USE OF CARBOCYANINE DYES IN THE ANALYSIS OF BACTERIAL LIPOPOLYSACCHARIDES (ENDOTOXINS).

II. THE LIPOPOLYSACCHARIDE OF Salmonella typhi

M. N. Mikheeva and L. I. Brutko

UDC 543.42.062.8

On the basis of the results of a study of the conditions for the photometric reaction forming an associate of a carbocyanine dye with the lipopolysaccharide of the typhoid bacillus Salmonella typhi$_{4446}$, a method is proposed for its spectrophotometric determination which excludes the use of an antioxidant. The proposed method is characterized by high sensitivity, good reproducibility, and simplicity of performance.

In the present paper we consider questions of the quantitative evaluation of the bacterial lipopolysaccharide (LPS) of the typhoid bacillus – Salmonella typhi – by a spectrophotometric method. Our aim was to study the conditions of the photometric reaction and to develop a procedure for the quantitative determination of this lipopolysaccharide.

We used the reagent described by Janda [1] and work which we had also used in preliminary investigations [2] – the carbocyanine dye 1-ethyl-2[3-(1-ethylnaptho[1,2-d]thiazolin-2-ylidene)-2-methylpropenyl]naphtho[1,2-d]thiazolium bromide.

We investigated the lipopolysaccharide of the typhoid bacillus S. typhi (strain 4446), which is a substance used in the production of the preparation "Pirogenal" [3].

The optical density measurements and the recording of the spectra were performed, with the protection of the solutions from daylight, at 22°C on a Gilford 240 recording spectrophotometer (USA).

It was found that a medium ensuring the formation of associates of the dye with the LPS was a mixture of water and ethanol containing 30% of ethanol in the final volume. The spectra of the dye in ethanol and of its associate with the LPS in aqueous ethanol are shown in Fig. 1.

The absorption maximum of the dye is located at 576 nm in ethanol and at 573 nm in aqueous ethanol containing 30% of ethanol in the final volume, and that of the associate with the LPS is present at 467 nm. The difference between the absorption maxima of the dye and the reaction product amounts to 106 nm, which fully satisfies the condition $\Delta\lambda_{max} \geq 100$ nm [4].

The amount of carbocyanine dye necessary for binding the LPS into the associate completely was determined experimentally from the maximum light absorption of the solutions. For this purpose, aqueous solutions of the LPS with a constant concentration (for example, 50 μg/ml) and a series of solutions of the dye in ethanol with increasing concentrations – 10, 20, 40, 60, 80, 100, 120, and 140 μg/ml – were prepared. It was established from the optical density values (D_{476}) found for the associate formed that the lowest concentration of dye ensuring the maximum absorption of light was 100 μg/ml in ethanol.

In view of the fact that the associates of the dye with the LPS are weak compounds [4], we studied their stability in time. For this purpose, the optical densities of the associates were measured over 30 min (from the moment of addition of the reagent) at 5-minute intervals. During the first 15 minutes the optical density of the associate increased somewhat (from 1.16 to 1.20) and then remained constant (1.20). The basic law of light absorption by the associate obtained was obeyed for concentrations of the LPS of from 0.8 to 50 μg/ml.

All-Union Scientific-Research Institute of Pharmacy, USSR Ministry of Health, Moscow.
Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 790-792, November-December, 1987. Original article submitted March 18, 1987; revision submitted July 10, 1987.

 0009-3130/87/2306-0654$12.50

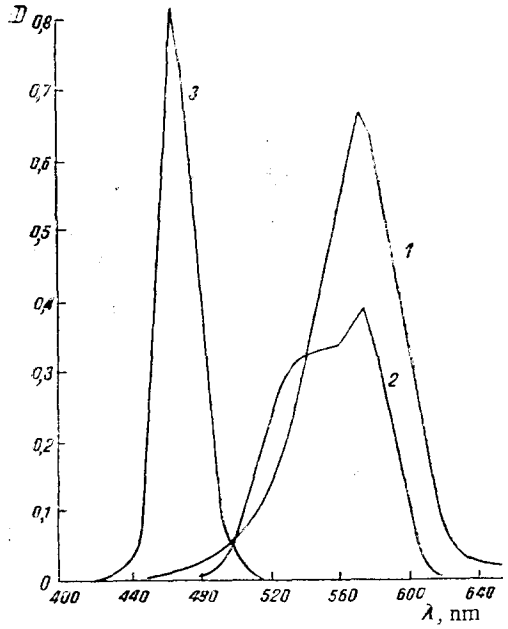

Fig. 1

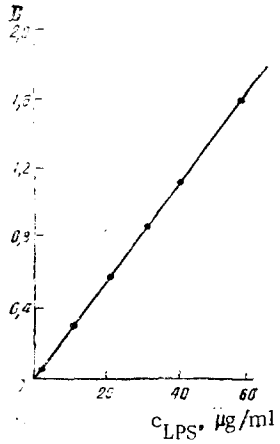

Fig. 2

Fig. 1. Absorption spectra: 1) the carbocyanine dye in 96% ethanol (λ_{max} = 576 nm); 2) the carbocyanine dye in 30% ethanol (λ_{max} = 573 nm); 3) associate of the dye with the LPS in aqueous ethanol containing 30% of ethanol in the final volume (λ_{max} = 467 nm).

