ISOLATION OF 4-AMINO-4-DEOXY-L-ARABINOSE FROM S AND R FORM BACTERIAL LIPOPOLYSACCHARIDES[a]

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1. Introduction

During the course of mild acid hydrolysis of the lipopolysaccharides (LPS) isolated from several *Salmonella* species, a positively-charged sugar which migrated faster than glucosamine on paper electropherograms was observed. Subsequent studies demonstrated that this sugar was either destroyed or underwent a molecular rearrangement, unless hydrolysis was accomplished under exceedingly mild acidic conditions. This amino sugar, which is detectable in a number of *Salmonella* and *Escherichia* cell wall LPS preparations, has been identified as 4-amino-4-deoxy-L-arabinose [1].

2. Materials and methods

Cell wall LPS used in this study were isolated from *Salmonella barielly* T2 and from *Salmonella minnesota* mR595 by the phenol-water [1], or by the phenol-chloroform-petroleum ether [2] method, respectively. Paper electrophoresis was carried out in a pyridine-acetic acid-water (100:40:860) buffer at pH 5.3 in apparatus as described by Kickhöfen and Warth [3]. Paper and thin layer chromatography were performed using the following solvents:

(A) ethyl acetate-pyridine-acetic acid-water (25:25:5:15);

(B) butanol-acetic acid-water (5:1:2);

(C) butanol-pyridine-water (6:4:3); and

(D) butanol-methyl ethylketone-NH_4OH-water (5:3:1:1).

Sugars were detected using either $AgNO_3$/NaOH or ninhydrin. Gas liquid chromatography was carried out at 190° after conversion of the sugars into their alditol forms following the method of Sawardeker et al. [4].

3. Results

3.1. *Liberation and detection of the amino sugar*

The amino sugar was obtained from LPS by hydrolysis in 0.5 N HCl at 37° for 7 to 16 hr. Paper electrophoresis proved useful for its detection (M_{GlcN}= 1.14) in the acid hydrolysates: The amino sugar turns yellowish-brown after the paper is dry, and can be stained with silver nitrate (turning grey/black) followed by alkali (deep black), or with ninhydrin (yellow/brown). However, paper electrophoresis has not been successful for its preparative purification since much of the amino sugar, after elution, was found to be degraded.

After its liberation from the *Salmonella minnesota* mR595 LPS, the purification of the amino sugar was accomplished by absorbing the 0.5 N HCl hydrolysate on a charcoal-celite column, followed by elution from the column with water. With this procedure, the amino sugar was eluted before contaminating hydrolysis products.

3.2. *Properties of the amino sugar*

Reduction of the amino sugar with $NaBH_4$ yielded two products which could be separated both electro-

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phoretically (M_{GlcN} = 1.14 and 1.24) and by paper chromatography. A spectral analysis of the ninhydrin reaction products from these two preparations showed that the ratio of the absorbance at 440 nm to that at 570 nm was 0.11 for the first, and 1.57 for the second. This indicated the latter was not an amino compound, but contained an *N*-ring.

N-Acetylation of the amino sugar was accomplished by the addition of acetic anhydride to the free amino sugar in $NaHCO_3$ buffer, followed by electrophoresis and isolation of the neutral material. Again, this procedure yielded two compounds which could be separated from each other on paper with solvents A, B and C.

The position of the amino group was determined by degrading the amino sugar. The acetylated amino sugar was oxidized with $NaIO_4$ followed by oxidation with NaIO. The oxidized product was deacetylated by hydrolysis in 2 N HCl at 100° for 2 hr and the mixture was subjected to paper electrophoresis. The area corresponding to the ninhydrin positive substance was eluted. This substance was an amino acid and was identified as serine by chromatography in 3 solvents, and by its retention time in the amino acid analyzer.

Reduction of the original amino sugar with ^3H-$NaBH_4$ prior to the periodate and hypoiodite oxidations yielded non-radioactive serine.

