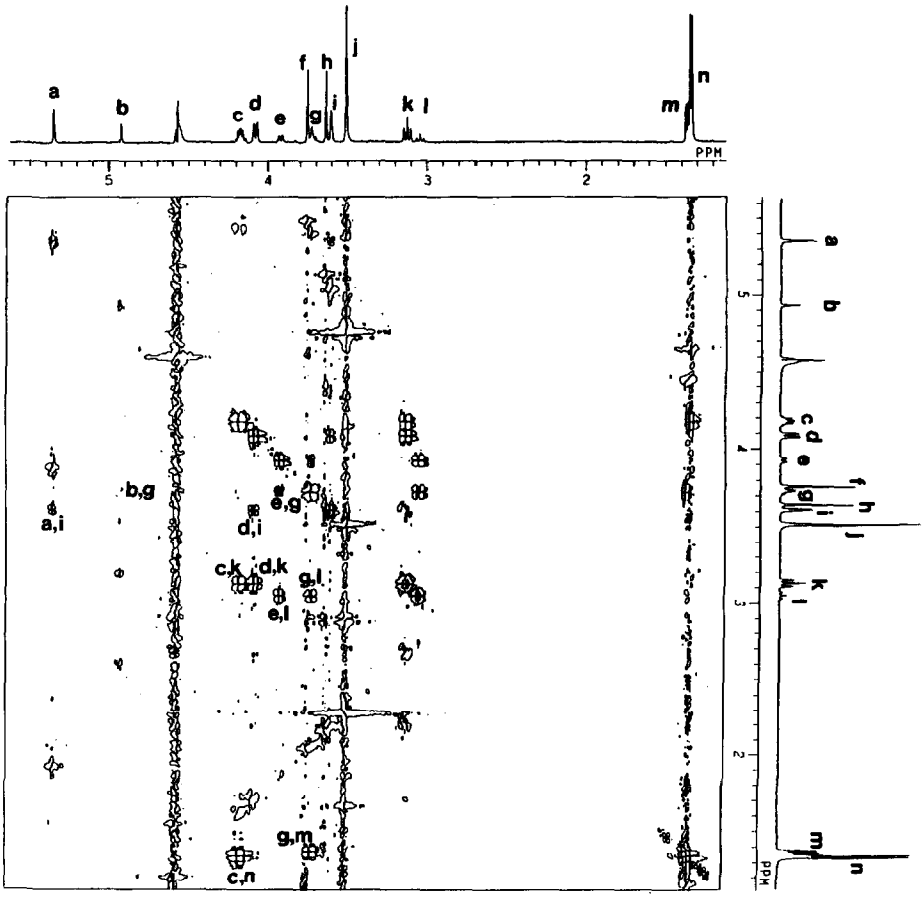


Fig. 5. DQF-COSY spectra of the sugar. The cross-peaks are indicated.

protons were identified by the NOESY spectra. The interrelationship of protons obtained from the NOESY spectra is shown in Table I. The coupling constant data (Table II) showed that the protons H-2 and H-3 are *cis*-oriented and that the

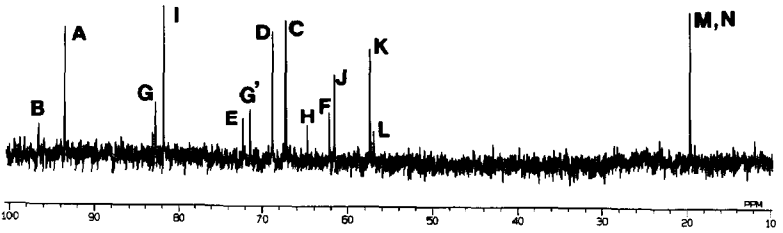


Fig. 6. The 1D 125-MHz ^{13}C NMR spectrum of the sugar. Peaks were assigned from the HMQC spectrum.

