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Paramagnetic Connections Contained in Cotton

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ABSTRACT: For the first time, a high resolution spectrum of electron paramagnetic resonance was obtained for the gossypol dialactol dimethyl ether bis-anion, and it was interpreted. Comparison of the intensity distributions of the four groups of lines of the obtained spectrum with those in the spectrum of the gossypol bis-anion indicate clear deviations from the binomiality in the spectrum of the bis-anion of the ether. The discovered fact and the manifestation of four groups of equidistant signals unambiguously indicate the triplet ground state of the studied bis-anions. It has been shown for the first time that the triplet ground states of two colliding two triplet structures in solution, bis-anion and oxygen visible from outside, lead to the formation of a donor-acceptor complex with charge transfer during the formation of the primary active oxygen form in the form of anion superoxide.

KEYWORDS: non-enzymatic processes, chiral centers, biosynthesis, defense systems, metabolite, antioxidant activity, hydrophilic and lipophilic medium, biradical, dioxodianhydrogossypol, pyrooxidant effect, redox cycle, electron delocalization, singlet oxygen.

I. INTRODUCTION

In the theory of the stability of biological objects, a special place is occupied by non-enzymatic chain oxidation processes of metabolites in the host-parasite system. If during enzymatic oxidation, products with chiral centers of only one species appear, then with non-enzymatic, racemates are always in a 1: 1 ratio. This means that 50% of the products of non-enzymatic oxidation cannot be used further, i.e. there is a loss (dissipation) of substances and energy expended on their biosynthesis. Therefore, the main task of protective systems is to prevent non-enzymatic oxidation of metabolites. To perform this task, antioxidants are used, which in higher and lower plants are represented by polyphenols. The antioxidant activity of some polyphenols, both in hydrophilic and lipophilic media, decreases after oxidation of hydroxyl groups in the initial structures [1]. For the main polyphenolic antioxidant of the family of malvace gossypol - it is noted that in some cases it exhibits a prooxidant effect [2]. It was suggested that this effect is due to the formation of free radical structures from the initial gossypol molecule [2,3]. The oxidation of all hydroxyl groups in gossypol leads to the formation of a stable biradical in the form of dioxodianhydrogossipole [3].

The multiplicity (mutual direction of the spins) of the ground state of unpaired electrons in biradicals determines their ability to “line up” in a chain, which is experimentally manifested in the electron spin resonance (ESR) spectra [1] and can affect the local viscosity surrounded by biological membranes. In addition, multiplicity determines the catalytic activity of biradical structures with respect to the most common natural biradical - molecular oxygen [4].

In this paper, for the first time, comparisons of the parameters of the obtained optical and paramagnetic absorption spectra of this new natural structure and the reasons for its prooxidant effect are discussed.

II. EXPERIMENTAL

For the study, the underground part (bark of the roots) of cotton of the variety Bukhara-6 was collected at the end of the vegetative (November-December) period in the Tashkent region of the Republic of Uzbekistan. Raw materials were dried to air-dry, and stored in the shade at room temperature.

To obtain the extract, the dried raw materials were ground using a mill (IKA) to a powder. Freshly distilled acetone per 20 g of powder was added to the obtained powder at room temperature — 20 ml; after 12 h, it was filtered with a paper filter.

The obtained samples in the form of yellow polycrystalline powders were tested for the absence of an EPR spectrum with maximum hardware gain on an ER-200D-SRC spectrometer with an operating frequency of 9.8 GHz.

Oxidation of gossypol. A suspension of these polycrystals in CC14 was left in petri dishes in contact with air at 150 ° C for 2 days until complete evaporation of CC14. This solvent is chosen as an environment in which the lifetime of ROS is much higher than in other organic solvents [7]. Samples of oxidized gossypol in the form of a powder with a brown tint and their solutions were used to record the EPR spectra at different temperatures. The EPR spectra were recorded in the regime of slow transmission under conditions far from saturation of the signals.

