THEORETICAL STUDY OF ANTIOXIDANT PROPERTIES OF THE 2.3-DIHYDRO-3.5-DIHYDROXY-6-METHYL-4(H)-PYRAN-4-ONE IS UNIQUE COMPOUNDS OF *BACILLUS SUBTILIS* IMV B-7023

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Stability of a redox-homeostasis in cells of microorganisms plays an important role in a set of biochemical processes. Its imbalance is accompanied by increasing of level of the reactive oxygen species (ROS) which can provide toxic effect on membrane lipids, proteins and nucleic acids [1].

One of the effective protection mechanisms of cells fro the aggressive oxidants in the majority of microorganisms, in particular the bacteria of genus *Bacillus*are functional protector complex which contain of enzymes and different low-molecular antioxidants. Bacilli are the widespread representatives of soil microflora; their antioxidant systems act as effective inhibitors stress agents at various crops of plants [2]. However, there is insufficient information about the mechanisms of inactivation of the ROS by the antioxidants of the metabolic complex of bacteria of the genus *Bacillus*.

Accordingly, an urgent question arises to the possible antioxidant behavior of the individual compounds synthesizedby bacilli. *Bacillus subtilis* IMV B-7023 strain, which is a part of complex highly effective bacterial preparation Azogran was the object of our investigation. By means of chromatographic methods the presence of 2.3-dihydro-3.5-dihydroxy-6-methyl-4(H)-pyran-4-one (DDMP) was identified in the culture medium of these bacteria (Table 1). This is unique metabolite belongs to the flavonoid fraction as its chemical structure is identical to flavonoid C ring (Fig. 1) [3].

Table 1

GC-MS-analysis of biological activity compounds present in ethanolic extract of the cell-free extract of *Bacillus subtilis* IMV B-7023 [4]

Biological activity compounds	Retention Time, min	Peak Area,%	Probability Identification, %
DDMP	4,40	10,47	95
5-hydroxymethyl-2-furancarboxaldehyde	4,94	39,22	62
Phenylacetic acid	5,06	18,88	87
4- Hydroxyphenylacetic acid	6,81	4,41	64

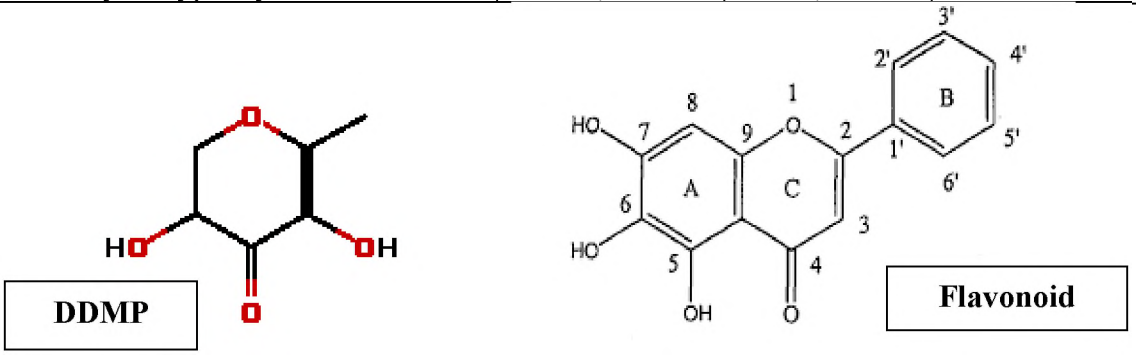

Fig. 1 Chemical structures of 2.3-dihydro-3.5-dihydroxy-6-methyl-4(H)-pyran-4-one and flavonoids [3]

Quantum-chemical analysis of antioxidant and antiradical properties of the DDMP was performed using the software package Gaussian 09W. All calculations were made in a vacuum at 298 K. The calculation results obtained in vacuum can be related to Antioxidative behavior of the compounds studied in nonpolar environment of the lipid-peptide membranes of living cells. It was established, that Hydrogen Atom Transfer Mechanism is implemented through 5-OH group. The Bond Dissociation Enthalpy (BDE) of this bond was 82.39 kcal/mol, and was lower than the BDE 3-OH of 107.41 kcal/mol. This indicates an average antiradical activity in relation to such radicals as $DPPH^{\cdot}$.

The SET-PT mechanism is characterized by 2 thermodynamic parameters: the adiabatic ionization potential (AIP) and the proton dissociation enthalpy (PDE), which describes the ability of a phenolic compound to give up H+. Low numerical values of PDE indicate easy proton dissociation from the antioxidant molecule. It should be noted that the course of the SET-PT mechanism mainly depends on PDE [5].

Determined, that DDMP can inactivate ROS by the electron-donor mechanism, which is accompanied by a splitting of a proton (SET-PT). At the same time, the dissociation enthalpy H^+ (PDE) for 5-OH group this compound was 209.8 kcal/mol and was slightly different from that for morin (210.9 kcal/mol), galangin (208.3 kcal/mol) and kaempferol (213.1 kcal/mol) (Table 2).Based on these results, DDMP can effectively scavenging the hydroxyl radical.

Table 2

Comparison of Proton dissociation enthalpy of 2.3-dihydro-3.5-dihydroxy-6-methyl-4(H)-pyran-4-one and different flavonoids

№	Compounds	PDE (5-OH), ккал/моль
1	Morin	210,9 [6]
2	Galangin	208,3 [7]
3	Kaempferol	213,10 [7]
4	DDMP	209,4

Thus, the dominant mechanism of ROS inactivation for DDMP was Single electron transfer-Proton Transfer (SET-PT).

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