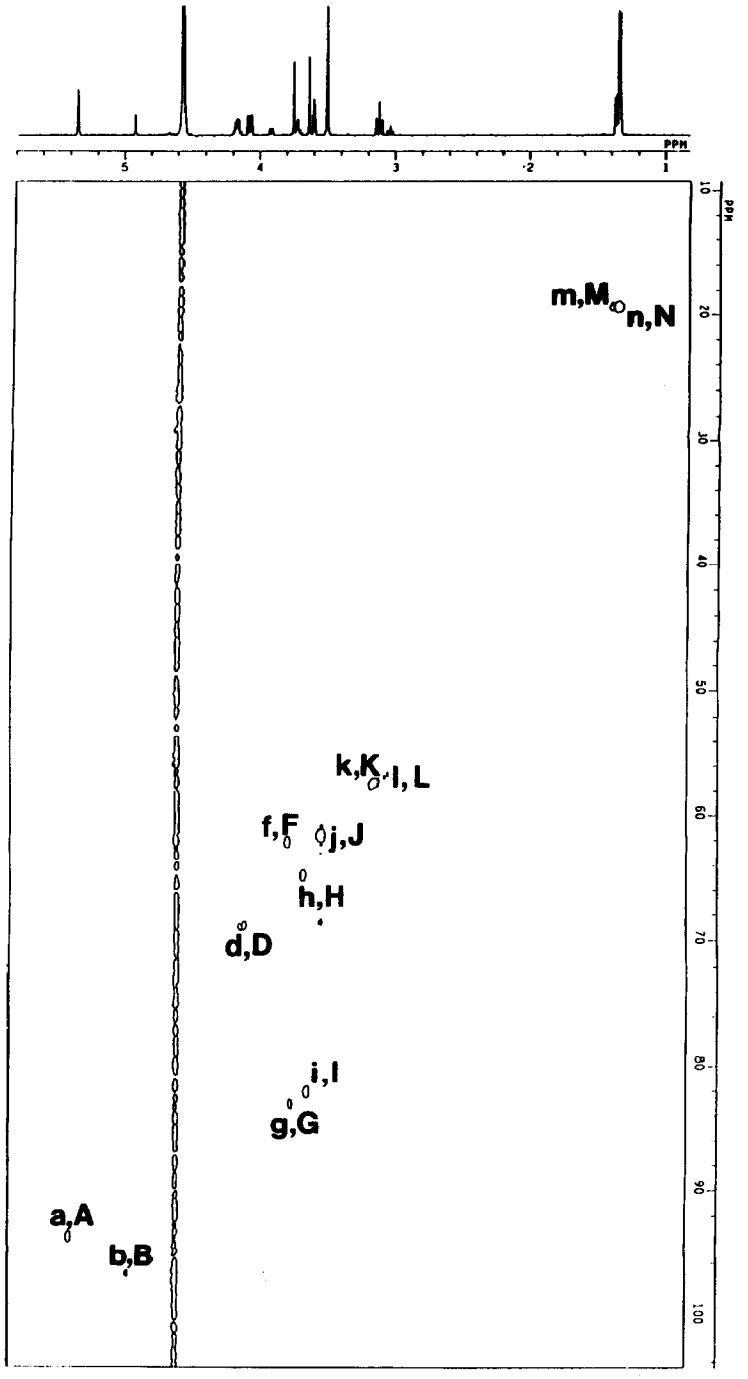


Fig. 7. HMQC spectra of the sugar: correlation of proton and carbon resonances.