III. RESULTS AND DISCUSSION

Glycosidation of gossypol is a means of detoxification during its biosynthesis and transportation in plant cells. As a result of the decrease in energy metabolism in cells due to external causes (thermal stress, salt, the introduction of a pathogen, etc.), there is a local decrease in the value of intracellular pH, which causes irreversible decomposition of water-soluble glycosylated polyphenols and accumulation of free aglycones in extracellular fluids. The presence of hydrophobic and hydrophilic groups in the structure of free gossypol contributes to its concentration on the boundaries of the sections of the aquatic environment - extracellular lipid formations of plants and fungi. The disappearance of stress conditions means returning the pH to normal values (pH> 7). Under these conditions, gossypol molecules (1) can take an anionic form (2) which dissolves in an aqueous medium (Fig. 1). Their combination into granule-like formations found inside the plant should prevent electrons of the same name in anionic forms (2) of gossypol. In the experiment, the presence of charges is indicated by the irreversible disappearance of the electron spin resonance (ESR) spectrum of a water-alkaline solution of gossypol [3] noted after a brief contact with air. The reason for the disappearance is the complete transfer of electrons from gossypol anions to oxygen molecules with the formation of superoxide anion oxygen. The ratio of the intensities of the components of the fine structure of the observed ESR spectrum is close to the binomial and remains constant as the temperature rises to 60 °C.

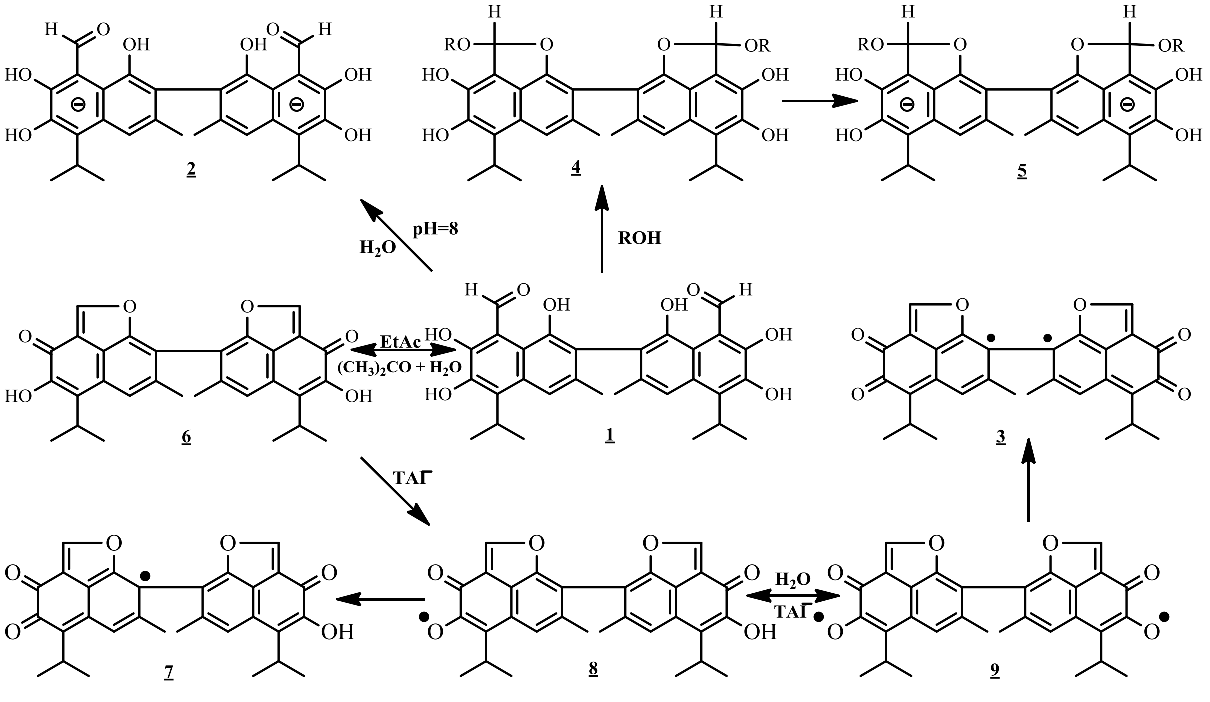

Fig.1. Formation of paramagnetic gossypol derivatives

Alkalinization of gossypol freshly prepared solution in ethyl alcohol leads to the appearance of a similar spectrum of ESD with signs of the presence of new signals. After exposure for 16 hours, a spectrum with a modified number of fine structure components is recorded in the specified sample. At the same time, the ratio of the intensities of the components of the new fine structure clearly does not correspond to the binomial distribution (Fig. 2).