Fig. 2. Calibration curve of the dependence of the optical density of the associate on the concentration of the lipopolysaccharide.

The procedure described permits the quantitative analysis of this lipopolysaccharide to be performed by a physicochemical method. The results of the quantitative determination of the LPS by the spectrophotometric method are given below:

LPS taken, μg	LPS found, μg
20.00	19.83 ± 0.6327
20.00	20.21 ± 0.6327
20.00	20.23 ± 0.6327
20.00	19.04 ± 0.6327
20.00	19.93 ± 0.6327

The relative error of the determination does not exceed ±3.19%. The specific absorption index is $2.91 \cdot 10^2$.

It was established experimentally that an optical density of the associate of 0.020 at a transverse action of the cell of 1 cm^2 corresponds to a concentration of the LPS of 0.8 μg/ml. Consequently, the nominal sensitivity of the reaction [5] is 0.8 μg.

EXPERIMENTAL

The bacterial preparation – the lipopolysaccharide of the typhoid bacillus Salmonella $typhi_{4446}$ – was obtained in the N. F. Gamaleya Institute of Epidemiology and Microbiology (supplied by E. K. Matkovskaya).

The lipopolysaccharide was isolated by enzymatic hydrolysis (trypsin) followed by phenol extraction, which ensures the maximum extraction of the substance and its freedom from protein impurities. The characteristics of the lipopolysaccharide – its elementary composition and the composition of the amino acid chain and of the polysaccharide – are given in [6].

The carbocyanine dye was obtained from GosNII KhimFotoProekt [All-Union State Scientific Research and Design Institute of the Photographic Industry] (Moscow).

Procedure. To 1.0 ml of an aqueous solution of the sample containing from 15 to 30 μg of the LPS were added 0.4 ml of distilled apyrogenic water and 0.6 ml of a solution of the reagent (a solution of 2.5 mg of the dye in 25 ml of ethanol is stable on storage in the dark at +4-6°C for 5-6 days). The reaction mixture was carefully stirred and the optical density was measured at 22°C 15 min after the addition of the reagent on a Gilford 240 spectrophotometer (ℓ = 1.0 cm) at 467 nm relative to a comparison solution consisting of 1.4 ml of apyrogenic distilled water and 0.6 ml of the solution of the reagent.

The amount of LPS in the sample was determined from the specific absorption index calculated previously.

A calibration curve (Fig. 2) was plotted on the basis of the proposed method. Each point on the curve represents the mean of five determinations.

SUMMARY

1. A procedure has been developed for the quantitative spectrometric determination of the bacterial lipopolysaccharide of Salmonella $typhi_{4446}$ which eliminates the use of an antioxidant.

2. The proposed procedure is characterized by high sensitivity, good reproducibility, and simplicity of performance.

LITERATURE CITED

1. J. Janda and E. Work, FEBS Lett., 16, No. 4, 343 (1971).
2. M. N. Mikheeva, S. A. Shmel'kova, and L. I. Brutko, Khim. Prir. Soedin., 370 (1986).
3. MRTU-[Interrepublican Technical Standard]-42 No. 406-71.
4. A. K. Babko and A. T. Pilipenko, Photometric Analysis [in Russian], Moscow (1968), pp. 45, 62.
5. V. B. Aleskovskii and K. B. Yatsimirskii, Physicochemical Methods of Analysis [in Russian], Leningrad (1971), p. 62
6. P. E. Budnitskaya, Experimental Investigations and the Clinical Use of Pirogenal [in Russian], Moscow (1961), p. 8.

NEW QUINONE FROM A MARINE SPONGE OF THE ORDER DICTYOCERATIDA

N. M. Rebachuk, V. A. Denisenko, and S. A. Fedoreev

UDC 547.567+593.4

Two pigments of quinoid nature, one of which has been identified as ilimaquinone, have been isolated from a hexane extract of a marine sponge Hyatella sp., family Spongiidae, order Dictyoceratida. The second was a new benzoquinone of the drimane series with the composition $C_{22}H_{32}O_5$, which has been called hyatoquinone. Its structure has been established on the basis of spectral characteristics and chemical transformations.

From a hexane extract of a freeze-dried preparation of the marine sponge Hyatella sp., family Spongiidae, order Dictyoceratida, we have isolated a fraction containing several substances of quinoid nature. The physicochemical properties of the main component of this fraction coincided completely with the properties given in the literature for ilimaquinone (I) [1].

The other quinone (I), which we have called hyatoquinone (II) proved not to have been described previously. Its spectral characteristics were largely similar to those of ilimaquinone, which indicated a closeness of their structures. The elementary analysis of hyato-

Pacific Ocean Institute of Bioorganic Chemistry, Far Eastern Scientific Center, USSR Academy of Sciences, Vladivostok. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 793-796, November-December, 1987. Original article submitted May 14, 1987.

 0009-3130/87/2306-0656$12.50