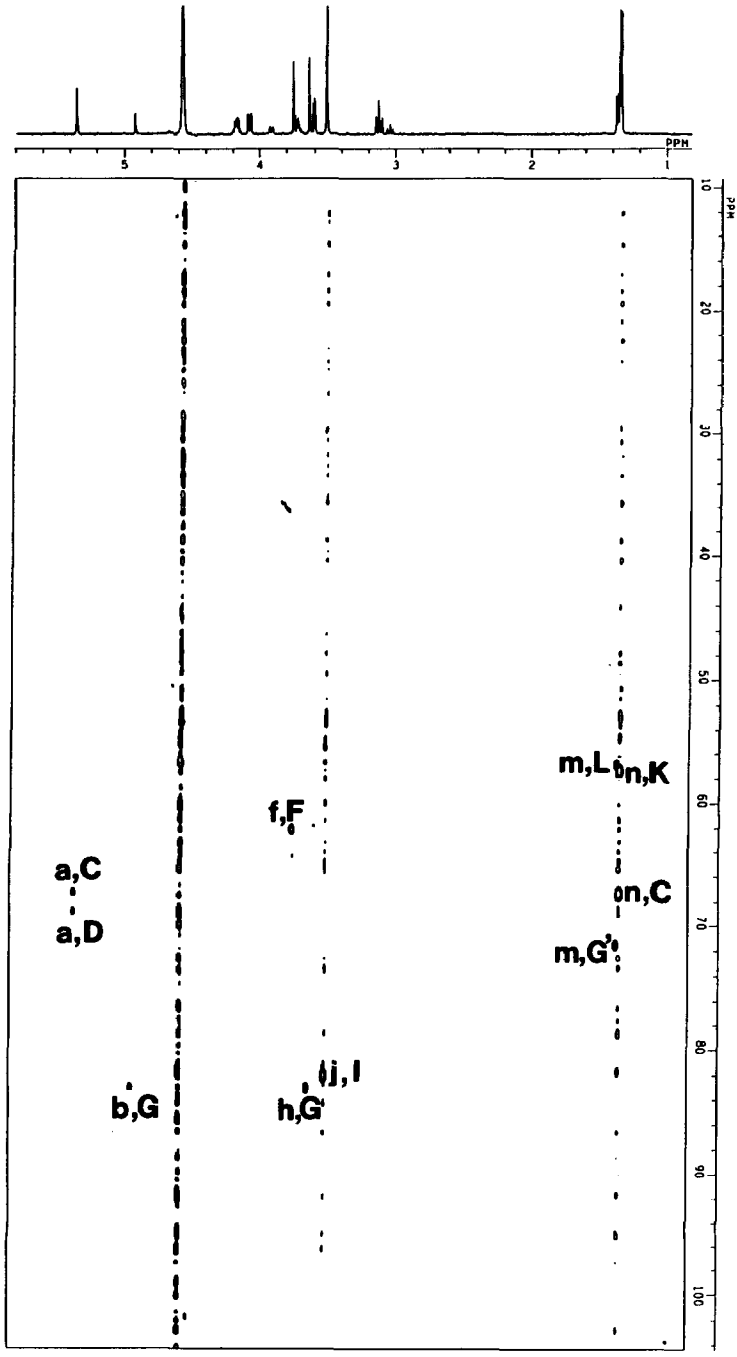


Fig. 8. HMBC spectra of the sugar. Correlation of a proton resonance with a carbon resonance 2–4 bonds distant was observed.

TABLE I

Pairs of protons identified by NOESY spectra

Series 1	Series 2
a–i,j	b–e,g
i–a,d,j	g–b,e,h,m
d–c,i,k	e–b,g
k–n,d	l–m
c–d,n	m–g,l
n–c,k	h,g
j–a,i	

TABLE II

Chemical shifts of protons and carbons, and coupling constant of protons

^{1}H	Peak	Ppm	J (Hz)	^{13}C	Peak	Ppm
H (C-1)	a (b)	5.35 (4.92)	$J_{a,i}$ 1.5	C-1	A (B)	93.6 (96.7)
H (C-2)	i (g)	3.60 (3.72)	$J_{i,d}$ 3.4	C-2	I (G)	81.9 (83.0)
H (C-3)	d (e)	4.08 (3.92)	$J_{d,k}$ 10.8	C-3	D (E)	68.9 (72.4)
H (C-4)	k (l)	3.12 (3.04)	$J_{k,c}$ 10.8	C-4	K (L)	57.5 (57.0)
H (C-5)	c (g′)	4.17 (3.72)	$J_{c,n}$ 6.4	C-5	C (G′)	67.3 (71.5)
H (C-6)	n (m)	1.34 (1.36)		C-6	N (M)	19.7 (19.7)
OCH_3	j (h)	3.41 (3.63)		OCH_3	J (H)	61.7 (64.9)

protons H-3 and H-4, and H-4 and H-5 are oriented in a *trans*-diaxial manner. This sugar therefore has the basic structure of mannose (rhamnose). The position of the NH_2 group was determined according to the ^{13}C NMR chemical shifts. The chemical shifts of C-2 to C-5 in mannose or rhamnose are usually 65–80 ppm, whereas the chemical shift of C-4(K, L) of the sugar was 57.0–57.5, i.e., at higher field. From these data, we concluded that the amino group was attached to C-4 and that the structure of the sugar is as in Fig. 9A. Among the signals in the ^{1}H and ^{13}C NMR spectra, signals f and F had no correlation with other protons or carbons. They were considered to be an impurity since, for the measurements of