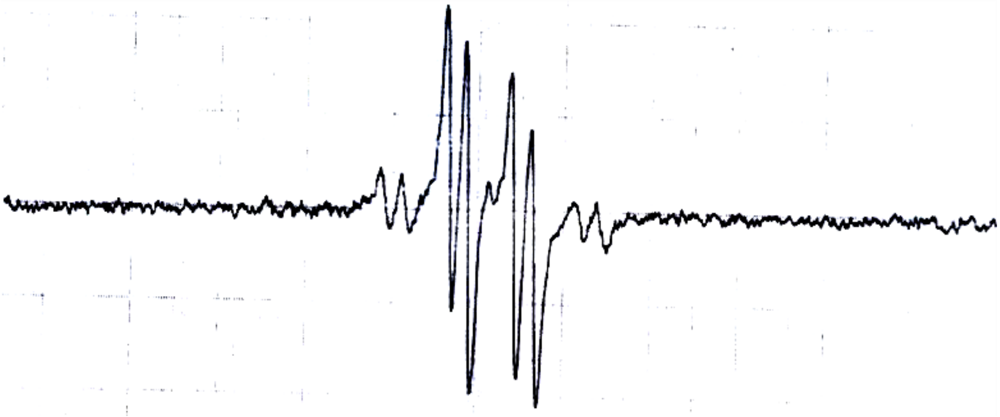

Fig.2. The spectrum of ESR ethanol-alkaline solution of gossypol after 16 hour exposure at pH 7.5.

Changes in the spectrum of ESR in time, noted in alcohol-alkaline solution, are due to the transition of gossypol from the aldehyde form (1) to lactic with the formation of gossypol dilactol diethyl ether (4), to which the spectrum transformed with time (Fig. 2).

The marked deviation from the binomial distribution is a sign of the presence of an intramolecular dynamic process [6]. This process may be the modulation of exchange interactions between paramagnetic centers due to the delocalization of unpaired electrons. These facts show that in the studied solutions there are doubly charged anions (2), the unpaired electrons of which are on non-overlapping additional orbitals in different parts of the binaphthyl skeleton of the structures under study. The splitting of ESR signals in the above spectra is topologically similar to the high-resolution spectrum of ESR of dioxodiohydrogossypol (3) at 70 ° C in castor oil solution, but with changes in the ratios of the splitting of the signals and, as a result, in the length of the spectrum. Castor oil was chosen because of the reliable absence of impurities of paramagnetic gossypol derivatives.

The appearance of superoxide anion oxygen in the plant as a result of electron transfer is a signal to launch a specialized enzyme SOD (superoxide dismutase). As a result, at the interfaces between the phases of the aqueous medium and extracellular lipids, a locally increased concentration of both gossypol and O2 is created in the inactive triplet state. In lipids, O_2 solubility is several orders of magnitude higher than that in an aqueous medium [7], and neutral gossypol is lipophilic. After the recoil of electrons, the neutral molecules of gossypol in a lipophilic medium can approach each other at distances that allow the “mesh” of isopropyl and other groups to form clusters of molecules. Oxygen is distributed throughout the volume of non-polar lipids of extracellular formations, represented mainly by triacylglycerides. It should be noted that in unsaturated lipid structures, their oxygenation occurs due to the formation of complexes with oxygen. In the experiment, this is manifested in the separation of the signals of the nuclei of ^{13}C unsaturated carbon atoms of the olein triacylglycerol fragments in the region of 120 ppm. in carbon tetrachloride. In pure triacylglycerols, these signals are slightly broadened and merge into one signal due to saturation of the medium with oxygen (Fig. 3). In the ESR spectra of the samples under study at temperatures above 350 °C, signals with narrower lines are recorded than at temperatures below 350 °C. The observed line broadening can be explained by the effect of unpaired electrons of molecular oxygen in the ground triplet state. Obviously, to a temperature of about 35 °C, these complexes retain oxygen inside the lipid formations of both the plant and its symbionts of the type of imperfect fungi. A number of researchers in the 70s of the last century noted a high concentration of oxygen in the tissues of a cotton plant infected with a wilt, but there was no explanation for this.

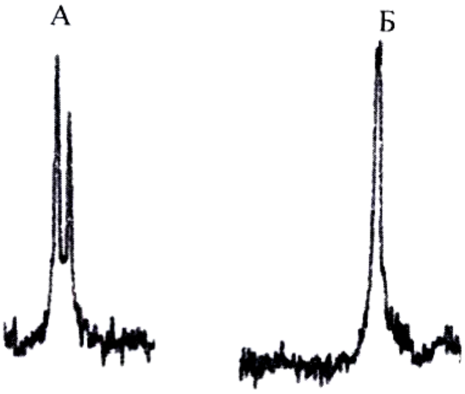

Fig.3. The signals of the nuclei of unsaturated carbon atoms ^{13}C oleic a fragment of triacylglycerides in the region of 120 ppm in the environment of carbon tetrachloride (a) and in pure triacylglycerols (b).

