USE OF METHYLENE BLUE FOR DETECTING PYROGENAL—BACTERIAL LIPOPOLYSACCHARIDE

L. E. Shchedrina and S. P. Potemkina

UDC 615.456.076.7

Pyrogenic substances are substances of bacterial origin of lipopolysaccharide nature causing a rise in body temperature, in addition to other changes, in warm-blooded animals and in Man [1].

The use of the LAL test for detecting bacterial pyrogens is known. The main reagent for the performance of this analysis is a lysat of sword-tail blood cells – amebocytes possessing the capacity for forming a gel in the presence of bacterial lipopolysaccharides [2]. There is information according to which bacterial pyrogens are capable of interacting in vivo and in vitro with animal and human blood cells [3]. A. A. Togaibaev et al. have proposed a spectrophotometric method using a vital dye – Methylene Blue – for the diagnosis of endogenous intoxication in patients [4]. It has been established that in pyoseptic diseases the absorption of the dye increases.

Lipopolysaccharides form a component part of the cell wall of Gram-negative bacteria; they are located on the surface of the outer membrane and largely determine the interaction of the bacterial cells with the medium in which they are present. We have undertaken an attempt to detect pyrogenal – a bacterial lipopolysaccharide isolated from S. typhi – by the method described above using Methylene Blue. Blood was taken from rabbits, and the reaction with Methylene Blue was carried out in the way described by Togaibaev et al. [4].

Procedure. Blood (5 ml) from a rabbit vein was collected in a test-tube containing 1 ml of 3.8% solution of sodium citrate, and, after stirring, the erythrocytes were separated by centrifugation at 3000 rpm for 10 min. From the erythrocytic mass, 1 ml was transferred into a test-tube containing 1 ml of a pyrogenal solution prepared in 0.9% sodium chloride solution. A control test-tube was charged with 1 ml of erythrocytic mass and 1 ml of 0.9% sodium chloride solution. The mixtures were stirred and thermostated at 37°C for 1 h. After this, 3 ml of a 0.025% solution of Methylene Blue prepared in 0.9% sodium chloride solution was added to each test-tube. After stirring, they were incubated at room temperature for 10-12 min. Then they were centrifuged at 3000 rpm for 10 min. Each supernatant liquid was transferred into a cell and its optical density at λ_{max} 660 nm was determined.

We studied the absorption spectra of the solutions under investigation in the presence and in the absence of pyrogenal (λ_{max} 660 nm). As can be seen from Fig. 1, when pyrogenal was present in the solution in a concentration of $3 \cdot 10^{-3}$ g/ml the optical density fell. The absorption spectra of the solutions under investigation were recorded on a Beckman DU-7 spectrophotometer (USA). The limit of detection is $0.5 \cdot 10^{-3}$ g of pyrogenal in 1 ml of solution.

The phenomenon studied may find use for the detection of bacterial lipopolysaccharides in various media.

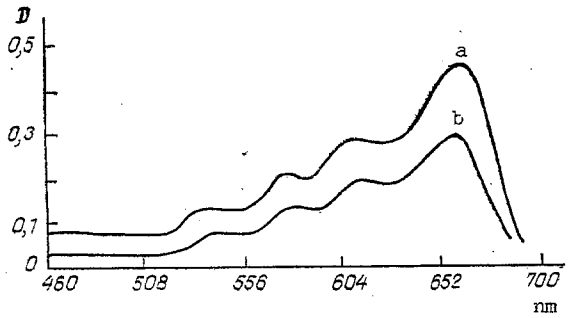

Fig. 1. Absorption spectrum of Methylene Blue: a) in a concentration of $2.5 \cdot 10^{-4}$ g/ml; b) with the addition of pyrogenal in a concentration of $3 \cdot 10^{-3}$ g/ml.

Scientific-Research Institute of Pharmacy, Ministry of Health of the Russian Federation, Moscow. Translated from Khimiya Prirodnykh Soedeninii, No. 1, pp. 156-157, January-February, 1993. Original article submitted March 16, 1992.

 0009-3130/93/2901-0132 $12.50

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INVESTIGATION OF THE FLAVONOID COMPOSITION OF *Scutellaria adenostegia*

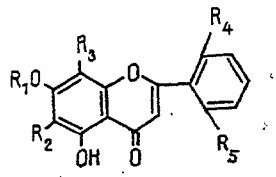
I. I. Chemesova, M. Iinuma, and A. L. Budantsev

UDC 547.972

The flavonoid composition of Scutellaria adenostegia Briq. (family Lamiaceae) has been studied. The material for investigation was collected under the natural conditions for the growth of the plant in the Tien-Shan (Talassian range, valley of the R. Itagar, 1.5 km above its mouth, 2000 m above sea level, roots, one-year shoots), and in the Pamir-Alai (Peter I range, environs of the village of Dzhelandy, 2100 m above sea level, roots, epigeal part).

The samples under investigation were subjected to extraction with aqueous ethanol (70%), and then, with the aid of chloroform and ethyl acetate, the extracted substances were separated into fractions containing components of similar polarity. The fractions so obtained were chromatographed on columns with various adsorbents (polyamide and silica gels L 100/160 and L 140/100 [sic]), using the eluents chloroform and ethanol and mixtures of them (ethanol–chloroform (1:100), (1:10), and (2:10)).

As a result of the separation of the chloroform fraction of the extracts of the roots, substances (I), (II), and (III) and a pale yellow crystalline precipitate that gave a single dark spot on chromatographs in various systems but was, according to its mass spectrum [m/z (%) 254 (M^+, 100%, 264 (M^+, 100%)], a mixture of two compounds difficult to separate by the usual chromatographic method were obtained. The acetylated mixture (Ac_2O + pyridine) was separated preparatively (PTLC): solvent: n-hexane–ethyl acetate (3:1), and the acetates (IV) and (VI) were obtained. The chromatographic separation of the ethyl acetate fraction of the extract of the roots led to the isolation of compound (VII), and that of the chloroform fraction from one-year shoots led to substances (IV) and (V). All the compounds were shown to be flavonoids by their IR spectra.



I. $R_1=R_3=R_4=R_5=H$; $R_2=OH$
II. $R_1=CH_3$; $R_2=R_3=CCH_3$; $R_4=R_5=OH$
III. $R_1=CH_3$; $R_2=H$; $R_3=OCH_3$; $R_4=R_5=OH$
IV. $R_1=R_2=R_3=R_4=R_5=H$
VI. $R_1=R_2=R_3=R_5=H$; $R_4=OCH_3$

Flavonoids (I) (mp 266°C, $C_{15}H_{10}O_5$), (II)* (mp 242°C, $C_{18}H_{16}O_8$), (IV) (mp 279°C, $C_{15}H_{10}O_4$), and (V) (mp 347°C, $C_{15}H_{10}O_5$) were identified on the basis of a comparison of their spectral characteristics and physical constants as bacalein, 2', 5,6'-trihydroxy-6,7,8-trimethoxyflavone, chrysin, and apigenin, respectively [1-3].

* Flavonoid (II) gave no depression of the melting point in admixture with a sample of 2',5,6'-trihydroxy-6,7,8-trimethoxyflavone kindly provided by Dr. T. Tomimori (Japan).

V. L. Komarov Botanical Institute, Russian Academy of Sciences, St. Petersburg. Pharmaceutical Institute, Gifu, Japan. Translated from Khimiya Prirodnykh Soedeninii, No. 1, pp. 157-159, January-February, 1993. Original article submitted November 26, 1991.

0009-3130/93/2901-0133 $12.50