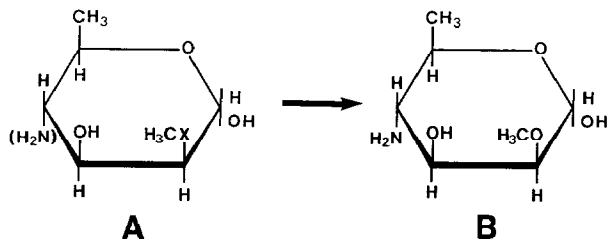


Fig. 9. A, Structure of the sugar deduced by NMR spectral analysis; and B, the final proposed structure of the sugar.

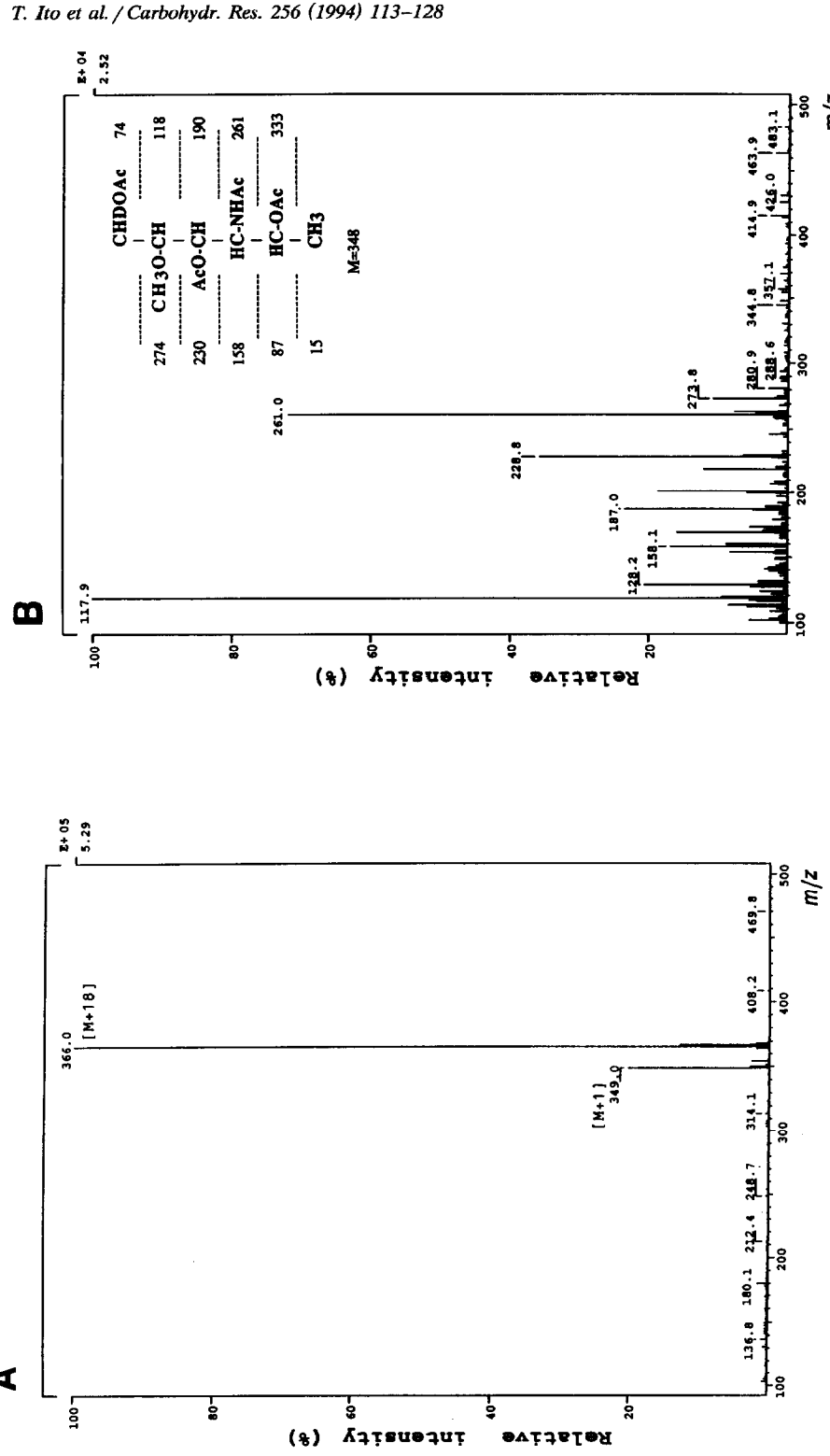


Fig. 10. Fragmentation pattern and mass spectra (A and B) of the sugar which had been reduced with sodium borodeuteride after *N*-acetylation, and then per-*O*-acetylated. A, Chemical ionization with ammonia; B, electron impact ionization at 50 eV. The fragmentation scheme of 4-acetamido-1,3,5-tri-*O*-acetyl-4,6-dideoxy-2-*O*-methyl-D-mannitol is shown.

NMR spectrum, we used the sugar before the final purification step with Amberlite CG50.

Identification of the sugar using mass spectra.—As shown in Fig. 1, the GLC–MS spectrum of this sugar in the form of the *N*-acetylated *O*-trimethylsilylated methyl glycoside, after chemical ionization with ammonia, showed a molecular ion peak at m/z 306(M + 1). When the mass number was calculated using the structure of the sugar proposed from the NMR spectra, it was predicted to be 177. Consequently, the structure of (C-2)–X–CH_3 is considered to be (C-2)–O–CH_3 (Fig. 9B). Fig. 10 shows the CI- and EI-mass spectra of the sugar. The sugar was reduced by $NaBD_4$ after *N*-acetylation, and converted into its peracetate. The CI-mass spectrum showed a molecular ion peak at m/z 349 and a pseudomolecular ion peak at m/z 366. The mass number in the CI-mass spectrum was identical to that of the mass number calculated from the proposed structure. Fragment ion peaks in the EI-mass spectrum agreed very well with that of the proposed structure. The main characteristic of the EI-mass spectrum is the fragment peak at m/z 261. This fragment is characteristic of 4-amino sugars. Fragment ion peaks at m/z 274, 261, 158, and 118, which were identical to the predicted fragment ion peaks from the proposed structure (Fig. 10B), were also observed. Based on these results, the structure of the sugar is 4-amino-4,6-dideoxy-2-*O*-methylmannose (Fig. 9B).