Acetone extracts from tissues of infected cotton plants of V.dahlia have a lighter color (with a yellow tint) than chloroform extracts. As is known, solutions of gossypol and dianhydrogossipol (6) in wet acetone have a yellow color. Obviously, the hydrophobicity of chloroform eliminates the reduction processes characteristic of phenoxyl radicals in the presence of water [8]. The primary radical in the oxidation of dianhydrogossipole (6) must be its phenoxyl mono radical (7). Further oxidation of the mono radical should give the phenoxy diradioxy dipole diradical (8).

These experimental facts suggest that the alternation of short-term stresses and normal conditions for the host-parasite system contributes to the saturation of extracellular lipid formations with molecular oxygen. The current situation is close to the conditions necessary for the development of non-enzymatic chain oxidation of lipids in this system.

The redox cycle of gossypol as the main antioxidant in cotton ensures its enzymatic oxidation and reduction. The enzyme (polyphenol oxidase) in the redox cycle accelerates only reversible chemical reactions. The end product of the non-enzymatic oxidation of dianhydrogossipol is a stable dioradiodixhydrogossipol biradical (3), in which the unpaired electrons of the biradical are protected by steric obstacles from contact with any reagent, especially with the enzyme (Fig. 1). In the enzymatic redox cycle of gossypol, such a stable product should not be formed, otherwise the energy and substance expended on its formation will be unused and the cycle will be interrupted due to the depletion of the initial gossypol pool. The assumption of continuous biosynthesis of gossypol in a plant does not change the conclusion that follows from the above facts about the restriction of the enzymatic oxidation of gossypol in the redox cycle to the formation of only dianhydrogossypol (6), as a substrate for reduction.

Analysis of cotton tissue sections using an electron microscope suggests that the transformation of lipophilic gossypol occurs at the interphase boundaries of immiscible liquids in cotton tissue. Clusters in the form of droplets, viewed in the pictures, change color during the development of the plant. The main content of these clusters is triacylglycerol and gossypol derivatives. Obviously, at the boundary between the aqueous medium and triacylglycerols, oxidation and reduction of dianhydrogossypol can occur, accompanied by a change in the color of cotton fabrics.

The relative stability of the primary phenoxyl form of the diangihydrogossipol biradical (9) can ensure the preservation of its pool in an infected plant. The question of the multiplicity of this form of the biradical has not previously been raised. If we start from the repulsion of unidirectional spins, the triplet state of the phenoxyl biradical can ensure its stability in the non-polar lipid environment. The excitation of the singlet state of the phenoxyl biradical by light can ensure its transition to carbon centered forms of the biradical with the structure of dioxodianhydrogossipole 3 due to the attraction of oppositely directed spins of unpaired electrons.

In this case, one should not expect the appearance of a singlet dioxodiagnohydrogossipole molecule with a double central bond due to the presence of bulky substituents preventing the flat configuration of the binaphtile skeleton rings. Obviously, the stability of dioxodianhydrogossipole 3 in its biradical form may be due to the “alignment” of the spins of unpaired electrons in one direction and cause an irreversible translation of the main cotton antioxidant into this form.

According to the principle of cyclic organization of metabolism, the release of radicals from the metabolite conversion cycle should be considered as the cause of pathology. However, if the radicals in this case become participants in another metabolic cycle, then a specific consideration of the transformations taking place is required. Therefore, we undertook a detailed study of the resonant forms of dioxodianhydrogossipol in order to obtain specific facts of their participation in the metabolism of the plant. To achieve this goal, the optical spectra of all resonant forms of the biradical are considered. The parameters of the spectra were calculated using quantum chemical calculations. It should be noted that the optical spectrum of the biradical obtained in the experiment (3) is the sum of the spectra of its most probable resonant forms. The presence of less probable forms indicates the appearance of deviations from the planar arrangement of the rings under the influence of the forces of the interspin interaction of unpaired electrons in the resonant form. Due to its point nature, the inter-spin interaction of electrons is the strongest within the structure and therefore leads to a distortion of the geometry in the planar arrangement of the rings, which is clearly visible in the model of this form. This reduces the degree of delocalization and the likelihood of distorted resonant form.

