FACTORS AFFECTING THE SPECTROPHOTOMETRIC DETERMINATION OF 2-OXO-3-DEOXY-D-MANNOOCTONIC ACID AND 3,6-DIDEOXYSUGARS IN BACTERIAL LIPOSACCHARIDES

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We have previously proposed the tetrahydroborate–2-TBA procedure [1] for the determination of 2-oxo-3-deoxy-D-mannooctonic acid (ODO) and of 3,6-dideoxyhexoses (3,6-DDHs) in a mixture. In the present paper we report the possibility of the use of this procedure for the analysis of hydrolyzates of bacterial lipopolysaccharides (LPS), in particular the LPS from the bacterium Yersinia pseudotuberculosis.

The hydrolysis of the LPS in 0.25 N sulfuric acid at 100°C for 15 min proved to be sufficient for the complete cleavage of the ODO and the 3,6-DDHs from the main chain of the LPS. More prolonged hydrolysis led to the decomposition of the ODO, while the 3,6-DDHs retained their reactivity with respect to 2-thiobarbituric acid (2-TBA).

The neutral monosaccharides (glucose, fucose, xylose, arabinose, fructose, and glucoheptose), amino sugars (glucosamine and N-acetylglucosamine), fatty acids (β-hydroxymyristic, levulinic, stearic, and palmitic acids), and proteins (trypsin, egg albumin) usually present in hydrolyzates of LPS in concentrations of 0.25 mg/ml do not give a color reaction with 2-TBA and do not inhibit the development of the colors due to ODO and 3,6-DDHs under the conditions of the proposed procedure.

2-Deoxyribose, which has not been detected in the majority of LPS investigated, may be present in DNAs accompanying the LPSs as impurities and be liberated by acid hydrolysis. Since the behavior of 2-deoxyribose in the tetrahydroborate–2-TBA procedure is similar to that of ODO, its presence increases the results for ODO and is not reflected in the figures for the 3,6-DDHs (in coloration, 1 μg of 2-deoxyribose is equivalent to 4.3 μg of ODO).

The presence of DNA in the LPS preparations increases the results of the determination both for ODO and for the 3,6-DDHs. The formation of a colored product with 2-TBA is due not only to the presence of 2-deoxyribose in the DNA but also, apparently, to an interaction of the reagent with the conjugated double bonds of the pyrimidine bases. In favor of this hypothesis is the fact that the preliminary reduction of a DNA hydrolyzate with sodium tetrahydroborate, in contrast to that of 2-deoxyribose, does not lead to the complete loss of the reactivity of the DNA to 2-TBA. Under the conditions of the tetrahydroborate–2-TBA procedure, 1 μg of DNA is equivalent in coloration to 0.0332 μg of ODO and 0.0268 μg of 3,6-DDHs. This influence can be taken into account by introducing a suitable correction for the DNA content. To calculate the concentrations of ODO and 3,6-DDHs in bacterial LPSs found by the tetrahydroborate–2-TBA procedure, we propose the following formulas:

$$C_{ODO} = C_{ODO_1} - 0.0332 \cdot C_{DNA}, \text{ and}$$

$$C_{3,6\text{-DDHs}} = C_{3,6\text{-DDHs}_1} - 0.0268 \cdot C_{DNA},$$

where C_{ODO} is the true concentration of ODO in an aliquot of the solution under investigation; $C_{3,6\text{-DDHs}}$ is the true concentration of 3,6-DDHs in an aliquot of the solution under investigation; C_{ODO_1} is the concentration of ODO calculated according to our previous paper [1]; $C_{3,6\text{-DDHs}_1}$ is the concentration of 3,6-DDHs calculated according to our previous paper [1]; C_{DNA} is the concentration of DNA in an aliquot of the solution under investigation determined by Spirin's method [3].

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The proposed tetrahydroborate–2-TBA procedure is applicable to the analysis of various bacterial LPSs. The standard deviation of the results of the determinations for ODO and for 3,6-DDHs amounts to 4%. The results of an analysis of a hydrolyzate of the LPSs obtained by the tetrahydroborate–2-TBA procedure and by the preparative separation of the ODO and 3,6-DDHs on a column of Dowex 1 (CO_3^{2-} form) [2] agree within the limits of the standard deviation shown.

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A NEW METHOD FOR OBTAINING METHYL β-L-RHAMNOPYRANOSIDE

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The method for obtaining methyl β-L-rhamnopyranoside described in the literature [1], which is based on the Koenigs–Knorr reaction, is a multistage process and is characterized by a low yield of the end-product (of the order of 1%). We have proposed a method which consists in obtaining methyl β-L-rhamnopyranoside as a by-product in the synthesis of methyl α-L-rhamnopyroside by the Fischer method [2].

Analysis by GLC and by chromato-mass spectrometry showed that after a solution of 98 g of α-L-rhamnose in 1 liter of methanol containing 1% of HCl had been left for 40 h, the reaction product contained 89% of methyl α-L-rhamnopyranoside and 11% of methyl β-L-rhamnopyranoside. After the methyl α-L-rhamnopyranoside (68.5 g) had been crystallized out by the usual method, the remaining mother syrup (21.6 g) contained 38% of methyl β-L-rhamnopyranoside. This product was acetylated in pyridine with acetic anhydride, and the resulting acetate (36.0 g) was crystallized from ethanol. After two crystallizations, the acetate of methyl β-L-rhamnopyranoside (6.1 g) was chromatographically pure and contained no methyl α-L-rhamnopyranoside. It melted at 154-155°C $[\alpha]_D^{20}$ + 44.3° (c 2.5; chloroform). Literature information [1]: mp 151-152°C, $[\alpha]_D^{18}$ +45.73°, $C_6H_8O_4$ (OCH_3) $(COCH_3)_3$.

The acetate of methyl β-L-rhamnopyranoside (6.0 g) was deacetylated with sodium methoxide in methanol. After recrystallization from ethanol, the yield was 2.8 g (3.1% on the initial rhamnose), mp 140-141°C, $[\alpha]_D^{20}$ +91.9°. Literature information [1]: mp 138-140°C, $[\alpha]_D^{20}$ +95.39°, $C_6H_{11}O_4$ (OCH_3).

For analytical GLC we used a Tsvet-106 instrument fitted with a flame-ionization detector and two columns (100 × 0.4 cm). As the stationary phase we used 10% of BDS on Chromosorb W (60-80 mesh). The temperature of the thermostat was 190°C, the rate of flow of argon 60 ml/min, and the retention time for the acetate of methyl α-rhamnoside 6.4 min and for the acetate of the β-rhamnoside 9.4 min.

The melting points were measured on a Boetius instrument and the specific rotations on a Perkin–Elmer 141 instrument.

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