DISCUSSION

We have found a novel sugar in the LPS of *V. cholerae* O1 serotype Ogawa. At first, we found an unknown sugar when we investigated the sugar compositions of LPSs of the Ogawa and Inaba serotypes. Sugars liberated from LPSs by methanolysis were converted into *N*-acetylated *O*-trimethylsilylated methyl glycosides. They were analyzed using GLC and GLC–MS. We detected well-known constituents of LPS of *V. cholerae*, such as glucosamine, perosamine, quinovosamine, glucose, heptose, and a small amount of mannose. At the same time, we detected the presence of a small amount of an unknown sugar only in the serotype Ogawa. The CI-mass spectrum of this unknown sugar showed a molecular ion peak at m/z 306 (Fig. 1). However, as the amount was very small, we could neither identify the material nor guess that the sugar was important in determining serotype specificity. We happened to find a new amino sugar during the purification of perosamine as a standard. The CI-mass spectrum of the new amino sugar was investigated after methanolysis in 1 M methanolic HCl at 80°C for 24 h and conversion into the *N*-acetylated *O*-trimethylsilylated methyl glycoside. The mass number was identical to that of the unknown sugar found in the LPS of strain NIH41 serotype Ogawa when it was methanolyzed in 1 M methanolic HCl (Fig. 1). The sugar was detected only in the Ogawa serotype (Fig. 2). In *V. cholerae* O1, the conversion of serotype, such as Ogawa into Inaba or Inaba into Ogawa, is known to occur during subculture in vitro, passage in vivo, or even during a pandemic[10–13]. We performed phenotypic conversion experiments in vitro, and obtained serotype-converted mu-