The presence of □-bonds in a fragment of a biradical with an unpaired electron on an oxygen atom prevents the delocalization of this electron by the aromatic system of conjugated double bonds. Nevertheless, the polarization of this aromatic system under the action of an unpaired electron provides a shift of the extreme absorption band to a position at 501 nm with considerable intensity. This result refers to the triplet state of the phenoxyl biradical of dianhydrogossipole. For the singlet state of this system, one should overcome the potential barrier in the form of the indicated следует-bonds. Obviously, the change in the environment affects the height of the indicated potential barrier. Adding chloroform to the acetone extract is accompanied by a change in color and the appearance of an absorption band in the 565 nm region, which is characteristic of the carbon centered form of the dianhydrogossiphide biradical, namely dioxodianhydrogossipole (3).

Obviously, a change in the dielectric properties of the medium in this case changes the mutual repulsion of unidirectional electrons in the triplet state in the direction of decrease. This allows electrons to pass into the aromatic system, which complicates the task of determining the structures to which the actually recorded optical spectrum belongs. The delocalization of electrons should lead to the appearance of four symmetric resonant forms of dioxodianhydrogossipole. Distortions in the flat arrangement of the aromatic system are accompanied by a weakening of the probability of occurrence of certain resonant forms. The transfer of electrons to the singlet state will lead to maximum polarization of the structure and the appearance of absorption at 605.6 nm. In the red powder of a freshly isolated biradical (3), this line is absent.

- For intermediates of cyclic enzymatic transformations, providing self-reproduction of the host-parasite system, Eyring [9] formulated the following basic requirements:
- • must be low molecular weight;
- • the energy required for their interconversions at each stage of the cycle should not exceed the thermal energy of the environment:
- • their appearance and disappearance in the cycle should be independent of each other.

The first two requirements are unconditionally fulfilled for gossypol: molecular weight 518 a.u. it is very far from high molecular weight, and its reversible transformations into a dianhydroform (m.v. 482) are reproduced at 250 °C [10]. The third requirement will not be satisfied in the case of the oxidation of the dianhydrogossipole to a stable biradical.

Thus, it follows from these requirements that the redox-cycle of gossypol in a plant should be limited to two substrates of gossypol and dianhydrogossypol.

The stable biradical obtained by nonenzymatic oxidation of dianhydrogossipol forms a complex with the oxidizer O_2 in its ground triplet state. This is due to the triplet state of the spins of the biradical and can be traced by changes in the width of the EPR lines of the biradical spectrum both in the environment of oxygenated lipids and in the solid state in air. The decomposition of the complex into the initial components requires energy expenditure, which is confirmed by the reversible disappearance of the observed broadening after reaching a temperature above 35 °C. The supplied thermal energy is transferred through the mechanism of spin-orbit interaction to the spins of the unpaired electrons of the biradical and molecular oxygen, which ensures the existence of the complex. The energy is spent on changing the quantum state of one of the spins, which leads to the disintegration of the complex. The choice of the indicated spin is carried out according to the rule of the "heavy atom": the change in the quantum state of the spin of the unpaired electron occurs at the "heaviest" of atoms, on which the unpaired electrons are concentrated. In this case, we have the spins of unpaired electrons on two carbon atoms in the biradical and on two oxygen atoms in molecular oxygen. Both pairs in the initial triplet state form a chain of unidirectional spins. In accordance with this rule, it must change the direction of the electron spin on one of the atoms of the O_2 molecule, as on the "heaviest" one. The result of a change in the quantum state of the spin will be the appearance of molecular oxygen in the singlet state of $^1O_2$, in which oxygen is most reactive and fungitoxic for imperfect fungi, including V.dahlia.

IV. EQUATIONS

1. It was established that dioxodianhydrogossipol in oxygenated lipids is a complexing agent for O_2. The quantitative composition of the biradical complex with O_2 depends on the air temperature.

2. The manifestation of the pro-oxidant action of gossypol may be due to the activation of molecular oxygen during the thermal decomposition of its complexes with dioxodiahydrogossipole.

3. It has been established that the complex of dioxodianhydrogossipole with O_2 dissolved in extracellular lipids during disintegration due to a rise in temperature above 30 °C catalyzes the transition of O_2 to the excited singlet state $^1O_2$ with fungicidal properties.

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