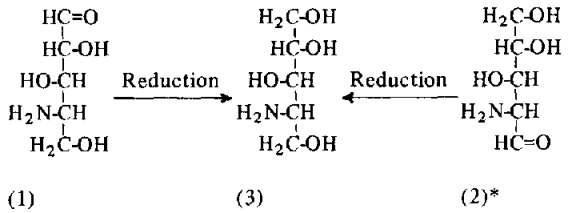
The serine obtained by degradation of the amino sugar was not oxidized by D-amino acid oxidase and, therefore, has an L-configuration. This was confirmed when its optical rotation was found identical to that obtained using authentic L-serine hydrochloride.

These results indicate that the amino group is attached to the carbon adjacent to a terminal CH_2OH group. This agrees with the finding that no acetaldehyde is formed after periodate oxidation of the reduced amino sugar.

3.3. *Identification of the amino sugar*

The above results suggest that the unknown amino sugar is a 5-amino hexose or a 4-amino pentose. None of these amino sugars were available for comparison with the isolated amino sugar. However, reduction of any 2-amino aldose results in the formation of an amino alditol identical to the compound obtained by reduction of the unknown amino sugar (M_{GlcN} 1.14). Thus, the following amino sugars were reduced with $NaBH_4$, and compared with the reduced amino sugar: 2-amino glucose, 2-amino galactose, 2-amino talose, 2-amino mannose, 2-amino arabinose and 2-amino lyxose (2). Of these, all the amino hexitols could be separated from the unknown reduced sugar by electrophoresis, but the amino pentitols migrated identically to the unknown compound. The unknown amino sugar alcohol (3) and the reduced amino pentitols were then compared by the following methods: paper chromatography in various solvents, gas liquid chromatography after *O* and *N* acetylation, and by column chromatography in the amino acid analyzer. It was found in all cases that the reduced 2-amino lyxose was identical with the reduced unknown amino sugar (table 1). On the other hand, 2-amino lyxose (2) itself could be distinguished from the unknown amino sugar by thin layer chromatography. These data indicate that the reduced unknown amino sugar (3) is identical to reduced 2-amino lyxose, i.e., reduced 4-amino arabinose.

Formula



(1) (3) (2)*

4. Discussion

The fact that the reduced amino sugar is identical to reduced 2-amino-2-deoxy-lyxose, and that after degradation of the C1-labelled reduced amino sugar, non-labelled L-serine was isolated, indicate that the new amino sugar is 4-amino-4-deoxy-L-arabinose (1).

Although this is the first known report of the biological occurrence of a 4-amino pentose, the chemical syntheses of 4-amino pentoses have been reported [5, 6]. These reports indicate that the instability of the 4-amino-L-arabinose, (particularly in a neutral or alkaline environment) and its tendency to form a pyrrolidine ring, are in accord with results

* 2-Amino-D-lyxose was used in our experiments; for simplicity, formula (3) shows the L-form.

Table 1
A comparison of the reduced amino sugar with authentic reduced amino pentoses.

Compound	Paper electrophoresis	Thin layer chromatography (Solvents)			GLC	Amino acid analyzer
	M[1]	A R_f[1]	B R_f[1]	C R_f[1]	R[2]	R[3]
Reduced unknown amino sugar	1.14	0.95	0.89	0.95	3.02	1.40
Reduced 2-amino lyxose	1.14	0.95	0.89	0.95	3.02	1.40
Reduced 2-amino arabinose	1.14	0.89	1.10	1.02	2.52	–

[1] Relative migration compared to glucosamine.
[2] Retention time on gas liquid chromatogram as compared to hexaacetyl sorbitol.
[3] Retention time compared to glucosamine.

obtained from the chemically synthesized 4-amino pentoses.

A survey of 31 smooth and rough LPS preparations representing 10 different species of *Salmonella* and 6 mutants of *Escherichia coli* was carried out. As judged by paper electrophoresis of the acid hydrolysate, arabinosamine was detectable in 29 of the *Salmonella* and 4 of the *Escherichia* preparations.

The apparent wide spread occurrence of 4-amino arabinose in the cell wall LPS preparations studied would indicate that it is an integral constituent of the LPS. This is supported by the observation that the amino sugar was found in preparations obtained by extraction of the bacteria with phenol water [1], or with phenol-chloroform-petroleum ether [2]. The possibility, however, cannot be ruled out that the 4-amino arabinose exists as a contaminant in the LPS preparations studied.

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