tant subclones from the Ogawa-type parent strains[14]. In this study, we investigated the occurrence of this amino sugar, using these parent and mutant strains (Fig. 2). As expected, the sugar disappeared when the serotype conversion occurred from Ogawa into Inaba. That is, although Ogawa strains, 47-4041 and P1418, contained the sugar (Fig. 2C,D), the sugar was not found in the LPS when these strains changed their serotypes to Inaba (mutants 47-4041-1 and P1418-201) (Fig. 2G,H). The sugar compositions of O-antigenic polysaccharides of strain NIH41 (Ogawa) and strain NIH35A3 (Inaba) were also investigated (data not shown). The sugar was found in the O-polysaccharide of strain NIH41 (Ogawa), but not in the O-polysaccharide of strain NIH35A3 (Inaba). These results showed that the sugar is localized on the O-polysaccharide portion of the LPS, and that it is most likely the antigenic determinant of the Ogawa serotype.

The conditions of acid hydrolysis used in this experiment were the same as used for the liberation of perosamine[9]. The purification procedure used was almost the same as that for fucosamine or quinovosamine. We used chromatography on Amberlite CG-50 to purify the sugar instead of a crystallization step from water–acetone according to the suggestion of S. Kondo. We determined the structure of the sugar using NMR and mass spectrometry.

In our ^{1}H and ^{13}C NMR study, we could not demonstrate the existence of the amino residue directly. However, the Elson–Morgan reaction indicated that the sugar was an amino sugar, supported by the fact that it adsorbed to a cationic resin, such as Dowex 50 (H^{+}). The existence of a nitrogen atom was clearly demonstrated by elemental analysis. The data presented in this paper show that the novel sugar liberated by acid hydrolysis from the LPS of *V. cholerae* O1 serotype Ogawa is 4-amino-4,6-dideoxy-2-*O*-methylmannose. The NOESY spectra indicated that the ratio of the α- to β-form was 3:1.

Some investigators have used methylation analysis in order to differentiate between LPSs of the Ogawa and Inaba serotypes. So far, no report on the existence of different methylated sugars to explain the difference between Ogawa and Inaba serotypes has been made. This can now be understood since methylated perosamine and methylated 2-*O*-methylperosamine would be indistinguishable.

The 2-*O*-methylperosamine reported in this paper is labile to acid. It was decomposed considerably by such mild acid conditions as 1 M methanolic HCl at 80°C for 24 h. This may be the reason why the sugar has not been identified until now.

EXPERIMENTAL

V. cholerae *strains used in this experiment.—V. cholerae* O1 biotype classical strain NIH41 (Ogawa), 47-4041 (Ogawa), and NIH35A3 (Inaba), and biotype eltor strain P1418 (Ogawa), K20 (Ogawa), and 78-651 (Inaba) were used. We used mutants expressing Inaba serotype, which were derived from the strains originally expressing Ogawa serotype determinants[10]. The two mutant strains, 47-4041-9

(Inaba) and P1418-201 (Inaba), were derived from parent strains 47-4041 (Ogawa) and P1418 (Ogawa), respectively.

LPS and O-polysaccharides.—For isolation of LPS, the organism was grown on a nutrient agar plate. Cells were collected, washed with phosphate-buffered saline (pH7.4), and then dried with acetone. The LPS was extracted from acetone-dried cells by the phenol–water method[15]. The crude extract was dissolved in water to give a 1% solution, and then centrifuged for 16 h at 100 000*g*. The sediment was suspended in 50 mM Tris · HCl and added with DNaseI and RNaseA to final concentrations of 25 and 20 μg/mL, respectively. The mixture was incubated at 37°C for 90 min. After centrifugation at 2000 rpm for 5 min, the mixture was recentrifuged at 100 000*g* for 16 h. The precipitate was lyophilized and used as purified LPS. O-Antigenic polysaccharides were prepared from the LPS by hydrolysis with aq 1% AcOH for 90 min at 100°C. The insoluble lipid-A was separated by centrifugation, washed twice with ether, then freeze-dried. After being dissolved in water, they were purified by column chromatography on Sephadex G100.

Isolation and detection of the sugar (2-O-*methylperosamine*).—Lipopolysaccharide (1.15 g) of *V. cholerae* O1 strain NIH41 was hydrolyzed in 120 mL of 10 M HCl at 90°C for 15 min. The hydrolysate was diluted with 5 vol of distilled water, then evaporated to dryness. It was purified according to the method for fucosamine, as described[16]. At first, it was subjected to chromatography on Dowex 50 (H^+) resin. Amino sugars were eluted from the column (4.4 × 15.5 cm) with 0.33 M HCl (flow rate of 40 mL/h; 18.4-mL fractions). Amino sugars were detected by the Boas modification of the Elson–Morgan reaction, using glucosamine as a standard[17]. The fraction containing the sugar was evaporated, and then applied onto a Dowex 50 (K^+) column (2.5 × 12.5 cm). The sugar was eluted from the column with 0.1 M potassium acetate (flow rate of 29 mL/h; 9.5-mL fractions). The sugar peak was collected and rechromatographed on Dowex 50 (H^+). After the column had been washed with water, it was eluted with 0.33 M HCl (flow rate of 16.7 mL/h; 9.5-mL fractions). Since the sugar was difficult to crystallize from water–acetone, we used a column of Amberlite CG-50 resin (4:6 mixture of H^+ and NH_4^+ forms). After washing with water, the column was eluted with 1% ammonium hydroxide. Purified sugars were obtained after freeze-drying.

For the detection of the sugar using LPSs of the *V. cholerae* O1 of various strains, the hydrolysate of LPS (22–25 mg) was separated using chromatography on a Dowex 50 (H^+) column (1.4 × 45 cm). Amino sugars were eluted from the resin with 0.33 M HCl (flow rate of ca. 10 mL/h; 9.5-mL fractions).

Chromatographic techniques.—TLC was performed with Kieselgel 60 plates (Merck, Germany), using 5:1:2 1-butanol–AcOH–water. Sugars were visualized using ninhydrin or Elson–Morgan reagent.

GLC of the carbohydrates was performed after methanolysis of LPS and their conversion into the corresponding *N*-acetylated trimethylsilyl methyl glycosides[18]. Amino sugars were reduced to alditols with $NaBD_4$ after *N*-acetylation, and converted into the alditol acetates[19].

A Hewlett–Packard 5890A gas chromatograph equipped with a Hewlett–Packard fused-silica capillary column HP-1 (0.32 mm × 25 m) or DB1301 (0.25 mm × 20 m) (J&W Scientific) was used for analysis.

Spectroscopic methods.—Gas–liquid chromatography–mass spectrometry (GLC–MS) analysis was carried out with a Finnigan Mat TSQ700 mass spectrometer equipped with a DB1301 column.

For NMR measurements, samples were dissolved in D_2O containing 4,4-dimethyl-4-silapentane-1-sulfonate sodium salt (DDS) as an internal standard. Spectra were measured at 40°C with a JEOL JNM-GSX 500 instrument; ^{13}C spectra were recorded at 125.8 MHz, and ^{1}H spectra at 500.2 MHz. Two-dimensional techniques such as DQF-COSY[20], NOESY[21], HMQC[22], HMBC[23], and HOHAHA[24] were used.

Chemicals.—Perosamine and quinovosamine used for standards were purified from the LPS of *V. cholerae* O1 strain NIH41 and *P. aeruginosa* P14, respectively, using the method described above. Cation-exchange resins Dowex 50W-X8 (200–400 mesh) and Amberlite CG-50 (Type 2, Organo Co. Ltd., Japan) were used for the separation of amino sugars. All other chemicals were of reagent grade.

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